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Citotoxicidade *in vitro* da ticlopidina em linhas celulares Hep G2 e Caco 2

Experiência profissionalizante nas áreas de Investigação, Farmácia Hospitalar e Farmácia Comunitária

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Dissertação para obtenção do Grau de Mestre em

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(ciclo de estudos integrado)

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Health Sciences

In vitro cytotoxicity of ticlopidine in Hep G2 and Caco 2 cell lines

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“Se não sais de ti, não chegas a saber quem és.”

José Saramago

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Last but not least, Manuel: "in a mad world, only the mad are sane".

Abstract

The present dissertation is divided into 3 parts.

In the Part I is described the research work conducted at the Health Sciences Research Center of the University of Beira Interior (CICS–UBI, Covilhã) with the aim of evaluating the cytotoxicity of ticlopidine and *Hypericum perforatum* extractin hepatic and intestinal epithelium cell lines.

Ticlopidine is a prodrug mainly used in the prophylaxis of thromboembolic complications in patients with thromboembolic disease, especially if they are aspirin-intolerant. It is associated with multiple drug interactions, mostly via cytochrome P450 (CYP)-inhibition.

H. perforatum has been widely used as an antidepressant, anti-inflammatory and antimicrobial agent, being considered, however, responsible for numerous herb-drug interactions due to the induction of CYP enzymes and P-glycoprotein expression.

Given the predisposition of both these compounds to induce interactions when co-administered with other substances, it was considered relevant to study their cytotoxicity, alone and in combination, in hepatic – Hep G2 – and intestinal epithelium – Caco 2 – cell lines. The cells were incubated with various concentrations of ticlopidine and/or *H. perforatum* extract at various periods of time and the putative cytotoxicity induced by each incubation condition was assessed through cellular viability (MTT) assays.

Overall, the results showed that ticlopidine may be cytotoxic in both Hep G2 and Caco 2 cells, depending on its concentration and period of incubation, and that the simultaneous incubation of cells with both the compounds promotes a similar pattern to that observed when cells are incubated with the extract alone and it is dose-, time- and cell line-dependent.

The Part II refers to the hospital pharmacy internship performed at the *Istituti Fisioterapici Ospedalieri*, in Rome, between February 2nd and April 29th 2015.

Finally, the Part III refers to the community pharmacy internship carried out in the Nunes Feijão Pharmacy, near Barreiro, between May 11th and August 10th 2015.

Keywords

Ticlopidine; *Hypericum perforatum*; Cytotoxicity; Hep G2 cells, Caco 2 cells.

Resumo alargado

A presente dissertação encontra-se dividida em 3 partes.

Na Parte I encontra-se descrito o trabalho de investigação realizado no Centro de Investigação em Ciências da Saúde da Universidade da Beira Interior (CICS-UBI, Covilhã) com o objetivo de avaliar a citotoxicidade da ticlopidina e de um extrato de hipericão em células hepáticas e do epitélio intestinal.

A ticlopidina é um pró-fármaco oral apresentado apenas na forma de comprimidos e utilizado essencialmente na profilaxia de eventos cardiovasculares *major* em pessoas com doença tromboembólica, especialmente se forem intolerantes ao ácido acetilsalicílico. Requer bioativação *in vivo* pelas enzimas do citocromo P450 (CYPs) para formar o seu metabolito ativo, que se liga irreversivelmente ao recetor P2Y12 nas plaquetas, inibindo a sua ativação e agregação induzidas pela adenosina difosfato (ADP) de uma forma tempo- e dose-dependente.

Tratando-se de um potente inibidor mecanismo-dependente de algumas CYPs, inibindo até o seu próprio metabolismo, a ticlopidina está associada a múltiplas interações farmacológicas e não farmacológicas. Além disso, apresenta diversas reações adversas hematológicas, pelo que o seu uso deve ser monitorizado nos primeiros três meses de tratamento. Os efeitos adversos são reversíveis após a retirada do fármaco.

O hipericão, uma das mais antigas e bem estudadas plantas medicinais, tem sido amplamente utilizado como agente antidepressivo, anti-inflamatório e antimicrobiano. Apresenta-se sob diversas formas farmacêuticas, sendo os extratos considerados como a mais importante. Barato e fácil de obter, torna-se uma alternativa às terapias farmacológicas comuns, sem apresentar efeitos adversos *major*.

Encontram-se identificados mais de 150 constituintes: a hipericina é considerada o composto farmacologicamente mais importante, enquanto à hiperforina é atribuída a responsabilidade pela maioria das interações medicamentosas. De facto, o hipericão é um potente indutor das CYPs e da expressão da glicoproteína-P (P-gp). De acordo com o efeito farmacológico demonstra diversos mecanismos de ação; enquanto antidepressivo, atua de uma forma semelhante aos inibidores seletivos da recaptação de serotonina, podendo originar um síndrome serotoninérgico ou interações farmacodinâmicas se coadministrado com fármacos que aumentam a sinalização deste neurotransmissor.

Dada a predisposição de ambos os referidos compostos para induzir interações quando coadministrados com outras substâncias considerou-se relevante estudar a sua citotoxicidade, sozinhos e em associação, em células hepáticas – Hep G2 – e do epitélio intestinal – Caco 2. As células foram incubadas com várias concentrações de ticlopidina e/ou de extrato de hipericão durante vários períodos de tempo e a citotoxicidade putativa induzida por cada condição de incubação foi avaliada através de ensaios de viabilidade celular (MTT).

No geral, a ticlopidina mostrou-se significativamente citotóxica em ambas as linhas celulares apenas na concentração de 200 μM; todavia, as células revelaram uma recuperação da viabilidade celular após 24 h de incubação.

Quanto ao hipericão, apresentou maior toxicidade na linha celular hepática, comparativamente aos resultados obtidos para a linha celular intestinal. Mais uma vez, as células recuperaram e voltaram a proliferar após 72 h de incubação.

Da incubação com ambos os compostos obtiveram-se resultados semelhantes aos obtidos na incubação apenas com hipericão, principalmente nas células Caco 2. Nas células Hep G2, a incubação com ambos os compostos mostrou-se menos citotóxica que a incubação com hipericão sozinho.

A recuperação da viabilidade celular apresentada por ambas as linhas celulares na incubação com os compostos sozinhos ou em combinação sugere, nas células Hep G2, a formação de conjugados de glutationa não tóxicos e, nas células Caco 2, um mecanismo de efluxo pela P-gp.

Em suma, os resultados obtidos mostraram que a ticlopidina pode ser citotóxica em ambas as linhas celulares Hep G2 e Caco 2, dependendo da sua concentração e do período de incubação, e que a incubação simultânea das células com ambos os compostos promove um padrão similar ao observado quando as células foram incubadas com o extrato sozinho, sendo a toxicidade dependente da dose, do tempo e da linha celular.

A Parte II refere-se ao estágio em farmácia hospitalar realizado nos *Istituti Fisioterapici Ospedalieri*, em Roma, entre 2 de fevereiro e 29 de abril de 2015.

Finalmente, a Parte III refere-se ao estágio em farmácia comunitária feito na Farmácia Nunes Feijão, perto do Barreiro, entre 11 de maio e 10 de agosto de 2015.

Palavras-chave

Ticlopidina; Hipericão; Citotoxicidade; Células Hep G2, Células Caco 2.

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List of Acronyms

5-HT	Serotonin
AD	*Anno Domini*
ADP	Adenosine diphosphate
ADR	Adverse drug reaction
AIFA	*Agenzia Italiana del Farmaco*
API	Active pharmaceutical ingredient
ATC	Anatomical Therapeutic Chemical
AUC	Area under curve
BMI	Body mass index
BSA	Body surface area
CCK	Cholecystokinin
C_{max}	Peak plasma concentrations
CNS	Central nervous system
CO_2	Carbon dioxide
COMT	Cathecol-*O*-methyltransferase
CYPs	Cytochrome P450 enzymes
DH	Day Hospital
DMEM-C	Dulbecco's Modified Eagle's Medium – high glucose complete
DMEM-I	Dulbecco's Modified Eagle's Medium – high glucose incomplete
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediamine tetraacetic acid
FBS	Fetal bovine serum
FDA	Food and Drug Administration
GABA	Gamma-aminobutyric acid
GSH	Glutathione
HbA1C	Glycated hemoglobin A1c
HCl	Hydrochloric acid
HMG-CoA	3-hydroxy-3-methyl-glutaryl-CoA
H. perforatum	*Hypericum perforatum*
IFO	*Istituti Fisioterapici Ospitalieri*
INFARMED	National Authority of Medicines and Health Products
IRCCS	*Istituti di Ricovero e Cura a Carattere Scientifico*
IRE	*Istituto Nazionale Tumori Regina Elena*
ISG	*Istituto Dermatologico San Gallicano*
LDL	Low-density lipoprotein
MAO	Monoamine oxidase

MPO	Myeloperoxidases
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NFP	Nunes Feijão Pharmacy
NHS	National Health System
OATP-B	Organic anion transporting polypeptide B
OTC	Over the counter
P-gp	P-glycoprotein
PAF	Platelet activating factor
PBS	Phosphate-saline buffer
RNF	*Rete Nazionale di Farmacovigilanza*
RPMI-C	Roswell Park Memorial Institute 1640 Medium complete
RPMI-I	Roswell Park Memorial Institute 1640 Medium incomplete
SSRI	Selective serotonin reuptake inhibitor
$t_{1/2}$	Elimination half-life
UMaCA	*Unità di Manipolazione di Chemioterapici Antiblastici*
UV	Ultraviolet
TRPA1	Transient receptor potential ankyrin1
VAT	Value-added tax
VEGF	Vascular endothelial growth factor
WHO	World Health Organization

Part I – *In vitro* cytotoxicity of ticlopidine in Hep G2 and Caco 2 cell lines

1. Introduction

1.1. Ticlopidine

1.1.1. Historical context and general characterization

Ticlopidine is a first generation thienopyridine [1], synthesized for the first time and patented in Germany in 1974 [2, 3]. Thienopyridines are heterocyclic compounds containing a thiophene ring fused to a pyridine ring [4].

It was first used in 1978 [5] and introduced in the German market in 1979 [6]. In the United States of America, ticlopidine was patented in 1977 [3] and received its market approval by the Food and Drug Administration (FDA) in October 1991 [7, 8], being available for general clinical use in December 1991 [9, 10]. Nevertheless, in Portugal, it was approved only since May 15th 1981 [11].

It was studied as an anti-inflammatory agent, but its potent antiplatelet effects were more remarkable [1], being therapeutically used in the prophylaxis of thromboembolic complications in patients with thromboembolic disease, especially if they are intolerant to aspirin [12].

The recommended dosage is 250 mg orally, twice a day with meals [6, 13]. Commercially, the unique available form is a 250 mg tablet of a hydrochloride salt [13, 14]. In Portugal, it is presented as coated tablets and film-coated tablets [14] and the commercial name is *Tiklyd*® [11].

Due to the reported idiosyncratic adverse reactions, especially blood disorders and drug induced liver injuries, attributed to ticlopidine, it was given a FDA black box warning for life threatening hematological adverse reactions [8, 15]. It is not genotoxic or cardiogenic [16].

It was referred by the Portuguese National Pharmacovigilance newsletter in 1997 due to an adverse reaction identified, a bone marrow depression [17], and in 1999 in the form of a reminder that its use must be monitored in the first 3 months of treatment (when adverse reactions are most likely to occur) [18].

1.1.2. Physical and chemical characteristics

Chemically, ticlopidine is the 5-(*o*-chlorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-c]pyridine [3].

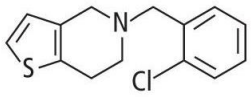

Figure 1. Chemical structure of ticlopidine (adapted from [19]).

Ticlopidine is a highly hydrophobic molecule [20], positively charged at physiological pH [21]. It exhibits low aqueous solubility and it improves the rheological properties of blood [22, 23]. Its commercially available formula, ticlopidine hydrochloride, is freely soluble in water and in methanol, moderately soluble in methylene chloride and ethanol and slightly soluble in acetone [13].

1.1.3. Pharmacokinetics

The thienopyridines, and thus ticlopidine, are oral prodrugs that require *in vivo* bioactivation, consisting in the conversion into a pharmacologically active metabolite containing a thiol moiety. This thiol metabolite binds specifically and irreversibly to the P2Y12 receptor, inhibiting platelet activation and aggregation in a time- and dose-dependent manner [6, 24].

Ticlopidine exhibits a non-linear pharmacokinetics [6]. When administered orally, approximately 85 % is absorbed, with a peak plasma concentration (C_{max}) after 2 h [6]. Bioavailability is decreased in about 18 % by co-administration with antacids and increased approximately 20 % if the drug is co-administrated with food [13, 24].

The median C_{max} after the first dose is 310 ng/mL, increasing to 990 ng/mL after 21 days of treatment [6]. The onset of activity of the recommended daily dose of ticlopidine (250 mg twice a day) is observed after 24 to 48 h, reaching its maximal activity within 3 to 5 days [6]. Previous authors verified that the delayed onset of action is related with its need for *in vivo* bioactivation [24].

The steady-state concentrations in the plasma are reached in 5 days in adults and in 2 to 3 weeks in elderly people [6]. The vast majority of ticlopidine (98 %) is bound to plasma proteins [6], mainly to serum albumin and lipoproteins [13]. The median elimination half-life ($t_{1/2}$) after multiple dosing is 29 h in adults, while in older patients it is of 4 to 5 days [6]. These data suggest that ticlopidine inhibits its own metabolism, probably by inhibiting the cytochrome P450 enzymes (CYPs) CYP2B6 and CYP2C19 [6].

Ticlopidine is rapidly and extensively metabolized in the liver, mainly through CYPs [6, 25]. Until now, at least 13 metabolites have been identified [8, 26]; among them are ticlopidine N-oxide, a hydroxy compound (non-sulfur-containing), a lactam [6, 27, 28] and dihydrothienopyridinium and thienopyridinium compounds [29].

The CYP2C19 and the CYP2B6 transform the parent drug in 2-oxo-ticlopidine, a thiolactone intermediate, by S-oxidation and epoxidation on the thiophene ring [6, 27]. However, the CYPs that intervene in the formation of the active metabolite, [1-(2-chlorobenzyl)-4-mercaptopiperidin-(3Z)-ylidene] acetic acid [28], from the 2-oxo-ticlopidine

intermediate remain unknown, as well as the ones which contribute to the *N*-dealkylation of ticlopidine to 2-chlorohippuric acid and tetrahydrothienopyridine [6].

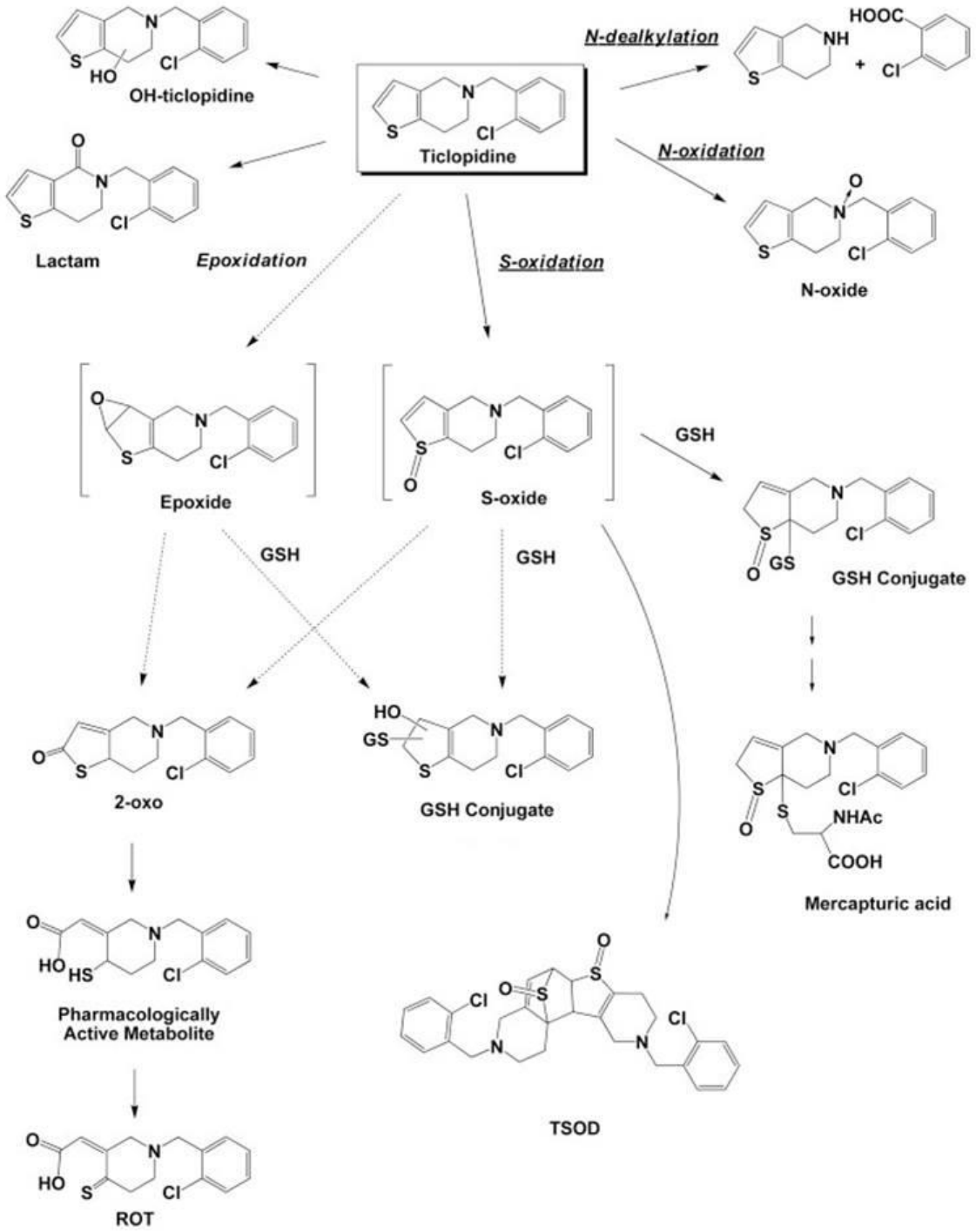

Figure 2. Metabolic pathways of ticlopidine (ROT: [1-(2-chloro-benzyl)-4-thioxo-piperidin-(3Z)-ylidene]-acetic acid, TSOD: ticlopidine S-oxide dimer, metabolites found *in vitro* only; adapted from [28]).

The reactive intermediates of ticlopidine formed by thiophene-epoxidation, *S*-oxidation and epoxidation of the chlorophenyl ring can be trapped by glutathione (GSH), leading to protein covalent bindings [25, 28]. Additionally, one of the *S*-oxide GSH-conjugates is transformed in the correspondent mercapturic acid [28].

In activated neutrophils, myeloperoxidases (MPO) also contribute to the metabolism of ticlopidine; the major metabolite attained by this pathway is a dehydro-ticlopidine [30]. Through this mechanism, the parent drug is oxidized by MPO to a reactive intermediate, thiophene-*S*-chloride, that can be rearranged to form 2-chloroticlopidine or be trapped by GSH to form a conjugate [30]. Although this pathway does not significantly contribute to the clearance of ticlopidine, the reactive intermediate is likely to be responsible for the ticlopidine-induced bone marrow toxicity [30].

The clearance of ticlopidine diminishes significantly with repeated dosage [6]. *N*-dealkylated and *N*-oxide metabolites appear rapidly in the urine [6, 28]**.** About 60 % of the dose is excreted in the urine and unchanged ticlopidine is present only in trace amounts [6, 25, 28]. Ticlopidine is a substrate of the P-glycoprotein (P-gp) and it is subject to intestinal absorption [4]. Also, almost 8 % of the unchanged drug is eliminated in the feces, through excretion in the bile and/or due to lack of absorption [6].

Table 1. Summary of pharmacokinetic characteristics of ticlopidine.

	Parameter	Values	References
Absorption	Oral absorption	≈ 85 %	[6]
	Time to peak plasma concentration	2 h	[6]
	Peak plasma concentration	310 ng/mL (adults) 990 ng/mL (elderly)	[6]
	Onset of activity	24-48 h	[6]
	Time to maximal activity	3-5 days	[6]
	Time to steady-state concentrations	5 days (adults) 2-3 weeks (elderly)	[6]
Distribution	Plasma protein bounding	98 %	[6]
Metabolism	Elimination half-life	29 h (adults) 4-5 days (elderly)	[6]
Excretion	Unchanged ticlopidine	Urine: traces Feces: 8 %	[6, 25, 28]
	Metabolites	Urine: 60 %	[6]

1.1.4. Pharmacodynamics

As mentioned, ticlopidine is a thienopyridine and, as such, it must undergo biotransformation to its active metabolite, [1-(2-chlorobenzyl)-4-mercaptopiperidin-(3Z)-ylidene] acetic acid. It irreversibly antagonizes the platelet P2Y12 receptor, inhibiting the adenosine diphosphate (ADP)-induced platelet activation and aggregation selectively and irreversibly [6, 19]. The active moiety of the active metabolite is a reactive thiol derivative [19, 24], which forms an irreversible disulfide bridge with the cysteine residues of the P2Y12 receptor [19, 31], preventing the binding of the P2Y12 agonist 2-methylthio-ADP [19]. This results in an ADP-induced adenylyl cyclase downregulation, affecting the platelet aggregation triggered by ADP and by other ADP-dependent substances [19, 24].

Thienopyridines inhibit shear- and platelet activating factor (PAF)-induced platelet aggregation and enhance the susceptibility of platelet aggregates to disaggregate [24, 32, 33]. They also decrease circulating levels of fibrinogen; inhibit erythrocyte aggregation, the expression of tissue factor on endothelial cells and fibronectin synthesis and stimulate nitric oxide production [24].

Additionally, due to the irreversible nature of the interaction with ticlopidine [26], platelets are inhibited even when no active metabolite is detected in plasma [19] and the platelet function is recovered only 11 to 13 days after discontinuation of the treatment [26].

The collagen- and the thrombin-induced platelet aggregation are also inhibited, but only to some extent, probably due to blockade of amplification [8, 34]. Ticlopidine also exhibits a vasomodulatory actions that apparently contribute to its therapeutic effects in ischemic syndromes and coronary events [35].

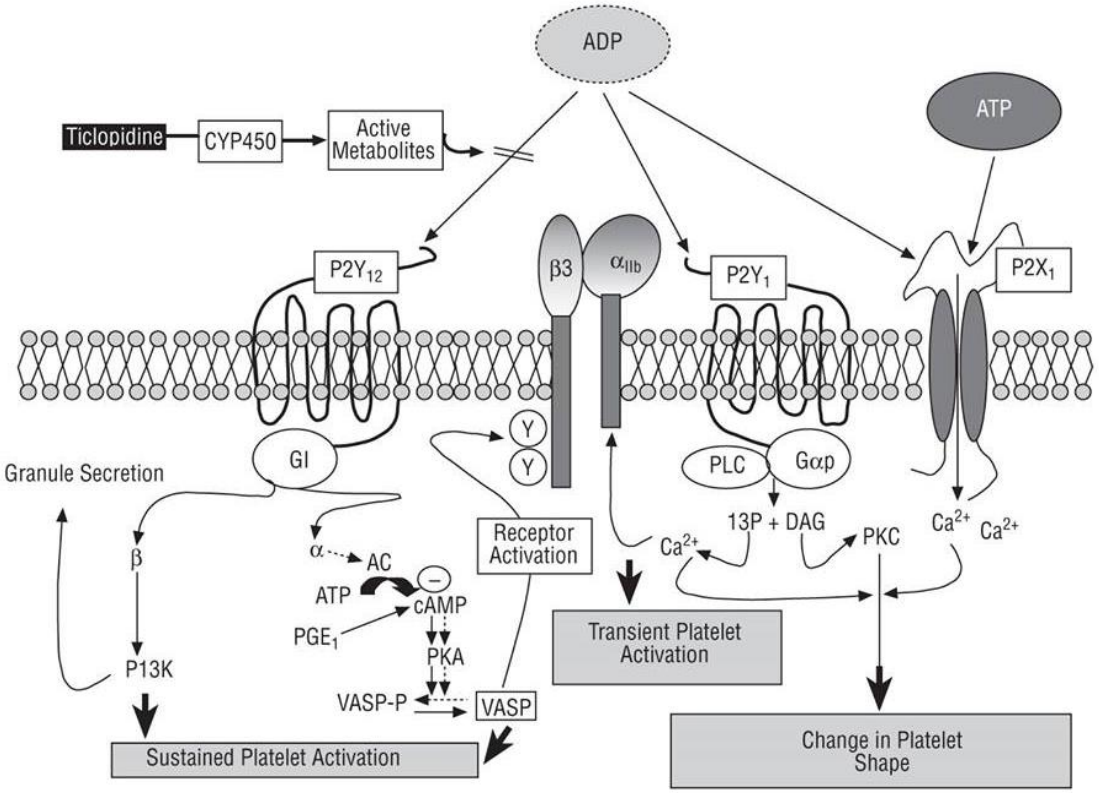

Figure 3. ADP platelet receptor signaling pathways and site of action of ticlopidine (adapted from [36]).

Recently, it has been found that ticlopidine activates the Ca^{2+}-permeable ion channel transient receptor potential ankyrin1 (TRPA1), which can thereafter trigger the release of serotonin (5-HT) and/or cholecystokinin (CCK) [37]. A case report from Matsui *et al*. (2004) also stated that ticlopidine decreased vascular endothelial growth factor (VEGF) levels in POEMS syndrome through inhibition of ADP-induced platelet aggregation, resulting in an α-granule release and a consequent reduction in VEGF releasing from platelets [38].

1.1.5. Therapeutic applications

Ticlopidine is indicated and widely used in the secondary prevention of major vascular events, namely stroke, in patients with a history of cerebrovascular, coronary or peripheral artery disease or at high risk for thrombotic stroke, especially if they are intolerant or allergic to aspirin [8, 24, 39].

It is also effective in the long-term management of ischemic stroke and claudication [40], as well as in the treatment of unstable angina, coronary artery bypass graft and acute occlusion after angioplasty [41].

Ticlopidine is contraindicated in patients with active bleeding, preexisting neutropenia or thrombocytopenia, history of thrombotic thrombocytopenic purpura or aplastic anemia, severe hepatic dysfunction or hypersensitivity to the drug [39].

1.1.6. Adverse and toxic effects

In general, drugs used at higher daily doses (more than 50 mg) exhibit an increased incidence of idiosyncratic drug reactions [27]. Also, drugs with considerable protein binding should be taken in lower doses [27]. The dose of ticlopidine is 250 mg twice a day and it circulates almost completely bond to plasma proteins (98 %), so it applies to both these cases.

The peak incidence of adverse reactions to ticlopidine is at 3 to 6 weeks of treatment and they become rare after 3 months [8, 42]. During the first 3 months, hematological monitoring should be performed every 2 weeks [8, 42].

The most commonly reported adverse effects are nausea, vomiting and diarrhea, frequently accompanied by abdominal cramp [9, 24], and a recent study showed that these adverse effects may be due to the activation of TRPA1, with the subsequent release of 5-HT and possibly CCK [37].

More serious and rarer effects include bleeding (minor or major), bone marrow suppression – neutropenia and agranulocytosis, thrombocytopenia (including thrombotic thrombocytopenic purpura), severe aplastic anemia, cholestatic alterations, colitis, hepatotoxicity, rash and arthritis [9, 11, 24]. Regarding the hepatotoxicity of ticlopidine, a previous study showed that it is probably due to a GSH saturation and depletion, leading to an increased concentration of reactive metabolites [43].

The high incidence of agranulocytosis due to the use of ticlopidine is probably related with its metabolism to reactive intermediates by activated neutrophils [29, 30] or with non-metabolized ticlopidine in the bone marrow [29].

Although, the adverse effects of ticlopidine are usually reversible after withdrawal of the drug [42].

1.1.7. Pharmacological and non-pharmacological interactions

Ticlopidine proved to be a potent mechanism-based inhibitor of the CYP2B6 and the CYP2C19, *in vitro* [6, 44, 45]. These authors also observed that the 2-oxo-ticlopidine (thiolactone metabolite) inhibits the CYP2B6 isoform similarly to ticlopidine [6, 45]. The parent drug also inhibits the CYP1A2 and the CYP2D6 [6]. A mechanism-based inhibition involves the metabolic activation of ticlopidine by the CYP2C19 and the CYP2B6 to a reactive intermediate, 2-oxo-ticlopidine, which can irreversibly modify the CYPs by forming a disulfide bond with an available cysteine, either after hydrolysis or after another cycle of CYP-dependent oxidation [45, 46]. Frequently, this results in drug-drug interactions, as the inactivated CYPs have to be replaced by newly synthesized ones [45].

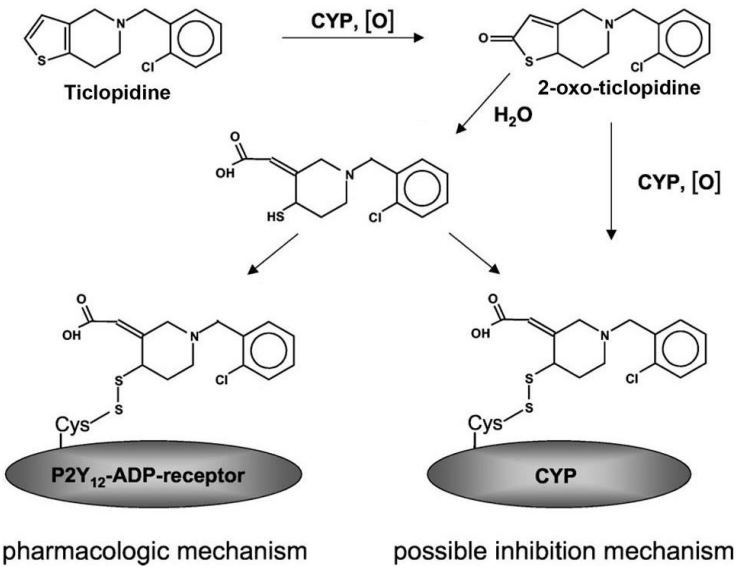

Figure 4. Possible mechanism for the irreversible inhibition of the CYP2B6 and the CYP2C19 by ticlopidine (adapted from [46]).

Table 2. Summary of the drug-drug interactions associated with ticlopidine and its downregulation of the expression of various CYPs.

Drug	Inhibited CYP	Effect of the interaction with ticlopidine	References
Theophylline	CYP1A2	Decreases the clearance and increases the elimination $t_{1/2}$	[6]
Phenytoin	CYP2C19	Inhibits the clearance and elevates the plasma concentrations, with consequent symptomatic toxicity	[6, 21, 47]
Omeprazole	CYP2C19	Significant decrease in the apparent clearance	[6]
Bupropion	CYP2B6	Inhibits its metabolism (possible occurrence of seizures)	[6, 48, 49]
Tramadol	CYP2B6 and/or CYP2D6	Decreases the renal clearance and inhibits the metabolism, with increased exposure to tramadol	[50]
Carbamazepine	CYP3A4	Increases the levels of carbamazepine	[42, 51]
Cyclosporin	CYP3A4	Reduces the blood levels of cyclosporin	[47, 52, 53]

Various interactions have been described in literature. In fact, ticlopidine interacts with digoxin, cimetidine, phenobarbital and propranolol [8, 11, 13]. Despite their competition for the CYP2C19 isoform, proton-pump inhibitors are commonly administered with ticlopidine for a prophylactic purpose, to help reduce the risk of gastrointestinal bleeding [54, 55].

Nonetheless, not all the pharmacological interactions with ticlopidine are adverse; some have synergistic effects. That is the case with aspirin, a nonsteroidal anti-inflammatory drug (NSAID), commonly used in dual antiplatelet treatment with ticlopidine [33, 56]. They may potentiate each other by the combination of different mechanisms of action [57]: while ticlopidine inhibits platelet aggregation induced by ADP and PAF, aspirin inhibits platelet aggregation induced by arachidonic acid – all the three mechanisms involved in platelet activation [32, 33]. However, this therapy is frequently associated with hemorrhagic adverse events, namely lower gastrointestinal tract bleeding [33, 58] and, in some cases, a triple therapy (ticlopidine, aspirin and warfarin) may be preferential, although the increased risk of hemorrhagic complications [56].

The concomitant administration of ergoloid mesylates with ticlopidine decreases plasma ticlopidine concentrations by inhibiting its absorption and, thus, its bioavailability [59]. Also, caffeine, the most widely used psychoactive substance and with extensively described neuroprotective properties, has its CYP1A2-catalysed metabolism inhibited by ticlopidine [60].

Regarding the herb-drug interactions, the concomitant use of *Ginkgo biloba* with ticlopidine increases the antithrombotic and antiplatelet effects of the drug, with reduced tendency for adverse events and no significant alterations in the pharmacokinetic parameters of ticlopidine [59, 61]. In fact, *Ginkgo biloba* has neuroprotective and antioxidant properties and it is a direct inhibitor of platelet aggregation [61].

1.2. *Hypericum perforatum*

1.2.1. General characterization

Hypericum perforatum (*H. perforatum*), also known as St. John's wort, is one of the oldest and best studied medicinal plants [62, 63]. It has been used since the Greek and the Roman civilizations [64] and it is commercially available in several forms: capsules, liquid extracts, oils, ointments and dried extracts, which are considered the most important preparations [63, 64].

H. perforatum has been widely used as an antidepressant, anti-inflammatory and antimicrobial agent [64, 65]. In fact, it displays an unusual combination of safety and effectiveness, as it has less side effects than some standard drug therapies, and it is also easy and cheap to obtain [64]. However, several studies showed that it can be responsible for numerous herb-drugs interactions, mainly due to the induction of some CYPs and the P-gp [66].

1.2.2. Bioactive compounds

Until now, more than 150 constituents have been identified in *H. perforatum*.

As shown in Figure 5, its main constituents are: naphthodianthrones (hypericin and pseudohypericin), xanthones, flavonoids and phloroglucinols (hyperforin and adhyperforin) [62]. Amongst them, hypericin is considered the most pharmacologically important compound and hyperforin is assumed as the responsible for most of the *H. perforatum* interactions [62, 63].

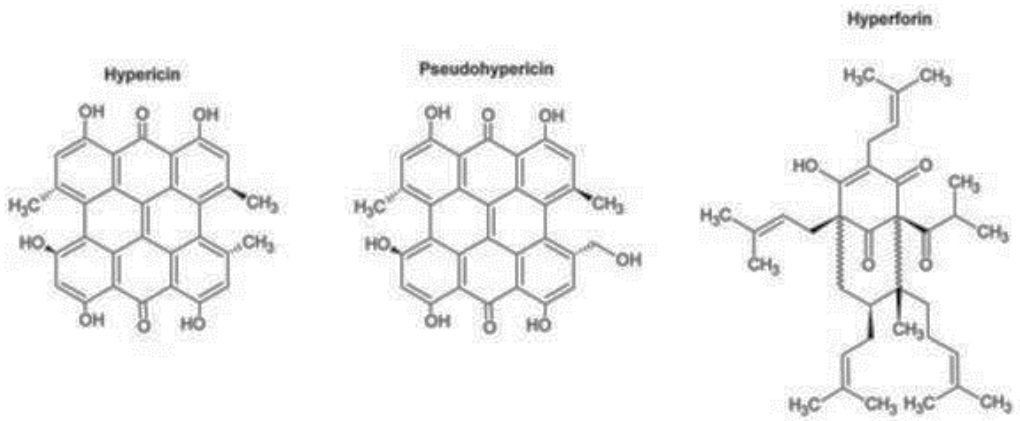

Figure 5. Chemical structures of hypericin, pseudohypericin, and hyperforin (adapted from [62]).

The concentrations of the bioactive compounds differ between the different parts of the plant. Most clinical studies have used *H. perforatum* extracts standardized to 0,2 % to 0,3 % hypericin. The required daily dose of dry extract to achieve antidepressant effects is 900 mg, ranging from 600 mg to 1200 mg, three times a day [62].

1.2.3. Pharmacodynamics

Despite the structural similarity between hypericin, pseudohypericin and hyperforin, these components are pharmacodinamically different [62].

H. perforatum extract and hyperforin alone inhibit the reuptake of several neurotransmitters, including 5-HT, noradrenaline, dopamine, glutamate and gamma-aminobutyric acid (GABA). These are involved in the regulation of mood, motivation and reward [62]. As an antidepressant, the proposed mechanisms of action also include inhibition of monoamine oxidase (MAO) by hypericin and by the flavonoids fraction of the extract, inhibition of cathecol-O-methyltransferase (COMT) and suppression of the release of interleukin-6 (which modulates the release of cortisol) [62, 63]. The subchronic treatment with *H. perforatum* triggers a downregulation of ß-adrenergic receptors [62, 63]. In the central nervous system (CNS), the extract may also interfere with the processes of polymerization of ß-amyloid, a peptide responsible for the onset and progression of Alzheimer's disease, thus preventing it [62].

The antiviral effects are associated with photoactivated hypericin. It requires an intact membrane or cell surface and it acts at the level of the anchor assembly or virus, binding to the lipid membrane by inhibition of viral fusion and syncytium formation [62, 65]. Other possible mechanisms are a direct action against the capsid of the virus by inhibiting its mobility, inhibition of protein kinase C phosphorylation of the receptor involved in CD4 and inhibition of tyrosine kinase [62]. Substances with antimicrobial, antibacterial and antifungal activity (essential oils, phloroglucinols, flavonoids and tannins) are also present in the extract, giving it antibacterial activity, even in topical use [62].

Previous studies also reported anticancer properties in *H. perforatum* extracts, due to its capacity to inhibit the nuclear factor κB and to block the endothelium migration in response to inflammatory cytokines [62].

1.2.4. Therapeutic applications

H. perforatum is used in the treatment of mild to moderate depression, demonstrating a similar activity comparing to other conventional antidepressants, without presenting its major side effects [62, 63]. It can also be effective in the treatment of generalized anxiety, somatoform, sleep, obsessive–compulsive and seasonal affective disorders [62, 63].

Additionally, various studies support its burn- and wound-healing, antimicrobial and antiviral properties and potential in the treatment of cancer and inflammatory disorders and its use as an antioxidant and neuroprotective agent [62]. In gastritis and gastric ulcer, *H. perforatum* extract is smoothing and it heals [62].

Table 3. Important biological compounds found in *H. perforatum* (adapted from [63]).

Active compounds	Examples	Action
Naphthodianthrones (lipophilic)	hypericin pseudohypericin	antidepressant, antiviral, photosensitizer
Phloroglucinols (lipophilic)	hyperforin adhyperforin	antidepressant, antibiotic
Flavonoids (lipophilic/hydrophilic)	quercetin hyperoside quercitrin isoquercitrin rutin	antidepressant, anti-inflammatory
Biflavonoids (lipophilic)	biapigenin	sedative, anti-inflammatory
Procyanidins (hydrophilic)	procyanidin catechin epicatechin	anti-inflammatory, antioxidant
Essential oils (lipophilic)	terpenes alcohols	n.d.
Amino acids (hydrophilic)	GABA	antidepressant
Phenylpropanes (hydrophilic)	caffeic acid chlorogenic acid	
Xanthons (lipophilic)	norathyriol	antidepressant

(n.d. – not determined)

1.2.5. Adverse and toxic effects

H. perforatum appears to be well tolerated [62, 63]. Nonetheless, previous studies reported some adverse effects, including: gastrointestinal complaints, nausea, rash, asthenia, dizziness, allergic reactions and, rarely, photosensitivity reactions [62, 63]; the latter are attributed to the presence of hypericin and pseudohypericin (naphthodianthrones) [62, 63].

Furthermore, confusion, restlessness, lethargy, dry mouth and psychotic events were also side effects reported to *H. perforatum* [62]. The serotonin syndrome was also already described as an adverse effect of this plant as it acts as a selective serotonin reuptake inhibitor (SSRI), increasing the serotonergic stimulation [62, 63].

1.2.6. Pharmacological interactions

According to literature, *H. perforatum* strongly increases the CYP3A4, CYP2E1, CYP2C19 and the P-gp expression. Moreover, several studies reported pharmacokinetic interactions when it is used in combination with drugs that are metabolized by these enzymes or transported by the P-gp [62, 66]. Additionally, pharmacodynamic interactions may arise when the extract is concomitantly used with drugs that increase serotonin signaling in the brain [66].

As a non-prescription medicine, it is frequently consumed/administrated in combination with other drugs, increasing the potential for the occurrence of several herb-drug interactions [62], some of them already identified as summarized and presented on Table 4.

Table 4. Pharmacological interactions with *H. perforatum* (adapted from [62]).

Prescribed drug		**Possible mechanism**	**Effects of the interaction with *H. perforatum***
CNS	*SSRIs, buspirone and bupropione*	Additive effects on 5-HT reuptake inhibition	Possible serotonin syndrome
	Benzodiazepines (alprazolam and midazolam)	Induction of CYP3A4 and CYP2C19	Reduction of the AUC and $t_{1/2}$, significant increase of oral clearance
	Amitriptyline and nortriptyline	Induction of CYP3A4 and CYP2C19	Reduced AUC
	Phenytoin	Induction of CYP2C19	Increased urinary excretion of phenytoin metabolites
	Zolpidem	Induction of CYP3A4	Decreased plasma concentration
	Clozapine	Induction of CYP1A2 and CYP3A4	Decreased AUC
General anesthetics	*Fentanyl, propofol and sevoflurane*		Delayed onset of action
Opioids	*Methadone and pethidine*	Induction of CYP2D2	Reduction of plasmatic concentration and abstinence syndrome
	Dextromethorphan and oxycodone	Induction of CYP3A4	Reduction of plasmatic concentration
NSAIDs	*Ibuprofen*	Increased expression of glycoprotein G	Reduction of plasmatic concentration
Corticosteroids	*Dexamethasone, prednisone and budesonide*	Induction of CYP3A4	Reduction of plasmatic concentration
Antihistamines	*Fexofenadine*	Induction of P-gp	Increased maximum plasma concentration and decreased oral clearance
Bronchodilators	*Theophylline*	Induction of CYP	Decreased plasma concentration

Table 4 (continued). Pharmacological interactions with *H. perforatum* (adapted from [62]).

Prescribed drug		**Possible mechanism**	**Effects of the interaction with *H. perforatum***
Cardiovascular	*Warfarin*	Particle formation with *H. perforatum* in aqueous solution, induction of CYP3A4	Loss of the anticoagulant effect, significant reduce in the pharmacologic effect of racemic warfarin
	Phenprocoumon	Induction of CYP3A4	Decreased plasma levels of phenprocoumon
	Nifedipine	Induction of CYP3A4 and CYP2C19	Induced metabolism with increased plasma concentrations of dehydronifedipine
	Verapamil	Induction of first-pass CYP3A4 metabolism	Reduced bioavailability of verapamil
	Digoxin	Induction of P-gp	Decreased intestinal absorption and reduction of plasma AUC and C_{max}
Hypolipidemic	*Atorvastatin*	Increases CYP3A4 and P-gp activity	Increased LDL, increased total cholesterol
	Simvastatin	Decreases plasma concentrations	Increased LDL
Hypoglicemic	*Gliclazide and tolbutamide*	Induction of CYP and P-gp	Decreased plasma concentrations of gliclazide and tolbutamide
Gastrointestinal	*Omeprazole, esomeprazole and pantoprazole*	Induction of CYP2C19	Decreased plasma concentration
Gastrointestinal	*Loperamide*	Induces a MAO inhibitor-drug reaction	Brief episode of delirium
Oral contraceptives	*Etinilestradiol and desogestrel*	Induction of CYP3A4	Reduction of plasmatic concentration, bleeding and pregnancies
	Etinilestradiol and noretindrone		Increased clearance of noretindrone and decreased $t_{1/2}$ of etinilestradiol; increased metabolism of both
Immuno-suppressants	*Cyclosporine and tacrolimus*	Induction of CYP and P-gp	Decreased plasma concentration and organ rejection
Antineoplastic	*Imatinib, irinotecan and docetaxel*	Induction of CYP3A4 and P-gp	Decreased plasma concentration, altered hepatic metabolism, decreased clinical efficacy
Antimicrobial	*Voriconazole*	Induction of CYP3A4, CYP2C19 and CYP2C9	Decreased AUC
	Erythromycin	Induction of CYP3A4	Increased metabolism (decreased AUC)
	Indinavir	Induction of CYP3A4	Decrease in AUC

2. Aims

Even though ticlopidine was synthesized for the first time more than 40 years ago, it is not an extensively studied drug. Due to its bone marrow toxicity, it was almost completely substituted by clopidogrel, a second generation thienopyridine, and most recently by new generation thienopyridines, as prasugrel, cangrelor and ticagrelor. *H. perforatum*, on the other hand, is a widely used herbal extract with plenty of pharmacokinetic and pharmacodynamic interactions documented, some of which with drugs also used in the cardiovascular system.

The main aim of this study was to evaluate and to determine the cytotoxicity of ticlopidine and *H. perforatum* hydroalcoholic extract in hepatic – Hep G2 – and intestinal – Caco 2 – cell lines incubated with various concentrations and for different periods of time. Moreover, the co-incubation of cells with both compounds also allowed the identification of a putative joint action promoted by these compounds in these cell lines.

For that purpose, MTT assays were performed, aiming to determine the cellular viability/cytotoxicity of the cells following their incubation with the mentioned compounds alone and combined.

3. Material and methods

3.1. Material

3.1.1. Compounds, solutions and culture media

- 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich Co. LLC)
- 70 % ethanol
- 100 % ethanol
- Dimethyl sulfoxide (DMSO, Sigma Aldrich Co. LLC)
- Dulbecco's Modified Eagle's Medium – high glucose complete (DMEM-C, Sigma-Aldrich Co. LLC)
- Dulbecco's Modified Eagle's Medium – high glucose incomplete (DMEM-I, Sigma-Aldrich Co. LLC)
- HCl 40 mM in isopropanol
- Hydroalcoholic extract of *H. perforatum* (0,3 % hypericin, EPO S.r.l.)
- Mixture of antibiotics and antimycotic (penicillin, streptomycin and amphotericin B, Sigma-Aldrich Co. LLC)
- Phosphate-buffered saline 1X (PBS 1X)
- Roswell Park Memorial Institute 1640 Medium complete (RPMI-C, Sigma-Aldrich Co. LLC)
- Roswell Park Memorial Institute 1640 Medium incomplete (RPMI-I, Sigma-Aldrich Co. LLC)
- Ticlopidine (Sigma-Aldrich Co. LLC)
- Trypan blue dye 0,4 %
- Trypsin-EDTA 0,25 % (Sigma-Aldrich Co. LLC)

3.1.2. Equipment

- Centrifuge Mikro 20 Hettich
- Centrifuge Sigma 3K18C Bioblock Scientific
- Greenhouse NuAire DHD Autoflow CO_2 Air-Jacketed Incubator
- Hemocytometer or Neubauer chamber
- Laminar flow cabinet NuAire Class II
- Optical microscope Olympus CK40
- Spectrophotometric microplate reader $Anthos_{2020}$

3.2. Cell lines

3.2.1. Hep G2 cells

The human hepatoma cell line, Hep G2, was isolated in 1979 by Aden *et al*. from a primary hepatoma of an 11-year old Argentine male [67] and it has been widely used for *in vitro* toxicity assays. These cells have an epithelial-like morphology, resembling the liver parenchymal cells, and synthesize and secrete many of the characteristic plasma proteins of normal human liver cells [67]. However, it has been stated that the overall drug metabolizing enzymatic activity is lower than in the normal liver cells [67]. Even though, it can predict non-metabolism mediated hepatotoxicity with an 80 % sensitivity and a 90 % specificity [68]. Additionally, it presents the advantages that these cells are easily maintained in culture (in Dulbecco's Modified Eagle's Medium [67]), stable, human derived, organ specific [68] and quite versatile: they are used in a variety of mutagenicity test systems and studies related with some mechanisms of viral infections and gene expression, transcription and cytotoxicity [67].

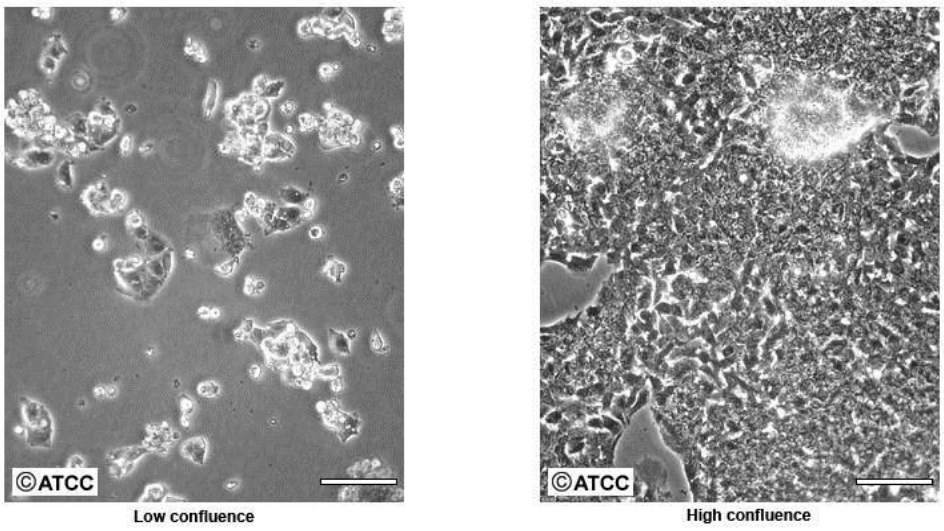

Figure 6. Hep G2 cells morphology under an optical microscope (scale bar = 100 µm, adapted from [69]).

In this study, Hep G2 cells were maintained in DMEM-C medium (DMEM medium supplemented with 10 % of fetal bovine serum (FBS) and 100 µg/mL of mixture of antibiotics and antimycotic). To perform the MTT assays, the cells were incubated in DMEM-I medium (which differs from DMEM-C due to the absence of FBS).

3.2.2. Caco 2 cells

The Caco 2 cell line was established by Jorgen Fogh in 1975 [70]; it is derived from heterogeneous human epithelial colorectal adenocarcinoma cells and it is the most commonly used and most extensively characterized cell-based model for the assessment of drug

absorption through the intestinal membrane. These cells spontaneously differentiate in normal culture conditions, with a progressive development of brush border, tight junctions and efflux and uptake transporters, ultimately resembling the human small intestinal mucosa cells. They express some CYPs, phase II conjugating enzymes and membrane efflux proteins, including the P-gp, enabling *in vitro* prediction of the likely gastrointestinal permeability of drugs. This cell line is also used to predict the solubility and bioavailability of drugs and the possibility of drug-drug or herb-drug interactions in the gut lumen [70].

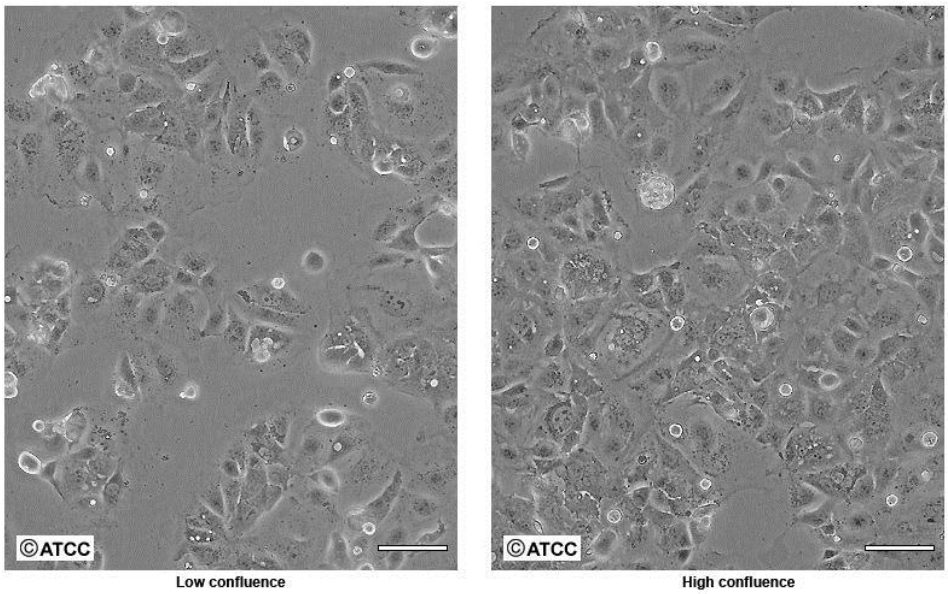

Figure 7. Caco 2 cells morphology under an optical microscope (scale bar = 100 µm, adapted from [71]).

Caco 2 cells were maintained in RPMI-C medium (RPMI medium supplemented with 10 % of FBS and 100 µg/mL of mixture of antibiotics and antimycotic). To perform the MTT assays, the cells were incubated in RPMI-I medium (which differs from RPMI-C due to the absence of FBS).

3.3. Methods

3.3.1. Cell cultures

Before starting to use the laminar flow cabinet, the UV light must be turned on for 15 min to sterilize the inside of the cabinet. About 5 min before starting to work, one should turn on the vertical flow and the light inside the cabinet, raise the front cover up until the indicated threshold, sprinkle the cabinet with 70 % ethanol and clean everything with paper laboratory towels.

All culture media and solutions must be pre-heated in a bath at 37 ºC. One should always wear gloves in the culture room, sprinkled with 70 % ethanol every time before putting the hands inside the cabinet. Also, all the material to be used inside the laminar flow cabinet must be sprinkled with 70 % ethanol before placing it inside the cabinet.

After the end of the work, one must pass bleach through the vacuum system, empty the residues reservoir, clean the cabinet with 70 % ethanol and paper laboratory towels, close it and turn on the UV light on for an additional 15 min.

3.3.1.1. Characterization and preparation of culture media

The basic components of culture media are water, inorganic salts, carbohydrates, vitamins, essential amino acids, lipids and various supplements (hormones, serum, proteins/peptides and/or antibiotics). If needed, tampon systems or pH indicators may be added.

In the maintaining of the cell cultures, the culture media is supplemented with FBS, because it contains vital elements for cell growth: growth factors, albumin, cell adhesion factors, Fe^{3+} ion carriers, antiproteases, etc.

The composition of each culture medium must be adequate to the cell line with which one is working, taking into account the nutritional needs of the cells.

When performing the assays (incubation with the compounds and MTT assays), FBS is withdrawn because it contains some not quantified components that may interfere with the studies.

The culture media used in this study, above referred, have a distinct composition and 2 of them are supplemented with serum:

- DMEM-C culture medium;
- DMEM-I culture medium;
- RPMI-C culture medium;
- RPMI-I culture medium.

3.3.1.2. Thawing and cell counting

The cells were removed from liquid nitrogen and thawed in a thermostatic bath at 37 ºC. Then, they were transferred to a 15 mL tube containing 4 to 5 mL of the appropriate culture medium and centrifuged at 230 rpm for 5 min. The supernatant was discarded, the pellet suspended and the volume equivalent to $1x10^6$ cells was added to a 25 cm^2 culture flask, making up a total volume of 5 mL with complete culture medium, adequate to the cell line.

The culture medium was replaced every 2 to 3 days and the cells were maintained inside in an incubator at 37 ºC, 5 % CO_2 and 95 % humidity.

To count the cells, after the obtaining of the cell pellet and its suspension in 1 mL of culture medium, 10 µL were added to a microtube containing 10 µL of trypan blue 0,4 %. The suspension was then placed in a hemocytometer – Neubauer chamber – and the viable cells (the ones that did not dye) were counted under an optical microscope in each of the four quadrants of the thinner grid. To calculate the cell concentration, the following formula was applied:

$$\text{number of cells/mL} = \text{cell average in the quadrants} \times 10^4 \times 2$$

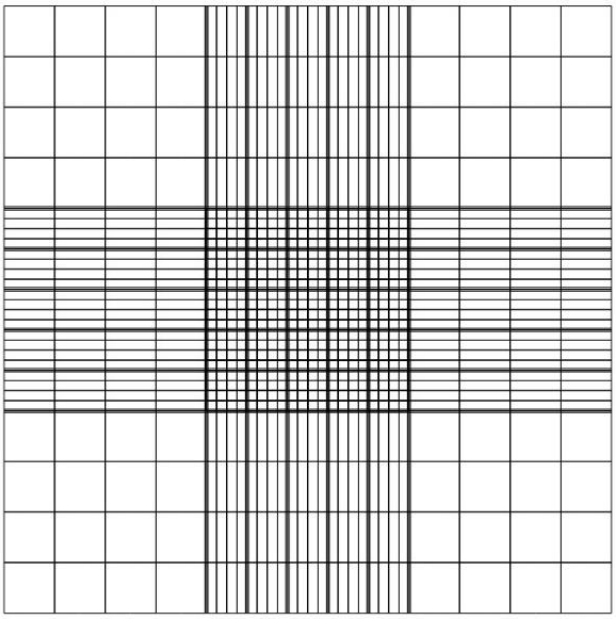

Figure 8. Grid layout of the Neubauer chamber (hemocytometer) under an optical microscope [72].

3.3.1.3. Trypsinization

When Hep G2 and Caco 2 cell lines reached 90-95 % and 85-90 % of confluence, respectively, a trypsinization procedure was performed. For that, the culture medium of the cells-containing flasks was discarded and the cells were washed with PBS 1X by adding this solution to the flask and gently stirring it over them. PBS 1X was discarded and trypsin-EDTA 0,25 % was added (2 mL in a 25 cm^2 culture flask, 4 mL in a 75 cm^2 culture flask and 8 mL in a 175 cm^2 culture flask), letting it to act for approximately 5 min inside an incubator at 37 ºC, 5 % CO_2 and 95 % humidity. The process was sped up with gently sharp raps to help the cells to detach from the bottom of the flask.

When the cells were no longer adhered to the culture flask, the trypsin-EDTA reaction was stopped by adding twice its volume of the appropriate complete culture medium. The cell suspension was recovered to a 15 mL tube and centrifuged at 230 rpm for 5 min. Then, the supernatant was discarded and the pellet was suspended in 1 mL of the appropriate complete culture medium. The suspension of cells was then transferred to one or more culture flasks, previously identified with the cell line designation, the passage number, the user acronym and the date. The number of cells per flask was $3x10^5$ in 25 cm^2 flasks (with 5 mL of culture medium), $2x10^6$ in 75 cm^2 flasks (with 15 mL of culture medium) and $1x10^7$ in 175 cm^2 flasks (with 30 mL of culture medium).

When the aim was to trypsinize the cells to a 96-well plate, the process was similar to the described above with the exception that, in the end, the cells were transferred directly to each well ($2,5x10^4$ and $2x10^4$ cells per well, for the Hep G2 and the Caco 2 cells, respectively).

3.3.1.4. Freezing

To freeze the cells, the process was similar to the described above for trypsinization. The difference was that, after the cell counting, $1x10^6$ cells were frozen in a cryopreservation tube, in adequate culture medium containing 10 % of DMSO.

3.3.2. Incubation of the cells with ticlopidine and/or *Hypericum perforatum*

3.3.2.1. Preparation of the solutions

To perform this study, the stock solutions of ticlopidine and *H. perforatum* extract were previously prepared, from which the intermediate and final solutions of both the compounds were prepared.

The stock solution of ticlopidine 10 mM was prepared in DMSO; successive dilutions, in the incomplete culture medium, were performed to obtain the working concentrations: 1, 10, 100 and 200 µM.

The stock solution of *H. perforatum* extract was prepared through the dilution of a hydroalcoholic extract in a 5 % DMSO and 0,1 % HCl 1 M solution to a final concentration of 10 mM. Similarly, successive dilutions in the appropriate incomplete culture medium were prepared: 1 and 10 µM.

3.3.2.2. Incubation procedure

Cells were incubated with various concentrations of ticlopidine and/or *H. perforatum* extract and for various periods of time. As mentioned, the concentrations tested for ticlopidine were: 1, 10, 100 and 200 µM. For the *H. perforatum* extract, the concentrations used were: 1 and 10 µM. Finally, for the combinations of both compounds, the concentrations used were: 1 + 1 and 10 + 10 µM. The periods of incubation studied were of 12, 24, 48 and 72 h.

Approximately 12 h before incubating the cells with the compounds, the culture medium was replaced for the same but without FBS – incomplete culture medium. After 12 h, the incomplete medium was discarded and the cells were incubated with the drug and/or the extract according to the defined concentrations and periods of incubation. At the end of the incubation with the compounds, the medium was discarded and MTT assays were performed.

The MTT assays were repeated for, at least, three independent experiments and each assay was carried out using 6 replicates of each experimental condition.

3.3.3. MTT assays

3.3.3.1. Background

The MTT assay is a spectrophotometric (colorimetric) assay that allows the evaluation of the effects of several compounds in cell proliferation/viability and, therefore, in their

metabolic activity. The metabolic reduction of MTT by the mitochondrial dehydrogenases associated to NADPH and NADH results in the production of water-insoluble formazan crystals (purple-blue/violet colored) inside the cells, which are quantified after its solubilization. This reduction occurs only when the reductases of the cells are active – and thus, the cells are viable – and so it is commonly used as a measure of cell proliferation/viability.

When determined compounds contact with the cells, the MTT assay allows the evaluation of their ability to inhibit cell proliferation/viability.

3.3.3.2. Experimental procedure

After the incubation of the cells with ticlopidine and/or *H. perforatum* extract, the culture medium was discarded and 120 µL of MTT 0,5 mg/mL were added to each cells-containing well (all this procedure was carried out protecting the MTT from the light). Then, the multiwell plate was wrapped in aluminum paper and incubated in an incubator at 37 ºC, 5 % CO_2 and 95 % humidity for approximately 2 h.

Next, the MTT solution in each well was discarded (to a specific container), each well was carefully washed twice with PBS 1X and 120 µL of HCl 40 mM in isopropanol were added to solubilize the formazan crystals. The content of each well was then transferred to a clean multiwell plate to minimize the interference of the color of the extract. Finally, the absorbance was read in a spectrophotometer (microplate reader) at 570 nm.

3.4. Statistical data analysis

Data were analyzed using the *Microsoft Office Excel 2013®*.

The percentage of viable cells after the incubation with the compounds in study was calculated through the absorbance's values obtained at the end of the MTT assay, according to the following formula:

$$\text{cell viability (\%)} = \frac{\text{average of the absorvances after icubation with drug/extract}}{\text{average of the control absorvances}} \times 100$$

Student's *t-test* was performed in order to compare the cellular viability of the control (cells not treated with any of the compounds) and the treated cells and to achieve putative significant differences between the groups. Statistical significance was considered when $p < 0.05$ (*).

4. Results and discussion

It is well known that most of the pharmacokinetic and pharmacodynamic interactions are due to the drugs or other substances which act as a substrate for the metabolizing enzymes, particularly CYPs. Additionally, the metabolism of a compound may be altered by the co-administration of another substance, which may be clinically significant [73]. Both ticlopidine and *H. perforatum* are metabolized by CYPs [6, 62, 66], suggesting a possible herb-drug interaction between them when co-administrated.

The transport across the gastrointestinal membrane also plays a key role in the biotransformation of compounds, namely through the P-gp, which is actively involved in the transport of molecules back into the gastrointestinal lumen. Several interactions occur due to the upregulation or inhibition of the transporter efflux pumps [73]. In fact, *H. perforatum* strongly increases the P-gp expression, which is the cause for many of the herb-drug interactions described in literature [66].

Hep G2 and Caco 2 cells were incubated with different concentrations of ticlopidine and *H. perforatum* hydroalcoholic extract, alone or in combination, and the cellular viability/cytotoxicity was evaluated.

In a first approach, it was required to choose the concentrations and incubation times to include in the subsequent assays. Based on literature (Table 5), the initial concentrations considered were: 1, 10, 50 and 100 μM for ticlopidine; 1 and 10 μM for *H. perforatum* and, for their combinations, 1 + 1 and 10 + 10 μM. However, ticlopidine concentrations were changed to 1, 10, 100 and 200 μM after the first assays because the analysis of the data revealed that the highest concentration used exhibited significant cytotoxicity only for the longer periods of incubation.

Table 5. Summary of the literature research to choose the ticlopidine concentrations to include in the study.

Concentration	Justification	Reference
1-3 μM	Therapeutic steady-state plasma concentrations	[47]
3 μM	Peak plasma concentrations of the active metabolites after a dose of 250 mg twice daily	[37]
4 μM	Main serum concentrations for the recommended maintenance dose (250 mg every 12 h) at steady-state	[74]
5 μM	Relevant concentrations of ticlopidine following a multiple dose administration	[75]
2,5-10 μM	Therapeutically relevant concentrations	[76]
> 10 μM	Dose-dependent cytotoxicity, namely concentration-dependent granulocytes toxicity	[29, 74]
50 μM	Identify all possible metabolites produced *in vitro*	[75]
60 μM	Concentration of the prodrug in the blood after oral or IV administration	[77]
> 100 μM	Lymphocytes cytotoxicity	[74]

Regarding the incubation times, they were chosen based on previous studies conducted by the research group. Therefore, for each compound and each concentration tested, the MTT assays were carried out after an incubation of 12, 24, 48 or 72 h with the compounds of interest.

The results are showed and discussed in the following sections.

4.1. Hep G2 cells

As mentioned, the Hep G2 cell line, although metabolically poor, allows the prediction of non-metabolism mediated hepatotoxicity [67, 68].

The results of the incubation of ticlopidine alone on Hep G2 cells are shown in Table 6 and in Figure 9. After 12 and 24 h of incubation, only 200 μM of ticlopidine decreased the cell viability. Furthermore, after 48 and 72 h of incubation, there was an increase in the relative percentage of cell viability for all concentrations tested, suggesting that ticlopidine is only slightly cytotoxic for these cells and, for incubations longer than 24 h (48 and 72 h) cells seemed to have the capacity to recover from the initial marginal cytotoxicity promoted by this drug.

Table 6. Relative percentage of the viable Hep G2 cells plus various concentrations of ticlopidine (1, 10, 100 and 200 μM) for different periods of incubation (12, 24, 48 and 72 h).

Incubation (h) / Concentration (μM)	12	24	48	72
0	100	100	100	100
1	101,09 ± 9,06	93,59 ± 11,00	140,15 ± 7,52	160,85 ± 4,04
10	85,54 ± 9,98	95,17 ± 7,47	130,21 ± 7,57	138,85 ± 2,50
100	82,65 ± 7,75	100,52 ± 10,61	151,50 ± 8,01	233,53 ± 4,35
200	69,14 ± 6,42	75,69 ± 8,13	138,98 ± 7,19	267,45 ± 4,46

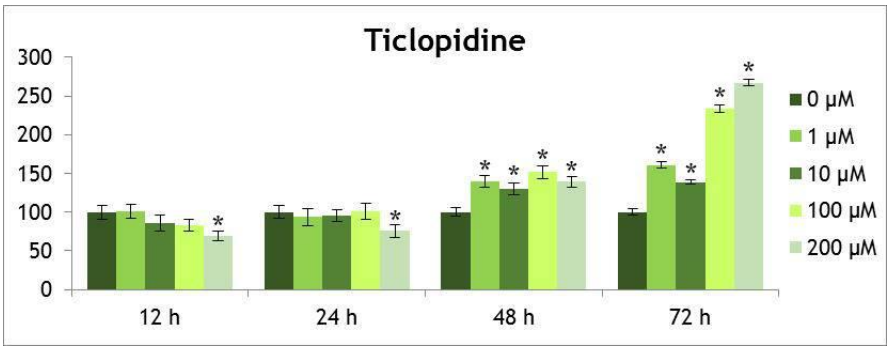

Figure 9. Graphical representation of the relative percentage of the viable Hep G2 cells after incubation with various concentrations of ticlopidine (1, 10, 100 and 200 μM) for different periods of incubation (12, 24, 48 and 72 h). (* $p < 0,05$)

Ticlopidine is almost completely metabolized by the CYPs in the liver. Despite Hep G2 cells are poor metabolizers, they synthesize and secrete many of the characteristic plasma proteins of normal human liver cells, thus degrading the parent drug into cytotoxic metabolites,

which may justify the decrease in the cellular viability after an incubation of 12 h with 200 μM of ticlopidine. Although, for longer periods of incubation, these cells seemed to recover from the initial toxicity, which may be supported by the fact that the levels of GSH are increased in cell cultures maintained in DMEM [67] and, as a consequence, the formed toxic metabolites may be trapped by this enzyme and, ultimately, be conjugated with it.

Maseneni *et al*. (2012) studied the toxicity of ticlopidine on human myeloid progenitor cells and concluded that this drug is dose-dependently cytotoxic starting at 10 μM, also verifying that pre-incubation of ticlopidine with the human recombinant CYP3A4 promoted the degradation of the drug into metabolites and increased its cytotoxicity [29]. However, Choi *et al*. (2011) observed that ticlopidine inhibited the activity of the CYP3A4 in a concentration-dependent manner at 35 μM [78] and Yang *et al*. (2011) also stated that ticlopidine inhibited the CYP2C9 and the CYP3A4 at 26 and 32.3 μmol/L, respectively [51].

It is proven that ticlopidine inhibits the expression of various CYPs, including the CYP3A4 and other isoforms involved in the biotransformation of the parent drug and of some of its metabolites [6, 44]. The results exhibit a decrease in the cytotoxicity along time, which may be a consequence of newly formed less toxic metabolites, despite the inhibition of CYPs expression.

In general, these results are somewhat distinct than those reported by these authors, as ticlopidine only exhibited a significant cytotoxicity at 200 μM. Another possibility is that, due to the increased levels of GSH in the Hep G2 cell line cultured in DMEM and given its high ability to form conjugates with the toxic metabolites, these GSH-conjugates permit the recovery and the higher proliferation of cells for longer periods of incubation.

Table 7 and Figure 10 display the results obtained for the incubation of Hep G2 cells with *H. perforatum* hydroalcoholic extract. As showed, generally, both concentrations of the extract promoted a significant decrease in the cellular viability of Hep G2 cells. In fact, 10 μM of the extract were cytotoxic for any of the concentrations in every period of time included while 1 μM promoted a decrease in the cellular viability until 48 h of incubation, indicating that *H. perforatum* is cytotoxic for this cell line in a concentration- and time-dependent manner.

Table 7. Relative percentage of the viable Hep G2 cells plus various concentrations of *H. perforatum* extract (1 and 10 μM) for different periods of incubation (12, 24, 48 and 72 h).

Incubation (h) / Concentration (μM)	12	24	48	72
0	100	100	100	100
1	75,10 ± 6,15	29,60 ± 6,95	76,53 ± 5,93	106,54 ± 1,27
10	24,51 ± 1,40	24,46 ± 1,90	29,10 ± 2,60	82,13 ± 0,84

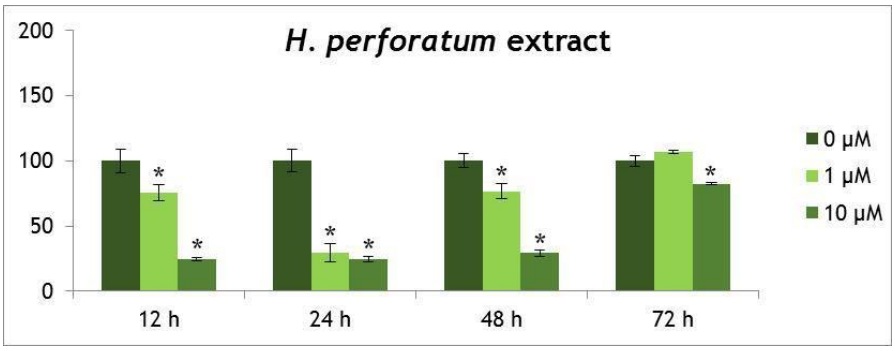

Figure 10. Graphical representation of the relative percentage of the viable Hep G2 cells after incubation with various concentrations of *H. perforatum* extract (1 and 10 µM) for different periods of incubation (12, 24, 48 and 72 h). (* $p < 0,05$)

Komoroski *et al.* (2004) verified that hyperforin, a constituent of the *H. perforatum* extract, is cytotoxic to human hepatocytes at 1,5 µM for an incubation of 48 h and that, at cytotoxic concentrations (up to 1 M), hyperforin induced the CYP3A4 activity and the CYP2C9 [79].

These results are consistent with the ones obtained for Hep G2 cells in this study: a possible explanation for this may be that *H. perforatum* extract is cytotoxic for both 1 and 10 µM, particularly for the last, but also promoted an inverse concentration-dependent induction of some CYPs involved in the metabolism of the compounds present in the extract. Thus, these compounds which are initially cytotoxic, metabolize and the resulting metabolites seem to be less cytotoxic for Hep G2 cells, as the cellular viability increased for the longer period of incubation (72 h).

Finally, the cytotoxicity of combinations of both the compounds in study was also assessed and the obtained results are shown in Table 8 and in Figure 11. The incubation with both ticlopidine and extract produced a similar pattern comparing to the data obtained for the incubation with *H. perforatum* alone; however, the relative values of cell viability are higher, revealing a lower cytotoxicity than for *H. perforatum* alone. A possible explanation may be the occurrence of a saturation of the metabolizing enzymes present in Hep G2 cells (CYPs), as both the compounds are metabolized by hepatocytes.

Table 8. Relative percentage of the viable Hep G2 cells plus various concentrations of the combination of ticlopidine and *H. perforatum* extract (1 + 1 and 10 + 10 µM) for different periods of incubation (12, 24, 48 and 72 h).

Incubation (h) / Concentration (µM)	12	24	48	72
0	100	100	100	100
1 + 1	97,18 ± 7,09	53,15 ± 14,81	66,42 ± 5,56	94,28 ± 1,41
10 + 10	36,76 ± 1,75	29,09 ± 2,21	31,95 ± 0,75	72,42 ± 0,50

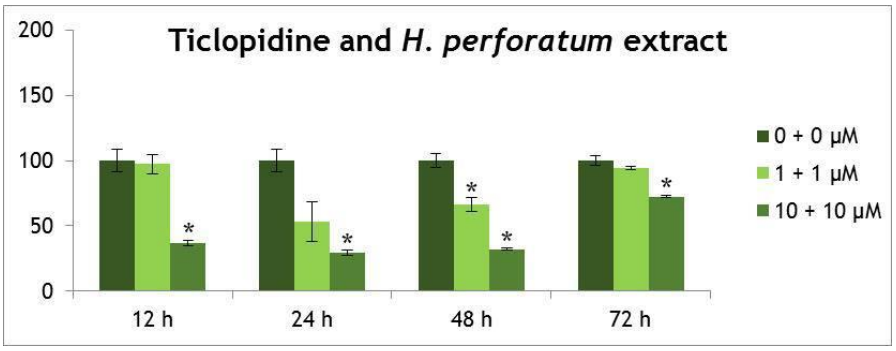

Figure 11. Graphical representation of the relative percentage of the viable Hep G2 cells after incubation with various concentrations of the combination of ticlopidine and *H. perforatum* extract (1 + 1 and 10 + 10 µM) for different periods of incubation (12, 24, 48 and 72 h). (* $p < 0,05$)

4.2. Caco 2 cells

The Caco 2 cell line enables the prediction of possible drug-drug or herb-drug interactions, also possessing the ability to express some metabolizing enzymes and the P-gp [70].

The relative cellular viability of Caco 2 cells after the incubation with ticlopidine is stated in Table 9 and in Figure 12. As showed, 200 µM of ticlopidine significantly decreased the cellular viability of these cells at any period of incubation. Also, contrarily to the observed in Hep G2, in Caco 2 cells longer periods of incubation generated higher cytotoxicity than the shorter ones, suggesting a major sensitivity of these cells for ticlopidine.

Table 9. Relative percentage of the viable Caco 2 cells plus various concentrations of ticlopidine (1, 10, 100 and 200 µM) for different periods of incubation (12, 24, 48 and 72 h).

Incubation (h) / Concentration (µM)	12	24	48	72
0	100	100	100	100
1	104,75 ± 5,76	94,74 ± 3,26	132,20 ± 4,93	86,79 ± 4,24
10	100,01 ± 3,44	104,30 ± 3,76	117,42 ± 5,41	84,92 ± 5,16
100	88,93 ± 4,97	84,78 ± 4,34	117,36 ± 4,93	92,54 ± 4,73
200	72,73 ± 6,40	64,10 ± 2,86	91,29 ± 3,11	76,43 ± 2,56

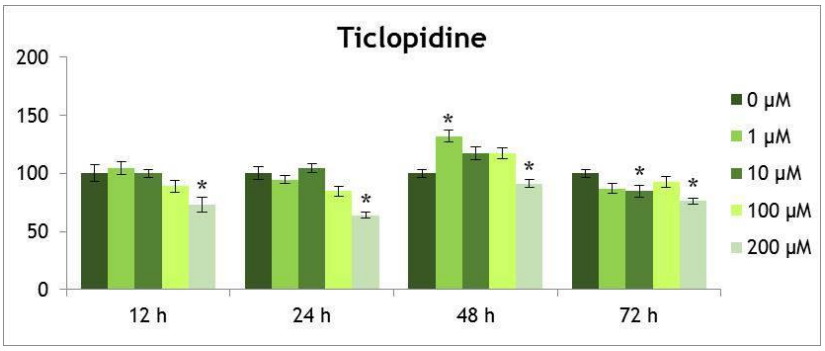

Figure 12. Graphical representation of the relative percentage of the viable Caco 2 cells after incubation with various concentrations of ticlopidine (1, 10, 100 and 200 µM) for different periods of incubation (12, 24, 48 and 72 h). (* $p < 0,05$)

Lu *et al.* (2006) stated that ticlopidine may be a substrate of the human organic anion transporting polypeptide B (OATP-B), involved in the absorption of anion drugs, and presenting the possibility that it may be intestinally absorbed [59]. In fact, it is indeed absorbed in the intestine and it is a substrate of the P-gp [4], meaning that it is effluxed by this protein. The P-gp, one of the most clinically important transmembrane transporters, is located on the apical surface of intestinal epithelial cells, which suggests an active role in drug elimination and absorption [66].

The results revealed that ticlopidine was cytotoxic after 24 and 72 h of incubation, but a recovery in the cellular viability was observed after 48 h. A possible justification for these findings may be that the drug is effluxed from Caco 2 cells – recovery in the cellular viability; however, it is again excreted to the cells and the cytotoxicity persists.

Ticlopidine is almost completely metabolized by CYPs in the liver and, in a much lower extent, by MPO in activated neutrophils. Only 8 % of the drug is excreted in the feces in its unchanged form, indicating that it is not metabolized in the intestine [6, 30]. Choi *et al.* (2010) observed that ticlopidine at 30 µM could not significantly inhibit the P-gp-mediated drug efflux [80]. Therefore, the revealed cytotoxicity of ticlopidine on Caco 2 cells is most likely due to the compound itself. The drug showed a significant toxicity only at 200 µM for all periods of incubation studied, suggesting that the time- and concentration- dependent cytotoxicity promoted by ticlopidine is due to its accumulation in these cells.

The results obtained for Caco 2 cells may be correlated with previous studies described in literature, as ticlopidine itself proved to be cytotoxic for human myeloid progenitor cells, for lymphocytes and for the Caco 2 cell line in a concentration-dependent manner [29, 74].

The data obtained for the incubation of Caco 2 cells with *H. perforatum* are in Table 10 and in Figure 13. As showed, 12 and 24 h of incubation with 10 µM of *H. perforatum* extract decreased the cellular viability of these cells. Moreover, a cellular viability recovery was observed after 48 and 72 h of incubation of cells with the extract. Also, 1 µM of the extract was not significantly cytotoxic at any period of incubation studied. In general, *H. perforatum* presented a significantly higher toxicity than ticlopidine.

Table 10. Relative percentage of the viable Caco 2 cells plus various concentrations of *H. perforatum* extract (1 and 10 μM) for different periods of incubation (12, 24, 48 and 72 h).

Incubation (h) / Concentration (μM)	12	24	48	72
0	100	100	100	100
1	106,36 ± 7,60	85,93 ± 6,30	154,49 ± 3,54	105,76 ± 3,52
10	69,71 ± 4,16	75,62 ± 4,59	92,72 ± 2,02	119,50 ± 7,56

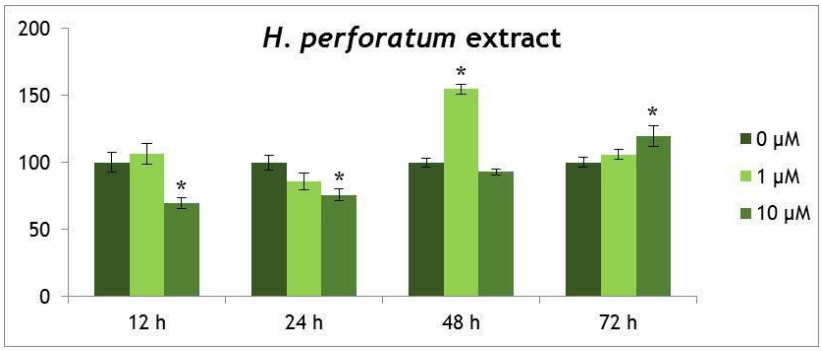

Figure 13. Graphical representation of the relative percentage of the viable Caco 2 cells after incubation with various concentrations of *H. perforatum* extract (1 and 10 μM) for different periods of incubation (12, 24, 48 and 72 h). (* $p < 0,05$)

In a study conducted by Tian *et al.* (2005), the authors verified that *H. perforatum* and hyperforin (1 μM) induced dose- and time-dependently the expression of the P-gp in the intestinal cell line LS 180 and observed a decrease in the cell viability at 75 μg/mL for incubations up to 48 h [81].

As such, the results obtained in this study for the incubation of Caco 2 cells with 10 μM of *H. perforatum* for 12 h were consistent with the above mentioned. However, considering the induction of the P-gp, one may suggest that the cytotoxic compounds were effluxed from the cells, which recovered from the initial cytotoxicity and exhibited a decrease in the relative cellular viability. This was observed for the 48 h of incubation with 1 μM concentration and 10 μM of the extract incubated for 72 h.

Finally, the results obtained for the combined incubation of ticlopidine and *H. perforatum* on Caco 2 cells are displayed in Table 11 and in Figure 14. A similar pattern to the observed for *H. perforatum* alone was observed, with an increase in the cell viability after 48 and 72 h. The incubation of these cells with 1 μM of both the compounds did not promote a decrease in the cellular viability of the cells while 10 μM of both the compounds decreased the relative cellular viability of Caco 2 cells for incubations shorter than 48 h. In fact, for periods of incubation longer than 24 h, the cellular viability was increased.

Table 11. Relative percentage of the viable Caco 2 cells plus various concentrations of the combination of ticlopidine and *H. perforatum* extract (1 + 1 and 10 + 10 µM) for different periods of incubation (12, 24, 48 and 72 h).

Incubation (h) / Concentration (µM)	12	24	48	72
0	100	100	100	100
1 + 1	95,77 ± 4,99	87,98 ± 6,86	118,13 ± 4,58	116,28 ± 3,86
10 + 10	62,31 ± 4,34	66,72 ± 4,71	90,31 ± 2,21	131,58 ± 8,89

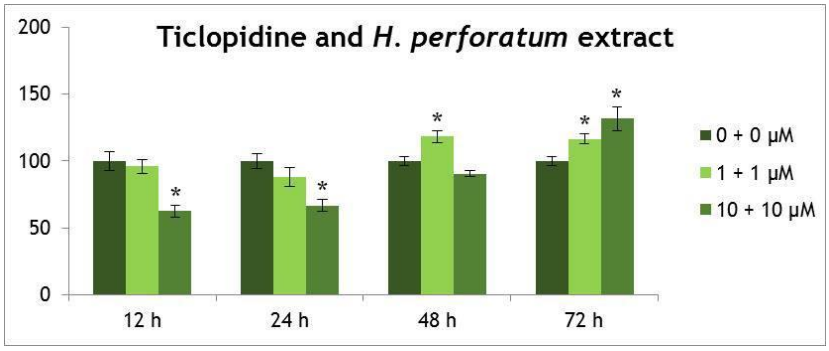

Figure 14. Graphical representation of the relative percentage of the viable Hep G2 cells after incubation with various concentrations of the combination of ticlopidine and *H. perforatum* extract (1 + 1 and 10 + 10 µM) for different periods of incubation (12, 24, 48 and 72 h). (* $p < 0,05$)

The *H. perforatum* extract has a very intense color (reddish-brown), which could have interfered in the reading of the absorbance in the spectrophotometer, even after being washed twice with PBS 1X and transferred to a clean multiwell plate. This may be a reason why the results obtained after the incubation with combinations of both the compounds are so similar to *H. perforatum* alone.

5. Conclusions and perspectives

Currently, ticlopidine is less and less used as a therapeutic option. Despite that, due to the adverse effects that it may induce, it remains of utmost importance to investigate the putative cytotoxicity promoted by this drug, alone and in combination with other concomitantly administrated compounds.

In this study, the cytotoxicity of this thienopyridine and/or of a *H. perforatum* hydroalcoholic extract was assessed, using a hepatic and an intestinal cell line.

Overall, on both Hep G2 cells and Caco 2 cells, only 200 μM of ticlopidine significantly decreased the cellular viability and, for incubations higher than 24 h, the cells showed the capacity, at least partial, to recover from the initial toxicity and to increase their proliferation.

Regarding the *H. perforatum*, the results showed that it promoted a significant cytotoxicity both at 1 and 10 μM on the Hep G2 and at 10 μM on the Caco 2 cells, thus showing a higher toxicity on the hepatic cell line. Again, both cell lines exhibited a recovery from this cytotoxicity as, after 72 h of incubation, the relative percentage of viable cells returned to values similar (Hep G2 cells) or higher (Caco 2 cells) than those in the controls, suggesting a putative role of GSH-conjugates on Hep G2 and an efflux mechanism by the P-gp on Caco 2 cells.

The incubation with both compounds showed a similar profile to the one corresponding to the incubation with *H. perforatum* extract alone, especially for Caco 2 cells. In Hep G2 cells, the incubation with both compounds showed a lower cytotoxicity comparing to the extract alone.

Given the overall results, in which the toxicity of ticlopidine and *H. perforatum* showed to be dose- and time- dependent for both Hep G2 and Caco 2 cells, it would be interesting to perform further studies, particularly *in vivo*, in order to confirm if the observed toxicity occurs in such conditions.

Finally, the remark that the results of this work were presented in a poster communication in the X Annual CICS Symposium, realized in the Faculty of Health Sciences of the University of Beira Interior (July 6th and 7th 2015), entitled "Cytotoxicity of ticlopidine and *H. perforatum* in hepatic and intestinal epithelium cell lines" (Attachment 1).

Part II – Internship report: hospital pharmacy

1. Introduction

The theoretical knowledge acquired during the four and a half years of the pharmaceutical graduation was applied to the real context of pharmaceutical activity and to promote the linkage between the scientific research and the professional activity focused in the patient [82], as the last semester of the integrated Master Degree in Pharmaceutical Sciences includes a curricular internship. This internship was partially done in a hospital pharmacy.

This report briefly describes the activities performed and the knowledge acquired during the hospital pharmacy internship, which took place between February 2nd and April 29th 2015.

Hospital pharmacy is defined in the Decree-Law #44204 of February 22nd 1962 as "the set of pharmaceutical activities carried out in hospital organizations or their connected services to provide care functions and services and to promote the scientific research and teaching" [83].

Presently, globalization is a real concept. As so, the hospital pharmacy internship was performed at the *Istituti Fisioterapici Ospitalieri* (IFO) in Rome, Italy, under the Erasmus+ program. Most of the time was spent at the non-sterile galenic preparations laboratory, sharing it with a Portuguese classmate and, later, with a Slovenian colleague.

It provided both professional and personal growth, through the knowledge of a distinct professional reality and the acquisition of new skills and responsibilities. At the personal level, it allowed a direct contact with a different culture and language.

2. Background

The *Agenzia Italiana del Farmaco* (AIFA), or the Italian Medicines Agency, is the Italian national authority for the regulation of drugs. It is an autonomous, transparent and cost-effective public organization in Italy, which acts under the supervision of the Ministry of Health and the vigilance of the Ministry of Health and the Ministry of Economy. It cooperates with the regional authorities, the National Institute of Health, several research institutes, scientific and patients' associations, health professionals, the pharmaceutical industry and the drugs' distributors [84]. The AIFA also cooperates with the *Istituti di Ricovero e Cura a Carattere Scientifico* (IRCCS), which are excellence hospitals with multiple biomedical research purposes and in the organization and management of health services; additionally, they provide internment, treatments and services in various medical specialties. Dermatology and oncology are two of the researched areas [85]; the IFO are IRCCS [86].

In Italy, the reimbursement of drugs is classified in three main classes:

- A: Drugs that are reimbursed by the National Health Service but, depending on the regional regulations, may be partially paid by the patient. It includes common drugs and those applied for chronic diseases [87, 88];
- C: Drugs that don't belong to the class A and are entirely paid by the patient. Drugs used for minor diseases and self-medication are included in this class [87, 88];
- H: Drugs that are of exclusive hospital use due to their pharmacologic characteristics, administration route, innovation or others. These drugs are totally paid by the National Health Service [87, 88].

3. General characterization of the *Istituti Fisioterapici Ospitalieri*

The *Istituti Fisioterapici Ospitalieri* (IFO) constitute a public corporation that manages two entities: the *Istituto Nazionale Tumori Regina Elena* (IRE), for the study and treatment of tumors, and the *Istituto Dermatologico San Gallicano* (ISG), specialized in dermatology; both were recognized as IRCCS in 1939. Since 2000, IRE and ISG are located in Via Elio Chianesi, 53 – 00144 Roma (Mostacciano), one of the most recent areas of the city [86].

The IRE operates in the clinical, research and training areas, with the main aim of achieving excellence in the prevention, diagnostic and treatment of tumors in the fields of epidemiology, etiology, neoplastic transformation and progression and experimental therapies [89]. The ISG used to be located near the city center, in Trastevere (where the external pharmacy is still located), and it is a national reference center for the diagnosis and treatment of dermatological and sexually transmitted diseases. The ISG activities are mostly focused on the immuno-inflammatory, oncological, allergic and infectious dermatological diseases [90].

3.1. IFO's pharmaceutical services: internal hospital pharmacy and external hospital pharmacy

3.1.1. Location, facilities and working hours

The internal pharmaceutical services of the IFO are located in the -3rd floor of the facilities, whilst the pharmacovigilance department is at the -1st floor. Additionally, the external pharmacy is located in Via di San Gallicano, 23 – 00153 Roma (Trastevere).

The internal pharmacy has many different areas: an area for the reception and verification of the orders; the storage area, which is divided in three parts (drugs, infusion therapies and medical devices); an area for dispensing drugs in the outpatient setting and a central area for the manipulation of cytotoxic drugs. Regarding its offices, it has the technical director office, where the narcotics cabinet is located, and the pharmacists, the nurse

coordinator and the administrative offices. It also has a room allocated to the clinical trials, where all drugs and related documentation are stored. Additionally, there is a social room, a bathroom and an external area for medicinal gases.

In the -1st floor, the pharmacovigilance department consists in two offices, one for the pharmacovigilance director and another where two pharmacists and one administrative assistant work.

The *Farmacia Ospedialiera Esterna San Gallicano*, the external pharmacy, acts as a community pharmacy and consists of a public attendance area, a storage area, an office (where the narcotics cabinet is), a galenic laboratory and a bathroom. The laboratory of non-sterile extemporaneous preparations is vey well equipped and it has more raw materials than usually in community pharmacies.

The working hours in the internal pharmacy and in the pharmacovigilance department are from 8.30 am to 3.30 pm from Monday to Friday and from 8.30 am to 1.00 pm on Saturdays. Besides, al least one pharmacist stays in the pharmacy till 8.00 pm.

The external pharmacy is opened from 8.30 am to 7.30 pm from Monday to Friday, closing from 1.00 pm to 4.00 pm on Monday and Friday to carry out galenic preparations. On Saturdays, the pharmacy is opened from 8.30 am to 1.00 pm.

3.1.2. Human resources

The IFO has a multidisciplinary team constituted by pharmacists, nurses and technical and administrative assistants.

The technical director of the internal pharmacy is Dr. Antonia Marina La Malfa, whilst Dr. Felice Musicco is the director of the external pharmacy and the pharmacovigilance department.

4. Organization and management

4.1. Provision

The hospital pharmacist is responsible for the management of the pharmaceutical inventory, ensuring that the patients receive the best treatment at the highest quality and lowest cost possible.

4.1.1. Hospital Therapeutic Handbook

The main purpose of the Hospital Therapeutic Handbook (*Prontuario Terapeutico Oespedaliero*) is to enclose the information of all drugs from all clinical and epidemiological diseases, giving the specialists the possibility to choose the most appropriate therapy based on scientific evidence. For each bioactive principle, the handbook contains information related with the administration route, limitations regarding its therapeutic indications, the patent state, the availability of equivalent products and its exclusivity, or not, of hospital use [91].

Each hospital must have its own Corporate Therapeutic Handbook (*Prontuario Terapeutico Aziendale*), based on the Regional Hospital Therapeutic Handbook, for the management of drugs according to the guidelines in the latter.

4.1.2. Selection system and acquisition

The drugs in the Corporate Therapeutic Handbook result of a review and selection carried out by the Hospital Therapeutic Commission (*Commissione Terapeutica Ospedaliera*), according to their efficacy, security, relation between cost and benefit and, as referred above, the regional guidelines.

The selection of the drugs to be acquired by the hospital is based on the ones present in the Corporate Therapeutic Handbook. There is a table-form with all medicines, technical data (batch number, quantity, price, etc.) and the responsible laboratory/pharmaceutical industry, as well as the supplier to which it can be ordered/acquired.

The internal system contains an inventory database of all products; through the analysis of the data, the quantity of each medicine and/or medical device to acquire is determined. Generally, the stock is enough for one month. After the request is completed, it must be validated by the technical director.

The technical assistants that work at the pharmacy are responsible for the logistics and the qualitative and quantitative control of the orders.

4.1.2.1. Drugs not included in the Corporate Therapeutic Handbook

Drugs not included in the Corporate Therapeutic Handbook may be acquired by the pharmacy in some particular cases, as in a therapeutic treatment started in a different hospital or a prescription done by an external specialist physician. In these cases, firstly, the pharmacist should approve its acquisition and then order it.

In the IFO, there are two order models used in these situations: one is a personalized model used for a determined patient of drugs belonging to the class H or that are very expensive (Attachment 2) and the other is for medicines not included in the class H and used in hospitalized patients (Attachment 3).

4.1.2.2. Narcotic and psychotropic drugs

The acquisition of narcotic and psychotropic drugs follows an accurate management of their consumption at the IFO, to avoid possible stock unavailability. There is an appropriate form – *Buono acquisto* (Attachment 4) – for each drug at the same dosage, with an identification number and different numbered modules. Each module has three sections: one to the hospital pharmacy, other to the supplier company and the last one must be sent by the supplier to the Regional Health Authority.

The section corresponding to the pharmacy is filled by a pharmacist and validated by the technical director; after that it is sent by mail or fax to the supplier company. It contains the following information:

- Name and address of the supplier company;
- Name and quantity of the medicine;
- The full name of the technical director and the address of the pharmacy;
- Date, stamp and signature of the technical director.

4.1.2.3. Drugs not registered in Italy

If a physician considers appropriate to subject a patient to a treatment with a medicine not authorized in Italy, but commercialized in a foreign country, it can be ordered by the hospital pharmacy. This process is regulated by the Italian Decree of the Health Ministry of February 11th 1997 [92].

In these cases, it must be ensured that no valid alternative is available in Italy and that the imported medicine will be exclusively used for the approved indications of the origin country and in accordance with the summary of product characteristics. The direct responsibility is attributed to the prescriber physician, who must attain, under the current legislation, the informed consent of the patient (or an authorized person, in cases of incapacity of the patient). The responsible for the import of the drug assures that the product is prepared accordingly with all the safety and quality requirements from the Italian Health Authority and, in the case of products for which it is mandatory in Italy to control its status (immunological products and hemo- or plasma derivatives), the imported batches are only accepted when accompanied by a copy of the status control certificate by the competent authorities [93].

The import may be done by a prescriber physician and validated by a pharmacist. The request – *Richiesta di farmaci esteri* (Attachment 5) – is an individual document, for stock replacement and for patients; in both cases, the durability should not be higher than a 30 days treatment for a single patient. It must be clarified if the medicine is to replace the stock or to a patient; in the latter, the patient identification and date of birth must be indicated, as well as the pathology associated. In the case of a stock replacement, the number of patients who will benefit from the treatment should be indicated. Both types of requests must be stamped by the hospital and sent by the prescriber physician or the pharmacist to the Health Ministry, accompanied by the following information/documents:

a) Drug identification (name and dosage form);
b) Producer company;
c) Holder of the market authorization;
d) Declaration of the authorization of the drug in its country of origin;
e) Quantity to import, with the specification that it corresponds to a therapeutic treatment that does not exceed 30 days;
f) General information about the patient(s);

g) Special needs that justify the use of an unauthorized medicine in Italy, in the absence of a valid therapeutic alternative;
h) The informed consent of the patient(s) to be subjected to the therapy;
i) Declaration of use of the medicine under the direct responsibility of the prescriber physician [92].

4.1.3. Reception and verification

The reception of the orders occurs in a specific area of the pharmacy, with direct outdoor access and easy connection to the various storage sections.

The first products to verify are cytotoxics, medicines requiring cold storage, narcotics, psychotropics and benzodiazepines and raw materials.

The technical assistants are the professional staff responsible for this task. They confirm, quantitatively and qualitatively, if the received products correspond to those ordered, comparing them with the transport document. They also verify if the products arrived in the predicted time, in proper conditions (package integrity, appropriate label, if it is the original package, cold status maintenance (if applied), batch number and expiration date). After this process, the technical assistant dates, signs and stamps the transport document and registers the products in the information system. Narcotics, psychotropics and trial medicines constitute an exception because they are also verified by a pharmacist.

4.1.3.1. Reception and verification of narcotic and psychotropic drugs

As mentioned, the technical assistants receive the narcotic and psychotropic drugs but its verification has to be carried out by a pharmacist, who confirms the proper conditions of the medicines and, after, dates, signs and stamps the transport document. Particularly, the pharmacist responsible for these substances confirms if the module is correctly filled by the provider company, as well as its identification, and if the requested medicines fulfill the quantitative and qualitative characteristics, the batch numbers and the expiration dates and the overall package status. The technical assistant copies the documents and delivers them to the pharmacist, who files them in the narcotics and psychotropics cabinet with the substances. Only the technical director and the responsible pharmacist have access to this cabinet. The medicines are stored in alphabetical order, attending to the first expiry/first out principle. All the substances inputs – reception – and outputs – dispensing – are manually registered by the responsible pharmacist in a proper document – *Registro di entrata e uscita delle sostanze e preparazione soggete alla disciplina degli stupefacienti e sostanze psicotrope* (Attachment 6).

4.2. Storage

The storage area is divided in three main sections: one for medicines, one for infusion therapies and another for medical devices. After the reception, the products are stored in their

proper section, according to their nature and storage criteria. Hygienic and environmental standards are always attained and all products are stored in a protected area, away from patients and visitors. The temperature, light and humidity conditions are extremely important characteristics. The storage area is clean, with no direct sunlight, a temperature kept under 25 ºC and a maximum humidity of 60 %.

Medicines that require a cold storage are the first ones to be stored and are kept in the refrigerators at a temperature ranging from 2 ºC to 8 ºC.

The narcotics and psychotropics are also immediately stored in their proper cabinet, as referred in the section above. The cytotoxics are stored in a cabinet near the preparation area, for its easier access. All products are alphabetically ordered, in terms of its active pharmaceutical ingredient (API) and identified with labels containing the respective barcode and API, except for the artificial nutrition and antiseptics. The first expiry/first out principle is always applied.

5. Distribution

An accurate distribution of the medicines, through an internal circuit, permits that the proper drug, in the correct dosage form, dose and quantity, is available in the services where and when it is needed and for the right patient.

The pharmacist is the responsible for the reviewing of all prescriptions, ensuring that no medical errors occur (incorrect dose regimens, contraindications, interactions, etc.). In case of identification of a mistake, the pharmacist should contact the prescriber physician in order to clarify the situation.

At the IFO, the two main distribution systems are:

- Direct dispensing, at discharge and of class H medicines;
- Dispensing to the hospital services.

Additionally, there are the experimental therapy dispensing and the narcotic and psychotropic drugs dispensing, which can also be included in the two mentioned systems.

The distribution in unitary dose is still uncommon in Italy; it is not practiced in the IFO at all.

5.1. Direct dispensing

Direct dispensing occurs when a medicine is dispensed directly to a patient.

5.1.1. Discharge

This distribution system applies to the following patients:

1. Patients that have been hospitalized, at least once, at the IFO or that had a consultation in the ambulatory regimen and need class A medicines (listed in the Hospital Therapeutic Handbook).

The hospital assures the continuation of the pharmacological treatment, through the direct dispensing of the medicines for the first treatment cycle (for the first 30 days, or less if the treatment is shorter) to the patient.

The patient goes to the internal hospital pharmacy and presents the prescription – *Modulo B* (Attachment 7), as well as his identification document. The pharmacist validates the prescription and dispenses the medicines to the patient, informing him of the appropriate administration, transport and storage. If any doubt about the prescription arises, the pharmacist should verify the patient treatment history in the information system. The pharmacist then signs, stamps and stores the prescription document, registering it in the information system.

These medicines are: antibiotics, antivirals, antimycotic and anti-emetic drugs, proton-pump inhibitors, HMG-CoA reductase inhibitors, heparin, human albumin and antidepressants.

2. Patients that require services from the Day Hospital, without the need to stay hospitalized.

   The Day Hospital is mostly required for the preparation of surgical interventions – the presurgical Day Hospital – and for the administration of antineoplastic drugs – the oncologic Day Hospital. It does not have an emergency character and patients have their consultations scheduled with the physician.

   The prescriptions are received via fax in the pharmacy and a pharmacist validates them and dispenses the medicines. A technical assistant confirms the presence of the "DH" (Day Hospital) indication in every label.

   When the patient can take the treatment at home, he goes to the pharmacy with the prescription – *Modulo A* (Attachment 8) – and his identification document. The pharmacist validates the prescription and dispenses the medicines, clarifying any doubts that may arise.

3. Patients in ambulatory regimen that need class H medicines or medicines not available in the community pharmacy.

   This distribution system is useful when the community pharmacies fail to ensure the drug needed. Additionally, it contributes to a better surveillance of certain chronic pathologies and prescribed therapies, allowing the patient to continue the treatment at home.

   The process is similar to the described above; the patient goes to the pharmacy with the prescription – *Modulo A* (Attachment 8) – and his identification document and the pharmacist validates the prescription and dispenses the medicines, registering it in the information system.

5.1.2. Class H drugs

Class H drugs include all medicines that, due to their pharmacological characteristics, administration mode or other reasons, are of exclusive hospital use or for use in specific ambulatory areas or that, due to their expensive cost, cannot be dispensed in community pharmacies.

These medicines are only available at the hospital pharmacy and the patient should present, additionally to the physician's prescription, the discharge proof and the regional health service card.

The cost of these drugs is supported by the local health unit of the patient residence area. Class H medicines are prescribed in an appropriate document – *Modulo A* (Attachment 8), according to the therapeutic indications, dosage forms, doses and administration mode for which the medicine is approved. Regarding the high cost class H drugs for severe pathologies, their prescription occurs exclusively in the hospital and by a specialist physician.

At the IFO, it is mandatory to send a report to the National Health Service every 3 months, with the identification of the patient and the quantities and therapeutic indications of the dispensed drugs. The majority of class H medicines dispensed at the IFO are the antiretrovirals.

5.2. Dispensing to the services

Each hospital service establishes, along with the pharmaceutical services, the maximal and minimal stocks for medicines and other pharmaceutical products needed, depending on their consumption and need.

The refill of stock requests are sent by the nurses from the services, evaluated by the pharmacists and dispensed by a nurse in the pharmaceutical services team. Weekly, this nurse fills a car with drawers, alphabetically ordered, with the medicines and pharmaceutical products in the required quantities. The car is then transported to the respective services.

The main advantage of this distribution system is that it allows a better control of the consumptions, avoiding accumulation or disruption of stocks in the services.

5.3. Dispensing of narcotic and psychotropic drugs

A prescriber physician fills the request document for narcotic and psychotropic drugs – *Modulo per l'approvvigionamento dei reparti delle aziende ospedaliera presso a la farmacia interna di medicinale a base di stupefacienti e sostanze psicotrope* (Attachment 9), which is dated and signed by the clinical director of the service. Each request contains only one drug, in the same dosage form and dose. The quantity can be expressed in units of dosage form (capsules, vials, etc.), weight units (grams, milligrams) or volume units (milliliters).

The complete document is verified by the responsible pharmacist, who checks if it is correctly filled and proceeds to the drug dispensing when no errors are detected. The drug is put in a bag, properly identified, and transported to the requesting service by a technical

assistant. The responsible pharmacist completes the request document and registers the output of the substances in a proper form – *Registro di entrata e uscita delle sostanze e preparazione soggete alla disciplina degli stupefacienti e sostanze psicotrope* (Attachment 6).

5.4. Dispensing of experimental therapy

The experimental therapy can be dispensed, by the responsible pharmacist, directly to the patient or to a hospital service.

In the latter, the technical assistant transports the experimental preparations in a proper cart (exclusively used for the transportation of cytotoxic medicines) and delivers them to a nurse at the service, along with the sheet of delivery – *Foglio di consegna*. The nurse verifies and signs the delivery document, which is then stored with a copy of the prescription.

6. Production

Production is one of the most important activities of the pharmaceutical services of the IFO. All the pharmaceutical formulations produced at the IFO are according to the ISO 9000 quality management system. Cytotoxic drugs are prepared in the internal pharmacy, while non-sterile galenic preparations are prepared in the external pharmacy.

6.1. Cytotoxic drugs

As an oncological institute, the preparation of cytotoxic medicines for cancer treatment is of major importance. The antineoplastic drugs present several mechanisms of action, such as alkylating agents, antimetabolites, cytotoxic antibiotics, monoclonal antibodies and others. This confers them carcinogenic, mutagenic and teratogenic potential and, therefore, numerous risks, not only for the patient, but also for the health professionals that prepare and administer these drugs. The exposure to cytotoxic drugs should be minimized, respecting the strict care demanded in their manipulation, transport and administration.

All the prescriptions must be validated by the responsible pharmacists before its preparation; the validation office is equipped with two computers, two label printers, a phone, some therapeutic handbooks, file cabinets and a fax. The cytotoxic drugs are prepared in the Antiblastic Chemotherapy Manipulation Unit (*Unità di Manipolazione di Chemioterapici Antiblastici* – UMaCA), a restricted area constituted by a central laboratory, an adjacent room with fridges and cabinets where the raw materials and the handling materials (serums, syringes, spikes, gloves, masks) are stored and two communication areas: one where the nurses clean and change lab coats before they enter and exit from the laboratory and another, with two double glass windows, where the materials go in – window 1 – and out – window 2. The UMaCA is a confined area where only authorized personnel have access. The laboratory is equipped with three vertical laminar flow chambers, where the cytotoxics are handled, a small area with

the sterilized products to use in the manipulation and another space where the copies of the prescriptions are stored.

Briefly, the cytotoxic medicines circuit follows these steps: reception, validation of the prescription, storage, preparation, dispensing and administration.

1. Reception: The physician sends the prescription via fax to the pharmaceutical services. A sequential number is attributed to each prescription, according to its arrival time and prioritizing the long-term therapies.
2. Validation of the prescription: The responsible pharmacist verifies the prescription, with special focus on the patient's name, height, weight and body surface area (BSA), clinical diagnostic, treatment cycle, medicine(s) and dose, administration route, estimated time of perfusion, service, date and prescriber signature, comparing it with the patient's data already registered in the information database.

   The dose is calculated taking into account the BSA or the weight of the patient. The pharmacist must confirm the operation in order to verify the accuracy of the prescribed dose.

   If the prescription is incomplete, inconsistent or illegible, the physician is contacted, usually by telephone, to review it and to make the appropriate corrections. The prescription is returned via fax to the corresponding service, where the physician corrects and signs it before sending it back to the internal pharmacy.

   After its validation, the pharmacist signs the prescription, inserts the data in the computer internal database and prints the label(s) for the therapy to be administered in the same day (some prescriptions consider the following days of therapy and have to be validated every day). The labels and the prescription are then sent to the manipulation laboratory through the glass window 1 (in).

   The information presented in the labels includes: the progressive number, the patient's name, the date of preparation of the cytotoxic drug, the requesting service, the identification of the pharmacist, the identification of the drug and its dose and volume, along with the dilution medium to be used (saline or glucose solution) and its correspondent volume. A different pharmacist monitors all the information contained in the label, confirms if it corresponds to the prescription and then signs it (Attachment 10).
3. Storage: Before delivering the prescriptions into the laboratory, the technical assistants copies them and files the copies by administration date.
4. Preparation: In the laboratory, the nurses prepare the cytotoxic therapies, not without controlling once again the data in the labels and their accordance with the prescription.

   They start to prepare the same day therapy and the stable drugs of successive days therapy, never forgetting to write the preparation time. For non-stable

medicines of successive days therapy, the nurse keeps the prescription and the necessary raw materials, taking in attention to whether they can be kept at room temperature or if they have to be stored in the fridge and if they need to be protected from direct light.

The manipulated products are prepared according to the information constant in the labels, in the prescriptions and in the summary of product characteristics in terms of pharmaceutical aspects.

Before sending the cytotoxic drugs to the services, the nurses control the preparation and the labels one last time, verifying their organoleptic characteristics and packaging. Then, they register the data in a proper file and sign it; the file is archived in the pharmacy with the copy of the prescription.

5. Dispensing: Dispensing is similar to what was previously described for the dispensing of experimental therapy.
6. Administration: The nurses at the services administer the correct drug to the correct patient.

During the internship, there was the opportunity to observe the preparation of several cytotoxic drugs. For the preparation of a new experimental cytotoxic, the pharmaceutical company provides a protocol which must be entirely fulfilled; some mandatory data include the date, start and end time of preparation and the signature of the nurse that prepared the drug. These documents are filed by the responsible pharmacist in the clinical trials room.

Additionally, in order to follow the whole circuit of the cytotoxic drugs within the hospital, some visits were made to the services where the cytotoxic therapies were administered to the patients.

Also, there was a request to complete a *Microsoft Office Excel®* table related to the stability of all the cytotoxic active principles used at the IFO, based on the summary of product characteristics for the respective commercial brand of each active principle. The collected data were:

- If the intact bottle should be kept in the fridge or at room temperature and/or protected from direct light;
- If the product should be kept in the fridge or at room temperature and/or protected from direct light after dilution (until administration);
- It there must be a light-protecting infusion set or a filter in the administration set;
- Relevant additional information (for example, interactions with polycarbonate- or aluminum-containing material).

6.2. Non-sterile galenic preparations

The non-sterile galenic preparations are prepared at the 2nd floor of the external pharmaceutical services, in Trastevere, where the ISG used to be located. Everyday, non-sterile galenic formulations for the IFO (and for the public) are prepared.

In general, the galenic preparations consist in dermatological ointments or creams (cold cream, salicylic vaseline, oleo-calcareous liniment) and solutions (strong lugol solution), with the occasional capsules or pharmaceutical papers preparation.

The pharmacists order the raw materials from the suppliers as they are needed (when the package is over or if it is a new raw material never used before). When the raw materials arrive, they are registered in a *Microsoft Office Access*® internal database, which attributes an internal serial number to each of them. This database also includes the protocols and procedures for each galenic preparation (ingredients, procedure, therapeutic indications and eventual notes to include in the label) and the registration of all galenic preparations done in the laboratory. Indeed, this database is a valuable and very useful work instrument.

The medical prescriptions from the IFO enter daily, by fax or e-mail. Additionally, the patients of the pharmacy can also request for galenic prescriptions to be prepared. All the prescriptions must be validated by the pharmacists, but it was a responsibility of the interns to prepare them.

First, the interns ensured that all the working environment was properly cleaned, with paper towels soaked in 70 % ethanol. Then, all the necessary raw materials were collected, as well as all the equipment and the packaging material. The galenic preparation data were filled in the preparation sheet – *Foglio di lavoro* (Attachment 11), which served as a base to register the preparation in the internal information database; it includes the date, the progressive number, information about the raw materials (quantity and internal serial number) and the signature of the professional who performs the galenic preparation. All the procedures of the galenic preparations follow the Good Preparation Standards (*Norme di Buona Preparazione*) and the Official Pharmacopoeia (*Farmacopea Ufficiale*).

Each raw material had an internal label, with the serial number attributed by the database, its identification, the supplier or manufacturer, the date of acquisition and the price.

After the preparation, the full package must contain a label with the identification and address of the pharmacy, prescriber physician and patient identifications, price, active principle and excipients (with quantities), progressive number, preparation date, expiry date and other relevant information (Attachment 12). The sale price is calculated as a sum of the raw materials and containers prices and fees. The galenic preparations destined to the IFO are put into a box and transported there by a hospital driver, accompanied by a transport guide. The transport guide and prescription copies are stored in the pharmacy for a minimum of three years.

7. Clinical trials

According to the Law #21/2014 of April 16th, a clinical trial is "any research in human subjects intended to discover or to identify the clinical, pharmacological or other pharmacodynamic effects of one or more investigational medicinal products, or to observe any adverse reactions of one or more medicinal products under research, or to study its absorption, distribution, metabolism and excretion in order to determine its safety and efficacy" [94]. Nevertheless, it is important to note that clinical trials involve complex legal (respect all the legal requirements), methodological (follow the Good Clinical Practice) and ethical (honor the Declaration of Helsinki) aspects.

As mentioned above, the IFO are IRCCS, which means they actively participate in research; most of the clinical trials carried out at the IFO are related with the oncology.

The representatives of the pharmaceutical industries visit the IFO to meet with the pharmacists, physicians and nurses and to discuss the putative participation of the hospital in their clinical trial studies. These meetings happen very frequently, almost every week.

During the hospital pharmacy internship, there was the opportunity to be present in one meeting where the advantages of subcutaneous rituximab over intravenous rituximab and trastuzumab were discussed and in a presentation of a clinical trial to study a new active principle.

7.1. Experimental medicines circuit

The experimental medicines circuit of a clinical trial at the IFO is briefly described in this section.

1. Start procedures: At the beginning, all clinical trials involving experimental medicines must be authorized by the hospital director, after the approval of the clinical trial protocol by the ethics committee and the AIFA. The secretariat of the ethics committee sends a copy of the authorization document to the director of the hospital pharmacy; this document has to be in the hospital pharmacy before the beginning of the clinical trial.
2. Sending of the experimental medicines by the supplier: The developer, which may be an individual, a society, an organization or a company, responsible for the clinical trial, sends the experimental medicine after the receiving of the authorization document from the ethics committee of the hospital. The medicines are exclusively sent to the hospital pharmacy, accompanied by a transport document, a declaration of its use only for a clinical trial and the identification of the physician from the hospital responsible for conducting the clinical trial. At the IFO, most of the pharmacists are aware of the current clinical trials; however, there is a responsible pharmacist, specialist in the field of relevance.

3. Reception of the experimental medicines in the pharmacy:
    a) First, a technical assistant receives the package containing the experimental medicines and verifies all the above mentioned documents, as well as the physical conditions of the package. If a problem is not observed, a reception declaration is completed – *Dichiarazione di ricevimento e invio DM per sperimentazione clinica* – with the date and hour of delivery and the storage conditions; the transport document is also dated and signed. The pharmacist is then notified that the package arrived.

       The responsible pharmacist, the only one authorized to open the package, starts by an accurate verification of its content, checking the qualitative and quantitative similarity between the content of the package and the transport document. Particularly, the pharmacist confirms the integrity, the expiry date, the proper conservation of the medicine and the accordance of the label with the applicable law, among other quality aspects.
    b) After, the pharmacist stores the medicines in the clinical trials room, separately from other drugs, in agreement with its storage conditions, listed in the document that accompanies the package and indicated by the developer (room temperature at 25 ºC or in the fridge between 2 ºC to 8 ºC, protected or not from direct light, etc.).

   All documentation is also stored in this room, including the transport document, a copy of the document that proves the dispatch of the experimental medicines by the supplier and a copy of the document of its reception, which is sent to the developer via fax or e-mail.

   Occasionally, the experimental medicines are not delivered according to the proper conditions. In these cases, the responsible pharmacist shall contact the developer, by fax or e-mail.
4. Conservation of the experimental medicines: The transport and storage of the experimental medicines must assure the appropriate conditions for their conservation. As mentioned, depending on the manufacturer's indications, the experimental medicines are stored in a proper room under 25 ºC or between 2 ºC to 8 ºC. This room is equipped with a temperature monitoring system and, daily, an administrative assistant checks and registers the temperature; these records are analyzed monthly. Additionally, the light conditions must also be taken into account, protecting from light the light-sensitive medicines.
5. Dispatch of the experimental medicines to the physician involved in the clinical trial (first delivery): The hospital pharmacy delivers the experimental medicines to the nurses assigned by the physician to participate in the clinical trial. Upon the first delivery, the pharmacist sends an e-mail to the responsible physician

notifying him about the date of delivery; this e-mail is stamped and attached with all the relevant documentation of the clinical trial. With the experimental medicine follows a copy of its reception document and its datasheet. Hereafter, the management of the experimental medicines is a responsibility of the physician.

The prescription of these medicines must always refer that they belong to a clinical trial. The responsible pharmacist validates the prescription, collects the medicine in question and completes the prescription with the drug name and batch number and dates and signs the document and the medicine box. The original is stored in the clinical trials room and a copy is sent to the developer.

At the IFO, the experimental medicines are also dispensed directly to the patient, through a medical prescription and upon presentation of the patient identification document.

6. Withdraw and destruction of the experimental medicines: The experimental medicines may be withdrawn by several motives, including the proximity of the expiry date, non-use by the departments, incorrect storage or on request by the developer. A document signed by the scientific responsible of the study is delivered to the pharmacy, certifying the withdraw and destruction of the experimental medicines.
7. Clinical trial record: The clinical trial record – *Registro delle sperimentazioni con farmaco della farmacia ospedaliera* – is a responsibility of the pharmacist. It includes:
    - The authorization to perform the clinical trial;
    - The declaration of dispatch of the experimental drugs and the document of their reception in the hospital pharmacy;
    - The document of delivery and reception in the departments;
    - The statement of clinical trial conclusion by the research responsible;
    - Proof of use of the experimental medicines by the physician;
    - Other important documents.

8. Pharmacovigilance

"Pharmacovigilance seeks to improve the quality and safety of medicines, in defense of the patients and the Public Health, through the detection, assessment and prevention of adverse drug reactions" [95].

In Italy, since 2001, the pharmacovigilance system is based on the Pharmacovigilance National Network – *Rete Nazionale di Farmacovigilanza* (RNF). This network ensures the collection, management and analysis of spontaneous reports of suspected adverse drug reactions (ADR); also, it warrants the willing and widespread dissemination of the AIFA information related to the safety of medicines, through a network that involves the AIFA,

regional and local health units, hospitals, IRCCS and the pharmaceutical industry. Since 2006, the pharmacovigilance activities have been strengthened with the consolidation of the national network and its connection to Eudravigilance (the European Medicines Agency pharmacovigilance system) and to the World Health Organization (WHO) Centre for International Drug Monitoring [96].

An ADR is defined as "a noxious and unintended response to one or more medicines" [95], in the usual dose administrated to humans for the prophylaxis, diagnosis, treatment or modification of the physiological function. In Italy, anyone can indicate a suspected ADR, not only health workers (physicians, pharmacists, nurses, etc.). To report an ADR, one must fill out the notification document and send it to the responsible of the hospital pharmacovigilance; alternatively, it can be printed from or filed at the AIFA's website.

As IRCCS, the IFO give special attention to the pharmacovigilance activities, having a specific department for that purpose, constituted by three pharmacists and an administrative assistant. During the internship, the intern accompanied the pharmacist that collects the signaling sheets – *Scheda unica di segnalazione di sospetta reazione avversa* (Attachment 13) – and additional information about the potentially identified ADR from the hospital services. After gathering all the required data, it is inserted in the RNF by the pharmacist.

Only the authorized pharmacist has access to the RNF, through his personal login credentials, and is able to insert the data in the Suspect Adverse Reaction Report. After that, the pharmacist has to send two distinct e-mails: to the health professional that reported the suspected ADR (generally a physician), containing a copy of the RNF report and RNF data regarding other similar ADRs previously identified, and to the pharmaceutical industry that commercializes the medicine.

In general, the RNF answers the ADR signaling in a short period of time and frequently asks for more detailed data about the patient or the pathology or even for diagnostic exams, as blood tests. In these cases, the pharmacist meets with the physician who signed the report in order to collect the additional information requested and sends it to the RNF. A similar procedure is applied to the pharmaceutical industries, which often ask for more detailed data.

During the stay in this department, the intern participated in this activity and also in the elaboration of a *Microsoft Office Excel*® file containing all the active principles prescribed by each physician. A research in the European Association of Hospital Pharmacists' website for scientific articles about pharmacovigilance was conducted, too.

9. Technical committees

The IFO services are composed by various technical committees:

- The ethics committee – *comitato etico*;
- The nosocomial infection committee – *comitato infezione ospedaliere*;
- The committee for the proper use of blood – *comitato per il buon uso del sangue*;

- The Therapeutic Handbook commission – *commissione del Prontuario Terapeutico*;
- The budget committee – *comitato di budget*.

10. Conclusions

Regardless the country, the main goal of a hospital pharmacist is to ensure the best healthcare possible to people who need and request the services.

Despite all the inherent difficulties of an international internship, which include a different language and a totally unknown health system, the knowledge and the experience achieved during the training of 3 months at the IFO was very enriching, allowing the intern the practical application of the theoretical knowledge previously acquired in the university.

It was an internship wherein the professional team worked mostly based on team work and sharing of knowledge, which helped to develop the professional skills and to prepare the intern as a future healthcare professional. Indeed, everyone at the IFO was always available to help and to answer any doubts, showing also a great curiosity in the differences between the functioning of the hospital pharmacy and the pharmacovigilance between Portugal and Italy.

Personally, the fact that one of the institutes works as an oncological hospital was very interesting, because it opened up new horizons and perspectives for future job opportunities.

Part III – Internship report: community pharmacy

1. Introduction

As stated, the last semester of the Integrated Master Degree in Pharmaceutical Sciences is a curricular internship. It is mandatory to perform at least part of it in a community pharmacy, which is still the main professional output for pharmacists in Portugal.

The community pharmacies constitute one of the most important communication routes with the patients, with their major purpose being "the provision of medicines in conditions that minimize the risks of their use and that allow the evaluation of the clinical outcomes so that the high morbidity and mortality associated with medication can be reduced" [97]. It is responsibility of the community pharmacist to promote the right to a treatment with quality, efficacy and safety, advising the patients on the rational use of drugs, monitoring them and ultimately establishing a trust relationship with the patients [97].

The community pharmacy internship was performed at the Nunes Feijão Pharmacy, near Barreiro, Portugal. Next, there is a brief report about the activities executed in the aforementioned pharmacy between May 11th and August 10th 2015, the culmination of 5 years of studies and challenges of knowledge.

2. General characterization of the pharmacy

2.1. Location, working hours and external appearance

The Nunes Feijão Pharmacy (NFP) is located in the José Gomes Ferreira street, #4B, Vila Chã, Santo António da Charneca (Barreiro). It is opened to the population Monday to Friday from 9 am to 1 pm and from 3 pm to 8 pm and Saturdays from 9 am to 1 pm and from 3 pm to 7 pm, in accordance with the Ordinance #14/2013 of January 11th (which alters the Ordinance #277/2012 of September 12th). Given the number of pharmacies existing in the municipality of Barreiro, the NFP is on permanent service (24 hours) every 18 days [98, 99].

As stated in the Decree-Law #307/2007 of August 31st, the NFP has some important information for its users displayed on the door:

- The identification of the technical director;
- The working hours;
- The permanent service and on call pharmacy scales of the municipality (printed from the Lisbon and Tagus Valley Regional Administration of Health website), as well as the addresses and phone contacts of the pharmacies;
- The provided pharmaceutical services;
- Eventual health screenings carried out in the pharmacy;

- The existence of a complaint's book [100].

On the outside, the NFP is identified by a big sign with its name and the characteristic green cross. When the pharmacy is on permanent service, these stay enlightened during the night [100].

To ensure the safety of the pharmacist during the permanent service, there is a lateral wicket to attend the clients after the normal working hours of the pharmacy. This way, the pharmacist can dispense the medicines to the public without their entrance in the facilities [99].

Concerning the storefront of the pharmacy, it is decorated in consonance with the current season, with advertising posters of cosmetic or parapharmacy products or promotional actions of such products.

2.2. Facilities

The mandatory facilities in every pharmacy are the public attendance room, the personalized attendance office, the storage, the laboratory and toilets. Additionally, the NFP has a room, where the permanent service pharmacist spends the night, equipped with a fridge and a microwaves, the technical director's office and an extra storage area [100, 101]. The public has access only to the attendance room and to the personalized attendance office, all the others are restricted to the pharmacy personnel.

The public attendance area of the NFP is characterized by a broad space with three connected but individual attendance counters, equipped with computers with the Sifarma 2000 installed, a specific software which easies and speeds up the attendance, without losing its quality. Dermocosmetic, family care, childcare, child health, infant feeding, podiatry, orthopedic and some natural products are exposed to the patients in this area. Inside, in drawers between the counters, are oral and dental care products, OTC (over the counter) products for the treatment of aches, colds, seasonal allergies, heartburn and small bottles of physiological serum and dermal anti-inflammatories.

The storage area is divided in the order's reception space, equipped with a computer (with the Sifarma 2000 software), a printer and a copy machine, and drawers, racks and the fridge. All products are stored in alphabetical order, from the lowest to the highest dose and following the first expiry/first out principle.

The extra products from larger orders that do not fit in the proper exposition shelves or drawers are kept in the lower ground floor, where there is a bigger storage space. The blood glucose measuring devices are also placed there, as well as old invoices, product losses and returns to the commercial representatives.

The laboratory, equipped with the necessary material for the preparation of galenic and extemporaneous preparations, is only used for the extemporaneous preparation of non-aqueous stable suspensions with purified water.

It is in the personalized attendance office that the patient's blood pressure and glucose and cholesterol levels are measured. To perform these services, there is a blood pressure monitor device, a blood glucose measuring device and the *Reflotron® Plus* system to measure total cholesterol. Of course, there are also the necessary lancets, strips and capillary tubes, 70 % ethanol, cotton and bandages. All the other screenings are also performed in this room, as the asthma and nutrition screenings.

The office of the technical director has an extra copy machine and it is where he performs eventual meetings and takes care of all the management procedures and logistic, administrative and accounting aspects.

2.3. Human resources

The NFP's team is composed only by women: the technical director and owner, Dr. Isabel Feijão, one pharmacist, Dr. Cátia Silva, and two pharmacy technicians, Andreia Rodrigues and Filomena Teles. It is a very cooperative and supporting team, which allows an excellent working environment.

The pharmacies should have more pharmacists than other auxiliary professionals, constituting the pharmaceutical and non-pharmaceutical boards of the pharmacy, respectively [100].

The main responsibility of the pharmacists is towards the health and well-being of the patients and citizens in general, promoting the right to a safe and an effective quality treatment (as stated before). They should also advice about the rational use of medicines, monitor their patients and perform other necessary pharmaceutical care, never forgetting the quality of the services [97].

2.4. Scientific documents and information support

The pharmacists and other health professionals working at the pharmacy must have sources of information about the medicines at their disposal, organized and continuously updated, in a paper or a digital library. During the dispensing, it is mandatory that the pharmacist accesses the information about the therapeutic indications, contraindications, interactions, dosage and administration and precautions with the use of the medicines. Nowadays, this information is available in the Sifarma 2000 software; however, the pharmacy must have a Therapeutic Handbook and the summaries of product characteristics for this purpose [97].

In the NFP, additionally, there are the 2010's Therapeutic Map, the National Therapeutic Index, the National Medicines' Form, the Therapeutic Symposium, the Portuguese Pharmacopoeia, the Portuguese Galenic Form, the Good Pharmaceutical Practices for the community pharmacy, the Pharmacists' Order's Code of Ethics and the Pharmacists' Order's by-laws, some Medical Dictionaries, the Non-prescription Drugs, the Nutritional Healing, among

others. Periodically, the pharmacy also receives some specialized magazines, as the Pharmacist News, the Portuguese Pharmacies and the PH.

As mentioned, the NFP's computers are equipped with the Sifarma 2000 software. It allows the pharmacist to access the information more quickly and diminishes the time to search for the medicine since, in cases of electronic prescription, it immediately gives the possible options. As such, the pharmacist has the opportunity to enhance and to improve the attendance time, providing the patient with a more accurate and clarified information.

Moreover, this software permits that each product has an individual record with all the information regarding its scientific data, stock movements, purchases, sales, prices and other relevant information, facilitating the stock management of every product. It is also possible to create individual files for the patients, with their relevant data, to improve the communication and to allow credit sales in case of urgency.

3. Organization and management

3.1. Provision

3.1.1. Orders management

The pharmacist responsible for the stock and order management must be aware of the most and less sold products in the pharmacy, taking into account their publicity, seasonality, demand, need and preference of the patients, as well as the most common health problems of the population surrounding the pharmacy.

According to the medication's by-laws (Decree-Law #176/2006 of August 30th), "pharmacies should always have available for sale at least three medicines with the same active ingredient, dosage form and dose, from the five with the lowest price of the correspondent homogeneous group" [102], so that the patients could have a freedom of choice. Finally, the existing space for storage is also taken into account.

With the current economic situation, a good management is crucial in every pharmacy. The pharmacist must choose the supplier that offers the best conditions and advantages and has to evaluate the most and less needed products in the pharmacy. In fact, the orders and stocks management are of the most important activities and with most impact on the management of the pharmacy.

3.1.1.1. Order submitting

The NFP receives orders from three suppliers: Botelho & Rodrigues, OCP and Udifar.

Botelho & Rodrigues is the primary supplier, to which 2 daily orders are sent through the Sifarma 2000: one before 1 pm and another before 8 pm. The daily orders are automatically generated by the Sifarma 2000, taking into account the shortages and the medicines sold between orders. This list is managed according to the minimum and maximum stocks for each

product, defined in each individual product file. If the maximum stock is zero, the system does not generate a request to order that product. Before the limit hours to send the order the pharmacist responsible for its submission analyses the list generated by the software and edits it according with the current needs of the pharmacy, sending the order after confirming all the products.

The punctual orders are sent to all the three suppliers, via Sifarma 2000 (instantaneous orders), the supplier's website or telephone call. OCP and Udifar are mostly used to order sold out or not commercialized products by Botelho & Rodrigues. These orders require stock confirmation by the supplier.

Some products are not provided to the intermediate suppliers by the manufacturing companies. In these cases, the pharmacy contacts directly with the respective company and orders the needed medicine (this happens, for example, with the anticonvulsant drug Zonegran, as observed during the internship).

The dermocosmetic, childcare and generic medicines, among others products, are mostly ordered directly to the commercial representative of the company, hence this method of purchase is more advantageous in terms of stock maintenance, merchandising material supply and financially.

3.1.1.2. Reception and verification of the order

After the delivery of the orders, the pharmacy technician responsible for entering the products in the Sifarma 2000 system looks for the invoice or dispatch note, which must accompany the order. He confers if the quantities are correct and if the products arrived in good conditions and verifies the shortages. During this process, the technician inserts the products in the system, always checking the expiring dates, and after that each product is stored in its proper place.

Some chemical products come with the analysis report, which must be filed in a specific dossier, destined to the purpose.

The order's reception and verification must be performed carefully in order to avoid stock errors or financial losses.

3.2. Price marking

"The price regime for the reimbursement of the prescribed and non-prescribed medicines is fixed by a decree-law" [102], and so these medications are received in the pharmacy with their selling price to the public already established. The selling price to the public of a medicine is composed by:

- The selling price to the supplier;
- The trading margin of the manufacturer;
- The trading margin of the supplier;
- The tax on the medicines sale;

- The value-added tax (VAT) [103].

The maximum trading margins, both for the manufacturers and for the pharmacies, are defined in the Decree-Law #112/2011 of November 29th. In the pharmacies case, these margins are divided according to the selling prices to the public: from less than 5€ to more than 50€. To each step is attributed a margin calculated over the selling price to the supplier [103].

The OTC products are priced in the pharmacy after their reception. The prices attributed to these products result from an internally defined margin on the selling price to the supplier and the VAT. The Sifarma 2000 generates labels that are printed after finalizing the reception of the order.

3.3. Expiring dates control

The expiring date of a medicine or a medicinal product is defined as the period beyond the manufacturer does not guarantee its stability, efficacy, safety and/or quality. This means that the product should not be dispensed to the public after this date or when this date is reached during the treatment. Expiring dates control in the pharmacy is a crucial process to guarantee the quality of all the products.

Every month, a list of the products which expiring date is reached during the next 3 months is printed in the NFP. The responsible pharmacy technician verifies all these products, removing the ones which effectively expire within a 3 months period; for these, a returning note to the supplier or manufacturer is generated. In some cases, the expiring dates may not correspond with the listed ones, being updated in the respective product file.

3.4. Storage

In order to facilitate the access to the products, it is of major importance that the storage is organized. In the NFP all products are grouped in alphabetical order, from the lowest to the highest dose and following the "first in/first out" principle – from the shortest to the largest expiring date.

As mentioned above, the NFP's storage is divided in:

- Drawers, mostly for the prescription medicines, distributed by dosage form:
    - Tablets and capsules;
    - Suspensions;
    - Eye drops;
    - Drops;
    - Creams and ointments;
    - Chemicals;
    - Injectable medicines;
    - Powders;
    - Products for external use;

  - Laxatives;
  - Suppositories;
  - Contraceptive pills;
  - Gynecological products;
  - Diagnostic strips and lancets;
  - Veterinary products;
- Racks for the syrups, ampoules, vitamins and other natural products and some childcare products;
- The fridge for the products that need to be stored between 2 ºC to 8 ºC.

The shelves of the public attendance area are filled with OTC products exposed, organized by their purpose – dermocosmetic, family care, childcare, etc. – and by brand. The same is observed to the products in the drawers between the counters.

In the lower ground floor is a wide storage area for the products that come in large orders and do not fit in the main floor: dermocosmetic, family care and childcare products, Ben-U-Ron and Ib-U-Ron, and also blood glucose measuring devices, office supplies and publicity and exhibitors that are currently not in use. Anytime one of these products is in shortage at the main floor, the stock is replaced with the units kept in the lower ground floor.

The old invoices, product losses and returns to the commercial representative, which must be collected directly by the manufacturer, are also kept in the lower ground floor.

To ensure the physical and chemical stability of all products, the pharmacy is equipped with thermo-hygrometers on both storage floors and in the fridge. These devices register the temperature and humidity values, which are monthly analyzed by the responsible pharmacist.

3.5. Returns

Medicines and other products may be sent back to the supplier or to the manufacturer in some justified cases, including:

- Recommended by the INFARMED (National Authority of Medicines and Health Products) or of the holder of the marketing authorization;
- Products not ordered but received in the pharmacy by mistake;
- Ordered products received in higher quantities than the requested;
- Damaged products;
- Products received with a short expiring date;
- Products with an incorrect bill price;
- Products of the pharmacy which expiry date is lower than 3 months.

The responsible pharmacy technician creates a return note in the “Returns management” option in the Sifarma 2000. The product’s code is inserted, along with the quantity to be returned and the motive of return. The identification of the pharmacy and the date are assumed by default by the software. The products are sent to the supplier or to the manufacturer with two copies of the return note (a third one is filed in the pharmacy) and with a copy of the

invoice. The suppliers collect the returns on their subsequent delivery, while the manufacturers only collect them when a commercial representative visits the pharmacy.

The return may or may not be accepted by the supplier or the manufacturer. If the return is accepted, the products may be substituted by similar ones or a credit note may be sent to the pharmacy. If the return is not accepted, the products are sent back to the pharmacy and a product loss is generated; these boxes must be kept for at least 5 years, in the lower ground floor.

4. Medicines and other healthcare products

A medicinal product is "any substance or combination of substances presented for treating or preventing diseases or their symptoms in human beings or that may be used or administered to human beings to establish a medical diagnosis or, by exerting a pharmacological, immunological or metabolic action, to restore, correct or modify physiological functions" [102]. However, not all the products in a pharmacy are encompassed by this definition. Actually, there are the reference medicinal products, the generic medicinal products, the medicinal products subjected to special legislation, as the narcotic and psychotropic drugs, but also dermocosmetic and hygiene products, phytotherapy medicines, medicines for veterinary use and medical devices.

The need to systematize the medicines by their therapeutic indications was verified, in order to allow the health professionals a better and faster identification of the products, given the therapies they were destined [104].

The pharmacotherapeutic classification system used in Portugal is based on the international ATC (Anatomical Therapeutic Chemical) classification, first developed by Norwegian researchers and then recommended as an international standard by the WHO, in order to measure drug use in different countries. This classification system is divided in accordance with the therapeutic effect of the predominant active substance of each medicine. The active substances are also ordered in subgroups depending on their therapeutic, pharmacological and chemical properties. Each group is divided in 5 levels [104, 105]. Additionally, each ATC code must correspond to a single administration route, dosage form and dose, meaning that, if a medicine presents more than one administration route and/or dosage form and/or dose, it will have as many ATC codes as necessary [105].

The pharmacotherapeutic classification system approved in Portugal is also listed in the Sifarma 2000, allowing the health professionals an easy and quick identification of the pharmacotherapeutic group to which every active ingredient belongs.

Table 12. The 5 levels of therapeutic classification [104-106].

Level	Group	Example	
		Portuguese classification	ATC classification
1	Anatomical main group	Group 4 – Blood	B
2	Therapeutic subgroup	4.3 - anticoagulant and antithrombotic drugs	B01
3	Pharmacological subgroup	4.3.1 – anticoagulant drugs	B01A
4	Chemical subgroup	4.3.1.3 – antiplatelet drugs	B01AC
5	Active ingredient	Ticlopidine	B01AC05

4.1. Medicines subjected to medical prescription

"The dispensing of medicines is the professional act in which the pharmacist, after evaluating the medication, gives medicines or medicinal products to patients through a medical prescription or a self-medication regimen or pharmaceutical statement, accompanied by all the needed information for the correct use of medicines", as stated in the Good Pharmaceutical Practices for the community pharmacy. During this act, "the pharmacist evaluates the dispensed medication, aiming to identify and to solve medicine's related problems and to protect the patient of possible negative outcomes associated with the medication" [97].

The medications are classified in medicines subjected to medical prescription and medicines not subjected to medical prescription. The firsts can also be divided in renewable prescription, special prescription and restricted prescription, only for use in certain specialized areas [102].

The medicines subjected to medical prescription are those that fulfill one of the following conditions:

- Directly or indirectly, may pose a risk to the patient's health, even when they are used for the purpose they are intended, if used without medical supervision;
- Directly or indirectly, may pose a risk to the health, when used often and in considerable quantities for other purposes than the ones for which they are intended;
- Substances, or preparations of substances, which activity or adverse reactions require further investigation;
- Intended for parenteral administration [102].

As the definition states, to be dispensed, these medicines must be in electronic or manual prescriptions following the models approved by the Dispatch #15700/2012 of November 30th (Attachmentes 14 to 16) [107]. The electronic prescriptions may be renewable, printed in 3 copies of the same prescription with a validity of 6 months, or unitary, with a validity of 30 days.

The manual prescriptions cannot be renewable, always having a validity of 30 days [108]. These should only be used in exceptional cases, as the electronic prescription system reduces the risk of errors during the prescription and the medicines dispensing and facilitates the

communication and the understanding between health professionals. It is mandatory that the prescriber physician justifies the reason why he/she is giving a manual prescription, marking one of the following exceptions provided in the prescription:

a) Failure of the computer system;
b) Prescribing doctor's inadaptation (confirmed and validated once a year by the respective professional Order);
c) Prescription at home;
d) Up to 40 prescriptions per month [108].

To be considered valid, an electronic prescription must contain its unique number; the prescribing place and the identification of the physician; the patient's name and the beneficiary number; the responsible financial entity; the reference to special regimens of reimbursement, if applicable, and the dispatch that establishes them; the international common denomination of the active substance; the dose, dosage form, package size and the number of packages; if appropriate, the trade name of the product; the date of prescription and the prescriber's signature [108]. Each prescribed product is accompanied by a code: the national code for the electronic prescription of medicines, when it is identified by the international common denomination, or the barcode of the trade product.

In cases of manual prescriptions, to be valid, these must contain all the items referred on the electronic prescriptions except the codes. Additionally, there must be a sticker identifying the place of prescription, if applicable; a sticker identifying the prescribing physician, as well as his medical specialty and telephone contact, and the above referred exception for manual prescription identified [108]. The manual prescriptions cannot be written in different handwritings or pen colors or have strikethroughs.

Each medical prescription contains a maximum of 4 medicines, with no more than 2 packages per medicine. If the medicine is presented in the unitary form, an exception could be present and up to 4 equal packages could be prescribed [109].

In some situations, the prescription can include the trade name of the product instead of the international common denomination of the active substance. These include:

- The prescription of a medicine with an active substance for which there is no reimbursed generic medicine or for which there is only the trade product and licenses;
- A technical justification of the prescriber regarding the medicine's replacement insusceptibility, with one of the following options:
    a) A medicine with a narrow therapeutic margin or index, according to information provided by the INFARMED;
    b) An established suspicion, previously reported to the INFARMED, of intolerance or adverse reaction to a medicine with the same active substance but identified by another trade name;
    c) A medicine to ensure the continuity of a treatment for longer than 28 days [108].

After verifying that the prescription fulfills all these aspects, the pharmacist proceeds to the dispensing of the product(s) following the Sifarma 2000 steps. During the attendance time, the pharmacist should advice and explain the medication to the patient, answering any question that may arise and making sure that the patient leaves the pharmacy with a full understanding of the medication.

One of the sale's steps is the print, in the back of the prescription, of the following information: the identification of the pharmacy; the date of dispensing of the medicine(s); the total price of each dispensed medicine, the reimbursement value and the value to be paid by the patient, as well as the total value of the prescription, the total value of reimbursement and the total value to be paid by the patient; the identification of the medicine(s), written and in barcode; the patient's statement "I declare that the *nn* medicine packages listed in the prescription were dispensed to me and that a professional provided me advice on its use" and one of the following possibilities, depending on the case:

- If the patient did not use the right of option: "I declare that I did not exert the right of option";
- If the patient used the right of option: "I declare that I exerted the right of option for the medication with price above the 5th cheaper";
- If the patient used the right of option in case of prescription with the technical justification of ensuring the continuity of a treatment for longer than 28 days: "I declare that I exerted the right of option for a medicine cheaper than the prescribed one to therapeutic continuity for longer than 28 days" [107].

The pharmacist must also add the pharmacy's stamp and sign the back of the prescription (Attachment 17) [107]. The medication is delivered to the patient, accompanied by the respective invoice and treatment guide and any relevant additional information.

In the NFP, the prescriptions are conferred daily; it is crucial that any possible errors are recognized as soon as possible to rapidly contact the patient and to correct the situation.

4.1.1. Generic medicines

A reference medicinal product, also mentioned as a brand medicine, is the "medication that was authorized on the basis of complete documentation, including results from pharmaceutical and pre-clinical and clinical trials" [102]. When the patent for these drugs ends, the generic medicines can be approved and introduced in the market. Generic medicinal products have "the same qualitative and quantitative composition in active ingredients, the same dosage form and their bioequivalence with the reference medicinal product has been demonstrated by appropriate bioavailability studies" [102].

All pharmacies should have available for sale at least three medicines with the same bioactive ingredient, dosage form and dose, from the five with the lowest price of the correspondent homogeneous group [102, 108]. For all the prescriptions with the international common denomination, the pharmacist asks the patient whether he prefers the brand medicine

or a generic one, explaining the differences between both; if the patient chooses the latter, he can still select the manufacturing laboratory or just pick the cheapest.

4.1.2. Reimbursement

"The National Health System (NHS) implemented a health policy people-focused and oriented to more and better health", aiming for "a national health system sustainable and well managed". "The drugs reimbursement system should be directed towards getting a better equity and more value for all citizens". As such, fairer measures were adopted for the access to medicines [110].

The reimbursement of medicines is cumulatively conditioned by the technical and the scientific demonstration of therapeutic innovation or equivalence, for the claimed therapeutic indications, and by the demonstration of economic advantage [110, 111]. The State's reimbursement corresponds to a percentage of the selling price to the public, according to the following levels:

- Level A: 90 % of the selling price of a medicine;
- Level B: 69 % of the selling price of a medicine;
- Level C: 37 % of the selling price of a medicine;
- Level D: 15 % of the selling price of a medicine [112].

The pharmaceutical groups and subgroups that take part of each level are listed in the Ordinance #195-D/2015 of June 30th [112]. In the case of medicines belonging to a homogeneous group, the reimbursement value is a percentage of its reference price (which may, or may not, be the same as the selling price to the public). The reference price for each homogeneous group is the average of the 5 cheapest prices in the market [111].

The patient's characteristics, prevalence of certain diseases and public health objectives may also be considered to establish the reimbursement values [111]. In fact, the reimbursement regimen is indicated in the prescription – R.C. space, with three possibilities:

- Empty: general regimen of reimbursement of the NHS;
- "R": pensioners regimen (covered by the Article 19th of the Decree-Law #106-A/2010 of October 1st) – the reimbursement of medicines belonging to the level A is increased in 5 % and in 15 % for the medicines in the levels B, C and D [110, 113];
- "O": other special regimen of reimbursement, as a function of certain pathologies or special patients' groups, mentioning the legal diploma; the reimbursement percentages may vary depending on the medication and, for some diseases, this reimbursement regimen is only valid if the prescription is executed by the specialist physician [113].

Table 13. Pathologies and medicines covered and values of reimbursement for the special regimen "O".

Pathology	%	Medicines covered	Prescribing physician	Legal diploma
Alzheimer's disease	37	Medicines listed in the Dispatch #13020/2011	Psychiatrists and neurologists	Dispatch #13020/2011 [114]
Bipolar disease	100	Lithium carbonate	Psychiatrists and neurologists	Dispatch #21094/1999 [115]
Ichthyosis	90	Medicines used in the treatment of ichthyosis	Dermatologists	Dispatch #5635-A/2014 [116]
Infertility	69	Medicines listed in the Dispatch #10910/2009	Doctors in the context	Dispatch #10910/2009 [117]
Inflammatory bowel disease	90	Medicines listed in the Dispatch #1234/2007	Specialist physicians	Dispatch #1234/2007 [118]
Lupus, hemophilia or hemoglobinopathies	100	Medicines reimbursed by the NHS	All	Dispatch #11387-A/2003 [119]
Non-oncological chronic pain	90	Medicines listed in the Dispatch #10280/2008	All	Dispatch #10280/2008 [120]
Oncological pain	90	Medicines listed in the Dispatch #10279/2008	All	Dispatch #10279/2008 [121]
Paramiloidosis	100	All medicines	All	Dispatch #4521/2011 [122]
Psoriasis	90	Keratolytic and anti-psoriatic medicines	All	Law #6/2010 [123]
Rheumatoid arthritis and ankylosing spondylitis	69	Medicines listed in the Dispatch #14123/2009	Specialist physicians	Dispatch #14123/2009 [124]

Although not listed in the table above, diabetes' patients also benefit of special reimbursement conditions, following the restructuring of the National Program for Diabetes Mellitus Prevention and Control, since 1998. This program emerged as an effort to improve the access to the indispensable devices for self-monitoring of metabolic control and for insulin administration [125]. The maximum prices for the test-strips, needles, syringes and lancets were established by the Ordinance #364/2010 of June 23rd, revoked by the Ordinance #222/2014 of November 4th [125, 126]. The State reimburses the test-strips in 85 % and the needles, syringes and lancets in 100 % of the selling prices [126]. The blood glucose measuring devices are also unpaid by the patients.

Additionally to the NHS reimbursement, some entities function as a complementary reimbursement system, which is the case of some health insurances (for example, Multicare, Tranquilidade, EDP-Sãvida, Medis-CTT), the *Caixa Geral de Depósitos* social services, the bankers' syndicates and the Lisbon's city hall social services, and as a special reimbursement system: some laboratories that reimburse their medicines (for example, Betmiga, Vesomni) and programs to promote the public health (for example, the 4HPV for the quadrivalent human papillomavirus vaccine). During the sale in the Sifarma 2000, the pharmacist inserts the complementary reimbursement, which will generate a document similar to the back of a

prescription for the NHS. This document is printed in the back of a copy of the prescription, where a copy of the beneficiary card of the respective complementarity system should also be included. When the special reimbursement is provided by the manufacturing laboratory or by a program to promote the public health, the Sifarma 2000 automatically assumes it (it is not manually inserted by the pharmacist) and a proper document is printed (Attachment 18).

Finally, the Regulatory Circular #13 of March 14th 2013 states that the reimbursement of medicines dispensed to civil service workers, armed forces militaries, public security polices and republican national guards becomes a responsibility of the NHS, with the exception to the cases provided in the Ordinance #1034/2009 of September 11th (occupational disease care for the armed forces) [127].

4.1.3. Special medicines

The special prescription medicines are those that contain substances classified as narcotic or psychotropic that may, if not correctly used, present a substantial risk of medicinal abuse or addiction or be used for illegal purposes [102]. For these reasons, these medicines are subjected to a much tighter control and can only be dispensed upon the presentation of a medical or veterinary prescription according to the approved model. Also, they may not be in the same prescription as medicines not considered narcotic or psychotropic [128].

In order to correctly dispensing these drugs, the pharmacist verifies the identification document of the acquirer, registering its number and date (to confirm its validity) and the acquirer's name, address and age. The patient's name and address are also registered, as well as the prescriber physician's name and the number of the prescription. All these proceedings are executed in a special box of the Sifarma 2000 and the software does not let the sale proceed without the above mentioned data [128]. It is prohibited the selling of narcotic or psychotropic medicines to individuals suffering from mental illnesses or to individuals under 18 years old – if the prescription is destined to a minor, it should be dispensed to his legal tutor [129].

Similarly to what happens with every other prescription, the acquirer must sign its back, confirming that the medicines were dispensed to him. The pharmacy keeps a copy of the prescription (front and back), filed with the narcotic/psychotropic documents that the Sifarma 2000 prints at the end of the sale (Attachment 19), for at least three years [128]. Every three months, the technical director of the pharmacy sends a list of the narcotic and psychotropic drugs dispensed in the pharmacy to the INFARMED. Additionally, once a year, he sends the records of the inputs and outputs of these medicines, which must be closed in the December 31st of every year [128].

4.2. Medicines not subjected to medical prescription

The medicines not subjected to medical prescription are those that do not fulfill the conditions mentioned in the previous section. With few exceptions, these are not reimbursable [102]. To be dispensed, these medicines can be prescribed by a physician (sometimes in just a

sheet identifying the prescribing place), advised by the pharmacist or even requested by the patient.

4.3. Suspended and credit sales

The suspended sales may be created when a patient does not want to or cannot purchase all the medicines of a prescription at one time. With the current economic environment, this situation is rather frequent, with the people not having enough money to buy all the prescribed medicines. When this happens, the prescription is kept in the pharmacy with the receipt of the medicines the patient purchased until the patient comes to buy the remaining medication. The price paid in these cases is the one with the reimbursement.

Additionally, and exceptionally, medicines subject to medical prescription can be dispensed, without presenting one, in proven urgency situations. The urgency dispensing is "the evaluation and dispensing of medication to a patient who needs it urgently", assuming that the pharmacist knows the pharmacotherapeutic profile of the patient [97]. Given the possibility, facilitated by the Sifarma 2000, of creating patient's profiles, it is possible to easily access the medicines they habitually use. In a case of urgency dispensing, the pharmacist confirms the usual pharmacotherapeutic therapy of the patient, dispensing him the necessary medicine through a suspended sale. The patient pays the full price of the medicine, receiving the reimbursement value when this situation is regularized with the prescription.

4.4. Advising and dispensing other healthcare products

4.4.1. Medicines and other products for veterinary use

A medicine for veterinary use is "any substance or combination of substances presented for treating or preventing diseases or their symptoms in animals or that may be used or administered to animals to establish a veterinary diagnose or, by exerting a pharmacological, immunological or metabolic action, to restore, correct or modify physiological functions". These medicines can be dispensed in pharmacies or other legally authorized entities. They are classified in medicines not subject to veterinary prescription, medicines subject to veterinary prescription and medicines for the exclusive use of veterinary doctors [130].

Medicines not subject to veterinary prescription are those that fulfill the following conditions:

- The administration is restricted to formulations requiring no particular knowledge or skills;
- Do not constitute a direct or indirect risk to the animal, to the person who administrates the product or to the environment;
- The summary of product characteristics does not include any warnings of potential serious side effects deriving from their correct use;

- No product containing the same active ingredient has been reported to have serious adverse reactions;
- The summary of product characteristics does not refer contraindications related to other veterinary products not subject to prescription;
- Do not require any special storage conditions;
- It is not known the existence of a risk concerning residues from their use;
- There are no known risks to human or animal health regarding the development of resistance to antimicrobials or anthelmintic substances [130].

The veterinary medicines that do not fulfill these conditions are subject to veterinary prescription. The medicines for the exclusive use of veterinary physicians can only be used by them and, therefore, cannot be dispensed in the community pharmacy [130].

Besides the medicines, other products for veterinary use may also be dispensed in the pharmacy. These are the "substances or mixtures of substances, with no therapeutic or prophylactic indications, for the animals, to promote the wellbeing and the hygiene and health conditions (…), for the veterinary diagnosis and for the environment surrounding the animals, particularly their facilities" and they include adjuvants for prophylaxis and treatment actions in animals and products with biocidal effect to be used in animals and facilities [131].

In the NFP, both the medicines and other products for veterinary use are stored in a proper drawer, separated from the products for the human beings usage. The stock is low and many products are only available if ordered for a specific request.

4.4.2. Dietary products

The dietary products for particular nutritional regimens are foodstuffs "that, due to their special composition or special manufacturing processes, are clearly distinguishable from normal consumption foods" and suitable to meet the special nutrition needs of certain groups of people, as people whose digestive processes are disturbed or with metabolic disorders, people in special physiological conditions who can benefit from a controlled consumption of determined substances in foodstuffs and infants and young children [132].

All foodstuffs intended for these purposes are formulated with a specific nutritional intent and follow the applicable law. Vitamins, mineral salts, amino acids and other substances may also be added. Included in the dietary products are pre-prepared products for infants, transitional milks, baby foods, foods and supplements of weight control or diabetes' treatment or for athletes and dietary foods for specific medical purposes.

The most sold in the NFJ are the dietary products destined to infant feeding, as milks and porridges. The milks are chosen according to the baby's age: infant milk from 0 to 6 months, transitional milk from 6 to 12 months and growth milk from 12 to 36 months. Additionally, some milks are supplemented to correct some dysfunctions: hypoallergenic, anti-cramp, antidiarrheal and anti-constipation milks, as well as lactose-free. The porridges can be dairy or non-dairy and with or without gluten.

4.4.3. Phytotherapy products and food supplements

"Non-conventional therapies are those departing from a different philosophical basis than conventional medicine and that implement specific diagnostic procedures and their own therapeutics", as it is the case of homeopathy and phytotherapy [133].

Herbal medicinal products are medicines "that exclusively contain one or more herbal substances or preparations as active ingredients" [102]. They include products to relieve gastrointestinal disorders, weight loss products, products for fatigue and tiredness and anti-anxiety and sleep-inducing products.

Food supplements, as vitamins, minerals, fatty acids, antioxidants and stimulants are also available in the NFP. These supplements are "foodstuffs intended to supplement the normal diet and that constitute concentrated sources of certain nutrients or other substances with nutritional or physiological effect, alone or in combination, marketed in dosage forms (…) intended to be taken in reduced quantities", as capsules, tablets, pills, powder sachets, liquid ampoules and other similar forms [134].

It is a competence of the pharmacist to advice the most adequate product in each situation, having the most complete knowledge about the composition of every product and taking into account any medication the patient might be taking and possible interactions that may arise with the phytotherapy product or the food supplement.

4.4.4. Cosmetic, dermocosmetic and hygiene products

A cosmetic product is any "substance or mixture intended to be placed in contact with the various superficial parts of the human body, namely epidermis, hair system, nails, lips and external genital organs, or with the teeth and the oral mucosa, with the purpose of cleaning, perfuming, changing the appearance, protecting, maintaining in good condition or correcting body odors". These products must not damage the human health when applied under the appropriate conditions. In Portugal, the responsibility to ensure the safety, efficacy and quality of cosmetic products and to supervise this market belongs to the INFARMED [135].

In the attendance area of the NFP, several types and brands of cosmetic products are exposed, including creams, ointments, sun protectors, make-up, childcare essentials, shampoos and conditioners and other hair care products of the manufacturers A-derma, Avène, Bioderma, D'Aveia, Klorane, La Roche-Posay, Mustela, REN, Uriage, Vichy and others.

Anytime it is necessary, the pharmacist advices the users, resorting of his wide knowledge to evaluate which products are the more adequate to the patient's characteristics. To offer the best advice possible, the pharmacist is always updating his knowledge, either through information about the products provided by the manufacturers or through his own autonomous research.

4.4.5. Medical devices

A medical device is "any instrument, apparatus, appliance, software, material or article used alone or in combination, including the software specifically intended (…) to be used for diagnostic or therapeutic purposes and necessary for the proper functioning of the medical device". Its "main intended action in the human body is not achieved by pharmacological, immunological or metabolic means, but its function may be assisted by such means" and it can be used for the diagnosis, prevention, monitoring, treatment, alleviation or compensation of a disease or an injury or handicap [136].

Considering the contact time of a medical device with the human body and the potential risks associated with the technical design and manufacturing, medical devices can be classified as:

- Class I: low risk;
- Classes IIa and IIb: medium risk;
- Class III: high risk [136].

The medical devices in stock in the NFP are dressing's products, as bandages, sterile and non-sterile gauzes and products to disinfect and to treat wounds; orthopedic products, as crutches, canes, elastic pulses/feet/elbows/knees; urine bags and collectors; needles and syringes and oral hygiene products, as tooth brushes.

4.5. Galenic preparations

It is a responsibility of the pharmacist to provide medicines to people, which may include their preparation. The pharmacist is the only health professional able to prepare galenic medicines in the community pharmacy, given his technical and scientific knowledge.

A galenic medicine is "any magistral or officinal formula prepared and dispensed under the supervision of a pharmacist". A magistral formula is "the medicine prepared (…) according to a prescription that specifies the patient to whom the drug is intended"; an officinal formula is "any medicine prepared in accordance with compendial indications (from a Pharmacopoeia or a Galenic Form) to be dispensed directly to the pharmacy's patients" [137].

As other medicines, galenic preparations can be dispensed through a separated medical prescription. It is mandatory that the physician writes the term "manipulate" in the prescription [109]. To be able for reimbursement, the galenic drug must be prescribed indicating the active substance (or substances) and its concentration, the approved excipients and the dosage form. It also has to fill at least one of the following conditions:

- Absence of the pharmaceutical specialty with the same active ingredient in the desired dosage form in the market;
- Therapeutic gap within the industrially manufactured medicines;
- The necessity to adapt doses or dosage forms to the therapeutic needs of specific populations (pediatrics, geriatrics) [138].

The reimbursement of galenic preparations is established in 30 % of the total value; the active principles and dosage forms subject to reimbursement are listed in the Dispatch #18694/2010 of November 18th. Prescriptions containing trade names cannot be reimbursed [138].

The selling price to the public of manipulated medicines is calculated as a sum of the fees, the raw materials' and the packaging materials' values, according to the formula:

$$(\text{fees} + \text{raw materials' value} + \text{packaging materials' value}) \times 1{,}3 + \text{VAT}$$ [139].

All the steps of the galenic manipulation – preparation, packaging, labeling and quality control – must be performed in an appropriate laboratory, inside the pharmacy, only used for this purpose. The area should be wide enough to avoid the contamination risk during the preparation and it must also be properly lit and ventilated, with adequate temperature and humidity conditions. Both the laboratory surfaces and the equipment should be easy to clean, disinfect or even sterilize and be kept in good state of cleanliness and conservation. The measuring devices must be calibrated and be periodically controlled to ensure their accuracy; the results of these periodic verifications should be registered and filed [140].

4.5.1. Raw materials

All the raw materials used in the preparation of galenic medicines must satisfy the demands of their respective Pharmacopoeia monographs. They should be acquired from the suppliers authorized by the INFARMED and be accompanied by the quality analysis report [140].

When the raw materials arrive to the pharmacy, the pharmacist ensures their quality by verifying the analysis report compliance with the monographs specifications, by verifying if the received raw material is the same as the ordered one and by verifying the integrity of the package, the hygiene conditions and the conservation demands [140].

All raw materials' packages must contain a label with the following information:

- Identification of the raw material;
- Identification of the manufacturer;
- Batch number;
- Storage conditions;
- Handling precautions;
- Expiration date [140].

The rejected raw materials (the ones that don't comply with the required parameters or after the expiry date) should be destroyed or returned to the supplier as soon as possible [140].

Regarding the packaging materials, they must not be incompatible with the medicine or alter its quality, meeting the requirements of the Portuguese Pharmacopeia or another prestigious reference book. Additionally, they must be stored in appropriate conditions for conservation [140].

4.5.2. Material and equipment

The minimum equipment required in the pharmacy to prepare galenic medicines and extemporaneous preparations is listed:

- Alcoholmeter;
- Glass and porcelain mortars;
- Analytical balance sensitive to the milligram;
- Thermostatically controlled water bath;
- Porcelain capsules;
- Glasses of various capacities;
- Metallic and non-metallic spatula;
- Glass funnels;
- Flasks of various capacities;
- Filter paper;
- Universal pH indicator paper;
- Stone for the preparation of ointments;
- Graduated pipettes of various capacities;
- Measuring cylinders of various capacities;
- Sieves with mesh size 180 µm and 355 µm (with bottom and lid);
- Thermometer (minimum scale to 100 ºC);
- Watch glasses [141].

4.5.3. Protocols and techniques

Before starting the preparation of the galenic medicine, the pharmacist must ensure its safety, concerning the dosage of the active ingredients and potential incompatibilities and interactions; the hygiene of the working area; the removal of unrelated products, materials or documents; the adequate temperature and humidity conditions; the availability of all the necessary raw materials, equipment, packaging materials and documents and that the raw materials are used following the first expiry/first out principle [140].

The weighing and volume measuring must be carried out by the responsible pharmacist and verified by his supervisor, who should also check the procedure and the package and label of the final product. All the procedures should be standardized so that, in case they are repeated, the reproducibility is guaranteed [140].

In the NFP, although the laboratory is provided with all the materials and equipment required by law, galenic medicines are not prepared due to the lack of request of this specific medication.

4.5.4. Quality control

After preparing a galenic medicine, "the pharmacist must ensure the quality of the preparation, observing for this purpose the good practices to be followed in the preparation of manipulated drugs in community pharmacy" and "verify the safety of the medicine with respect to the doses of the active substances and to the existence of interactions that undermine the action of the drug or the patient's safety" [137].

The pharmacist should proceed to all the necessary verifications to ensure the good final quality of the galenic drug. At least, the organoleptic characteristics should be checked and some not destructive assays shall be performed, according to the dosage form. The semi-finished product must satisfy the requirements of the general monograph of the Portuguese Pharmacopoeia for the respective dosage form and the final mass or volume must be measured to certify its compliance with the prescribed quantity [140].

4.5.5. Documents and registrations

The documents are part of the quality assurance system of the medicines prepared in the pharmacy and aim to establish general and specific procedures, by recording the data referring to the preparation and control processes and by enabling the quality assessment of the prepared medication. Ultimately, they allow the pharmacist to reconstitute the history of each preparation. All documents must be elaborated, signed and dated by the technical director or under his supervision; he also validates all the modifications. All documents are filed in the pharmacy for a minimum period of three years [140].

The minimum required registrations correspond to the records of verifications and calibrations of the measuring devices and the following data, referring to the carried out preparations, in the preparation document:

- Name of the galenic medicine;
- Name and address of the patient (if it is a magistral formula or a preparation for a specific patient);
- Name of the prescriber (if any);
- Batch number attributed to the prepared product;
- Composition of the medicine, indicating the raw materials, quantities used and the batch numbers;
- Description of the protocol;
- Registration of the verifications' results;
- Description of the packaging;
- Date and rubric of the pharmacist who prepared the galenic medicine and of the supervisor [140].

Last but not least, an accurate label is of major importance, as it must contain all the necessary information to the patient:

- Patient's name (if it is a magistral formula);
- Formula of the galenic medicine (as prescribed by the doctor or as described in literature);
- Batch number assigned to the prepared product;
- Expiration date;
- Storage conditions;
- Eventual special instructions, essential for the use of the medicine, as "shake before use", "external use" (red background); "keep out of children's reach", etc.;
- Administration route;
- Identification of the pharmacy;
- Identification of the technical director [140].

4.5.6. Extemporaneous preparations

Not all the medicines are stable in aqueous solution. In these cases, to not compromise their quality, they are commercialized as a powder or a granule suspension, packed in sealed vials. At the time of the sale, they are reconstituted with purified water in the quantity indicated in the vial.

The pharmacist must explain to the user the posology of the medicine, referring that it must be stored in the fridge and stirred before administration. Depending on the product, the expiry date can vary between 12 h to 14 days.

5. Pharmacist-patient-medication relationship

5.1. Ethical issues

To create a trust relationship with the patients, the pharmacist should adapt his dialogue, having the ability to listen, to question, to relate and to negotiate, in a friendly, but fast and effective, way. This exercise should be done autonomously, both at the technical and at the scientific level, in order to fulfill his role as a preserver and a promoter of public health [142].

The pharmacist is responsible for the promotion of a safe, effective and rational use of medication assuring that, during the dispensing of medicines, the patient receives accurate and correct information about its use. He must be able to evaluate and to interpret medical prescriptions, inform the patient and clarify any doubt he may have about medicines for human or veterinary use and also advice, monitor and control the dispensing of any product [97, 142].

"The pharmacist shall keep informed from the scientific, ethical and legal points of view and assume an appropriate level of competence to provide an efficient practice" [97]. As the last health professional to contact with patients, the pharmacist plays a key role in their

adherence to the therapy, making them understand the medication, its advantages and possible side effects, always being available to help solving any issues.

It is a right of every patient to have a private dialogue with the pharmacist; the distance between the individual attendance counters must be enough to guarantee the privacy of every attendance. The measure of physical and chemical parameters is performed in the personalized attendance office, to keep the patient comfortable and to respect his privacy [97].

In short, "in the relations with the patients the pharmacist must observe the strictest correction, scrupulously fulfilling his professional duty and bearing in mind that he is in the service of public health and patients" [142].

5.2. Pharmacovigilance

In Portugal, the National Pharmacovigilance System is coordinated by the INFARMED and constituted by four regional units. It monitors the safety of the medicines authorized in the national market, evaluates potential problems related to ADRs and implements security measures whenever necessary [95].

Anyone can report a suspected ADR: health professionals as pharmacists, pharmacy technicians, physicians, nurses and patients. In the INFARMED website, there is a proper spot to do that. The required information is the description of the ADR, the identification of the medicine, information about the person who suffered the reaction and the contact of the person who notifies [95].

If the suspected ADR is reported to the pharmacist, he must try to attain a more complete information, namely: description of the ADR (signs and symptoms) and its duration, severity and progression; relation of the signs and symptoms with medicines' intake; suspected medicine, start and suspension date of intake, batch, administration route and therapeutic indication; other medicines the patient might be taken (including medicines not subjected to medical prescription) [97].

Taking into account that the pharmacist is the last health professional in contact with the patient, he has the increased responsibility of paying attention to the signs and symptoms that patients might present after the dispensing of medication, adopting a proactive attitude in the notification of suspected ADRs.

5.3. Recycling expired medicines

"As a health worker and in accordance with his responsibility towards society, stemming from his professional practice, the pharmacist must act on actions to safeguard a humane, healthy and ecologically balanced environment" [142]. To face the specific nature of the medication as a waste, the VALORMED was created in 1999. It is a nonprofit corporation, responsible for the management of waste from empty packages and unused medicines [143].

The community pharmacies have the role to regularly disclose the communication and information campaigns cyclically produced by the VALORMED. Pharmacists and pharmacy technicians, as health workers, should actively participate in the sensitization of patients for good environmental practices, stating the need to deliver empty containers and medicinal products out of use in proper containers in the pharmacy [144].

In the NFP, the patients adhere quite a lot to the recycling of medication, leaving their out of use medicines in the pharmacy's container almost daily. When full, this container is closed and replaced by an empty one; after its closing, it is identified with the name of the pharmacy and the Pharmacies National Association's code, weighted, dated and signed by the responsible for its closing. Some of the pharmacy's suppliers are also responsible for the collection of the VALORMED containers, taking them to the processing site where its safe treatment is performed.

6. Self-medication

Self-medication is the responsible use of medicinal products not subject to medical prescription, intended to the relief and treatment of temporary, and not serious, health complaints, with the optional assistance or counseling of a healthcare professional. It is limited to well-defined clinical situations and conducted in accordance with the established specifications for those medicines [145, 146].

The pharmacist must guide the patient to the use or not of the requested medicine, contributing to an appropriate and rational self-medication. For this practice, the pharmacist evaluates the patient's needs:

- Makes sure that the patient has enough information to properly assess the specific health problem, including information about the problem, its symptoms, for how long they persist and any medicines already taken;
- Assess whether the symptoms may, or may not, be associated with a serious illness; if so, the patient should be adviced to request a physician's appointment;
- For smaller disorders, gives to the patient adequate information, dispensing medicines only in case of manifest necessity [97].

Self-medication is not recommended in specific populations, as babies, pregnant woman and breastfeeding women. Ultimately, it allows to relieve the pressure on the NHS, freeing up resources that can be applied in situations of greater need and contributing to an increased civic conscience of citizens who are willing to participate in the managing of their own health [146]. The Dispatch #17690/2007 of July 23rd lists the possible situations of self-medication [145].

During the self-medication process, the patient should be careful and consult a pharmacist or a physician in the following situations:

- If the symptoms persist;
- If the symptoms get worse or in case of relapse;

- If there is sharp pain;
- After using medicines, if no results show;
- In case of adverse effects or reactions;
- Whenever suspecting of a serious situation;
- If he suffers from other diseases;
- If he is using other medicines [146].

If the pharmacist suspects of a severe pathology, not referred in the above mentioned list, or if he considers that the patient should be examined more precisely, it is his function to forward the patient to a physician to be examined.

7. Other services

In order to promote the health and the well-being of people, pharmacies can provide some pharmaceutical services, namely domestic support, first aid care, administration of medicines, use of auxiliary diagnostic and therapeutic means, administration of vaccines not included in the National Vaccination Plan, pharmaceutical care programs, information campaigns and collaboration in health education programs. At least for the first aid care, the administration of medicines and vaccines and the use of auxiliary diagnostic and therapeutic means, the pharmacy must have adequate and autonomous facilities. Additionally, the type of service and its price must be disclosed and visibly exposed in the pharmacy [147].

The services performed in the personalized attendance office of the NFP are the anthropometry (height and weight) and body mass index (BMI) measurement and the control of the physical and chemical parameters blood pressure, capillary blood glucose and total cholesterol.

7.1. Anthropometry and body mass index

Obesity is a chronic disease in which body fat excessively accumulated is capable of affecting the health status. It has a high prevalence worldwide and the WHO declared it a global epidemic of the XXI century. The National Anti-obesity Program was created to counteract the growth rate of the prevalence of overweight and obesity in Portugal, aiming to help the reducing of weight in obese people and in people who have particular risk to develop this pathology. It should be noted that visceral obesity is associated with metabolic disorders such as type 2 diabetes and dyslipidemia and cardiovascular diseases such as hypertension, coronary heart disease and cerebral vascular disease [148].

The prevalence of overweight and obesity in the adult Portuguese population has been assessed using the BMI, which is calculated by dividing the weight, in kilograms, by the height, in meters:

$$\text{weight} / \text{height}^2$$ [148].

A BMI ≥ 25 is considered overweight, while a BMI ≥ 30 is considered obesity, with few exceptions [148].

The NFP's patients can control their anthropometry values – weight and height, and their BMI by using the scale available for that purpose; they only need to insert a 50 cents coin and it gives all these values digitally and in a paper.

7.2. Blood pressure

High blood pressure is considered a major individual risk factor for developing cardiovascular diseases: cerebrovascular disease, heart failure and other cardiovascular complications. The diagnosis and control of hypertension assumes particular importance in Portugal, as cerebrovascular disease is the leading cause of disability and death [149].

In practice, hypertension is diagnosed as the persistent elevation in several measurements at different occasions of the systolic blood pressure (≥ 140 mmHg) or the diastolic blood pressure (≥ 90 mmHg), for people aged 18 years or more, not subject to anti-hypertensive drug therapy and who have no concomitant acute disease or are pregnant. The measurement should be performed in a convenient environment, with the patient seated and relaxed for at least 5 min. The patient must not smoke or ingest stimulants previously, his arm shall be naked and an appropriately sized cuff should be used [150].

The NFP's personalized attendance office is equipped with a blood pressure measuring device. When a patient requests to measure his blood pressure, the pharmacist guides him to this office, inviting him to sit and rest a few minutes and investigating if the patient took any stimulants or smoked in the last hour. After that, the blood pressure is measured, through a payment of 50 cents.

7.3. Capillary blood glucose and total cholesterol

In the general population, the diagnosis of diabetes is based on the following parameters and values for venous plasma:

- Fasting blood glucose ≥ 126 mg/dL (or ≥ 7 mmol/L);
- Classic symptoms + casual blood glucose ≥ 200 mg/dL (or ≥ 11,1 mmol/L);
- Glucose ≥ 200 mg/dL (or ≥ 11,1 mmol/L) at 2 h, in the proof of glucose oral tolerance with 75 g of glucose;
- Glycated hemoglobin A1c (HbA1c) ≥ 6,5 % [151].

This diagnostic must be confirmed with a second analysis, one or two weeks later [151].

The incidence of both types of diabetes, 1 and 2, has been increasing in the last decades, with the contribution of genetic and environmental factors such as obesity and sedentary lifestyles. The main complications of this metabolic disease are cardiovascular diseases, neuropathy, nephropathy, retinopathy and amputation of the lower limbs [152].

Some people are considered at higher risk of developing diabetes, namely overweight or obese; aged more than 45 years; leading a sedentary life; with a family history of diabetes or previous cardiovascular disease, hypertension or dyslipidemia, and women who previously developed gestational diabetes [152].

Dyslipidemia, as hypertension, is a major risk factor for cardiovascular morbidity and mortality [149]. Its diagnosis is carried out by laboratory tests on blood, under fasting for 12 h, of total cholesterol, triglycerides, high-density lipoproteins cholesterol and low-density lipoproteins cholesterol and it must be confirmed by a second laboratory evaluation performed after a minimum of 4 weeks. Dyslipidemias may be related to the interaction between a genetic predisposition and environmental factors or other diseases: secondary dyslipidemias [153].

To keep an accurate control of both of these diseases, patients should regularly measure their capillary blood glucose and total cholesterol levels. With this public health concern in mind, it is possible to do that in the NFP.

For the measurement of capillary blood glucose, there is a blood glucose measuring device similar to the ones given to users. A drop of blood of one of the patient's fingers is collected, after disinfecting the finger and pricking it with a lancet. Then, it is inserted in the test-strip and the value is shown in the screen of the measuring device.

To measure the total cholesterol, there is the *Reflotron® Plus*, a spectrophotometric device. After disinfecting and pricking with a lancet one of the patient's fingers, a capillary tube is filled with the blood sample, which is inserted in the appropriate test-strip. After a few minutes, the reading device shows the value on its screen.

All the obtained values are registered in a personal card offered by the pharmacy, so that the patients can perform the follow-up of their health status.

8. Accounting and management

For the pharmacy to receive the reimbursement values of the medicines dispensed, the prescriptions must be conferred and organized at the end of every month. Then, they are sent to the Invoices Conference Center, where they are re-conferred and, if everything is correct, the Portuguese State pays the reimbursement values to the pharmacy.

When dispensing medicines, the pharmacist verifies the authenticity and the validity of the prescription; after that, he re-verifies the prescription and the back print. In case he detects any error, the pharmacist corrects it, justifying, and signs and stamps the back of the prescription.

To easy the invoicing process, the print in the back includes a successive numeric code, constituted by the sequence number of the prescription, the batch and the series; each batch has 30 prescriptions. Along the month, the prescriptions are organized by reimbursement organism and batch, in ascending order of the prescription's number.

When the month ends, the invoicing is closed for the referent month and the following documents are sent to the Invoices Conference Center:

- Invoice (in duplicate), with the summary of the total batches per reimbursement organism, the sum of the selling prices to the public, the sum of the reimbursement values and the sum of the values paid by the patients;
- Debit/credit notes (in duplicate), if any prescriptions of the previous month were returned to correction;
- Batches' summary list, with the name and the pharmacy's code, as well as the correspondent month and year, the total number of sheets and the batches' description (how many prescriptions and their respective values);
- Batches' identification sheets, with the name and the pharmacy's code, as well as the correspondent month and year, the reimbursement organism, the batch number, the amount of prescriptions and medicines' codes and the total values: selling price to the public, reimbursement and paid by the patient;
- Prescriptions [154].

These documents must be sent until the 10th day of the month following the one they refer to. In the Invoices Conference Center, all prescriptions are conferred. If any errors (in the prescription, in the dispensed medicines, in reimbursement organism) or values' differences are detected, the prescriptions may be returned to the pharmacy for correction – they are re-invoiced in the following month – or the values are immediately corrected [154].

The information regarding the complementary reimbursement systems is sent to the Pharmacies National Association, where the correspondent prescriptions are conferred so that the pharmacy can receive the values of these reimbursements.

9. Conclusions

During the four and a half years of the pharmaceutical studies, especially in the last two and a half years, a lot of theoretical information about diseases and the appropriate therapies is learnt, which does not exactly prepare the student for the reality of the professional life. Thus, the curricular internship is very important in the last semester and constitutes major gain in the training and preparation for the real pharmaceutical activity.

During the internship in the community pharmacy, there was the contact with the full medication's circuit, from the ordering to dispensing, as well as with the pharmaceutical advice, the clinical follow-up and the measurement and control of various biochemical parameters, not forgetting the accounting and management aspects.

In fact, integrated in a cooperation team of health professionals, not only the pharmacist's values were improved, but also the sociological ones. There were many opportunities to apply the theoretical knowledge previously acquired to real situations, always with the help of the colleagues.

Through the contact with the public and the advising, the pharmacist performs his most important role: to promote the gradual improvement of the public health.

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Attachments

1 – Poster presented in the X Annual CICS Symposium

CYTOTOXICITY OF TICLOPIDINE AND *HYPERICUM PERFORATUM* IN HEPATIC AND INTESTINAL EPITHELIUM CELL LINES

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Ticlopidine, a thienopyridine prodrug, is mainly used in the prophylaxis of thromboembolic complications in patients with thromboembolic disease, particularly if they are allergic to aspirin. It is widely associated with multiple drug interactions, mostly via cytochrome P450 inhibition, which, along with the high incidence of bone marrow toxicity, have contributed to the decrease of its use.

Hypericum perforatum has been widely used as an antidepressant, anti-inflammatory and antimicrobial agent. However, in literature, there are described numerous herb-drugs interactions due to the induction of CYP enzymes and P-glycoprotein expression by various components of this plant.

The purpose of this study was to evaluate and to determine the cytotoxicity of ticlopidine and *H. perforatum* hydroalcoholic extract at different concentrations, as well as their combined action in hepatic – Hep G2 – and intestinal epithelium – Caco 2 – cell lines. For that, cell viability (MTT) assays were performed, aiming to determine the relative cellular viability of these cells after its exposure to the compounds.

The results showed that ticlopidine promoted cytotoxicity, dose- and time-dependent in both Hep G2 and Caco 2 cells and *H. perforatum* decreased the relative cellular viability in these cells, particularly in Hep G2 cells. Furthermore, the incubation of cells with both compounds showed a similar pattern to that observed when cells were incubated with the extract alone but a potential joint action is suggested.

Overall, this study extended the current knowledge of the ticlopidine actions on cells and highlights its potential relevance on organisms, particularly when co-administrated with *H. perforatum*.

Keywords: Ticlopidine; Hypericum perforatum; Cytotoxicity; Hep G2 cells, Caco 2 cells.

Acknowledgements: The authors acknowledge to "Programa Operacional Regional do Centro 2007-2013 QREN (Programa Mais Centro)" with the project CENTRO-07-ST24-FEDER-002012 entitled "Therapeutic drug monitoring on age related diseases", COMPETE program and Portuguese Foundation for Science and Technology through the project (PEst-OE/SAU/UI0709/2014). The authors also want to acknowledge to Dr. Sara Silva for her laboratory assistance.

Cytotoxicity of ticlopidine and *Hypericum perforatum* in hepatic and intestinal epithelium cell lines

Catarina Martins, Eugenia Gallardo and Ana Martinho

CICS-UBI - Health Sciences Research Centre, University of Beira Interior, Covilhã, Portugal.

Introduction

- Ticlopidine is mainly used in the prophylaxis of thromboembolic complications in patients with thromboembolic disease, especially if they are allergic to aspirin [1].
- It is widely associated with multiple drug interactions, mostly via cytochrome P450 (CYPs), which along with the high incidence of bone marrow toxicity have contributed to a decrease in its use [1].
- *Hypericum perforatum* (*H. perforatum*) is widely used as an antidepressant, anti-inflammatory and antimicrobial agent and it is responsible for numerous herb-drugs interactions due to the induction of both CYPs and P-glycoprotein expression [2].

Aim

To evaluate the cytotoxicity of ticlopidine and *H. perforatum* hydroalcoholic extract, alone and in combination in hepatic and intestinal cell lines through MTT assay.

Methods

Hep G2 and Caco 2 cells were incubated with:

- Ticlopidine (1 µM, 10 µM, 100 µM and 200 µM);
- *H. perforatum* hydroalcoholic extract (1 µM and 10 µM);
- Ticlopidine + *H. perforatum* hydroalcoholic extract (1/1 µM and 10/10 µM).

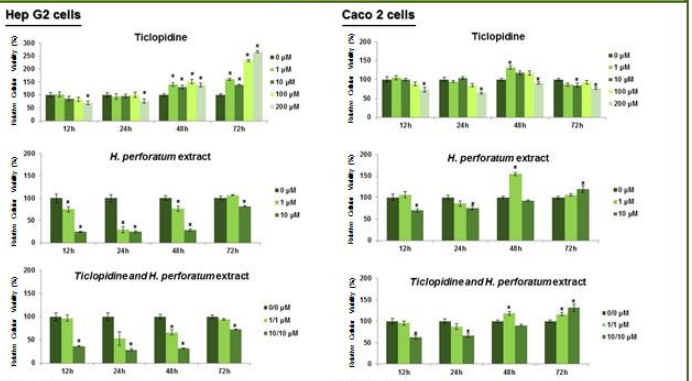
MTT assays were performed after various periods of incubation (12h, 24h, 48h and 72h).

Conclusions

Ticlopidine may be cytotoxic in both Hep G2 and Caco 2 cells, depending on its concentration and period of incubation.

The simultaneous incubation of cells with both compounds promotes a similar pattern to that observed when cells are incubated with the extract alone and it is dose-, time- and cell line-dependent.

Results



- **Ticlopidine**
  - After 12 and 24h, only 200 µM of ticlopidine decreased the relative cellular viability;
  - After 48 and 72h, the cellular viability is increased for all concentrations studied.
- ***H. perforatum* extract**
  - The cellular viability is diminished for both concentrations used until 48h of incubation and for 10 µM after 72h.
- **Ticlopidine + *H. perforatum* extract**
  - The cellular viability shows a similar pattern to that observed when cells are incubated with the extract alone. However, a minor cytotoxicity is observed.

- **Ticlopidine**
  - 200 µM is cytotoxic for any period of incubation;
  - For longer periods of incubation, ticlopidine promote a small decrease in the cellular viability.
- ***H. perforatum* extract**
  - The cellular viability is diminished at 10 µM after 12, 24 and 48h and increased at 1 and 10 µM after 48 and 72h, respectively.
- **Ticlopidine + *H. perforatum* extract**
  - The cellular viability shows a similar pattern to that observed when cells are incubated with the extract alone;
  - After 48 and 72h, an increase in the cellular viability is observed.

References

[1] Farid, N. A., *et al.*, Metabolism and Disposition of the Thienopyridine Antiplatelet Drugs Ticlopidine, Clopidogrel, and Prasugrel in Humans. J Clin Pharma, 2010. 50: p. 126-142.
[2] Russo, E., *et al.*, *Hypericum perforatum*: Pharmacokinetic, Mechanism of Action, Tolerability, and Clinical Drug-Drug Interactions. Phytother. Res. 2014. 28: p. 643-655.

Acknowledgements

"Programa Operacional Regional do Centro 2007-2013 QREN (Programa Mais Centro)" with the project CENTRO-07-ST24-FEDER-002012 entitled "*Therapeutic drug monitoring on age related diseases*", COMPETE program and Portuguese Foundation for Science and Technology through the project PEst-OE/SAU/UI0709/2014. Dr[a] Sara Silva for her laboratory assistance.

2 – Personalized request of class H or high cost medicines for a determined patient

ISTITUTI FISIOTERAPICI OSPITALIERI *(mod farma11)*

MODULO PER RICHIESTA PERSONALIZZATA FARMACI non inseriti nel PTA di fascia H ed alto costo

DATA_____
REPARTO RICHIEDENTE (timbro e codice CDC) _____
FARMACO RICHIESTO

- o Altro (indicare)_____ qtà richiesta _____

Inoltrata richiesta di inserimento in PTA: o si ◊o no ◊

TERAPIA PREVISTA (dose die e durata)

_____

_____

INIZIALI DEL/I PAZIENTI _____
Diagnosi:

_____

_____ Cod AIFA _____
Timbro e Firma del Responsabile del CDC

_____

parte sottostante a cura del Servizio di Farmacia

Farmaco di fascia : o H; costo della terapia:_____

o Proposta e accettata sostituzione con _____
Firma del Farmacista

_____

o Allegata prescrizione del reparto o ordine numero:_____

data e firma per ricevuta del Reparto (il farmaco si ritira presso il settore ricezione merci della farmacia tel. 5129)

_____

3 – Personalized request for medicines not included in class H for hospitalized patients

ISTITUTI FISIOTERAPICI OSPITALIERI *(mod farma5)*

MODULO PER RICHIESTA FARMACI PER PAZIENTI RICOVERATI, NON INSERITI NEL PTA (esclusi i farmaci di fascia H)

DATA_____

REPARTO RICHIEDENTE (timbro e codice CDC) _____

FARMACO RICHIESTO_____

TERAPIA PREVISTA (dose die e durata)

_____

_____

INIZIALI DEL PAZIENTE _____

Si tratta di:

- o Proseguimento terapia domiciliare
- o Altro (specificare)

_____

Dichiaro che la presente terapia non è sostituibile con medicinali inserti nel PTO.

Timbro e Firma del Medico

_____

parte sottostante a cura del Servizio di Farmacia

Farmaco di fascia : o A, o C, o galenico; costo della terapia:_____

o Inoltrato a Farmacia Esterna S. Gallicano (fax 0658543637)
o Proposta e accettata sostituzione con _____
Firma del Farmacista

_____
o Allegata prescrizione del reparto

data e firma per ricevuta del Reparto (il farmaco si ritira presso la stanza Farmaci Sperimentali della farmacia tel. 5129 ed è disponibile generalmente il giorno successivo alla richiesta)

_____

4 – Request for the acquisition of narcotic and psychotropic drugs

BUONO ACQUISTO n° B B 0060954

SEZIONE PRIMA
(rimane alla farmacia richiedente)

Si richiede alla ditta

la cessione del sottospecificato prodotto

qualità

quantità

dalla farmacia richiedente sottoindicata

data

per la farmacia richiedente

BUONO ACQUISTO n° B B 0060954

SEZIONE SECONDA
(rimane alla ditta cedente)

Si richiede alla ditta

la cessione del sottospecificato prodotto

qualità

quantità

dalla farmacia richiedente sottoindicata

data

per la farmacia richiedente

BUONO ACQUISTO n° B B 0060954

SEZIONE TERZA
(da inviare a cura della ditta cedente all'Autorità sanitaria regionale nella cui circoscrizione ha sede la farmacia)

La ditta

cede il sottospecificato prodotto

qualità

quantità

alla farmacia richiedente sottoindicata

data

per la ditta che cede

5 – Request for the acquisition of medicines not registered in Italy

MODULO DI RICHIESTA PER L'AUTORIZZAZIONE ALL'IMPORTAZIONE DI MEDICINALI REGOLARMENTE REGISTRATE IN ITALIA E TEMPORANEAMENTE CARENTI SUL TERRITORIO NAZIONALE (AI SENSI DEL DM 11/05/2001) OVVERO DI MEDICINALI EMO O PLASMA DERIVATI LEGALMENTE IN COMMERCIO NEL PAESE DI PROVENIENZA MA NON REGISTRATI IN ITALIA (AI SENSI DEL DM 07/09/2000)

Il sottoscritto Medico curante Dr. _____;
operante presso il Reparto/ Divisione di _____ dell'Ospedale/ASL _____;

considerato che il medicinale _____ risulta:

- o regolarmente registrata in Italia ma temporaneamente carente sul territorio nazionale *;

ovvero

- o non registrato in Italia ma legalmente in commercio nel Paese di provenienza (solo per le specialità medicinali emo o plasma derivate)*;

CHIEDE

l'autorizzazione ad importare dall'estero il seguente medicinale:

Principio attivo _____
Nome commerciale _____
Forma farmaceutica _____
Dosaggio e via di somministrazione _____
Quantità _____

- o per n. pazienti* _____

ovvero

- o per scorta reparto* _____

Indicazione terapeutica/diagnostica per la quale verrà utilizzato il medicinale _____
_____
_____
Paese di provenienza presso il quale il medicinale è regolarmente autorizzata alla immissione in commercio:
_____
Titolare estero e n. dell'autorizzazione all'immissione in commercio nel paese di provenienza _____
Ditta estera produttrice _____
Eventuali intermediari _____

A tal fine dichiara che non sono disponibili al momento in Italia valide alternative terapeutiche e che tale medicinale:

- verrà utilizzato esclusivamente per le indicazioni approvate nel paese di provenienza e in accordo con il relativo riassunto delle caratteristiche del prodotto.
- verrà impiegato sotto la diretta responsabilità dello scrivente medico curante dopo aver ottenuto, ai sensi della normativa vigente, il consenso informato dei pazienti o, in caso di minori o incapaci, di chi esercita la patria potestà.

A cura del responsabile dell'importazione viene assicurato che il prodotto è preparato secondo i requisiti di sicurezza e qualità equivalenti a quelli richiesti dall'Autorità Sanitaria Italiana e che, in caso di prodotti per i quali è previsto in Italia l'obbligo di controllo di stato (medicinali immunologici e medicinali emo o plasma derivati), i lotti importati saranno accettati solo se corredati di copia del Certificato di controllo di stato rilasciato dalle Autorità competenti.

Data _____

Il Medico Curante
(firma per esteso e timbro)
_____

Il Dirigente del Servizio Farmaceutico
(firma per esteso e timbro)
_____

* (barrare la voce applicabile)

6 – Register of the input and output of narcotic and psychotropic drugs

L'autorità sanitaria locale AZIENDA USL ROMA 77

TIMBRO

Firma

DOCUMENTAZIONE DI ENTRATA O DI USCITA				Entrata		Uscita		Giacenza		Annotazioni
mento	N.	Data	Origine o Destinazione							
0			(a)	(b)		(b)		(b)		
			da pag. _____ Riporti							

7 – Prescription – *Modulo B*

ISTITUTI FISIOTERAPICI OSPITALIERI
ISTITUTO REGINA ELENA
ISTITUTO SAN GALLICANO
MODULO **"B"** PAZIENTI IN DIMISSIONE FILE F (COMUNICAZIONE PROT. 124 DIREZIONE SANITARIA AZIENDALE)

REPARTO (DESCRIZIONE) _____CODICE _____
DATI DEL PAZIENTE:

NOME E COGNOME:	MEDICO DI BASE:
DATA DI NASCITA:	LUOGO DI NASCITA:
ASL DI APPARTENENZA:	CODICE SANITARIO REGIONALE:
CODICE FISCALE:	LUOGO DI RESIDENZA (REGIONE, PROVINCIA E COMUNE):

DATI SANITARI

FARMACO DISPENSATO E POSOLOGIA	**QUANTITA' ESPRESSA IN UNITA'** (CAPSULE, FIALE, ETC)	**NOTA CUF**	**GG DI TERAPIA (MAX 7)**	**QUANTITA' CONSEGNATA (A cura del Farmacista)**
1_				
2_				
3_				
4_				
5_				

DATA DISPENSAZIONE:_____ IL FARMACISTA:_____

TIMBRO E FIRMA MEDICO: _____ CAPO SALA: _____

FIRMA DEL PAZIENTE PER RICEVUTA: _____

MODULI DA CONSEGNARE IN COPIA IN FARMACIA TRIMESTRALMENTE ENTRO IL 7 APRILE, 7 LUGLIO, 7 OTTOBRE, 7 GENNAIO. RIF. LETTERA 124/ds.

La presente va consegnata al medico di base per opportuna conoscenza.

- Il paziente viene identificato in ordine di preferenza da:
  - Codice fiscale
  - Cognome, nome e data di nascita
  - Codice sanitario regionale
    (pertanto uno degli identificativi deve essere sempre presente)
  - Provincia e comune di residenza
  - ASL di residenza
- Le informazioni sanitarie indispensabili sono:
  - Data di erogazione del farmaco
  - Farmaco consegnato
  - Quantità del farmaco consegnata/somministrata in unità posologiche (compresse, fiale)

Si delega il Sig._____
documento riconoscimento n.
_____ rilasciato il
_____ da _____ a
ritirare i farmaci prescritti.

Data_____firma_____

8 – Prescription – *Modulo A*

ISTITUTI FISIOTERAPICI OSPITALIERI
ISTITUTO REGINA ELENA
ISTITUTO SAN GALLICANO
MODULO UNIFICATO "A" FarmED ***4 versione*** (COMUNICAZIONE PROT. 124 DIREZIONE SANITARIA AZIENDALE poi modificata. prot. 01/afff/06 03/01/2006)

[] AMBULATORIO DEL CDC (DESCRIZIONE) _____ CODICE ____
[] PAC DEL CDC (DESCRIZIONE) _____ CODICE ____
[] DH (solo in caso di cicli di cura domiciliari programmati)_____ CODICE_____

DATI DEL PAZIENTE:

NOME:	COGNOME:
DATA DI NASCITA:	LUOGO DI NASCITA:
ASL DI APPARTENENZA (obbligatorio solo se Regione Lazio):	CITTADINANZA:_____ ESENZIONE TICKET (Specificare):_____
CODICE FISCALE/STP:	LUOGO DI RESIDENZA (REGIONE, PROVINCIA E COMUNE):

DATI SANITARI

FARMACO DISPENSATO	QUANTITA' Richiesta IN UNITA' (CAPSULE, FIALE, ETC)	QUANTITA' Consegnata IN UNITA' (CAPSULE, FIALE, ETC)
1_		
2_		

Tossicità _____ **[si] [no]**
N.B. ***In caso di tossicità compilare e allegare la scheda ministeriale di reazione avversa***

Diagnosi:_____ Mon. AIFA: **[si] [no]**

TIMBRO e FIRMA del MEDICO Prescrittore:

Dati relativi alla consegna (tutti obbligatori)
DATA DELLA DISPENSAZIONE:_____

TIMBRO E FIRMA DEL Medico/Farmacista/Capo Sala: _____
(il Farmacista e il Capo Sala consegnano il farmaco come da prescrizione/cartella clinica)

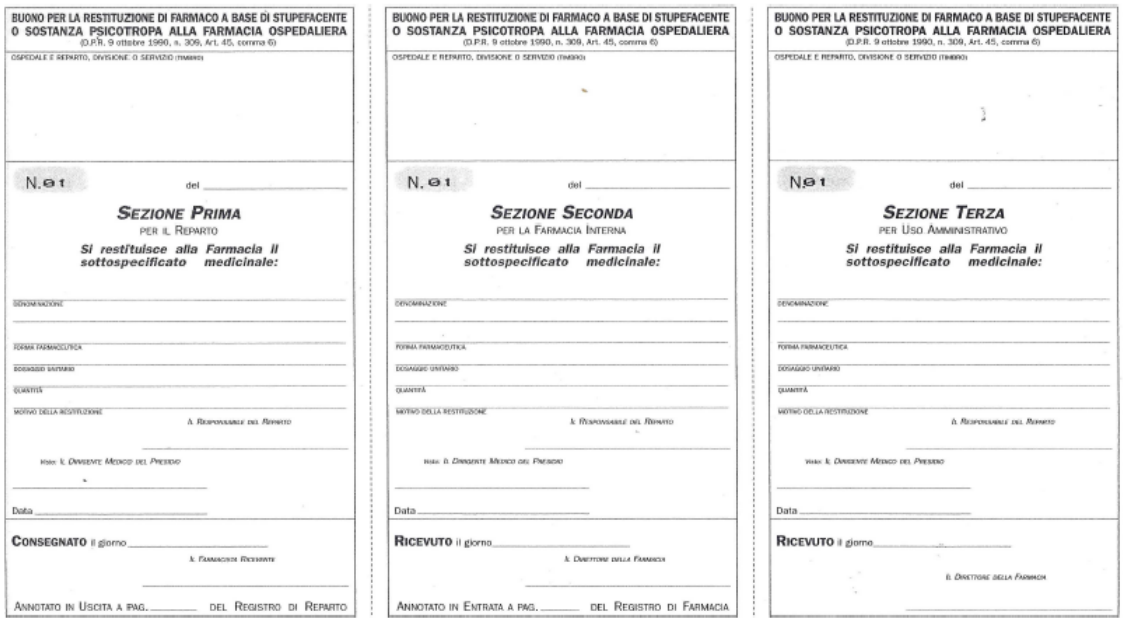
FIRMA DEL PAZIENTE PER RICEVUTA: _____

MODULO DA TRASMETTERE IN COPIA al Servizio IFO "FarmED" ***fax 2850*** *MENSILMENTE ENTRO IL 3 del MESE SUCCESSIVO A QUELLO DI DISPENSAZIONE A CURA DEL RESPONSABILE DEL CDC*

- Il paziente viene identificato da:
  - Codice fiscale (obbligatorio)
  - Cognome, nome e data di nascita (obbligatori)
  - Cittadinanza (obbligatorio)
  - Provincia e comune di residenza (obbligatorio)
  - ASL di residenza (obbligatorio se Regione Lazio)
- Le informazioni sanitarie indispensabili sono:
  - Data di erogazione del farmaco
  - Farmaco consegnato
  - Quantità del farmaco consegnata/somministrata in unità posologiche (compresse, fiale)

Parte sottostante a cura del servizio FarmED
Id scarico FarmED:_____ _____
Prescrizione AIFA: [] note _____

9 – Request for narcotic and psychotropic medicines



BUONO PER LA RESTITUZIONE DI FARMACO A BASE DI STUPEFACENTE O SOSTANZA PSICOTROPA ALLA FARMACIA OSPEDALIERA
(D.P.R. 9 ottobre 1990, n. 309, Art. 45, comma 6)

OSPEDALE E REPARTO, DIVISIONE O SERVIZIO (timbro)

N. 01 del _____

SEZIONE PRIMA
PER IL REPARTO
Si restituisce alla Farmacia il sottospecificato medicinale:

DENOMINAZIONE
FORMA FARMACEUTICA
DOSAGGIO UNITARIO
QUANTITÀ
MOTIVO DELLA RESTITUZIONE
Il Responsabile del Reparto

Visto: Il Dirigente Medico del Presidio

Data _____

CONSEGNATO il giorno _____
Il Farmacista Ricevente

ANNOTATO IN USCITA A PAG. _____ DEL REGISTRO DI REPARTO

BUONO PER LA RESTITUZIONE DI FARMACO A BASE DI STUPEFACENTE O SOSTANZA PSICOTROPA ALLA FARMACIA OSPEDALIERA
(D.P.R. 9 ottobre 1990, n. 309, Art. 45, comma 6)

OSPEDALE E REPARTO, DIVISIONE O SERVIZIO (timbro)

N. 01 del _____

SEZIONE SECONDA
PER LA FARMACIA INTERNA
Si restituisce alla Farmacia il sottospecificato medicinale:

DENOMINAZIONE
FORMA FARMACEUTICA
DOSAGGIO UNITARIO
QUANTITÀ
MOTIVO DELLA RESTITUZIONE
Il Responsabile del Reparto

Visto: Il Dirigente Medico del Presidio

Data _____

RICEVUTO il giorno _____
Il Direttore della Farmacia

ANNOTATO IN ENTRATA A PAG. _____ DEL REGISTRO DI FARMACIA

BUONO PER LA RESTITUZIONE DI FARMACO A BASE DI STUPEFACENTE O SOSTANZA PSICOTROPA ALLA FARMACIA OSPEDALIERA
(D.P.R. 9 ottobre 1990, n. 309, Art. 45, comma 6)

OSPEDALE E REPARTO, DIVISIONE O SERVIZIO (timbro)

N. 01 del _____

SEZIONE TERZA
PER USO AMMINISTRATIVO
Si restituisce alla Farmacia il sottospecificato medicinale:

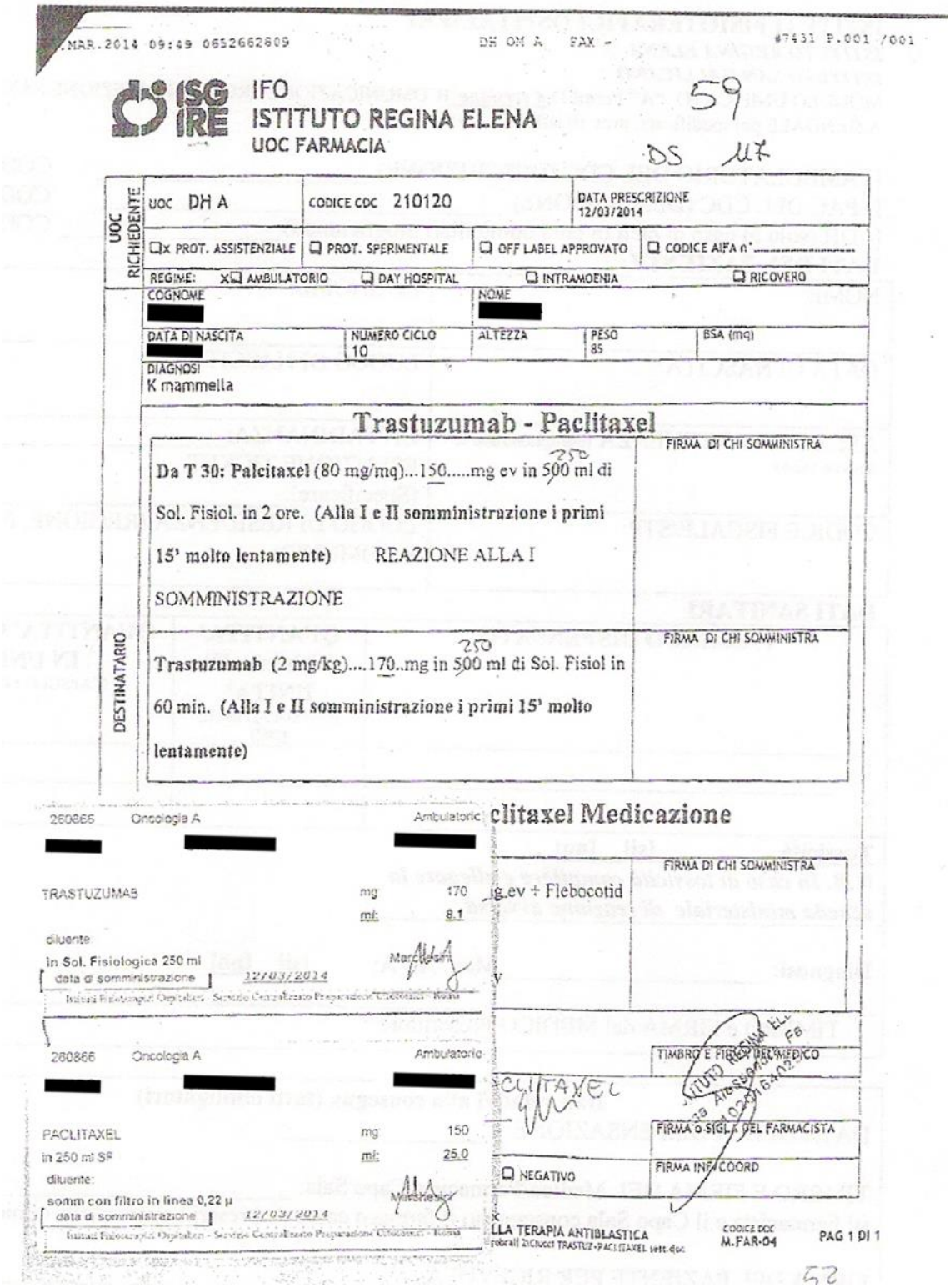
DENOMINAZIONE
FORMA FARMACEUTICA
DOSAGGIO UNITARIO
QUANTITÀ
MOTIVO DELLA RESTITUZIONE
Il Responsabile del Reparto

Visto: Il Dirigente Medico del Presidio

Data _____

RICEVUTO il giorno _____
Il Direttore della Farmacia

10 – Prescription and labels after validation



.MAR.2014 09:49 0652662609 DH OM A FAX - #7431 P.001 /001

IFO
ISTITUTO REGINA ELENA
UOC FARMACIA

59
DS 117

UOC RICHIEDENTE

UOC DH A	CODICE CDC 210120	DATA PRESCRIZIONE 12/03/2014

☒ PROT. ASSISTENZIALE ☐ PROT. SPERIMENTALE ☐ OFF LABEL APPROVATO ☐ CODICE AIFA n°.....

REGIME: ☒ AMBULATORIO ☐ DAY HOSPITAL ☐ INTRAMOENIA ☐ RICOVERO

DESTINATARIO

COGNOME	NOME			
[redacted]	[redacted]			
DATA DI NASCITA	NUMERO CICLO	ALTEZZA	PESO	BSA (mq)
[redacted]	10		85	

DIAGNOSI
K mammella

Trastuzumab - Paclitaxel

	FIRMA DI CHI SOMMINISTRA
Da T 30: Palcitaxel (80 mg/mq)...150.....mg ev in 250 ml di Sol. Fisiol. in 2 ore. (Alla I e II somministrazione i primi 15' molto lentamente) REAZIONE ALLA I SOMMINISTRAZIONE	
Trastuzumab (2 mg/kg)....170..mg in 250 ml di Sol. Fisiol in 60 min. (Alla I e II somministrazione i primi 15' molto lentamente)	

260866 Oncologia A Ambulatorio
[redacted]

TRASTUZUMAB mg 170
ml: 8.1
diluente
in Sol. Fisiologica 250 ml
data di somministrazione 12/03/2014
Marchesini

260866 Oncologia A Ambulatorio
[redacted]

PACLITAXEL mg 150
in 250 ml SF ml: 25.0
diluente:
somm con filtro in linea 0,22 µ
data di somministrazione 12/03/2014
Marchesini

clitaxel Medicazione

	FIRMA DI CHI SOMMINISTRA
g ev + Flebocotid	

TIMBRO E FIRMA DEL MEDICO

FIRMA O SIGLA DEL FARMACISTA

☐ NEGATIVO FIRMA INF. COORD

LLA TERAPIA ANTIBLASTICA
Foglio di Clinici TRASTUZ-PACLITAXEL sett.doc

CODICE DOC M.FAR-04 PAG 1 DI 1

52

11 – Preparation sheet

FARMACIA S. GALLICANO

FOGLIO LAVORO

FOGLIO DI LAVORO della Prep: _____ **(vedi scheda tecnica)**

Nuova preparazione [] **Se nuova indicare modus operandi:**
_____
_____

DATA PREPARAZIONE __/__/___ **Nr. PROGRESSIVO** ____/_____

[] NOME MEDICO _____ NOME PAZIENTE_____
DATA DI REDAZIONE DELLA RICETTA__/__/____ **[] ORDINE ospedale** _____

Componenti	Quantità (peso o volume)	Rif. Interno/ n. lotto	Note	Sostanza finita (segno X)
		__/___		
		__/___		
		__/___		
		__/___		
		__/___		
		__/___		
		__/___		
		__/___		

SIGLA DEL PREPARATORE____

12 – Label of a galenic preparation

FARMACIA SAN GALLICANO
Via San Gallicano, 23 - 00153 Roma Tel: 065895764

Minoxidil lozione 2% – 100 ml

con CICLOSILICONE PENTAMERO

Paziente: [redacted] - **Medico prescrittore:** [redacted]

Data di preparazione: 01/10/2014 - **Data di scadenza:** 01/04/2015

Composizione
Minoxidil base.....2 g
Ciclosilicone pentamero 15 g
Etanolo 96° q.b. a 100 ml

Prezzo praticato: 13,93 € (M: 4,61€; O: 6,32€; D.A. ---; C: 3,00€)
N° progressivo: 1330

M24 'rev0 29/07/14

Precauzioni ed avvertenze
Tenere fuori dalla portata dei bambini - Lavarsi abbondantemente le mani dopo l'utilizzo - Non utilizzare il preparato oltre la data di scadenza riportata sulla confezione - Conservare in luogo fresco ed al riparo dalla luce - Non disperdere il flacone nell'ambiente - Segnalare al medico o al farmacista gli eventuali effetti collaterali - **USO ESTERNO**

13 – Datasheet for the signaling of a suspected adverse drug reaction

SCHEDA UNICA DI SEGNALAZIONE DI SOSPETTA REAZIONE AVVERSA (ADR)

(da compilarsi a cura dei medici o degli altri operatori sanitari e da inviare al Responsabile di farmacovigilanza della struttura sanitaria di appartenenza)

1. INIZIALI DEL PAZIENTE ☐☐	2. DATA DI NASCITA	3. SESSO	4. DATA INSORGENZA REAZIONE	5. ORIGINE ETNICA	CODICE SEGNALAZIONE

6. DESCRIZIONE DELLA REAZIONE ED EVENTUALE DIAGNOSI* ** se il segnalatore è un medico*

7. GRAVITA' DELLA REAZIONE:

◊ **GRAVE**
- ☐ DECESSO
- ☐ OSPEDALIZZAZIONE O PROLUNGAMENTO OSPED.
- ☐ INVALIDITA' GRAVE O PERMANENTE
- ☐ HA MESSO IN PERICOLO DI VITA
- ☐ ANOMALIE CONGENITE/ DEFICIT NEL NEONATO

◊ **NON GRAVE**

8. EVENTUALI ESAMI DI LABORATORIO RILEVANTI PER ADR: *riportare risultati e date in cui gli accertamenti sono stati eseguiti*

9. ESITO
- ◊ RISOLUZIONE COMPLETA ADR IL __/__/__
- ◊ RISOLUZIONE CON POSTUMI
- ◊ MIGLIORAMENTO
- ◊ REAZIONE INVARIATA O PEGGIORATA
- ◊ DECESSO IL __/__/__
  - ☐ dovuto alla reazione avversa
  - ☐ il farmaco può avere contribuito
  - ☐ non dovuto al farmaco
  - ☐ causa sconosciuta
- ◊ NON DISPONIBILE

10. AZIONI INTRAPRESE: *specificare*

In caso di sospensione compilare i campi da 16 a 19

INFORMAZIONI SUL FARMACO

11. FARMACO(I) SOSPETTO (I) *nome della specialità medicinale**

A) _____ **12. LOTTO**_____ **13. DOSAGGIO/DIE** _____

14. VIA DI SOMMINISTRAZIONE _____ **15. DURATA DELL'USO:** DAL _____ AL_____

B) _____ **12. LOTTO**_____ **13. DOSAGGIO/DIE** _____

14. VIA DI SOMMINISTRAZIONE _____ **15. DURATA DELL'USO:** DAL _____ AL_____

C) _____ **12. LOTTO**_____ **13. DOSAGGIO/DIE** _____

14. VIA DI SOMMINISTRAZIONE _____ **15. DURATA DELL'USO:** DAL _____ AL_____

**** Nel caso di vaccini specificare anche il numero di dosi e/o di richiamo e l'ora della somministrazione***

16. IL FARMACO E' STATO SOSPESO?	A: sì / no	B: sì / no	C: sì / no
17. LA REAZIONE E' MIGLIORATA DOPO LA SOSPENSIONE?	A: sì / no	B: sì / no	C: sì / no
18. IL FARMACO E' STATO RIPRESO?	A: sì / no	B: sì / no	C: sì / no
19. SONO RICOMPARSI I SINTOMI DOPO LA RISOMMINISTRAZIONE?	A: sì / no	B: sì / no	C: sì / no

20. INDICAZIONI O ALTRO MOTIVO PER CUI IL FARMACO È STATO USATO:
A:
B:
C:

21. FARMACO(I) CONCOMITANTE(I), DOSAGGIO, VIA DI SOMMINISTRAZIONE, DURATA DEL TRATTAMENTO

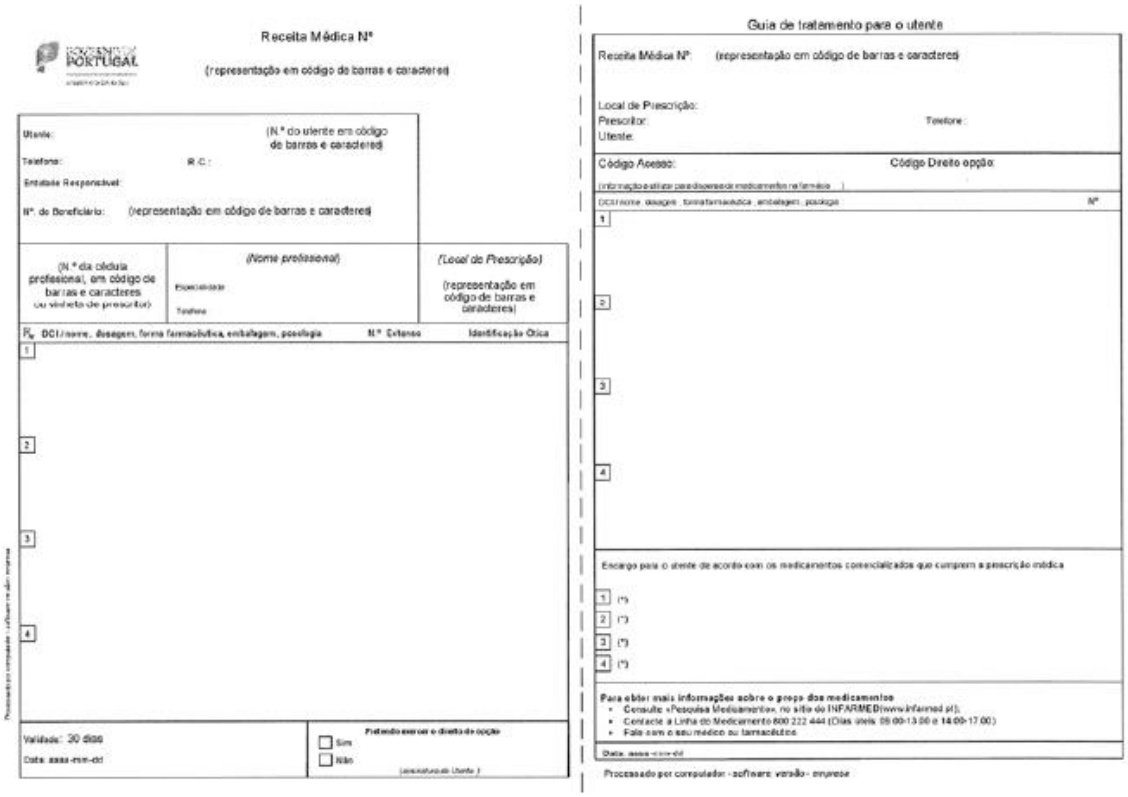
22. USO CONCOMITANTE DI ALTRI PRODOTTI A BASE DI PIANTE OFFICINALI, OMEOPATICI, INTEGRATORI ALIMENTARI, *ECC. (specificare):*

23. CONDIZIONI CONCOMITANTI PREDISPONENTI *(se il farmaco sospetto è un vaccino riportare l'anamnesi ed eventuali vaccini somministrati nelle 4 settimane precedenti alla somministrazione)*

INFORMAZIONI SULLA SEGNALAZIONE

24. QUALIFICA DEL SEGNALATORE		**25. DATI DEL SEGNALATORE**	
O MEDICO DI MEDICINA GENERALE	O PEDIATRA DI LIBERA SCELTA	NOME E COGNOME	
O MEDICO OSPEDALIERO	O FARMACISTA	INDIRIZZO	
O SPECIALISTA	O ALTRO	TEL E FAX	E-MAIL
26. DATA DI COMPILAZIONE		**27. FIRMA DEL SEGNALATORE**	
28. CODICE ASL		**29. FIRMA DEL RESPONSABILE DI FARMACOVIGILANZA**	

14 – Model of electronic prescriptions



Receita Médica Nº

(representação em código de barras e caracteres)

Utente: (Nº do utente em código de barras e caracteres)

Telefone: R.C.:

Entidade Responsável:

Nº. de Beneficiário: (representação em código de barras e caracteres)

(Nº da cédula profissional, em código de barras e caracteres ou vinheta de prescritor) | (Nome profissional) Especialidade Telefone | (Local de Prescrição) (representação em código de barras e caracteres)

Nº	DCI / nome, dosagem, forma farmacêutica, embalagem, posologia	Nº Extenso	Identificação Ótica
1			
2			
3			
4			

Validade: 30 dias

Data: aaaa-mm-dd

Pretendo exercer o direito de opção: ☐ Sim ☐ Não

(assinatura do Utente)

Guia de tratamento para o utente

Receita Médica Nº: (representação em código de barras e caracteres)

Local de Prescrição:

Prescritor: Telefone:

Utente:

Código Acesso: Código Direito opção:

(informação a utilizar para dispensa de medicamentos reformados)

DCI / nome, dosagem, forma farmacêutica, embalagem, posologia Nº

1

2

3

4

Encargo para o utente de acordo com os medicamentos comercializados que cumprem a prescrição médica

1 (*)

2 (*)

3 (*)

4 (*)

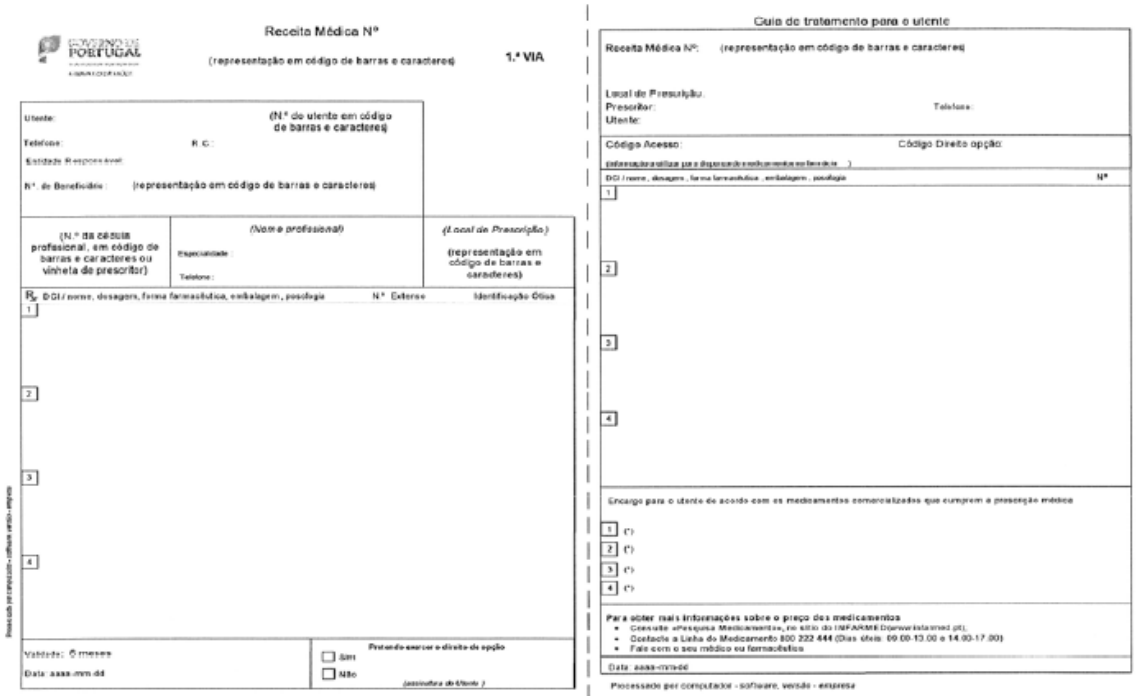
Para obter mais informações sobre o preço dos medicamentos

- Consulte «Pesquisa Medicamento», no sítio do INFARMED (www.infarmed.pt),
- Contacte a Linha do Medicamento 800 222 444 (Dias úteis: 09:00-13:00 e 14:00-17:00)
- Fale com o seu médico ou farmacêutico

Data: aaaa-mm-dd

Processado por computador - software: versão - empresa

15 – Model of renewable electronic prescriptions



Receita Médica Nº

(representação em código de barras e caracteres)

1.ª VIA

Utente:

(Nº de utente em código de barras e caracteres)

Telefone:

R.C.:

Entidade Responsável:

Nº. de Beneficiário:

(representação em código de barras e caracteres)

(Nº da cédula profissional, em código de barras e caracteres ou vinheta de prescritor)

(Nome profissional)

Especialidade:

Telefone:

(Local de Prescrição)

(representação em código de barras e caracteres)

Rx DCI / nome, dosagem, forma farmacêutica, embalagem, posologia Nº Extenso Identificação Ótica

1

2

3

4

Validade: 6 meses

Data: aaaa-mm-dd

Pretendo exercer o direito de opção

☐ Sim

☐ Não

(assinatura do Utente)

Guia de tratamento para o utente

Receita Médica Nº: (representação em código de barras e caracteres)

Local de Prescrição:

Prescritor: Telefone:

Utente:

Código Acesso: Código Direito opção:

(informação prática para a dispensa de medicamentos sujeitos a receita médica)

DCI / nome, dosagem, forma farmacêutica, embalagem, posologia Nº

1

2

3

4

Encargo para o utente de acordo com os medicamentos comercializados que cumprem a prescrição médica

1 (*)

2 (*)

3 (*)

4 (*)

Para obter mais informações sobre o preço dos medicamentos

- Consulte «Pesquisa Medicamentos» no sítio do INFARMED (infarmed.pt);
- Contacte a Linha do Medicamento 800 222 444 (Dias úteis: 09.00-13.00 e 14.00-17.00)
- Fale com o seu médico ou farmacêutico

Data: aaaa-mm-dd

Processado por computador - software, versão - empresa

16 – Model of manual prescriptions

Receita Médica Nº

PORTUGAL

99999999999999999

Utente

Nº de Utente

Telefone:

R.C.:

Entidade Responsável

Nº de Beneficiário

RECEITA MANUAL

Excepção legal

a) Falência informática

b) Inadaptação do prescritor

c) Prescrição no domicílio

d) Até 40 receitas/mês

Vinheta do Prescritor

Especialidade

Telefone

Vinheta do Local de Prescrição

Rx DCI/ Nome, dosagem, forma farmacêutica, embalagem N.º Extenso

1

Posologia

2

Posologia

3

Posologia

4

Posologia

Validade: 30 dias

Data: ____ / __ / __

Assinatura do Prescritor

Pretendo exercer o direito de opção

Sim

Não

(assinatura do Utente)

17 – Back of the prescription after dispensing

FARMACIA NUNES FEIJAO - SANTO ANTONIO CHARNECA
Dir. Téc.:Dra.Isabel C.P.R.B.Nunes Feijao
Reg. C.R.C. 501907513

CAPITAL SOCIAL: 5.000 Euros
Nº de Contribuinte: 501907513
DOCUMENTO PARA FACTURAÇÃO
99x - R/L/S:12/9/8
Rec.: 3021000019002471815
Ben.:

R02gcG6z9jtL - VENDA - 110264 (21) 05/09/15

Prod PVP	PRef	Qt	Comp	Utente
1) *5351358* - Esomeprazol Tolife, 20 mg x 56 comp g				
9,96	8,37	1	3,10	6,86
2) *3418597* - Carvedilol Coronat MG, 6,25 mg x 60 c				
6,09	4,57	1	3,15	2,94
3) *5398177* - Tation, 5 mg x 30 comp lib prol				
4,23	0,00	1	2,92	1,31
T: 20,28		3	9,17	11,11

Declaro que: Me foram dispensadas as 3 embalagens de medicamentos constantes na receita e prestados os conselhos sobre a sua utilização.
Direito de Opção:
1,2 Exerci o direito de opção para o medicamento com preço superior ao 5.º preço mais barato.

Ass. do Utente [redacted]

Nunes Feijão FARMÁCIA

18 – Example of a special reimbursement system (4HPV)

FARMACIA NUNES FEIJAO
R.JOSE GOMES FERREIRA,4B -VILA CHA
2835-463 SANTO ANTONIO CHARNECA
501907513
NIF:501907513
Dra.Isabel C.P.R.B.Nunes Feijao
Tel.:212160562

WQ Prevenção 4HPV 4HPV
Data: 01-09-2015 (CATARINA)

Rec./Lote/Série: 1/1/16 Venda nº:109861

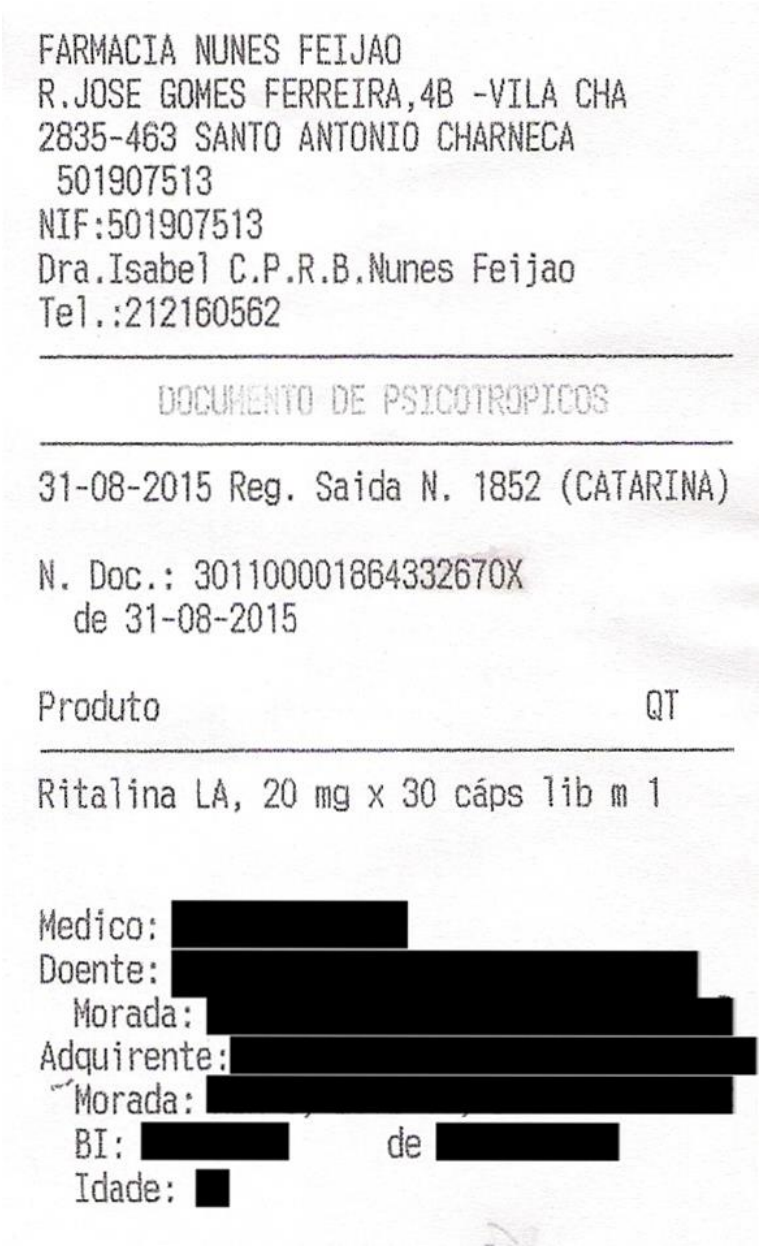
Ben.: NºRec.:301100001897

Produto						
	PVP	PRef	Qt	Comp	Liquído	IVA
Gardasil x 1 susp inj IM seringa						
	119,81	0,00	1	24,81	95,00	6%

Total(Euros): 119,81 1 95,00

[redacted]

19 – Dispensing document for narcotic and psychotropic medicines



FARMACIA NUNES FEIJAO
R.JOSE GOMES FERREIRA,4B -VILA CHA
2835-463 SANTO ANTONIO CHARNECA
501907513
NIF:501907513
Dra.Isabel C.P.R.B.Nunes Feijao
Tel.:212160562

DOCUMENTO DE PSICOTROPICOS

31-08-2015 Reg. Saida N. 1852 (CATARINA)

N. Doc.: 3011000018643326 70X
de 31-08-2015

Produto QT

Ritalina LA, 20 mg x 30 cáps lib m 1

Medico: [redacted]
Doente: [redacted]
Morada: [redacted]
Adquirente: [redacted]
Morada: [redacted]
BI: [redacted] de [redacted]
Idade: [redacted]