p38 MAP kinase inhibition ameliorates cisplatin nephrotoxicity in mice

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Ramesh, Ganesan, and W. Brian Reeves. p38 MAP kinase inhibition ameliorates cisplatin nephrotoxicity in mice. Am J Physiol Renal Physiol 289: F166–F174, 2005. First published February 8, 2005; doi:10.1152/ajprenal.00401.2004.—Cisplatin is an important chemotherapeutic agent but can cause acute renal injury. Part of this acute renal injury is mediated through tumor necrosis factor-α (TNF-α). The pathway through which cisplatin mediates the production of TNF-α and injury is not known. Cisplatin activates p38 MAPK and induces apoptosis in cancer cells. p38 MAPK activation leads to increased production of TNF-α in ischemic injury and in macrophages. However, little is known concerning the role of p38 MAPK in cisplatin-induced renal injury. Therefore, we examined the effect of cisplatin on p38 MAPK activity and the role of p38 MAPK in mediating cisplatin-induced TNF-α production and renal injury. In vitro, cisplatin caused a dose-dependent activation of p38 MAPK in proximal tubule cells. Inhibition of p38 MAPK activation led to inhibition of TNF-α production. In vivo, mice treated with a single dose of cisplatin (20 mg/kg body wt) developed severe renal dysfunction at 72 h [blood urea nitrogen (BUN): 154 ± 34 mg/dl; creatinine: 1.4 ± 0.4 mg/dl], which was accompanied by an increase in kidney p38 MAPK activity and an increase in infiltrating leukocytes. However, animals treated with the p38 MAPK inhibitor SKF-86002 along with cisplatin showed less renal dysfunction (BUN: 55 ± 14 mg/dl; creatinine: 0.3 ± 0.02 mg/dl, P < 0.05), less severe histological damage, and fewer leukocytes compared with cisplatin+vehicle-treated animals. Serum levels of TNF-α, sTNFRI, and sTNFRII also increased significantly in cisplatin-treated mice compared with SKF-86002-treated mice (P < 0.05). Kidney mRNA levels of TNF-α were significantly increased in cisplatin-treated mice compared with either SKF-86002- or saline-treated animals. The hydroxyl radical scavenger DMTU (100 mg·kg body wt⁻¹·day⁻¹) prevented the activation of p38 MAPK by cisplatin both in vitro and in vivo. DMTU also completely prevented cisplatin-induced renal injury (BUN: 140 ± 27 vs. 22 ± 2 mg/dl, P < 0.005) and the increase in serum TNF-α (33 ± 7 vs. 4 ± 2 pg/ml, P < 0.005) and kidney TNF-α mRNA in vivo. We conclude that hydroxyl radicals, either directly or indirectly, activate p38 MAPK and that p38 MAPK plays an important role in mediating cisplatin-induced acute renal injury and inflammation, perhaps through production of TNF-α.

tumor necrosis factor-α; acute renal failure; antioxidants; gene expression; cytokines; protein kinases

CISPLATIN IS A HIGHLY EFFECTIVE chemotherapeutic agent used to treat wide variety of solid tumors (20). However, 25–35% of patients experience a significant decline in renal function after the administration of a single dose of cisplatin (38). Several mechanisms, including oxidants, inflammation, genotoxic damage, and cell cycle arrest, have been incriminated in cisplatin nephrotoxicity (5, 26, 33, 35–37). However, little is known about the role of MAPK in cisplatin-induced acute renal injury. The MAPK pathways are a series of parallel cascades of serine/threonine kinases that are activated by diverse extracellular, physical and chemical stresses and regulate cell proliferation, differentiation and survival (6, 31). The three major MAPK pathways terminate in the ERK, p38, and JNK/SAPK enzymes. The ERK pathway is activated typically by extracellular growth factors and has been linked to cell survival. The p38 and JNK/SAPK pathways are activated by a variety of stresses, e.g., oxidants, UV irradiation, hyperosmolality, and inflammatory cytokines, and have been linked to cell death (6, 45). Cisplatin is known to activate p38, ERK, and JNK/SAPK in various cell lines (30, 39, 44), including renal epithelial cells (2, 28). In many human cancer cell lines, cisplatin preferentially activates p38 MAPK compared with JNK or ERK. Moreover, inhibition of p38 MAPK in ovarian carcinoma cells increases their resistance to cisplatin-induced apoptosis (23), suggesting that activation of p38 MAPK contributes to cell death in response to cisplatin. Cisplatin was recently shown to activate all three MAPKs in kidney both in vitro and in vivo (2). However, no data are available on the role of p38 MAPK in cisplatin-induced acute renal injury. Therefore, one goal of the current study was to examine the role of p38 MAPK in cisplatin-induced renal dysfunction.

We (35–37) and others (7, 42, 43) demonstrated a role for inflammation in cisplatin-induced acute renal failure. In particular, cisplatin increases renal TNF-α production (7, 36, 42) and inhibition of TNF-α production reduces cisplatin nephrotoxicity (35, 36). p38 MAPK is involved in TNF-α production in a variety of models of inflammation (13, 15, 19, 46). However, the role of p38 MAPK in mediating cisplatin-induced TNF-α production has never been evaluated. Therefore, the second goal of the current study was to determine the role of p38 MAPK in cisplatin-induced TNF-α production both in vitro and in vivo.

Finally, there is substantial evidence supporting the role of reactive oxygen species (ROS) in mediating cisplatin-induced renal injury (3–5, 24). ROS are known to activate p38 MAPK in a variety of tissues (12, 18, 32) including kidney (43). Tsuruya et al. (43) recently demonstrated that ROS are involved in the production of TNF-α in response to cisplatin. However, the role of p38 MAPK in this process was not examined. Therefore, the third goal of the current studies was to determine the role of ROS in the activation of p38 MAPK and TNF-α production in response to cisplatin both in vitro and in vivo.

MATERIALS AND METHODS

Cell culture. Immortalized mouse proximal tubule cells TKPTS (8) cells kindly provided by Dr. E. Bello-Reuss and Dr. J. Megyesi were developed from immortalized mouse proximal tubule cells TKPTS (8) cells kindly provided by Dr. E. Bello-Reuss and Dr. J. Megyesi were...
cared in DMEM/F12 supplemented with glutamine, 7.5% FBS, and antibiotics, grown to confluence, and maintained at 37°C in 5% CO₂.

All experiments were carried out in serum-containing medium. Cells were treated with different concentrations of cisplatin for the indicated times and cells were harvested at the end of incubation for Western blot and RNA isolation. For inhibition of p38 MAPK, cells were incubated with SB-203580 (90 μM) beginning 30 min before the cisplatin addition. This concentration of inhibitor was chosen on the basis of preliminary studies of MAPK inhibition in TKPTS cells. Dimethylthiourea (DMTU; 20 mM) was added 30 min before cisplatin addition.

Animals and drug administrations. Experiments were performed using 10- to 12-wk-old male C57BL/6 mice weighing 25–30 g. Mice were maintained on a standard diet and water was freely available. Cisplatin was dissolved in saline at a concentration of 1 mg/ml. Mice were given a single intraperitoneal injection of either saline, cisplatin (20 mg/kg body wt) plus vehicle, cisplatin and SKF-86020, DMTU, or DMTU and cisplatin. SKF-86020 was dissolved in DMSO-Ethanol-saline (30:30:40 by volume) and injected subcutaneously at a dose of 20 mg/kg body wt⁻¹·day⁻¹. DMTU (100 mg·kg⁻¹·day⁻¹) was dissolved in saline and injected intraperitoneally. The initial doses of SKF-86020 and DMTU were administered 30 min before the cisplatin. Cisplatin-treated animals were administered the vehicles for SKF-86020 or DMTU on the same schedule.

Animals were killed 72 h after cisplatin injection and blood and kidney tissues were collected. Kidney tissues were processed for histology, p38 activity, and RNA isolation. All animal protocols conformed with the American Physiological Society’s Guiding Principles in the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State College of Medicine.

Renal function. Renal function was assessed by measurements of blood urea nitrogen (BUN; VITROS DT60II Chemistry slides, Orthoclinical Diagnostics) and serum creatinine (DZ072B, Diazyme Labs).

Quantitation of mRNA by real-time PCR. Real-time PCR was performed in an Applied Biosystem 7700 sequence detection system; 1.5 μg of total RNA was reverse transcribed in a reaction volume of 20 μl using Omniscript reverse transcriptase kit (Qiagen) and random primers. The product was diluted to a volume of 150 μl and either 2-μl (actin) or 10-μl (all others) aliquots were used as templates for amplification using SYBR Green PCR amplification reagent and gene-specific primers. The primer sets used were: actin (forward: CATGATGACTGCCATCGCT; reverse: CATGATGACTGCCATCGCT); TNF-α (forward: GCATGATGCACGAGTTG; reverse: AGATCCATCACGAGTTG); IL-1β (forward: 5'-CTCCATGACCTTGTGTCAGAT; reverse: 5'-TGATCGATATCGCTTACAGCTG); TNF-α (forward: 5'-GATCACATTTACGCTG; reverse: 5'-CTTCAAGGCGAAGACAG); MCP-1 (forward: 5'-ATGAGGCTTCCTGCTG; reverse: 5'-GCTTGGATGTCCTGGAGAGA); TGF-β1 (forward: 5'-TGACGTCAATGGAGTGTAT; reverse: 5'-GCTTCAATGGATGGTAGG); heme oxygenase-1 (forward: AGCATGCCCAAGTTTG; reverse: AGCTCAATGCTGGAGAGA). The amount of RNA was normalized to actin amplified in a separate reaction.

TNF-α, soluble TNFR1 and TNFR2 quantitation by ELISA. The levels of TNF-α, sTNFR1, and sTNFR2 were quantitated using an ELISA kit (Quantikine Mouse TNF-α kit, Quantikine Mouse sTNFR1 and sTNFR2 kit, R&D System, Minneapolis, MN) according to the manufacturer’s instructions.

**p38 MAPK assay in kidney tissue extract.** p38 MAPK activity was measured by immunoprecipitation and phosphorylation of ATF-2 (p38 MAPK assay kit, Cat. No. 9820, Cell Signaling Technologies, Beverly, MA). Briefly, kidney was homogenized in 6 ml of lysis buffer and incubated on ice for 10 min. The homogenate was centrifuged at 10,000 rpm for 5 min and the supernatant was transferred to a fresh tube; 400 μg of supernatant protein in 300-μl volume were immunoprecipitated using immobilized p38 antibody beads. The mix-

![Fig. 1. Effect of cisplatin on p38 MAPK activity. A: TKPTS cells were treated with either saline or cisplatin in the indicated concentrations for 12 h. Cells were harvested and p38 MAPK activity was determined using an immuno-complex kinase assay as described in MATERIALS AND METHODS. B: mice were injected with saline or cisplatin (20 mg/kg) and kidneys were harvested after 72 h. p38 MAPK activity was determined in the kidney homogenate. C: quantitation of p38 MAPK activity by densitometry of phospho ATF-2. *P < 0.001 vs. saline.](image-url)
ture was centrifuged and washed with lysis buffer twice and kinase buffer twice. The pellet was resuspended in 50 μl of kinase buffer containing 200 μM ATP and 2 μg of ATF-2 fusion protein and incubated for 30 min at 30°C. The reaction was arrested by adding SDS-sample loading buffer and then separated on a 4–12% polyacrylamide gel. Proteins were transferred onto a PVDF membrane and probed with anti-phospho ATF-2 antibody. Phospho ATF-2 was visualized using ECL reagents.

Histology and immunohistochemistry. Kidney tissue was fixed in buffered 10% formalin for 12 h and then embedded in paraffin wax. For assessment of injury, 5-μm sections were stained with PAS. Tubular injury was assessed in PAS-stained sections using a semi-quantitative scale (16, 17, 34) in which the percentage of cortical tubules showing epithelial necrosis was assigned a score: 0 = normal; 1 = <10%; 2 = 10–25%; 3 = 26–75%; 4 = >75%. Sections were scored independently by two investigators who were blinded to the treatment of the animal. To quantitate leukocyte infiltration, sections were stained with naphthol AS-D choroacetate esterase (Sigma kit no. 91C), which identifies neutrophils and monocytes. Twenty-five 40 fields of esterase-stained sections were examined for quantitation of leukocytes. For ATF-2 immunohistochemistry, sections were deparaffinized and rehydrated. Antigen retrieval was performed by heating the sections in 10 mM sodium citrate buffer (pH 6.0) at 95°C for 10 min. The sections were washed and incubated with 1% hydrogen peroxide in 90% methanol for 10 min to block endogenous peroxidases. Sections were then blocked with 5% goat serum/1% BSA in PBS, followed by incubation overnight at 4°C with an anti-phospho ATF-2 antibody (diluted 1:50 in blocking buffer). Slides were washed and then incubated with a biotinylated secondary antibody for 1 h followed by ABC reagent. Color was developed with a metal-enhanced DAB reagent (Pierce), counterstained with hematoxylin, and mounted.

Renal platinum content. Platinum content was measured in kidneys removed 72 h after cisplatin injection and in cells harvested after 6 h of cisplatin treatment. Tissues and cells were digested in a mixture of Ultrex trace metal grade nitric acid and hydrochloric acid for 1 h at 100°C. The digested samples were diluted with high-purity water and subjected to inductively coupled plasma/mass spectroscopy (Perkin Elmer Elan 6000 ICP/MS) to determine the platinum content.

Fig. 2. Immunolocalization of phosphorylated ATF-2. Kidneys were harvested from mice injected with saline (A, D) or cisplatin (B) or cisplatin and SKF-86002 (C). Phosphorylated ATF-2 was detected by immunohistochemistry as described in MATERIALS AND METHODS. A: saline-treated kidney, ATF-2 antibody. B: cisplatin-treated kidney, ATF-2 antibody. C: cisplatin and SKF-86002-treated kidney, ATF-2 antibody. D: saline-treated kidney, no primary antibody. Brown nuclear staining is seen mainly in cisplatin-treated kidneys and was largely prevented by SKF-86002. Magnification: ×1,000.

Fig. 3. Effects of p38 MAPK inhibition on TNF-α production. TKPTS cells were treated with saline or 100 μM cisplatin (CP) for 12 h in the presence or absence of SB-203580. Cellular TNF-α mRNA (A) and TNF-α protein in the culture supernatant (B) were determined as in MATERIALS AND METHODS. Similarly, mice were treated with saline, cisplatin, or cisplatin and SKF-86002 and killed after 72 h. Renal TNF-α mRNA (A) and protein content (B) was determined. Values are means ± SE or n = 5–8. *p < 0.05 vs. saline. +p < 0.05 vs. cisplatin.
Table 1. Effect of SKF-86002 on serum levels of TNF-α and TNF receptors

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TNF-α, pg/ml</th>
<th>sTNFR1, pg/ml</th>
<th>sTNFR2, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>ND</td>
<td>684 ± 11</td>
<td>7,197 ± 1,455</td>
</tr>
<tr>
<td>Cisplatin plus vehicle</td>
<td>39.2 ± 10</td>
<td>7,098 ± 2,919†</td>
<td>16,116 ± 1,984†</td>
</tr>
<tr>
<td>Cisplatin and SKF-86002</td>
<td>14.2 ± 1.9*</td>
<td>1,818 ± 183*</td>
<td>9,095 ± 177*</td>
</tr>
<tr>
<td>n = 5–8</td>
<td>n = 4–8</td>
<td>n = 5–8</td>
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</tbody>
</table>

Values are means ± SE. ND, not detectable. *P < 0.05 vs. cisplatin. †P < 0.01 vs. saline.

Statistical methods. All assays were performed in duplicate. The data are reported as means ± SE. Statistical significance was assessed by unpaired, two-tailed Student’s t-test for single comparison or ANOVA for multiple comparisons.

RESULTS

Cisplatin induces p38 MAPK activation in proximal tubule cells in vitro and in mouse kidney in vivo. Figure 1 illustrates the effect of cisplatin on the activity of p38 MAPK. We measured p38 MAPK enzyme activity by substrate phosphorylation as well as the phosphorylation status of p38 enzyme by Western blot analysis. Cisplatin resulted in a dose-dependent increase in both enzyme activity and phospho-p38 levels (not shown) in vitro. In vivo, p38 MAPK activity was also markedly increased when measured at 72 h after cisplatin injection. These results confirm the recent report by Arany et al. (2) demonstrating increased phosphorylation of p38 MAPK in cisplatin-treated TKPTS cells and mouse kidney. Immunohistochemical localization of phosphoATF-2 was used to determine the site of p38 MAPK activation in vivo. We used phosphoATF-2 rather than phospho p38 MAPK as a marker of p38 MAPK activation in vivo since the p38 MAPK inhibitor used in this study, SKF-8602, does not inhibit the phosphorylation of p38 MAPK. As shown in Fig. 2, kidneys from saline-treated mice demonstrated little or no ATF-2 phosphorylation. In contrast, kidneys from cisplatin-treated mice showed extensive ATF-2 phosphorylation, primarily in a nuclear distribution as expected for a transcription factor. The phosphoATF-2 staining was seen mainly in proximal tubular cells of the cortex. Glomeruli and blood vessels were not stained. Kidneys from mice treated with cisplatin and SKF-8602 had less phosphoATF-2 staining, confirming that the inhibitor was active in vivo and that phosphoATF-2 staining reflected p38 MAPK activity.

Inhibition of p38 MAPK blocks TNF-α expression in vivo and in vitro. p38 MAPK activation leads to TNF-α production in number of cell types (13, 15, 19, 27, 46). To determine whether p38 MAPK mediates TNF-α expression in renal proximal tubule cells in response to cisplatin, TKPTS cells were treated with cisplatin along with the p38 MAPK inhibitor SB-203580 for 12 h. As shown in Fig. 3, cisplatin induced an 8- to 10-fold increase in TNF-α mRNA levels. SB-203580 significantly blunted the cisplatin-induced TNF-α mRNA expression and reduced TNF-α protein secretion into the culture supernatant. SB-203580 did not affect the cellular uptake of cisplatin (222 ± 22 vs. 257 ± 20 ng Pt/mg protein, cisplatin vs. cisplatin + SB-203580, P = NS). Similarly, mice treated with cisplatin had increased amounts of renal TNF-α protein and mRNA which were significantly reduced by the p38 inhibitor SKF-86002 (Fig. 3B). SKF-86002 also significantly reduced the cisplatin-induced increases in serum levels of TNF-α, sTNFR1 and sTNFR2 (Table 1).

p38 Inhibition reduces cisplatin-induced functional and structural renal damage. To examine the role of p38 MAPK in cisplatin-induced kidney dysfunction, cisplatin was administered alone or in combination with SKF-8602 to mice. As shown in Fig. 4, cisplatin injection produced severe renal failure. Administration of SKF-8602 along with cisplatin significantly reduced both BUN (154 ± 34 vs. 55 ± 14 mg/dl, P < 0.05) and creatinine (1.4 ± 0.4 vs. 0.3 ± 0.02 mg/dl, P < 0.05) levels compared with cisplatin and vehicle. Vehicle alone did not affect either urea or creatinine values (not shown). The improvement in renal function was accompanied by less severe histological damage and reduced leukocyte infiltration. As shown in Fig. 5, cisplatin treatment resulted in severe tubular injury reflected by cast formation, loss of brush-border membranes, sloughing of tubular epithelial cells, and dilation of tubules. These changes were minimal in SKF-86002-treated animals. Semiquantitative scores of tubular necrosis were significantly lower in cisplatin plus SKF-86002-treated animals than in mice which received cisplatin and vehicle (Fig. 5D). As noted previously (35–37), cisplatin-induced renal injury is accompanied by the influx of inflammatory cells. Treatment with SKF-86002 significantly reduced the number of infiltrating leukocytes (Fig. 5D). The improvements in renal function and histology were not due to an inhibition of platinum uptake in the kidney by SKF-8602. The platinum content of kidneys of mice treated with cisplatin plus SKF-86002 was similar to mice treated with cisplatin plus vehicle (8,113 ± 710 vs. 9,350 ± 1,001 μg Pt/kg tissue, P > 0.05, n = 3).

SKF-86002 inhibits the upregulation of cytokine and adhesion molecule expression induced by cisplatin. Cisplatin injection results in the upregulation of a number of proinflammatory cytokines and chemokines in the kidney (36). Accordingly, we tested whether inhibition of p38 MAPK reduced the expression of these cytokines. Kidneys were harvested 72 h after treatment with cisplatin alone or in conjunction with SKF-86002. Cytokine and chemokine gene expression was determined by RT-
PCR. TNF-α, IL-1β, MCP-1, ICAM-1, and TGF-β1 were upregulated after cisplatin injection (Fig. 6). TNF-α expression was reduced significantly ($P < 0.05$) by SKF-86002. Although the expression of the other genes was also reduced by SKF-86002, these differences did not reach statistical significance.

Effect of hydroxyl radical scavenger on cisplatin-induced p38 MAPK activation and nephrotoxicity. The results presented above (Figs. 1 and 2) show an increase in p38 MAPK activity in response to cisplatin in the kidney and in a proximal tubule cell line. Because cisplatin is known to generate ROS (22, 24), and ROS can activate p38 MAPK (12, 18, 32), we examined the effect of a hydroxyl radical scavenger, DMTU, on cisplatin-induced renal dysfunction and p38 MAPK activation. Consistent with the results in Fig. 1, cisplatin caused a dose-dependent increase in p38 MAPK phosphorylation (not shown) and activity in proximal tubule cells. The addition of 20 mM DMTU to TKPTS cells completely prevented p38 MAPK activation (Fig. 7). Similarly, kidneys from cisplatin-treated mice showed increased p38 MAPK activity that was completely prevented by the administration of DMTU (100 mg·kg body wt$^{-1}$·day$^{-1}$). DMTU also protected against cisplatin-induced renal dysfunction and histological damage (Fig. 8). Finally, DMTU markedly blunted the increase in serum TNF-α concentrations and the increase in kidney TNF-α mRNA in cisplatin-treated mice (Fig. 9). Heme oxygenase-1, known to be upregulated in cisplatin nephrotoxicity (1), was measured as a marker of oxidative stress. DMTU completely prevented the cisplatin-induced upregulation of HO-1 (Fig. 10).

**DISCUSSION**

Inflammatory mechanisms contribute to the pathogenesis of several forms of acute renal failure, including cisplatin nephrotoxicity.
rotoxicity. The production of TNF-α in response to cisplatin is a central element in renal injury (36). Inhibition or deletion of TNF-α reduces cisplatin-induced renal dysfunction, epithelial cell necrosis and apoptosis and infiltration of leukocytes (35, 36). The pathways by which cisplatin induces TNF-α production are poorly understood. p38 MAP kinase is activated in cisplatin nephrotoxicity and mediates the production of TNF-α in a number of other settings. However, the role p38 MAPK in cisplatin nephrotoxicity and in cisplatin-induced TNF-α production, in particular, is unknown. The current studies were performed to address these issues.

We determined that p38 MAPK activity is increased in response to cisplatin both in vitro and in vivo. Arany et al. (2) recently reported that cisplatin increased p38 MAPK phosphorylation in TKPTS cells, the same cells used in the present study, and in kidney of cisplatin-treated mice. Our studies, which measured actual enzyme activity in addition to phosphorylated enzyme, confirm their findings. In addition, using immunocytochemistry, we determined that p38 MAPK was activated in cells of the proximal tubule in vivo, the predominant site of cisplatin-induced injury. A significant finding of our study is that pharmacological inhibition of p38 MAPK resulted in a substantial reduction in cisplatin nephrotoxicity in vivo as assessed by either functional (Fig. 4) or structural (Fig. 5) parameters. These results indicate that p38 MAPK signaling contributes to cisplatin-induced renal injury in vivo. Similarly, p38 MAPK is believed to contribute to the tumoricidal activity of cisplatin in cancer cells (23). p38 MAPK activation has also been observed after renal ischemia-reperfusion (27, 29, 47) and likely plays a role in injury in that setting. Furuichi et al. (11) reported that inhibition of p38 MAPK reduced renal ischemia-reperfusion injury. Similarly, brief periods of ischemia that precondition the kidney to resist subsequent ischemic injury are associated with a marked reduction in p38 MAPK activation (29). Thus p38 MAPK activation may be a common element in the mechanism of acute renal injury.

Arany et al. (2) found that inhibition of ERK, but not p38 MAPK, reduced cisplatin-induced cell death in vitro. The effects of MAPK inhibition in vivo were not examined in that study. In the present study, we focused on the role of p38 MAPK on cisplatin nephrotoxicity in vivo. It is likely that direct cytotoxicity, as studied in vitro, is only one component of a complex mechanism of renal injury in vivo (40). There-
fore, our finding that p38 MAPK is involved in nephrotoxicity in vivo does not contradict the results of Arany et al. (2). Rather, p38 MAPK may be involved in other processes, such as inflammation or hemodynamic alterations, which are not recapitulated by in vitro models.

TNF-α is produced in response to cisplatin and plays an important role in the pathogenesis of renal injury. Inhibition of TNF-α production or action reduces nephrotoxicity and reduces the infiltration of leukocytes within the kidney (35–37). Therefore, we examined whether the protective effect of p38 MAPK inhibition in cisplatin nephrotoxicity is associated with a reduction in TNF-α production and a reduction in renal inflammation. Indeed, we found that cisplatin led to the up-regulation of both TNF-α mRNA and protein in TKPTS cells in vitro and in kidney in vivo and that p38 MAPK inhibition markedly reduced TNF-α mRNA and protein levels in both settings. Inhibition of p38 MAPK also markedly reduced the influx of inflammatory cells into the kidney. Although cisplatin is known to stimulate p38 MAPK activity in a variety of cells (30, 39, 44), and leads to Fas ligand expression in ovarian cancer cells (23), this is the first report, to our knowledge, of cisplatin stimulating TNF-α production through p38 MAPK. p38 MAPK has previously been implicated in TNF-α production after renal ischemia (11, 27) and in a murine model of lupus (15). Thus p38 MAPK may be an important mediator of TNF-α production in a variety of forms of renal injury. The mechanism whereby p38 MAPK increases TNF-α production is cell and stimulus specific. p38 MAPK has been reported to increase TNF-α production through effects on transcription, mRNA stability, and translation (10, 21, 41). Further studies will be required to determine the precise mechanism for p38 MAPK stimulation of TNF-α production in proximal tubule cells in response to cisplatin.

Oxidants are potent activators of MAPKs, including p38 MAPK (12, 18, 32). Cisplatin stimulates the production of ROS, including hydroxyl radicals. The formation of hydroxyl radicals in cisplatin-treated kidney cells, both in vitro and in vivo, involves the release of iron from the heme groups of cytochrome P-450 2E1 (22). Earlier studies by Matsushima et al. (24) showed that DMTU, a hydroxyl radical scavenger, prevented accumulation of malondialdehde, tubular damage, and renal dysfunction in cisplatin toxicity. In another study, DMTU reduced cisplatin-induced apoptotic cell death and the expression of death receptors (TNFR1 and Fas) and their ligands in the kidney (43). However, a possible role for p38 MAPK in that process was not examined. In the present study, we confirmed that DMTU reduced cisplatin-induced renal injury and TNF-α production. We also demonstrated that DMTU prevented the activation of p38 MAPK by cisplatin. This finding, along with the demonstration that p38 MAPK is required for cisplatin-induced TNF-α production, is consistent
with the view that hydroxyl radicals stimulate p38 MAPK, which in turn, increases TNF-α production.

The mechanism whereby hydroxyl radicals activate p38 MAPK in kidney cells is not known. In macrophages, oxidant stress activates p38 MAPK via src (18). Arany et al. (2) reported recently that cisplatin-induced activation of ERK was mediated through src. Although cisplatin also stimulated p38 MAPK in that study, the effect of src inhibition on p38 MAPK activity was not reported. We believe that different mechanisms mediate the activation of p38 MAPK and ERK in response to cisplatin. In this regard, we noted that DMTU completely inhibited the activation of p38 MAPK by cisplatin (Fig. 7), but did not prevent the phosphorylation of ERK (preliminary studies). Alternatively, or in addition to src, oxidants might activate p38 MAPK via ASK1. ASK1 is a member of the MAPKKK family and activates both the p38 MAPK and the JNK signaling pathways (14). Under normal conditions, ASK1 forms an inactive complex with thioredoxin. In the presence of oxidant stress, cysteine residues of thioredoxin are oxidized, resulting in dissociation of thioredoxin from ASK1 and activation of ASK1 (25). Further studies will be required to determine the elements of the signaling pathway upstream of p38 MAPK activated in response to cisplatin.

In conclusion, we demonstrated that cisplatin stimulates TNF-α production in the kidney through a mechanism that involves the production of hydroxyl radicals and the subsequent activation of p38 MAPK. Inhibition of p38 MAPK not only reduces TNF-α production but also provides marked protection against cisplatin-induced renal injury in vivo. Inhibitors of p38 MAPK are in clinical trials (9). Accordingly, p38 MAPK may be an attractive target for the prevention of cisplatin nephrotoxicity.

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REFERENCES


