CARDIOTOXIC EFFECTS OF CHEMOTHERAPEUTIC AGENTS WITH SPECIAL EMPHASIS ON CISPLATIN

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Abstract
Cisplatin (CPT) is a long-standing widely used chemotherapeutic alkylating agent. Its main mechanism of action is by damaging the DNA, causing strand breaks which inhibit cell proliferation. In addition, they also alter ion channel function, intracellular calcium, and free radical production. Although these additional mechanisms of action may contribute to their chemotherapeutic action, they frequently cause adverse reactions, and originate mechanisms of drug resistance. In this review, we focus on the properties of the adverse effects of CPT on the heart. In isolated hearts a biphasic effect of CPT on inotropism can be described, which is positive at concentrations below 5 μM and negative at higher concentrations. These effects could be explained by the ability of CPT to promote changes in Cav1.2 channels and intracellular Ca^{2+} in isolated cardiomyocytes. The biphasic inotropic behavior of the heart to CPT has good correlation with its actions on Cav1.2 currents and intracellular Ca^{2+}.These findings suggest that CPT has several sites of impact on the heart, and that Cav1.2 channels and intracellular Ca^{2+} dysfunction are key players in the heart's adverse reactions to CPT.
Keywords: Chemotherapeutic agents, cardiotoxicity, cisplatin, Cav1.2 channels. Intracellular Ca^{2+}

Resumen
El cisplatino (CPT) es un quimioterápico alquilante antiguo muy usado. El principal mecanismo de acción es por daño del ADN ocasionando rupturas del mismo que inhiben la proliferación celular. Además, pueden alterar la función de canales iónicos, calcio intracelular y producción de radicales libres. Si bien estos mecanismos de acción adicionales pueden contribuir a su acción quimioterapéutica, frecuentemente causan reacciones adversas y originan resistencia a estas drogas. En esta revisión, nos centraremos en las propiedades de los efectos adversos del CPT en el corazón la cual puede describirse por un efecto inotrópico positivo para dosis menores a 5 μM e inotrópico negativo para dosis mayores. Estos efectos pueden explicarse por la capacidad del CPT de promover cambios en los canales Cav1.2 y calcio intracelular en cardiomiocitos. El efecto bifásico observado en el inotropismo cardíaco por CPT se correlaciona bien con las acciones observadas en las corrientes por Cav1.2 y calcio intracelular. Esto sugiere que el CPT tiene varios sitios de impacto en el corazón y que actores centrales en las reacciones adversas cardíacas al CPT pueden ser al menos la disfuncionalidad que este fármaco promueve sobre los canales Cav1.2 y el calcio intracelular.
Palabras clave: Quimioterápicos, Cardiotoxicidad, cisplatino, canales Cav1.2, calcio intracelular

Received: August 23, 2023. Accepted in the final form: September 12, 2023

Introduction

Chemotherapeutic agents are drugs used to treat cancer by inhibiting the growth and proliferation of cancer cells. They are classified according to their mechanism of action and include alkylating agents, antimetabolites, topoisomerase inhibitors, antibiotics, mitotic inhibitors, and protein kinase inhibitors [1]. Alkylating agents work directly on DNA to keep the cell from reproducing itself, while antimetabolites restrict DNA and RNA synthesis [1]. Topoisomerase inhibitors interfere with the action of topoisomerase enzymes, which control the manipulation of the DNA structure necessary for replication [1]. Antibiotics are chemo treatments made from natural products produced by species of the soil fungus Streptomyces [1]. Mitotic inhibitors work by inhibiting cell division [1]. Protein kinase inhibitors target specific proteins that are involved in the growth and division of cancer cells [1]. Chemotherapy drugs can be administered in different ways, including intravenous (IV), oral, intramuscular (IM) injection, subcutaneous (SC) injection, and intrathecal therapy [1]. The method of drug administration is based on the actual disease diagnosed and the agent's effectiveness. Combination chemotherapy, which involves the use of two or more drugs, is often employed to increase the cancerous cell-killing effectiveness [1]. Chemotherapy drugs can be toxic and require safe handling [1]. They can cause both short-term and long-term side effects and people have different sensitivities to these damaging effects [2], which can vary depending on the individual, type of cancer and type of chemotherapy drug used [2]. Common side effects of chemotherapy include fatigue, hair loss, easy bruising and bleeding, infection, anemia, nausea and vomiting, appetite changes, constipation, diarrhea, mouth, tongue, and throat problems such as sores and pain with swallowing, peripheral neuropathy or other nerve problems, as numbness, tingling and pain, and skin and nail changes including dry skin and color change. Some chemo drugs can cause important and sometimes irreversible side effects, like heart or nerve damage or fertility problems [2]. One of the most important and limiting side effects of chemotherapeutic drugs is their cardiotoxicity. It is highly relevant, as cancer has an increased prevalence in the elderly population, which has also increased cardiovascular diseases. Because of this, a new specialization has emerged in the last years, termed cardio-oncology [3]. Some chemotherapy drugs are more likely to cause heart problems than others. Anthracycline drugs, such as doxorubicin, daunorubicin, and epirubicin, are most commonly associated with changes in the heart muscle.Other chemotherapy agents that may cause heart damage include cyclophosphamide, ifosfamide, CPT, busulfan, mitomycin, paclitaxel, fluorouracil, and others [3]. Cardiotoxicity can be prevented by screening and modifying risk factors, aggressively monitoring for signs and symptoms as chemotherapy is administered and continuing follow-up after completion of a course or the entire treatment. Prompt measures such as discontinuation or modification of chemotherapy or use of appropriate drug therapy should be initiated based on changes in monitoring parameters before the patient exhibits signs and symptoms of cardiotoxicity [4]. It is important to note that not all chemotherapy drugs cause cardiac effects, and the risk of cardiac effects can vary depending on the individual, type of cancer, and type of chemotherapy drug used. This review will focus on the cardiotoxicity of cisplatin (CPT). We will review the literature and we will present findings from our laboratory, using isolated hearts and cells from animal models.

Alkylating agents and cisplatin

Alkylating agents are a class of drugs that are used to treat cancer by inhibiting the transcription of DNA into RNA and thereby inhibiting protein synthesis [5]. They work by substituting alkyl groups for hydrogen atoms on DNA, resulting in the formation of cross-links within the DNA chain and thereby resulting in cytotoxic, mutagenic, and carcinogenic effects [5]. Alkylating agents are divided into two types: those that react directly with biological molecules and those that form a reactive intermediate, which then reacts with the biological molecules [6]. These types are termed SN1 and SN2, respectively [6]. The alkylating agents react with water and are inactivated by this hydrolysis [6]. They are also inactivated by reactions with thiols, such as glutathione. The reaction of alkylating agents with glutathione can be increased by glutathione S-transferase enzymes, as is discussed later in the sections on mechanisms of cellular resistance. Alkylating agents also undergo microsomal and

other types of xenobiotic metabolism. The primary mode of action for most alkylating drugs is via cross-linking of DNA strands, though they can also affect directly or indirectly other cellular structures and produce the desired effects on target cells or side effects [5, 6]. They can be classified as either monofunctional alkylating agents or bifunctional alkylating agents, depending on whether they form one or two covalent bonds with DNA, respectively [7]. Alkylating agents prevent cells from dividing and replicating by damaging the DNA [5, 6, 7]. They work in all phases of the cell cycle and attack DNA in both cancerous and healthy cells. However, cancer cells are among the most affected because they are among the most rapidly dividing cells [5, 6, 7]. In summary, alkylating agents are a class of drugs that are used to treat cancer by inhibiting the transcription of DNA into RNA preventing protein synthesis. However, as with all chemotherapeutic agents, they have multiple sites of action which can lead to unwanted side effects.

Cisplatin is a platinum-based chemotherapy drug that belongs to the class of alkylating agents. It slows down the growth of cancer cells damaging the DNA of dividing cells, binding especially to guanine and adenine purine bases with non-cycle dependency, causing intra and inter-strand DNA crosslinking and breakdown [8]. It can be used alone or in combination with other treatments, especially in those rapidly dividing neoplasms found in solid tumors and some hematological types of cancer [8]. The toxic effects of CPT on cells are not only a consequence of covalent adduct formation between CPT and DNA but also with RNA and many proteins. The processes that determine the molecular mechanisms of toxicity are usually associated with an undesired effect and problem of CPT treatment, which is the development of drug resistance [9]. Fig. 1 shows CPT 3D structure.

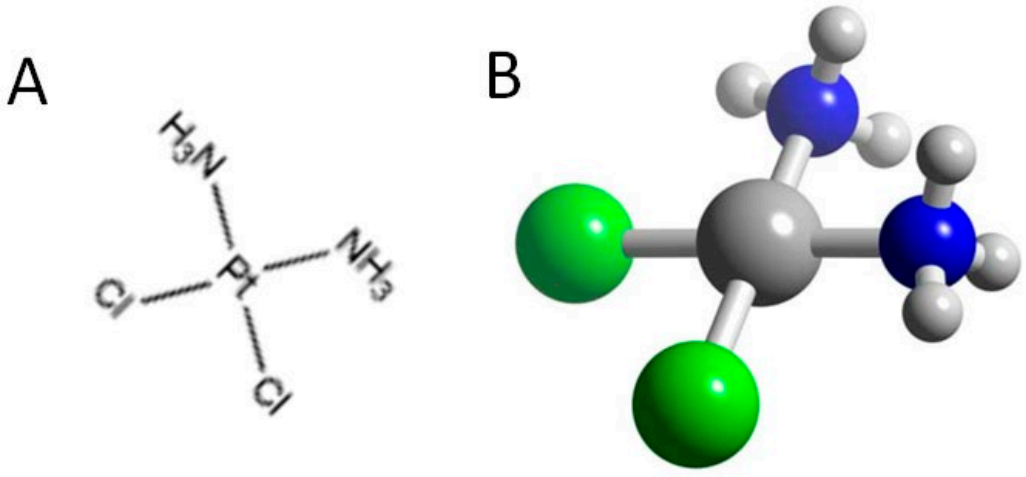

Figure 1. Structure of Cisplatine. (A). The planar structure shows platinum in the middle in coordination with two chloride and two ammonia. (B). The 3D structure of CPT showing Platinum as a grey sphere in the middle, with two chloride ions in green and two nitrogens in blue. The small grey spheres represent hydrogen atoms.

Cispaltin-induced cardiotoxicity

CPT is known to have cardiac toxicity [10, 11]. CPT cardiotoxicity can range from silent and symptomatic arrhythmias to cardiomyopathy and sudden death [10]. CPT-induced paroxysmal supraventricular tachycardia (PSVT) is rare but has been mentioned in a few case reports [10]. CPT is unique in that it can cause late cardiovascular complications such as hypertension, left ventricular hypertrophy, myocardial ischemia, and myocardial infarction as long as 10 to 20 years after treatment [11]. Acute cardiovascular toxicity by CPT is hypothesized to be caused by direct damage to the vascular endothelium [12]. In general, CPT can affect the electrophysiology of the heart in various ways, including: i) Cardiac arrhythmias: they may be due to the direct effect of the drug on cardiac ion channels, leading to increased QT dispersion or other mechanisms that cause inhomogeneity of ventricular recovery [10]. In addition, electrolyte imbalances may also play a role in the pathogenesis of CPT-induced arrhythmias, in particular altered intracellular and extracellular potassium and magnesium concentrations, which may contribute to its cardiotoxicity [10]. ii) Changes in cardiac contractility: acute exposure to CPT has been shown to have

a biphasic effect on cardiac contractility, presumably by blocking L-type calcium channels (Cav1.2) [13]. This can affect the heart's ability to contract and pump blood effectively, and finally iii) Cardiotoxicity: CPT-induced cardiotoxicity can include electrocardiographic changes, arrhythmias, myocarditis, cardiomyopathy, and congestive heart failure [10, 14]. These effects can impact on the electrophysiology of the heart and lead to changes in heart rate, rhythm, and contractility. It is important to note that the effects of CPT on the electrophysiology of the heart are complex and can involve multiple pathways. CPT-induced cardiotoxicity can be either due to a direct toxic action on cardiac myocytes or due to the production of reactive oxygen species (ROS) and the subsequent induction of oxidative stress and switch to a prothrombotic condition [10]. Not all patients who receive CPT will experience cardiac toxicity, and the risk of cardiac effects can vary depending on the individual, type of cancer, and type of chemotherapy drug used [15]. Further research is needed to fully understand the mechanisms by which CPT affects the electrophysiology of the heart and its potential impact on cardiac function.

Effect of cisplatin in isolated hearts

Acute exposure to CPT in guinea pig isolated hearts promotes changes in heart rate, heart rhythm and inotropic state. Fig.2 shows the records and the dose-response curves with data and the best fit of a Hill equation for inotropic state. Inotropy had a bell shape dependence with increasing concentrations of CPT. The heart rhythm was altered increasing the propensity to arrhythmias with increasing concentrations of CPT. Interestingly, the inotropic state exhibited a bell shape dose-response behavior, similar to that described for heart rate [13]. These findings suggest that i) CPT probably has more than one mechanism of action to explain the results observed; and ii) some mechanisms might have a common molecular explanation to explain the bell-shape effects observed in heart-rate and the inotropic state, as they happen in a similar range of CPT concentrations. One of the likely targets could be the L-type calcium channels (Cav1.2), as they are critical during phase 0 in the sinoatrial action potential that establishes the heart rate and also during the plateau and phase 2 of a ventricular action potential, triggering calcium induced calcium release (CICR) through the ryanodine receptor type 2 [16]. We explored next the effects of acute exposure of CPT on L-type calcium channels (Cav1.2).

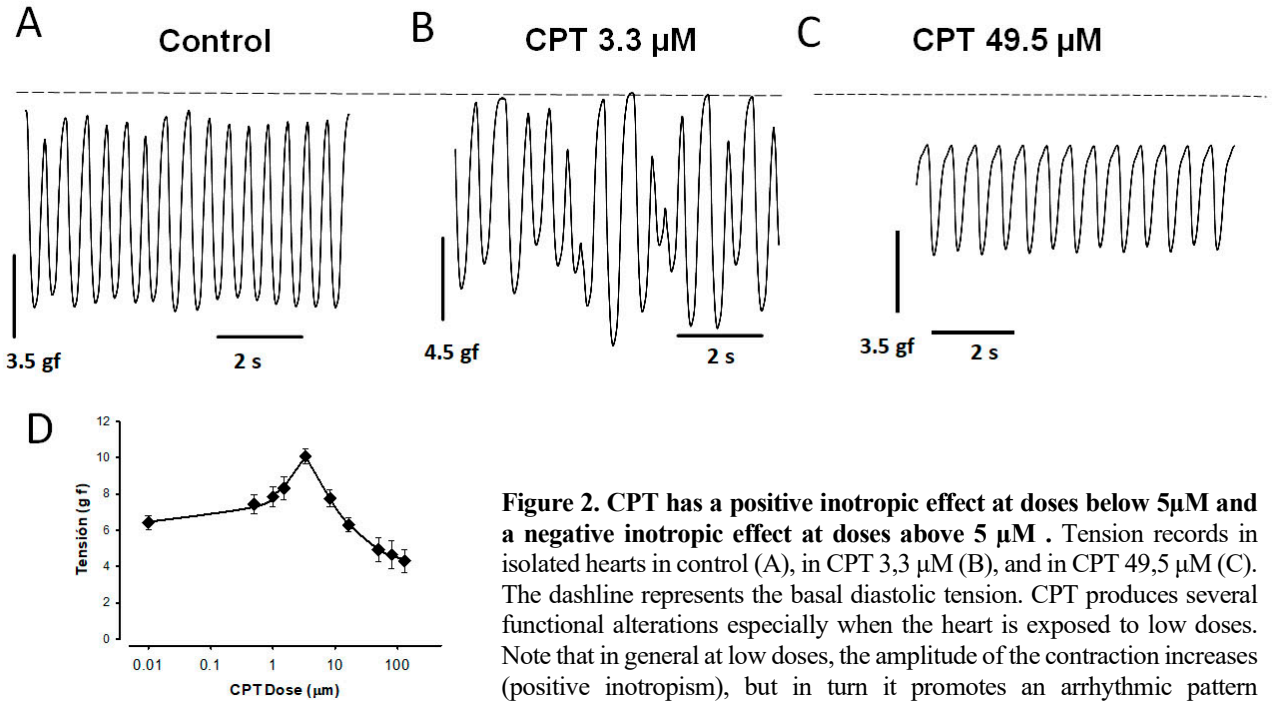

Figure 2. CPT has a positive inotropic effect at doses below 5µM and a negative inotropic effect at doses above 5 µM . Tension records in isolated hearts in control (A), in CPT 3,3 µM (B), and in CPT 49,5 µM (C). The dashline represents the basal diastolic tension. CPT produces several functional alterations especially when the heart is exposed to low doses. Note that in general at low doses, the amplitude of the contraction increases (positive inotropism), but in turn it promotes an arrhythmic pattern observed as irregular contractions (*middle, up*). (D). CPT in isolated hearts has a positive inotropic effect for doses that are less than 5 µM. At higher doses, the negative inotropic effect is consistently observed but does not seem to alter the cardiac function as much as the lower concentration does. The solid lines are the best fit of a Hill equation for positive inotropism (IC 50 = 0.75 µM) and for negative inotropism (IC 50 = 12 0.75 µM).

Effect of cisplatin on voltage gated calcium channels

The effect of CPT on voltage-gated calcium channels (VGCCs) can be beneficial in the treatment of neoplasms and/or promote adverse effects. The combination of the calcium channel blocker verapamil and the cytotoxic chemotherapeutic agent vincristine had synergistic antimetastatic effects [17], showing that their blocking properties might be beneficial in some treatments. However, in most of the cases, the effects of CPT on calcium channels are associated with adverse effects. Several reports have shown that CPT affects several types of VGCCs in different cells of the organism. CPT has been shown to modulate VGCCs in dorsal root ganglion (DRG) neurons, altering intracellular calcium homeostasis. It also alters the function and expression of N-type VGCCs in sensory neurons, without causing morphological damage [18]. In addition, it has been reported that CPT induces calcium ion accumulation in inner hair cells by causing functional alterations in calcium channels and exocytosis [19]. The pharmacological inhibition of VGCCs by nimodipine reduced the increase in calcium induced by CPT, suggesting its involvement in the response to CPT and its association with CPT resistance [20]. Thus, the impact on VGCCs would promote alterations in intracellular calcium dynamics as has been reported in many different cells, and it is important to understand it not only in terms of basic science but also in translational science, as it could explain the resistance to CPT in several tumors. These later studies suggest that CPT can affect calcium channels, which may contribute to its toxic effects. However, more research is needed to fully understand the mechanisms by which CPT affects calcium channels and how this contributes to its cardiotoxicity. In the heart the VGCCs that are most relevant are the L-type calcium channels (Cav1.2). We used isolated cardiomyocytes from guinea pig ventricles exposed to different concentrations of CPT (Fig. 3). CPT changed Cav1.2 currents increasing them at concentrations lower than 5 µM, but blocking them at concentrations higher than 5 µM. It is interesting that these results are somehow consistent with what we found regarding the impact of CPT in isolated heart function (see Fig. 2) [13]. It also implies that CPT has at least two different mechanisms by which it impacts L-type calcium channels (Cav1.2) in the heart. At doses below 5 µM it enhances their current as described for other VGCCs [18]. However, at higher doses it diminishes the currents through Cav1.2 channels. Despite a direct block of the channel like the one we found for environmental pollutants cannot be discarded [21, 22], we think that an indirect modification of the channel by CPT is more likely as the reversibility of the effect is hard to observe. Given the central role that VGCCs and intracellular calcium have in cardiac function, these observations suggest that in the heart, CPT might be affecting VGCCs and intracellular calcium dynamics to explain the disturbances in heart function reported before.

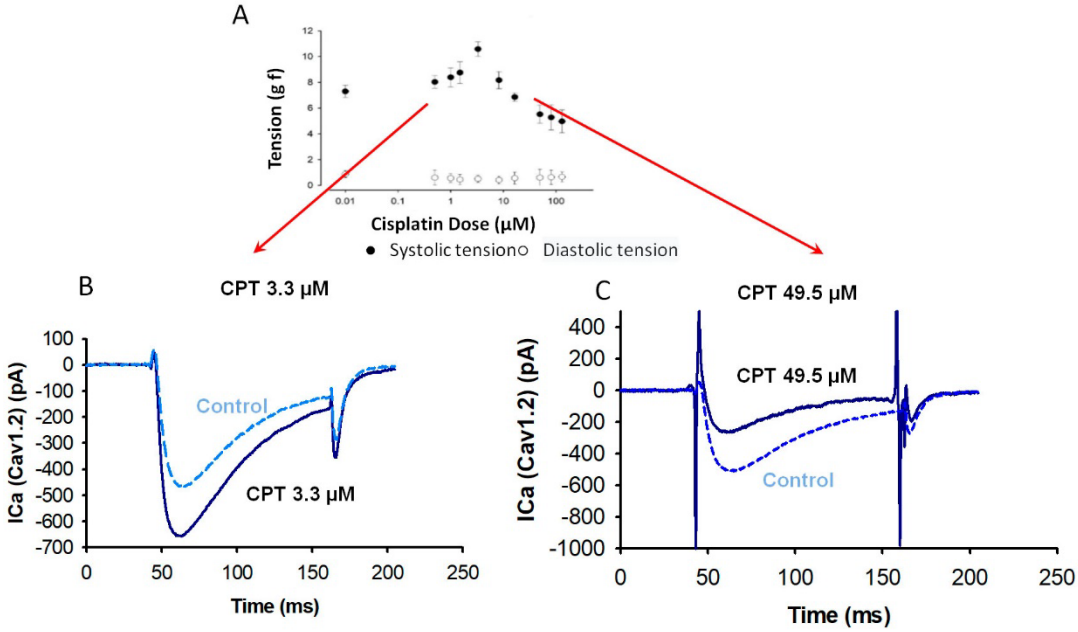

Figure 3. The CPT effect observed in cardiac contractility is well correlated with the effects observed in L-type Ca^{2+} currents. (A) CPT affects mostly the systolic tension in a biphasic way, though the diastolic tension also slightly increases with CPT. (B) Calcium currents through Cav1.2 channels were recorded for those concentrations of CPT showing an initial increase at doses below 5 µM. (C) Increasing concentrations of CPT above 5 µM, showed a inhibition of Cav1.2 currents.

Effect of cisplatin on intracellular calcium

Several studies have investigated the relationship between CPT and intracellular calcium, with relevant findings regarding its role as a chemotherapeutic agent, adverse reactions and resistance to CPT. It has been reported that lowering intracellular calcium using an intracellular calcium chelator like BAPTA-AM, sensitizes breast cancer MCF-7 cells to sub-toxic doses of CPT [23]. Moreover, calcium efflux from the endoplasmic reticulum (ER) regulates CPT-induced apoptosis in human cervical cancer HeLa cells [24]. Regarding CPT adverse effects, CPT-related calcium release from the site of intracellular calcium storage in the early phase causes oxidative stress in renal cells [25]. It was found in inner hair cells that CPT induces the accumulation of calcium ions causing functional alterations in calcium channels and exocytosis [19]. The mechanisms underlying CPT resistance involve changes in calcium channels and an alteration of calcium homeostasis in tumor cells [26]. These studies suggest that CPT can affect intracellular calcium levels and that manipulating calcium levels may affect the sensitivity of cancer cells to CPT. However, more research is needed to fully understand the relationship between CPT and intracellular calcium and how it can be used to improve cancer treatment. In the heart, CPT affects intracellular calcium levels through various mechanisms. As we have previously shown either through enhancement or inhibition of L-type calcium channels, acute exposure to CPT has been shown to have a biphasic effect on cardiac contractility by enhancing and inhibiting these channels [13]. This blockade can disrupt the normal influx of calcium ions into cardiac cells, affecting intracellular calcium levels. CPT can also have a direct effect on cardiac sodium channels inducing cardiac arrhythmias, leading to an increase in QT dispersion or other mechanisms that cause inhomogeneity of ventricular recovery [10]. These effects can indirectly impact on intracellular calcium levels by altering the electrical activity of the heart. CPT can also impair intracellular calcium handling in the heart leading to sarcoplasmic reticulum calcium release dysfunction, (a key player for intracellular calcium handling), contributing to cardiac damage and arrhythmias. CPT treatment can worsen this impairment, particularly in the presence of a ryanodine receptor 2 gene mutation [27]. It is important to note that CPT effects on intracellular calcium levels in the heart are complex and can involve multiple pathways. Further research is needed to fully understand the mechanisms by which CPT affects intracellular calcium in the heart. We used isolated cardiomyocytes loaded loaded with the calcium-sensitive rhodamine dye, incubated at various concentrations of extracellular CPT (see Fig. 4). At concentrations of CPT below 5 μM, there was an increase in intracellular calcium with CPT, which is consistent with the positive inotropic effect and the agonist effect on L-type calcium channels (Cav1.2) [13]. The opposite was observed for CPT concentrations above 5 μM. These results suggest that the positive inotropic effect of CPT is related to its agonist effect in Cav1.2 channels, with subsequent increase of CICR, and that the negative inotropic action of CPT is related to its antagonist effect on the same channels with CICR inhibition.

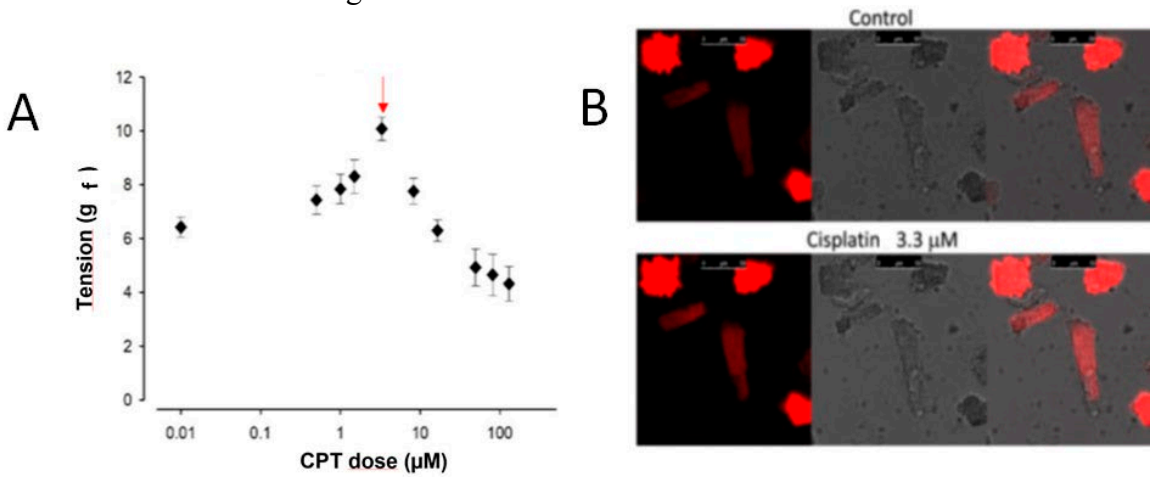

Figure 4. The increase in systolic tension correlates well with an increase in intracellular Ca^{2+} (A). CPT in isolated hearts has a positive inotropic effect for doses that are less than 5 μM (up to red arrow). At higher doses, the negative inotropic effect is consistently observed but does not seem to alter the cardiac function as much as the lower concentration does. (B) Intracellular Ca^{2+} measurements in isolated cardiomyocytes loaded with a Rhodamine Calcium sensitive dye in fluorescent, transilluminated and merged conditions (from left to right). In control situation (above) the intensity of fluorescence is less than with cisplatin 3.3 μM (below).

Concluding remarks

CPT is a widely used chemotherapeutic agent that belongs to the group of alkylating agents. Though its primary mechanism of antitumoral action involves the interaction of these type of compounds with DNA, promoting strand breaks and interference with cell proliferation, these agents also have other direct and indirect effects on several pathways that might contribute to the antineoplastic effect, the resistance pathways that became activated over years of treatment or adverse effects in several organs and tissues. A critical organ that can be affected by these chemotherapeutic agents is the heart. In this review, we have shown that CPT is able to affect the heart interacting either directly or indirectly with Cav1.2 channels, promoting several changes in cardiac function. The range of doses used to treat patients usually varies from 0.5 to 15 μM [1, 8, 9], doses that are within the range of CPT doses explored in this report. CPT also affects other ion channels present in cardiomyocytes, though not as much as Cav1.2 channels [28], and it also affects organelles critical for cardiac function like mitochondria [29]. Most likely, cardiac toxicity by CPT involves the latter plus the mechanism described in more detail in this review. The mechanism described could also be extended to adverse reactions or resistance mechanisms to CPT in other cells. Further research is needed to fully understand the mechanisms by which CPT and alkylating agents affect ion channels and intracellular calcium in different cells and its potential impact on the heart.

Acknowledgments: This work was supported by CSIC I+D 91 to GF and PEDECIBA to FS and GF. We thank Dr. Alicia Mattiazzi for her kindness helping in the final stages of this manuscript.

Ethical agreements: All the experiments were performed in protocols approved by international standards by the Honorary Uruguayan Committee of Experimental Sciences (Exp 070153-000572-17)

Author Contributions: Florencia Savio analyzed and performed some experiments, Romina Cardozo performed and helped analyzing the experiments, Gariel Krygier edited the manuscript, Gonzalo Ferreira analyzed and performed the experiments, wrote and edited the manuscript, and developed the original idea.

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Gabriel Krygier is the Chairman of the Department of Clinical Oncology at the University Clinical Hospital from the School of Medicine, UdelaR, Montevideo, Uruguay. He is specialized in mastoiogy, uro-oncology, and melanoma. During his former years as an oncologist, he acquired a deep background in epidemiological/statistical and clinical studies and had a postdoc specialization at the Breast Cancer International Research Group in Edmonton, Canada. He also participated in several research studies at the MD Anderson Cancer Center in Houston, TX, USA. He has published more than 65 papers in peer-reviewed journals. He is an active member of several international, regional, and local medical and clinical Oncology societies.

Gonzalo Ferreira is the chairman of the Department of Biophysics at the School of Medicine, Universidad de la República, Montevideo, Uruguay. He is a physician with a magister and Ph.D. in Biophysics from the Program for the Development of Basic Sciences in Uruguay (PEDECIBA). During his studies, he installed the first patch-clamp setup in Uruguay and recorded gating currents from Calcium channels from cardiomyocytes being mentored by Drs. Gustavo Brum and Gonzalo Pizarro. He continued his studies with Dr. Eduardo Rios at Rush University in Chicago, USA where he adapted this technique to heterologous expressed Calcium channels trying to develop kinetic models of the channel. During this period he gained expertise in the molecular biology of ion channels and intracellular calcium measurements. He had a second postdoc with Dr. Larry Salkoff at Washington University in Saint Louis where he studied Slo channels, especially the Slo2 (K(Na)) channels expressed in oocytes. During this time, he started a collaboration with Dr. Latorre's laboratory in Chile, especially related to BK channels, which was later extended to Trp channels. Upon his return to Uruguay in 2006, he focused on how several pollutants and pharmacological agents can have an impact on heart function through ion channels and intracellular Calcium. He also tested several new therapeutic approaches related to ion channel function in biological membranes and intracellular Calcium. He contributed to developing several projects to improve the infrastructure for education (EVA, 2008), scientific journals (AnFaMed), and research (Confocal and Epifluorescence Unit with Dr. Brum) at the Universidad de la República. He also developed collaborations with Dr. Alberto Darszon's laboratory in Mexico (topic sperm physiology and biophysics), Dr. George Bloom from the University of Virginia, USA (topic Alzheimer's disease and intracellular calcium and ion channels), Dr. Gregg Gundersen from Columbia University, USA (topic perinuclear proteins, intracellular calcium and ion channels). Since 2015 he has maintained a tight collaboration with Dr. Garth Nicolson (coauthor of the mosaic fluid model), Director of the Institute for Molecular Medicine, California, USA, and more recently with Dr. Luis Sobrevía from the Pontificia Universidad Católica de Chile. Some of his former students are professors abroad and at Universidad de la República. His lab currently has 10 students. As he believes that love and science are fundamental human conditions for the future of mankind, he tries to stay in touch with most of the mentors, students, and colleagues that he had the fortune to meet during his career. https://www.researchgate.net/profile/Gonzalo-Ferreira-2, https://scholar.google.com/citations?user=nkigf5EAAAAJ&hl=en