Universidade de Aveiro Departamento de Biologia
2020

Rita de Jesus Peixoto

BETA-BLOQUEADORES E TRATAMENTO DE CANCRO: APLICAÇÕES E EFEITOS AMBIENTAIS

BETA-BLOCKERS AND CANCER TREATMENT: APPLICATIONS AND ENVIRONMENTAL EFFECTS

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Eco-Toxicologia e Análise de Risco, realizada sob a orientação científica do Doutor Miguel Oliveira, Investigador Auxiliar do Departamento de Biologia da Universidade de Aveiro, e da Doutora Maria de Lourdes Gomes Pereira, Professora Associada com Agregação do Departamento de Ciências Médicas da Universidade de Aveiro.

o júri

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agradecimentos

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palavras-chave

Beta-bloqueadores, propranolol, cancro, aplicações terapêuticas, efeitos, organismos não-alvo, níveis.

resumo

Os fármacos são de considerável importância para a vida humana desde a nascença até à terceira idade. Com o envelhecimento, a necessidade dos fármacos para melhorar a qualidade de vida e controlar doenças crónicas frequentemente aumenta. Além disso, certas doenças como o cancro têm afetado cada vez mais humanos, reforçando a relevância dos fármacos para melhorar a qualidade de vida e a longevidade, apesar dos seus potenciais efeitos adversos a doses elevadas.

O consumo aumentado de fármacos promove a sua libertação ambiental. Compostos parentais ou os seus metabolitos são excretados e libertados nos sistemas de esgotos. A indústria, as instituições médicas e os efluentes domésticos e são as principais fontes que introduzem fármacos no ambiente aquático, uma vez que o tratamento dos sistemas de esgotos continua ineficaz na remoção da maioria destas substâncias biologicamente ativas. Assim, estes compostos podem ser encontrados nos ecossistemas aquáticos, resultando numa exposição contínua dos organismos aquáticos.

O cancro é uma das principais causas de morte a nível mundial. Muitas abordagens usadas para tratar o cancro também afetam células saudáveis. São necessários tratamentos mais eficazes para diminuir a toxicidade das células normais e melhorar as taxas de sucesso dos tratamentos. O uso de beta-bloqueadores no cancro tem sido alvo de estudo pela sua ação antagonista no sistema adrenérgico. Além de regular processos como a pressão arterial, frequência cardíaca e força ou reatividade das vias aéreas, os beta-bloqueadores bloqueiam mecanismos que desencadeiam tumorigénese, angiogénese e a metastização. O propranolol, um dos beta-bloqueadores mais prescritos, tem uma elevada taxa de consumo e uma baixa taxa de remoção nas estações de tratamento de águas residuais que se têm refletido no aumento dos seus níveis ambientais, onde podem causar efeitos sub-letais em organismos não-alvo.

O objetivo desta dissertação foi estudar o papel dos beta-bloqueadores em geral, e do propranolol em particular, no tratamento do cancro e os seus efeitos em organismos não-alvo. A revisão da literatura disponível revelou que o propranolol pode ser usado como complemento no tratamento de diversos tipos de cancro devido à sua capacidade de melhorar os resultados através da diminuição das taxas de proliferação das células cancerígenas. Contudo, devem ser realizados testes *in vitro* adicionais de modo a permitir a compreensão da proteção dos beta-bloqueadores em doentes oncológicos.

No ambiente aquático, esta classe de medicamentos pode causar efeitos deletérios na biota. O propranolol, por exemplo, tem afetado uma grande variedade de organismos desde microalgas a peixe. Os dados disponíveis demonstram que o propranolol afeta processos moleculares, bioquímicos e fisiológicos que podem resultar em alterações em termos de comportamento, reprodução e desenvolvimento. A informação recolhida revela ainda a necessidade de desenvolver estudos a longo prazo, bem como estudos com exposição de combinações com outros contaminantes presentes no ambiente aquático, para permitir a compreensão dos efeitos através de uma perspetiva ambientalmente mais relevante, uma vez que os organismos estão expostos no ambiente a propranolol e outros contaminantes durante as suas vidas. No entanto, não estão disponíveis estudos nesta abordagem. Os estudos existentes são referentes a investigação que avalia efeitos após exposições a curto-prazo a um único contaminante.

keywords

Beta-blockers, propranolol, cancer, therapeutic applications, effects, non-target organisms, levels.

abstract

Pharmaceuticals are of considerable importance for human life from birth to old age. As aging occurs, the need for pharmaceuticals to improve life quality and control chronic diseases often increases. Additionally, certain diseases like cancer have been increasingly affecting humans reinforcing the relevance of pharmaceuticals to improve life quality and longevity, despite their potential pernicious effect at high doses.

The increased consumption of pharmaceuticals promotes its environmental release. Parent compounds or their metabolites are excreted and released into the sewage systems. Industrial, medical facilities, and domestic effluents are the primary sources that lead to the presence of pharmaceuticals in the aquatic environment as sewage system treatments are still ineffective in the removal of most of these biologically active substances. Thus, these compounds can be found in aquatic ecosystems resulting in continuous exposure of aquatic organisms.

Cancer is one of the leading causes of death worldwide. Several approaches used to treat cancer also affect normal cells and, thus, there is a need for more effective treatments that decrease the toxicity to normal cells and increase the success rates of treatment. The use of beta-blockers in cancer has been studied for their antagonist action on the adrenergic system. Besides regulating processes such as blood pressure, heart rate, and airway strength or reactivity, beta-blockers block mechanisms that trigger tumorigenesis, angiogenesis, and tumor metastasis. Propranolol, one of the most prescribed beta-blockers, has a high consumption and a low removal rate in the wastewater treatments plants that have been reflected in its increased environmental levels where it may cause sub-lethal effects in non-target organisms.

The aim of this dissertation was to study the role of beta-blockers in general, and propranolol in particular, in cancer treatment and its effects on non-target organisms. The review of the available literature revealed that propranolol might be used as a complement for the treatment of several types of cancer due to its ability to improve cancer outcomes by decreasing cancer cell proliferation rates. Nonetheless, additional *in vitro* studies should be performed to fully understand the protective role of beta-blockers in cancer patients.

Once in the aquatic environment, this class of drugs can cause pernicious effects to biota. Propranolol, for example, has been reported to affect a wide range of organisms from microalgae to fish. Available data demonstrates that propranolol affects molecular, biochemical, and physiological processes that may result in behavior, reproduction, and developmental alterations. The collected information also revealed the need to perform long-term studies, as well as studies with combined exposure with other contaminants present in the aquatic environment, to allow understanding of effects through a more environmentally relevant perspective, as organisms are exposed in the environment to propranolol and other contaminants throughout their lives. Nonetheless, there are no available studies addressing this approach, with most research assessing effects after short-term exposures to a single contaminant.

Table of Contents

Table of Figures

Table of Tables

Abbreviatures

BBs – Beta-blockers

cAMP – Cyclic adenosine monophosphate

CREB – cAMP-responsive element binding

CV – Cardiovascular

EROD – Ethoxyresorufin O-deethylase

GABA – Gamma-aminobutyric acid

GSK3 – Glycogen synthase kinase 3

GST – Glutathione-*S*-transferases

LPO – Lipid peroxidation

LVEF – Left-ventricular ejection fraction

PKB – Protein kinase B

SOD – Superoxide dismutase

TnI – Troponin I

VEGF – Vascular endothelial growth factor

WWTP – Wastewater treatment plants

Chapter I
General Introduction

General Introduction

Worldwide projections indicate that by 2050, the number of people aged at least 60 years old will be much higher than those aged 10 to 24 years (2.1 billion versus 2.0 billion) (Rudnicka *et al*., 2020). The process of aging is often associated with an increasing number of diseases, which decrease the quality of life of the elderly. In addition, diseases like cancer have been increasingly affecting humans. Multiple chronic conditions are particularly different in cancer patients since more than 60% of cancer patients suffer from three or more chronic illnesses (Lund *et al*., 2020). The arise of comorbidities in the elderly is associated with increased mortality, higher rates of hospital admissions, the worse status of physical function and greater use of health services (Kim *et al*., 2018). Pharmaceuticals play a key role in reestablishing human health, despite their potential pernicious effect in high doses. The use of these substances has allowed improved life quality and longevity. However, after the use of pharmaceuticals, the parent compounds or their metabolites are excreted and released into the sewage systems. The primary sources leading to the occurrence of pharmaceuticals in the aquatic environment are industrial, medical facilities, and domestic effluents. Despite the increased improvement of sewage system treatments, they are still ineffective in the removal of most of these biologically active substances, which are released in the wastewater treatment plant effluents, and/or retained in sludge, often used as fertilizer, reaching agricultural fields. As hydrophilic and long half-life compounds are not effectively removed by wastewater treatment plants, these compounds easily persist in aquatic ecosystems, resulting in continuous exposure of aquatic organisms (Dong *et al*., 2013; Fent *et al*., 2006). Thus, pharmaceuticals can be found in surface water, groundwater, drinking water, seawater, wastewater, sediment and soil.

Beta-blockers are the most widely used class of pharmaceuticals used worldwide to regulate blood pressure, heart rate and airway strength or reactivity. Recent studies have shown that the use of beta-blockers increases survival rates in patients with hypertension and coronary heart disease, given their protective role in the cardiac muscle (Kaboli *et al.*, 2007; Sorbets *et al.*, 2019).

Among the diseases that concern humans, the most common is cancer. Cancer is a group of abnormalities characterized by unmeasured growth of cells, which leads to the development of tumors. At the top of leading causes of death worldwide, cancer develops in more than 18 million individuals every year, of which about 9.6 million die (WHO, 2018). Currently, a considerable amount of research is being performed to develop new and more effective treatments to cure patients and promote a better quality of life. The available knowledge has identified that beta-adrenergic receptors are associated with mechanisms that trigger tumorigenesis, angiogenesis and tumor metastasis. Thus, beta-blockers were also considered for cancer treatment (Peixoto *et al*., 2020; Zhou *et al*., 2016). Propranolol is one of the most prescribed beta-blockers. Its high consumption rate is reflected in its increased environmental levels. This pharmaceutical has a low removal rate in wastewater treatments plants (20%), reaching the environment where it may cause sub-lethal effects in non-target organisms (Di Lorenzo *et al*., 2019; Wöhler *et al*., 2020).

The aim of this dissertation was to study the role of beta-blockers in general, and propranolol in particular, in cancer treatment and its effects to non-target organisms.

This dissertation is divided into four chapters. Chapter I is a general introduction to the topic which presents beta-blockers as a therapeutic class of drugs. Chapter II presents a review of the use of beta-blockers in cancer treatments, describing their general mechanism of action and how they can act on different cancer tissues, as well as traditional pharmaceuticals used in chemotherapy. Chapter III presents a review of the reported effects of the beta-blocker propranolol to aquatic biota and environmental levels. Chapter IV comprises the final considerations and future perspectives on the applications and effects of beta-blockers.

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Chapter II
Beta-Blockers and Cancer: Where Are We?

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Beta-Blockers and Cancer: Where Are We?

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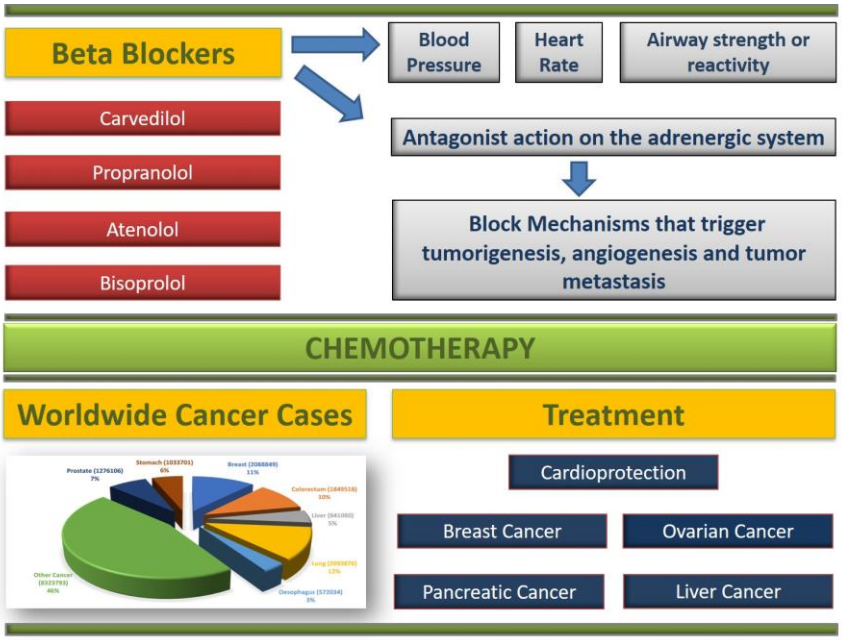
Graphical Abstract



Beta-Blockers and Cancer: Where Are We?

Abstract
Cancer is one of the leading causes of death worldwide. After diagnosis, cancer treatment may involve radiotherapy, chemotherapy, and surgery. Several of the approaches used to treat cancer also attack normal cells and, thus, there is the need for more effective treatments that decrease the toxicity to normal cells and increase the success rates of treatment. The use of beta-blockers in cancer has been studied for their antagonist action on the adrenergic system through inhibition of beta-adrenergic receptors. Besides regulating processes such as blood pressure, heart rate, and airway strength or reactivity, beta-blockers block mechanisms that trigger tumorigenesis, angiogenesis, and tumour metastasis. This study presents a literature review of the available studies addressing cancer treatments and beta-blockers. Overall, data suggest that propranolol may be used as a complement for the treatment of several types of cancer due to its ability to improve cancer outcomes by decreasing cancer cell proliferation rates. Nonetheless, additional *in vitro* studies should be performed to fully understand the protective role of BBs in cancer patients.
Keywords: beta-blockers; therapeutic application; cancer

1. Introduction

Cancer, a group of abnormalities characterized by unmeasured growth of cells leading to the development of tumors, is a global public health problem, at the top of the leading causes of death in wealthy countries (CDC, 2020). The global cancer burden is significant and increasing. According to the National Center for Health Statistics of the United States of America, the most commonly diagnosed cancers in men are prostate, lung, and colorectal cancer, whereas, in women breast, lung, and colorectal cancer are the most common [1]. Figure 1 presents the distribution of the estimated cancer cases worldwide (18,078,957), per types of cancers.

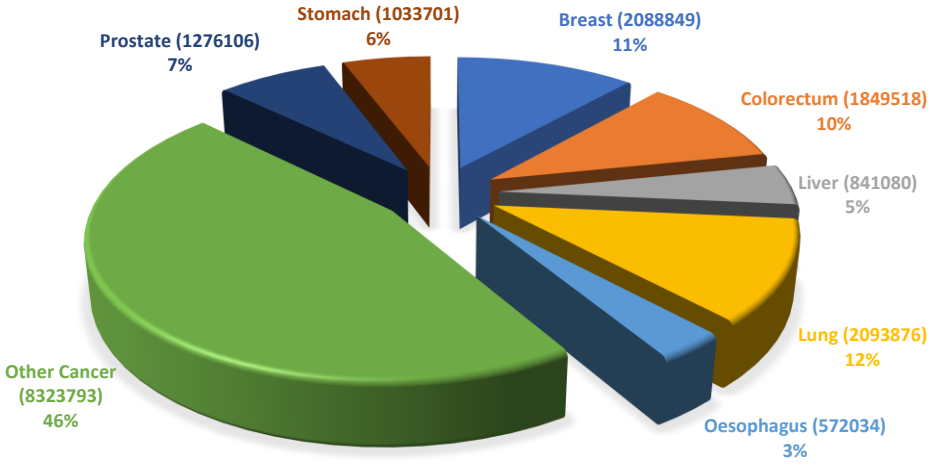


Figure 1. Distribution of the estimated number of worldwide cancer cases in 2018 (18,078,957) per type of cancer. Data includes all types of cancers, all ages and both sexes (Adapted from Global Cancer Observatory—World Health Organization [2]).

It is estimated that each year 9.6 million people die from cancer, and that a quarter of those deaths are related to lung cancer. The five-year survival rate for patients diagnosed with cancers is lower for pancreas (9%), increasing for liver (18%), esophagus (19%), and lung (19%) cancers [3]. During their lifetime, one in five men and one in six women worldwide will develop a type of cancer [4]. Once diagnosed, the treatment of patients may involve different approaches that include radiotherapy, chemotherapy, and surgery. Primary prevention, screening and early diagnosis, multimodal treatment and survival and palliative care are the spectrum of cancer control interventions. There are significant differences in terms of cost of treatment, with estimates of 25,000 Canadian dollars for melanoma, thyroid, and testicular cancers and 60,000 Canadian dollars for leukemia. Lifetime treatment costs may range from 55,000 Canadian dollars for lung and liver cancers to over 110,000 Canadian dollars for leukemia, lymphoma and breast cancer [3].

2. Beta-Blockers

The expression of specific receptors (proteins able to bind ligands (e.g., catecholamines) and transducing extracellular signals across the plasma membrane) and the activation of intracellular signaling pathways is a key process of cells. These specificities enable cells to interact and adapt to the surrounding environment. Beta-blockers (BBs) are commonly considered cardioprotective drugs used in various diseases (e.g., hypertension or coronary artery disease) due to their antagonist action on the adrenergic system through inhibition of beta-adrenergic receptors [5–9]. BBs have been considered for cancer treatment due to their antagonist action on receptors associated with mechanisms that trigger tumorigenesis, angiogenesis, and tumor metastasis, which may allow the decrease of the enormous costs of cancer treatments, as well as short survival rates [10].

BBs were first discovered in 1906 by Sir Henry Hallett Dale, awarded with a Nobel prize for his discovery. However, it was only in 1948 that Raymond Perry Ahlquist observed that adrenergic receptors could be divided into two types (alpha- and beta-receptors). In 1967, Alonzo M. Lands observed that, depending on the tissue, BBs could act by two different pathways, culminating in the differentiation of beta-adrenergic receptors into two subtypes: beta-1 and beta-2 subtypes. Meanwhile, it was discovered that some BBs may act on both pathways, acting on both receptor subtypes. An example of this type of drugs is propranolol, the prototype of the first invented BBs and the one with the most collected experience and clinical indications [11].

The adrenergic receptors, members of the superfamily of cell surface receptors that carry out signaling via coupling to guanine nucleotide binding proteins (G-proteins) can be divided into 2 types: alpha-receptors (associated with “excitatory” functions such as vasoconstriction) and beta-receptors (associated with “inhibitory” functions like vasodilatation and excitatory effects in the myocardium) [12–17]. Beta-receptors are divided into three subtypes: beta-1-receptors (commonly associated with the heart), beta-2-receptors (responsible for vascular and airway relaxation), and beta-3-receptors (present in the cells of brown adipose tissue from rats) [18,19]. In this perspective, an agent able to inhibit the response of the adrenergic receptors is an adrenergic antagonist, whereas, a molecule stimulator of response (e.g., catecholamines) is an adrenergic agonist [17]. Thus, based on the affinity to the beta-subtype receptors, BBs can be

considered as “beta-1 selective” or “cardioselective” (as the beta-1 subtype is the predominant one in the heart) when exhibiting a higher affinity for beta-1 subtype than for beta-2 (e.g., atenolol, celiprolol, metoprolol, bisoprolol, and nebivolol) or “nonselective BBs” if acting on both beta-1 and beta-2 receptors (e.g., propranolol, sotalol, carvedilol, labetalol, and timolol) [20,21]. Some selective or nonselective BBs are also antagonists of the alpha-1 receptors (carvedilol and labetalol) and alpha-2 receptors (celiprolol) and have the capacity of increasing nitric oxide release (nebivolol and carvedilol), causing a vasodilatory activity [11,22–25].

The use of this group of molecules of different pharmacokinetic and pharmacodynamic properties has been considered in the treatment of different pathologies like hypertension, cancer, and migraine, suggesting a protective effect that may span far beyond the cardiovascular (CV) system [26,27]. The known effects of BBs are diverse. BBs regulate, among other functions, processes like blood pressure, heart rate and airway strength or reactivity [17]. These pharmaceuticals are used worldwide, and its consumption has been increasing, over time, in older patients as BBs are considered to play a protective role in the cardiac muscle. Furthermore, it is believed that patients with hypertension have increased survival rates when taking BBs [28]. However, recent meta-analyses (that do not take age into account) state that BBs are unsuitable for the treatment of hypertension as first-line therapy [29]. BBs have been associated with lower mortality rates in the 5 years following an episode of myocardial infarction in patients with stable coronary heart disease [30]. However, in patients with myocardial infarction without heart failure, BBs showed no beneficial effect when used beyond 1 year after the episode [31]. Studies have, however, suggested side effects of BBs. According to a study performed by the Action to Control Cardiovascular Risk in Diabetes, diabetic patients taking BBs have significantly higher CV disease event rates and increased incidence of hypoglycemia [8,32].

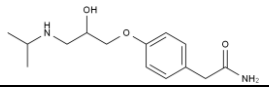
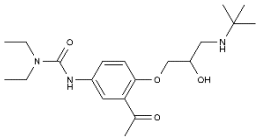
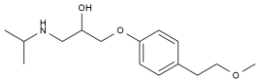
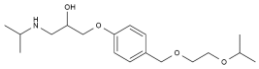
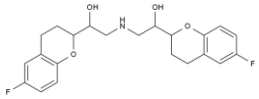
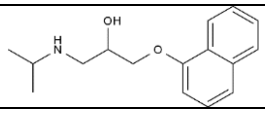
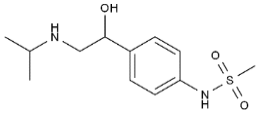
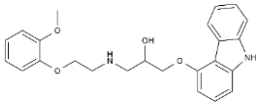
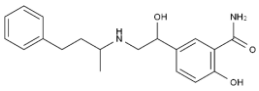
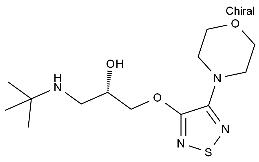
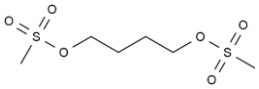
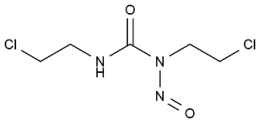
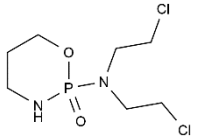
The use of BBs is contraindicated in patients with asthma, reactive airway disease, acute decompensated heart failure with systolic dysfunction, heart block and sick sinus syndrome, even in the therapeutic dose range [33].

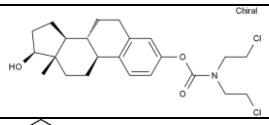
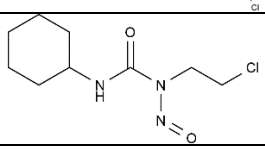
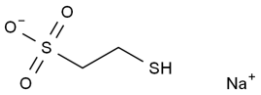
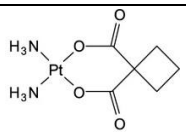
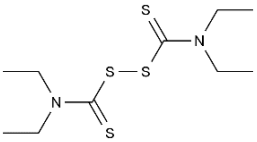
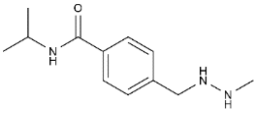
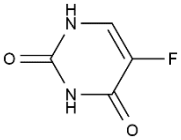
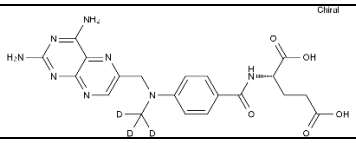
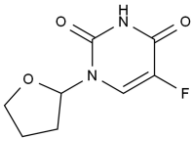
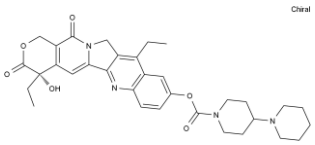
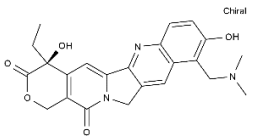
3. Antineoplastic Agents and Cardiotoxicity

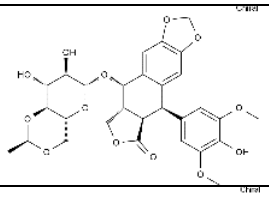
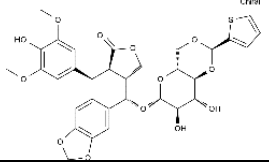
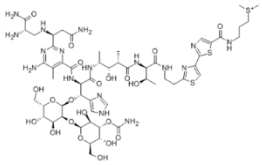
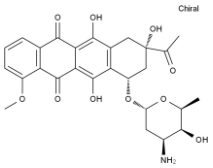
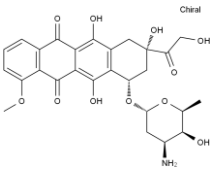
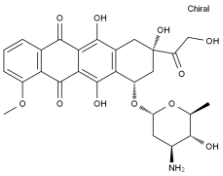
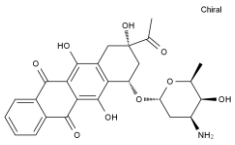
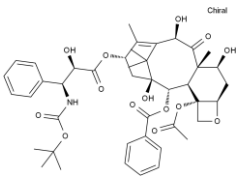
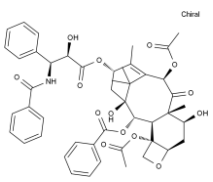
Regardless the type of cancer, cancer treatment aims to control or even terminate the uncontrolled growth of cancer cells [34]. In the last years, new antineoplastic agents have been emerging, presenting advantages in terms of safety, availability, and lower cost when compared to those widely used [35]. These agents play essential roles in triggering, controlling, and modifying cancer cell mechanisms regarding its proliferation, differentiation, and survival [36].

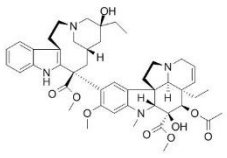
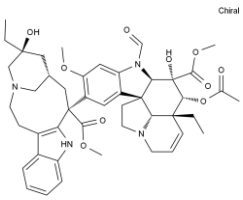
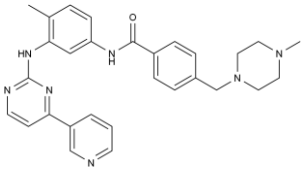
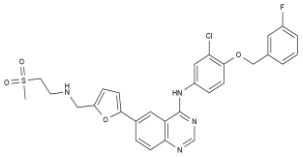
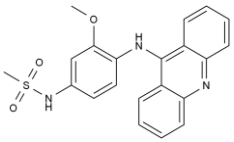
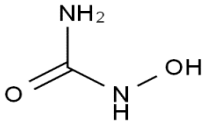
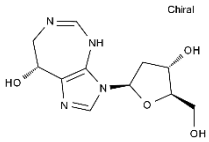
Antineoplastic agents can be divided into nine groups (Table 1): alkylating, alkylating-related, antimetabolite, topoisomerase-1 inhibitors, topoisomerase-2 inhibitors, DNA-intercalating agents, agents that interfere with tubulin, tyrosine-kinases inhibitors, and others [37].

Table 1. Examples of frequently used beta blockers and antineoplastic agents.

Pharmaceutical	Class	Chemical Formula	Structure	CAS
Atenolol	BB-B1	$C_{14}H_{22}N_2O_3$		29122-68-7
Celiprolol	BB-B1	$C_{20}H_{33}N_3O_4$		56980-93-9
Metoprolol	BB-B1	$C_{15}H_{25}NO_3$		51384-51-1
Bisoprolol	BB-B1	$C_{18}H_{31}NO_4$		66722-44-9
Nebivolol	BB-B1	$C_{22}H_{25}F_2NO_4$		99200-09-6
Propranolol	BB-NS	$C_{16}H_{21}NO_2$		525-66-6
Sotalol	BB-NS	$C_{12}H_{20}N_2O_3S$		3930-20-9
Carvedilol	BB-NS	$C_{24}H_{26}N_2O_4$		72956-09-3
Labetalol	BB-NS	$C_{19}H_{24}N_2O_3$		36894-69-6
Timolol	BB-NS	$C_{13}H_{24}N_4O_3S$		26839-75-8
Busulfan	AA-Alk	$C_6H_{14}O_6S_2$		55-98-1
Carmustine	AA-Alk	$C_5H_9Cl_2N_3O_2$		154-93-8
Cyclophosphamide	AA-Alk	$C_7H_{15}Cl_2N_2O_2P$		50-18-0

Estramustin	AA-Alk	$C_{23}H_{31}Cl_2NO_3$		2998-57-4
Lomustine	AA-Alk	$C_9H_{16}ClN_3O_2$		13010-47-4
Mesna	AA-Alk	$C_2H_5NaO_3S_2$		19767-45-4
Carboplatin	AA-AlkRel	$C_6H_{12}N_2O_4Pt$		41575-94-4
Cisplatin	AA-AlkRel	$Cl_2H_6N_2Pt$		15663-27-1
Disulfiram	AA-AlkRel	$C_{10}H_{20}N_2S_4$ or $((C_2H_5)_2NCS)_2S_2$		97-77-8
Procarbazine	AA-AlkRel	$C_{12}H_{19}N_3O$		671-16-9
Fluorouracil	AA-AntMet	$C_4H_3FN_2O_2$		51-21-8
Methotrexate	AA-AntMet	$C_{20}H_{22}N_8O_5$		59-05-2
Tegafur	AA-AntMet	$C_8H_9FN_2O_3$		17902-23-7
Irinotecan	AA-Top-1Inh	$C_{33}H_{38}N_4O_6$		97682-44-5
Topotecan	AA-Top-1Inh	$C_{23}H_{23}N_3O_5$		123948-87-8

Etoposide	AA-Top-2Inh	$C_{29}H_{32}O_{13}$		33419-42-0
Teniposide	AA-Top-2Inh	$C_{32}H_{32}O_{13}S$		29767-20-2
Bleomycin	AA-DNAIntAg	$C_{55}H_{84}N_{17}O_{21}S_3^+$		11056-06-7
Daunorubicin	AA-DNAIntAg	$C_{27}H_{29}NO_{10}$		20830-81-3
Doxorubicin	AA-DNAIntAg	$C_{27}H_{29}NO_{11}$		23214-92-8
Epirubicin	AA-DNAIntAg	$C_{27}H_{29}NO_{11}$		56420-45-2
Idarubicin	AA-DNAIntAg	$C_{26}H_{27}NO_9$		58957-92-9
Docetaxel	AA-IntTub	$C_{43}H_{53}NO_{14}$		114977-28-5
Paclitaxel	AA-IntTub	$C_{47}H_{51}NO_{14}$		33069-62-4

Vinblastine	AA-IntTub	$C_{46}H_{58}N_4O_9$		865-21-4
Vincristine	AA-IntTub	$C_{46}H_{56}N_4O_{10}$		57-22-7
Imatinib	AA-TyrKinInh	$C_{29}H_{31}N_7O$		152459-95-5
Lapatinib	AA-TyrKinInh	$C_{29}H_{26}ClFN_4O_4S$		231277-92-2
Amsacrine	AA-Other	$C_{21}H_{19}N_3O_3S$		51264-14-3
Hydroxyurea	AA-Other	$CH_4N_2O_2$		127-07-1
Pentostatin	AA-Other	$C_{11}H_{16}N_4O_4$		53910-25-1

BB—Beta Blocker; B1—Beta 1 selective; NS—Non-Selective; AA—Antineoplastic Agent; Alk—Alkylating, AlkRel—Alkylating Related; AntMet—Antimetabolite; Top-1Inh—Topoisomerase-1 inhibitor; Top-2Inh—Topoisomerase-2 inhibitor; DNAIntAg—DNA-intercalating agents; IntTub—Interfere with tubulin; TyrKinInh—Tyrosine-kinase inhibitors.

Anthracyclines (e.g., doxorubicin, daunorubicin, epirubicin, and idarubicin) are widely used for the treatment of breast cancer and lymphoma [38]. They act on cancer cells by intercalating between DNA base pairs, disrupting the DNA chain, and they stabilize the topoisomerase 2-alpha complex, preventing the association of disrupted DNA strands and, thus, lead to cell death [39,40]. However, its use has been associated with cardiomyocyte injury, due to the formation of reactive oxygen species (ROS) and inhibition of cardiomyocytes' topoisomerase 2-beta, and their death associated with double-stranded DNA that lead to apoptosis through the activation of p53 pathway [41,42]. These

effects may cause arrhythmias, symptomatic heart failure, and asymptomatic left-ventricular dysfunction which can be quantified through measurement of left-ventricular ejection fraction (LVEF). A decline in LVEF is a strong indicative of heart failure following anthracycline treatment and is more commonly detected in the elderly [41,43]. Arrhythmias may be caused not only by anthracyclines use but also by cisplatin, taxanes, vinca alkaloids, platinum, arsenic, thalidomide, antimetabolites, and interleukin-2 (IL-2) [44]. In a pediatric population, the risk of heart failure may remain high, even decades after treatment with anthracyclines [45].

Cisplatin is an alkylating-related agent that stands between two adjacent guanines followed by an adjacent guanine and adenine, inhibiting DNA replication and transcription and leading to cell death [46]. It is one of the most extensively used drugs for cancer treatment [47,48]. In testicular cancer treatment, a 90% cure rate has been reported in patients using this drug which is also used in the treatment of head and neck, cervical, breast, lung, ovarian, gastric, and bladder cancers [49]. Despite the success of cisplatin in cancer treatment, the reported side effects (e.g., nephrotoxicity, neurotoxicity, and ototoxicity) limit its use [50,51]. Nausea, vomiting, and myelosuppression are other common side effects [52]. Over the last years, cardiotoxic events have been reported for cisplatin treatments, including silent and symptomatic arrhythmias, angina, myocarditis, pericarditis, diastolic disturbances, cardiac ischemia, acute myocardial infarction, thromboembolic events, and chronic heart failure [53]. Considering that treatment with cisplatin is not as strictly monitored as those with other anticancer drugs (e.g., anthracyclines), some cardiotoxic effects may have been undetected or overlooked [49].

Arrhythmia is the most common cardiotoxic effect occurring in treatments with cisplatin (18–32% of patients) [44,54]. Tachycardia, which may be defined as a heart rhythm disorder in which the heart produces electrical signals that lead to faster heart beats (above 100 beats per minute), is the most recurrent type of arrhythmia resulting from treatment with cisplatin. Sinus bradycardia, a clinical term for a heartbeat slower than normal (below 60 beats per minute) [55,56] has also been reported. The cisplatin induction of hypomagnesaemia (Mg serum levels lower than 1.7mg/dL) may be responsible for these side effects as well as tremors and ataxia [49].

In order to decrease the toxicity induced by cancer treatment (chemo- and radiotherapy) to normal cells, new approaches have emerged, such as targeted molecular therapies, aiming at treatments focused on specific targets. New monoclonal antibodies have been approved due to their favorable tolerability profiles and reduced secondary effects. These molecules may also be safely combined with widely used chemotherapeutic agents or radiotherapy [57]. Trastuzumab is a monoclonal antibody used to treat breast cancer. However, its use has been associated with cardiotoxicity, injuring cardiomyocytes, and increasing the risk of heart failure. In a cohort study with 16,456 patients, 4325 of them receiving trastuzumab as treatment for breast cancer, the rate of heart failure incidence was 8.3%, against the 2.7% observed in patients not treated with trastuzumab. The risk was higher in patients treated with anthracyclines and trastuzumab, followed by patients receiving trastuzumab-based chemotherapy and then by patients receiving anthracyclines treatment. As expected, older patients exhibited increased risk of cardiotoxicity. These data are consistent with the fact that older patients presented comorbidities such as hypertension and valve disease. Radiation therapy, however, did not have any influence in the risk of heart failure [58].

The vascular endothelial growth factor (VEGF) receptor inhibitors (antibodies and kinase inhibitors) are anticancer drugs due to their anti-angiogenesis properties. However, cardiac side effects are known to be caused by these agents. They may also cause hypertension, endothelial dysfunction, and increased platelet aggregation. The use of bevacizumab in cancer therapeutics has been related with increased risk of congestive heart failure, but only in breast cancer patients. In a combination therapy including bevacizumab and taxanes, congestive heart failure risk is reported to increase significantly [54].

3.1. Cardioprotection during Cancer Therapy

Traditional cardiac risk factors include hypertension, dyslipidemia, smoking, and diabetes mellitus. Additionally, cumulative dose, age, radiotherapy of the left side of the chest, previous exposure to cardiotoxins, and co-administration of anthracyclines and trastuzumab or taxanes and bevacizumab are factors that may increase the risk of cancer treatment-associated cardiotoxicity [54,59].

There are multiple formulations which may mitigate toxic effects of anticancer therapy by altering the drug properties or by protecting cells against its cardiotoxic effects.

The use of liposomal formulations has been considered to improve drug targeting and to reduce toxic effects of doxorubicin, once inside a liposome. As a phospholipid bilayer vesicle, it carries doxorubicin presenting advantages in terms of immunogenicity and toxicity [60]. In a study with 509 metastatic breast cancer patients, who received either liposomal doxorubicin or conventional doxorubicin, investigators assessing LVEF concluded that progression-free survival rate was higher for patients taking liposomal doxorubicin (7.8 months) than for those taking conventional doxorubicin (6.9 months). Although the use of liposomal doxorubicin lead to less secondary effects, both showed similar efficacy [54,61].

Vitamin E has a cardioprotective effect due to its antioxidant properties [54,62]. However, in a human-based study with 13 patients on chemotherapy, no difference in cardiac protection was found between patients treated with and without vitamin E [54,63].

The use of BBs has been proposed to improve relapse-free and overall survival in patients being treated for multiple types of cancer [35]. The induction of endogenous beta agonists (such as catecholamines) is associated with mechanisms that trigger tumorigenesis, angiogenesis, and tumor metastasis. Those mechanisms include the activation of genes associated with metastasis and inflammation, activation of cell proliferation pathways and upregulation of pro-angiogenic factor and VEGF [12]. Considering that mediation via beta-2 receptor seems to be partly responsible for those mechanisms, a non-selective beta-1 and beta-2 receptor antagonist like propranolol should be a more promising potential anti-cancer agent than selective beta-1 receptor antagonists [64]. However, this assumption is dubious since a meta-analysis came to the conclusion that the use of propranolol did not cause any significant difference in cancer specific death rate, overall death rate or relapse-free survival rate between patients taking propranolol and those who did not [65].

Nevertheless, propranolol demonstrates high safety and good tolerability profiles, being recommended for first-line therapy in some CV diseases. Its first indication was for the treatment of angina but soon it was discovered that propranolol was also effective

when used for other CV conditions such as hypertension, myocardial infarction, and arrhythmias [66].

3.2. BBs and Breast Cancer

The number of successful breast cancer treatments has been increasing in the recent years, partially due to early detection of cancer, better treatment options and multidisciplinary healthcare teams [67]. The use of aromatase inhibitors such as anastrozole, letrozole and exemestane, for the treatment of post-menopausal women, and tamoxifen, for pre-menopausal women, is an available therapy [68]. Secondary effects of these drugs such as impairment of cognitive function—perception, planning, and memory—and psychomotor speed have been reported in some women also experiencing depressive symptoms [69–71].

The consumption of BBs during chemotherapy treatment helps improving relapse-free survival in breast cancer women, but not overall as seen in a populational study [72]. Also, breast cancer patients taking BBs before diagnosis presented a significant lower rate of metastasis [35,73–75].

A recent review of eight studies revealed that patients receiving treatment for breast cancer with an anthracycline with or without trastuzumab and using BBs presented a significant reduction in heart failure incidence when compared to those not using BBs, which supports the use of these medicines as cardioprotective in patients receiving cardiotoxic treatments [35,76].

A patient with stage III HER2-negative breast cancer type, treated with 1.5 mg/kg/day propranolol for 18 days and with the daily dose reduced over subsequent 7 days, after the treatment period, had the tumor removed surgically [77]. The tissue was collected for analysis to compare pre- and post-treatment tissues. Ki-67 (a pro-proliferative protein) expression decreased with treatment, an indication of tumor proliferation altered by propranolol administration. Bcl-2 (a pro-survival marker) expression decreased after propranolol administration whereas p53 protein (a pro-apoptotic protein) expression increased approximately 2.5-fold [77]. These findings were validated in a study with MDA-MB-231 breast cancer cell line exposed to propranolol (40 µM) and doxorubicin (3 µM) where propranolol reduced the rate of cells arrested in the G_2/M phase of the cell cycle, showing that cells died or were in the process of dying. These results support the hypothesis that BBs may have some antagonist action on breast cancer cell proliferation. Following 6-hour treatment with propranolol, p53 protein expression in MDA-MB-231 cells markedly increased. Thus, propranolol led to increased levels of cleaved initiator caspase 9 and execution caspases 3 and 6, so propranolol may lead to apoptosis of breast cancer cells. The investigators concluded that BBs may decrease breast tumors proliferation. However, additional studies are needed to fully understand the anticancer mechanisms underlying propranolol [77].

The potential of carvedilol to prevent cardiotoxicity during chemotherapy treatments was assessed in a trial test involving 1,122 patients aged 18 years or older [78]. The primary endpoint was a decrease of at least 10% in LVEF and the secondary the levels of troponin I (TnI) higher than 0.04 ng/mL. High TnI levels associated with LVEF reduction are indicative of cardiac events [78]. Patients were being treated for HER2-negative breast cancer and the treatment included doxorubicin, cyclophosphamide and paclitaxel.

Patients with previous heart failure symptoms, cardiomyopathy, coronary artery disease, mitral aortic disease, and chemo- or radiotherapy history; patients previously treated with angiotensin-converting enzyme inhibitors and BBs; and patients with contraindication to the use of BBs were excluded. Carvedilol and placebo were administered with the beginning dose of 3.125 mg, ascending to 6.25 mg, then 12.5 mg and the maximum dose of 25 mg, every 12 h until completion of chemotherapy. During the follow-up, 27 patients (14%) had a decrease of at least 10% in LVEF, 14 of those were receiving carvedilol, whereas the other 13 were in the placebo group. TnI levels increased in both groups from baseline until the end of the follow-up but its levels were attenuated in the carvedilol-treated group. The investigators concluded that there were no significant changes in LVEF between groups, however, the TnI elevation and further attenuation by carvedilol use suggests that carvedilol may have a protective role against myocardial injuries [79]. This protection is probably due to the antioxidant pharmacological properties and subsequent carvedilol protection against free radicals [78].

Using *in vitro* cell evaluation, the hypothesis that BBs reduce the proliferation rates of breast tumors when collected in the year prior to diagnosis was assessed. The expression of beta-1, beta-2, and beta-3 adrenergic receptors was measured in breast cancer tissue in contrast to normal breast tissue [80]. Tissues were collected from 404 breast cancer patients. Beta-1 and beta-3 receptors were significantly more expressed in breast cancer tissue than in normal breast tissue, however, there were no differences detected in beta-2 receptors expression. Cells collected from patients in stage I breast cancer who used BBs showed a significant decrease in Ki-67 compared to non-users. The same was observed for stage II breast cancer patients. Moreover, a significant decrease in tumor proliferation was observed in stage I breast cancer patients taking nonselective BBs, but the same was not found in stage II, III, or IV breast cancer patients. To corroborate these results, the investigators administered propranolol, a nonselective BB, to a HER-2 negative breast cancer patient [80]. Ki-67 index was evaluated pre- (through biopsy) and post- (after surgical resection) treatment with propranolol for 25 days (1.5 mg/kg/day). In the post-treatment period, Ki-67 was 23% lower than in the pre-treatment tissue. Therefore, the investigators concluded that propranolol may significantly decrease tumor proliferation. To fully understand the mechanism underlying propranolol, *in vitro* testing consisting in 24h exposure of SK-BR-3 cells to 18 µM propranolol (EC_{50} for the cell line) was performed [80]. Test results demonstrated decreased phosphorylation of multiple mitogenic activated protein kinases and cyclic adenosine monophosphate (cAMP) responsive element binding protein (CREB), and increased phosphorylation of protein kinase B (PKB), glycogen synthase kinase 3 (GSK3) and p53. These data suggested that propranolol lead to a decrease in cancer cell proliferation and an increase of cell stress [80].

3.3. BBs and Ovarian Cancer

Ovarian cancer is the eighth most common cancer in women and its survival rate after 5 years of diagnosis is only approximately 40% [81]. There is, thus, the need to improve ovarian cancer outcomes and to provide better treatment options. In this perspective, BBs could be considered for their action on the adrenergic system [8].

In a population-based study, 9420 patients being treated for ovarian cancer were administered BBs to test if there was an improvement in survival. Patients were given cardioselective BBs or nonselective BBs. During the follow-up (maximum 5 years), 2918

patients (47%) died, with 2051 deaths (70%) due to ovarian cancer. The use of BBs was associated with increased mortality. However, the decreased survival among the users of BBs may be explained by the fact that older patients, consequently having increased prevalence of CV diseases and other comorbidities, were mostly the ones taking BBs [9]. Even though previous studies have shown that BBs have no beneficial protective effect on patients under cancer treatment, the impact of nonselective BBs on survival was higher than cardioselective BBs [9,82,83]. The investigators did not consider confounding covariates such as body mass index, hypertension, CV comorbidities, or other comorbidities. This lack of information was a limitation in this study as CV comorbidities may be an explanation for the increased mortality rates of BBs users [9].

3.4. BBs and Pancreatic Cancer

Pancreatic cancer is, currently, the fourth cause of cancer-related death due to the lack of therapeutic strategies. Only a small percentage (5%) of patients with this type of cancer survive for 5 years, the lowest rate of survival among all cancer patients. Adenocarcinoma represents more than 85% of all pancreatic cancers and is the most lethal one [84].

The development of pancreatic cancer is associated with induction of the sympathetic nervous system, which leads to an increase in catecholamines stimulation. Several studies indicate that BBs, particularly the nonselective ones such as propranolol, may inhibit the damage induced by catecholamines stimulation of the adrenoreceptors in pancreatic cancer patients [85–91].

In a retrospective cohort, the association between BBs exposure and cancer-specific mortality was assessed. Patients recently diagnosed with pancreatic adenocarcinoma (2,394) were, during the 4 years of follow-up, given a certain type of BB in the generally prescribed dose for adults. During the follow-up, 91% of patients (2187) died (2054 of pancreatic cancer, 33 of CV disease, and 100 of other causes) and the median survival was 5.1 months for all the patients. Of the patients, 522 were treated with nonselective beta-blocker propranolol or cardioselective BBs metoprolol, atenolol, or bisoprolol and the rest of the patients with a combination of BBs formulas and alpha-1 blockers or other anti-hypertensive agents. BBs use, when compared with nonuse, was associated with an overall reduced cancer-specific mortality rate. Due to poor use of nonselective BB propranolol, authors listed no significant differences between nonselective and cardioselective BBs use. Authors concluded that BBs may inhibit progression of pancreatic adenocarcinoma and may be a complement for current therapies in order to prevent cell damage in pancreatic cancer patients [91]. In this study, other medications used by patients, such as other antihypertensive drugs, antidepressants, anxiolytics, antipsychotics, aspirin, nonsteroidal anti-inflammatories, statins, digoxin, metformin, insulin, and other hypoglycemic agents were considered. Blockers of angiotensin II receptors, serotonin and norepinephrine reuptake inhibitors, diuretics, nonsteroidal anti-inflammatory drugs and antipsychotics were associated with increased rate of cancer-specific mortality, whereas the use of anxiolytics was associated with a statistically significant reduced rate of cancer-specific mortality. Since anxiolytics (such as benzodiazepines) act by reducing norepinephrine release through potentiation of gamma-aminobutyric acid (GABA) at the GABA receptors, it is assumed that their influence in the central nervous system may be related with less damage caused by catecholamines,

leading to a decreased rate of cancer-specific mortality, following the reduced rate of cancer-specific mortality seen in those individuals [92–94].

3.5. BBs and Liver Cancer

Liver cancer accounts for the second deadliest mortality rate caused by malignant cancers in men. Liver cancer develops quickly but it is asymptomatic [95].

Propranolol has been administered to patients with liver cancer and liver cirrhosis as a prevention for esophageal and gastric variceal hemorrhage. Variceal bleeding is one of the major causes of death in cirrhotic patients. In cirrhosis, portal pressure initially increases as a consequence of resistance to blood flow within the liver. This resistance is due mainly to fibrous tissue and regenerative nodules in the hepatic parenchyma. Therefore, propranolol should be able to reduce portal vein pressure, exhibiting an anticancer effect [96].

In a cell-based study (with HepG2 and HepG2.2.15 liver cancer cells and HL-7702 normal human liver cells), it was demonstrated that propranolol (80 μM) inhibits the proliferation of liver cancer cells (HepG2 and HepG2.2.15) but not normal cells (HL-7702). At the same time point, progressive propranolol concentrations lead to a pronounced inhibitory effect of liver cancer cell proliferation. Treatment with 160 μM resulted in shrinkage of cell lines HepG2 and HepG2.2.15 and 320 μM propranolol resulted in shrinkage of all three cell lines. Both beta-1 and beta-2 receptors were expressed on the membrane of the three cell lines, but expression was higher on HepG2 and HepG2.2.15 cells compared with HL-7702 cells. When treated with propranolol, both beta-1 and beta-2 receptors showed decreased expression on HepG2 and HepG2.2.15 cells. Therefore, the investigators concluded that, although beta-adrenergic receptors are more highly expressed in liver cancer cells than in normal liver cells, propranolol reduced their expression, inhibited proliferation, and induced apoptosis in liver cancer cells [97].

4. Conclusions

The use of propranolol in breast cancer patients may have promising effects based on its ability to reduce the proliferation of cancer cells and its potential ability to cause apoptosis in those cells. Studies have shown that nonselective BBs, for their action of both beta-1 and beta-2 adrenergic receptors, are able to reduce Ki-67 expression, an indication of decreased proliferation rate. Increased relapse-free survival and decreased rate metastasis and heart failure incidence were also found in the breast cancer patients evaluated.

Regarding pancreatic cancer, the use of BBs was associated with a reduction of cancer-specific mortality rate. However, no differences were found between patients treated with cardioselective and nonselective BBs.

The literature review performed in this study suggests that propranolol may be used as a complement for the treatment of several types of cancer, due to its ability to improve treatment outcomes, decreasing the proliferation rates of cancer cells, although this information needs to be clarified with further studies with well-defined target groups and placebo controlled conditions. A first approach may involve *in vitro* studies designed to provide responses to the potential mechanistic action of these drugs and their interaction with other pharmaceuticals, on different types of cancer cells.

Considering that the conventional chemotherapy affects cancer cells but may also attack healthy cells, smart nanocarrier-based drug delivery systems, consisting of a controlled drug release (e.g., by liposomes), which direct the drug release to the specific cancer site [98] are being developed. As phospholipid-based nanocarriers, liposomes improve pharmacokinetics, biodistribution, solubility, and stability and control release and site-specific delivery of the anti-cancer drugs, causing less side effects than conventional systems [99]. Therefore, nanocarriers could be studied in order to understand if their use decreases cardiac toxic effects related to anticancer chemotherapy.

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Chapter III

Propranolol and Aquatic Environment: Levels and Known Effects

Propranolol and Aquatic Environment: Levels and Known Effects

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Propranolol and aquatic environment: Levels and known effects

Abstract

After the use of pharmaceuticals, the parent compounds or their metabolites are excreted and released into the sewage systems. Industrial, medical facilities, and domestic effluents are the primary sources that lead to the presence of pharmaceuticals in the aquatic environment. Sewage system treatments are still ineffective in the removal of most of these biologically active substances, which leads to their persistence in aquatic ecosystems and results in continuous exposure of aquatic organisms.

Beta-blockers, which are used worldwide to regulate blood pressure, heart rate and airway strength or reactivity, have also been considered for cancer treatment. These class of pharmaceuticals has a low removal rate in wastewater treatments plants, reaching the environment in amounts that may cause sub-lethal effects in non-target organisms. Propranolol, for example, has been reported to affect organisms from microalgae to fish. Available data demonstrates that propranolol affects molecular, biochemical, and physiological processes that may result in behavior, reproduction, developmental alterations.

The collected information demonstrates the importance to perform long-term studies, as organisms are exposed in the environment to propranolol and other substances at concentrations that may compromise the survival of the organisms and populations.

Keywords: Propranolol; Beta-Blockers; Non-target organisms; Effects; Levels

1. Occurrence of Pharmaceuticals in the environment

1.1. Emission pathways and occurrence

Pharmaceutical compounds are being released daily into the aquatic environment, either as the parent compound (not metabolized form) or as metabolites (active or inactive), directly and indirectly. The sewage system (industrial, medical facilities and domestic facilities) is the primary route of entry of pharmaceuticals into the environment, as parent compound (non-metabolized form) or as metabolites (active or inactive) (Oliveira *et al*., 2015). These compounds may survive the passage through wastewater treatment plants (WWTP), which are responsible for the collection and accumulation of drugs discharged into wastewater (Li *et al.*, 2020). The fractions in solution may be disposed of as effluents into water bodies or reused for irrigation in areas with high pressure of water resources, whereas fractions in the sludge may reach agricultural fields as a result of sludge use to improve soil properties due to its richness in organic matter or disposed of to landfills (Oliveira *et al.*, 2015). Other relevant sources, such as manure associated to animal production, have been identified (Li *et al.*, 2019).

Nowadays, in Europe, approximately 5,000 pharmaceuticals are in use and, in the United States, the number increases to 10,000 (Li *et al.*, 2020). As a result of continuous high consumption and ineffective wastewater treatment systems, these substances are being increasingly detected in the environment, despite the short half-life of most pharmaceuticals, leading to continuous exposure of aquatic organisms to these biologically active substances and, resulting in potential biological impacts (Fent *et al*.,

2006). Currently, analgesics, antidepressants and anti-hypertensives are at the top of the most detected pharmaceuticals in the environment (aus der Beek *et al*., 2016).

Pharmaceuticals are frequently found in sewage sludge, resulting from the treatment of domestic, industrial and hospital wastewater. Due to its characteristics of fertilizer and low economic impact, sewage sludge is usually applied in agricultural and forestry activities which, along with the use of effluents for irrigation in areas with high pressure on water resources, significantly contributes to the contamination of agricultural soils, groundwater and crops, which later reaches humans via consumption water and the food chain (Xu *et al*., 2019) and other environmental matrices (Pérez-Lemus *et al*., 2019). In consequence, sewage irrigation will bring contamination to the groundwater and crops, which will later be transferred to humans via the food chain (Xu *et al*., 2019).

1.2. Fate in the environment and risks for organisms, ecosystems and human health

Once in the aquatic environment, drugs may remain in the water column or undergo mineralization processes (Nunes *et al.*, 2019). In the water column, drugs may be degraded as a result of biotic factors (e.g. action of microorganisms) and abiotic factors (e.g. photodegradation by sunlight or ultraviolet radiation) (Khaleel *et al*., 2019). The incomplete mineralization of pharmaceuticals results in the formation of new products, which may have completely different properties from the original pharmaceutical and thus, completely different environmental fate, bioavailability and biological effects (Khaleel *et al*., 2019). Considering that pharmaceuticals are used for specific pharmacological and physiological functions in a target organism, its presence in the environment is currently recognized as an emerging topic of concern (Oliveira *et al*., 2015) due to the potential consequences to non-target organisms and humans, despite their design to act on specific metabolic pathways. The potential targets of the presence of pharmaceuticals range from bacteria, microalgae, invertebrates to fish (Li *et al*., 2019). As biologically active substances, pharmaceuticals may impact resident species, even at low concentrations. Reported effects include alterations in growth, metabolism, reproduction and behavior (Duarte *et al*., 2020; E. Fabbri & Franzellitti, 2016).

Effects on aquatic wildlife have been reported at various levels, including cytotoxicity, neurotoxicity, oxidative stress and individual behavior alternation (H. Yang *et al.*, 2020). Some of the effects already described include the spawning induction of mussels, by the antidepressant fluoxetine, and feminization of fishes induced by 17α-ethinylestradiol, a substance present in birth control pills (Wang *et al*., 2019). Early larval development of the Mediterranean mussel *Mytilus galloprovincialis* has been shown to be affected by several pharmaceuticals with different therapeutic effects (Balbi *et al.*, 2016; Estévez-Calvar *et al*., 2017; R. Fabbri *et al.*, 2014). Altered levels of mRNA from genes involved in relevant physiological functions, like oxidative status, immune function, lipid metabolism and stress responses, have been reported in gilthead seabream (*Sparus aurata*) exposed to drugs as gemfibrozil, a lipid regulator (Teles *et al*., 2016). Fish behavior, specifically its self-preservation instinct, has been reported to be affected by drugs like benzodiazepines (Mackuľak *et al*., 2019), associated with altered levels of neurotransmitters in the brain (Mackuľak *et al*., 2019). These effects may be enhanced as a result of bioaccumulation and biomagnification events frequently reported also for other

contaminants. For example, carbamazepine has been reported to ascend through aquatic food chains (Ding *et al.*, 2015).

The presence of pharmaceuticals in the aquatic environment, even at the reportedly low concentrations of ng/L and µg/L range, may cause effects on human health (Nielsen & Roslev, 2018), through different pathways. Through aquatic organisms used as a source of human food (e.g. fish and shellfish), drugs may reach humans and, therefore, pose a threat to human health. The potential effects may be associated with endocrine disruption, which may lead to infertility, abnormal prenatal and childhood development (Cao *et al.*, 2020; Praveena *et al*., 2019). However, exposure to drugs present in the environment may impact human health. A wide variety of antibiotics – macrolides, quinolones, sulfonamides and tetracyclines that can be found in the environment and human food sources of aquatic origin such as fish and shellfish may promote antimicrobial resistance, a major problem worldwide (Xie *et al*., 2019). Although not occurring in the environment at levels as high as other classes, anticancer substances like capecitabine, imatinib, fluorouracil and cyclophosphamide that are used to kill tumor cells, but lack selectivity leading to the damage in both tumor and healthy cells, may cause several pernicious effects (e.g. cytotoxic, genotoxic, mutagenic and teratogenic effects), even at low concentrations (Cristóvão *et al.*, 2020).

Diclofenac, a non-steroidal anti-inflammatory drug, is one of the most prescribed drugs, used to treat inflammation and relieve pain (Duarte *et al*., 2020). Diclofenac was listed in the Watch List of Commission Implementing Decision 2015/495, which establishes the substances which require environmental monitoring (Sathishkumar *et al*., 2020). Diclofenac has been the target of many ecotoxicological studies in order to fully understand the potential risks of aquatic organisms.

Beta-blockers are a class of therapeutic agents highly prescribed for the cardiovascular system but with different studies suggesting potential application for the treatment of several types of cancers (Peixoto *et al*., 2020). Thus, levels of these drugs in the environment may be expected to increase, which may have consequences to aquatic biota (Khan *et al*., 2018). As a result of increasing concern on the occurrence of beta-blockers in the aquatic environment, the study of their potential effects on non-target organisms has been emerging.

2. Beta-Blockers – Propranolol

2.1. Characteristics

Beta-blockers are antagonists of beta-adrenergic receptors, being involved in the control of physiological processes, such as blood pressure, heart rate and airway strength or reactivity (Oliver *et al*., 2019). Beta-blockers compete with catecholamines for the binding site on beta-adrenergic receptors, exhibiting greater affinity for beta-1 adrenergic receptor, beta-2 adrenergic receptor or equal affinity to both receptor subtypes (Do Vale *et al*., 2019).

Propranolol, the first invented beta-blocker, and the one with the most collected experience and clinical indications, is considered a non-selective beta-blocker due to its antagonism in beta-1 and beta-2 adrenergic receptors (Peixoto *et al*., 2020). In addition to being administered in the treatment of cardiovascular diseases, such as high blood pressure, coronary artery disease and arrhythmias, propranolol is also prescribed as an anxiolytic. This pharmaceutical may also act as a selective serotonin reuptake inhibitor

(SSRI), due to its antagonistic action on serotonin reuptake receptors (Do Vale *et al*., 2019; Nielsen & Roslev, 2018). Furthermore, propranolol may also be used as a complement to anticancer therapy in the breast, ovarian, pancreatic and liver cancers, among other functions (Peixoto *et al*., 2020). As a result of its high consumption, it resulted in its environmental release and, currently, propranolol is frequently detected in water bodies (Nielsen & Roslev, 2018).

2.2. Metabolism

Highly lipophilic, propranolol is completely absorbed when administered orally. However, only 25% of the drug reaches the systemic circulation due to liver removal (Srinivasan, 2019). Propranolol is highly metabolized by the liver, mainly by cytochrome P450 isoenzymes 1A2 and 2D6 (Kiriyama *et al*., 2008) and is excreted by humans in more than 80% via urinary metabolites (Di Lorenzo, Di Cicco, *et al*., 2019; Godoy *et al*., 2015). According to its pharmacokinetics, 17% undergoes glucuronidation, 41% side-chain oxidation and 42% ring oxidation. One of the primary transformation products is 4-OH-propranolol – a parent compound with potency equal to propranolol, but shorter half-life due to hydroxylation by cytochrome P450 isoenzyme 2D6 (Brown & Wong, 2016). The plasma half-life of propranolol is 3-6 hours, although long-acting preparations reach a half-life of 8-11 hours (Srinivasan, 2019).

2.3. Occurrence in environmental compartments and biota

2.3.1. Environmental compartments

Approximately 20% of propranolol is removed by WWTPs, leaving a great amount of free substance in the aquatic systems (Di Lorenzo, Di Cicco, *et al*., 2019). The continuous release of propranolol may result in the permanent exposure of non-target organisms (Duarte *et al*., 2020). Furthermore, propranolol has shown potential for bioaccumulation and bioconcentration in the aquatic biota (Nielsen & Roslev, 2018). Reported levels of propranolol in aquatic systems vary according to the water body and the location (Table 1), with the highest reported level in surface waters in the range of ng/L.

Table 1. Reported levels of propranolol in distinct environmental compartments

Surface water		
Location	**Concentration (ng/L)**	**Reference**
France	12	(Vulliet & Cren-Olivé, 2011)
Lagos State, Nigeria	≤12	(Ebele, Oluseyi, Drage, Harrad, & Abou-Elwafa Abdallah, 2020)
Valencia, Spain (Pego-Oliva marsh)	≤16.6	(Vazquez-Roig, Andreu, Blasco, & Picó, 2012)
Tianjin, China	0.25	(Xu, Sun, Zhang, & Alder, 2019)
Germany	3.5	
Groundwater		
South Korea (rural agricultural groundwater)	≤0.098	(Lee *et al*., 2019)
Barcelona (center of the city)	6.11 – 17.9	(López-Serna *et al*., 2013)
France	18	(Vulliet & Cren-Olivé, 2011)

Lagos State, Nigeria	≤1	(Ebele *et al.*, 2020)
Drinking Water		
France	≤2	(Vulliet & Cren-Olivé, 2011)
Lagos State, Nigeria	≤1	(Ebele *et al*., 2020)
Seawater		
Belgium (North Sea)	24	(Mezzelani, Gorbi, & Regoli, 2018)
China (Jiulong River estuary)	0.8	
Spain (Atlantic Ocean)	≤5.9	
Portugal (Douro River estuary)	3.2	
United Kingdom (Tyne estuary)	110	
United Kingdom (estuaries)	56	
China (Jiaozhou Bay)	≤0.03	(Peng *et al*., 2019)
European countries (rivers)	≤590	(Di Lorenzo, Castaño-Sánchez, *et al*., 2019)
Barcelona (rivers)	0.4 – 18.3	
Sweden (North Sea)	1.0 – 24	(E. Fabbri & Moon, 2016)
Germany (rivers and streams)	≤590	
South Korea (Mankyung)	≤40.1	
Wastewater		
United States (effluents)	1900	(Di Lorenzo, Castaño-Sánchez, *et al.*, 2019)
Canada (influent)	9.0 – 1650	(MacLeod, Sudhir, & Wong, 2007)
Canada (effluent)	8.0 – 987	
Germany (effluent)	25 – 730	
Tianjin, China (hospital wastewater)	110 – 158	(Xu *et al*., 2019)
Swedish (influent)	50 – 180	
Swedish (effluent)	32 – 172	
France (influent)	266	
France (effluent)	203	
Portugal (influent)	0.21	
Portugal (effluent)	0.17	
Sediments		
Location	**Concentration (ng/g)**	**Reference**
Valencia, Spain (Pego-Oliva marsh)	≤2.1	(Vazquez-Roig *et al.*, 2012)
Not defined	≤0.9	(Franzellitti *et al*., 2019)
Soil		
Valencia, Spain (Pego-Oliva marsh)	≤0.4	(Vazquez-Roig *et al*., 2012)

Propranolol is one of the drugs with the greater persistence in soils, higher than others such as carbamazepine, fluoxetine and diclofenac (Carter *et al*., 2014) that have already been reported as capable of impacting soil organisms (M. Oliveira *et al*., 2018).

For example, propranolol is able to accumulate in radish leaf and bulb (Carter *et al*., 2014). However, the levels detected in these products have not been considered dangerous for human consumption (Carter *et al*., 2014).

Exposure to pharmaceuticals, including propranolol, is already documented for several species like duckweed, fish, frogs and vultures (Wöhler *et al.*, 2020).

2.3.2. Interaction with aquatic biota

Propranolol, among other beta-blockers, has been reported to inhibit the efficiency of photosynthesis in the green algae *Desmodesmus subspicatus*, with a 24h EC_{50} (the concentration that gives half-maximal response) of 4.1 mg/L (Y. Yang *et al*., 2019). In terms of effects on algae growth, a 24h EC_{50} of 24 mg/L has been reported for green algae *Scenedesmus vacuolatus* (Ding *et al*., 2015).

Propranolol has been pointed by the scientific community as a potential modifier of different processes, such as growth, metabolism, reproduction and behavior on several aquatic organisms (e.g. crustaceans, oyster, mussels, fish) (Duarte *et al*., 2020). The concentrations of propranolol reported in the environment, in the ng/L range, are not expected to be lethal to most aquatic invertebrates. In a study with *Daphnia magna,* a planktonic crustacean species widely used as model organism in ecotoxicological tests due to its sensitivity to water contamination by many pollutants and its role in an aquatic food chain (Sivula *et al.*, 2018), 1000 and 10,000 µg/L propranolol led to death of the animals after the first 24h of exposure, whereas 0.1 and 1 µg/L resulted in the onset death only after 48h incubation (Nielsen & Roslev, 2018). Sublethal effects have been reported at low exposure concentrations, in the range of ng/L or µg/L (Nielsen & Roslev, 2018). The concentrations of 0.1 and 1 µg/L propranolol decreased the swimming distance, and 1000 and 10,000 µg/L caused a significant inhibition of swimming. A 48h EC_{50} of 2.76 and 4.20 mg/L was estimated for behavior tests – swimming distance and swimming time, respectively (Nielsen & Roslev, 2018), which is much higher than the concentration of propranolol usually found in freshwater (≤0.59 µg/L) (Ebele *et al*., 2017). The EC_{50} assessed in this study shows similarity to previous studies for acute toxicity tests of propranolol (L. L. D. de Oliveira *et al*., 2016; Godoy *et al*., 2015; Varano *et al.*, 2017), which also tested different propranolol concentrations in *D. magna* and observed differences in terms of mobilization. A 72h starvation-survival test allowed the estimation of 0.79 mg/L as the EC_{50} for this parameter (Nielsen & Roslev, 2018). This value is also in the same range as other results by Varano *et al*. (2017), which reported EC_{50} of 0.59 and 0.88 mg/L for both trials performed in *D. magna* (Varano *et al.*, 2017). Starvation-survival tests exhibit the ability of organisms to survive in the absence of food, and extended starvation is expected to exacerbate the effects of propranolol exposure (Nielsen & Roslev, 2018). Organisms like bivalves, which have high filtration rates and tend to bioaccumulate xenobiotics (Khan *et al*., 2018) may be more prone to toxic effects. Environmentally relevant concentrations of propranolol (0.5 and 1 µg/L) have been reported able to affect the number of eggs released by Japanese medaka (*Oryzias latipes*), after 4 weeks exposure (Xu *et al*., 2019).

Cyclopoids, of the order of copepods, are omnivorous organisms that feed on organic detritus, microbes, protists, fungi and algae (Suárez-Morales, 2015) and are widely distributed in freshwater ecosystems. Data obtained by Di Lorenzo *et al*. (2019), which assessed the acute effects of propranolol on the freshwater cyclopoid *Diacyclops*

crassicaudis crassicaudis, allowed an estimation of a 96h LC_{50} (concentration lethal for 50% of the study population) of 27.0 mg/L, which led to the assumption, by the authors, that propranolol poses a risk for the survival of this species (Di Lorenzo, Castaño-Sánchez, *et al*., 2019).

The potential effects of propranolol on early larval development of *Mytilus galloprovincialis* were assessed in a concentration range of 0.01 - 1000 µg/L by Franzellitti *et al*. (2019) through the standardized 48-h post-fertilization embryotoxicity assay. Increasing concentrations of propranolol led to decreased percentages of normally developed embryos. The 48h EC_{50} obtained was 1.34 ± 0.43 µg/L with effects detected from 10 up to 1000 µg/L. The proportion of viable embryos at 48h post-fertilization was significantly reduced by 10 – 1000 µg/L (Franzellitti *et al*., 2019). This study supports the idea that, in the range of concentrations found in the aquatic environment, propranolol can cause effects in marine populations, leading to progressive development arrest or mortality of species, especially at the higher concentrations. These effects might be explained by the antagonism of adrenergic receptors, which impair the physiological function of catecholamines in the development of mussel embryo (J.-L. Yang *et al*., 2014).

Effects of propranolol in the development of sea urchins have been observed. A developmental arrest was observed in urchin embryos, following exposure to 5 µg/L propranolol (lowest observed effect concentration [LOEC]) (Ribeiro *et al.*, 2015).

The neotropical fish species *Phalloceros harpagos,* highly representative of tropical freshwater ecosystems (Matus *et al*., 2018) has also been reported to be sensitive to propranolol exposure. After a 96h exposure, animals exposed to 0.001 mg/L were less agitated than controls and those exposed to 0.0001 mg/L presented a decrease of liver glycogen store. No hepatotoxicity was found in fish exposed to 0.0001, 0.001, 0.01, 0.1 and 1.0 mg/L propranolol (Matus *et al*., 2018).

Similar effects in terms of reduced anxiety have also been observed in juvenile fish of *Danio rerio,* a well-established biological model fish species, after acute exposure to 0.0006, 60 and 20 mg/L propranolol. Estimated LOEC was 125 µg/L for zebrafish development (Mitchell & Moon, 2016).

The consequences of long-term exposure of *Argyrosomus regius*, a top predator fish species, to propranolol, were assessed after 30 days exposure to two different concentrations of propranolol considered environmentally relevant: 0.3 and 15 µg/L (Duarte *et al*., 2020). The study showed no significant morphometric changes, in terms of length and weight, but reported bioconcentration of propranolol in fish muscle, for both concentrations tested (Duarte *et al*., 2020). However, its bioconcentration in the muscle of fish is diminished, probably due to the ability of fish to rapidly and efficiently metabolize propranolol, as *observed in vitro* (Baron *et al*., 2017; Connors *et al.*, 2013; Gomez *et al*., 2010). At the sub-individual level, other parameters were evaluated. Different fish tissues were dissected – liver, brain, muscle, and heart. No significant effects on antioxidant enzymes catalase and superoxide dismutase (SOD), the activity of biotransformation enzymes ethoxyresorufin O-deethylase (EROD) and glutathione-*S*-transferases (GST), lipid peroxidation (LPO) and DNA damage were found in liver. Similarly, there were found no significant differences in acetylcholinesterase activity in the brain. Significant differences from control were only found in terms of DNA damage in fish muscle (Duarte *et al*., 2020). These results are in agreement with others previously published, at which no differences were found in LPO levels in crucian carp (*Carassius auratus*) (Ding *et al*.,

2016) and no significant differences were observed in terms of cholinesterase activity in freshwater fish *Phalloceros harpagos* (Pereira *et al*., 2018).

Overall, propranolol has been shown to affect aquatic organisms. The degree of effects, as well as other environmental contaminants, is associated with length, concentration, type of exposure and species. The results so far demonstrate the ability of this drug to affect molecular, biochemical, and physiological endpoints that may culminate in behavioral alterations. Propranolol plays a role in the mobilizing hepatic glycogen, regulating important processes for energy storage, such as food intake and blood glucose (E. Fabbri & Moon, 2016). However, based on the available study, propranolol does not appear to induce lethal effects at environmentally relevant concentrations.

3. Concluding remarks

The available data suggest that propranolol may be pernicious to aquatic organisms. The fact that new applications of propranolol lead to increased environmental levels raises concerns associated with the presence of this pharmaceutical in the environment. Furthermore, there is an array of pharmaceuticals in the environment, a considerable part of which share toxicological properties with propranolol and, thus, with a high probability of inducing additive or synergistic effects in simultaneous exposures. Although some of the reported effects of propranolol were induced by concentrations that are not environmentally relevant, effects like behavior, that may compromise the survival of the organisms and populations, have been detected in low and environmentally relevant concentrations. The analysis of the available literature makes clear that there is a need to perform long-term studies, as organisms are exposed in the environment to these substances throughout their lives and different generations.

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Chapter IV

General Discussion and Future Perspectives

General Discussion and Future Perspectives

Considering that each chapter includes a specific discussion of its content, this general discussion is intended to be a critical analysis of the whole dissertation, bearing in mind the guidelines initially defined.

The overall goal of the use of pharmaceuticals in humans is to improve human health and promote well-being. Due to our current lifestyle and increased life-expectancy, often associated with an increased number of diseases, pharmaceutical products are an integral part of our daily routines. A considerable number of active substances are currently available in the market, and intense research is focused on finding more effective treatments for diseases like cancer. Used for specific pharmacological and physiological functions in a target organism, and expected to be persistent enough to reach the target site before becoming inactive, the presence of these pharmaceuticals in the environment is a problem of concern once these compounds reach the environment, as the wastewater treatment plants are often ineffective in their removal. Thus, the use of pharmaceuticals should take into account the desired effects, side effects and potential effects on the environment.

Among the diseases, most aggressive and lethal to humans is cancer. A variety of treatments are being tested to treat cancer patients more efficiently, often using a combination of drugs. The pharmaceuticals most commonly used in cancer treatments lack specificity towards cancer cells, affecting also normal cells.

Beta-Blockers (BBs), a group of molecules of different pharmacokinetic and pharmacodynamic properties, have been considered in the treatment of different pathologies like hypertension, cancer, and migraine, suggesting a protective effect that may span far beyond the cardiovascular (CV) system (Fumagalli *et al*., 2020; Knight *et al.*, 2018). The available studies, reviewed in chapter II, show that BBs are a promising complement for chemotherapy. These pharmaceuticals have shown the ability to decrease the metastasis rates and heart failure incidence during anthracycline and trastuzumab therapy for breast cancer (Choy *et al*., 2016; Gujral *et al*., 2018). Carvedilol seems to play a protective role against myocardial injuries (Avila *et al.*, 2018). Nonselective BBs have shown to be able in decreasing tumour proliferation in stage I of breast cancer (Montoya *et al*., 2017). Propranolol induces apoptosis of breast cancer cells by increasing levels of initiator and execution caspases and reducing cell-cycle arrest (Montoya *et al*., 2019). When compared to cardioselective BBs, nonselective BBs increase survival in ovarian cancer patients. In pancreatic cancer patients, BBs reduce cancer-specific mortality rate and prevent damage to healthy cells (Udumyan *et al*., 2017). As observed for breast cancer, propranolol showed an important role also in the treatment of liver cancer, as it is able to inhibit the proliferation of cancer cells and induce apoptosis without affecting normal cells (Wang *et al*., 2018).

Overall, propranolol, that acts on both beta-adrenergic receptors is the most studied beta-blocker in terms of cancer treatments, having been used in different types of cancer. This pharmaceutical is useful in cancer treatments, probably due to the association of beta-2 receptor with mechanisms that lead to metastasis, inflammation, activation of cell proliferation pathways and upregulation of pro-angiogenic factor and VEGF (Guimaraes & Moura, 2001). However, little information about the exactly anticancer mechanism of action underlying propranolol and other beta-blockers is

available, which could be obtained by *in vitro* studies with different types of cancer cells. This is one of the research approaches that could considerably improve the current knowledge of the mechanisms associated with BBs effects on cancer cells. This approach could be valuable to address interactions with other chemotherapy agents and other classes of pharmaceuticals, as cancer patients are often being treated for other illnesses.

Considering the lack of selectivity in conventional chemotherapy, healthy cells may also be attacked. Improvements in the application of treatments may be applied. Targeted therapies, such as monoclonal antibodies or nanocarriers, could be considered for their specificity to act on particular targets, as the case of cancer cells. Monoclonal antibodies are targeted molecular therapy that have favorable tolerability profiles and reduced secondary effects. They are beneficial for their high specificity and affinity for the specific antigen exposed in cancer cells and, therefore, they do not affect healthy cells (Carvalho *et al*., 2016). The improvement in nanotechnology can also be used for a more efficient treatment of several diseases like cancer. Smart nanocarrier-based drug delivery systems are applied for their controlled drug release. These molecules direct the drug release to the specific cancer site and, therefore, cause fewer side effects than conventional anticancer drugs (Din *et al.*, 2017; Hossen *et al*., 2019).

As observed for other substances, the increased consumption of beta-blockers like propranolol will promote its increased environmental levels, with potential pernicious effects for different species. The available studies presented in Chapter III show that an increase in the environmental levels of propranolol may have serious environmental consequences, as suggested by the reported sub-lethal effects to non-target organisms, namely on growth, metabolism, behavior, reproduction and development (Duarte *et al*., 2020; Franzellitti *et al*., 2019). Additionally, the ability of propranolol to bioconcentrate in fish muscle and bivalves may be dangerous for humans through diet. The potential effects to humans may be associated with endocrine disruption, which may lead to infertility, abnormal prenatal and childhood development (Cao *et al*., 2020; Praveena *et al*., 2019). The review of the available literature regarding the effects of propranolol on aquatic organisms show that there are considerable knowledge gaps on effects at environmentally relevant concentrations. Furthermore, studies with long-term exposures, more representative of the conditions to which animals are exposed in the environment should be performed, as the available literature only refers to effects occurring from acute exposures. Given the wide array of pharmaceuticals in aquatic ecosystems, additional studies should also address the effects of mixtures of drugs, including propranolol, as a considerable part of them shares toxicological properties with propranolol, increasing the possibility of additive or synergistic effects.

As a final remark, it should be highlighted that *in vitro* assays, to study the effects of propranolol on different human and fish cell lines, were planned to be performed in this dissertation. These assays would allow the study of the mechanisms of action of propranolol in different cell lines and establish toxicity thresholds. These were not performed due to the COVID-19 pandemic event, which lead to the lockdown of the university labs, forcing an alteration of the structure of the dissertation to a critical literature review. The review showed that the originally planned dissertation would provide needed information to the scientific community and thus, should be considered in as future work.

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