Note

Grape Seed Proanthocyanidins Extract Prevents Cisplatin-induced Cardiotoxicity in Rats

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Cisplatin-treated rats experienced a significant elevation of serum activities of lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT) and creatine kinase (CK). These effects were accompanied by significant increases in the levels of malondialdehyde (MDA) and nitric oxide (NO), and decreases in the glutathione (GSH) content, and the activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in cardiac tissues. Pathological examination revealed that cisplatin caused significant cardiac damage in rats. Grape seed proanthocyanidins extract (GSPE) administration produced amelioration in biochemical indices of cardiotoxicity, lipid peroxidation, oxidative stress, as well as histological change when compared to group cisplatin alone. GSPE were shown to be potential candidates to ameliorate cisplatin-induced cardiotoxicity.

Keywords: grape seed proanthocyanidins extract, cisplatin, cardiotoxicity, oxidative stress

Introduction

Chemotherapy is an important intervention in oncotherapy, and cisplatin is one of the common clinical chemotherapy drugs for its definite curative effect. However, as the dose of the cisplatin increases, its toxic and side effects such as nephrotoxicity increase as well.

Earlier studies have also reported that cisplatin therapy is usually associated with cardiotoxicity (Chvetzoff et al. 1998, Pai and Nahata 2000). Cardiac events, reported in many case reports, may include electrocardiographic changes, arrhythmias, myocarditis, cardiomyopathy and congestive heart failure. Combinations of cisplatin with other anticancer drugs as methotrexate, 5-fluorouracil, bleomycin and doxorubicin are associated with lethal cardiomyopathy (El-Awady et al. 2011).

Some experiments revealed that cisplatin could break the metabolic balance of oxygen free radicals which resulted in oxidative stress (Satoh et al. 2003). Cisplatin-induced nephrotoxicity is closely associated with an increase in lipid peroxidation in kidney tissues (Antunes et al. 2000). The drug is able to induce active oxygen species, such as superoxide anion and hydroxyl radical (Wozniak et al. 2004). It was also shown that cisplatin-based chemotherapy induces a fall in patient plasma concentrations of various antioxidants (Weijl et al. 1998). This may lead to the failure of the antioxidative defense mechanism against free radical-mediated organ damage and the genotoxicity of cisplatin that may lead to the induction of secondary malignancies in other normal tissues (Wozniak et al. 2004). Moreover, mitochondrial damage by cisplatin has increasingly been studied as a mediator of toxicity in normal tissues in animals receiving cisplatin. Gastrointestinal toxicity (Yanez et al. 2003), ototoxicity (Devarajan et al. 2002), and nephrotoxicity (Park et al. 2002) have all been attributed to the mitochondrial damages caused by cisplatin. However, the exact mechanisms of cisplatin-induced toxicity have not yet been clearly elucidated. Understanding these mechanisms could lead to the
development of new protective interventions

There is an increasing amount of evidence that administration of antioxidants may be effective in ameliorating cisplatin-induced toxicity (Ali and Al 2006, Yagmurca et al. 2007). GSPE is the main polyphenols in the grape, which possess strong antioxidant properties, free radical-scavenging ability that is to scavenge peroxyl and hydroxyl and other radicals because of the hydroxyl parts in its molecule structure (Hassan et al. 2013, Pataki et al. 2002). Therefore, this study aims to investigate the protective effect of GSPE against cisplatin-induced cardiotoxicity in vivo.

Materials and Methods

Chemical and drugs Cisplatin (freeze-dried powder for dissolved in saline solution for injection) was obtained from Shandong Qilu Pharmaceutical Factory; grape seed procyanidins (purity is more than 95% as analyzed by UV, in which procyanidolic oligomers and procyanidin B2 contents are more than 60% and 1.8% respectively as analyzed by HPLC) were purchased from Tianjin Peak Natural Product Research Development Co., Ltd (Zhao et al. 2014).

Animals and experimental design Sixty male 8-week-old Sprague-Dawley rats, weighing 150 – 200 g, were purchased from the Laboratory Animal Center, China Military Academy of Sciences. Rats were kept in ventilated room under controlled laboratory conditions of normal 12 h light-dark cycle and room temperature (25 ± 2°C). Food and water were provided ad libitum.

After one week of acclimation rats were randomly assigned to four different groups, 15 animals each: the control group, the cisplatin group, the GSPE group, the GSPE + cisplatin group. The whole experiment lasted 15 days. The cisplatin group and the GSPE + cisplatin group were treated with single dose cisplatin injection intraperitoneally (7.5 mg/kg body weight) in the 10th day. The GSPE group and the GSPE + cisplatin group were treated with daily GSPE injection intraperitoneal (400 mg/kg body weight) throughout the experiment course. The corresponding groups were injected with normal saline or distilled water with the same schedule. The dose of GSPE used in this research was selected on the basis of previous published researches (Abir et al. 2009, Yousef et al. 2009, Zhao et al. 2014). Six days after cisplatin administration, rats were executed to collect blood from the femoral artery. Serum was separated in aliquots for further analyses. Hearts were rapidly excised, trimmed of connecting tissue, and washed off all residual blood with ice-cold 0.9% NaCl solution. Harvested some hearts were then stored in sealed bottles at −80°C until used in the biochemical analyses. The rest hearts were isolated and fixed in 10% formalin for histopathological analyses. The sections were evaluated under a light microscopy.

Materials and Methods

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Determination of oxidative stress markers Cardiac tissues were homogenized and diluted to 10% with phosphate buffer (pH 7.4). The activity of SOD and the content of NO, MDA and GSH were measured using SOD, NO, MDA and GSH assay kits purchased from Nanjing Jiancheng Bioengineering Institute, Nanjing, China. The activities of GSH-Px were measured at 340 nm using GSH-Px assay kit purchased from Beyotime Institute of Biotechnology, Haimen, China.

Histopathological examination The hearts samples that fixed in 10% formalin (v/v) were processed (dehydrated in graded concentrations of alcohol, immersed in xylene) and embedded in paraffin. Sections were cut at 5 μm thicknesses on a rotary microtome, mounted and then stained with hematoxylin and eosin (H&E). The sections were evaluated under a light microscope.

Statistical analysis The statistical analysis was done with a SPSS program (Version 16.0). Values were compared by one-way analysis of variance (ANOVA) and post hoc Duncan test to determine the differences among all the groups. The results were expressed as the mean ± standard error of mean (SEM) in each group, and a statistical probability of $P < 0.05$ was considered to be significant.

Result

Observation of general index observation At the beginning of GSPE gavage, both the GSPE and the GSPE + cisplatin groups had a minor anorexia. In addition, several rats showed light sternutation, which subsided gradually. It may be because of the rats’ in adaptation on GSPE. After cisplatin injection, the cisplatin group and the GSPE + cisplatin group showed anorexia and became less active. The rat fur curled and darkened, the rats were less active days after cisplatin injection, and worsened on the fourth day. In the cisplatin group, losing hair and bleeding were observed in several rats. In addition, rats were less active comparing to other groups.

Serum cardiac enzymes The changes in the serum cardiac enzyme activities are shown in Table 1. The enzyme activity assays showed the activities of serum LDH, ALT, AST, CK increased significantly, by 57%, 11.81%, 14.5% and 42.27%, respectively, after single administration of cisplatin (7.5 mg/kg body weight), in comparison to the control group ($P < 0.05$). It suggested that cisplatin (7.5 mg/kg. body weight) could induce the cardio toxicity in rat. In contrast, GSPE protected group showed a clear decrease in cardiac enzyme activity ($P < 0.05$), by 16.47%, 3.63%, 9.98% and 26.79% for LDH, ALT, AST and CK, respectively. It suggested that GSPE could alleviate the cisplatin-induced cardiotoxicity to some degree.

Biochemical markers of oxidative stress Table 2 summarizes the alternations in the biochemical indicators of oxidative stress,
namely activities of SOD and GSH-Px, and the content of MDA, GSH and NO in cardiac tissues of treatment and control groups.

a) Effect on lipid peroxidation

The MDA level, as a well-known biomarker of overall oxidative damage to cellular integrity, such as membrane lipids, was determined to investigate the effect of cisplatin-caused oxidative stress. Table 2 showed that the myocardial MDA level of lipid peroxidation, significantly increased in the cisplatin group, 74.83 ± 14.49, comparing to control group, 56.53 ± 8.71, (P < 0.05). Administration of GSPE diminished the cisplatin-induced MDA level in heart tissues. These results indicated that the administration of GSPE prevented cisplatin-induced lipid peroxidation in heart tissues.

The present data indicated that SOD activity in heart tissue significantly decreased in cisplatin group (P < 0.05). However, GSPE significantly elevated the SOD activity decreased by cisplatin in the GSPE protective group. This further suggested the GSPE may protect myocardial toxicity induced by cisplatin.

b) Effect on GSH content and GSH-Px activity

Both the GSH content and GSH-Px activity in cardiac tissue significantly decreased in the cisplatin group comparing to the control group (P < 0.05). While GSPE recovered the GSH content and GSH-Px activity decreased by cisplatin. These results showed that cisplatin can reduce the GSH content and GSH-Px activity, but the antioxidant properties of GSPE alleviated oxidative stress.

c) Effect on NO content

Table 2 indicated that NO content in the cisplatin group was significantly lower than in the control group (P < 0.05), which was reversed significantly by GSPE (P < 0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Cisplatin</th>
<th>GSPE</th>
<th>GSPE + Cisplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/ mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>56.53 ± 8.71a</td>
<td>74.83 ± 14.49b</td>
<td>51.9 ± 6.68b</td>
<td>59.59 ± 13.18a</td>
</tr>
<tr>
<td>cisplatin</td>
<td>74.83 ± 14.49b</td>
<td>9.94 ± 0.69a</td>
<td>8.96 ± 0.43a</td>
<td>137.35 ± 11.33a</td>
</tr>
<tr>
<td>GSPE</td>
<td>9.94 ± 0.69a</td>
<td>9.94 ± 0.69a</td>
<td>9.94 ± 0.69a</td>
<td>8.96 ± 0.43a</td>
</tr>
<tr>
<td>GSPE + Cisplatin</td>
<td>9.94 ± 0.69a</td>
<td>9.94 ± 0.69a</td>
<td>9.94 ± 0.69a</td>
<td>8.96 ± 0.43a</td>
</tr>
</tbody>
</table>

Observation of histomorphology The histomorphology results showed that cardiac muscle fibers in control group was intact in order and no abnormal change on myocardial interstitium (Fig.1A). However, in the cisplatin group, myocardial cells showed a dissolved multifocal sarcoplasm and were in small focal or massive necrosis. Intercellular space was significantly expanded. Multiple mononuclear lymphocyte infiltration was detected (Fig.1B), which suggested that cisplatin can bring about serious injury in the heart tissues. Cardiac muscle fibers were more intact in the GSPE + cisplatin group than in the cisplatin group (Figs. 1C and 1D). Myocardial cell cytoplasm was abundant, cell membrane was intact, myocardial level of heart organization was clear and shown no pathological change. In the GSPE + cisplatin group, the injury of rat’s heart was less severe; cardiac muscle fibers were more intact; the intercellular space was smaller and less inflammatory cell infiltration in comparison with the cisplatin group, which showed that GSPE improve the cardiac trauma induced by cisplatin.

Discussion

Earlier studies have reported that cisplatin therapy is usually associated with cardiotoxicity (Chvetzoff et al. 1998, Pai and Nahata 2000). The cardiotoxic reaction may include electrocardiographic changes, arrhythmias, myocarditis, cardiomyopathy and congestive heart failure (Al-Majed et al. 2006, Shanmugasundaram et al. 2002). In addition, Myocardial enzymes including AST, ALT, LDH, and CK are often used clinically as relative testing index in hypoxic-ischemic myocardial damage (Guan 2009). Myocardial enzymes are enzymes catalyzing

Table 1. Effect of GSPE (400 mg/kg body weight) on the change in the cardiac enzyme, namely serum LDH activity, ALT activity, AST activity and CK activity in male rats treated with cisplatin (7.5 mg/kg body weight)

<table>
<thead>
<tr>
<th>Groups</th>
<th>LDH (U/L)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>CK (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>1075.00 ± 215.93</td>
<td>52.5 ± 2.64</td>
<td>149.42 ± 22.22</td>
<td>1961.09 ± 811.60</td>
</tr>
<tr>
<td>cisplatin</td>
<td>1688.18 ± 128.69</td>
<td>58.7 ± 4.42</td>
<td>171.08 ± 15.91</td>
<td>2790.00 ± 965.44</td>
</tr>
<tr>
<td>GSPE</td>
<td>1013.00 ± 245.07</td>
<td>47.0 ± 4.59</td>
<td>149.08 ± 25.36</td>
<td>1939.09 ± 626.68</td>
</tr>
<tr>
<td>GSPE + Cisplatin</td>
<td>1410.36 ± 401.14</td>
<td>50.7 ± 6.46</td>
<td>154.00 ± 15.08</td>
<td>2042.64 ± 461.40</td>
</tr>
</tbody>
</table>

Note. Data are expressed as means ± SEM. N=15 for each experimental group. Different letters (a, b, c) indicate values that differ significantly between them (P < 0.05).

Table 2. Protective effect of GSPE (400 mg/kg body weight) on the change in the biochemical indicators of oxidative stress, namely SOD, GSH-Px activity and MDA, GSH and NO content in cardiac tissues of cisplatin-induced rats (7.5 mg/kg body weight)

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (units/mg protein)</th>
<th>MDA (nmol/ mg protein)</th>
<th>GSH (μmol/ mg protein)</th>
<th>GSH-Px (μmol/ mg protein)</th>
<th>NO (μmol/ mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>1267.94 ± 76.68a</td>
<td>56.53 ± 8.71a</td>
<td>9.99 ± 0.84a</td>
<td>151.32 ± 10.4a</td>
<td>35.83 ± 5.32a</td>
</tr>
<tr>
<td>cisplatin</td>
<td>889.54 ± 122.91b</td>
<td>74.83 ± 14.49b</td>
<td>7.91 ± 0.71b</td>
<td>120.15 ± 10.57b</td>
<td>46.28 ± 10.38b</td>
</tr>
<tr>
<td>GSPE</td>
<td>1073.19 ± 166.26c</td>
<td>51.9 ± 6.68b</td>
<td>9.94 ± 0.69a</td>
<td>154.33 ± 11.48a</td>
<td>35.04 ± 9.59c</td>
</tr>
<tr>
<td>GSPE + Cisplatin</td>
<td>1009.65 ± 127.39c</td>
<td>59.59 ± 13.18a</td>
<td>8.96 ± 0.43a</td>
<td>137.35 ± 11.33a</td>
<td>36.58 ± 11.88a</td>
</tr>
</tbody>
</table>

Note. Data are expressed as means ± SEM. N=15 for each experimental group. Different letters (a, b, c) indicate values that differ significantly between them (P < 0.05).
metabolism and regulating the electrical activity of myocardial cells. When acute myocardial infarction occurred, blood supply in coronary artery decreased sharply. As a result, many cardiac enzymes were released into blood from apoptotic cells and accumulated in blood. In this study, we observed the condition of rats and tested the activity of myocardial enzyme in serum. Results showed that administration of cisplatin (7.5 mg/kg body weight) made the rats' condition frail obviously. Cisplatin also significantly increased the activity of cardiac enzymes in serum. Besides, cardiac injury was found through histopathology with damaged myocardial cells and myofibrils in rats of cisplatin group. Granular-vacuolar degeneration was found under the light microscope. Many lymphocyte entered inflammatory infiltration. The results indicated that cisplatin induced cardiotoxicity in rat.

Some researches stated that cisplatin's side effects were related to oxidative stress (Satoh et al. 2003). In this present study, cisplatin treatment significantly increased the MDA and NO content, and decreased the GSH content, GSH-Px and SOD activities in cardiac tissue compared to the control group, indicating that cisplatin induced oxidative stress in cardiac tissue, and caused lipid peroxidation.

Some research showed that cisplatin’s side effects could be at least partially prevented by using natural products or synthetic antioxidants such as quercetin, alpha-tocopherol, L-ascorbic acid, selenium and zinc and so on (Aldemir et al. 2014, Kilarkaje 2014). GSPE was chosen as the protective agent in this experiment. Procyanidins not only inhibits the growth of various cancer cells such as digestive system neoplasms, respiratory tumors, urogenital neoplasms (Feng et al. 2014, Li et al. 2011), but also antagonize toxicity of chemotherapy agents (Mao et al. 2015). This makes procyanidins possible to act as protective agents during the course of chemotherapy. It has been reported that GSPE conferred cardio protection against exogenous H_2O_2 and antimycin A-induced oxidant injury by reactive oxygen species scavenging and iron chelating (Shao et al. 2003). More reports stated that procyanidins could play a part in the cardiac protective effect by suppressing of oxidative stress (Li et al. 2009).

Results from this experiment showed that GSPE was able to decrease the raised serum cardiac enzymes activities induced by cisplatin. Besides, pretreatment with GSPE (400 mg/kg body
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weight) significantly decreased MDA and NO content, inhibited depletion of GSH and increased the activity of SOD and GSH-Px in cardiac tissue. Moreover, the pathological change caused by cisplatin was significantly milder in the GSPE protective group. These observations indicate that GSPE has protective and therapeutic effects on cisplatin induced oxidative stress in cardiac tissue, which is consistent with a previous research regarding the protective effect of GSPE against testicular toxicity in rat (Zhao et al. 2014).

The possible explanation for the protective effects of GSPE against cisplatin-induced cardiotoxicity is its ability to react with the oxygen metabolites. While many oxygen radicals are generated due to the chemotherapy, the body may not be able to cope with this change due to the limited antioxidants protection and the reduced level of the body’s antioxidants. GSPE, a combination of biologically active bioflavonoids including oligomeric proanthocyanidins, can reduce the free radicals level and lipid peroxidation, so as to protect cisplatin-induced degeneration and necrosis of cardiac muscle fiber cells. In addition, published researches have shown that procyanidins and polyphenol can enhance sensitivity of A549 cell line to cisplatin and other anticancer drugs. (Ohnuma et al. 2011, Zhang et al. 2015). This indicates that GSPE might be an effective concomitant agent not only to reduce cisplatin cardiac toxicity but also to enhance its anticancer effect.

Conclusion

This study demonstrates that GSPE can reduce the attack of free radicals, alleviate the lipid peroxidation and reduce the lipid peroxide generation by improving antioxidant activity and promoting radicals scavenging. Consequently, GSPE can antagonize the cardiotoxicity caused by cisplatin.

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References


