Reduction of Cisplatin Toxicity and Lethality by Sodium Malate in Mice

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The effects of oral treatment with sodium malate, an active ingredient of Juzen-taiho-to, on the nephrotoxicity, bone marrow toxicity, hepatotoxicity and gastrointestinal toxicity caused by i.p. administration of 9 doses of 3.0 mg/kg/d cisplatin (CDDP) (on days 3, 4, 5, 6, 7, 8, 10, 11 and 12) were examined in ddY mice inoculated with sarcoma 180 (S-180) cells on day 1 of the study. The CDDP-induced increases in blood urea nitrogen, serum creatinine, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminases and relative stomach weight and the decreases in food intake and body weight were inhibited nearly to the control levels without reducing the antitumor activity of CDDP against S-180 by the oral treatment with sodium malate of 12 doses of more than the equimolar amount of CDDP (on days 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14 and 15). However, the CDDP-induced decreases in white blood cell and platelet counts and relative spleen and thymus weight could not be inhibited completely by combination with sodium malate, even at a dose of twice the equimolar amount of CDDP. The sodium malate-induced reduction of CDDP-induced nephrotoxicity and hepatotoxicity was observed after oral administration, as well as with i.p., s.c. and i.v. administration, and the effect was almost the same for each route of administration. Sodium malate also reduced the toxicity induced by high doses of CDDP (4.5, 6.0, 7.5, 9.0 and 12.0 mg/kg/d) at doses of twice the equimolar amount of CDDP. Sodium malate at a dose of 10.68 mg/kg/d (twice as high as carboplatin, CBDCDA) did not reduce the nephrotoxicity, bone marrow toxicity, hepatotoxicity and gastrointestinal toxicity caused by i.p. administration of 9 doses of 15.0 mg/kg/d CBDCDA on days 3, 4, 5, 6, 7, 8, 10, 11 and 12 in ddY mice inoculated with sarcoma 180 (S-180) cells on day 1 of the study. From this study, it was suggested that sodium malate could become a useful agent for the reduction of CDDP-induced toxicity, particularly nephrotoxicity and hepatotoxicity.

Key words sodium malate; cisplatin; nephrotoxicity; bone marrow toxicity; carboplatin; Kampo medicine

Cisplatin (CDDP)[1,2] is an anticancer agent with excellent anticancer effects on various solid cancers, including testicular tumors and ovarian cancer, by inhibiting DNA synthesis[3,4] or inducing apoptosis.[5–10] This drug is now widely used all over the world, including Japan. However, severe renal disorders,[11–16] bone marrow disorders[17,18] and gastrointestinal disorders[19,20] such as vomiting pose problems and prevent adequate therapeutic effects. The subacute toxicity of long-term multiple administrations of CDDP is particularly problematic, so that the development of countermeasures would be highly desirable. Several methods have been employed in an attempt to reduce the nephrotoxicity as a dose-limiting side effect[21–25] of CDDP, but these problems have not yet been completely solved. We assumed that some Chinese medicines could possibly reduce the toxicity of CDDP and showed that Juzen-taiho-to, a formula frequently used to improve physical conditions during recuperation, could inhibit the CDDP-induced nephrotoxicity, bone marrow toxicity and gastrointestinal toxicity almost completely without reducing the antitumor effect of CDDP. We also demonstrated that one of the nephrotoxicity reducing agents in Juzen-taiho-to is sodium malate.[26] In this study, therefore, we examined the sodium malate-induced reduction of the CDDP toxicity and the toxicity of a derivative of CDDP, carboplatin (cis-diammine(1,1-cyclobutane dicarboxylate) platinum(II); CBDCDA).

MATERIALS AND METHODS

Animals Five-week-old, ddY male mice (average weight, 25 g) were obtained from Japan SLC, Inc. (Hamanatsu, Japan) and kept in rooms with controlled temperature (23 ± 0.5 °C), humidity (50 ± 5%), and 12-h light/12-h dark cycles. They were fed commercial mouse chow (MF: Oriental Yeast Co., Ltd., Tokyo, Japan) and had free access to tap water. The animals were used for the experiments after one week of acclimation (average weight, 30 g).

Chemicals CDDP and CBDCDA were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The solutions of CDDP and CBDCDA were prepared with sterile 0.9% saline at concentrations of 0.5 mg/kg and 2.5 mg/ml, respectively. Sodium malate was obtained from Wako Pure Chemical Industries, Ltd. (Toko).

Treatment of Animals We used a modification of the previously reported method[26–28] for the treatment of the animals. The animals in the test groups (n = 10) were inoculated subcutaneously in the left thigh on day 1 of the study with sarcoma 180 (S-180) cells (10⁶/mouse). The mice then i.p. received CDDP (3.0, 4.5, 6.0, 7.5, 9.0 or 12.0 mg/kg) on days 3, 4, 5, 6, 7, 8, 10, 11 and 12. Sodium malate was given orally to the mice on days 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 14 and 15. The doses of sodium malate were 0.223, 0.445, 0.89, 1.78 and 3.56 mg/kg/d, equivalent to 1/8, 1/4, 1/2, 1 and 2 times of the equimolar amount of CDDP. The mice in the control group were treated with water (p.o.) and 0.9% saline (i.p.). On day 17, the mice were anesthetized with ether to collect blood from the inferior vena cava with a heparinized syringe, after which we immediately counted the number of red blood cells (RBC), white blood cells (WBC) and platelets (PLT). After centrifugation of the remaining blood, the serum was analyzed for blood urea nitrogen (BUN), serum creatinine, serum glutamic-oxaloacetic transaminase (sGOT) and serum glutamic-pyruvic transaminase (sGPT). The liver, kidneys, spleen, thymus, stomach (organ and contents) and

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tumor were resected and weighed. The 24-h urine volume and 24-h food intake were measured on day 14. The reduction of the toxicity of CBDC by sodium malate was examined using CBDA (15.0 mg/kg) showing an antitumor effect similar in magnitude and characteristics to CDDP (3 mg/kg). Moreover, the effect of sodium malate (3.56 mg/kg) administered either intraperitoneally, intravenously or subcutaneously was examined according to the above protocol.

Measurement of Functions RBC, WBC and PLT counts were made using a Celltac 4150 (Nihon Koden, Ltd., Tokyo). BUN, serum creatinine, SGOT and SGPT were measured spectrometrically with a COBAS FARA (Baxter, Ltd., Tokyo) using assay kits for urea nitrogen-HR II, creatinine-HA test Wako, GOT-FA test Wako and GPT-FA test Wako (Wako Pure Chemical Industries, Ltd., Tokyo), respectively.

Antitumor Activity The inhibition of the antitumor effect was calculated using the following formula:

\[
\text{Inhibition} (\%) = (1 - B/A) \times 100
\]

where \( A \) is the mean tumor weight in the control group and \( B \) is that in the drug-treated groups.

Histopathological Examinations Mice were sacrificed on day 17 under ether anesthesia by exsanguination following decapitation. Kidneys, liver, spleen, thymus and stomach were resected and immediately cut into slices of about 5 mm in a side. Bone marrow cells were extracted from the left femur. Subsequently, the slices were fixed in 10% buffered formalin (Wako Pure Chemical Industries, Ltd.), embedded in paraffin according to conventional methods, stained with hematoxylin and eosin, and observed under optical microscopy.

Statistics The Student's \( t \)-test was used to evaluate the significance of differences between the experimental groups.

RESULTS

Effects of Sodium Malate on the Toxicity Induced by 3.0 mg/kg of CDDP Nephrotoxicity: Figure 1 shows the effects of sodium malate on CDDP-induced nephrotoxicity. BUN and serum creatinine were significantly increased to about 3 and 2 times the corresponding control levels, respectively, while urine volume was significantly decreased to about 42% of the control level observed after i.p. treatment with 3.0 mg/kg/d of CDDP alone in 9 dosings, whereas the values of the CDDP alone groups were 97.5±13.1 mg/dl, 0.69±0.33 mg/dl, 5.1±0.9 ml/100 g, and 1.54±0.18 \((\times 10^{-2})\), respectively, for BUN, serum creatinine, urine volume and relative kidney weight. However, compared to the control values, no significant changes in relative kidney weight were observed in any of CDDP-treated mice; the control values were 28.5±1.1 mg/dl, 0.47±0.06 mg/dl, 8.3±1.1 ml/100 g, and 1.39±0.11 \((\times 10^{-2})\), respectively, for BUN, serum creatinine, urine volume and relative kidney weight. Sodium malate at a dose of 0.223 mg/kg (1/8 times the equimolar amount of CDDP) significantly inhibited the increase in BUN, and a dose of 0.89 mg/kg (1/2 times the equimolar amount) led to values comparable to the control levels. Creatinine showed almost the same value in the control after combined treatment at 0.89 mg/kg. Regarding the urine volume, significant differences from the CDDP alone groups were observed after combined treatment with more than 1.78 mg/kg (equimolar amount) of sodium malate. Sodium malate alone did not cause any significant changes in these parameters at any of the doses tested.

Bone Marrow Toxicity: Figure 2 shows the effects of sodium malate on CDDP-induced bone marrow toxicity. WBC count, PLT count and relative spleen and thymus weight decreased significantly, to about 33%, 27%, 56% and 25% of the respective control levels. RBC, WBC and PLT

Fig. 1. Effect of Sodium Malate on Cisplatin(CDDP)-Induced Nephrotoxicity

Sodium malate (0.223—3.56 mg/kg/d×12) was administered p.o. to mice s.c. inoculated with S-180 cells 30 min before CDDP i.p. injection (3.0 mg/kg/d×9) once a day. BUN and serum creatinine levels and relative kidney weight were determined 14 d after CDDP injection. and the 24-h urinary volume was measured 14 d after CDDP injection. Each value is the mean±S.E. (n=10). Significant differences from the CDDP (−) groups, *: p<0.05, **: p<0.01 and ***: p<0.001. Significant differences from the CDDP alone group, #: p<0.05, ##: p<0.01 and ###: p<0.001.
counts and the relative spleen and thymus weight of mice treated with CDDP alone were 10.3±0.5 \times 10^6/mm^3, 2.1±0.4 (10^7/mm^3), 2.2±0.3 (10^7/mm^3), 1.98±0.22 (\times 10^{-5}) and 0.41±0.10 (\times 10^{-3}), respectively, while these values in untreated mice were 9.4±0.4 (10^6/mm^3), 6.4±0.5 (10^7/mm^3), 8.2±0.8 (10^7/mm^3), 3.53±0.39 (\times 10^{-5}) and 1.63±0.20 (\times 10^{-3}), respectively.

Although treatment with sodium malate equimolar to CDDP or at higher doses significantly inhibited the decreases in WBC count and PLT count, these values did not return to the control levels, even after treatment with twice the equimolar amount. Similarly, the relative spleen and thymus weight were significantly reduced by combined treatment with sodium malate in an amount equimolar to CDDP, but not even after treatment with an amount twice the equimolar amount did it return to the value in the sodium malate alone group. Sodium malate alone did not significantly change those parameters at any of the doses tested.

Hepatotoxicity: Figure 3 shows the effect of sodium malate on hepatotoxicity induced by 3.0 mg/kg CDDP given 9 times. Administration of CDDP alone increased sGOT and sGPT values to about 3 and 5 times the control values, although significant changes in relative liver weight were not observed. sGOT, sGPT and the relative liver weight in mice treated with CDDP alone were 67.6±9.5 U/l, 46.6±8.5 U/l and 5.05±0.75 (\times 10^{-5}), respectively, while the corresponding values in untreated mice were 19.2±1.3 U/l, 9.8±1.2 U/l and 5.11±0.32 (\times 10^{-2}), respectively.

Significant hepatotoxicity was reduced to a level similar to that in the sodium malate alone group after combined treatment with sodium malate at a dose of more than 0.89 mg/kg.

Other Toxicities: The administration of CDDP alone decreased body weight and food intake to 64% and 24% of the control values, respectively, and significantly increased the relative stomach weight to about 4 times the control value. Body weight, food intake and relative stomach weight of mice treated with CDDP alone were 22.5±0.9 g, 8.4±0.9 (\times 10^{-2}), respectively, while the corresponding values in untreated mice were 34.8±0.7 g, 3.4±0.7 g and 2.2 (\times 10^{-3}), respectively.

Body weight, food intake and relative stomach weight were significantly improved after combined treatments with sodium malate of 1/2, equivalent and 1/8 times the equimolar amount of CDDP. These factors showed almost the same values as in the sodium malate alone group after combined treatment with sodium malate of twice the equimolar amount of CDDP for body weight and food intake, and of an equimolar amount of CDDP for relative stomach weight (Fig. 4).

Histopathological Examination: Kidney: In the CDDP-treated group, moderate or severe degeneration and necrosis of the epithelium of cortical tubules were particularly observed, which were sporadically accompanied by dilation of the tubule and cast. In the interstitium, although edema was
observed in some animals, no marked cellular infiltration was observed. Medullary changes were mostly of minor degree. On the other hand, the sodium malate co-treated group showed almost normal findings except for mild edema and cellular infiltration in the interstitium. Although the findings in the sodium malate alone group were almost normal, a mild disorder of the cortical tubule was observed in some mice.

Liver: In the CDDP-treated group, a mild degeneration of hepatic cells was observed, which was locally accompanied by comparatively intense necrosis. While most findings in the sodium malate co-treated group resembled those in the normal group, hepatocellular nucleus size showed certain variations. In the sodium malate alone group, findings were almost the same as in the normal group.

Bone Marrow Cell: In the CDDP-treated group, a marked decrease in cell count, particularly in megakaryocytes and juvenile hematopoietic cells were observed, but the differences between the normal group and the sodium malate co-treated group were not significant. A mild decrease in cell count was observed in both the sodium malate treated group and the normal group.

Additionally, in the animals showing histological changes...
attributable to sodium malate, changes in megakaryocytes ranging from small and immature cells to large and polynuclear cells were frequently observed.

Spleen: In the CDDP-treated group, marked atrophy was observed throughout the spleen, but the disappearance of hematopoietic loci in the red pulp was particularly marked. There were almost no differences between the sodium malate co-treated group and the normal group. In the sodium malate alone group, mild atrophy was observed in both the white and red pulp.

Thymus: In the CDDP-treated group, a marked decrease in the number of cells was observed in both the cortex and the medulla, which did not stop the basic construction of the thymus. In the sodium malate co-treated group, structural disturbances were observed at the border between the cortex and the medulla, yet resembled the findings in the normal group. In the sodium malate alone group, on the other hand, changes such as mixed atrophy of the cortex and cystosis of the medulla were observed.

Stomach: In the CDDP treatment group, only histologically mild atrophy of the mucous epithelium was observed. The sodium malate co-treated group showed almost the same findings as the normal group. In the sodium malate alone group, on the other hand, mild atrophy of mucosa and degeneration of epithelium were observed.

**Antitumor Activity** We demonstrated that treatment with CDDP alone dose-dependently inhibited the growth of S-180 cells (ED_{50}: 2.30 mg/kg), with the inhibitory rate reaching a peak (80.9%) at a dose of 3.0 mg/kg. Table 2 summarizes the antitumor activity of CDDP and/or sodium malate against S-180. There was no significant difference between the CDDP-treated group and the sodium malate plus CDDP-treated group, although at a dose of 3.56 mg/kg, corresponding to twice the equimolar amount of CDDP; the antitumor effect was slightly (about 11%) reduced compared to the CDDP alone group. Sodium malate alone also had no obvious antitumor effect on S-180 at any of the doses tested.

**Effect of Sodium Malate on Lethal Toxicity of 3.0—12.0 mg/kg of CDDP** Figure 5 shows the effects of sodium malate on the lethal toxicity induced by 3.0, 4.5, 6.0, 7.5, 9.0, and 12.0 mg/kg of CDDP. During the first 20 d, all the mice receiving 3.0 mg/kg of CDDP survived, while about 40% died after day 21. Meanwhile, CDDP alone at a dose of 4.5, 6.0, 7.5, 9.0 and 12.0 mg/kg was very toxic, so that all the mice receiving CDDP at these doses died within 18, 15, 13, 9 and 8 d after the initial injection of CDDP, respectively, without showing any obvious increase in the volume of S-180 cells inoculated in the left thigh.

Treatment with sodium malate at a dose of twice the equimolar amount of CDDP completely countered the lethal toxicity of 3.0 and 4.5 mg/kg of CDDP and efficiently prolonged the survival time of the S-180-bearing mice receiving 6.0, 7.5, 9.0 and 12.0 mg/kg of CDDP.

The BUN, WBC, relative stomach weight and body weight on day 17 in the animals treated with 5.34 mg/kg of sodium malate (twice the equimolar amount) and 4.5 mg/kg of sodium malate were 7.0, 1.1 and 7.1, respectively.

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**Table 1. Pathological Changes by CDDP and/or Sodium Malate (SM)**

<table>
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<td>- ±</td>
<td>- ± + + + +</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>tubular dilation/cast formation;</td>
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<tr>
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<td>10 0 0</td>
<td>8 1 0</td>
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<td>6 1 3 0</td>
<td>10 0 0</td>
<td>4 3 3 0</td>
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</table>

- , negative or within the borderline of normal variation; ±, slightly positive; +, positive; ++, strongly positive.
Table 2. Effect of Sodium Malate on Antitumor Activity of CDDP Against S-180

<table>
<thead>
<tr>
<th>Dose of sodium malate (mg/kg, p.o.)</th>
<th>Sodium malate alone</th>
<th>Sodium malate plus CDDP</th>
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<td>Tumor wt. (g)</td>
<td>Antitumor activity (%)</td>
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<tr>
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<td>1.15±0.17</td>
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<tr>
<td>0.223</td>
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<td>0.445</td>
<td>1.14±0.17</td>
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<td>1.78</td>
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<tr>
<td>3.56</td>
<td>1.14±0.15</td>
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</table>

S-180 cells (10⁶ cells/mouse) were inoculated s.c. on day 1 and antitumor activity was determined on day 17. Sodium malate (0-3.56 mg/kg/d×12) was administered p.o. 30 min before 3.0 mg/kg CDDP i.p. injection 9 times. Each value is the mean±S.E. (n=10). Significant difference from the non-treated control group, **p<0.001. Significant difference from only the sodium malate treated group, ***p<0.001. There was no significant difference between only CDDP-treated group and sodium malate and CDDP-treated groups.

CDDP are shown in Fig. 6. As shown in Fig. 5, all the mice receiving 4.5 mg/kg of CDDP alone died within 17 d, preventing the collection of a complete body of data, so the data obtained with 3.0 mg/kg of CDDP were used as a reference. Combined treatment with sodium malate at a dose twice the equimolar amount of 4.5 mg/kg of CDDP inhibited the increase in BUN, showing almost the same value as that after treatment with sodium malate alone. On the other hand, the depressed leukocyte count recovered to only 3.9±0.7 ×10³/mm³, or approximately 50% of the control. Even after co-treatment with sodium malate, this did not improve. Antitoxic effects pertaining to the relative stomach weight and body weight were very weak.

Effect of Sodium Malate Classified by Routes of Administration The effects of sodium malate on the CDDP-induced nephrotoxicity (BUN), bone marrow toxicity (WBC) and loss of body weight after the oral, intravenous, intraperitoneal and subcutaneous administration of sodium malate are shown in Fig. 7.

Effectiveness did not depend on the route of administration. However, effectiveness after intravenous administration tended to be weaker than that after oral administration. No
route of administration had any influence on the antitumor effect of CDDP (Fig. 8).

Effect of Sodium Malate on the Toxicity Induced by 15.0 mg/kg of CBDDCA. The inhibition of CBDDCA toxicity by sodium malate is shown in Fig. 9. CBDDCA given 9 times at a dose of 15.0 mg/kg caused a mild increase in BUN, but the decreases in platelet and WBC counts were severe compared with those after CDDP. Combined treatment with sodium malate at a dose of twice the equimolar amount (10.68 mg/kg/d) of CBDDCA inhibited the toxicity of CBDDCA only slightly; the difference was not significant.

Additionally, sodium malate had no effect on the antitumor effect of CBDDCA (Fig. 10).

DISCUSSION

CDDP has excellent effects on testicular tumors, urological cancers (bladder cancer, prostatic cancer), gynecological cancers (ovarian cancer, uterus cancer) and squamous cell carcinomas such as head and neck cancer and esophageal carcinomas, but it also has severe side effects including renal disorders, bone marrow suppression (leukopenia, thrombocytopenia, anemia), gastrointestinal disorders (severe nausea, vomiting, anorexia, diarrhea), auditory disorders (otalgia, hearing impairment), and general malaise, limiting its use. Currently, forced diuresis by sufficient water supply and administration of diuretics (furosemide, mannitol), in an attempt to counter the nephrotoxicity, while nausea and vomiting are treated with...
serotonin blockers. The effectiveness of these measures is not yet adequate, calling for the development of effective agents to inhibit CDDP-induced toxic reactions. We found that the Chinese medicine, JuZen-taiho-to, reduces the toxicity of CDDP well, and showed that one of the active ingredients of JuZen-taiho-to may be sodium malate. In this study, we found that treatment with sodium malate at a dose 1/4 times the equimolar amount (0.445 mg/kg) of CDDP significantly, and treatment with sodium malate at a dose twice the equimolar amount (3.56 mg/kg) almost completely, inhibited the CDDP-induced nephrotoxicity (Fig. 1). Histopathological examination of the kidneys showed almost no renal disorders, including the edema, tubular degeneration/necrosis, tubular dilation/cast formation observed after administration of CDDP alone, after treatment with sodium malate at a dose twice the equimolar amount (3.56 mg/kg). This coincides roughly with the findings in the untreated group.

Regarding the bone marrow disorders, the antitoxic effect of an equimolar dose of sodium malate was weak, although significant effects were observed at higher than the equimolar dose (1.78 mg/ml) of CDDP. Nevertheless, a complete reduction to the control level was not achieved, even after treatment with sodium malate at a dose twice the equimolar amount of CDDP, providing complete inhibition of nephrotoxicity (Fig. 2). However, histopathological examinations of bone marrow cells, spleen and thymus did not reveal any of the disorders observed in the CDDP group, such as atrophy and the suppression of hemopoiesis.

Regarding hepatotoxicity, body weight, food intake and relative stomach weight, similarly to the nephrotoxicity, the related toxicity was reduced to the level of the untreated group by combined treatment with sodium malate at a dose twice the equimolar amount of CDDP. Histopathological examination also showed that these CDDP-induced disorders were improved almost completely to the control level. Thus, although sodium malate effectively inhibited such CDDP-induced toxicities such as nephrotoxicity, hepatotoxicity and a decrease in body weight, its inhibition of bone marrow toxicity was poor.

CDBCA is a platinum derivative obtained by replacing 2 chlorine ligands of CDDP with 1,1-cyclobutane-dicarboxylate to reduce nephrotoxicity. We therefore examined whether sodium malate also inhibits the toxicity of carboplatin. The results showed that sodium malate had no influence on any of the CDBCA-induced disorders such as bone marrow toxicity (Fig. 5). Although many mechanisms of action of CDBCA are still uncertain, it is considered that CDBCA may conceivably develop its antitumor effect through the DNA-binding form, which is almost identical with that of CDDP. Unlike CDDP, in CDBCA, the ligand 1,1-cyclobutane-dicarboxylate is probably replaced directly
by the N-7 position of the guanine residue of DNA. For CBDDCA, it is known that the active intermediate (diaquo form), which is formed by the replacement of 1,1-cyclobutanedicarboxylate with OH\(^-\) ions or H\(_2\)O, is almost never formed in blood or tissue. Because the ligand of CBDDCA, that is, 1,1-cyclobutanedicarboxylate, and sodium malate are probably both dicarboxylic acids with similar ligand forming properties, there is a low possibility that both ligands are replaced in the body. Thus, sodium malate could not inhibit the toxicity of CBDDCA.

Furthermore, we found that sodium malate does not inhibit CDDP-induced bone marrow disorders, suggesting that the mechanism of the onset of CDDP-induced toxicity could differ between kidney and bone marrow, and that although both toxicity forms are based on the same mechanism, the distribution of sodium malate in the tissue might be different between both organs. Combining these findings, we are presently investigating the mechanism of action of sodium malate on CDDP-induced toxicity.

Sodium malate is an active constituent isolated from Juzen-taiho-to by monitoring the nephrotoxicity induced by CDDP. Juzen-taiho-to almost completely inhibited most of the CDDP-induced toxicity, including nephrotoxicity and bone marrow toxicity, at a dose 10 times or more of the usual clinically used dose.\(^{26,27}\) However, the pattern of inhibition of toxicity by sodium malate is different for each toxicity form. Sodium malate was effective against nephrotoxicity and heptatoxicity but ineffective against bone marrow toxicity. Based on the results of this study, the inhibition of CDDP-induced toxicity by Juzen-taiho-to is apparently not attributable to a single substance. This suggests that the substance responsible for the inhibition of nephrotoxicity and heptatoxicity is sodium malate, but that bone marrow toxicity may be inhibited by a component different from sodium malate. Reportedly, Juzen-taiho-to activates the immune\(^{42-45}\) and hematopoietic systems\(^{46,47}\), so the responsible components could be polymers.\(^{48}\) It was therefore assumed that these polymer components in Juzen-taiho-to may also be involved in the inhibition of CDDP-induced bone marrow toxicity.

In the present study, it was found that, regardless of the route of administration, treatment with sodium malate inhibited nephrotoxicity and heptatoxicity without affecting its antitumor effect at a dose twice the equimolar amount of CDDP. The kinetics and the mechanism of action of sodium malate should be examined in the future; however, from this study, it was suggested that sodium malate can be a useful inhibitor of CDDP-induced toxicity, particularly nephrotoxicity and heptatoxicity.

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