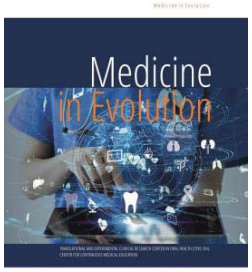
A detailed study on the antitumor effects of consecrated drugs - digoxin and labetalol



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Abstract

Cardiotonic glycosides and beta-blockers are drug classes intensely known for their benefits in cardiovascular diseases, having therapeutic utility in certain conditions and in pregnant women. Due to their established actions, in recent years attention has been directed towards the antitumor effect of cardiotonic glycosides and non-selective beta-blockers. Thus, the aim of the present study was to highlight the anticancer activity of digoxin and labetalol, both in vitro and in vivo, to continue evaluating their effects and to study in more detail their mechanisms of antitumor action. Analyzing the data, it can be say that digoxin, but also non-selective beta-blockers, including labetalol, are promising anticancer agents.

Keywords: cardiotonic glycosides, digoxin, beta-blockers, labetalol, anticancer effect

INTRODUCTION

Digoxin is part of the class of cardiotonic glycosides derived from Digitalis plant species. It is one of the most used drugs in therapeutic practice, being known for its beneficial effects in heart diseases such as heart failure and cardiac arrhythmias. The mechanism of action consists in its positive inotropic effect (increases the contractility of the myocardium), the increase of blood volume and blood pressure and, in addition, the reduction of heart rate [1]. The positive cardiac inotropic effect is exerted by inhibiting the Na+/K+ ATPase pump, resulting in an increase intracellular concentration of calcium ions [2]. According to the classification of the risks of pharmaceutical preparations, stipulated by the FDA (Food and Drug Administration), digoxin is part of risk category C. Digoxin can be administered during pregnancy, if the potential benefits justify the potential risks. Cardiomyopathy in pregnancy can be catastrophic for the mother's health, accounting for up to 11% of maternal deaths. Therefore, the cardiotonic glycoside can be administered to pregnant women who have persistent symptoms of heart failure, despite the treatment instituted with beta-blockers or other cardiovascular drugs. During pregnancy, digoxin can also be used to treat maternal tachycardia, an arrhythmia with rapid ventricular response [3]. Digoxin is a substance that easily crosses the placenta, but in normal doses, it has a minimal negative effect on the child.

Another category of drugs analyzed in terms of administration among pregnant women with cardiomyopathy is that of beta-blockers, synthetic drugs, included in risk class C. Beta-blockers are considered safe drugs during pregnancy; however, some studies suggest that they may limit intrauterine growth. Selective beta-1 compounds are preferred to be used, such as metoprolol, compared to non-selective ones that can stimulate uterine contractions. However, labetalol, a non-selective beta-blocker (alpha and beta-blocker), is routinely administered to pregnant women for the treatment of hypertension and cardiomyopathy, preserving uteroplacental blood flow. Although it has a favorable safety profile, labetalol induced several adverse effects in pregnant women, such as: bradycardia, arterial hypotension, or maternal hepatotoxicity [4,5]. After birth, newborns of mothers who have treated with beta-blockers must be monitored for up to 3 days to evaluate the potential adverse effects that may occur [6].

Recently, attention has focused on the potential anticancer effects of existing drugs in therapy for the treatment of various pathologies. Specific cardiovascular medication has demonstrated its effectiveness in heart diseases even in certain physiological states, such as pregnancy, but today it is desired to study in detail their toxic effects and especially their anticancer properties through in vitro and in vivo studies.

Cancer is considered a major health problem that affects the entire population of the world. In the last decades, numerous studies have been conducted to establish the mechanisms of carcinogenicity, but especially to identify the antitumor potential of natural compounds [7].

Standard cancer treatments consist of chemotherapy, radiotherapy, and surgery. The basic goal of anticancer therapy is to kill the tumor cell without affecting the healthy cell, but in the case of standard therapy, this is not fully achieved, as the healthy cells are also affected and numerous adverse effects occur such as anemia, peripheral neuropathy, and loss of appetite [8]. Starting from these inconveniences, it was desired to develop a treatment as effective as possible, with targeted action and with reduced side effects [9]. Thus, the study of natural compounds in the treatment of carcinomas began; observing that they are better tolerated, with few adverse reactions, and can be administered even in particular physiological situations, such as pregnancy. An example of an intensively studied natural compound is betulinic acid, a pentacyclic triterpene, which exhibits numerous biological

activities, including antitumor effects against several types of cancer cells. In addition, several conventional antitumor agents are derived from natural sources such as Taxol, vinca alkaloids [10]. Studies in recent years have suggested that cardiac glycosides and beta-blockers may exhibit antitumor activity [11-13].

Aim and objectives

The aim of this study was to highlight the anticancer activity of cardiotonic glycosides and non-selective beta-blockers, more precisely digoxin and labetalol, both in vitro and in vivo, in order to continue the study of their effects and in detail the mechanisms of action the basis of their antitumor activity.

MATERIALS AND METHODS

Systematic searches were performed on PubMed, Google Scholar to identify relevant studies on the anticancer effect of cardiotonic glycosides and beta-blockers, especially digoxin and labetalol respectively. Data related to the antitumor activity of cardiotonic glycosides and β-blockers through in vitro and in vivo studies were extracted.

The searches in the specialized literature were carried out using the following terms: cardiotonic glycosides, digoxin, beta-blockers, labetalol, cancer, anticancer/antitumor effect, in vitro/in vivo studies. The titles and abstracts of the identified studies were checked in detail to finally exclude irrelevant studies. Relevant articles were assessed to determine whether they were eligible or should be removed. References from eligible articles were screened to further pick out potentially relevant studies.

RESULTS AND DISCUSSIONS

The initial search revealed a number of 3378 results for cardiotonic glycosides and 18167 for beta-blockers in the PubMed database from the past 10 years, which were then sorted to select information specific to our study, as can be seen in figure 1.

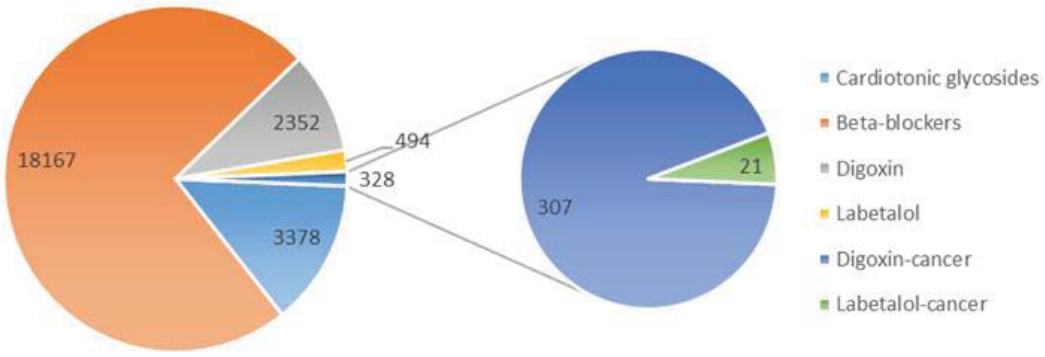

Figure 1. Diagram of systematic database searches

In the following, we will present the articles from which we began our research to analyze the anticancer effect of digoxin and the beta-blocker, labetalol.

Now, worldwide, cancer is one of the main causes of mortality. More precisely, cancer is the second cause of mortality in the population under 70, immediately after cardiovascular pathologies [14].

In recent years, research studies have focused on investigating the anticancer potential of several molecules used in therapy to treat various pathologies. Thus, cardiotonic glycosides and especially digoxin came to the fore, as promising molecules in the treatment of cancers,

their targeted effect being investigated at the molecular level [15]. Digoxin has attracted attention regarding its potential antitumor activities, highlighting its capacity to inhibit cancer cell proliferation and induce apoptosis [16].

Moreover, there is evidence that beta-blockers, both the non-selective ones (labetalol, propranolol, carvedilol) and the selective ones (nebivolol, atenolol), show activity in the treatment of cancer. Most studies have highlighted the effect of non-selective molecules, especially propranolol [17-19].

The cytotoxic effect of digoxin was exposed by our research group through *in vitro* studies on melanoma cells and *in ovo*, in association with betulinic acid, known for its antitumor activity [20]. From these first studies carried out, we want to continue closely investigating the antitumor mechanism of digoxin in skin cancer. This can be accomplished by evaluating and summarizing its antitumor potential in other types of cancer.

Cardiotonic glycosides - digoxin - candidates for cancer treatment. Preclinical studies

Cardiotonic glycosides are molecules that show antitumor properties against lung cancer at relatively low concentrations [21,22]. Digoxin has demonstrated that inhibits the development of the primary tumor and, in addition, inhibits the metastasis of tumor cells from the breast to the lung, by implantation in severe combined immunodeficiency mice [23]. It was observed, at the molecular level, that digoxin decreases NDRG1 (N-Myc Downstream Regulated 1) and VEGF (Vascular endothelial growth factor) by inhibiting HIF-1α (Hypoxia-inducible factor 1-alpha) in lung adenocarcinoma cells (A549) under low oxygen conditions [24]. Other studies have strengthened the potential of digoxin's antitumor activity on lung cancer. The study led by Lin revealed that digoxin inhibited the proliferation, migration, and colony formation of A549 cancer cells and was found to suppress Src (Proto-oncogene tyrosine-protein kinase) activity and its protein expression in a dose (50-500 nM) and time (2-24 hours) dependent manner and, moreover, it decreases the activity of EGFR (Epidermal growth factor receptor) and STAT3 (Signal Transducer Moreover, activator of transcription 3) [25]. Another study, carried out on human non-small cell lung cancer cells (A549 and H1299), showed that digoxin induces autophagy in the two cancer lines by inhibiting the phosphorylation of Akt (Protein kinase B), mTOR (Mechanistic target of rapamycin) and p70S6K (Ribosomal protein S6 kinase beta-1) [26].

On the other hand, digoxin inhibits the proliferation of lung cancer by hampering the expression of subunit α-1 and exerts discriminatory antitumor activity in lung cancer cells with STK11 (Serine/threonine kinase 11) mutation; mutation considered a new biomarker in the treatment of lung cancer for cardiotonic glycosides [27].

Lately, different scientific research groups have suggested that cardiotonic glycosides have the potential to inhibit the proliferation of breast cancer, with a selective effect only on cancer cells. It has been shown that digoxin together with other glycosides such as: peruvoside, strophanthidin, ouabain, oleandrin and lanatoside C suppress the development of breast cancer [21, 28-30]. Glioblastoma is considered one of the most aggressive carcinomas in the world, often relapsing even after chemotherapy and surgery [31]. In this direction, digoxin has been shown to target HIF-1α in human glioma stem cells and induce apoptotic effects in brain cancer [32,33].

The antitumor effect of digoxin was studied on neuroblastoma xenografts from mice, as well as Lewis's lung and colon cancer. SH-SY5Y neuroblastoma grafts were inhibited in the highest proportion of 44%, respectively 19% for Neuro-2a, while lung and colon cancer grafts were less sensitive. Digoxin revealed an inhibitory effect (50% at 53 ng/ml) on angiogenesis *in vitro* on bovine endothelial cells and *in ovo* through the chicken chorioallantoic membrane assay [34]. On the other hand, it was observed that the administration of low and long-term

doses of digoxin, digitoxin and ouabain inhibits the expression of the PSA gene (Prostate-Specific Antigen) by changing the expression of the PDEF gene (Prostate-derived Ets factor) in human prostate cancer cell lines (LNCaP) [35]. Following a systematic screening of 2000 drugs, it was revealed that five cardiotonic glycosides, such as: digoxin, digitoxin, peruvoside, strophanthidin and ouabain, cause the death of anoikis-resistant PP-C1 prostate cancer cells. In addition, digitoxin and ouabain produced apoptosis in prostate cancer cells (PC3) by reducing the expression of Hoxb-13, hepatocyte nuclear factor-3α, hPSE/PDEF and SURVIVIN [36].

The most used cardiotonic glycosides, with the most proven actions in the treatment of cancer, are digoxin and digitoxin. The structural difference between digitoxin and digoxin is an additional hydroxyl group on digoxin, which changes the pharmacokinetic and pharmacodynamic of the molecule. Therefore, digitoxin is a more lipophilic substance, metabolized mainly in the liver and with a longer half-life than digoxin [37].

According to the above, table 1 shows the mode of action of digoxin and digitoxin on cancer cells.

Table 1. Digoxin and digitoxin and their mode of action in cancer cells

Cardiotonic glycosides	Mechanism of action	References
Digoxin	inhibits HIF-1alpha synthesis	[32]
	inhibits androgen-dependent/independent mechanism	[38]
	inhibits Src signaling pathways	[39]
Digitoxin	↓ anti-apoptotic proteins Bcl-xL and Bcl-2	[40]
	↑ cytochrome c release and Caspase activation	[41]
	inhibits topoisomerase I	[42]
	↑ Ca2+ uptake	[43]
	inhibits p53 synthesis	[44]
	inhibits general protein synthesis	[45]
	caspase 9 mediated apoptosis	[46]
	MAPK pathway mediated apoptosis	[47]

The presented preclinical investigations have suggested that cardiac glycosides, including digoxin, may exert anticancer activity. As well as the preclinical studies, there are also numerous clinical studies that reinforce the idea of studying in depth the anticancer effect of cardiotonic glycosides.

Cardiotonic glycosides - digoxin - candidates for cancer treatment. Clinical studies

It is well known that cardiotonic glycosides are effective in the treatment of cardiovascular diseases. But as we can see, this drug class is increasingly shaping its anti-tumor effect, an effect also supported in clinical studies. Several cardiotonic glycosides have been included in clinical trials, including digoxin, Anvirzel (aqueous extract of *Nerium oleander*), PBI-02504 (CO_2 extract of *Nerium oleander*), and UNBS-1450 (semisynthetic derivative). The first results of the phase I studies were promising. More precisely, Anvirzel has shown that it has an anticancer effect with a safe and effective administration up to 1.2 mL/m^2/day. This pharmaceutical form has been clinically studied for its effect on non-small cell lung cancer in combination with chemotherapy medication [48,49]. Regarding the PBI-

05,204 extract, the maximum tolerated doses evaluated (0.6–10.2 mg/day) in phase I studies were shown to be effective and it was recommended to proceed to phase II studies in the treatment of colon cancer, rectum, breast, and bladder. Following these studies, the safety, pharmacokinetics, and pharmacodynamics of the product were monitored, and the most tolerated dose of 0.2255 mg/kg was identified [50].

Digoxin has so far been included in 32 clinical studies to evaluate the antitumor effect in several types of cancer (breast, prostate, pancreatic, etc.), alone or in combination with other immunotherapeutic drugs [51]. A clear example is the association of digoxin with cisplatin in head and neck cancer, where a stronger effect of the combination was observed than in the case of using the compounds separately [52].

Cardiotonic glycosides, especially digoxin and digitoxin, have been intensively evaluated regarding their anticancer potential in numerous preclinical and clinical studies, figure 2 shows this information.

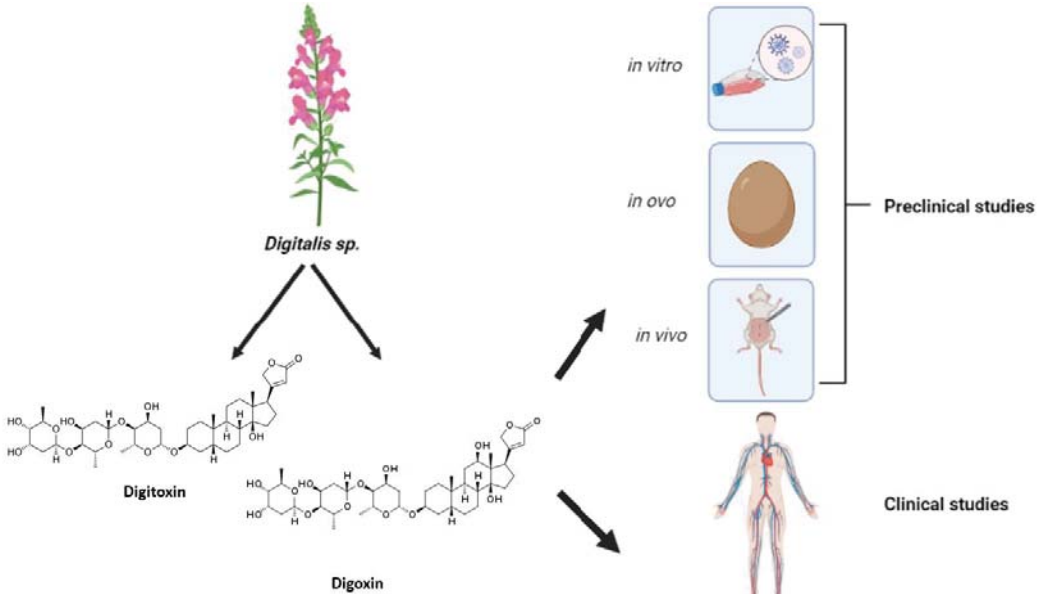

Figure 2. Digoxin and digitoxin - preclinical and clinical studies

Digoxin, a compound long used in cardiovascular diseases, has shown potential in the treatment of cancer. We want to supplement the data obtained by our research group and those from the literature with other more detailed studies, especially regarding the mechanism of digoxin's cytotoxic action at the level of skin cancer cells. Moreover, we wish to continue studying another drug used in cardiac pathologies, labetalol, considered safe in pregnancy, regarding its antitumor action.

A first study carried out by our research team showed that labetalol does not show cardio and hepatotoxicity *in vitro* on healthy cells, willing to deepen the effect of the beta-blocker on cancerous cell lines [53].

Beta-blockers - labetalol - candidates for cancer treatment. Preclinical studies

Beta-blockers are a heterogeneous pharmacotherapeutic class that presents multiple benefits in cardiovascular diseases leading to the reduction of mortality caused by these pathologies [54]. Furthermore, to the proven benefits, in recent years this drug class has sparked interest in studying its antitumor effects.

Regarding the effect on cancer cells, propranolol and other β-blocker drugs have been observed to reduce MAPK activity in pancreatic carcinoma [17,55,56]. It has also been

reported that propranolol decreases the viability and migration of breast cancer lines, to the greatest extent when co-administered with metformin. Thus, it was concluded that the two drugs decrease tumor development, improving survival, an effect observed after studying two models of triple breast cancer. Besides these, the evidence suggests that non-selective beta-blockers, more precisely propranolol, potentiate the anti-angiogenic and antitumor effects of chemotherapy medication [17,57]. In breast cancer biopsies isolated from patients who received propranolol, changes in cancerous proliferation were observed. More precisely, on the MDA-MB-231 cell line, it was demonstrated that propranolol after 24 hours of treatment produces changes in cell viability, observed with the help of flow cytometry [58].

To support the *in vitro* effect of beta-blockers, additional studies and especially clinical studies are needed.

Beta-blockers - labetalol - candidates for cancer treatment. Clinical studies

In a phase II, placebo-controlled, triple-blind study, the research group noted that the administration of propranolol before surgical removal of breast cancer was associated with an important decrease in the expression of metastasis markers [59]. Thus, this evidence supporting the survival benefits of beta-blockers should pave the way for a phase III clinical trial. The study by Watkins et al., which included a large number of patients (>1400), evaluated the effect of β-blockers in ovarian cancer. Beta-blockers showed an increase in overall survival compared to patients who were not given the drugs. Moreover, it was reported that this increase in survival was characteristically associated with non-selective beta-blockers [60].

Another study conducted on the Swedish population indicated that patients with pancreatic cancer who received beta-blockers had a lower mortality specific to adenocarcinoma [61]. In another type of cancer, prostate cancer, it was observed that beta-blockers decrease pathology-specific mortality, results that were obtained after 4 observational studies that included 16825 patients [62].

Also, the administration of non-selective beta-blockers was correlated with a longer survival in patients with metastatic melanoma compared to those who were administered selective beta-blockers [63,64]. Beta-blockers have shown their benefits in several types of cancer; however, new preclinical and clinical studies are needed to establish the utility of this drug class in cancer management. Beta-blockers have been evaluated for their antitumor potential in various preclinical and clinical studies, figure 3 outlines this.

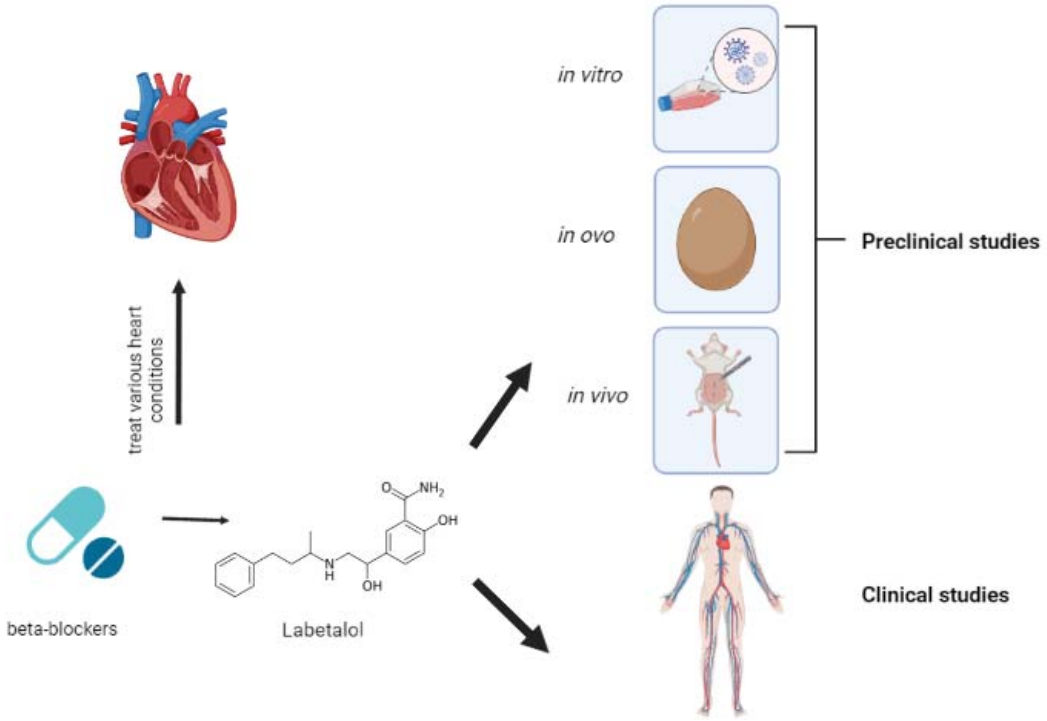

Figure 3. Beta-blockers- labetalol - preclinical and clinical studies

CONCLUSIONS

Cardiotonic glycosides and beta-blockers have been used in the treatment of cardiovascular pathologies, but studies in recent years on cancer cell lines and animal systems have revealed other new therapeutic actions, supported by clinical studies. Analyzing the data, it can be noted that digoxin, but also non-selective beta-blockers, including labetalol, are promising anticancer agents according to the information from the specialized literature. The antitumor effect of labetalol has not been intensively debated, but the beneficial effect of propranolol in the treatment of cancer, which is part of the same class of non-selective beta-blockers, has been highlighted. Therefore, our research group will focus on the study of the labetalol molecule. Although there is clear evidence for both substances, further studies are needed to support their cytotoxic effects and to understand in detail their mechanism of action.

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