Wistar rats were cotreated with cisplatin (5 mg/kg, i.p.) and loss and ROS production of PTCs. In the in vivo study, male rats were divided into 5 groups: control, cisplatin (20 mg/kg, i.p.), cisplatin (20 mg/kg, i.p.) and edarabone (1 or 5 mg/kg, i.v.). Effects of edarabone on the kidney were examined 5 days after treatment. Cisplatin resulted in renal dysfunction, renal tubular damage, mitochondrial damage (assayed by histochemical staining for respiratory chain complex IV), renal protein oxidation (examined by Western blot analysis using a specific antibody for carbonyl group-containing proteins), and tubular apoptosis (determined by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling staining). The above changes were attenuated by edarabone treatment. Thus, edarabone exhibited cytoprotective effects in PTCs and renoprotective effects against cisplatin. Our findings suggest that ROS, in particular hydroxyl radicals, are involved in cisplatin nephropathy and that edarabone may be potentially useful in protecting the kidneys and prevention of acute renal failure.

ABSTRACT
Accumulating evidence suggests that enhanced peroxidative damage caused by reactive oxygen species (ROS) may contribute to the pathogenesis of cisplatin-induced acute renal failure. Nevertheless, little is known about the involvement of oxygen radicals in cisplatin nephropathy. In this study, we investigated the effects of a novel free radical scavenger, 3-methyl-1-phenyl-pyrazolin-5-one (MCI-186; edarabone), on murine proximal tubular cell (PTC) damage induced by exposure to cisplatin in vitro and on renal function in an in vivo model of cisplatin-induced acute renal failure. Edarabone inhibited cisplatin-induced (40 μM, 24 h) cytotoxicity in a concentration-dependent manner (10^-9 to 10^-3 M). Edarabone also attenuated cisplatin-induced mitochondrial transmembrane potential loss and ROS production of PTCs. In the in vivo study, male Wistar rats were cotreated with cisplatin (5 mg/kg, i.p.) and edarabone (1 or 5 mg/kg, i.v.). Effects of edarabone on the kidney were examined 5 days after treatment. Cisplatin resulted in renal dysfunction, renal tubular damage, mitochondrial damage (assayed by histochemical staining for respiratory chain complex IV), renal protein oxidation (examined by Western blot analysis using a specific antibody for carbonyl group-containing proteins), and tubular apoptosis (determined by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling staining). The above changes were attenuated by edarabone treatment. Thus, edarabone exhibited cytoprotective effects in PTCs and renoprotective effects against cisplatin. Our findings suggest that ROS, in particular hydroxyl radicals, are involved in cisplatin nephropathy and that edarabone may be potentially useful in protecting the kidneys and prevention of acute renal failure.

Cisplatin is a simple platinum complex, comprising a central platinum atom, a chloride atom and ammonia molecule in the cis-position, and is effective in the treatment of a wide variety of neoplastic diseases (Lebwohl and Canetta, 1998). Although effective, cisplatin is associated with many adverse drug reactions, such as renal damage, gastrointestinal dysfunction, auditory toxicity, and peripheral nerve toxicity (Coolen et al., 1994). Nephrotoxicity in particular is a major complication and a dose-limiting factor for cisplatin therapy (Safirstein et al., 1986). Administration of cisplatin is frequently associated with renal insufficiency and tubular dysfunction. The possible involvement of peroxidative damage caused by a reactive oxygen species (ROS) has been suggested in the pathogenesis of cisplatin-induced renal failure (Matsushima et al., 1998). In particular, the hydroxyl radical is highly reactive among oxygen radicals. Once excessive hydroxyl radicals are released, lipid peroxidation, which causes changes in the fluidity and permeability of membranes, is induced (Schmidley, 1990). Several antioxidants and oxygen radical scavengers have been reported to be effective in protection against these injuries (Rao et al., 1999; Davis et al., 2001; Hara et al., 2001). The roles of hydroxyl radicals and other free radical species in cisplatin nephrotoxicity have not been fully elucidated, however.

The novel free radical scavenger, 3-methyl-1-phenyl-pyrazolin-5-one (MCI-186; edarabone), has been shown to trap both hydroxyl radicals and prevent iron-induced peroxidative injuries (Murota et al., 1990). It has also been shown to have protective effects against cerebral and myocardial ischemia.
in rats. Pharmacological studies have suggested that the anticerebral ischemic action of edarabone is related to its antioxidant action (Watanabe et al., 1994a; Wu et al., 2000). Oxidative stress is also involved in the pathogenesis of glomerular and tubular injuries in various acute renal diseases (Baud and Ardaillou, 1986). Nevertheless, the usefulness of this compound in acute renal disease has not been investigated.

The present study was designed to examine whether edarabone would prevent cisplatin-induced cytotoxicity in cultured renal epithelial cells. Furthermore, we investigated whether edarabone provided protection against cisplatin-induced deterioration of renal function and structural injury in vivo. Our results indicate that edarabone may be of value in preventing cisplatin-induced cytotoxicity and decline of renal function. The beneficial effect of edarabone relates to reducing the mitochondrial damage, ROS generation and lipid, protein, and nucleic acid oxidation, ultimately resulting in preservation of tubular cell structure.

Materials and Methods

In Vitro Studies

Cell Culture. Murine renal proximal tubular epithelial mProx cells (PTCs; kindly provided by Dr. T. Sugaya, Discovery Research Laboratory, Tanabe Seiyaku Co., Osaka, Japan) were used. These PTCs were microdissected out from C57BL/6J adult mouse kidney and immortalized and transfected with the SV40 large T antigen. These cells were stained by cytokeratin, but not by α smooth muscle actin, and showed albumin reabsorption (Takaya et al., 2003). Thus, this cell line expressed proximal tubular phenotype. PTCs were cultured in Dulbecco’s modified Eagle’s medium supplemented with 4 mM glutamine, 5% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C under a 95% room air/5% CO2 gas mixture on a 1% gelatin-coated dish. Cultured cells from the 4th to 30th passages were used for the experiments.

Cell Viability Assays. Cell viability was examined by trypan blue exclusion for living cells and the tetrazolium salt (WST-1) degradation assay for mitochondrial viability. For trypan blue exclusion, cells were seeded in 12-well plates followed by exposure to medium containing phosphate-buffered saline or 20 μM cisplatin (Sigma-Aldrich Japan Co., Tokyo, Japan) for 24 h with or without edarabone (a gift from Mitsubishi Pharma Corp., Osaka, Japan). Nonadherent cells were removed, and adherent cells were harvested by trypsin-EpDI digestion and stained with 0.4% trypan blue for 5 min at 37°C.

Measurement of Mitochondrial Membrane Potential (∆Ψm) and Hydrogen Peroxide Production. To assess changes in ∆Ψm, we used the potentiometric, fluorescent dye 5',6'-tetrachloro-1,1',3',3'-tetraethylbenzimidazolyl-carbocyanine iodide (JC-1; Molecular Probes, Inc., Eugene, OR) (Di et al., 1995). We determined changes in intracellular ROS levels by measuring the oxidative conversion of cell-permeable 2',7'-dichlorofluorescein diacetate (DCFH-DA; Molecular Probes, Inc.) to fluorescent dichlorofluorescein (DCF), by confocal laser scanning microscopy or fluorescence-activated cell sorting (FACS) (Mancini et al., 1998). Cells were seeded in six-well plates followed by exposure to medium containing phosphate-buffered saline or 20 μM cisplatin for 6 h with or without edarabone. Cells were then incubated with 5 μg/ml JC-1 or 20 μM DCFH-DA in medium for 15 min at 37°C in the dark. Both floating and adherent cells were collected and pelleted by centrifugation at 500g for 5 min. In each sample, a minimum of 10,000 cells was subjected to FACS using a FACs Calibur (Nippon Becton Dickinson Co., Tokyo, Japan) and CellQuest software (Nippon Becton Dickinson). Intracellular distribution of the dye was assessed by confocal laser-scanning microscopy TCS-NT (Leica-Microsystems Co., Tokyo, Japan).

In Vivo Studies

Animal Experiments. Male Wistar rats (300 ± 30 g b.wt.; Charles River Japan, Inc., Kanagawa, Japan) were maintained on standard chow and tap water ad libitum. The experimental protocol was approved in advance by the Ethics Review Committee for Animal Experimentation of the Kawasaki Medical School (Kurashiki, Japan). Rats were assigned to four groups as follows: group 1, saline-treated controls (n = 7); group 2, cisplatin-treated rats (n = 7); group 3, rats treated with cisplatin plus edarabone at a dose of 1 mg/kg (n = 7); and group 4, rats treated with cisplatin plus edarabone at a dose of 5 mg/kg (n = 7). Under light ether anesthesia, animals were injected intraperitoneally with 1.0 ml of saline or 5 mg/kg cisplatin (Matsushima et al., 1998; Ramesh and Reeves, 2002). At the same time, 0.1 ml of saline or 1 or 5 mg/kg edarabone was administered by a single intravenous injection (Kawai et al., 1997). Animals from each group were placed into metabolic cages for 24 h of urine collection for measurement of N-acetyl-β-d-glucosaminidase (NAG) excretion and creatinine clearance (Ccr) at 4 days after injection of cisplatin or saline. After 5 days, animals were humanely killed using pentobarbital anesthesia. Blood samples were obtained for measurement of blood urea nitrogen (BUN) and creatinine, and the kidneys were dissected out. Surgically removed kidney specimens were immediately frozen in liquid nitrogen and unfixed cryostat sections (5-μm thick) were prepared for cytochrome c oxidase staining. Another kidney cross-section was collected in 4% neutral buffered paraformaldehyde for histopathological evaluation. The remaining portions of the kidney were frozen in liquid nitrogen and stored at −80°C for DNA and protein isolation.

Histopathology. The tissues collected in paraformaldehyde were processed, embedded in paraffin, sectioned (4-μm thick), and stained with H&E. A pathologist performed a semiquantitative analysis of the kidney sections in a blinded fashion. Changes observed were limited to the tubules, especially the proximal straight S3 portion, the main site of cisplatin toxicity, such as cell swelling, vacuolization, necrosis, and desquamation. Tubular lesions were graded as follows: 0, no damage; 1+, lesion areas<50%; 2+, lesion areas>50% with or without focal involvement of the S3 portion; and 3+, lesion areas 100% with diffuse involvement of the medullary rays. The mean score for each group was calculated.

Cytochrome c Oxidase Staining. To confirm mitochondrial activity in vivo, cytochrome c oxidase (COX) staining methods (Seligman et al., 1968) were used. The percentages of COX-positive areas, with the exception of the glomerulus, were measured by using an image analyzer (MCID image analyzer; Fuji Film, Tokyo, Japan). Both floating and adherent cells were collected and pelleted by centrifugation at 500g for 5 min. In each sample, a minimum of 10,000 cells was subjected to FACS using a FACs Calibur (Nippon Becton Dickinson Co., Tokyo, Japan) and CellQuest software (Nippon Becton Dickinson). Intracellular distribution of the dye was assessed by confocal laser-scanning microscopy TCS-NT (Leica-Microsystems Co., Tokyo, Japan).

Western Blot Analysis. Kidney samples were homogenized in lysis buffer containing 0.25 M sucrose, 50 mM diithiotreitol, 3 mM HEPES (pH 7.9), 500 μM EDTA, 1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride, 0.8 μM aprotinin, 21 μM leupeptin, 36 μM bestatin, 15 μM pepstatin A, 14 μM (4-guanidino)butane, and 1% Triton X-100. After centrifugation (8000g, 10 min, 4°C), the supernatants were used for Western blotting. Protein concentrations were determined using a protein concentration assay kit (QuantiPro BCA assay kit; Sigma-Aldrich). Western blot analysis for oxidative protein was performed with the Oncor Oxylab kit, according to the manufacturer’s protocol (Oncor, Gaithersburg, MD), as described previously (Keller et al., 1993). For the 4-hydroxy-2-nonenal (HNE) immunoblot, proteins (10 μg) were separated in a 12.5% SDS-polyacrylamide gel and then transferred onto a polyvinylidene difluoride membrane.
The membranes were incubated for 1 h with anti-HNE monoclonal antibody (1.0 μg/ml; Japan Institute for the Control of Aging, Fuku-
rai, Shizuoka, Japan) followed by incubation with peroxidase-conju-
gated goat anti-mouse IgG antibody (0.1 μg/ml) for 1 h. Bands were visualized using the ECL-plus Western blotting detection system (Amersham Biosciences, Inc., Tokyo, Japan).

Assessment of DNA Oxidative Injury. The amount of 8-hy-
droxy-deoxyguanosine (8-OHdG) in DNA was measured using a com-
petitive enzyme-linked immunosorbent assay kit (8-OHdG Check, Japan Institute for Control of Aging) (Toyokuni et al., 1997).

Genomic DNA from the kidneys was extracted using DNAzol reagent (Invitrogen Oriental Co., Tokyo, Japan). After hydrolyzing with DNA nuclease and alkaline phosphatase, 10 μg of DNA were used for enzyme-linked immunosorbent assay.

The number of apurinic/apyrimidinic (AP) sites in DNA was de-
tected using a DNA damage quantification kit (Kumamoto Immuno-
chemical Laboratory Co., Kumamoto, Japan). Purified DNA (1 μg) was incubated with N′-aminoxyethyl-carbonyl-hydrazino-d-bi-
tin, which binds specifically to AP sites, for 1 h at 37°C. After fixing in 96-well plates overnight at room temperature, the plates were incubated with peroxidase-conjugated streptavidin in 1 h at 37°C. After color development with substrate solution, absorbances were measured at 660 nm.

Assessment of Apoptosis in Vivo. Apoptotic nuclei were de-
tected with the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) method, using an apoptosis detection kit (in situ apoptosis detection kit; TaKaRa Biomedicals) accord-
ing to the protocol specified by the manufacturer. The analysis was performed in a blinded fashion. Random areas were viewed at a magnification of ×100 and scored for the number of apoptotic nuclei present in cortical renal tubules. The mean number of stained cells in at least 20 random fields was expressed as the number of TUNEL-positive cells.

Statistical Analysis. All data are presented as mean ± S.E.M. The Student’s t test was used for comparisons between two groups. 

Results

In Vitro Studies

Cell Viability. To test whether edarabone protected against cisplatin-induced cell death, cell viability was exam-
ined by trypan blue exclusion. The number of living PTCs decreased by treatment with 20 μM cisplatin for 24 h. Edarabone treatment significantly attenuated cisplatin-induced cytotoxicity in a dose-dependent manner. Treatment with 10−4 M edarabone increased cell viability by 45.0% (cisplatin: 38.6 ± 4.3%; cisplatin + 10−4 M edarabone: 83.6 ± 5.2%; P < 0.01; n = 6) (Fig. 1A). Mitochondrial viability was determined by degradation of tetrazolium salt with mitochondria dehydro-
genation enzyme. The results also revealed that cisplatin significantly decreased mitochondria dehydrogenation enzyme activity. Incubation with various concentrations of edarabone resulted in a concentration-dependent increase in survival rate (Fig. 1B). Treatment with 10−4 M edarabone significantly increased mitochondrial dysfunction induced by cisplatin (cisplatin: 55.1 ± 9.1%; cisplatin + 10−4 M edarabone: 84.9 ± 12.6%; P < 0.01; n = 6).

∆Ψm Change and Reactive Oxygen Species Production. The ∆Ψm is an important indicator of mitochondrial function and dysfunction. To assess mitochondrial damage induced by cisplatin, we monitored changes in ∆Ψm using the fluorescent dye JC-1. FACS analysis showed that high ∆Ψm was maintained in control cells (68.9%) (Fig. 2). After cispla-
tin treatment, the rate of high ∆Ψm was decreased (5.4%), and the rate of low ∆Ψm was increased (46.5%). By coincu-

Fig. 1. Cell viability assay. A, cell survival rate determined from trypan blue staining after 20 μM cisplatin treatment for 24 h with or without edarabone (10−3 to 10−7 M). *, P < 0.01 versus cisplatin (+) + edarabone (−). B, mitochondrial dehydrogenation enzyme activity determined from WST-1 assay after 20 μM cisplatin treatment for 24 h with or without edarabone (10−3 to 10−7 M). The trypan blue exclusion and WST-1 assays were both performed in triplicate and repeated at least twice. Data are expressed as mean percentages ± S.E.M., relative to noncisplatin-treated cells. *, P < 0.01 versus cisplatin (+) + edarabone (−).

Fig. 2. ∆Ψm change. PTCs were incubated with 20 μM cisplatin with or without 10−4 M edarabone for 6 h. The compound JC-1 was used as a probe for ∆Ψm. Flow cytometry analysis of the cells 6 h after exposure to 20 μM cisplatin with or without 10−4 M edarabone were shown. The abscissa shows 530-nm emission values that reflect low ∆Ψm cells. The ordinate shows 580-nm emission values that reflect high ∆Ψm cells. Each experiment was performed in triplicate and repeated at least twice. Each panel represents the results from a total 10,000 input cells.

In Vivo Studies

Morphological Evaluation. Compared with controls at day 5, renal tissue sections from the cisplatin group exhibited increased evidence of acute structural damage, characterized by tubular necrosis, degeneration, casts, and red blood cell
extravasation (Fig. 4). These changes usually involved the entire S3 segments in the outer stripe of the medulla zone. Semiquantitative assessment of the histological lesions revealed a significantly higher score in cisplatin-treated rats versus controls at day 5 (Table 1; 2.7 ± 0.2 versus 0.1 ± 0.1, P < 0.05). Edarabone treatment (5 mg/kg) significantly lowered the semiquantitative score compared with cisplatin treatment only (cisplatin + 5 mg/kg edarabone: 1.7 ± 0.3, P < 0.05 versus the cisplatin group).

Assessment of Renal Function. Injection of cisplatin induced a significant increase in plasma BUN levels at day 5 (Table 2). Administration of edarabone significantly prevented the increase in BUN in cisplatin-treated animals. Creatinine levels also improved following edarabone treatment. Five days after cisplatin administration, a marked and significant decrease in Ccr was observed in the cisplatin treatment group versus controls (0.05 ± 0.01 ml/min/100 g b.wt. versus 0.93 ± 0.05 ml/min/100 g b.wt., P < 0.05). As shown in Table 2, Ccr was significantly higher in the edarabone-treated group compared with the cisplatin-treated group. Urinary NAG excretion, a marker of tubular damage, also increased in the cisplatin group but was significantly decreased by edarabone administration at a dose of 5 mg/kg (1.01 ± 0.15 versus 0.48 ± 0.07 IU/day, P < 0.05).

Assessment of Renal Mitochondrial Function. To investigate the involvement of mitochondrial function in the pathogenesis of cisplatin-induced renal dysfunction, we analyzed COX activity by histochemical COX staining (Fig. 5A). Histochemical staining for COX yielded an intense, fine granular pattern in the tubular cells in controls. Proximal tubular COX reactivity was markedly decreased in the cisplatin-treated compared with the control group. The edarabone-treated group exhibited equivalent COX reactivity to controls, however. The COX-positive area was significantly increased by edarabone treatment (Fig. 5B; cisplatin, 44.7%; cisplatin + edarabone 5 mg/kg, 79.6%).

Analysis of Oxidatively Modified Proteins, Lipids, and Nucleic Acids. To characterize the effects of edarabone on the level of protein oxidation in the cytosolic and mitochondrial fractions of the kidney, we measured levels of carbonyl groups in specific proteins by Western blot analysis. As shown in Fig. 6A, more carbonyl groups were present in cytosolic protein from the cisplatin-treated rats. Edarabone (5 mg/kg) reduced the protein oxidation by cisplatin in cytosol extracts. We also examined renal HNE content as an index of lipid peroxidation by Western blotting. The HNE content was increased by cisplatin treatment (Fig. 6B). The increase was completely prevented by treatment with 5 mg/kg edarabone. DNA damage caused by cisplatin-induced ROS was examined by investigating the 8-OHdG and AP sites in DNA. Cisplatin treatment resulted in a significant increase in 8-OHdG and the number of AP sites compared with controls (Table 3). The amount of these increases were reduced by edarabone treatment in a dose-dependent manner.

Quantification of Apoptosis in Vivo. The degree of apoptosis in the kidney was assessed using the TUNEL assay at day 5 after administration of cisplatin. As shown in Fig. 7A, significantly increased numbers of apoptotic nuclei were seen in the cisplatin group, but there were few apoptotic nuclei in the edarabone-treated group. Figure 7B shows the results of quantification of apoptotic nuclei after 5 days. The cisplatin-treated and cisplatin + edarabone-treated groups showed a 10.0- and 2.5-fold increase in apoptotic nuclei, respectively, over the saline-treated group.
Effects of edarabone on cisplatin-induced nephrotoxicity in rats

Values are mean ± S.E.M.

<table>
<thead>
<tr>
<th>Group</th>
<th>BUN (mg/dl)</th>
<th>Cre (ml/min/100 g b.wt.)</th>
<th>Ccr (U/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.6 ± 1.9</td>
<td>0.26 ± 0.01</td>
<td>0.93 ± 0.05</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>166.1 ± 27.7</td>
<td>2.93 ± 0.61</td>
<td>0.05 ± 0.00</td>
</tr>
<tr>
<td>Cisplatin + Ed (1)</td>
<td>53.0 ± 8.3</td>
<td>1.23 ± 0.19</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>Cisplatin + Ed (5)</td>
<td>36.0 ± 5.5</td>
<td>0.81 ± 0.12</td>
<td>0.30 ± 0.03</td>
</tr>
</tbody>
</table>

*P < 0.05 versus cisplatin group.

**P < 0.05 versus control group.

Discussion

Peroxidative damage caused by ROS has been implicated in the pathogenesis of cisplatin-induced renal failure. ROS induce modification of disulfide bonds of protein, oxidation of phospholipids, and DNA injury in cells such that ROS are considered cytotoxic. ROS contain a superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), singlet oxygen (¹O₂) or hydroxyl radical (·OH). Superoxide anion is the most common oxygen radical and is a molecular species generated in large amounts in the human body. Nevertheless, its reactivity is very low such that it is not involved in modification of protein or lipid. In contrast, the hydroxyl radical is one of the most reactive and aggressive chemical species. Once excessive hydroxyl radicals are released, lipid peroxidation, which causes changes in the fluidity and permeability of membranes, is induced. No enzymes exist to eliminate the hydroxyl radical from the cell. Edarabone, a free radical scavenger that traps hydroxyl radicals, was developed for the potential treatment of cardiovascular disease, cerebrovascular ischemia, and cerebral edema. This compound has been registered in Japan for treatment of acute brain infarction. In the present study, we demonstrated that edarabone treatment protected kidneys against cisplatin-induced nephrotoxicity, both in vitro and in vivo. The present study suggested that enhanced peroxidative damage caused by ROS, especially the hydroxyl radical, might contribute to the pathogenesis of cisplatin-induced acute renal failure.

Mitochondrial dysfunction is a central component of cisplatin nephrotoxicity to proximal tubules in vivo (Gordon and Gattone, 1986; Davis et al., 2001) and in vitro (Brady et al., 1993; Zhang and Lindup, 1994). Renal PTCs, which accumulate significantly greater amounts of cisplatin in vivo and in vitro than other nephron segments (Goldstein and Mayor, 1983), undertake substance transport by con-
Mitochondria normally generate small quantities of superoxide by continuously converting 1 to 2% of consumed oxygen. Therefore, they are an important source of ROS. Mitochondria are generally protected from oxidative damage in several ways, including the activities of Mn-containing superoxide dismutase and Se-containing glutathione peroxidase. Nevertheless, expression of the superoxide dismutase and glutathione peroxidase genes is down-regulated by cisplatin (Rao et al., 1999; Huang et al., 2001; Saad et al., 2001). The present study also demonstrated that cisplatin causes lipid peroxidation. Edarabone was reported to inhibit iron-dependent lipid peroxidation and to depress the lipoxygenase pathway (Abe et al., 1988; Watanabe et al., 1994a; Watanabe and Egawa, 1994b). Our data also indicate that edarabone is active in inhibiting lipid peroxidation, suggesting that edarabone might protect against cisplatin-induced functional disorders of renal metabolism.

Despite its toxic effects, cisplatin can induce DNA damage through the generation of active oxygen species, such as the superoxide anion and hydroxyl radicals (Çrul et al., 1997). Hydroxyl radicals have very strong oxidative power and, therefore, can injure almost all bases and induce breakdown. It is known that 8-OHdG and AP site increase as markers of oxidative DNA injury (Lu et al., 2001). In this study, we analyzed 8-OHdG and AP sites in extracted DNA from renal cortical slices. Edarabone administration resulted in a suppression of 8-OHdG and AP sites increase. We infer from this...
that oxidative damage to DNA was diminished by edarabone treatment in cisplatin nephropathy, indicating that edarabone might protect against cisplatin-induced distorted gene expression in the kidneys.

Recent studies have shown that cisplatin causes apoptotic cell death in renal tubule cells (Lieberthal et al., 1996; Lau, 1999). It has also been reported that activation of mitochondrial pathways were important in apoptosis induced by cisplatin (Davis et al., 2001; Park et al., 2002). We confirmed mitochondrial permeability transition by cisplatin exposure in vitro. This led to release of cytochrome c, activation of caspase 9, and entry into the execution phase of apoptosis (Park et al., 2002). Edarabone inhibited the cisplatin-induced decrease of ΔΨm. It should be noted that mitochondrial dysfunction could induce necrotic cell death. Histological analysis of apoptotic and necrotic cell death in prevention of cisplatin-induced renal dysfunction by edarabone is currently under investigation in our laboratory.

Although edarabone did reduce the renal damage as evidenced on histopathology, significant damage is still present. Another pathway, which is not affected by edarabone, is thought to cause the cisplatin-induced nephrotoxicity. The free radical scavenger, edarabone, can trap OH only, so that another ROS such as O2 or H2O2 may cause renal damage. Recent findings suggest that O2 can modulate nuclear factor-κB (NF-κB) activation (Schreck et al., 1991). NF-κB is a transcription factor implicated in the inducible regulation of a wide range of genes involved in inflammatory and immune responses (Baueerle and Henkel, 1994; Barnes and Karin, 1997). Transcription of the intercellular adhesion molecule-1, inducible nitric oxide synthase, and tumor necrosis factor-α genes is regulated at the NF-κB site, and these genes have been shown to be up-regulated and to participate in renal injury (Deng et al., 2001; Ramesh and Reeves, 2002). So there is a possibility that these factors play a significant role in cisplatin-induced renal damage. Another reason that edarabone did not provide complete protection may be its short half-life. Edarabone has a half-life of about 6 to 8 hours. Because of this short half-life, the cisplatin-induced nephropathy was not completely blocked by a single intravenous injection of edarabone.

In conclusion, we have demonstrated in the present study that edarabone attenuated cisplatin-induced PTC damage in vitro and acute renal failure in vivo. In addition to its excellent antioxidant effects, the promising features of edarabone are that it is lipophilic, readily accessible to tissue, and thus an effective tissue level can be maintained with a single intravenous bolus injection. Our results also suggest that ROS induced by mitochondrial injuries contribute to renal damage after cisplatin treatment. ROS are involved in many other acute and chronic renal injuries, suggesting that edarabone treatment might be beneficial in various renal diseases.

References


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