Protective Effect of the Ethanol Extract of the Roots of *Brassica rapa* on Cisplatin-Induced Nephrotoxicity in LLC-PK₁ Cells and Rats

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A study was conducted to determine whether the ethanol extract of the roots of *Brassica rapa* (EBR) ameliorates cisplatin-induced nephrotoxicity in terms of oxidative stress, as characterized by lipid peroxidation, reactive oxygen species (ROS) production, and glutathione (GSH) depletion in LLC-PK₁ cells. Pretreatment of cells with EBR prevented cisplatin-induced decreases in cell viability and cellular GSH content. The effect of EBR was then investigated in rats given EBR for 14 d before cisplatin administration. A single dose of cisplatin (7 mg/kg, i.p.) caused kidney damage manifested by an elevation in blood urea nitrogen (BUN), serum creatinine, and urine lactate dehydrogenase (LDH) levels. Also, renal tissue from cisplatin-treated rats showed a significant increase in malondialdehyde (MDA) production, and in the activities of aldehyde oxidase (AO) and xanthine oxidase (XO). Moreover, a significant decrease in the activities of antioxidant enzymes, such as, glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) was observed in cisplatin-treated rats versus saline-treated normal group. In contrast, rats given EBR showed lower blood levels of BUN and creatinine, and of urinary LDH. Moreover, EBR prevented the rise of MDA production and the induction of AO and XO activities. This extract also recovered the reduced activities of GPx, SOD and CAT. Taken together, our data indicate that the ethanol extract of the roots of *Brassica rapa* (EBR) has a protective effect against cisplatin-induced nephrotoxicity because it attenuates oxidative stress.

Key words  *Brassica rapa*; cisplatin; nephrotoxicity; oxidative stress

Cisplatin (*cis*-diaminedichloroplatinum II) is one of the most effective chemotherapeutic agents and plays a major role in the treatment of a variety of human solid tumors including those of the head, neck, testis, ovary, and breast.1) However, the nephrotoxicity of cisplatin limits the efficacy of this important anticancer drug.2) Although the pathogenesis of cisplatin-induced acute renal failure (ARF) has been the subject of many studies, the molecular mechanisms that underlie its toxicity are poorly understood. Some investigators have suggested that free radicals play an important role in cisplatin-induced nephrotoxicity;3) and another proposed that reactive oxygen species (ROS), particularly the superoxide radical, play an important role in cisplatin-induced renal injury.4) It has also been reported that cisplatin-induced nephrotoxicity is closely associated with an increase in lipid peroxidation in the kidney.5,7) In addition, cisplatin has been found to inhibit antioxidant activity8) and to induce GSH depletion, which has been proposed to be involved in nephrotoxicity.9,10) Some evidence suggests that free radical scavengers and antioxidants ameliorate cisplatin-induced nephrotoxicity.11) The antioxidant DPPD (N,N’-diphenyl-p-phenylenediamine) reduced nephrotoxicity in cisplatin-treated rats, inhibited an increase in lipid peroxide level, and reduced the inhibition of p-aminohippurate transport *in vitro*.12,13) Actually, amifostine has been clinically approved as a cytoprotective agent during cisplatin therapy. It is known to act via a mechanism that involves the scavenging of free radicals, the donation of hydrogen ions to free radicals, the depletion of oxygen, and the binding to active derivatives of antineoplastic agents.14,15)

Thus, as a part of our on-going screening program to evaluate the protective potentials of natural compounds, we investigated the nephroprotective activity of the ethanol extract of the roots of *Brassica rapa in vitro* and *in vivo*. The group *Brassica rapa* includes many significant crops such as the Chinese cabbage; in Italy this group is mainly represented by turnip tops [*B. rapa* L. subsp. *sylvestris* (L.) *Janchi var. esculent* *Hort.*], which are used as a cooked vegetable and are known as “cime di rapa.” In Korea, *Brassica rapa* is cultivated commercially in GangHwa County, Kyunggi Province. In previous studies on *Brassica rapa*, flavonoids (isorhamnetin, kaempferol, and quercetin glycosides),16) phenylpropanoid derivatives,16) indole alkaloids,17) and sterol glucosides17) were identified. The only report on the minor components of *B. rapa* involved the determination of glucosinolates, a group of secondary metabolites of β-thioglucose, which is characteristics of the genus of *Brassica*.17) It is generally assumed that flavonoid components are of great importance in view of their biological effects of the human metabolism,18) but no report has been issued on the protective effect of *B. rapa* on cisplatin-induced nephrotoxicity. Therefore, we investigated whether the ethanol extract of *Brassica rapa* can ameliorate cisplatin-induced nephropathy by cultured renal epithelial cells and rats.

MATERIALS AND METHODS

Plant Material and Preparation of the Ethanol Fraction *Brassica rapa* was collected from GangHwa Country, Incheon, Korea during September 2005. The plant was iden-
tified by Dr. Hae-Gon Chung, one of the authors. A voucher specimen (#05157) has been deposited at the Laboratory of Natural Product Chemistry, Kyung-Hee University. Fresh root (100 g) was cut and extracted three times with EtOH (3 × 1 l). Extract solutions were filtered and dried using a rotatory evaporator under reduced pressure to give the EtOH extract (10.6 g).

**Chemicals** Medium 199, fetal bovine serum (FBS), penicillin, and streptomycin were obtained from Life Technologies Inc. (Grand Island, NY, U.S.A.). Cisplatin, xanthine, methyl nicotinamide chloride, l-lactate, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), RNase, leupeptin, aprotinin, phenylmethyl-sulfonylfluoride (PMSF), epinephrine, reduced glutathione (GSH), oxidized GSH (GSSG), 2-vinylpyridine, N-acetyl-l-cysteine (NAC), 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), 2,7-dichlorofluorescein diacetate (DCFH-DA), and the serum creatinine kit were purchased from Cayman Chemical Co. (Ann Arbor, MI, U.S.A.). BUN kit was purchased from Asan Pharm (Seoul, Korea). Glutathione peroxidase kits was purchased from Cayman Chemical Co. (Ann Arbor, MI, U.S.A.).

**Cell Culture and Sample Treatment** LLC-PK₁ cells, a cultured renal epithelial cell line derived from the porcine kidney, were obtained from the American Type Culture Collection. LLC-PK₁ cells were maintained as monolayer cultures in Medium 199 supplemented with 5% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin (complete culture medium). Two days before experiments, cells were seeded at 1 × 10⁶ cell/cm² on culture area. Cells were maintained throughout in a 5% CO₂ humidified atmosphere at 37 °C.

**MTT Assay for Cell Viability** Cell respiration (an indicator of cell viability) was determined by measuring the mitochondrial-dependent reduction of MTT to formazan. Briefly, cells were seeded in wells containing 100 μl of Medium 199 supplemented with 5% FBS in 96-well plates and stabilized for 24 h. After pretreatment with EBR at 25, 50, and 100 μg/ml for 3 h, 50 μM cisplatin was added. After incubation for a further 48 h, cells were washed once before adding 50 μl of FBS-free medium containing MTT 5 mg/ml and incubation for 4 h at 37 °C. Medium was then discarded and the formazan blue that had formed in cells was dissolved in DMSO 100 μl/well. Optical densities were measured at 540 nm.

**Determination of ROS Generation** 2,7-Dichlorofluorescein diacetate (DCFH-DA) was used to measure the levels of ROS induced by cisplatin. LLC-PK₁ cells were harvested and suspended in Medium 199 containing 0.2% fetal bovine serum. After pretreatment with 100 μg/ml EBR for 3 h, 50 μM cisplatin was incubated for 6 h, and then 20 μM DCFH-DA were added and incubated for 30 min at 37 °C. DCFH-DA was taken up by the cells and, on deacetylation, formed a non-fluorescent DCFH, which formedDCF when oxidized. Fluorescence intensities were measured by flow cytometry (Cytomics FC500 Series, Beckman Coulter, CA, U.S.A.).

**Animals and Experimental Design** Sprague–Dawley male rats weighing 100—120 g were purchased from the Korean Experimental Animal Co. and maintained under constant conditions (temperature: 20 ± 2 °C, humidity: 40—60%, and a 12 h light/dark cycle for two weeks or more). Twenty-four hours before the experiment the animals were allowed water only. In view of diurnal enzyme activity variations, animals were sacrificed at a fixed time (10:00—12:00 a.m.). These experiments were approved by the University of Kyungsu Animal Care and Use Committee. All procedures were conducted in accordance with the “Guide for Care and Use of Laboratory Animals” published by the National Institute of Health.

The rats were divided into six groups; each group containing six rats. Acute renal failure was induced by injecting (i.p.) cisplatin at a single dose of 7 mg/kg, which is well documented to induce nephrotoxicity in rats.¹⁰ EBR (each fraction: 50, 100 and 200 mg/kg) were dissolved in 10% Tween 20 and diluted with saline. Group 1 (normal) received a single dose i.p. injection of 1 ml isotonic saline following oral pretreatment with 1 ml saline for 14 d. Group 2 (EBR) received a single dose i.p. injection of 1 ml isotonic saline following oral pretreatment with 200 mg/kg EBR for 14 d. Group 3 (cisplatin) received a single dose i.p. cisplatin following oral pretreatment with 1 ml saline for 14 d. Group 4 (cisplatin+EBR 50) received a single dose cisplatin following oral pretreatment with 50 mg/kg EBR for 14 d. Group 5 (cisplatin+EBR 100) received a single dose cisplatin following oral pretreatment with 100 mg/kg EBR for 14 d. Group 6 (cisplatin+EBR 200) received a single dose cisplatin following oral pretreatment with 200 mg/kg EBR for 14 d.

**Biochemical Assay** Rats were sacrificed at fourth day after cisplatin administration. Blood was collected and centrifuged for 10 min at 3000 rpm to measure serum BUN, creatinine. BUN and creatinine were measured using commercial kits according to the manufacturers’ instructions and results are expressed as mg/dl. Urine was collected for 24 h at fourth day after cisplatin administration to measure lactate dehydrogenase (LDH) levels. Briefly, this urine was added to buffer containing 6.3 mm NAD and 50 mm l-lactate (pH 8.9) and increased absorbance at 340 nm was then monitored. The kidneys of rats were removed surgically, washed with physiological saline solution and cleared of fatty tissue. Tissues were homogenized and prepared for assays as described previously.¹⁹ The upper clear part of tissue homogenates was used for the measurements. The protein contents of these clear supernatants were examined using the method devised by Lowry et al.²⁰ Malondialdehyde (MDA), glutathione (GSH) levels, xanthine oxidase/dehydrogenase (XO/XD), aldehyde oxidase (AO), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) enzymes activities were measured in supernatants. MDA levels were measured using the thiobarbituric acid reactive substances (TBARS) method.²¹ GSH was assayed using an enzymatic recycling procedure adapted to a microplate reader.²² Results are expressed as μmole GSH/g protein. XO and XD activities were determined by measuring uric acid formation from xanthine at 295 nm.²³ AO activity was determined by measuring 2-pyridone formation at 300 nm.²⁴ SOD activity was determined using the method described by Misra and Fridovich²⁵ at room temperature. SOD activity is defined as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which was set equal to 1 unit. CAT activity was determined by measuring the absorbance decrease of hydro-
gen peroxide (H$_2$O$_2$) at 240 nm. GPx activity was measured by following changes in NADPH absorbance at 340 nm using Cayman kits.

**Data and Statistical Analysis** Data are reported as means±S.D. Statistical analysis was performed by analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test and p-values of <0.05 were considered statistically significant. The numbers of independent experiments performed are provided in the figure legends.

**RESULT**

**Effect of EBR on Cisplatin-Induced Cytotoxicity in LLC-PK$_1$ Cells** To assess the effects of EBR on cisplatin-induced cytotoxicity in LLC-PK$_1$ cells, the cells were preincubated with EBR (25, 50, 100 μg/ml) for 3 h, and then treated with 50 μM cisplatin for 48 h. As shown in Fig. 1, EBR significantly protected the cells from the decrease in viability by exposure to 50 μM cisplatin (Fig. 1). The protective effect of EBR was concentration dependent: highest protection (86.4%) was obtained at an EBR concentration of 100 μg/ml. In addition, cisplatin-induced cytotoxicity was completely blocked by pretreatment with the antioxidant N-acetyl-L-cysteine (NAC), indicating that cisplatin-induced cytotoxicity in LLC-PK$_1$ cells acts via ROS generation.

**Effect of EBR on Cisplatin-Induced ROS Response in LLC-PK$_1$ Cells** To determine whether ROS is involved in the cytoprotective effect of EBR, we next measured levels of ROS within cells using an ROS-sensitive fluorometric probe, 2,7-dichlorofluorescein (DCF). As shown in Fig. 2A, the basal level of DCF-sensitive ROS in LLC-PK$_1$ cells was rarely detectable. Following treatment with 50 μM cisplatin a significant amount of ROS about 52.2% was generated (Fig. 2B). Pretreatment of LLC-PK$_1$ cells with 100 μg/ml EBR for 3 h or 5 mM NAC for 1 h significantly reduced cisplatin-induced ROS generation about 6.5% or 7.8%, respectively (Figs. 2C, D).

**Protective Effect of EBR on MDA Production and GSH Depletion in Cisplatin-Treated LLC-PK$_1$ Cells** To further determine the involvement of oxidative stress in cisplatin-induced cytotoxicity, we measured MDA levels and GSH contents in LLC-PK$_1$ cells. Cisplatin treatment produced a significant (p<0.05) elevation in MDA levels and reduced GSH contents versus non-treated controls. Moreover, pretreatment with EBR for 3 h significantly decreased cisplatin-induced MDA levels compared with cisplatin only treated cells (Fig. 3A). In addition, GSH level reduction was significantly prevented by EBR pretreatment (Fig. 3B).

**Effect of EBR on Cisplatin-Induced BUN and Creatinine, and Urine LDH Levels in Rats** BUN and creatinine, and urine lactate dehydrogenase (LDH) levels were used as a markers of renal injury. ARF was induced by a single admin-

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**Figure 1.** The Effect of EBR on Cisplatin-Induced Cytotoxicity in LLC-PK$_1$ Cells

Cells were treated with various concentrations (25, 50, 100 μg/ml) of EBR for 3 h and then cisplatin (50 μM) was added and cells were incubated for 48 h. Non-treated (None) values were obtained in the absence of cisplatin or EBR. N-acetyl-L-cysteine (NAC) 5 mM was used as a positive control. The values are means±S.D. from three independent experiments. *p<0.05 vs. the non-treated group; b p<0.05 vs. the cisplatin-treated group; significances of differences between treatment groups were evaluated using ANOVA followed by Dunnett’s test.

**Figure 2.** The Effect of EBR on Cisplatin-Induced ROS Response in LLC-PK$_1$ Cells

Generation of ROS induced by cisplatin and the effects of pretreatment with EBR or NAC detected by flow cytometry. Cells were treated with 100 μg/ml EBR for 3 h or 5 mM NAC for 1 h and then cisplatin (50 μM) was added, and the cells were incubated for 6 h. Non-treated values were obtained in the absence of cisplatin or EBR. (A) Non-treated values. (B) Treatment with cisplatin alone. (C) Pretreatment with EBR prior to cisplatin. (D) Pretreatment with NAC 5 mM as a positive control prior to cisplatin.
istration of cisplatin (7 mg/kg, i.p.). At fourth day, treatment with EBR prior to cisplatin significantly reduced BUN and serum creatinine, and urine LDH levels in a dose-dependent manner, indicating that cisplatin-induced ARF was significantly attenuated by pretreatment with EBR (Table 1).

**Protective Effect of EBR on Oxidative Stress in Cisplatin-Administered Rats** To determine the involvement of oxidative stress in vivo, we measured MDA production and GSH contents in rat kidneys; MDA production in renal tissue was used as a measure of lipid peroxidation. Figure 4A shows results in rats sacrificed 4 d after cisplatin injection. Injection of cisplatin (7 mg/kg, i.p.) increased the formation of MDA levels in kidney by 51.6% compared to saline-treated normal groups (p<0.05). This increase was significantly inhibited in a dose-dependent manner by the oral administration of EBR at 50, 100 and 200 mg/kg for 14 d in rats. The results of pretreatment with EBR on cisplatin-induced changes in the level of renal GSH are shown Fig. 4B. Cisplatin treatment significantly reduced kidney GSH contents versus saline-treated normal group (p<0.05), and pretreatment with EBR also significantly prevented this reduction in renal GSH at high dose (100, 200 mg/kg) (Fig. 4B).

**Effect of EBR on Oxidant and Antioxidant Enzyme Activities in Cisplatin-Administered Rats** Since cisplatin administration was found to lead to an imbalance between the production and catabolism of oxidants, we tested whether EBR alters cisplatin-induced changes in oxidant and antioxidant statuses by assaying for enzymes related to oxidative processes. We found significant increases in two main oxidant parameters, namely kidney tissue aldehyde oxidase (AO) and xanthine oxidase (XO) activity. In cisplatin-administered rats, AO and XO levels were significantly higher than those in the saline-treated normal group (Table 2). XO/XD ratio in an antioxidant parameter was significantly lower in cisplatin-treated group compared to saline-treated normal group. The enzyme activities were assayed using an enzymatic recycling procedure. The values shown are means±S.D. a, b

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**Table 1. The Effect of EBR on Renal Injury in Cisplatin-Administered Rats**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>LDH (wrobleski unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>19.7±5.63</td>
<td>0.80±0.10</td>
<td>28.7±3.16</td>
</tr>
<tr>
<td>EBR</td>
<td>200</td>
<td>21.5±4.78</td>
<td>0.92±0.12</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>7</td>
<td>79.4±9.43</td>
<td>1.73±0.15(*)</td>
</tr>
<tr>
<td>Cisplatin+EBR</td>
<td>50</td>
<td>67.3±8.57</td>
<td>1.65±0.11(*)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>51.9±5.42</td>
<td>1.46±0.13(*)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>48.3±9.21</td>
<td>1.31±0.10(*)</td>
</tr>
</tbody>
</table>

BUN, creatinine and LDH level were assessed at the fourth day after cisplatin injection. To measure LDH urine was collected for 24 h at the fourth day. Then, blood sample was obtained after sacrifice rat to measure BUN and creatinine. Values are expressed means±S.D. The number of animal used for each group was 6. a, b vs. the saline-treated normal group; p<0.05 vs. the cisplatin-treated group; significances of differences between treatment groups were evaluated using ANOVA followed by Dunnett’s test.
discovered, although recently, free radicals have been shown to cause cisplatin nephrotoxicity, the factors responsible are largely unclear. The development of therapies designed to inhibit cisplatin-induced toxicity is important. The nephrotoxicity of cisplatin limits the usefulness of this drug. Therefore, exploring the nephro-protective effects of EBR is prudent.

Table 2. The Effect of EBR on Changes in Activities of Oxidant Enzymes in Cisplatin-Administered Rats

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>2-Pyridone nmol/mg protein/min</th>
<th>2-Pyridone nmol/mg protein/min</th>
<th>2-Pyridone nmol/mg protein/min</th>
<th>2-Pyridone nmol/mg protein/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>12.9 ± 0.72</td>
<td>0.221 ± 0.008</td>
<td>0.095 ± 0.019</td>
<td>0.30 ± 0.03</td>
</tr>
<tr>
<td>EBR</td>
<td>13.2 ± 0.33</td>
<td>0.232 ± 0.007</td>
<td>0.102 ± 0.018</td>
<td>0.31 ± 0.04</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>16.2 ± 0.59</td>
<td>0.018 ± 0.009</td>
<td>0.208 ± 0.015</td>
<td>0.92 ± 0.16</td>
</tr>
<tr>
<td>Cisplatin + EBR</td>
<td>15.9 ± 0.78</td>
<td>0.034 ± 0.016</td>
<td>0.193 ± 0.017</td>
<td>0.85 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>14.3 ± 0.42&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.088 ± 0.007&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.138 ± 0.019&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.61 ± 0.10&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>13.8 ± 0.57&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.096 ± 0.013&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.127 ± 0.018&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.57 ± 0.13&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The enzyme activities were assessed from renal tissue at fourth day after cisplatin administration. The assay procedure was described in the experimental methods. Values are expressed as mean ± S.D. The number of animal used for each group was 6. *p < 0.05 vs. the saline-treated normal group; **p < 0.05 vs. cisplatin-treated group; significance of differences between treatment groups was evaluated using ANOVA followed by Dunnett’s test.

Table 3. The Effect of EBR on Changes in Activities of Antioxidant Enzymes in Cisplatin-Administered Rats

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>GPx (oxidized NADPH nmol/mg protein/min)</th>
<th>SOD (unit/mg protein/min)</th>
<th>CAT (H/O&lt;sub&gt;2&lt;/sub&gt; nmol/mg protein/min)</th>
<th>XD (uric acid nmol/mg protein/min)</th>
<th>Type conversion ratio (NO/XD + XO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>246.3 ± 12.8</td>
<td>25.4 ± 3.36</td>
<td>187.9 ± 4.9</td>
<td>12.3 ± 10.3</td>
<td>0.007 &lt; 0.009</td>
</tr>
<tr>
<td>EBR</td>
<td>256.9 ± 15.6</td>
<td>26.8 ± 4.21</td>
<td>182.8 ± 5.6</td>
<td>12.3 ± 10.3</td>
<td>0.015 ± 0.019</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>117.6 ± 15.7</td>
<td>13.9 ± 2.90</td>
<td>92.3 ± 5.4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>12.1 ± 10.3</td>
<td>0.019 ± 0.019</td>
</tr>
<tr>
<td>Cisplatin + EBR</td>
<td>123.9 ± 13.2&lt;sup&gt;+&lt;/sup&gt;</td>
<td>16.3 ± 4.17</td>
<td>100.8 ± 8.6&lt;sup&gt;+&lt;/sup&gt;</td>
<td>12.2 ± 10.3</td>
<td>0.04 ± 0.03c</td>
</tr>
<tr>
<td></td>
<td>164.3 ± 10.3&lt;sup&gt;+&lt;/sup&gt;</td>
<td>21.9 ± 1.26</td>
<td>140.5 ± 7.8&lt;sup&gt;+&lt;/sup&gt;</td>
<td>12.4 ± 10.3</td>
<td>0.10 ± 0.03c</td>
</tr>
<tr>
<td></td>
<td>171.9 ± 12.2&lt;sup&gt;+&lt;/sup&gt;</td>
<td>22.2 ± 2.08</td>
<td>149.3 ± 8.9&lt;sup&gt;+&lt;/sup&gt;</td>
<td>12.6 ± 10.3</td>
<td>0.23 ± 0.03c</td>
</tr>
</tbody>
</table>

The enzyme activities were assessed from renal tissue at fourth day after cisplatin administration. The assay procedure was described in the experimental methods. Values are expressed as mean ± S.D. The number of animal used for each group was 6. *p < 0.05 vs. the saline-treated normal group; **p < 0.05 vs. cisplatin-treated group; significance of differences between treatment groups was evaluated using ANOVA followed by Dunnett’s test.

The nephrotoxicity of cisplatin limits the usefulness of this important chemotherapeutic agent. Although several studies have been performed to elucidate the molecular mechanisms that cause cisplatin nephrotoxicity, the factors responsible are not fully understood, although recently, free radicals have been proposed to participate in this process.

Different strategies have been proposed to inhibit cisplatin-induced toxicity. The development of therapies designed to prevent the damaging actions of free radicals may influence the progression of oxidative renal damage and the acute renal damage induced by cisplatin. Some investigators have recommended that antioxidant vitamins be supplemented to attenuate nephrotoxicity. In addition, it has been proposed that some foods, such as black grape, which contains resveratrol can provide significant protection against cisplatin-induced nephrotoxicity. Epidemiological studies have shown that brassica vegetables, including cabbages, kale, Brussels sprouts and broccoli can play protective roles against several cancers. These protective effects of brassicas against cancer are plausible due to their relatively high contents of glucosinolates, which are broken down by hydrolysis to species like indoles and isothiocyanates that are able to influence antioxidant response element, and thereby influence several processes related to chemical carcinogenesis.

Weiner and Jacobs reported that the S3 segment in the straight part of proximal tubules is a major location of the renal disorder induced by cisplatin. Therefore, using the porcine kidney-derived culture epithelial cell line LLC-PK<sub>1</sub>, which has proximal tubule-like features, we examined the protective effects of EBR on cisplatin-induced cytotoxicity. In the present study, EBR (25, 50, 100 µg/ml) protected LLC-PK<sub>1</sub> cells from cisplatin-induced damage in a dose-dependent manner, but EBR alone did not affect the cell viabilities of LLC-PK<sub>1</sub> cells, even at 300 µg/ml. Moreover, we observed that cisplatin increased the production of MDA and depleted GSH contents, which is in-line with the effects of ROS overproduction. However, EBR suppressed these cisplatin-induced effects in a dose-dependent manner. Of the various mechanisms suggested for cisplatin-induced cytotoxicity, much attention has been focused on intracellular redox status. It has been demonstrated that intracellular GSH, the main determinant of intracellular redox status, is depleted by cisplatin. In the present study, the cisplatin-induced reduction of intracellular GSH was significantly recovered in the presence of EBR. These findings suggest the usefulness of cultured cells as an in vitro model system reflecting conditions in vivo. However, the kidney consists of various cells, and we believe that an in vivo experimental system is necessary for investigating the kidney as a whole, and therefore, we also used a rat model.

Whilst investigating the nephro-protective effects of EBR in vivo, we found that the administration of EBR attenuated cisplatin-induced renal damage characterized by increases in BUN, creatinine, and LDH. Lipid peroxidation is a major feature of cisplatin-induced nephrotoxicity and may be caused by cisplatin-induced GSH depletion. ROS, such as, hydrogen peroxide, the superoxide anion, and hydroxyl radi-
cals, are generated under normal cellular conditions and are immediately detoxified by endogenous antioxidants, like GSH, catalase, and superoxide dismutase, but excessive ROS accumulations by cisplatin cause an antioxidant status imbalance and lead to lipid peroxidation and GSH depletion.\textsuperscript{,}\textsuperscript{3,32}

Our data show that cisplatin-induced MDA production was significantly decreased by EBR treatment \textit{in vivo}, and EBR also slightly attenuated cisplatin-induced GSH depletion in rats. EBR treatment significantly inhibited increases in the activities of AO and XO and ratio of XO/XD, and also slightly attenuated cisplatin-induced GSH depletion in kidneys of rats. EBR treatment significantly inhibited increases in the activities of AO and XO and ratio of XO/XD, and thus protects cells against ROS. The antioxidant activity of phenolics, which act as reducing radicals and lead to lipid peroxidation and GSH depletion.\textsuperscript{8,32}

The antioxidant activity of phenolics, which act as reducing agents and hydrogen donors, has been studied also in relation to polyphenol content.\textsuperscript{15} After a liquid–liquid extraction and fractionation procedures, most flavonoids were identified by means of HPLC-DAD/MS techniques.\textsuperscript{16} Further pharmacological examination study on the flavonoids isolated from \textit{B. rapa} will provide more clarified information on the active component.

It has been suggested that cisplatin is able to generate ROS and that it inhibits the activities of antioxidant enzymes in renal tissue, e.g., of GPx, SOD and CAT.\textsuperscript{8,28} In the present study, the activities of GPx, SOD and CAT were found to be reduced in the kidneys of rats treated with cisplatin. The selenium containing enzyme GPx, scavenges hydroperoxides and lipid peroxides, and thus protects cells against ROS. Moreover, it is evident from the present study that EBR pretreatment restored cisplatin induced impairments in GPx, SOD, and CAT activities. Based on available evidence we conclude that the ethanol extract of the roots of \textit{Brassica rapa} (EBR) eliminates oxidative stress and benefits renal function.

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