D-Ribose Ameliorates Cisplatin-Induced Nephrotoxicity by Inhibiting Renal Inflammation in Mice

Masaaki Ueki,1,2 Masaki Ueno,3 Jun Morishita2 and Nobuhiro Maekawa2

1Department of Anesthesia, Nishiwaki Municipal Hospital, Nishiwaki, Hyogo, Japan
2Department of Anesthesia and Perioperative Medicine, Kobe University, Kobe, Hyogo, Japan
3Department of Pathology and Host Defense, Faculty of Medicine, Kagawa University, Kita-gun, Kagawa, Japan

Cisplatin is one of the most potent chemotherapeutic anticancer drugs, but it can produce side effects such as nephrotoxicity. Inflammatory cytokines, chemokines and adhesion molecules have important roles in the pathogenesis of cisplatin-induced nephrotoxicity. D-Ribose is a naturally occurring five-carbon monosaccharide that is found in all living cells, and has anti-inflammatory effects in renal ischemia/reperfusion injury. The purpose of this study was to determine the protective effects of D-ribose on cisplatin-induced nephrotoxicity. Forty-eight mice were randomly divided into four groups: control, cisplatin, cisplatin + ribose, and ribose. Mice were given cisplatin (20 mg/kg body weight, intraperitoneally) with or without D-ribose (400 mg/kg body weight, intraperitoneally, immediately after cisplatin injection). At 72 h after cisplatin injection, we measured serum and renal tumor necrosis factor (TNF)-α and renal monocyte chemoattractant protein (MCP)-1 concentrations by enzyme-linked immunosorbent assay; renal expression of intercellular adhesion molecule (ICAM)-1 mRNA by real-time polymerase chain reaction; serum blood urea nitrogen and creatinine; and histological changes. Cisplatin increased serum and renal TNF-α concentrations, renal MCP-1 concentration, and renal ICAM-1 mRNA expression. Treatment with D-ribose attenuated the increase in serum and renal TNF-α concentrations, renal MCP-1 concentration, and renal ICAM-1 mRNA expression. Consequently, cisplatin-induced renal dysfunction and renal tubular necrosis were attenuated by D-ribose treatment. This is believed to be the first time that protective effects of D-ribose on cisplatin-induced nephrotoxicity via inhibition of inflammatory reactions have been investigated. Thus, D-ribose may become a new therapeutic candidate for the treatment of cisplatin-induced nephrotoxicity.

Keywords: chemokine; cisplatin; D-ribose; nephrotoxicity; tumor necrosis factor α

Introduction

Cisplatin (cis-diaminedichloroplatinum II) is a chemotherapeutic agent used for treatment of malignant tumors (Pabla and Dong 2008). However, severe nephrotoxicity after cisplatin treatment is a dose-limiting adverse reaction, and approximately 20% of patients develop evidence of nephrotoxicity following high-dose cisplatin chemotherapy (Yao et al. 2007). Although several therapeutic strategies have been suggested to prevent this condition, no specific treatments are currently recommended, except for vigorous hydration with normal saline (Launay-Vacher et al. 2008). Therefore, new and effective therapeutic strategies are needed for the prevention of cisplatin-induced nephrotoxicity.

The mechanisms involved in cisplatin-induced nephrotoxicity are not fully understood. The signaling mechanisms responsible for cisplatin-induced nephrotoxicity appear to be multifactorial, involving inflammation, oxidative stress and apoptosis (Yao et al. 2007). Recent studies suggest that inflammation may play an important pathophysiological role in cisplatin-induced nephrotoxicity (Deng et al. 2001; Ramesh and Reeves 2002; Dong and Atherton 2007). In particular, tumor necrosis factor (TNF)-α plays a key role in cisplatin-induced nephrotoxicity (Ramesh and Reeves 2004; Li et al. 2011). Furthermore, TNF-α is known to cause further inflammatory reactions via stimulating the production of cytokines and/or chemokines including monocyte chemoattractant protein-1 (MCP-1) and intercellular adhesion molecule (ICAM)-1 (Ramesh and Reeves 2002; Dong and Atherton 2007). Therefore, modulation of the renal inflammatory reaction after cisplatin injection may help to prevent cisplatin-induced nephrotoxicity.

Rare sugars are defined as monosaccharides that exist...
in nature. We have focused their attention on the biological functions of rare sugar in medicine (Ueki et al. 2007, 2008). We have reported rare sugar D-allose ameliorates cisplatin-induced nephrotoxicity in mice (Miyawaki et al. 2012). Rare sugars are present only in limited quantities; therefore, we screened some monosaccharides which have inhibitory action of activation of TNF-α. D-ribose is another naturally occurring five-carbon monosaccharide that is found in all living cells. It is the sugar moiety of adenosine triphosphate (ATP), therefore, it has been investigated as a potential metabolic supplement for the heart (Pauly and Pepine 2000). We have previously demonstrated that D-ribose has anti-inflammatory effects in renal ischemia/reperfusion injury (Nishiyama et al. 2009; Sato et al. 2009). These anti-inflammatory effects have suggested new approaches to development of renoprotective strategies for use during cisplatin chemotherapy.

In this study, we investigated whether D-ribose ameliorated cisplatin-induced renal injury in mice.

Materials and Methods

Animals and reagents

Male 8-9-week-old C57BL/6 mice weighing 20-25 g were purchased from CLEA Japan Inc. (Tokyo, Japan). This study was approved by the Institutional Animal Care and Use Committee (permission number: P100501) of Kobe University, Japan. It was carried out according to Kobe University Animal Experimental Regulations. Cisplatin and D-ribose were purchased from Sigma-Aldrich (St. Louis, MO, USA). Pentobarbital sodium was purchased from Dainippon Sumitomo Pharma Co. Ltd. (Osaka, Japan).

Animal preparation and treatment

Mice were randomly divided into four groups. The cisplatin group received a single dose of cisplatin [20 mg/kg body weight, intraperitoneally (i.p.)] (n = 12); the cisplatin + D-ribose group received 100 or 400 mg/kg D-ribose i.p. immediately after cisplatin injection (n = 12); the D-ribose group received D-ribose alone (n = 12); and the control group received an equivalent volume of saline (n = 12). Cisplatin was dissolved in saline at a concentration of 2 mg/ml. The dose of cisplatin used has been shown to produce severe renal injury in mice (Ramesh and Reeves 2002). After treatment, mice were acclimated in a special pathogen-free environment and provided with food and water ad libitum under a 12 h light/12 h dark cycle. Maximal renal injury was observed 72 h following i.p. injection of 20 mg/kg cisplatin (Ramesh and Reeves 2004; Kang et al. 2009). Animals were sacrificed 72 h after cisplatin injection under general anesthesia with sodium pentobarbital (50 mg/kg) and the kidneys removed.

Enzyme-linked immunosorbent assay (ELISA) for serum TNF-α, renal TNF-α and renal monocyte chemoattractant protein-1 (MCP-1) concentrations

On the day of sacrifice, 72 h after cisplatin injection, blood was collected from the femoral artery and centrifuged (2,500 × g for 5 min) and sera were stored at −80°C until analysis. Serum blood urea nitrogen (BUN) and creatinine levels were measured commercially (Special Reference Laboratories, Tokyo, Japan) and used as indicators of renal dysfunction.

Measurement of renal function

Femoral artery blood samples collected 72 h after cisplatin injection were centrifuged (2,000 × g for 5 min) and sera were stored at −80°C until analysis. Serum blood urea nitrogen (BUN) and creatinine levels were measured commercially (Special Reference Laboratories, Tokyo, Japan) and used as indicators of renal dysfunction.

Renal histology

Kidneys were removed 72 h after cisplatin injection, placed in 10% formalin, and embedded in paraffin wax according to a standard protocol. The samples were cut on a microtome into 4-µm sections, which were stained with hematoxylin and eosin for general histological examination. Tubular injury was assessed using a semi-quantitative scale (Ramesh and Reeves 2004). Scores were assigned according to the percentage of cortical tubules having epithelial necrosis, as follows: 0, 0%; 1, < 10%; 2, 10-25%; 3, 26-75%; or 4, > 75%. An independent assessor blinded to the treatment conditions examined 10 fields per kidney at × 400 magnification and then assigned necrosis severity scores.

Statistical analysis

All values are expressed as mean ± standard deviation (s.d.). Statistical significance was ascertained using analysis of variance, followed by individual comparisons with Scheffe’s post hoc test. Differences with a value of p < 0.05 were considered to be significant.

Results

D-Ribose reduces serum TNF-α, renal TNF-α and renal MCP-1 concentrations after cisplatin injection

TNF-α is a proinflammatory cytokine and is known to play an important role in cisplatin-induced renal injury (Ramesh and Reeves 2002). Therefore, we evaluated serum TNF-α concentrations by ELISA after cisplatin injection. Our previous data showed that serum TNF-α concentration
increased gradually 1 day after cisplatin injection and markedly peaked after 3 days (Miyawaki et al. 2012). Serum TNF-α concentration was markedly increased in cisplatin-treated mice compared to control animals (59.5 ± 8.0 vs. 5.2 ± 3.3 pg/ml, \( p < 0.01 \); Fig. 1). When D-ribose was administered at 400 mg/kg 72 h after cisplatin injection, serum TNF-α concentration was significantly decreased in comparison with that in the cisplatin group (33.0 ± 10.3 pg/ml, \( p < 0.01 \); Fig. 1), whereas 100 mg/kg D-ribose had no such effect. Thus, 400 mg/kg D-ribose was chosen as the optimal dose for inhibiting TNF-α production, which is part of the systemic inflammatory response induced by i.p. injection of cisplatin in mice.

Inflammatory reactions, such as upregulation of proinflammatory cytokines and chemokines, are major pathophysiological mechanisms in cisplatin-induced nephrotoxicity (Ramesh and Reeves 2002; Dong and Atherton 2007). To examine whether D-ribose could inhibit the increases in renal TNF-α and MCP-1 concentrations induced by cisplatin injection, we examined the renal TNF-α and MCP-1 concentrations 72 h after cisplatin injection. The renal TNF-α and MCP-1 concentrations in the cisplatin group significantly increased compared with those in the control mice (57.3 ± 3.6 and 444.1 ± 18.3 pg/g tissue, respectively, \( p < 0.01 \); Fig. 2A, 2B). D-Ribose (400 mg/kg) significantly inhibited the increase in renal TNF-α and MCP-1 concentrations seen in the cisplatin group 72 h after cisplatin injection (38.8 ± 3.8 and 347.3 ± 31.6 pg/g tissue, respectively, \( p < 0.01 \); Fig. 2A, 2B). These findings demonstrate that D-ribose reduces cisplatin-induced inflammatory mediators in the injured kidney. There were no significant differences in these variables between the control and D-ribose groups.

**D-Ribose inhibits ICAM-1 mRNA expression after cisplatin injection**

To determine whether D-ribose was capable of inhibiting the infiltration of neutrophils into the injured kidney, we measured the mRNA expression of ICAM-1 by quantitative real-time RT-PCR. After cisplatin injection, ICAM-1 mRNA expression in the kidney in the cisplatin group was significantly higher than that in the control group. In contrast, D-ribose significantly inhibited cisplatin-induced ICAM-1 mRNA expression (\( p < 0.01 \), Fig. 3).

**D-ribose ameliorates renal dysfunction after cisplatin injection**

The protective effect of D-ribose on cisplatin-induced nephrotoxicity was monitored by estimating the level of BUN and creatinine in the sera 72 h after cisplatin and/or D-ribose treatment. Cisplatin administration significantly elevated BUN and serum creatinine levels compared with those in control mice (Table 1). Treatment with D-ribose (400 mg/kg) significantly decreased cisplatin-induced elevations in BUN and creatinine levels (\( p < 0.01 \), Table 1), whereas 100 mg/kg D-ribose had no effect on serum creatinine levels. BUN and creatinine levels after treatment with D-ribose alone were not significantly different from those of control mice (Table 1).

**D-Ribose reduces renal damage after cisplatin injection**

The control group did not show any morphological changes (Fig. 4A). In contrast, extensive renal tubular injury was observed in the cisplatin group 72 h after treatment, including tubular cell necrosis, loss of brush border membrane, tubular dilatation, and inflammatory cell infil-
Treatment with D-ribose significantly reduced the cisplatin-induced renal tubular damage 72 h after cisplatin injection (0.92 ± 0.60 in the cisplatin + D-ribose group versus 3.18 ± 0.59 in the cisplatin group, p < 0.01; Fig. 4C and 4E).

**Discussion**

The present study demonstrated that (i) D-ribose reduced cisplatin-induced renal injury, as assessed by functional and histological measurements; and (ii) D-ribose inhibited not only cisplatin-induced upregulation of renal TNF-α and MCP-1 proteins, but also of ICAM-1mRNA expression, suggesting that D-ribose acts through inhibition of serum TNF-α production to reduce cisplatin-induced nephrotoxicity.

Cisplatin is one of the simplest and most effective chemotherapeutic agents and is widely used for treatment of various types of solid tumors. Cisplatin accumulation in renal cells causes nephrotoxicity, which is the main disadvantage of the clinical use of cisplatin (Arany and Safirstein...
D-Ribose in Cisplatin-Induced Nephrotoxicity (2003). After administration, cisplatin is taken up in renal proximal tubular cells at high concentrations, and toxicity is both time and dose dependent (Yao et al. 2007). Multiple mechanisms, including oxidative stress, DNA damage, apoptosis, and more recently, inflammation, have been implicated in the pathogenesis of cisplatin-induced nephrotoxicity (Arany and Safirstein 2003; Yao et al. 2007; Pabla and Dong 2008). Cisplatin induces a cascade of inflammatory reactions. Several cytokines and chemokines, such as TNF-α, interleukin-1β, MCP-1, and macrophage inflammatory protein (MIP)-2, are upregulated in the kidneys during cisplatin-induced nephrotoxicity. TNF-α is responsible for the production of other cytokines and chemokines, leading to inflammatory renal injury, and is a key player in the cisplatin-induced inflammatory response (Deng et al. 2001; Ramesh and Reeves 2002). The role of TNF-α in cisplatin-induced nephrotoxicity has been extensively studied by using inhibitors of TNF-α or blocking antibody, producing less severe renal damage with reducing TNF-α concentration (Ramesh and Reeves 2002).

Previously, we reported that rare sugar D-allose attenuates cisplatin-induced nephrotoxicity by suppressing the production of TNF-α (Miyawaki et al. 2012). Rare sugars are defined as monosaccharides that exist in nature but are present only in limited quantities. However, the use of these rare sugars for humans remains limited, since the biological function and bioavailability of rare sugars is still poorly understood. Since this result strongly suggests that monosaccharides may have an inhibitory effect on the activation of TNF-α, we screened some familiar monosaccharides except for rare sugars to determine whether they actually have such an effect. Among familiar monosaccharides, D-ribose is a naturally occurring five-carbon monosaccharide that is found in all living cells. D-ribose not only has potential as a metabolic supplement for the heart (Pauly and Pepine 2000), but also exerts anti-inflammatory effects against conditions such as renal ischemia/reperfusion injury (Nishiyama et al. 2009; Sato et al. 2009). In this study, the inhibitory effect of activation of TNF-α with D-ribose on cisplatin-induced nephrotoxicity is almost same potency compared with that of D-allose (Miyawaki et al. 2012). The possibility of applying D-ribose as a pharmaceutical drug may be high because D-ribose is already present in

Table 1. Renal function data.

<table>
<thead>
<tr>
<th>Group</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>21.1 ± 4.3</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>CDDP*</td>
<td>87.1 ± 17.3</td>
<td>0.26 ± 0.06</td>
</tr>
<tr>
<td>CDDP + ribose400c</td>
<td>40.2 ± 7.1</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>CDDP + ribose100d</td>
<td>71.2 ± 4.7</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>Ribosee</td>
<td>23.7 ± 0.7</td>
<td>0.06 ± 0.01</td>
</tr>
</tbody>
</table>

*BUN: blood urea nitrogen; CDDP: cisplatin
*Equivalent volume of saline
^Single dose of cisplatin (20 mg/kg, i.p.)
D-Ribose (400 mg/kg, i.p.) immediately after cisplatin injection
D-Ribose (100 mg/kg, i.p.) immediately after cisplatin injection
Ribose (400 mg/kg, i.p.) alone.
Mean ± s.d. (n = 7/group)
*p < 0.05 compared with control group; **p < 0.01 compared with control group; ^p < 0.05 compared with CDDP group; ***p < 0.01 compared with CDDP group.

![Fig. 3. Effect of D-ribose on ICAM-1 mRNA expression 72 h after cisplatin injection.](image)

Mean ± s.d., n = 5 per group. Control: equivalent volume of saline; CDDP: single dose of cisplatin (20 mg/kg, i.p.); CDDP + ribose: D-ribose (400 mg/kg, i.p.) immediately after cisplatin injection; Ribose: D-ribose alone. **p < 0.01 compared with control group. ***p < 0.01 compared with CDDP group.
humans. Further experimental studies into the effects of D-ribose and D-allose in human are required in order to identify potential pharmaceutical applications. Furthermore, in the present study, a single dose of 400 mg/kg D-ribose inhibited the increase in serum and renal TNF-α concentrations 72 h after cisplatin injection. We used 400 mg/kg D-ribose and found that it safely and effectively suppressed cisplatin-induced renal inflammation in mice; animals treated with D-ribose alone showed no specific side effects or toxicity. Meanwhile, in previous report regarding the metabolism of D-ribose in man, a marked reduction in blood glucose concentration occurred after 20 and 10 gram of D-ribose infusion, presumably by inhibiting the enzyme phosphoglucomutase (Segal and Foley 1958). Further research is required to clarify the other side effects of D-ribose (400 mg/kg) in human.

The precise mechanisms underlying the suppression of nephrotoxicity by D-ribose are not fully elucidated. Cisplatin administration causes nuclear factor (NF)-κB activation with subsequent inflammatory reactions responsible for renal injury (Sung et al. 2008; Kang et al. 2009). In this study, we did not examine whether D-ribose inhibited NF-κB activation. Further studies to assess the effects of D-ribose administration on NF-κB activation in cisplatin-induced nephrotoxicity are warranted.

TNF-α stimulates the production of other cytokines and chemokines, such as MCP-1 and MIP-2 (Banas et al. 1999). MCP-1 and MIP-2 are chemotactic for a variety of leukocytes, including neutrophils, monocytes and natural killer cells (Rovin and Phan 1998). Inflammatory cell infiltration into damaged kidney tissue may be an important process in cisplatin-induced nephrotoxicity. Infiltration of macrophages into kidney tissue is increased 24-72 h after cisplatin administration (Lu et al. 2008). Our observation that D-ribose inhibited the increase in renal tissue MCP-1 concentration and renal ICAM-1 mRNA expression 72 h after cisplatin injection suggests that an increase in chemokines is sufficient to account for increased leukocyte infiltration in the kidneys after cisplatin injection.

As noted above, injection of cisplatin mainly produces proximal tubular necrosis. Consequently, live and dead cells slough off into the lumen of the proximal tubules, resulting in obstruction, fluid back-leakage, and/or cast formation, as well as renal epithelial cell apoptosis and necrosis (Bonegio and Lieberthal 2002; Arany and Safirstein 2003). Casts reduce the glomerular filtration rate (GFR) and lead to renal dysfunction (Thadhani et al. 1996). These changes are associated with the loss of renal functions as revealed by the observed abnormalities in BUN and creatinine. Clinical evidence of acute kidney injury has been demonstrated with elevated levels of serum BUN and creatinine. Marked elevation of serum BUN and creatinine (indicating decreased GFR) in cisplatin-treated compared with control mice confirmed significant acute renal injury in our study. Induction of cisplatin-induced nephrotoxicity is assumed to be a rapid process involving a reaction with proteins in the renal tubules (Montine and Borch 1990). This renal damage occurs within 1 h after cisplatin administration; therefore, it is important that the protective agents be present in the renal tissue before damage occurs. Treatment with D-ribose immediately after cisplatin injection prevented increases in serum BUN and creatinine as well as tubular damage, which was demonstrated histologically by the preservation of renal architecture. Cisplatin
administration increases renal infiltration of leukocytes and macrophages within 72 h (Sung et al. 2008), which plays an important role in the pathophysiology of acute renal injury (Kang et al. 2009). In this study, we demonstrated the preservation of renal histology and a reduction in necrosis in D-ribose-treated mice. These data suggest that the marked morphological protection by D-ribose is sufficient to inhibit the increase of serum BUN and creatinine levels in cisplatin-induced nephropathy.

There were two limitations in the present study. First, the question of potential interference of D-ribose with the chemotherapeutic efficacy of cisplatin is a potential limitation. This is a difficult question to answer. Further studies to assess the effects of D-ribose administration on the chemotherapeutic efficacy of cisplatin are warranted. The second limitation was the timing of D-ribose administration. In a clinical setting, we can administer D-ribose before cisplatin injection as premedication. Further experimental studies are needed to clarify the effects of D-ribose administration before cisplatin injection as premedication.

We have demonstrated that D-ribose attenuated cisplatin-induced nephropathy and therefore represents a potential therapeutic strategy for renal injury caused by cisplatin.

Acknowledgments

All authors report that there was no third-party sponsor or funding involved in the collection, analysis and interpretation of data, the writing of the manuscript, or in the decision to submit the manuscript for publication.

Conflict of Interest

All authors have no conflict of interest.

References