D-Allose Ameliorates Cisplatin-Induced Nephrotoxicity in Mice

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Cisplatin (cis-diamminedichloroplatinum II) is a potent antineoplastic agent widely used to treat various forms of cancer. However, its therapeutic use is limited because of dose-dependent nephrotoxicity. Inflammatory mechanisms may play an important role in the pathogenesis of cisplatin nephrotoxicity. D-allose is an aldo-hexose present in nature that recently has been demonstrated to inhibit production of inflammatory mediators in septic kidneys. The purpose of this study was to determine the protective effects of D-allose on cisplatin-induced nephrotoxicity. Cisplatin (20 mg/kg) was administered by intraperitoneal injection to mice in the cisplatin group and the cisplatin plus D-allose group, as was normal saline to control group mice. D-allose was intraperitoneally administered immediately after cisplatin injection. Serum and renal tumor necrosis factor (TNF)-alpha concentrations, renal monocyte chemoattractant protein-1 (MCP-1; a chemotactic factor for monocytes), renal function, histological changes and renal cortex neutrophil infiltration were determined 72 h after cisplatin injection. The serum TNF-alpha concentration in the cisplatin plus D-allose (400 mg/kg body weight) group significantly decreased in comparison with that in the cisplatin group. The renal TNF-alpha and MCP-1 concentrations in the cisplatin plus D-allose group significantly decreased in comparison with those in the cisplatin group. Neutrophil infiltration in the cisplatin plus D-allose group was significantly lower than that in the cisplatin group. Cisplatin-induced renal dysfunction and renal tubular injury scores were attenuated by D-allose treatment. These results reveal that D-allose attenuates cisplatin-induced nephrotoxicity by suppressing renal inflammation. Hence, D-allose may become a new therapeutic candidate for treatment of cisplatin-induced nephrotoxicity.

Keywords: acute kidney injury; cisplatin; D-allose; monocyte chemoattractant protein-1; tumor necrosis factor alpha

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other significant detrimental effects. It has been demonstrated that D-allose has anti-inflammatory effects (Ueki et al. 2007, 2008). These results have suggested new approaches to development of renoprotective strategies for use during cisplatin chemotherapy.

The present study was designed to investigate the possible protective effect of D-allose for cisplatin-induced nephrotoxicity in mice.

Materials and Methods

Animal 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 and was carried out according to the Kagawa University Animal Experimental Regulations (Kagawa, Japan). Cisplatin was purchased from Sigma Aldrich (St. Louis, MO, USA). Pentobarbital sodium was purchased from Abbott Laboratories, (Chicago, IL, USA). Lidocaine hydrochloride was purchased from AstraZeneca (Wilmington, DE, USA). D-allose was generously donated by the Rare Sugar Research Center (Kagawa University, Kagawa, Japan).

Animal preparation and treatments

Mice were randomly divided into three groups: control, cisplatin and cisplatin + D-allose groups. Cisplatin was dissolved in saline at a concentration of 2 mg/ml. Cisplatin (20 mg/kg) was administered by intraperitoneal injection to animals in the cisplatin and cisplatin + D-allose group, as was normal saline to control group mice. This dose of cisplatin produces severe renal injury in mice (Megyesi et al. 1998; Ramesh and Reeves 2002). Normal saline was intraperitoneally administered to the cisplatin group and D-allose was administered to the control and cisplatin + D-allose groups immediately after cisplatin injection. D-allose was dissolved in saline to accomplish individualized doses of 100 mg/kg at a concentration of 12.5 mg/ml and 400 mg/kg body weight at a concentration of 50 mg/ml. After treatment, mice were acclimated in a specific pathogen free-grade environment and provided food and water ad libitum in a 12-h light/12-h dark cycle. Maximal renal injury was observed at 72 h following intraperitoneal injection of 20 mg/kg cisplatin (Ramesh and Reeves 2004; Kang et al. 2009). Under general anesthesia induced using sodium pentobarbital (50 mg/kg body weight) after 1 ml of lidocaine hydrochloride as a local anesthetic, kidneys were removed by 72 h after cisplatin injection (n = 7 per group) and animals were sacrificed.

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

On the day of sacrifice, pre, 24 h and 72 h after cisplatin injection, blood was collected from the femoral artery and centrifuged (2,500 × g, 10 min, 4°C); sera were collected and stored at −80°C until used. The kidney was removed and homogenized with a homogenizer. Homogenates were then sonicated for 20 s and centrifuged (2,500 × g, 10 min, 4°C). The supernatants were kept at −80°C until the measurement of the cytokine concentration. Enzyme-linked immunosorbent assays (ELISAs) were carried out using a TNF-alpha ELISA kit (R&D Systems, Minneapolis, MN) and MCP-1 ELISA kit (Invitrogen Corporation, Camarillo, CA), according to the manufacturers’ protocols. All assays were performed in duplicate.

Measurement of renal function

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

Renal Histology

Kidneys were removed, placed in 10% formalin, and embedded in buffered 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 (H&E) for general histological examination. Tubular injury was assessed using a semi-quantitative scale (Ramesh and Reeves 2004; Liu et al. 2006). 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 treatment conditions examined 10 fields per kidney under higher-power magnification (∗100) and then assigned necrosis severity scores. In addition, the number of neutrophils per high-power field (∗400) was counted by investigators blinded to treatment conditions, and the average number in 10 fields of each sample was recorded.

Statistical analysis

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

Results

D-allose reduces serum TNF-alpha, renal TNF-alpha and renal MCP-1 concentrations after cisplatin injection

As shown in Fig. 1A, the serum TNF-alpha concentration increased gradually 1 day after cisplatin injection and markedly peaked at 3 day after cisplatin injection. Serum TNF-alpha concentration was markedly increased in cisplatin-treated mice compared to control animals (Fig. 1B). When D-allose was administered at 400 mg/kg body weight, by 72 h after cisplatin injection, the serum TNF-alpha concentration was significantly decreased in comparison with that in the cisplatin group, whereas the 100 mg/kg body weight dose showed no such effect (Fig. 1B). Thus, the dose at 400 mg/kg body weight D-allose was chosen as optimal for inhibiting TNF-alpha production, part of the systemic inflammatory response induced by intraperitoneal injection of cisplatin in mice. Furthermore, to investigate the effect of D-allose on TNF-alpha and MCP-1 concentrations in kidney associated with the inflammatory response induced by cisplatin, we compared the renal TNF-alpha and MCP-1 concentrations at 72 h after cisplatin injection. The renal TNF-alpha and MCP-1 concentrations in the cisplatin + D-allose group were significantly lower than those in the cisplatin group (Fig. 2A, 2B).
D-allose protects against renal dysfunction after cisplatin injection

A single dose of cisplatin significantly increased serum BUN and creatinine 72 h after injection; treatment with D-allose significantly ameliorated these elevations (Table I).

D-allose reduces renal damage after cisplatin injection

Extensive renal tubular injury was observed 72 h after cisplatin injection, including tubular cell necrosis, loss of brush border membrane, tubular dilatation and inflammatory cell infiltration (Fig. 3B). Treatment with D-allose significantly attenuated these effects (Fig. 3C, D).

D-allose reduces inflammatory neutrophil infiltration after cisplatin injection

Cisplatin injection produced a significant increase in the number of infiltrating neutrophils present in renal corti-
The renoprotective effects in mice of D-allose administered immediately after a single intraperitoneal injection of cisplatin were evaluated. Cisplatin-only-treated mice had elevated serum BUN and creatinine levels, more renal tubular necrosis, and increased serum and renal TNF-alpha levels of the cisplatin-treated animals compared to the D-allose-treated animals (Table 2).

**Discussion**

The renoprotective effects in mice of D-allose administered immediately after a single intraperitoneal injection of cisplatin were evaluated. Cisplatin-only-treated mice had elevated serum BUN and creatinine levels, more renal tubular necrosis, and increased serum and renal TNF-alpha levels of the cisplatin-treated animals compared to the D-allose-treated animals (Table 2).

**Table 1. Renal function data.**

<table>
<thead>
<tr>
<th>Group</th>
<th>BUN (mg/dl)</th>
<th>Cr (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.6 ± 0.7</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>79.1 ± 4.8**</td>
<td>0.27 ± 0.05**</td>
</tr>
<tr>
<td>Cisplatin + D-allose400</td>
<td>55.7 ± 17.7**</td>
<td>0.19 ± 0.04**</td>
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BUN; blood urea nitrogen. Mean ± s.d. (n = 7/group).

**p < 0.01 compared with control group.

**p < 0.01 compared with cisplatin group.

Fig. 2. Changes in TNF-alpha and MCP-1 concentrations in the kidney after cisplatin injection.

(A) Change in renal TNF-alpha concentration at 72 h after cisplatin injection. Mean ± s.d., n = 7 per group. Control; control group that received an equivalent volume of normal saline: Cisplatin; animals received a single dose of cisplatin (20 mg/kg, ip): Cisplatin + D-allose400; animals received allose (400 mg/kg, ip) immediately after cisplatin injection. **p < 0.01 compared with controls. ##p < 0.01 compared with cisplatin-treated.

(B) Change in renal MCP-1 concentration at 72 h after cisplatin injection. Mean ± s.d., n = 7 per group. Control; control group that received an equivalent volume of normal saline: Cisplatin; animals received a single dose of cisplatin (20 mg/kg, ip): Cisplatin + D-allose400; animals received allose (400 mg/kg, ip) immediately after cisplatin injection. **p < 0.01 compared with controls. ##p < 0.01 compared with cisplatin-treated.
concentrations compared to cisplatin + D-allose-treated animals, suggesting that D-allose may protect against cisplatin-induced nephrotoxicity.

Cisplatin is an effective antineoplastic agent used to treat cancer. Cisplatin accumulation in renal cells causes nephrotoxicity, the main disadvantage of clinical use of cisplatin (Arany and Safirstein 2003). The highest concentrations of cisplatin in the kidney occur in the proximal tubule, 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 as contributors to 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-alpha, Interleukin (IL)-1beta, MCP-1, and macrophage inflammatory protein (MIP)-2, to name a few, have been found to be upregulated in the kidney during cisplatin-induced nephrotoxicity. TNF-alpha is responsible for 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). TNF-alpha engages cell surface receptors (TNF receptor 1 and TNF receptor 2) to induce a variety of cellular responses, ranging from inflammation to cell death (Pabla and Dong 2008). TNF-alpha can induce apoptosis of renal epithelial cells (Peralta Soler et al. 1996). In TNF-alpha-deficient mice, production and secretion of proinflammatory cytokines and chemokines were attenuated, and this was associated with amelioration of acute kidney injury during cisplatin treatment (Ramesh and Reeves 2002). Similar studies have involved TNF-alpha inhibition by pharmacological inhibitors (Ramesh and Reeves 2004; Kang et al. 2009; Li et al. 2011).

In the present study, D-allose inhibited an increase in
As noted above, injection of cisplatin produces mainly proximal tubular necrosis. Consequently, live and dead cells slough into the lumina of proximal tubules, resulting in obstruction, fluid back-leakage and/or cast formation from 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). Marked elevation in the current study of serum BUN and creatinine (indicating decreased GFR) in cisplatin-treated compared with control mice confirmed significant acute renal injury. Induction of cisplatin-induced nephrotoxicity is assumed to be a rapid process involving reaction with proteins in the renal tubules (Montine and Borch 1990). Because this renal damage occurs within 1 h after cisplatin administration, it is important that the protective agents are present in renal tissue before damage occurs. Treatment with D-allose immediately after cisplatin injection prevented increases in serum BUN and creatinine as well as tubular damage, demonstrated histologically by the relative 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 the in pathophysiology of acute renal injury (Kang et al. 2009). In this study, preservation of renal histology and a reduction in necrosis and renal neutrophil infiltration in D-allose treated mice were demonstrated. The observation that D-allose decreased renal MCP-1 concentration is sufficient to account for the decreased of neutrophil infiltration. These results are consistent with previous reports that D-allose reduced the number of neutrophils after ischemia/reperfusion in liver and kidney (Hossain et al. 2004; Ueki et al. 2007).

The precise mechanisms underlying cisplatin nephrotoxicity are not fully elucidated. On the use of D-allose in other animal injury, another experimental research demonstrated that D-allose inhibited the ischemic injury of inner retina by suppressing the production of hydrogen peroxide after ischemia (Hirooka et al. 2006) and D-allose attenuated cerebral ischemia/reperfusion injury by anti-oxidative effects (Nakamura et al. 2011). D-allose may suppress other pathways independent of TNF-alpha. Oxidative stress with increased generation of reactive oxygen species also seems to play a crucial role (Behling et al. 2006; Yao et al. 2007). Among aldohexoses, D-allose was shown to have a potent inhibitory effect on production of reactive oxygen species (ROS) by stimulated neutrophils (Murata et al. 2003). Potent inhibitory action on production of ROS by D-allose may reduce cisplatin-induced nephrotoxicity. Further experimental studies are required to elucidate the antioxidant effects of D-allose in cisplatin-induced renal toxicity. The current results strongly suggest that monosaccharides like D-allose inhibit activation of neutrophils and may reduce cisplatin-induced nephrotoxicity. However, biological function and physiological implications of D-allose have been little known. D-allose is not toxic to rats in regarding the safety of D-allose as a nutritional substrate (Iga et al. 2010). Further experimental studies into the effects of D-allose and other monosaccharides on cisplatin-induced nephrotoxicity are warranted.

We have demonstrated that D-allose attenuated cisplatin-induced TNF-alpha and MCP-1 production, neutrophil infiltration, and kidney dysfunction. The current study highlights the importance of TNF-alpha pathways in cisplatin-induced nephrotoxicity. Blockade of TNF-alpha production and/or action may be effective for reducing cisplatin-induced nephrotoxicity. However, further studies are needed to determine the precise mechanisms underlying this suppression of TNF alpha by D-allose.

**References**


D-Allose in Cisplatin-Induced Nephrotoxicity

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