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Effect of lipoic acid on cisplatin-induced cardiotoxicity and inflammation in cardiomyocytes

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ABSTRACT

Aims: Cisplatin (CPL) is a potent chemotherapeutic agent widely used to treat various cancers. However, CPL causes toxicity in various tissues, including the heart. Lipoic acid (LPA) is a thiol compound with antioxidant, anti-inflammatory, and anti-apoptotic properties. Although LPA has been reported to have protective effects in various cardiac diseases, the mechanisms underlying its cardio-protective effects have not been elucidated. This study aimed to establish a CPL-induced cardiotoxicity model in the H9c2 cardiomyocyte cell line, to understand the mechanisms underlying this cardiotoxicity, and to investigate the effect of LPA on cardiotoxicity.

Methods: H9c2 cardiomyocyte cells as control (CNT), CPL (40 µm), and LPA-1 (300 µm LPA, and 40 µm CPL), LPA-2 (500 µm LPA, and 40 µm CPL) in combination along with CIS were used. In the analyses made, glutathione (GSH) and glutathione peroxidase (GSHPx) enzyme activity, lipid peroxidation [malondialdehyde, (MDA)] levels, inflammation markers interleukin (IL) -1β, IL-6, and tumor necrosis factor (TNF) -α levels, total oxidant/antioxidant (TOS and TAS) status levels, reactive oxygen species (ROS) and caspase activity (Casp 3 and 9) in the cells were determined.

Results: CIS treatment caused cardiomyocyte cell toxicity and increased Casp 3, Casp 9, ROS, IL-1β, IL-6, TNF-α, TOS, and MDA levels while decreasing GSHPx, GSH, and TAS levels. Increased inflammation and impaired oxidant/antioxidant balance in cardiomyocyte cells after CPL treatment were regulated by LPA treatment.

Conclusion: LPA treatment was found to have a protective effect against CPL-induced cardiotoxicity in cardiomyocyte cells.

Keywords: Apoptosis, lipoic acid, cardiotoxicity, cisplatin, oxidative stress

INTRODUCTION

Cisplatin (CPL) is a platinum based compound widely used as a chemo-therapeutic agent against various human cancers.[1] However, despite the anticancer efficacy of CPL, its toxicity in various tissues limits its safety in chemotherapy.[2] CPL, which has anti-cancer activity by inducing reactive oxygen species (ROS) that trigger cell death and DNA damage, causes toxicity by inducing the same effects on non-cancer cells.[3-5] As it is known that CPL treatment has significant side effects such as cardiotoxicity, ototoxicity, nephrotoxicity, and hepatotoxicity, intensive experimental and clinical studies are underway to discover new agents to reduce toxicity in these tissues.[1,3,4,6] Although CPL-induced cardiotoxicity is not frequently observed as an acute effect, it is an important side effect observed in the long-term survival of patients receiving CPL chemotherapy.[7] Therefore, the identification of agents that reduce drug toxicity during and after chemotherapy is very important for patients. CPL treatment can cause cardiotoxic effects in patients, such as congestive heart failure or even sudden cardiac death.[4] By clarifying the multifactorial physio-pathological mechanisms underlying the cardiotoxic effects of CPL, it may be able to reduce its side effects during treatment. In the literature, the results of studies related to CPL-induced cardiotoxicity have not fully elucidated the mechanisms of damage. Evidence suggests that inflammation[7,8] oxidative stress (OS)[6,9,10] and apoptosis[11] are the main factors in CPL toxicity. CPL has been shown to induce apoptosis by depleting glutathione (GSH) and deactivating glutathione peroxidase (GSHPx).[7] It has been reported that CPL may cause mitochondrial dysfunction and, consequently, activate the apoptotic pathway by increasing intracellular ROS levels.[2,4] The effects of adding compounds with anti-apoptotic, antioxidant, and anti-inflammatory properties to CPL chemotherapy on slowing CPL-induced myocyte damage have been investigated. Still, experimental studies should be continued as the results are uncertain.

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Lipoic acid (LPA), known to have anti-apoptotic, antioxidant, and anti-inflammatory properties, is a thiol compound found naturally in plants and animals.[12] As LPA is an important factor in enzymes that scavenge reactive oxygen species, the antioxidant properties of LPA have recently attracted considerable interest.[13] LPA has been reported to be effective in preventing and treating OS in many models or clinical conditions.[12,14] In addition, recent studies have shown that LPA is also protective against cardiac oxidative damage induced by cardiovascular disease and cardiotoxic drugs.[14,15] Therefore, LPA may serve as a protective agent against the risk factors of cardio-vascular disease. Although the ability of LPA to directly scavenge ROS appears to be partly responsible for its cardio-protective effects, it is not known whether the cardio-protective effects of LPA also occur through other mechanisms, such as the induction of endogenous antioxidant enzymes such as GSH and GSHPx in the myocardium, the reduction of inflammatory markers such as tumor-necrosis-factor-alpha (TNF-α), interleukin (IL) -1β, and IL-6, and caspase activation.

The limited number of studies in the literature showing that CPL-induced myocardial damage is improved by LPA application focused more on oxidative parameters. In contrast, the present study analyzed inflammatory markers, apoptotic caspase pathways, and OS parameters, providing more reliable results. In this study, H9c2 cells were used as an experimental model of CPL-induced cardiotoxicity, which was induced by CPL in these cells. CPL-induced damage in H9c2 cells and the regulatory effects of LPA on this damage were evaluated by analyzing caspase activation (Casp 3 and 9) and inflammation markers (TNF-α, IL-1β, and IL-6 levels). In addition to GSH and GSHPx levels, total antioxidant/oxidant status (TAS and TOS) was analyzed to investigate OS. Moreover, lipid peroxidation as malondialdehyde (MDA) levels were analyzed to determine lipid peroxidation.

METHODS

The current study has no study with human and human participants. The study is not subject to ethics committee approval. All procedures were carried out in accordance with the ethical rules and the principles.

Chemicals

LPA (Cat; 437692) and CPL (Cat; PHR1624) were purchased from Sigma Aldrich C. (USA). Caspase 3 (Cat; SRBT-81638), Caspase 9 (Cat; SRBT-82090), IL-1β (Cat; SRBT-83324), IL-6 (Cat; SRBT-83168), reactive oxygen species (Cat; 201-11-0305), and TNF-α (Cat: SRBT-82883) were obtained from Sun. Red Biotech Comp (SRB) Ltd (China). Total antioxidant/oxidant capacity (TAS-Pro No: RL0017 and TOS-Pro No: RL0024) ELISA kit was purchased from RelAssay (Turkiye).

Cell Culture

The manufacturer's instructions prepared the growth medium for the cells used in the study. Dulbecco's-modified-eagle's-medium (DMEM, Cat; L0064, Biowest, France) was supplemented with 10% Fetal-bovine-serum (FBS, Cat; SV30160.03, Cytiva, Turkiye) and 1% antibiotics (Cat; LMA 4118, Biosera, Turkiye). Cells previously purchased from ATCC company and reached 6-8 passages were used. The growth medium of the cells was prepared following the experimental procedure for the H9c2 embryonic cardiomyocyte cells taken from the nitrogen tank. The H9c2 embryonic cardiomyocyte cells were passaged and divided into four groups after reaching 80-85% confluence, and then this process was repeated. Cells in T25 (flask with 25 cm^2 surface area) culture flasks were cultured in an incubator (95% air, 5% CO2, and 37 °C). According to the experimental procedure, treatments were applied to the cells according to the groups. Depending on the experimental group, the following treatment procedure was used. Cells were washed with fresh 1x phosphate-buffered saline (PBS) (Cat; LM-S2043/500, Biosera, Turkiye) at the end of the incubation period, and trypsin-EDTA (0.25%) (Cat; LM-T1720/100, Biosera, Turkiye) was used to detach the cells from the bottom of the flask. Analyses were performed for all groups after completion of the experimental steps.

Experimental Groups

The cardiomyocyte cell line was divided into four groups.

CNT (n: 6), H9c2 cells in this group were not treated with any treatment and were incubated (24 h).

CPL (n: 6), H9c2 cells in this group were treated with 40 µm CPL and were incubated (24 h).[4]

LPA-1 (n: 6), H9c2 cells in this group were pre-treated with 300 µm LPA[16,17] 3 hours before 40 µm CPL treatment, followed by 40 µm CPL treatment and were incubated (24 h).

LPA-2 (n: 6), H9c2 cells in this group were pre-treated with 500 µm LPA[18] 3 hours before 40 µm CPL treatment, followed by 40 µm CPL treatment and were incubated (24 h).

Cell Homogenate Preparation Steps

Following the kit instructions, the cells for each group were added to separate Eppendorf tubes and centrifuged (1000 RPM, 5 min). The following steps were followed: Using a pipette, the supernatants were removed from the top of the Eppendorf tubes, and the cell pellets underneath were diluted in PBS (pH 7.4) to a concentration of $1x10^6$ cells/ml. The H9c2 embryonic cardiomyocyte cell structure was lysed by freeze thaw repetition, and the mixture was centrifuged at 4 °C (3000 RPM, 20 min.) after removing the cytoplasmic components. Any supernatants were removed by automated pipetting and transferred to new sterile tubes for further analysis.

Analysis

Caspase, reactive oxygen species, and inflammation markers levels in the cardiomyocyte cells: Caspase, ROS, and inflammation markers (TNF-α, IL-1β, and IL-6) levels in cardiomyocyte cell supernatants were determined (ELISA kit). For analyses according to the kit protocol following the instructions of the commercially purchased company, the following steps were followed: supernatants were incubated (37°C) according to the specified protocols, using a pipette, the supernatant and standard samples were transferred into 96-well plates, standard and supernatant samples placed on 96-well plates were incubated (60 min), washing steps were applied and staining solutions were added and incubated (15 min). At the end of all these procedures, a stop solution was added, and an ELISA spectrophotometer was used to read the absorbance values (Bio Tek EL808™).[19,20]

Total antioxidant/oxidant status levels in the cardiomyocyte cells: Supernatants of the samples were used for TAS analyses, and the following steps were followed for TAS analyses according to the kit protocol and the instructions of the

commercially purchased company. The method is based on reducing the colored azinobis radical by antioxidants to a colorless reduced form. Sample supernatants, kit standard, and dH20 were mixed with 200 µl Reagent 1 buffer in 96 well plates and incubated according to the protocol (5 min). The first absorbance of the sample was measured at 660 nm with an ELISA reader, then 30 µl of Reagent-2 buffer was added, and the mixtures were incubated (37°C, 5 min). After incubation, the second absorbance value of the sample was measured at 660 nm with an ELISA reader. The kit standard equivalent to 1 mmol/L Trolox was used to calculate the data for each sample.

Supernatants of the samples were used for TOS analyses, and the following steps were followed for TOS analyses according to the kit protocol and the instructions of the commercially purchased company. The ferrous ion-dianisidine complex is oxidized to ferric ions by oxidizing agents in the sample. A coloured complex is produced by the ferric ion in an acidic medium with xylenol orange, which is related to the amount of oxidant molecules (colour intensity associated with the amount of oxidant molecules). For the dilution step, Standard-2 (5 µl) and distilled water (1 ml) were added to an Eppendorf tube, vortexed (20 seconds), then 5 µl of this solution was added to another Eppendorf tube, and by adding 1 ml of distilled water, 20 mm H2O2 was prepared. Afterward, 200 µL of Reagent-1 buffer was transferred to each well of 96-well plates, and the first TOS absorbance of the sample was measured at 530 nm with an ELISA reader. Next, 10 µl Reagent-2 was added, and samples were incubated (37°C, 5 min). After incubation, the second TOS absorbance value of the sample was measured at 530 nm with an ELISA reader. The kit standard (µmol H2O2 equivalents/L) was used to calculate the data for each sample.[19]

Glutathione, glutathione peroxidase, and lipid peroxidation, levels in the cardiomyocyte cells: Lipid peroxidation (malondialdehyde, MDA) levels of cardiomyocyte cells were analyzed by spectrophotometer according to the method described by Placer et al.[21] In the experiment, cardiomyocyte cell groups were diluted 1/9 (2.25 ml) with thiobarbituric acid (TBARS) solution. A mix of 1/9 of TBARS and 0.25 ml phosphate buffer was used as a blind. The samples and the blind were kept in boiling water at 100°C in a water bath for 20 minutes.[22] Then they were then cooled with tap water. Afterwards, 3500 RPM centrifugation was performed for 5 min. After centrifugation, the top pink coloured liquid was collected with a pipette (500 µl). The liquid was read against the blind in a spectrophotometer (V-730 UV, spectrophotometer Japan) at 532 nm wavelength in a cuvette with 1 cm light transmission. The standard used was 1,1,3,3,3 tetraethoxypropane solution prepared in the same proportions. The results were given as µmol/g protein.

Cardiomyocyte cell GSH levels were determined using the Sedlak and Lindsay[23] technique. Cardiomyocyte cell GSHPx levels were measured by the Lawrence and Burk[24] technique (V-730 UV spectrophotometer Japan). The solutions required for GSH determination were 10% trichloroacetic acid (TCA) solution and Tris-II buffer. Tris-II buffer (pH: 8.9 and 0.4 M) was prepared by dissolving 48.46 grams of tris-hydroxymethyl-aminomethane in 1 L of distilled water (final volume 1 L), and the pH was adjusted to 8.9 with hydrochloric acid (HCl).

A pipette added cells to falcon tubes for GSH analysis (10^6 cells per ml). Afterwards, 0.1 ml cardiomyocyte cell homogenate fluid and 0.4 ml trichloro-acetic-acid (TCA) solution were transferred to a separate Eppendorf tube. Then, it was mixed on a vortex for 20 seconds and centrifuged (3000 RPM, 5 min). Centrifugation was performed to separate proteins. Then, the following steps were followed: After centrifugation, 0.4 ml supernatant was taken into a clean glass tube, and 2,0 ml Tris-II buffer and 0.1 ml DTNB (0.099 g dissolved in 25 ml methanol) solution were added. The yellow color formed after the added solutions were read with a spectrophotometer at a wavelength of 412 nm in a cuvette with a light transmission of 1 cm against distilled water. And GSH analyzed values were µmol/g protein calculated as.

Solutions required for GSHPx determination: Tris-I buffer solution (pH: 7.6 and 50 mm,), GSH solution, CHPO (cumene hydro peroxide) solution, 10% TCA solution, Tris-II buffer (pH: 8.9 and 0.4 M), DTNB [5.5 dithiobis (2 nitro–benzoic-acid)] solution. Tris-I buffer (pH: 7.6 and 50 mm) was prepared by dissolving 6,057 grams TRIS-hydrochlorid and 0,372 grams EDTA in 1 litre of distilled water (final volume 1 litre), and the pH was adjusted to 8.9 with hydrochloric acid (HCl).

In the experiment, 0.5 ml cardiomyocyte cell homogenate fluid, 0.3 ml Tris-I HCl buffer, and 0.1 ml CHPO (except control) solution were mixed. 0.1 ml GSH (5 s intervals for each tube) was added (in an Eppendorf tube) and kept at room temperature for 10 minutes, and 1.0 ml TCA (5 s intervals for each tube) was added. Centrifuged at 2500 RPM for 5 minutes. After centrifugation, the upper 0.1 ml supernatant was taken into a clean glass tube, and 2 ml Tris-II buffer and 0.1 ml DTNB were added. Waiting time was 5 minutes at room temperature. The yellow color formed after the added solutions were read with a spectrophotometer at a wavelength of 412 nm in a cuvette with a light transmission of 1 cm against distilled water. The GSHPx content of the samples was determined by spectrophotometry at a wavelength of 412 nm, and GSHPx values were (international units) IU/g protein calculated as.

Statistical Analysis

Data analyses were performed with SPSS (ver. 17.0, software, USA) software, and all data were expressed as mean ± standard deviation (SD). A one-way ANOVA, Post-hoc Tukey test was used to evaluate all data showing statistically significant differences between groups. A value of $p<0.05$ was considered statistically significant.

RESULTS

Effect of LPA on Caspase, Reactive Oxygen Species, Total Oxidant Status Levels in Cardiomyocyte Cells

LPA treatment modulated the increase in ROS, TOS, Casp 3, and Casp 9 levels in CPL-treated cardiomyocyte cells (Figure 1). A significant increase in ROS (Figure 1A), TOS (Figure 1B), Casp 3 (Figure 1C), and Casp 9 (Figure 1D) levels was observed in the CPL-treated group was compared to CNT, LPA-1, and LPA-2 groups ($p\leq0.05$). The increase in ROS, TOS, Casp 3, and Casp 9 levels after CPL treatment in cardiomyocytes was regulated by LPA treatment. Significant results were obtained in H9c2 embryonic cardiomyocytes pre-treated with 300 and 500 µm LPA for ROS, TOS, Casp 3, and Casp 9 levels. However, the CPL-induced and disturbed oxidant/antioxidant balance was further regulated by 500 µm LPA.

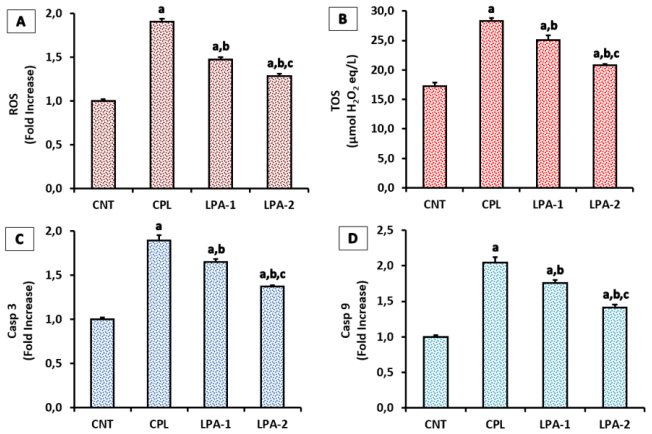

Figure 1. Effect of LPA on ROS (A), TOS (B), Casp 3 (C), and 9 (D) levels in H9c2 embryonic cardiomyocytes after CPL-induced cytotoxicity (mean±SD) ([a]p≤0.001 vs CNT group, [b]p≤0.001 vs CPL group, [c]p≤0.001 vs LPA-1 group)

LPA: Lipoic acid, ROS: Reactive oxygen species, CPL: Cisplatin, SD: Standard deviation, CNT: Cardiomyocyte cells as control

Effect of LPA on Inflammation in Cardiomyocyte Cells

LPA treatment modulated the increase in TNF-α, IL-1β, and IL-6 levels in CPL-treated cardiomyocyte cells (Figure 2). A significant increase in IL-1β (Figure 2A), IL-6 (Figure 2B), and TNF-α (Figure 2C) levels was observed in the CPL-treated group was compared to CNT, LPA-1, and LPA-2 groups (p≤0.05). The increase in TNF-α, IL-1β, and IL-6 levels after CPL therapy in cardiomyocytes was regulated by LPA therapy. Significant results were obtained in H9c2 embryonic cardiomyocytes pre-treated with 300 and 500 μm LPA for TNF-α, IL-1β, and IL-6 levels. However, the CPL-induced and disturbed inflammatory cytokines balance was further regulated by 500 μm LPA.

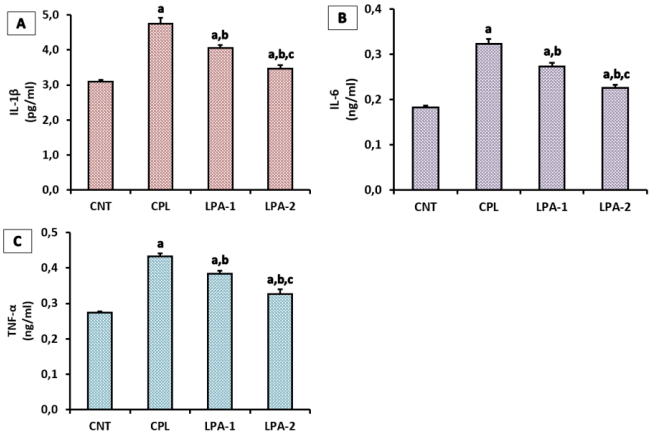

Figure 2. Effect of LPA on IL-1β (A), IL-6 (B), and TNF-α (C) levels in cardiomyocytes after CPL-induced cytotoxicity (mean±SD) ([a]p≤0.001 vs CNT group, [b]p≤0.001 vs CPL group, [c]p≤0.001 vs LPA-1 group)

LPA: Lipoic acid, TNF: Tumor necrosis factor, CPL: Cisplatin, SD: Standard deviation, CNT: Cardiomyocyte cells as control

The LPA therapy regulated the cisplatin-induced changes in total antioxidant status, lipid peroxidation, glutathione peroxidase, and glutathione levels: LPA therapy regulated the increase in lipid peroxidation (MDA) and disturbance of the antioxidant balance (GSH, GSHPx, and TAS) levels in CPL-treated cardiomyocyte cells (Figure 3). A significant decrease in GSH (Figure 3A), GSHPx (Figure 3B), and TAS (Figure 3D) levels was observed in the CPL-treated group compared to CNT, LPA-1, and LPA-2 groups (p≤0.05). A significant increase in MDA (Figure 3C) levels was observed in the CPL-treated group compared to CNT, LPA-1, and LPA-2 groups (p≤0.05). Elevated MDA and reduced GSH, GSHPx, and TAS levels following CPL therapy in cardiomyocytes were regulated by LPA therapy. Significant results have been achieved in cardiomyocytes pre-treated with 300 and 500 μm GAL for GSH, GSHPx, and MDA levels. However, the CPL-induced and disturbed oxidant/antioxidant balance was further regulated by 500 μm LPA.

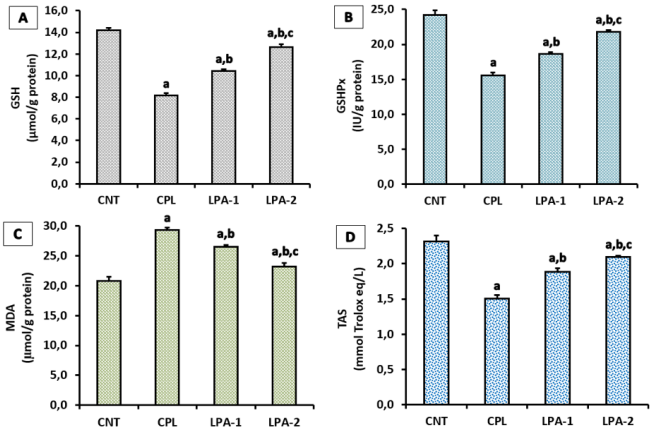

Figure 3. Effect of LPA on GSH (A), GSHPx (B), MDA (C), and TAS (D) levels in cardiomyocytes after CPL-induced cardiotoxicity (mean±SD) ([a]p≤0.001 vs CNT group, [b]p≤0.001 vs CPL group, [c]p≤0.001 vs LPA-1 group)

LPA: Lipoic acid, GSH: Glutathione, GSHPx: Glutathione peroxidase, MDA: Malondialdehyde, TAS: Total antioxidant, CPL: Cisplatin, SD: Standard deviation, CNT: Cardiomyocyte cells as control

DISCUSSION

Although CPL is a potent chemotherapeutic agent, significant side effects such as cardiotoxicity limit its use.[1,4] In this context, it is important to add protective compounds to CPL chemotherapy to reduce toxic effects in patients who need to receive CPL chemotherapy. In this study, H9c2 cells were treated with CPL (40 μm), and drug-induced cardiotoxicity was assessed. In addition to changes in OS parameters and inflammatory markers in CPL-treated cells, whether CPL induces apoptosis in cells was investigated by measuring caspase levels. Recent studies in the literature have reported that CPL therapy is associated with cardiotoxicity.[4,25,26] It has been proposed that CPL may induce ROS and OS, apoptosis, and inflammation in cells, leading to cardiotoxicity.[27,28] CPL has also been reported to react rapidly with thiol containing molecules, leading to depletion of glutathione and related antioxidants and accumulation of ROS.[29] Research has reported that CPL increases ROS levels and lipid peroxidation while decreasing the activity of catalase, GSH, and superoxidedismutase (SOD) enzymes.[30,31] In this study, in agreement with the literature, we showed that MDA levels increased in the CPL-treated groups in H9c2 myocyte cells compared to the CNT group. At the same time, GSH and GSHPx enzyme levels decreased. With the observed decrease in cardiac GSH levels and increase in MDA levels, the reduction in GSHPx enzyme activity may be evidence of OS induced by CPL therapy. Indeed, similar studies have reported a significant decrease in antioxidant capacity with a substantial increase in MDA levels in the heart and other tissues following CPL therapy.[32-35] The increase in MDA levels can be attributed to the rise in ROS caused by CPL.

Several antioxidant molecules have been tested in studies that predict that they may prevent toxicity by reducing CPL-induced OS. In this study, we applied two doses of 300 μM and 500 μm LPA to investigate the efficacy of LPA in preventing cardiotoxicity. Our results showed that CPL treatment led to a significant increase in inflammatory markers, apoptosis, and OS, as well as a decrease in antioxidant capacity. Conversely,

in the H9c2 cardiomyocytes we pre-treated, we found that LPA reduced cardiac damage by regulating these parameters. Cao et al.[12] showed that LPA could induce several endogenous antioxidants in H9c2 cardiomyocytes and that LPA-mediated enhancement of cellular defences significantly conferred resistance to ROS-induced cardiac cell injury. LPA has been reported to have beneficial effects against nephrotoxicity[36] and neurotoxicity[13,37] by suppressing CPL-induced OS. Another study reported that LPA reduced sodium nitroprusside-induced damage in H9c2 cells by downregulating ROS.[18] Consistent with the literature, this study demonstrated that in H9c2 myocyte cells, MDA levels increased in the CPL-treated groups compared to the CNT group. Accordingly, GSH and GSHPx enzyme activities decreased. We found that antioxidant enzyme activities increased and MDA levels decreased in the LPA-1 and LPA-2 groups treated with LPA compared to the CPL group. Thus, our study demonstrated that LPA might protect cardiomyocytes against oxidative damage. There are only a few studies on the effect of LPA on cardiomyocyte damage caused by CPL.[32,36] Hussein et al.[36] found that the CPL-induced cardiotoxicity model in rats caused a significant decrease in GSH and SOD and a significant increase in MDA and NO in cardiac tissue. On the other hand, they reported that LPA administration caused improvement in these parameters. Similarly, El-Awady et al.[32] reported that the CPL-induced decrease in GSH and SOD and the increase in MDA in rat heart tissue were ameliorated by LPA administration. In our study, we observed a significant improvement in the LPA groups (LPA-1 and LPA-2) with two different doses of LPA pre-treatment. The ROS, MDA, and TOS increase with CPL treatment was significantly reduced in both the LPA-1 and LPA-2 groups. This significant improvement was more pronounced in the LPA-2 group. Furthermore, in our study, we found that although TAS, GSH levels, and GSHPx activities decreased in the CPL group compared to the CNT group, these values increased substantially in the LPA groups compared to the CPL group. The limited number of studies in the literature showing that CPL-induced myocardial damage is improved by LPA application focused more on oxidative parameters (two studies).[32,36] In contrast, the present study analyzed inflammatory and apoptotic markers in addition to OS parameters, providing more reliable results.

CPL has been reported to increase the expression of pro-inflammatory signalling molecules such as chemokines and cytokines by stimulating certain cellular signalling pathways.[38] These increased signaling molecules have also been reported to mediate CPL toxicity in many tissues.[1,2,6,8] Most previous publications have found that the increase in CPL toxicity is significantly paralleled by an increase in TNF-α, IL-1β, and IL-6 values.[1,6,27,38] In the physio pathological process of CPL-induced cardiotoxicity, we predict the activation of pro-inflammatory cytokines in cardiomyocytes. To this end, we examined the levels of inflammation markers after the application of CPL to H9c2 embryonic cardiomyocytes. In this study, we found a significant increase in TNF-α, IL-1β, and IL-6 values in the CPL treatment groups compared to the CNT group. The effects of LPA in reducing inflammation-related damage have been reported in many tissues.[13,22,39] In this study, we have shown that LPA can protect cardiomyocytes from inflammatory damage. In our study, we observed that TNF-α, IL-1β, and IL-6 values were markedly decreased in the LPA pre-treated LPA-1 and LPA-2 groups compared to the CPL group. We also found that a dose of 500 μM LPA was more effective. These results suggest that the cardiotoxic effects of CPL chemotherapy may be reduced by LPA treatment.

It has been reported that the accumulation of CPL in the cell during chemotherapy reduces the activity of the antioxidant system, increases ROS production and lipid peroxidation, and also promotes cell apoptosis through the activation of various pro-apoptotic pathways.[4,9,11] Previous studies have shown that mitochondrial dysfunction due to increased ROS and subsequent activation of the apoptotic pathway is involved in the pathogenesis of CPL toxicity.[11,28] Casp 3 and 9 activation is an irreversible step that induces apoptosis. Therefore, these caspases are often used in studies to assess apoptosis.[19,20] Inflammatory responses, OS, and apoptosis were detected after CPL treatment in this study. We examined Casp 3 and 9 levels after CPL treatment of cardiomyocyte cells. We found a significant upregulation of Casp 3 and 9 in the CPL-treated groups compared to the CNT group. Previous studies suggested that CPL caused upregulation of Casp 3 and downregulation of Bcl-2.[40,41] Another study found that Casp 9 and Casp 3 activity was significantly increased after CPL application in H9c2 cells.[11] LPA has been shown to have pharmacological potential in the regulation of various cellular and molecular processes, such as apoptosis and autophagy.[17,27] LPA is a potent regulator of different molecular and cellular processes, including apoptotic and autophagic processes.[17] Qi et al.[42] found that LPA decreased the expression of inflammation markers (TNF-α, IL-1β, and IL-6 levels), increased cell viability in an in vitro model, and decreased ROS and apoptosis in cardiac ischemia/reperfusion model in vivo/in vitro models. They also reported that it increased the expression of anti-apoptotic protein Bcl-2 and suppressed the levels of apoptotic protein Bax. In our study, we observed a significant improvement in the LPA pre-treatment groups (LPA-1 and LPA-2 groups). Casp 3 and 9 levels were significantly reduced in the LPA-1 and LPA-2 groups compared to the CPL group. The improvement was more pronounced in the LPA-2 group. As a result, using the H9c2 embryonic cardiomyocyte cell line, we were able to show that LPA prevents a CPL-induced increase in OS and inflammation in cardiac tissue and prevents the induction of caspases.

CONCLUSION

In conclusion, this study showed that LPA could increase antioxidant capacity in CLP-induced injury in cultured cardiomyocytes, which is consistent with the literature. However, it is also the first study to report that LPA could reduce the levels of inflammatory markers and down-regulate caspase 3 and 9 levels in CLP-induced cardiotoxicity. By establishing the protective effects of LPA, such as suppression of inflammation and apoptosis and enhancement of antioxidant capacity, using LPA for cardio-protection in various forms of drug toxicity-induced cardiac damage, including CPL toxicity, can be recommended.

ETHICAL DECLARATIONS

Ethics Committee Approval

The current study has no study with human and human participants. The study is not subject to ethics committee approval.

Informed Consent
Because the study has no study with human and human participants, no written informed consent form was obtained.

Referee Evaluation Process
Externally peer-reviewed.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Financial Disclosure
The authors declared that this study has received no financial support.

Author Contributions
All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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