Protective Effects of Capsaicin against Cisplatin-Induced Nephrotoxicity in Rats

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Cisplatin-induced nephrotoxicity is related to an increase in lipid peroxidation and oxygen free radicals in a kidney. In the present study, we investigated the effect of the dietary antioxidants, capsaicin (Cap), against cisplatin-induced lipid peroxidation and nephrotoxicity in rats. Nephrotoxicity induced by treatment with a single dose of cisplatin (5 mg/kg body weight i.p.). The animals were divided into 4 groups. Cap (10 mg/kg/d) was given by gavage from the same day of cisplatin injection. Cisplatin administration resulted in significant increases in the kidney weight as a percentage of the total body weight, urine volume, serum creatinine, and blood urea nitrogen by about 132, 315, 797, and 556% in comparison with the control rats, respectively (p<0.05). Also, the renal tissue from the cisplatin-treated rats showed significant decreases in the kidney glutathione (GSH) content and superoxide dismutase (SOD) activity and a significant increase in malondialdehyde (MDA) production in comparison to the values at 0 h (p<0.05). Seven days after Cap plus cisplatin treatments, the renal damage induced by cisplatin recovered to a significant statistically level. In addition, Cap prevented the rise of MDA and the reduction of SOD activities. These results suggest that Cap has protective effects against cisplatin-induced nephrotoxicity and lipid peroxidation in rats.

Key words capsaicin; cisplatin; nephrotoxicity; lipid peroxidation; free radical

Cisplatin (cis-diaminedichloroplatinum II, CDDP) is extensively used for the treatment of several cancers. Its major side effect; namely, nephrotoxicity, is a dose-limiting factor in CDDP therapy. The exact mechanisms of nephrotoxicity induced by CDDP are still not fully elucidated. However, lipid peroxidation and free radical generation in the renal tubular cells have been suggested to be responsible for CDDP-induced renal failure. Zhang et al. showed that CDDP significantly depletes glutathione (GSH) and increases lipid peroxidation in the mitochondria prepared from the rat renal cortical slices. In addition, the activities of superoxide dismutase (SOD) and catalase in the rat kidney tissues are significantly suppressed by CDDP. Capsaicin (Cap) is the major pungent ingredient of red hot peppers. It has been used as a tool in the study of pain sensations which are caused by stimulation of Cap receptor or vanilloid receptor 1, an ion channel protein expressed by nociceptive primary afferent neurons. On the other hand, Cap potentially inhibits the peroxidation of various lipids and generates reactive oxygen species in rat peritoneal macrophages. However, the mechanism responsible for the potent inhibitory effects of Cap on lipid peroxidation in vivo is still unclear.

Several studies have examined the effects of decreased lipid peroxidation and nephrotoxicity induced by CDDP using various agents including antioxidants. The purpose of the present study was to investigate whether the administration of Cap exerts any protective effect against cisplatin-induced lipid peroxidation and nephrotoxicity in rats.

MATERIALS AND METHODS

Chemicals Cap with a purity of 98.6% was kindly supplied by Maruishi Pharmaceutical Co., Ltd. (Osaka, Japan). CDDP was purchased from Sigma (MO, U.S.A.). All other chemicals and reagents used were of analytical grade.

Animals Male Sprague-Dawley rats weighing 200 to 250 g were purchased from Nippon SLC Inc. (Shizuoka, Japan). The rats had free access to standard rat chow and water ad libitum, were individually housed in metal cages, and kept in a room maintained at 23±2°C with a 12 h/12 h light/dark cycle. The study adhered to the guidelines of Osaka University of Pharmaceutical Sciences for the experimental use of animals.

Animal Treatments The rats were divided at random into four groups of 4 or 5 animals each. The first group (control) received a vehicle (5% carboxymethyl cellulose sodium solution (CMC-Na), 5 ml/kg body wt., p.o.) used for Cap. The second group received Cap (10 mg/kg/d, p.o.) in 5% CMC-Na (5 ml/kg), and the third received 5% CMC-Na for 6 consecutive days injected with CDDP (5 mg/kg in physiological saline solution, i.p.). The fourth group received Cap (10 mg/kg/d, p.o.) in 5% CMC-Na for 6 consecutive days after CDDP injection (5 mg/kg, i.p.). For all groups, Cap or vehicle was given twice daily. The selected Cap concentration and the dose administration schedule without inducing any rat intestinal damage were chosen using data from our preliminary experiments.

On day 7 after CDDP administration, the animals were anesthetized with diethyl ether, blood samples were taken, and then the plasma was separated. The plasma was used to measure the indices of nephrotoxicity. The kidneys were re-
moved, washed with ice-cold saline, and blotted with a piece of filter paper. Then, they were weighed, decapsulated and homogenized with a Potter-Elvehjem homogenizer in ice-cold 20 mM phosphate buffer (pH 7.4, 1:5 wt./vol.) solution. The homogenate was centrifuged at 3000×g for 10 min at 4°C. The supernatant was collected and stored frozen at −80°C; this sample was tested for the activities of SOD and products of lipid peroxidation. For GSH analysis, a portion of kidney tissues were homogenized in 5% ice-cold 5-sulfosalicylic acid dehydrate (Sigma, MO, U.S.A.) to yield a 10% (w/v) homogenate and centrifuged at 8000×g for 10 min at 4°C. The supernatant was collected and stored frozen at −80°C for glutathione assay. The changes in urinary volume were measured at 12-h intervals, and the changes in the body weight were also determined throughout the experiments.

Biochemical Assays The plasma creatinine and blood urea nitrogen (BUN) were measured according to the methods of Bonsnes and Taussky\(^\text{15}\) and Searcy et al.\(^\text{16}\) respectively, using diagnostic kits (Wako Pure Chemicals Industries Ltd., Osaka, Japan). Tissue-reduced total GSH content and SOD activity were determined according to the methods of Esterbauer et al.\(^\text{17}\) and McCord and Fridovich\(^\text{18}\) and the tissue lipid peroxidation in terms of Malonaldehyde (MDA) production was determined according to the method of Estabauer et al.\(^\text{19}\) Protein determinations were performed using the method of Lowry et al.\(^\text{20}\)

Statistical Analysis The experimental data are expressed as mean±S.D. The data were analyzed by the Student t-test. The differences were considered to be statistically significant when \(p<0.05\).

RESULTS

The effects of Cap on CDDP-induced nephrotoxicity were evaluated by the changes in body weight. The control group and those that received only Cap showed a relatively constant increase in the body weight throughout the experimental periods (data not shown). A statistically significant difference in weight loss was detected 7 d after CDDP injection in the CDDP-treated group as compared to the control groups (\(p<0.05\)) (Fig. 1). The decrease in body weight in the CDDP-treated animals shown in Fig. 1 was not inhibited by oral treatment with Cap.

No differences in the kidney weight as a percentage of the total body weight were observed between the Cap and control groups (Table 1). Treatment of the normal rats by CDDP resulted in a 132% increase in kidney weight as a percentage of the total body weight as compared with the control group (\(p<0.05\)) (Table 1). Also, the administration of Cap along with CDDP decreased the kidney weight to normal levels (\(p<0.05\) as compared to the CDDP-treated group) (Table 1).

Table 1. Effect of Cap on the CDDP-Induced Nephrotoxicity in Normal Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Kidney weight as % of the total BW</th>
<th>Urine volume (µl·ml/kg)</th>
<th>Serum creatinine (mg/dl)</th>
<th>Blood urea nitrogen (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.363±0.020</td>
<td>30.4±1.1</td>
<td>0.34±0.03</td>
<td>20.7±0.9</td>
</tr>
<tr>
<td>Cap</td>
<td>0.345±0.020</td>
<td>45.3±3.2*</td>
<td>0.52±0.24</td>
<td>17.7±1.3*</td>
</tr>
<tr>
<td>CDDP</td>
<td>0.478±0.046*</td>
<td>95.5±8.5*</td>
<td>2.71±0.82*</td>
<td>115.0±29.4*</td>
</tr>
<tr>
<td>CDDP+Cap</td>
<td>0.385±0.032*</td>
<td>68.7±14.7*</td>
<td>0.63±0.06*</td>
<td>36.6±5.5*</td>
</tr>
</tbody>
</table>

Data are expressed as mean±S.D. (\(n=5\)). *Significantly different from the control group (\(p<0.05\)). # Significantly different from the CDDP-treated group (\(p<0.05\)).

Treatment with CDDP resulted in significant increases in the urine volume, serum creatinine, and BUN by about 315, 797, and 556% in comparison with the control rats, respectively (Table 1). The fourth group exhibited a significant decrease in the urine volume, serum creatinine, and BUN by about 28, 77, and 62% in comparison with the CDDP-treated normal rats, even though they were not as low as the values of the control group (\(p<0.05\)) (Table 1).

CDDP treatment produced a significant (\(p<0.05\)) reduction in the kidney GSH content and SOD activity and a significant elevation in the kidney MDA production at all time points in comparison with the values at 0 h, respectively (Figs. 2—4). CDDP plus Cap treatment increased the renal GSH but not significantly. However, such treatment revealed significant increases in the SOD activity in the animals sacrificed at 24, 48, and 72 h after CDDP injection (Fig. 3), and
significant decreases in the kidney MDA production in the animals killed at 6 h after CDDP plus Cap treatment as compared with those treated with CDDP alone (Fig. 4).

DISCUSSION

In this study, the CDDP-induced kidney damage was characterized by significant increases in the kidney weight as a percentage of the total body weight, urine volume, serum creatinine, and BUN in comparison with the control group (Table 1). Morphologic alterations in the kidneys after injection of CDDP (5 mg/kg) were characterized by selective proximal tubular damage including a moderate degree of necrosis and a formation of proteinaceous casts of the tubular cells (data not shown). Also, CDDP treatment resulted in significant decreases in the kidney GSH content and SOD activity and a significant increase in the MDA production in comparison with the values at 0 h, and the SOD activity significantly decreased and the kidney MDA production significantly increased in CDDP-treated rats that received Cap reflected the protective effect of Cap against the CDDP-induced nephrotoxicity (Fig. 1). From the results of the present study the renal GSH content and the SOD activity significantly decreased and the renal MDA production significantly increased in CDDP treated rats compared to the values at 0 h, and the SOD activity only was remarkably improved compared to the results of the renal GSH and MDA content by co-treatment with Cap. Our studies demonstrated that Cap is potent in protecting against free radical damage induced by CDDP. CDDP gener-

shown to be protective against CDDP-induced nephrotoxicity. Also, various free radical scavengers have been shown to be effective in protection against CDDP-induced nephrotoxicity, and treatment with such agents provides significant protection against CDDP-induced acute renal failure. Cap and its analogues have more effective antioxid-

dance radicals. On the other hand, many antioxidants have been shown to be protective against free radical damage induced by CDDP. CDDP gener-

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ates active oxygen species such as superoxide anion and hydroxyl radical \(^\text{16,29}\) and stimulates renal lipid peroxidation. \(^\text{3,30—32}\) The free radical scavengers SOD are reported to provide partial protection against CDDP-induced structural and functional alteration in rats. \(^\text{4}\) And also, Nishikawa et al reported that the cationic superoxide dismutase derivative rapidly and selectively accumulates in and around proximal tubule cells and effectively dismutates superoxide radicals \textit{in situ}. \(^\text{23,25}\) On the other hand, Cap showed the radical scavenging activity by measuring in the oxidation of lipid \textit{in vitro} system. \(^\text{9,25}\) Moreover, Cap was reported to be effective against the oxidant stress by countering the depleted antioxidant molecules and antioxidant enzyme in erythrocytes and liver of rats. \(^\text{24}\) The exact roles of free radicals in CDDP-induced nephrotoxicity and the mechanisms for the beneficial effects of free radical scavengers have not been fully proved. \(^\text{2}\) However, this suggests that Cap protects against oxidative damage induced by CDDP due, at least in part, to its radical scavenging capacity. Furthermore, it was reported that the radical scavenging site of Cap is the C7-benzyl carbon \(^\text{10}\) and Cap inhibited the oxidation almost as effectively as \(\alpha\)-tocopherol in liposomal membrane. \(^\text{9}\) Although little is known about such direct interactions, Cap might prevent the CDDP-induced acute renal failure through attenuation of the renal tubular damage or enhancement of the regenerative response in the damaged tubular cells.

In conclusion, Cap markedly reduced the CDDP-induced nephrotoxicity and suggests that Cap confers protection against the oxidative damage associated with CDDP. This mechanism may be reasonably attributed to its radical scavenging activity, to its SOD activating property, and/or to its regulatory activity on renal function. Further studies on tumor-bearing animals are necessary to clarify whether Cap under the conditions used in the present investigation, may not affect the therapeutic efficacy of CDDP.

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REFERENCES