Selection of agents for prevention of cisplatin-induced hepatotoxicity

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Abstract

The objective of this study was to explore the optimal combination of agents used along with cisplatin for protection of hepatotoxicity. Animal experiment was carried out based on the orthogonal design L\textsubscript{8} (2\textsuperscript{7}) setting seven factors with two different levels of each, and eight groups of mice were needed. The agents tested in this study were zinc, selenium, fosfomycin, sodium thiosulfate (STS), N-acetyl-cysteine (NAC), methionine and taurine. Mice were supplemented by gavage with various combinations of agents as designed in the orthogonal table once a day for nine days beginning two days before cisplatin administration. 3.5 mg/kg body weight of cisplatin was given intraperitoneally once a day for five days simultaneously. After cessation of cisplatin administration, the agents were supplemented continuously for two days. Activities of alanine aminotransferase (ALT) in serum, levels of glutathione (GSH) and malondialdehyde (MDA) in liver were analyzed after cessation of supplementation. Results showed zinc, fosfomycin and methionine were the effective factors for protection of weight loss; fosfomycin and methionine were the effective factors for prevention of decreased liver ratio; selenium, fosfomycin and STS were the effective factors for prevention of increased ALT activities in serum. On the other hand, methionine was the only effective factor for prevention of decreased GSH levels in liver; zinc, selenium and fosfomycin were the effective factors for prevention of increased MDA levels in liver. Based on the data observed in this study, the optimum combinations of agents were selenium, fosfomycin, methionine and taurine, and zinc, selenium, STS and methionine. In conclusion, each agent used in this study could play a beneficial role for prevention of cisplatin hepatotoxicity, however, none could play the crucial role. The potentiated actions for prevention of cisplatin hepatotoxicity could be achieved via combined use of these agents.

Keywords: Cisplatin; Side effects; Protective agents; Oxidative stress; Hepatotoxicity

1. Introduction

Cisplatin (\textit{cis}-diamine-dichloroplatinum) is a prominent member of the effective broad-spectrum antitumor drugs. However, its clinical usage is restricted due to some adverse side effects, such as ototoxicity and nephrotoxicity [1–3]. The cisplatin-induced ototoxicity and nephrotoxicity have been very well studied in both clinical and animal researches, however hepatotoxicity has been rarely paid attention to. Recent studies in our laboratory and others around the world suggested hepatotoxicity is also a major dose-limiting side effect in cisplatin-based chemotherapy [4–8].

Continued aggressive high-dose cisplatin chemotherapy necessitates the investigation of ways for prevention of the dose-limiting side effects that inhibit the cisplatin administration at tumoricidal doses. Until now a large number of studies have been focused on the ways for prevention of cisplatin side effects via supplementation of preventive agents simultaneously [9]. Findings in these studies suggested the side effects of cisplatin could be protected by drugs and micronutrients with different chemical nature [10–15]. Although the mechanism underlying the side effects induced by cisplatin are not understood clearly, it was considered to be attributed to the combination of multi-ways [5,16–19], such as the generation of reactive oxygen species (ROS), which could interfere with the antioxidant defense system and result in oxidative damage in different tissues [7,20–22], and reaction with thiols in protein and glutathione, which could cause cell dysfunction. On the other hand, it has been proposed that the antitumor activity of cisplatin is due to its ability to form adducts with DNA, which could cause cross-linking of DNA strands. As the antitumor activity and side effects in cisplatin-based chemotherapy are

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mediated in part by different mechanisms, the actions on selective inhibition of certain side effects could be achieved while the antitumor activity is not altered [23]. Further, as the side effects in cisplatin-based chemotherapy were thought to be induced by multi-ways, it could be speculated that a potentiated action could be achieved via combined use of preventive agents with different chemical nature, however, until now little is known regarding the combined actions of these agents. In this study, therefore, we attempted to explore the optimum combination of these agents for prevention of the cisplatin hepatotoxicity.

2. Materials and methods

2.1. Animals

Forty-eight male albino mice weighing 30.0 ± 1.0 g, purchased from the animal laboratory of China Medical University, were used. Animal room was kept at a temperature of 20 ± 2°C with a 12 h light/dark cycle and a relative humidity of 50–60%. Free access to food and water was allowed at all the time. Mice were housed five per cage in the sterilized plastic cages with wood shaving bedding. The Institutional Animal Care and Use Committees in China Medical University approved the protocol of this animal experiment.

2.2. Experimental procedure

After one-week adaptation, mice were divided into nine groups randomly with five mice in each experimental group and eight mice in control. In order to investigate the optimum combination of preventive agents used along with cisplatin, an orthogonal experiment design of L₈ (2⁷) was carried out [24]. Agents and their levels tested in present study were listed in Table 1, and selected based on the papers listed in the reference. Each agent was dissolved in distilled water to its desired concentration, and mixed with each other, then supplemented by gavage immediately to mice. Cisplatin was dissolved into isotonic saline solution by multi-ways, it could be speculated that a potentiated action could be achieved via combined use of preventive agents with different chemical nature, however, until now little is known regarding the combined actions of these agents. In this study, therefore, we attempted to explore the optimum combination of these agents for prevention of the cisplatin hepatotoxicity.

Table 1. Agents and levels in orthogonal design L₈ (2⁷) (mg/kg body weight)

<table>
<thead>
<tr>
<th>Levels</th>
<th>Zn</th>
<th>Se</th>
<th>Fos</th>
<th>NAC</th>
<th>STS</th>
<th>Met</th>
<th>Tau</th>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>2</td>
<td>25</td>
<td>1.5</td>
<td>300</td>
<td>500</td>
<td>1000</td>
<td>200</td>
<td>400</td>
</tr>
</tbody>
</table>

Notes: Zn, zinc sulfate; Se, sodium selenite; Fos, fosfomycin; NAC, N-acetyl-cysteine; STS, sodium thiosulfate; Met, methionine; Tau, taurine.

2.3. Reagents and laboratory wares

All reagents used in present study are analytical grade. All glasses and plastic wares were washed with detergent and acid, and rinsed with redistilled water. Water used in this study was doubly distilled. 5,5'-dithiobis 2-nitrobenzoic acid was purchased from Sigma Chemical Co., St. Louis, MO, USA, and the others were the products from the Chemicals Company in Shanghai, People’s Republic of China.

2.4. Analysis procedures

2.4.1. Weight

Animal weight was measured and evaluated between the first day of cisplatin administration and the final day of preventive agent supplementation.

2.4.2. Liver ratio

Liver was removed and weighed immediately. Liver ratio was calculated as the following formula, organ ratio (%) = organ weight (g) × 100/body weight (g).

2.4.3. Alanine aminotransferase (ALT) in serum

Activities of ALT in serum were determined in accordance with the method provided by the diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, People’s Republic of China).

2.4.4. Malondialdehyde (MDA) in liver

Levels of MDA were assayed by the method of Satoh [25]. Briefly, 10% (weight/volume) homogenate of liver made by 5% trichloroacetic acid solution was centrifuged by the reaction of TBA with MDA and heated in a boiling water bath for 30 min. The pink-colored chromogen formed by the reaction of TBA with MDA was measured at 532 nm. The results were expressed as MDA nmol/mg protein. Contents of protein in the supernatant were measured by Lowry’s method.

2.4.5. Glutathione (GSH) in liver

Levels of GSH in liver were assayed by the method of Beutler et al. [26]. Briefly, 10% (w/v) homogenate made by 5% trichloroacetic acid solution was centrifuged at 3500 rpm for 10 min. 0.2 mL supernatant was mixed with 0.67% 2-thiobarbituric acid (TBA) and 20% trichloroacetic acid solution, and heated in a boiling water bath for 10 min. The yellow-colored substance formed by the reaction of GSH and DTNB was measured at 412 nm. The results were expressed as GSH mg/g tissue weight.
2.5. Statistical analysis

The mean value of five mice in each group was calculated and used as the measure of every dependent variable in each group. Analysis of range was used to evaluate the effects of independent variables on dependent variables. Significant difference was analyzed by variance analysis. The sum of squared errors (SSE) was estimated by the difference between the sum of squares due to total variation and the total sum of squares due to independent variables [24]. The statistical significance was defined as $p < 0.05$.

3. Results

As shown in Table 2, mice weight, liver ratio and GSH levels in liver decreased significantly, and ALT activities in serum and MDA levels in liver increased significantly in group of mice treated with 3.5 mg/kg body weight of cisplatin compared to mice in control.

As shown in Table 3, zinc, fosfomycin and methionine were the factors that could ameliorate significantly weight loss; fosfomycin and methionine were the factors that could ameliorate significantly liver ratio; selenium, fosfomycin and STS were the factors that could ameliorate significantly ALT activities in serum. On the other hand, as shown in Table 4, the optimum preventive action was shown in groups 7, 6 and 3 based on the changes of weight loss, and those are in groups 3, 6 and 7 based on the changes of liver ratio, and in groups 7, 3 and 4 based on the changes of ALT activities in serum. Thus, the optimum action for prevention of cisplatin hepatotoxicity was observed generally in groups 3 and 7.

As shown in Table 5, methionine was the only factor that could ameliorate significantly GSH levels in liver; zinc, selenium and fosfomycin were the factors that could ameliorate significantly MDA levels in liver. On the other hand, as shown in Table 6, the optimum preventive action was shown in groups 7, 6 and 3 based on the changes of GSH levels in liver, and those were in groups 5, 7 and 3 based on the changes of MDA levels in liver. Thereby, the optimum action for prevention of oxidative damage in liver was also observed generally in groups 3 and 7.

4. Discussion

Hepatotoxicity in this study was gauged by weight loss, liver ratio and ALT activities in serum, and the hepatic oxidative damage was evaluated by levels of GSH and MDA in liver. Recent studies have been focused on the ways for protection of cisplatin hepatotoxicity [2,8,20–22]. However, little is reported regarding the combined actions of agents against cisplatin hepatotoxicity. To our knowledge, our paper is the first report to explore the optimum combination of agents used for prevention of cisplatin hepatotoxicity.

The findings disclosed that each agent used in this study could play a beneficial role for prevention of cisplatin hepatotoxicity, however none could play the crucial role. Although fosfomycin can benefit nearly all indicators examined in this study, the preventive effect in every group supplemented with fosfomycin was not better than that in the other. However, a potentiated effect could be observed when these agents were used together. As the optimum combination for prevention of hepatic toxicity and oxidative damage was same, our findings supported the hypoth-
Table 4
Orthogonal test (1)

<table>
<thead>
<tr>
<th>Group</th>
<th>Independent variables</th>
<th>Dependent variables (mean ± S.D.)&lt;sup&gt;a&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Zn</td>
<td>Se</td>
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<tr>
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</tr>
<tr>
<td>8</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Notes: S.D., standard deviation.

<sup>a</sup> The mean value of five mice received the same combination of agents.

Table 5
Analysis of range and variance (2)

<table>
<thead>
<tr>
<th>Levels</th>
<th>Agents</th>
<th>L.GSH (mg/g wt)</th>
<th>L.MDA (nmol/mg prot.)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>m&lt;sub&gt;1&lt;/sub&gt;</td>
<td>m&lt;sub&gt;2&lt;/sub&gt;</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>2.70</td>
<td>2.74</td>
</tr>
</tbody>
</table>

Notes: L.GSH, levels of glutathione in liver; L.MDA, levels of malondialdehyde in liver. m<sub>1</sub> represents the mean value of different dependent variables corresponding to the first level of every factor, and m<sub>2</sub> is the mean value of different dependent variables corresponding to the second level of every factor. R represents the difference between m<sub>1</sub> and m<sub>2</sub>; F denotes the variance, F = MS(factor)/MSe (MS: mean square).

* p less than 0.05.
** p less than 0.01.

Table 6
Orthogonal test (2)

<table>
<thead>
<tr>
<th>Group</th>
<th>Independent variables</th>
<th>Dependent variables (mean ± S.D.)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zn</td>
<td>Se</td>
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<td>8</td>
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</tr>
</tbody>
</table>

Notes: S.D., standard deviation.

<sup>a</sup> The mean value of five mice received the same combination of agents.

Selenium is an antioxidant as to be the essential element of glutathione peroxidase. Results of extensive laboratory studies have suggested that selenium is an effective agent for reducing cisplatin toxicity, thereby allowing administration of high doses of cisplatin and resulting in an improved antitumor efficacy [27–30]. Another possible mechanism underlying its protective action was assumed due to the formation of cisplatin–selenol complex in the body [31,32]. Based on the findings in this study, selenium was suggested to be an effective factor for protection of the hepatic toxicity and oxidative damage induced by cisplatin. Zinc is an essential trace element which can induce the synthesis of metallothionein (MT) in liver and kidney. MT is a protein with low molecular weight, and one-third of its amino acids are cysteine residues therefore, can easily trap and sequester metal ions with its thiol. MT was reported to be the main mechanism underlying the action of zinc against cisplatin toxicity [33,34]. Zinc is also an antioxidant and can prevent production of free radicals by transition metals. Based on the findings in this study, zinc was suggested to be an effective factor for protection of the hepatic toxicity and oxidative damage induced by cisplatin.

Methionine, a sulfur-containing amino acid, has been shown to provide a strong action for protection of ototoxicity and nephrotoxicity in cisplatin-based chemotherapy [35–38]. Methionine was reported to have an excellent safety profile, and may act as both a direct and indirect antioxidant [39]. Results reported by Cloven et al. [40] showed methionine could provide cytoprotection against cisplatin toxicity without significant compromise of antitumor activity. Based on the findings in this study methionine was suggested to be an effective factor for protection of cisplatin hepatotoxicity, and the only effective factor.
for protection of depletion of GSH in liver. Study reported by Campbell et al. [41] showed methionine could markedly reduce weight loss and mortality in rats treated with cisplatin. Therefore, our findings were in accordance with their observations. Taurine, the major intracellular free beta-amino acid, is known to be an antioxidant and a membrane-stabilizing agent. Unlike most amino acids, taurine is not metabolized or incorporated into protein but remains free in the intracellular fluid [42,43]. Results of recent studies showed it could attenuate the accumulation of platinum in kidney and counteract with the deleterious effects of cisplatin on the renal tubular function [42,44]. It has been reported expression of taurine transporter (TauT) was significantly reduced by cisplatin and forced over-expression of TauT could attenuate cisplatin-induced down-regulation of taurine uptake and protect renal tubular cells from apoptosis [45]. In this study, however, it failed to show any effective actions of taurine for protection of cisplatin hepatotoxicity. NAC as the precursor of glutathione is one of the important low molecular weight antioxidants in the body. It has been reported that NAC can protect the nephrotoxicity and ootoxicity of cisplatin [46–49]. It was suggested NAC could completely blocked cisplatin dependent intracellular GSH oxidation, ROS generation and apoptosis [50,51]. However, recent studies disclosed cisplatin requires metabolic activation to become nephrotoxic, and the activation is proposed to be via the metabolism of a glutathione-platinum conjugate to a cysteiny1-glycine-platinum conjugate, which is further processed to a cysteine conjugate. Preincubating cisplatin with NAC could result in a transient increase of cisplatin toxicity toward renal proximal tubular cells, and it was attributed to the increased uptake of platinum as the conjugate of NAC–cisplatin in the cells [52,53]. In this study, we failed to testify any beneficial actions of NAC for protection of cisplatin hepatotoxicity.

Fosfomycin is a natural broad-spectrum antibiotic with an epoxide structure. It has been reported to be a cytoprotective agent for protection of cisplatin toxicity without inhibition of the tumoricidal actions [54–57]. Fosfomycin can be administered orally or parenterally in a wide range of doses. It does not bind to plasma proteins and has a good distribution volume, therefore it can reach high concentrations in the interstitial fluid. It was proposed that the possible mechanism underlying the protective action of fosfomycin could be attributed to stabilize the lysosome membrane in tubular cells, favor phagocytosis and act as an immunomodulator. Based on the findings in this study, it was suggested that fosfomycin is the strongest factor for protection of cisplatin hepatotoxicity. STS is a potent antidote against the formation of the platinum–thiosulfate complex in the extracellular fluid, therefore it could promote the urinary cisplatin excretion and rescue dysfunction of the proximal tubules [61,62]. Based on the findings in present study, STS is also an effective factor for prevention of cisplatin hepatotoxicity.

The observation in this study highlights the usefulness of combined use of preventive agents against cisplatin hepatotoxicity. Although, we have no immediate explanation for the results observed in present study, and the mechanisms underlying the combined actions of these agents were assumed to be complicated, there is a possibility that as the trace metal element, zinc and selenium can induce the synthesis of MT in the liver, moreover based on the findings in present study, methionine can ameliorate the depletion of GSH in the liver induced by cisplatin, it seemed that MT and GSH play the crucial and cooperative role for prevention of cisplatin toxicities [33,63]. It has been reported that the active metabolites of cisplatin can react quickly with the thiol groups in glutathione and small proteins such as MT, then in high molecular weight proteins such as albumin through covalent link. Thereby, the levels of GSH and MT can play an important role in switching the mode of cell death induced by cisplatin [64]. On the other hand, it has been reported that intracellular levels of GSH and induction of MT were directly involved in the resistance to cisplatin in tumor cells [63,65,66]. Therefore, the cooperative effects of GSH and MT might be the main mechanism underlying the combined action of these agents observed in present study.

In conclusion, our findings would provide a more promising strategy for prevention of hepatotoxicity in cisplatin-based chemotherapy. However, our study is a pilot study and the results are preliminary, additional studies using animals and patients are needed.


