A Novel Free Radical Scavenger, Edarabone, Protects Against Cisplatin-Induced Acute Renal Damage in Vitro and in Vivo

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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^{-5} 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 (Cooley 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.

ABBREVIATIONS: ROS, reactive oxygen species; MCI-186, 3-methyl-1-phenyl-pyrazolin-5-one, edarabone; PTC, proximal tubular cell; WST-1, tetrazolium salt; ΔΨm, mitochondrial membrane potential; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolyl-carbocyanine iodide; DCF, dichlorofluorescein; DCFH-DA, 2′,7′-dichlorofluorescein diacetate; FACS, fluorescence-activated cell sorting; NAG, N-acetyl-β-d-glucosaminidase; Ccr, creatinine clearance; BUN, blood urea nitrogen; COX, cytochrome c oxidase; HNE, 4-hydroxy-2-nonenal; 8-OHdG, 8-hydroxy-deoxyguanosine; AP, apurinic/apyrimidinic; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; NF-κB, nuclear factor-κB.
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 gene. 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 15 μg/ml streptomycin in 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 six-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 EDTA digestion and stained with 0.4% trypan blue for 5 min at 37°C. The numbers of cells excluding trypan blue were counted in a hemocytometer and expressed as a percentage of viable cells compared with vehicle-treated cells. To assess mitochondrial viability, cells were plated in 96-well plates and incubated with 20 μM cisplatin for 24 h. WST-1 measurement was performed according to the manufacturer’s protocol (cell proliferation assay system; TaKaRa Biomedicals, Tokyo, Japan).

Measurement of Mitochondrial Membrane Potential (ΔΨm) and Hydrogen Peroxide Production. To assess changes in ΔΨm, we used the potentiometric, fluorescent dye 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine 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 administrated 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 segment in the medullary rays; 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). Ten consecutive fields from the renal cortex of each rat were randomly selected and evaluated at a magnification of 100×.

Western Blot Analysis. Kidney samples were homogenized in lysis buffer containing 0.25 M sucrose, 50 mM diethiothreitol, 3 mM HEPES (pH 7.9), 500 μM EOTA, 1 mM 4-(2-aminoethyl)benzene-sulfonyl fluoride, 0.8 μM apratigon, 21 μM leupeptin, 36 μM bestatin, 15 μM pepstatin A, 14 μM (4-quinodimino)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 Oxyblot kit, according to the manufacturer’s protocol (Oncor, Gaithersburg, MD), as described previously (Keller et al., 1993). For the 4-hydroxy-2-nonenal (HNE) immuno blot, 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- 
rroi, 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 
competitive 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/apyrimidic (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'-aminooxymethyl-carbonyl-hydrazino-d-bi-
otin, 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 for 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 detect-
tion 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. 
Statistics were performed using StatView (SAS Institute, Inc., Cary, 
NC) on a Macintosh computer. Significance was defined as P < 0.05.

**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. Edar- 
abone 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 dehy-
drogenation enzyme. The results also revealed that cisplatin 
significantly decreased mitochondria dehydrogenation en-
zyme 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 decreased mitochondrial dysfunction induced by 
cisplatin (cisplatin: 55.1 ± 9.1%; cisplatin + 10⁻⁴ M edar- 
abone: 84.9 ± 12.6%; P < 0.01; n = 6).

**ΔΨᵐ Change and Reactive Oxygen Species Production.** The 
ΔΨᵐ is an important indicator of mitochondrial function and dysfunction. To assess mitochondrial damage 
induced by cisplatin, we monitored changes in ΔΨᵐ using the 
fluorescent dye JC-1. FACS analysis showed that high ΔΨᵐ 
was maintained in control cells (68.9%) (Fig. 2). After cispla-
tin treatment, the rate of high ΔΨᵐ was decreased (5.4%), 
and the rate of low ΔΨᵐ was increased (46.5%). By coincu-
boration with edarabone, the ΔΨᵐ was maintained at high 
levels (47.1%), and the rate of low ΔΨᵐ was not increased 
(1.3%).

To determine intracellular ROS generation induced by cis-
platin and the oxygen radical elimination ability of edar-
abone, cisplatin-treated PTCs with or without edarabone 
were incubated with DCFH-DA, and oxidation of intracellu-
lar DCF was measured by confocal laser-scanning micro-
scopy and FACS. No ROS production was evident in the control 
cells (Fig. 3), whereas enhanced ROS production was 
observed in cisplatin-treated cells. Edarabone treatment re-
duced ROS production in cisplatin-treated PTCs. FACS an-
alysis showed that cisplatin caused a significant increase in 
ROS generation (1.4 versus 74.5%). Preincubation of PTCs 
with 10⁻⁴ M edarabone significantly reduced ROS levels 
(22.3%).

**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
Cisplatin with or without 10 μM edarabone was used as a probe for intracellular peroxide formation. ROS in the cells induce oxidation of DCFH, yielding the fluorescent product DCF. Changes in DCF fluorescence in cells were shown. The cells were analyzed by flow cytometry. The ordinate shows the number of cells.

Each experiment was performed in triplicate and repeated at least twice.

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.

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.

![Fig. 3. ROS production in vitro. PTCs were incubated with 20 μM cisplatin with or without 10 μM edarabone for 6 h. The compound DCFH-DA was used as a probe for intracellular peroxide formation. ROS in the cells induce oxidation of DCFH, yielding the fluorescent product DCF. Changes in DCF fluorescence in cells were shown. The cells were analyzed by flow cytometry.](image)

![Fig. 4. Light microscopy of kidneys stained with H&E at day 5 after cisplatin (5 mg/kg) administration. Extensive tubular damage and intra-tubular cast formation in the outer stripe of the outer medulla was observed in the cisplatin group. In contrast, tubular damage was reduced by treatment with 1 or 5 mg/kg edarabone.](image)

### TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Histopathological Classification</th>
<th>Average Score</th>
</tr>
</thead>
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<tr>
<td>Control</td>
<td>7</td>
<td>0 +1 +2 +3</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>7</td>
<td>0 0 2 5</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>Cisplatin + Ed (1)</td>
<td>7</td>
<td>0 1 3 3</td>
<td>2.3 ± 0.3</td>
</tr>
<tr>
<td>Cisplatin + Ed (5)</td>
<td>7</td>
<td>0 3 3 1</td>
<td>1.7 ± 0.3</td>
</tr>
</tbody>
</table>

Ed (1), edarabone 1 mg/kg; Ed (5), edarabone 5 mg/kg.  
* P < 0.05 versus control group.  
+ P < 0.05 versus cisplatin group.
TABLE 2
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>
<th>Urinary NAG Excretion</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>
<td>0.26 ± 0.02</td>
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<tr>
<td>Cisplatin</td>
<td>166.1 ± 27.7</td>
<td>2.93 ± 0.61</td>
<td>0.05 ± 0.0^a</td>
<td>1.01 ± 0.15^a</td>
</tr>
<tr>
<td>Cisplatin + Ed (1)</td>
<td>53.0 ± 8.3b</td>
<td>1.23 ± 0.19 ^a</td>
<td>0.18 ± 0.02^ab</td>
<td>0.69 ± 0.12^a</td>
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<tr>
<td>Cisplatin + Ed (5)</td>
<td>36.0 ± 5.5^ab</td>
<td>0.81 ± 0.12^ab</td>
<td>0.30 ± 0.03^ab</td>
<td>0.48 ± 0.07^ab</td>
</tr>
</tbody>
</table>

Ed (1), edarabone 1 mg/kg; Ed (5), edarabone 5 mg/kg.
^a P < 0.05 versus control group.
^b P < 0.05 versus cisplatin group.

Fig. 5. Histochemistry of the COX respiratory complex. A, COX staining in the kidneys of rats treated with saline, cisplatin (5 mg/kg) or cisplatin + edarabone 5 mg/kg at day 5. A marked decrease in tubular reactivity of COX was observed in the cisplatin group, whereas COX levels were comparable in the edarabone and control groups. Magnification: 100 ×. B, percentage of the COX-positive area measured by image analyzer. Values are means ± S.E.M. * P < 0.05 versus cisplatin-treated group. Cont, control group; Cis, cisplatin group; Cis + Ed (1), cisplatin + 1 mg/kg edarabone group; Cis + Ed (5), cisplatin + 5 mg/kg edarabone 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 (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), singlet oxygen (O$_2^+$) 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 kidneys. Mitochondrial DNA does not bind histone protein, and thus, it is readily susceptible to oxidative stress. Accumulation of oxidative damage in mitochondrial DNA may affect mitochondrial respiratory function and cause cellular dysfunction. In this study, we observed that mitochondria dehydrogenation enzyme activity was deteriorated by cisplatin in PTCs and that mitochondrial complex IV activity (determined by COX staining) was also reduced. In vitro assays have revealed the generation of superoxide anion and hydroxyl radical during interaction of cisplatin with DNA (Masuda et al., 1994). We found that treatment with edarabone, which has hydroxyl radical but not superoxide anion scavenging activity, significantly ameliorated the mitochondrial dysfunction induced by cisplatin both in vitro and in vivo. These data indicate that hydroxyl radicals are important in the mechanism that underlies cisplatin-induced renal injury.

Despite its toxic effects, cisplatin can induce DNA damage through the generation of active oxygen species, such as the superoxide anion and hydroxyl radicals (Crul et al., 1997). Hydroxyl radicals have very strong oxidative power and, therefore, can injure almost all bases and induce breakdown. It has been suggested that cisplatin-induced cytotoxicity may be due to peroxidation of cell membranes (Montine and Borch, 1988). The main content of cell membrane is lipid, and thus lipid peroxidation in renal tissue might cause nephrotoxicity. Several investigators have shown that cisplatin nephrotoxicity is associated with lipid peroxidation in renal cortical slices and that antioxidants reverse cisplatin-induced lipid peroxidation (Rao et al., 1999; 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.
that oxidative damage to DNA was diminished by edarabone treatment in cisplatin nephropathy, indicating that edarabone might protect against cisplatin-induced doserelated 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 in proximal tubular epithelial cells via activation of mitochondrial pathways. J Am Soc Nephrol 15:138–145.


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