Rutin trihydrate attenuated cisplatin- induced cardiac toxicity in isolated perfused rat's hearts

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Abstract

Background

The present study aims to investigate the protective effect of rutin against cisplatin induced toxic effects on the mechanical performance of the myocardium, histopathology, and oxidative stress in isolated perfused rat hearts.

Methods

Cardiotoxicity of cisplatin was assessed at three dosage levels (1, 7, and 14 mg/l) in the isolated perfused rat hearts. The toxic effect of cisplatin was assessed on left ventricular pressure (LVP), heart rate (HR), dp/dt(max), dp/dt (min), perfusion pressure, pressure-time index, contractility index and duration of diastole. Measurements were carried out one minute before perfusion of cisplatin and 60 minutes after perfusion.

Results

Cisplatin reduced significantly ($p < 0.05$) in a dose-dependent manner LVP, dp/dt(max), dp/dt(min) and pressure- time index. Perfusion of rutin trihydrate (1 μ M/l), 10 minutes before administration of cisplatin and throughout the experiment significantly ($p < 0.05$) attenuated the detrimental effects of cisplatin on cardiac parameters. Cisplatin caused degeneration and necrosis of cardiac muscle cells, while rutin reduced these changes and restored normal heart histology. Moreover, cisplatin reduced the myocardium concentration of reduced glutathione and increased the level of malondialdehyde, whereas rutin almost reversed these changes.

Conclusion

Cisplatin-induced dose-dependent impairment of several parameters of cardiac function and produced histopathological alterations in isolated rat hearts. These harmful effects of cisplatin were ameliorated by rutin trihydrate. These findings suggest the potential protective effects of rutin trihydrate against cisplatin-induced cardiotoxicity.

Background

Cisplatin is a broad spectrum anti malignant drug. It is widely used in in the management of testicular, ovarian, non-small cell lung cancer, gastric and esophageal cancers, and hematological malignancies [1]. The clinical use of cisplatin is significantly limited by its adverse effects and toxicities affecting the gastrointestinal, renal, neurological, and hematological systems [2].

Growing number evidences have revealed that cisplatin produced multiple cardio toxic effects such as unstable angina pectoris [3], cardiac arrhythmias [4], bradycardia [5], acute myocardial infarction [6], hypotension [7], atrioventricular block [8], cardiac failure [9] and cardiomyopathy [10].

Increase in oxidative stress and decrease in antioxidant enzymes are linked with cardio-toxic effects of cisplatin [11]. Rutin has been reported for beneficial protective effects against variety of drug induced toxicities including doxorubicin and cisplatin induced cardiac toxicity and memory deficits [12–14]. These protective effects of rutin are related to its profound antioxidant and anticancer properties. Considering this hypothesis, the efficacy of rutin against cisplatin-induced cardiac toxicity was investigated. It is also an established fact that therapeutic effect of cisplatin is significantly increased with the increased dose [15], but at the cost of severe adverse effects [16]. Keeping this in mind the dose dependent cardiotoxicity of cisplatin was explored. The data related to prophylactic effect of rutin against dose dependent cisplatin-induced cardiotoxicity is scarce. Therefore, the focus of our study was to evaluate the protective effect of rutin on cisplatin induced derangements on mechanical performance of the myocardium, histopathology, and oxidative stress in isolated perfused rat hearts.

Materials And Methods

Mechanical performance of the heart

Male rats of the Albino Wistar strain (250-350 g body weight, King Saud University breed), five groups (8 rats each) were used. The experiments were conducted following the ethical guidelines for investigation in laboratory animals, and the College of Medicine, KSU; Institutional Review Board (IRB) approved the protocol of the study. The rats were anesthetized with urethane and the hearts were removed and perfused with Krebs-Henseleit solution (composition in mM: NaCl 118.4, KCl 4.7, MgSO₄ 1.2, K₂HPO₄ 1.2, NaHCO₃ 25.0, CaCl₂ 2.5, glucose 11.5) gassed with 95% O₂, 5% CO₂ at a constant flow rate of 10 ml/ min and maintained at 37⁰C with LE Thermostat (Panlab, HARVARD APPARATUS, USA). A saline-filled latex balloon connected via a polyethylene tube to a pressure transducer (MLA844, ADInstruments, Spain) was inserted into the left ventricle, and a baseline enddiastolic pressure was set at 5–10mmHg. The pressure transducer was connected to ADInstrument Powerlab 8/30

via a bridge amplifier (ADInstruments, Sydney Australia). The perfusion pressure was monitored by a second pressure transducer (MLA844, ADInstruments, Spain) connected to ADInstrument Powerlab 8/30 via a bridge amplifier (ADInstruments, Sydney Australia). All hearts were allowed to perfuse for 15 minutes to achieve stabilization. Following the stabilization period, the parameters recorded are Left ventricular pressure (LVP), heart rate (HR), dp/dt (max), dp/dt (min), perfusion pressure, pressure-time index, contractility index and duration of diastole. Results were analyzed using Labchart pro 7 software. Measurements were carried out at one minute before perfusion of cisplatin (Ebewe Pharma, Austria) and 60 minutes after perfusion. In a separate group, rutin trihydrate (Sigma- Aldrich GmbH, Germany) was perfused for 10 minutes before administration of cisplatin and throughout the experiment. A third sham group was perfused with Krebs-Henseleit solution for 60 minutes. Responses obtained 60 minutes after

perfusion were expressed as percentages in relation to those obtained at one minute before perfusion of cisplatin.

Histopathological examination

At the end of the experiment, specimens of the ventricles of control, cisplatin 14mg/l and rutin and cisplatin groups were dissected, divided into two portions, one for detecting the underlying structural changes and the other for endogenous antioxidants analysis. The first specimens were then fixed in 10% buffered formalin and processed with paraffin wax. For histopathological feature examination, 5 µm sections were stained with hematoxylin and eosin for the analysis using a light microscope.

Analysis of endogenous antioxidants

The other heart specimens were washed by PBS then further divided and stored at -80°C till used for quantification of (GSH and MDA) the antioxidant / pro-oxidant variables. On the day of quantification, each specimen was weighed, homogenized in relevant specified buffers, centrifuged, and processed following the methodology of sample preparation for each estimate.

Reduced Glutathione (GSH) Assay

The principle is based on the reaction of reduced GSH with 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) (E. Merck Ltd., Bombay, India), according to the method of Owens and Belcher [17]. The absorbance was measured by Shimadzu double beam spectrophotometer (UV200S, Japan) at 412 nm. The amount of GSH present was calculated using a standard solution of GSH containing 1 mg of GSH/ml of 3% metaphosphoric acid. The increase in the extinction at 412 nm was proportional to the amount of GSH present and was expressed as nmol/g wet tissue.

Malondialdehyde (MDA) Assay

The principle is based on the reaction of MDA with thiobarbituric acid (TBA) (E. Merck Ltd., Bombay, India), according to the method described by Buege and Aust [18]. The concentration of the MDA-TBA complex was being quantified spectrophotometrically at 532 nm and expressed as nmol/g wet tissue.

Statistical analysis

Data reported as mean $\pm$ SEM. One-way analysis of variance followed by Tukey's multiple comparison tests as a post hoc test was used to analyze the data. A p-value of 0.05 or less was taken as a criterion for statistically significant differences. Data were analyzed using Graph Pad Prism 5 software.

Results

1-Effect of cisplatin (1, 7, and 14 mg/l) on parameters of mechanical performance of the heart Table 1 depicts the effects of different concentrations of cisplatin (1, 7 and 14 mg/l) on left ventricular pressure,

dp/dtmax, dp/dtmin, heart rate, contractility index and pressure- time index. Eight isolated hearts were perfused with Krebs solution containing different concentrations of cisplatin for 60 minutes. The response obtained at minute 60 is calculated as a percentage of the response obtained at the first minute before perfusion of hearts with cisplatin. All concentrations of cisplatin-induced a statistically significant ($p < 0.01$) and dose-dependent reduction in left ventricular pressure. Cisplatin at 7 and 14 mg/l concentrations, significantly ($p < 0.05$) reduced dp/dt max and dp/dt min. Cisplatin exerted a bell-shaped concentration-response relationship on heart rates. Cisplatin at 1 mg/l increased the heart rate significantly ($P < 0.01$), while 7 mg/l slightly reduced the heart rate. All three doses of cisplatin increased significantly ($p < 0.01$) the contractility index, whereas, Cisplatin 7 and 14 mg/l significantly ($p < 0.05$) reduced pressure- time index.

Table 1

Effects of cisplatin (1, 7, and 14 mg/l) on parameters of mechanical performance of the isolated rat heart.

Time/parameters	LVP %	+dp/dt %	- dp/dt %	HR %	Contractility index %	Pressure- time index %
One min before	100	100	100	100	100	100
Untreated 60 min after	94 ± 9	101 ± 7	92 ± 8	101 ± 7	105 ± 3	92 ± 12
Cisplatin 1 mg/l, 60 min after	70 ± 6**	97 ± 12	78 ± 7	145 ± 11**	138 ± 5**	74 ± 8
Cisplatin 7 mg/l, 60 min after	69 ± 5**	77 ± 3*	72 ± 5*	85 ± 6	140 ± 6**	68 ± 7*
Cisplatin 14 mg/l, 60 min after	65 ± 4**	72 ± 5*	69 ± 4*	104 ± 7	151 ± 7**	57 ± 7*
Cisplatin was perfused for 60 minutes. Measurement of parameters of mechanical performance was carried out one minute before and 60 minutes after administration of cisplatin. Parameters were expressed as percentages of values after 60 minutes versus those at one minute before administration of cisplatin. Sham experiment was intended to show the mechanical performance of the heart in absence of drugs after 60 minutes. Eight isolated rats were used in each group.						
* $p < 0.05$; ** $p < 0.001$						

2-Effects of rutin trihydrate (1 μ M) on parameters of mechanical performance altered by cisplatin

Table 2 shows the effects of rutin trihydrate (1 μ M) on alterations of the mechanical performance of isolated rat hearts induced by cisplatin (14 mg/l). Rutin trihydrate (1 μ M) was perfused 10 minutes before administration of cisplatin. Rutin (1 μ M) significantly ($p < 0.05$) reversed the reduction of left ventricular pressure induced by perfusion of hearts with 14 mg/l cisplatin for 60 minutes and attenuated the

reduction in dp/dt max and dp/dt min exerted by cisplatin. There was a trend for the heart rate to increase, when hearts were perfused for 60 minutes with cisplatin (14 mg/l), but this increase did not attain statistical significance. Perfusion of hearts with rutin (1 μ M) significantly ($p < 0.05$) reduced heart rate. After 60 minutes of perfusion, cisplatin (14 mg/l) significantly ($p < 0.05$) increased contractility index. Perfusion of hearts with rutin (1 μ M) significantly ($p < 0.01$) reversed this increase in contractility index and significantly ($p < 0.01$) reversed the reduction in pressure- time index induced by cisplatin. When hearts were perfused for 60 minutes with cisplatin(14 mg/l), there was a tendency for the duration of diastole to decrease, but perfusion of hearts with rutin (1 μ M) significantly ($p < 0.05$) increased period of diastole. There was a trend for the perfusion pressure to increase, when hearts were perfused for 60 minutes with cisplatin (14 mg/l), but this increase was statistically non-significant. Perfusion of hearts with rutin (1 μ M) significantly ($p < 0.01$) reduced perfusion pressure.

Table 2

Effects of rutin trihydrate (1 μ M) on parameters of mechanical performance altered by cisplatin (14 mg/l) in isolated hearts.

Time/parameters	LVP %	+dp/dt %	- dp/dt %	HR %	Contractility index	Pressure-time index	Diastolic duration	Perfusion pressure
One min before	100	100	100	100	100	100	100	100
Untreated 60 min after	94 $\pm$ 9	101 $\pm$ 7	92 $\pm$ 8	101 $\pm$ 7	105 $\pm$ 3	92 $\pm$ 12	116 $\pm$ 7	166 $\pm$ 16
Cisplatin 14 mg/l, 60 min after	65 $\pm$ 4**	72 $\pm$ 5*	69 $\pm$ 4*	104 $\pm$ 7	151 $\pm$ 7**	57 $\pm$ 7*	99 $\pm$ 12	117 $\pm$ 7
Cisplatin 14 mg/l + Rutin 1 μ M, 60 min after	84 $\pm$ 3##	85 $\pm$ 6 [#]	83 $\pm$ 4 [#]	86 $\pm$ 6 [#]	101 $\pm$ 7##	82 $\pm$ 5##	139 $\pm$ 17 [#]	84 $\pm$ 2 [#]
Rutin trihydrate was administered 10 minutes before administration of cisplatin(14 mg/l) to 8 isolated hearts. Parameters were expressed as percentages of values after 60 minutes versus those at one minute before administration of cisplatin.								
*p < 0.05 significantly different from untreated								
**p < 0.01 significantly different from untreated								
#p < 0.05 significantly different from cisplatin 14 mg/l, 60 minutes after.								
##p < 0.01 significantly different from cisplatin 14 mg/l, 60 minutes after.								

Histological Examination

Figure 1: A specimen of the control group showed single, oval, and centrally located nuclei of cardiomyocytes with regularly arranged cardiac myofibers and no histopathological lesion observed in the control group. While cisplatin group showed degenerated muscle cells, necrosis of cardiac myofibers

and dissolution of nuclei. The cardiac myofibres in this group were found to be in a disarrayed pattern compared to the control muscle (Fig. 2). Another specimen of cisplatin specimen showed degenerated muscle cells with interstitial edema (Fig. 3). Addition of rutin to hearts perfused with cisplatin 14 mg/l showed a reduction in the toxic changes and the normal heart histology was significantly restored (Fig. 4).

Antioxidants Analysis

Perfusion of hearts with cisplatin 14 mg/l significantly ($p < 0.01$) reduced the concentration of glutathione, while perfusion of hearts with both rutin and cisplatin approximately restored the concentration of the reduced form of glutathione (Fig. 5). Whereas, perfusion of hearts with cisplatin greatly and significantly ($p < 0.01$) increased the concentration of malondialdehyde. Addition of Rutin to the perfusate containing cisplatin greatly reduced the tissue concentration of malondialdehyde (Fig. 6).

Discussion

Cardiac dysfunction can be demonstrated by hemodynamic changes in various parameters of cardiac function. Parameters of cardiac function assessed in this study included left ventricular pressure, heart rate, dp/dt (max), dp/dt (min), perfusion pressure, pressure-time index, contractility index and duration of diastole. Data from this study revealed that cisplatin reduced significantly left ventricular pressure, dp/dt(max), dp/dt (min) and pressure -time index in the isolated rat heart in a dose-dependent manner after perfusion for 60 minutes. In the present study, histopathological findings of heart tissues depicted that cisplatin administration induced degeneration and necrosis of cardiac muscle with the dissolution of nuclei. These observations correlate well with the alterations of hemodynamic and mechanical function induced by cisplatin. Moreover, cisplatin increased lipid peroxidation in heart tissue and reduced antioxidant activity.

Numerous injurious effects of cisplatin on the heart have been reported. Cisplatin has been shown to produce creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) leakage, a progressive reduction in total carnitine and ATP and depletion of glutathione content in cardiac tissue [10]. It is also reported to produce a dose-dependent decrease in contractile force, heart rate, and coronary flow [19] and induce oxidative and nitrosative stress [10, 20]. Furthermore, it caused apoptosis [21], deterioration of diastolic cardiac function [22] oxidative stress, inflammation, and histopathological alterations [10].

Several mechanisms have been reported to explain the harmful effects of cisplatin on the myocardium. Cisplatin can generate reactive oxygen species, such as superoxide anion and hydroxyl radical [23]. These reactive oxygen species are associated with an increase in lipid peroxidation [24]. Lipid peroxidation of the cardiac membrane results in leakage of lactate dehydrogenase and creatinine kinase from cardiac myocytes [19]. Moreover, cisplatin induced a fall in plasma concentrations of various antioxidants [25]. This may lead to failure of the anti-oxidative defense mechanism against free radical-mediated organ damage. These effects are evident in the current study. Cisplatin increased lipid peroxidation, which is indicated by increased tissue concentration of malondialdehyde and reduced

tissue antioxidant activity, which is indicated by reduced tissue reduced glutathione concentration. Glutathione has a direct antioxidant function by reacting with free radicals. This activity is useful in the detoxification of peroxidized lipids. Thus, it is likely that cisplatin-induced detrimental effect on the hemodynamic and mechanical function and histology of the myocardium, by increasing lipid peroxidation and reduction of glutathione tissue concentration.

To overcome cisplatin-induced cardiotoxicity, numerous efforts have been made in the past [26]. In the present study, rutin trihydrate reversed the harmful effects of cisplatin on left ventricular pressure, $dp/dt(max)$, $dp/dt(min)$, contractility index, pressure- time index, heart rate, duration of diastole and perfusion pressure. Moreover, cisplatin restored the normal histology of the myocardium, inhibited lipid peroxidation, and increased reduced glutathione concentration. Coronary blood flow is impeded during systole, the duration of diastole is an important determinant of coronary blood flow and subendocardial perfusion. Rutin trihydrate by prolonging the duration of diastole and reducing perfusion pressure could greatly improve coronary blood flow and subendocardial perfusion.

Cisplatin causes cardio-toxic effects by oxidative stress and decrease in antioxidant enzymes [11]. Rutin has been previously reported for beneficial protective effects against variety of drug induced toxicities including doxorubicin and cisplatin induced cardiac toxicity [12–14]. The protective effects of rutin trihydrate observed in our current study mostly likely occurred due to its profound anti-oxidant properties. This protective effect of rutin trihydrate could be attributed to its antioxidant property. Rutin trihydrate has been shown to enhance superoxide dismutase (SOD) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) activity [27] and reduced lipid peroxide content increased by cisplatin in kidneys of rats [28–29]. Moreover, rutin is reported to have anti-inflammatory and anti-apoptotic effects. It inhibited NF κ B and TNF- α pathway mediated inflammation and caspase-3 cell apoptosis [30].

Conclusion

Cisplatin-induced dose-dependent impairment of several parameters of cardiac function, induced histopathological alterations, and reduced antioxidant activity. These harmful effects of cisplatin were ameliorated by rutin trihydrate. Cisplatin the ability of rutin to ameliorate cisplatin-induced cardiotoxicity could be attributed to its antioxidant effects.

The attenuation of the detrimental effects of cisplatin by rutin trihydrate could have eventual clinical implications in terms of more rational usage of this agent. Rutin trihydrate appears to be a potential candidate to ameliorate cardiotoxicity associated with cisplatin in rats. Hence, it would be worthwhile studying the effects of rutin trihydrate supplementation in cisplatin-treated cancer patients, in the hope of reducing cisplatin-induced cardiotoxicity.

Abbreviations

CK-MB: creatine kinase-MB; DTNB: 5,5-dithiobis-(2-nitrobenzoic acid); $+dp/dt(max)$: Rate of maximum change of left ventricular contraction; $-dp/dt(min)$: rate of minimum change of left ventricular relaxation; GSH: Reduced glutathione; LDH: lactate dehydrogenase; LVP: left ventricular pressure; MDA: Malondialdehyde; TNF- α : Tumor necrosis factor alpha; TBA: thiobarbituric acid

Declarations

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Authors' contributions

IAB is the main author, contributed in funding, methodology, analyzed the data, wrote and revised the manuscript. OY contributed in the methodology and helped in data analysis and revising the manuscript. RL contributed in the experimental work and data compilation and manuscript writing. SF contributed in the experimental work and data compilation. FV contributed in the literature review and data analysis. SA contribute in data compilation, analysis and literature review and data.

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Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article.

Ethics approval and consent to participate

The experiments were conducted following the ethical guidelines for investigation in laboratory animals, and the College of Medicine, King Saud University Riyadh.

Consent for publication

Not applicable

Competing Interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Control group showed single, oval and centrally located nuclei of cardiomyocytes with regularly arranged cardiac myofibers. No histopathological lesion observed in control group.

Figure 2

Cisplatin Group depicts degenerated muscle cells, necrosis of cardiac myofibers, and dissolution of nuclei in cardiac myofibers. The cardiac myofibers in this group were found to be in disarrayed pattern compared to the control muscle.

Figure 3

Cisplatin Group showing degenerated muscle cells and interstitial edema (arrows) (cisplatin 14mg/l).

Figure 4

Rutin+cisplatin group shows reduction in the toxic changes and the normal heart histology is significantly restored. (Cisplatin 14mg/l, rutin 1 μ M).

Figure 5

Effect of rutin (1 µM) on reduction of reduced glutathione concentration induced by 14mg cisplatin following perfusion for 60 minutes. Tissues from 8 isolated hearts were used to quantify reduced glutathione. $p < 0.01$ significantly different from control, # $p < 0.01$ significantly different from cisplatin 14mg/l.

Figure 6

Effect of rutin (1µM) on increase of MDA concentration induced by 14mg cisplatin following perfusion for 60 minutes. Tissues from 8 isolated hearts were used to quantify malondialdehyde. $p < 0.01$ significantly different from control, # $p < 0.01$ significantly different from cisplatin 14mg/l.