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Protection by a radical scavenger edaravone against cisplatin-induced nephrotoxicity in rats

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Abstract

Acute renal failure is a dose-limiting factor of cisplatin chemotherapy. Here, we show the protective effect of edaravone, a recently developed radical scavenger for clinical use, against cisplatin-induced renal dysfunction in rats. A marked increase in blood urea nitrogen and creatinine in serum, and histological changes including vacuolation, necrosis and protein casts were observed in proximal renal tubules at the fourth day after cisplatin injection (5–10 mg/kg). Repeated injection of edaravone (1–10 mg/kg, i.v. twice a day for 3 days) reversed the cisplatin-induced elevation of blood urea nitrogen and creatinine, and morphological changes in a dose-dependent manner. In particular, the protective effect of edaravone was almost complete at 10 mg/kg. Moreover, the compound was still fully effective, when it was administered only at the second day after cisplatin injection. On the other hand, the glutathione content in renal tissues lowered at the fourth day after cisplatin injection, which was reversed by the late treatment with edaravone. These findings suggest that the clinically available radical scavenger edaravone is potentially useful for the prevention of cisplatin-induced renal toxicity.

Keywords: Cisplatin; Renal toxicity; Radical scavenger; Blood urea nitrogen; Creatinine; Glutathione

1. Introduction

Although cisplatin is an effective chemotherapeutic agent for a wide variety of solid tumors, its use is often limited by the serious adverse effects such as nephrotoxicity (Leonard et al., 1971; Madias and Harrington, 1978). Thus, the prevention of nephrotoxicity is considered to be of clinically great importance in the cancer chemotherapy with cisplatin. The renal toxicity induced by cisplatin is restricted primarily to the S3 segment of the proximal tubules (Blachley and Hill, 1981); however, the mechanism of action remains unclear. It has been reported that free radicals, including superoxide anion and hydroxyl radicals generated by cisplatin, are associated with the nephrotoxicity (Hannemann and Baumann, 1988; Kruidering et al., 1997; Sugihara et al., 1987). Several free radical scavengers or antioxidants have been shown to protect renal tissues against cisplatin-induced nephrotoxicity (Appenroth et al., 1997; Braunlich et al., 1997; Davis et al., 2001; Husain et al., 1998; Liu and Baliga, 2000; Matsushima et al., 1998; Mishima et al., 1999; Nishikawa et al., 2001; Yuhas and Culo, 1980). However, most of them are not clinically available. Moreover, the effective doses of these compounds are so high as to produce other undesirable side actions.

Edaravone is a neuroprotective agent that was approved for the acute therapy of embolic stroke by the Japanese Ministry of Health, Labor and Welfare in 2001. This compound has potent free radical scavenging and antioxidant actions without causing any serious side effects (Kawai et al., 1997; Watanabe et al., 1994).

The purpose of the present study was to evaluate the ability of edaravone to protect renal tissues against cisplatin-induced toxicity at therapeutically relevant doses.

2. Materials and methods

The present study was reviewed by the ethics committee regarding animal experiments at the Faculty of Medicine, Kyushu University and was performed according to the

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Guidelines for Animal Experiments in the Faculty of Medicine, Kyushu University, and the law (No. 105) and notification (No. 6) of the Japanese government.

2.1. Chemicals

Edaravone (MCI-186, norphenazone, 3-methyl-1-phenyl-pyrazolin-5-one) was kindly provided by Mitsubishi Pharma (Tokyo, Japan). Cisplatin was purchased from Sigma (St Louis, MO). Edaravone was dissolved in 1 N NaOH, and the pH was adjusted to 7.0 with 1 N HCl. Cisplatin was dissolved in saline to give a 1 mg/ml solution. Glutathione was purchased from Wako (Osaka, Japan). Other reagents used were of analytical grade.

2.2. Animals

Male Wistar rats weighing 200–230 g were purchased from Kyudo (Kumamoto, Japan). Animals were housed in a room maintained on a 12-h light/dark condition (lights on at 7:00 a.m.) at the temperature of 23 ± 2 °C. Food and water were given ad libitum.

2.3. Cisplatin-induced acute renal failure

Animals were randomly divided into four groups, consisting of five to seven animals. They were injected intraperitoneally with physiological saline or cisplatin at a dose of 5, 7.5 or 10 mg/kg. Rats were sacrificed at 72 h after cisplatin administration. Blood specimens were collected and plasma was separated by centrifugation for the determination of serum creatinine and blood urea nitrogen.

2.4. Assessment of the effect of edaravone on cisplatin-induced nephrotoxicity

Cisplatin was injected intraperitoneally at a single dose of 7.5 mg/kg. Edaravone was administered intravenously via tail vein twice a day for 3 days. Three doses of edaravone (1, 3 and 10 mg/kg) were used. Control group was injected with saline. The first administration of edaravone was carried out immediately after cisplatin injection. In a set of experiments where the timing of edaravone administration was examined, edaravone (10 mg/kg) was injected twice a day at the first, second or third day after cisplatin injection. At the fourth day after cisplatin injection, blood samples were collected and kidneys were removed. Renal dysfunction was assessed by serum concentrations of creatinine and blood urea nitrogen, and histochemical observation of the kidney slices.

2.5. Determination of serum creatinine and blood urea nitrogen

Serum creatinine and blood urea nitrogen concentrations were determined by Jaffe's method and the diacetylmonoxime method, respectively, using assay kits purchased from Wako.

2.6. Histochemical examination of renal damage

The rat kidney was sectioned in blocks and fixed in 20% formalin, then dehydrated in graded concentrations of alcohols, and embedded in paraffin. The kidney block was cut into 2-μm sections and stained with periodic acid-Schiff reagents. The morphometric examination was performed in a blinded manner.

2.7. Determination of glutathione

The tissue concentration of glutathione was determined by high-performance liquid chromatography (HPLC) with fluorescent detection, according to the method of Neuschwander-Tetri and Roll (1989) with modifications. Briefly, glutathione was separated by a reversed-phase C18 column, and subjected to postcolumn fluorescent derivatization with *o*-phthalaldfehyde under alkaline condition. The HPLC system consisted of four independent solvent-delivery pumps (CCPM-II; Tosoh, Tokyo, Japan), which deliver mobile phase (0.6 ml/min) and reagents, including 0.1% *o*-phthalaldehyde (0.12 ml/min), 2.5 N NaOH (0.12 ml/min) and 4 M H_3PO_4 (0.14 ml/min), an automatic sample injector (AS-8020-LV; Tosoh), a guard column (5.0 × 4.0 mm, inside diameter) packed with C18 resin, a reversed-phase separation column (150 × 4.0 mm, inside diameter, Wakosil-II 5C18 HG, Wako), a thermostatic reactor (RE-8020; Tosoh), a fluorescence spectromonitor (RF-550; Shimadzu, Kyoto, Japan), and a chromatorecorder (C-R4A, Shimadzu). The mobile phase was 0.1 M sodium phosphate buffer (pH 2.5) and methanol (96:4 v/v) containing 400 mg/l sodium octanesulfonate.

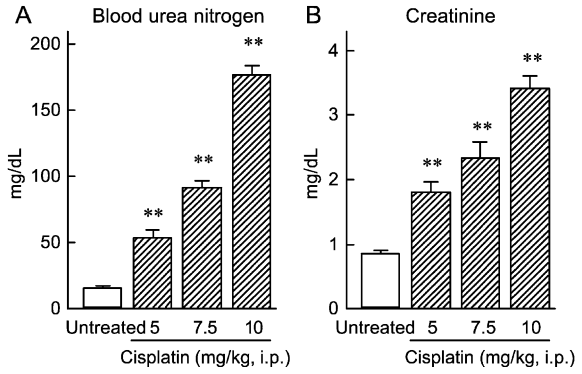

Fig. 1. Dose-dependent renal toxicity induced by cisplatin in rats, as determined by plasma levels of blood urea nitrogen and creatinine. Blood urea nitrogen and creatinine were measured at the fourth day after cisplatin injection. Each column represents the mean ± S.E. of five to seven animals. * * $P < 0.01$ vs. untreated.

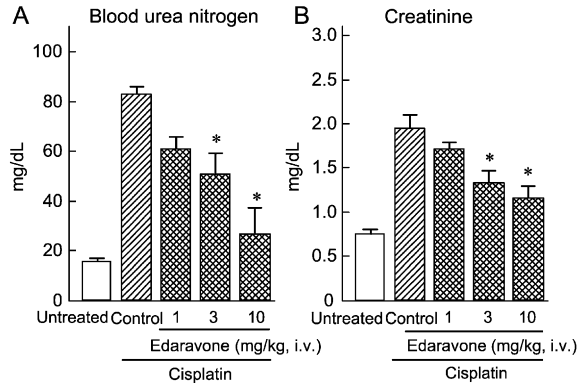

Fig. 2. Dose-dependent protective effect of edaravone on cisplatin-induced renal toxicity in rats. Edaravone was injected i.v. twice a day for 3 days starting immediately after cisplatin treatment (7.5 mg/kg, i.p.). Blood urea nitrogen and creatinine were measured at the fourth day after cisplatin injection. Each column represents the mean ± S.E. of seven animals. * $P < 0.05$ vs. control.

2.8. Statistical analysis

Data were expressed as mean ± S.E. and analyzed statistically using unpaired independent Student's t test. The 0.05 level of probability was used as statistical significance.

3. Results

3.1. Cisplatin-induced renal toxicity in rats

As shown in Fig. 1, cisplatin at 5–10 mg/kg produced a dose-dependent elevation of blood urea nitrogen and creatinine. A marked increase in the serum markers was observed at the fourth day after cisplatin administration. At 10 mg/kg, cisplatin caused the most marked increase in blood urea nitrogen and creatinine concentrations, but two out of seven animals died within 72 h after cisplatin injection. Therefore, the effect of edaravone on the renal toxicity was evaluated in rats treated with 7.5 mg/kg of cisplatin.

3.2. Dose-dependent protective effect of edaravone against cisplatin-induced renal toxicity

Edaravone, when treated at 1–10 mg/kg (i.v.) twice a day for 3 days, reduced the cisplatin-induced elevation of serum

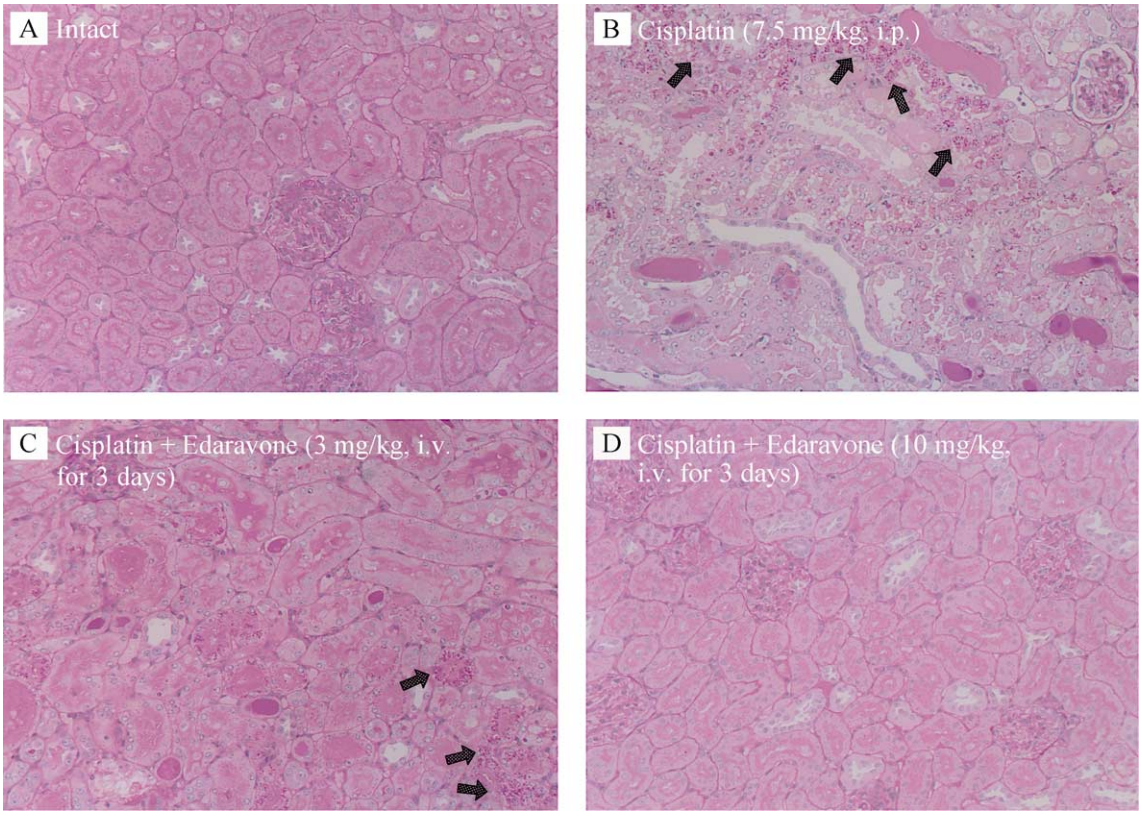

Fig. 3. Representative photographs showing the protective effect of edaravone on cisplatin-induced renal injury in rats. Cisplatin (7.5 mg/kg) was injected i.p. and kidney was removed at 72 h thereafter. Edaravone was injected i.v. twice a day for 3 days, starting immediately after cisplatin treatment. (A) intact; (B) cisplatin + saline; (C) cisplatin + edaravone (3 mg/kg, i.v., for 3 days); (D) cisplatin + edaravone (10 mg/kg, i.v., for 3 days). Renal sections of 2-μm thick were prepared after fixation of the renal block with 20% formalin, followed by dehydration with graded concentrations of alcohol, then stained with periodic acid-Schiff reagents (× 200 magnification). Arrows represent protein casts.

levels of blood urea nitrogen and creatinine in a dose-dependent fashion, and the significant effect was observed at doses of 3 and 10 mg/kg (Fig. 2). Histochemical observations after staining of renal sections with periodic acid-Schiff reagents revealed the remarkable vacuolation, necrosis, desquamation of epithelial cell and protein cast in renal tubules of cisplatin (7.5 mg/kg)-treated control animals (Fig. 3B). It was noteworthy that the repeated administration of edaravone (3 or 10 mg/kg for 3 days) dramatically improved the cisplatin-induced renal toxicity, in which few histological damage was observed in renal tubules and glomeruli (Fig. 3C,D). Thus, it is suggested that the reduction by edaravone of cisplatin-induced elevation of blood urea nitrogen and creatinine is associated exclusively with the cytoprotective action on renal tissues.

3.3. Therapeutic time-window for the edaravone-induced renal protection

Subsequently, we determined the timing of edaravone treatment in producing the significant renal protection. Edaravone (10 mg/kg) was injected twice a day at the first, second or third day after cisplatin treatment. It was quite unexpected that the significant reversal of cisplatin-induced renal damage was observed, when edaravone was injected only at the second day (Fig. 4), but no significant protection was observed in groups treated with edaravone at the first day or the third day.

3.4. Effect of edaravone on cisplatin-induced decrease in renal glutathione

To determine whether the radical formation is enhanced after cisplatin injection, we measured the concentrations of glutathione in renal tissues after cisplatin injection. As shown in Fig. 5A, the glutathione content began to lower at the third day after cisplatin injection (7.5 mg/kg), and the significant reduction was observed at the fourth day after injection. Interestingly, edaravone (10 mg/kg, i.v.) treated twice a day only at the second day after cisplatin injection completely reversed the reduction in the glutathione content determined at the fourth day after cisplatin treatment (Fig. 5B).

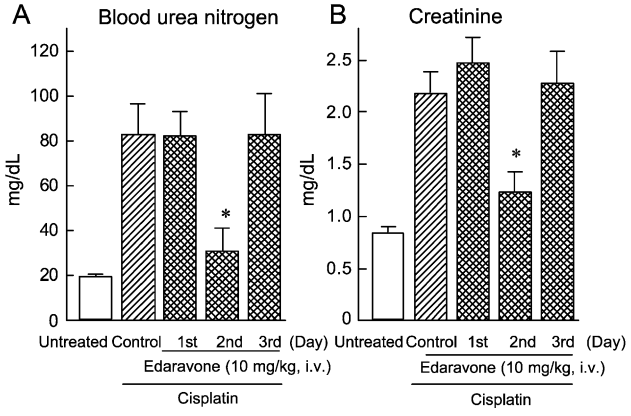

Fig. 4. Influence of dosing period on the protective effect of edaravone on cisplatin-induced renal toxicity in rats. Edaravone was injected i.v. twice a day on the first, second or third day of cisplatin treatment (7.5 mg/kg, i.p.). Plasma blood urea nitrogen and creatinine were measured 72 h after cisplatin injection. Each column represents the mean ± S.E. of seven animals. * $P < 0.05$ vs. control.

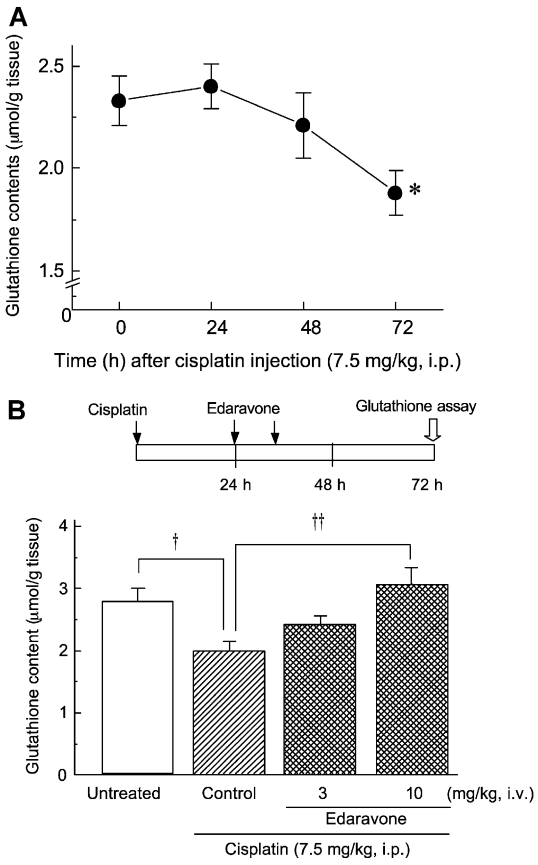

Fig. 5. Time course of changes in glutathione concentration in the rat kidney after cisplatin injection (A) and its reversal by delayed treatment with edaravone (B). Cisplatin (7.5 mg/kg) was injected i.p. in both cases. Two doses of edaravone (3 and 10 mg/kg) were injected i.v. twice a day only at the second day after cisplatin treatment (B). Tissue glutathione contents were measured at the first, second, third and fourth day (A) or at the fourth day (B) after cisplatin injection by using HPLC-fluorescence detection. Each point or column represents the mean ± S.E. of seven animals. * $P < 0.05$ vs. values at time 0; † $P < 0.05$, †† $P < 0.01$.

4. Discussion

The present study describes for the first time that the clinically available radical scavenger edaravone dramatically inhibited the cisplatin-induced in vivo nephrotoxicity in rats. Cisplatin is a platinum complex that consists of two carrier ligands of ammonia and two leaving groups of chloride. Its anticancer activity is attributable to the conversion of cisplatin to a di-ucl-acquo complex of cisplatin, which forms an

interstrand cross-link with double-strand DNA to prevent DNA synthesis (Zwelling et al., 1979). However, the mechanisms underlying the cisplatin-induced nephrotoxicity have not been fully understood. Several investigators have shown that the reactive oxygen species or free radicals are closely related to the nephrotoxicity induced by cisplatin (Hannemann and Baumann, 1988; Kruidering et al., 1997; Zwelling et al., 1987). Indeed, several antioxidants such as WR-2721 (Amifostine), superoxide dismutase, ebselen, 2,3-dimercaptosuccinic acid, vitamins E and C are reported to attenuate cisplatin-induced renal toxicity (Appenroth et al., 1997; Braunlich et al., 1997; Davis et al., 2001; Husain et al., 1998; Liu and Baliga, 2000; Matsushima et al., 1998; Mishima et al., 1999; Nishikawa et al., 2001; Yuhas and Culo, 1980). It has also been reported that the inordinate or aberrant generation of reactive oxygen species contributes to the initiation and/or maintenance of acute tubular necrosis (Nath and Norby, 2000). In the present study, we evaluated the potential of edaravone for the prevention of cisplatin-induced nephrotoxicity. It has been reported that edaravone inhibits the oxidation of linoleic acid induced by hydrogen peroxide and ferrous ions, retards the lipid peroxidation during the autooxidation of rat-brain homogenate, reduces the alloxan-induced lipid peroxidation in rats, and suppresses the hydroxylation of salicylate induced by hydroxyl radicals (Kawai et al., 1997; Watanabe et al., 1994). Moreover, edaravone attenuates the formation of superoxide anion by the xanthine oxidase–hypoxanthine system (Wu et al., 2000). The doses of this compound used in the present study were comparable to those reported previously to produce free radical-scavenging actions. Thus, it is suggested that the protective effect of edaravone observed in the present study is due primarily to the inactivation of free radicals. However, the doses of edaravone used in the present study were somewhat higher than the clinically tolerable dose (1.5 mg/kg, i.v.).

Cisplatin-induced nephrotoxicity is associated with the increase in serum levels of creatinine and blood urea nitrogen. The nephrotoxicity is reported to be maximum at 3–5 days after cisplatin injection to the experimental animals (Singh, 1989). It is also notable that edaravone was effective, even when it was treated twice a day at the second day after cisplatin injection. Thus, the radical production may be fully elevated during the second day of injection. To ascertain the idea, we examined the time course of changes in tissue concentration of glutathione, since reactive oxygen species including hydroxyradical induce glutathione depletion (Andreoli et al., 1986; Towell and Wang, 1987; Trocino et al., 1995). The glutathione concentration in renal tissues began to decrease at the third day after cisplatin injection, and the change was significant at the fourth day. Edaravone (10 mg/kg), when administered twice a day only at the second day after cisplatin injection, again completely reversed the reduction of renal glutathione determined at the fourth day. At present, we cannot explain precisely why such a delayed treatment with edaravone is effective in protecting renal tissues against cisplatin toxicity. In the present study, the glutathione content began to decrease at the third day after cisplatin injection, suggesting that glutathione is used to scavenge reactive oxygen species generated lately after cisplatin injection. Treatment with edaravone at the second day reversed the decrease in glutathione content. Taken together, it is suggested that the production of oxygen free radicals is enhanced during the second day of cisplatin injection, which leads to the degeneration of renal tubules.

In conclusion, cisplatin-induced renal toxicity was reversed by a clinically available free radical scavenger edaravone. Moreover, the compound was fully effective, even when it was administered lately on the next day (at 24 and 36 h) of cisplatin treatment. Therefore, it is potentially useful for the prevention or cure of renal toxicity during cisplatin chemotherapy.

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