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Effect of astaxanthin in imatinib mesylate-induced cardiotoxicity

İmatinib Mesilat Kaynaklı Kardiyotoksisitede Astaksantinin Etkileri

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ABSTRACT

Aim: Imatinib mesylate is a tyrosine kinase inhibitor and is approved as a standard first-line therapy of chronic myeloid leukemia. Oxidative stress, as well as intracellular calcium overload and mitochondrial dysfunction, play an important role in chemotherapy-induced cardiotoxicity. The underlying pathophysiological mechanism associated with imatinib-induced cardiotoxicity is not well understood. In the present study, we investigated alterations in calcium influx, oxidative stress and apoptosis through transient receptor potential melastatin 2 (TRPM2) channels. Also, we aimed to investigate if there is a modulator role of astaxanthin in cardiomyocytes during imatinib mesylate-induced cardiotoxicity.

Materials and methods: The cells were divided into seven main control groups: imatinib, imatinib+antranilic acid, imatinib+astaxanthin, imatinib+antranilic acid+astaxanthin, astaxanthin and astaxanthin+antranilic acid groups. Cells in the groups were stimulated with cumene hydroperoxide and inhibited with antranilic acid in related experiments for activation and inactivation of TRPM2 channels, respectively. We measured cytosolic calcium, intracellular reactive oxygene, mitochondrial depolarization, caspase 3 and caspase 9 levels.

Results: The apoptosis values were significantly lower in the astaxanthin and the imatinib+astaxanthin group than in the imatinib group of cardiomyocytes ($p< 0.001$). The cell viability values were significantly higher in the imatinib+astaxanthin+antranilic acid ($p<0.001$) and the imatinib+astaxanthin ($p<0.05$) groups, than in the imatinib group.

Conclusions: As a result, we found that TRPM2 channels were found in cardiomyocyte cells and they were activated by reactive oxygen species. Also, we showed that overactivated TRPM2 channels are associated with increased cytosolic free calcium, oxidative stress and apoptotic cell injury in imatinib mesylate-induced cardiotoxicity, whereas astaxanthin could have a modulator role in this instance.

Keywords: İmatinib mesylate, TRPM2, oxidative stress, cardiomyocyte

ÖZ

Amaç: İmatinib mesilat bir tirozin kinaz inhibitörüdür ve kronik miyeloid löseminin standart bir birinci basamak tedavisi olarak onaylanmıştır. Kemoterapiye bağlı kardiyotoksisitede oksidatif stresin yanı sıra hücre içi kalsiyum aşırı yüklenmesi ve mitokondriyal disfonksiyon önemli rol oynar. İmatinib ile indüklenen kardiyotoksisitenin neden olduğu altta yatan patofizyolojik mekanizma tam olarak anlaşılamamıştır. Bu çalışmada, geçici reseptör potansiyel melastatin 2 (TRPM2) kanalları üzerinden kalsiyum akışı, oksidatif stres ve apoptozdaki değişimleri araştırdık. Ayrıca, imatinib mesilat kaynaklı kardiyotoksisite sırasında astaksantinin kardiyomiyositlerde modülatör rolü olup olmadığını araştırdık.

Gereç ve Yöntemler: Hücreler, kontrol, imatinib, imatinib + antranilik asit, imatinib + astaksantin, imatinib + antranilik asit + astaksantin, astaksantin ve astaksantin + antranilik asit grupları olmak üzere yedi ana gruba ayrıldı. Gruplardaki hücreler, ilgili deneylerde TRPM2 kanallarının aktivasyonu ve inaktivasyonu için sırasıyla kümen hidroperoksit ile uyarıldı ve antranilik asit ile inhibe edildi. Sitosolik kalsiyum, hücre içi reaktif oksijen, mitokondriyal depolarizasyon, kaspaz 3 ve kaspaz 9 seviyeleri ölçüldü.

Bulgular: Apoptoz değerleri astaksantin ve imatinib + astaksantin grubunda, imatinib grubundaki kardiyomiyositlerden anlamlı olarak daha düşüktü ($p<0.001$). Hücre canlılığı değerleri imatinib + astaksantin + antranilik asit ($p<0.001$) ve imatinib + astaksantin ($p <0.05$) gruplarında imatinib grubundan anlamlı olarak daha yüksekti.

Sonuç: Sonuç olarak, TRPM2 kanallarının kardiyomiyosit hücrelerinde bulunduğunu ve reaktif oksijen türleri ile aktive edildiğini bulduk. Ayrıca, aşırı aktifleştirilmiş TRPM2 kanallarının, imatinib mesilat ile indüklenen kardiyotoksisitede artmış sitosolik serbest kalsiyum, oksidatif stres ve apoptotik hücre hasarı ile ilişkili olduğunu, buna karşın astaksantinin bu aşamada modülatör bir rol oynayabileceğini gösterdik.

Anahtar kelimeler: İmatinib mesilat, TRPM2, oksidatif stres, kardiyomiyosit

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INTRODUCTION

Chronic myeloid leukemia (CML) is a myeloproliferative disease which develops from a translocation between chromosomes 9 (Abl) and 22 (Bcr). The mutant Bcr_Abl protein (active tyrosine kinase) can induce malignancies and is associated with CML [1]. Imatinib mesylate is a tyrosine kinase inhibitor and it is approved as a standard first-line therapy of CML [2]. Imatinib mesylate-induced cardiotoxicity including left ventricular dysfunction and heart failure has been shown before in previous studies [3,4]. In addition to limited clinical data on cardiotoxicity rates, imatinib-induced cardiac dysfunction is difficult to diagnose in early stages. Also, the underlying pathophysiological mechanism associated with imatinib-induced cardiotoxicity is not well understood. It is thought that imatinib is one of the cardiotoxic agents affecting mitochondrial function [5].

Antioxidant therapies have been evaluated in clinical trials with patients at risk of cardiovascular events. In most studies, antioxidants have failed to show any cardiovascular benefits since patients are not selected based on the presence of confirmed oxidative stress. Astaxanthin (xanthophyll carotenoid) is accepted as a potent antioxidant and with its anti-inflammatory properties, it has potential as a therapeutic agent in cardiovascular disease [6]. As the involvement of mitochondrial dysfunction and oxidative stress in cardiotoxic responses have been shown, we decided to examine the effects of imatinib on calcium signaling, apoptosis, mitochondrial depolarization levels and oxidative stress in cardiomyocytes. Also, we wanted to evaluate if there is a modulator effect of astaxanthin through TRPM2 channels in imatinib-induced cardiotoxicity.

MATERIALS AND METHODS

Reagents

Caspase-3 substrate (AC-DEVD-AMC) and Caspase-9 substrate (AC-LEHD-AMC) were provided from Enzo (Lausen, Switzerland). Dulbecco's modified Eagle's medium, Trypsin–EDTA, Fetal Bovine Serum and penicillin-streptomycine and Dimethyl sulfoxide, Dihydrorhodamine-123 (DHR 123) were provided from Sigma-Aldrich (St. Louis, MO), Fura-2 (AM) calcium florescent dye was bought from Calbiochem (Darmstadt, Germany). APOPercentage assay with releasing buffer were purchased from Biocolor (Belfast, Northern Ireland). Pluronic® F-127 was obtained from Biovision (San Francisco, USA). A mitochondrial stain 5,50, 6,60-tetrachloro-1,10,3,30-tetraethylbenzimidazolyl carbocyanine iodide (JC-1) and Probenecid were obtained from Santa Cruz (Dallas, Texas, USA). MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was obtained from Thermo Fischer (Waltham, MA, USA).

Cell culture

AC16 (Human cardiomyocyte cell line) was obtained from ATCC. Cardiomyocytes were cultured in DMEM containing 10% fetal bovine serum. Cardiomyocytes were seeded in 8-10 flasks at a density of 1×106 cells per flask. Cells were incubated in T25 flasks at 37°C at 5% CO2 in a humidified incubator. After cells have reached 75–85% confluence, cells were incubated with the chemical compounds described in the groups section. Cells were examined daily for evidence of contamination and after treatments, the cells were detached with %0.25 Trypsin EDTA for analysis and split into the sterile falcon tubes for analysis.

Study Groups

Cardiomyocytes were cultured at 37°C and divided into seven main groups.

Group 1 (Control): Cardiomyocytes were not incubated with Imatinib (IMTNB), Antranilic Acid (ACA) and Astaxanthin (ASTX) but were kept in a flask in the same condition for 72 h.

Group 2 (IMTNB): Cardiomyocytes were incubated with 50 µM Imatinib for 24 hrs.

Group 3 (IMTNB+ACA): Cardiomyocytes were incubated with 50 µM Imatinib for 24 hrs and then incubated with Antranilic acid (ACA, 0.04 mM, 30 min).

Group 4 (IMTNB+ASTX): Cardiomyocytes were incubated with 50 µM Imatinib for 24 hrs and then incubated with 40 µM Astaxanthin for 12 hrs.

Group 5 (IMTNB+ASTX+ACA): Cardiomyocytes

were incubated with 50 μM Imatinib for 24 hrs, then incubated with 40 μM Astaxanthin for 12 hrs and then incubated with Antranilic acid (ACA, 0.04 mM, 30 min).

Group 6 (ASTX): Cardiomyocytes were incubated with 40 μM Astaxanthin for 12 hrs.

Group 7 (ASTX+ACA): Cardiomyocytes were incubated with 40 μM Astaxanthin for 12 hrs and then incubated with Antranilic acid (ACA, 0.04 mM, 30 min).

In related experiments (except for calcium signaling), the cells were farther treated with Cumen hydroperoxyde (CMPx) (0.1 mM, 10 min) for activation of TRPM2 channel before related analysis and they were also inhibited the TRPM2 blocker ACA (0.04 mM, 30 min) before related analysis during 1.2 mM extracellular calcium. During calcium signaling analysis (Fura-2/AM), cells were stimulated on 20th cycles with 0.1 mM CMPx in the existence of 1.2 mM Calcium and calcium free buffer in extracellular environment.

Evaluation of intracellular free calcium concentration ([Ca2+]i)

After cell were incubated with the chemical compounds described in the groups section, cells were detached with %0.25 Trypsin–EDTA from T25 flasks, then centrifuged (100G, 5 min). The medium was taken off and changed with HEPES-buffered saline [HBS; 5 mM KCl, 145 mM NaCl, 10 mM D-glucose, 1 mM MgCl2, 1 mM CaCl2, (1.2 mM). 10 mM HEPES and 0.1% (w/v) BSA; pH 7.4] containing 5 μM Fura-2 AM (Ca2+-sensitive fluorescent ratiometric dye) and 0.05% (w/v) Pluronic F-127 and cells were incubated for 1 hour at 37˚C in the dark. The loaded cardiomyocyte cells were washed twice with HBS and covered with 1 ml of HBS supplemented with 2.5 mM probenecid for at least 20 minutes at 37˚C in the incubator (in the dark) to allow for Fura-2 AM de-esterification. Cells were seeded in clear flat-bottom 96-well (black) culture trays (Grainer Cell Star, Life Sciences USA) at a density of 3×104 cells/each well. Fluorescence emission intensity at 510 nm was determined in individual wells using a plate reader equipped with an automated injection system (SynergyTM H1, Biotek, USA) at alternating excitation wavelengths of 340 and 380 nm every 3 seconds for 50 acquisition cycles (cycle: 3 s; gain: 120) in response to agonists (CMPx, 0.1 mM) added by the automated injector. [Ca2+]i in cells was expressed as the average emission at 510 nm in each wells in response to excitation at 340 nm / 380 nm normalized to initial fluorescence emission obtained during the first 20 cycles. Measurement of [Ca2+]i was performed according to the previous study [7].

Evaluation of intracellular reactive oxygen species production

DHR-123 (Dihydrorhodamine 123) is a cell-permeable nonfluorescent reactive oxygen species (ROS) indicator can easily pass the cell membranes where it is oxidized to cationic rhodamine 123 which localizes in the mitochondria and exhibits green fluorescence. The Rh123 fluorescence intensities were determined by the previously described method using an automatic microplate reader (SynergyTM H1, Biotek, USA). Excitation and emission wave lengths of the analyses were 488 nm and 543 nm, respectively [8].

Assay for programmed cell death (apoptosis), caspase-3 / -9 activities

The apoptosis assay was executed with a commercial kit by Biocolor Ltd. (Northern Ireland) that according to the manufacturer instructions as method previously described [8,9]. The detection of apoptosis by spectrophotometry (SynergyTM H1, Biotek, USA) was achieved at 550 nm.

The activities of caspase-3 and -9 were measured by the previously described method [10]. The cleavages of caspases' substrates were determined by the microplate reader (SynergyTM H1, Biotek, USA) with 360 nm (excitation wavelength) and 460 nm (emission wavelength). The apoptosis and caspase values were given as fold change over the pre-treatment level.

Analyses of the Potential of Mitochondrial Membrane

The potential of mitochondrial membrane was determined by a cationic fluorescent dye which can pass through the cell membrane. This dye accumulates in the normally respiring mitochondria and reduction the red-to-green fluorescence

intensity in the medium shows mitochondrial depolarization. The membrane potential changes were evaluated by previously described method [11]. JC-1 fluorescence was measured by a single excitation wavelength (488 nm) with dual emission [green (520 nm)and red (596 nm)] using the microplate reader (SynergyTM H1, Biotek, USA). JC-1 values were assessed as fold change relative to untreated control cells.

The potential of mitochondrial membrane was determined by a cationic fluorescent dye which can pass through the cell membrane. This dye accumulates in the normally respiring mitochondria and reduction the red-to-green fluorescence intensity in the medium shows mitochondrial depolarization. The membrane potential changes were evaluated using the previously described method [11]. JC-1 fluorescence was measured by a single excitation wavelength (488 nm) with dual emission [green (520 nm)and red (596 nm)] using the microplate reader (SynergyTM H1, Biotek, USA). JC-1 values were assessed as fold change relative to untreated control cells.

Cell viability (MTT) assay

The MTT method for measuring the mitochondrial activity of living cells was used in this study to demonstrate the effects of IMTNB and ASTX on cell viability. Cardiomyocytes were incubated with the chemical compounds described in the Experimental Design Section and then performed by previously described method by an automatic microplate reader (SynergyTM H1, Biotek,USA) [12].

Statistical analyses

All results were presented as means ± standard deviation (SD). Differences between groups were analyzed with one-way ANOVA. Statistical analyses were calculated using GraphPad Prism version 7.04 for windows (GraphPad Software, San Diego California, the USA). P<0.05 was considered significant.

RESULTS

Effects of imatinib and astaxanthin on cytosolic calcium levels in cardiomyocytes: The effect of imatinib and astaxanthin administrations on cytosolic calcium levels in cardiomyocyte cells are shown in figure 1A / 1B. The TRPM2 channel antagonist antranilic acid (ACA) was used in in-vitro model to evaluate the receptors involved in Ca2+ increase through TRPM2 channels. As shown in figure 1B, the Ca2+ concentration in cardiomyocytes was higher in the imatinib than in the control (p<0.001). The Ca2+ concentration was lower in the astaxanthin+ACA compared to the control (p<0.05). Also, cytosolic Ca2+ concentration was lower in the imatinib+ACA, imatinib+astaxanthin and imatinib+astaxanthin+ACA than in the imatinib (p<0.001). In addition, in cytosolic Ca2+ concentration, there is no statistically significant difference between imatinib+astaxanthin+ACA compared to the imatinib+astaxanthin group.

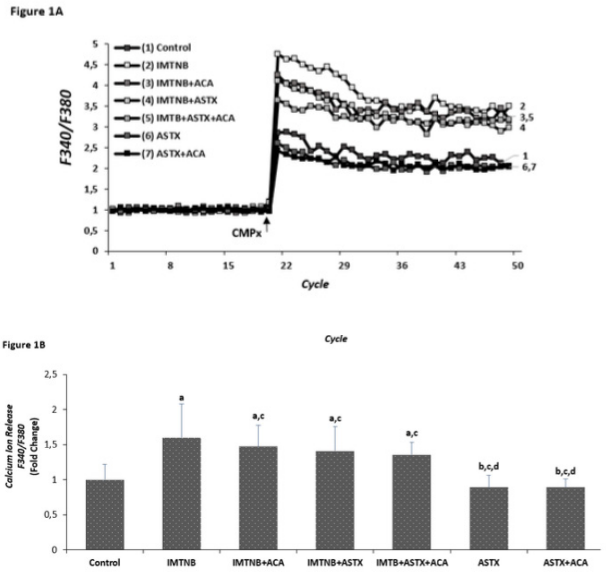

Figure 1A / 1B . The effect of Imatinib (50 µM, 24 hrs) and Astaxantin (40 µM, 12 hrs) on cytosolic calcium levels in cardiomyocyte cells. Cardiomyocytes are stimulated by Cumene hydroperoxide (CMPx 0.1 mM and on 20th cycle) but they were inhibited with Antranilic Acid (ACA 0.1 mM for 30 min) (mean ± SD and n=3). ap<0.001 and bp<0.05 vs control, cp≤0.001 vs IMTNB, dp<0.001 vs IMTNB+ASTX and fp<0.05 vs ASTX .

Effects of imatinib and astaxanthin on apoptosis, ROS and MTT (Cell Viability) levels in cardiomyocytes

Effects of imatinib and astaxanthin administrations on apoptosis levels are shown in figure 2A. The values were higher in the imatinib group than in the control group (p<0.001). The apoptosis values were significantly lower in the astaxanthin and the imatinib+astaxanthin than in the imatinib group of cardiomyocytes (p<0.001). Also, the values were significantly lower in the imatinib+astaxanthin+ACA

when compared with the imatinib+astaxanthin (p<0.001).

Intracellular ROS production of groups are shown in figure 2B. The ROS production values were higher in the imatinib group than in the control (p<0.001). The values were significantly lower in the imatinib+ACA (p<0.001), the imatinib+astaxanthin (p<0.001) and the imatinib+astaxanthin+ACA (p<0.001) than in the imatinib. Also, the ROS production was markedly lower in the imatinib+astaxanthin+ACA when compared to the imatinib+astaxanthin (p<0.001).

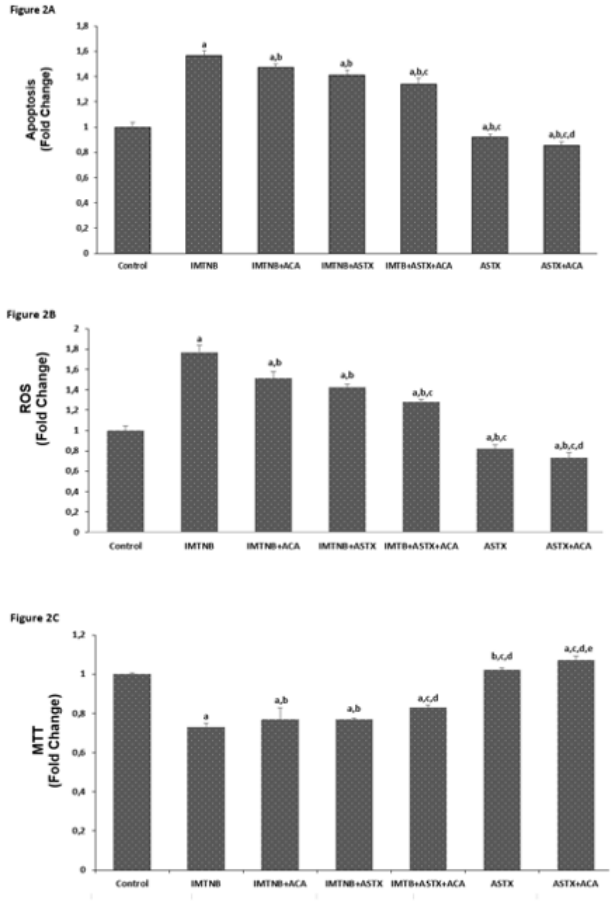

Figure 2A / 2B / 2C. The effect of Imatinib (50 µM, 24 hrs) and Astaxantin (40 µM, 12 hrs) on Apoptosis (A), ROS (B), and MTT (C) levels in the Cardiomyocyte cells. Cardiomyocyte are stimulated by Cumene hydroperoxide (CMPx 0.1 mM for 10 min) but they were inhibited by Antranilic Acid (0.04 mM, 30 min) (mean ± SD and n=10). 2A/2B : ap<0.001 vs control, bp<0.001 vs IMTNB, cp<0.001 vs IMTNB+ASTX and dp<0.001 vs ASTX .2C: ap<0.001 vs control, bp<0.05 and cp<0.001 vs IMTNB, dp<0.001 vs IMTNB+ASTX and ep<0.001 vs ASTX

MTT (cell viability) values in the groups are shown in figure 2C. MTT values were lower in the imatinib than in the control (p<0.001). The values were significantly higher in the imatinib+ACA (p<0.05), the imatinib+astaxanthin+ACA (p<0.001) and the imatinib+astaxanthin (p<0.05) than in the imatinib. In addition, MTT values were higher in the imatinib+astaxanthin+ACA when compared to the imatinib+astaxanthin (p<0.001).

Effects of imatinib and astaxanthin on intracellular ROS production in cardiomyocytes

Effects of imatinib and astaxanthin on caspase 3, caspase 9 activities, mitochondrial depolarization levels in cardiomyocytes

Mitochondrial membrane depolarization levels, caspase-3 and -9 activities of groups are shown in figure 3A,3B,3C respectively. It has been shown that caspase 3 and 9 activities have an important role in the mitochondrial apoptotic pathways. Also, they are associated with mitochondrial cytochrome c release during the apoptotic cascade.

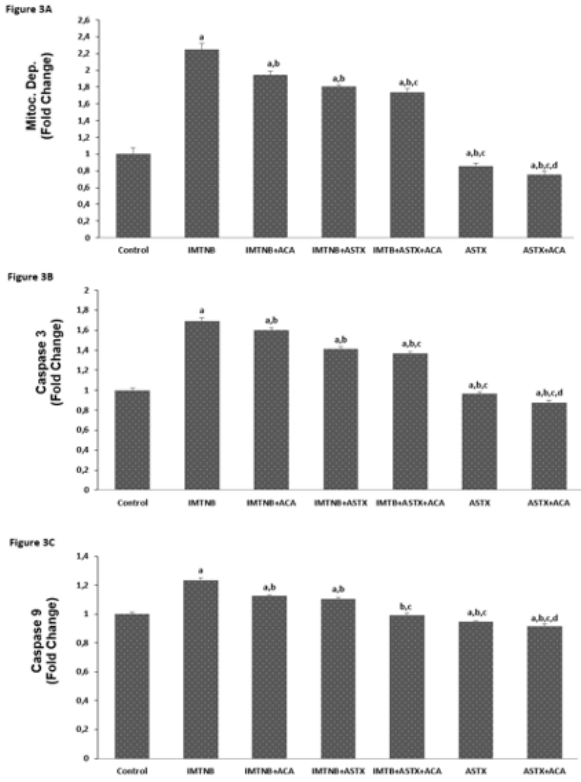

Figure 3A /3B / 3C. The effect of Imatinib (50 µM, 24 hrs) and Astaxantin (40 µM, 12 hrs) on Mitochondrial Depolarization (A), Caspase 3 (B) and Caspase 9 (C) levels in the Cardiomyocyte cells. Cardiomyocyte are stimulated by Cumene hydroperoxide (CMPx 0.1 mM for 10 min) but they were inhibited by Antranilic Acid (ACA 0.04 mM for 30 min) (mean ± SD and n=10). 3A: ap<0.001 vs control, bp<0.001 vs IMTNB, cp<0.05 vs IMTNB+ASTX and dp<0.001 vs ASTX. 3B/3C: ap<0.001 vs control, bp<0.001 vs IMTNB, cp<0.001 vs IMTNB+ASTX and dp<0.001 vs ASTX .

The mitochondrial depolarization, caspase 3, caspase 9 activities values were higher in the imatinib group than in the control ($p<0.001$). The mitochondrial depolarization, caspase 3, caspase 9 values were significantly lower in the imatinib+ACA ($p<0.001$), the imatinib+astaxanthin ($p<0.001$) and the imatinib+astaxanthin+ACA ($p<0.001$) than in the imatinib. Also, the mitochondrial depolarization, caspase 3, caspase 9 values production was markedly lower in the imatinib+astaxanthin+ACA when compared to the imatinib+astaxanthin (Mit. dep. $p<0.05$; caspases $p<0.001$).

DISCUSSION

In our study, we observed that imatinib increased oxidative stress, cytosolic calcium levels and apoptosis in cardiomyocytes. Imatinib mesylate is one of the first tyrosine kinase inhibitor and represents a revolution in the management of patients with chronic myeloid leukemia [13]. Cardiotoxic effect of imatinib was reported first in 2006 by Kerkala et al. Authors collected clinical data from patients who developed left ventricular dysfunction after few months of imatinib therapy. They reported that imatinib had detrimental effects on cardiomyocytes in culture and ultra-structured evaluations revealed mitochondrial dysfunction and prominent membrane whorls in myocytes [3]. Existing data strongly suggests that alterations in endoplasmic reticulum stress pathways leading to mitochondrial function impairment, has an essential role in the cause of imatinib -induced cardiotoxicity [5]. In a study by Perik et al., it was been suggested that only patients with a history of cardiac disease should have cardiac monitorization during imatinib therapy [14]. However, in a clinical trial for imatinib mesylate, Cohen et al. reported left ventricular dysfunction and congestive heart failure in patients without a prior history [15]. These studies revive questions regarding the potential cardiotoxic effects of imatinib mesylate therapy. A more recent report has noted that imatinib mesylate related adverse effects are associated with oxidative stress and mitochondrial dysfunction [16]. Furthermore, previous study showed that imatinib could target cardiomyocytes and mitochondrial dysfunction and cell death could be aggravated by the presence of oxidative stress [17]. Reactive oxygen species and oxidative stress parameters seem to play an important role in chemotherapy-induced cardiotoxicity, leading to cardiac dysfunction [18]. Mitochondria has an important role in cell survival as well as apoptosis. Also, it has been known that increased cytosolic calcium levels stimulate respiratory chain activity and oxidative stress which lead to release proapoptotic factors [19]. It remains controversial whether left ventricular dysfunction with imatinib mesylate is associated with myocyte death or myocyte dysfunction. Barr et al. has suggested that imatinib can alter intracellular calcium levels and has a potential to cause cardiomyopathy resulting from cell death [20]. Calcium plays a crucial role in cardiomyocyte homeostasis and survival. Transient receptor potential (TRP) family are one of the important plasma membrane transporters of calcium ions. Transient receptor potential channels influence cell death rates and affect different pathologies including cardiovascular, neurological, metabolic or neoplastic disorders [21]. TRP melastatin 2 (TRPM2) is the second member of the TRP melastatin subfamily and is expressed in many cell types including brain, heart and endothelial cells [22]. It has been demonstrated that TRPM2 channels are involved in several physiological processes, such as oxidative stress and apoptosis [23]. Wang et al. reported that oxidative stress activated TRPM2 channels and then by positive feedback, it further induces intracellular ROS production and causes loss of mitochondrial membrane potential [24]. In another study, it has been shown that calcium entry via TRPM2 is important in maintaining mitochondrial function and reducing oxidative stress in cardiomyocytes [25]. Also, the results of the previous studies showed that, TRPM2 channels are mainly associated with calcium overload, mitochondrial dysfunction and apoptosis signaling pathway [26].

In our study we observed that TRPM2 channels are present in cardiomyocytes and they are stimulated by cumene hydroperoxide, whereas blocked by antranilic acid respectively. The results of the present study demonstrated that imatinib mesylate increased oxidative stress, calcium entry and apoptosis in cardiomyocytes. In addition, we observed that caspase 3, caspase 9 and intracellular ROS production values were decreased by astaxanthin administration in

cardiomyocytes through modulation of TRPM2 channels.

In the light of the data from previous studies, it has been known that left ventricular dysfunction and many other conditions that predispose heart failure are associated with oxidative stress [27]. Several studies have demonstrated beneficial effects of a therapy with antioxidant agents against endothelial dysfunction, ischemia-reperfusion injury or cardiotoxic agent-induced myocardial damage [28,29]. Astaxanthin is a xantophyll carotenoid that is found in a variety of living organisms especially in the marine environment. It has antioxidant and anti-inflammatory effects. In a review about astaxanthin in cardiovascular disease, it has been reported that biomarkers of oxidative stress and inflammation are decreased by astaxanthin supplementation [30]. Chemotherapy-induced cardiotoxicity is a life-threatening complication which limits the clinical use of chemotherapeutic agents. Also understanding the molecular mechanisms of chemotherapy induced cardiotoxicity is necessary to improve effective preventive strategies. To the best of our knowledge, there is no study that examines the effect of using a combination of imatinib mesylate and astaxanthin on apoptosis, oxidative stress and calcium influx through TRPM2 channels in cardiomyocytes. The present study demonstrated that astaxanthin modulates imatinib-induced oxidative stress and apoptosis through TRPM2 channels. Also, we observed that astaxanthin suppressed mitochondrial depolarization levels and had protective effect on the apoptosis as indicated by caspase 3 and caspase 9 values in myocytes.

In conclusion, understanding the pathophysiological mechanism, as well as an increased awareness of chemotherapy-induced cardiotoxicity, could improve clinical care of cancer patients. Astaxanthin can be a useful preventive agent and TRPM2 channels can be potential therapeutic targets in patients with imatinib-induced cardiotoxicity.

Study limitations

In the present study, we did not evaluate concentration response to distinguish effects of different toxic levels of imatinib on the molecular mechanism studied. Additionally, we were not able to perform an electrophysiological study and evaluate if imatinib and astaxanthin administrations also change the expression of TRPM2 channels in cardiomyocytes.

Conflict of Interest: No conflict of interest was declared by the authors

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