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# Oral Sustained-release Cisplatin Preparation for Rats and Mice

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## Abstract

A new oral sustained-release solid-dispersion preparation of cisplatin (*cis*-diamminedichloroplatinum(II); cisplatin) has been developed for administration to small experimental animals such as mice. This preparation was obtained by formulating cisplatin with the water-insoluble polymer ethylcellulose and with stearic acid in different ratios.

In-vitro dissolution studies showed that cisplatin release characteristics were zero-order for the formulation cisplatin–ethylcellulose–stearic acid (1 : 10 : 5) and levels equilibrated 7 h after the start of the experiment. The availability of cisplatin from this preparation was evaluated both in rats and mice. The cisplatin preparation (20 mg $kg^{-1}$) was administered orally to rats and the resulting curve of serum cisplatin levels against time was compared with that obtained after intravenous infusion (20 mg $kg^{-1}$) to rats. By comparing the areas under serum concentration–time curves (AUCs), the bioavailability of cisplatin was estimated to be 31%. The mean residence time (MRT) of cisplatin solid dispersion was $6{\cdot}13 \pm 0{\cdot}43$ h, whereas the MRT of cisplatin administered by intravenous infusion was $3{\cdot}89 \pm 0{\cdot}05$ h. Serum cisplatin levels were maintained above 0·3 mg $mL^{-1}$ (believed from our clinical studies to be the minimum effective concentration) for 24 h. The curve of serum cisplatin level against time suggested that cisplatin was released from the solid dispersion preparation in a sustained-release fashion. Similar levels were also maintained in mice for 24 h. The MRT of the cisplatin preparation was 10·16 h in mice, which is longer than that obtained after oral administration of the physical mixture. The serum free-cisplatin concentration was determined to be 0·10 mg $mL^{-1}$ in mice serum in which the total cisplatin concentration was 0·30 mg $mL^{-1}$. The free fraction of cisplatin in mice serum was the same as that in human patient serum. Pathological examination showed that this new sustained-release oral cisplatin preparation did not have any side effects on the gastrointestinal tract.

These results suggest usefulness of this new solid-dispersion preparation for oral cisplatin therapy in lung cancer patients.

Cisplatin is an antineoplastic agent developed in 1965 (Rosenberg et al) which has been used for the therapy of ovarian, lung, bladder, breast, head and neck, and testicular cancers (Loehrer & Einhorn 1984; Muggia 1991). Its clinical use is, however, limited by severe toxic effects which include renal failure (Pinzani 1994). Acute and cumulative renal toxicity associated with histological damage has been shown in studies both on animals and on man (Offerman et al 1984; Daugaard et al 1986). Several theories about the pathophysiological mechanism underlying this toxicity have been suggested (Los et al 1991). As with its therapeutic effects, toxicity seems to be proportional to the cisplatin dose delivered (Egorin et al 1984; Marina et al 1993). As an approach to solving problems of cisplatin toxicity, continuous intravenous infusion therapy with a low dose of cisplatin was attempted by our group and good clinical effects were obtained (Ike et al 1996). On the basis of these clinical results, we attempted to prepare an oral sustained-release cisplatin preparation using a microporous capsule made from ethylcellulose containing Carbopol as a gel-forming material which retains cisplatin within the capsule (Houjou et al 1996). After oral administration of this capsule to rabbits (10 mg $kg^{-1}$), serum cisplatin levels were maintained above 0·4 mg $mL^{-1}$ for 24 h. In addition, pathological examination revealed no toxicity to the kidneys or gastrointestinal tract. As a next step, the pharmacological efficiency of the oral sustained-release cisplatin preparation must be evaluated with an experimental therapeutic study. Tumour-loaded mouse systems have been widely used for this purpose (Ike et al 1991; Sugiyama et al 1995). Our capsule is, however, too large for administration to mice. As an alternative oral sustained-release preparation for mice, a solid-dispersion system was judged most appropriate. Cisplatin, ethylcellulose and stearic acid were, therefore, used to prepare an oral sustained-release cisplatin preparation. Although polymers such as Eudragit, which have been used by others as a pharmaceutical additive (Phuapradit et al 1995) were also considered, ethylcellulose was thought to be the best candidate for a sustained-release preparation of cisplatin, because ethylcellulose is a water-insoluble polymer and cisplatin is an extremely water-soluble low-molecular-weight compound. Ethylcellulose has also long been used as a pharmaceutical additive in many countries. An oral sustained-release cisplatin preparation was, therefore, prepared using ethylcellulose and in-vitro and in-vivo evaluation studies were performed using rats and mice.

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## Materials and Methods

Commercially available lyophilized cisplatin was obtained from Nippon Kayaku (Tokyo, Japan); cisplatin (50 mg) was dissolved in saline (100 mL). Ethylcellulose (7G grade) was obtained from Shin-etsu Chemical Industry (Tokyo, Japan). Stearic acid and triton X-100 were obtained from Nacalai

Tesque (Kyoto, Japan). Carmellose sodium was obtained from Wako Pure Chemical Industries (Osaka, Japan). Male rats and male ddY mice used in the study were obtained from Nippon SLC (Hamamatsu, Japan). All other materials were commercial products of reagent grade.

*Solid-dispersion preparation*

olid dispersions of three different weight ratios (cisplatin-ethylcellulose-stearic acid 1 : 10 : 10, 1 : 10 : 5 and 1 : 10 : 2) were prepared according to the method reported elsewhere (Takada et al 1989). As an example, the solid dispersion of 1 : 10 : 5 cisplatin, ethylcellulose and stearic acid was prepared by dissolving ethylcellulose (7G grade; 100 mg) in ethanol (2 mL) by adding very slowly with constant magnetic stirring. After complete dissolution of the ethylcellulose, cisplatin (10 mg) and stearic acid (50 mg) were added to the polymer solution and stirring continued until the drug had dissolved completely. After complete dissolution of cisplatin and stearic acid the mixture solution was cast on to a teflon plate (2·0 × 2·0 cm). The film (solid dispersion) was obtained by drying in a desiccator overnight. The next day, the film was ground in a mortar to afford finely powdered solid dispersion. Particle diameter was estimated as 200 $\mu$m by use of a micrometer with a microscope. The 1 : 10 : 10 and 1 : 10 : 2 solid dispersions were prepared in the same manner.

*Physical mixture preparation*

Ethylcellulose (7G grade; 100 mg) cisplatin (10 mg) and stearic acid (50 mg) were blended by trituration in a mortar.

*Dissolution study of the solid-dispersion preparations*

Dissolution tests on the sustained-release cisplatin-loaded solid dispersions were performed on a reduced scale. The test solid dispersion (16 mg containing 1 mg cisplatin), contained in a bag made of tissue paper (1·0 × 1·0 cm), and JP #13 1st fluid (pH 1·2; 5 mL) were introduced into a 20-mL vial containing a 104-mm stirring paddle; the rotation speed was 250 rev. $min^{-1}$ as described in our previous report (Houjou et al 1996). The dissolution medium was degassed by sonication at room temperature and maintained at 37°C throughout the test period. To simulate transit from gastric to intestinal pH, samples of 1st fluid were replaced after 1 h by JP 2nd fluid (pH 6·8). To determine the amount of cisplatin released from the test solid dispersion, samples (0·5 mL) of the dissolution medium were removed for analysis every hour and replaced with fresh dissolution medium.

*Drug assay*

The platinum content of the samples obtained in the dissolution experiment was determined by atomic absorption spectrophotometry with a Shimadzu (Kyoto, Japan) AA-640-12 atomic absorption spectrometer with a GFA-4A graphite furnace atomizer (wavelength, 265·9 nm; spectral width, 3·8 nm; lamp current, 12 mA). The furnace temperature programme consisted in drying at 200°C for 45 s, ashing at 900°C for 30 s, atomizing at 2700°C for 4 s and cooling for 30 s. Platinum concentrations were linearly related to absorbance in the range 0·1 to 50·0 mg $mL^{-1}$. Samples were quantified by comparison with authentic standards.

*In-vivo animal studies*

Five rats, 250 ± 10 g, were fasted overnight for at least 12 h. 30 min before drug administration blood samples (0·2 mL) were removed from the jugular vein after incision by direct puncture with a syringe fitted with a 26-gauge needle. For intravenous (i.v.) infusion of cisplatin, a disposable catheter (1·9 mm o.d.) was inserted into the femoral vein. The cisplatin solution (0·5 mg $mL^{-1}$) was infused for 2 h by use of an SP220i variable-speed infusion syringe-pump (World Precision Instruments, Florida, USA). A total of 10 mg $kg^{-1}$ of cisplatin was administered to each rat. Samples of blood (0·2 mL) were collected from the right jugular vein 1, 2, 2·5, 3, 5, 9 and 12 h after the start of infusion.

The cisplatin-loaded solid dispersion was orally administered to another group of five rats at a dose of 10 mg $kg^{-1}$. Before drug administration, 0·2-mL blood samples (blanks) were obtained from the right jugular vein. For administration of cisplatin-loaded solid dispersion the test solid dispersion (40 mg) was suspended in carmellose sodium solution (1·0% w/v; 3·0 mL). A stainless-steel zonde (0·9 mm i.d., 1·0 mm o.d., 8 cm length) was introduced into the stomach of the rat and the suspension of the solid dispersion (3·0 mL) was administered. Samples of blood (0·2 mL) were collected from the jugular vein 1, 3, 6, 10, 12 and 24 h after drug administration. Serum was obtained by centrifugation of blood samples at 3000 rev. $min^{-1}$ for 30 min. Serum samples were immediately frozen in a deep freezer at − 80°C until analysis. Serum cisplatin concentrations were measured by atomic absorption spectrophotometry as described above. Thawed serum samples were diluted with double-distilled deionized water containing triton X-100 (1·0% v/v). Standard serum cisplatin samples were prepared by adding known amounts of cisplatin to blank serum. The calibration was linear over the range 0·1–2·0 mg $mL^{-1}$.

Studies on mice were performed to compare the pharmacokinetics of cisplatin with those obtained in rats, because tumour-bearing mice will be used for experimental therapeutic studies. Twenty mice, 305 g, were fasted overnight for at least 12 h. Each group consisted of five mice. Groups 1, 2 and 3 received 4·8 mg, 9·6 mg and 14·4 mg cisplatin-loaded solid dispersion, respectively. Group 4 mice received 14·4 mg of the physical mixture. Suspensions were prepared in carmellose sodium solution (1·0% w/v; 0·8 mL). The cisplatin doses administered were 10 mg $kg^{-1}$ for group 1, 20 mg $kg^{-1}$ for group 2 and 30 mg $kg^{-1}$ for group 3. The cisplatin dose for group 4 was 30 mg $kg^{-1}$. A stainless-steele zonde (0·9 mm i.d., 1·0 mm o.d., 8 cm length) was introduced into the stomach of the mice and the suspension of the solid dispersion or the physical mixture (0·8 mL) was administered. Blood was collected from the abdominal aorta by direct puncture, under ether anaesthesia, with a syringe fitted with a 25-gauge needle; samples were taken 1, 3, 6, 9, 12 and 24 h after drug administration. An additional control group consisting of 5 mice was also used. Serum samples from controls were obtained in the same manner as from the experimental groups. Serum was obtained by centrifuging the blood samples, and serum cisplatin concentrations were measured by atomic absorption spectrophotometry as described above.

*Data analysis*

A non-compartmental pharmacokinetic analysis was applied to the data. The terminal elimination rate constant, $K_e$, was determined by linear regression of at least three data points from the terminal portion of the serum concentration–time plots using a non-compartmental pharmacokinetic analysis program, Harmony (Yoshikawa et al 1995). The area under the serum con-

centration-time curve after administration, AUC, was calculated using the linear trapezoidal rule up to the last measured serum concentration. The area under the first-moment curve after administration, AUMC, was also calculated using the linear trapezoidal rule up to the last measured serum concentration. The terminal elimination half-life, $t_{\frac{1}{2}}$, was determined by dividing ln 2 by $K_e$. The mean residence time, MRT, was calculated as AUMC/AUC.

*Statistics*

All values are expressed as means ± s.e.m. Statistical differences were assumed to be reproducible when $P < 0.05$ (two-sided *t*-test).

## Results

*In-vitro dissolution study*

The dissolution profiles of cisplatin from the solid-dispersion preparations of cisplatin, ethylcellulose and stearic acid in the ratios 1 : 10 : 10, 1 : 10 : 5 and 1 : 10 : 2 are shown in Fig. 1, where the amount of cisplatin dissolved is represented as the percentage of the amount of cisplatin used for the dissolution experiment. The dissolution profile of the solid dispersion cisplatin–ethylcellulose–stearic acid, 1 : 10 : 5, showed zero-order release characteristics for cisplatin. Equilibration occurred 7 h after the dissolution study was started; thereafter, almost all the cisplatin was released. The percentage dissolution of the other two solid-dispersion preparations were, on the other hand, below 100% after 24 h, even though the formulated amounts of stearic acid were increased in one and reduced in the other. Approximately 85% cisplatin was released from the 1 : 10 : 10 preparation and 60% from the 1 : 10 : 2 preparation. The amount (%) of cisplatin released from these two solid dispersions was lower than that from the 1 : 10 : 5 preparation and the rate of release of cisplatin from the 1 : 10 : 10 preparation was slow during the first 2 h. The 1 : 10 : 5 solid dispersion was, therefore, selected for in-vivo experiments.

*In-vivo studies*

To study the effect of the formulation on the bioavailability of cisplatin, cisplatin-loaded solid dispersion (cisplatin–ethylcellulose–stearic acid 1 : 10 : 5) and the physical mixture of cisplatin, ethylcellulose and stearic acid (1 : 10 : 5) were orally administered to rats. The cisplatin dose was 10 mg $kg^{-1}$. As a reference, cisplatin solution was administered to five rats by intravenous infusion (10 mg $kg^{-1}$) for 2 h. The profile of mean serum cisplatin concentration against time (Fig. 2) shows that the peak level (2·84 ± 0·82 mg $mL^{-1}$) was obtained at the end of the infusion period. Thereafter, serum cisplatin levels declined rapidly with a terminal elimination half-life of 10·0 ± 25·78 h. To estimate the absolute bioavailability of cisplatin, cisplatin-loaded solid dispersion was orally administered (10 mg $kg^{-1}$) to another five rats. The resulting profile of mean serum cisplatin concentration against time is also shown in Fig. 2. The mean peak level (0·46 ± 0·03 mg $mL^{-1}$) appeared 6 h after administration and serum cisplatin levels of approximately 0·3 mg $mL^{-1}$ were maintained for 12 h. Comparison of these profiles of serum cisplatin levels against time, suggests that cisplatin is released from the solid-dispersion preparation in a sustained-release fashion. The pharmacokinetic parameter values of cisplatin obtained in rats after two different administration modes were calculated according to a non-compartmental analysis method. The results are shown in Table 1. The area under the curve of serum concentration against time curve (AUC) obtained after intravenous infusion of 2·5 mg cisplatin was 11·7 ± 2·62 mg h $mL^{-1}$, and that obtained after oral administration of the same amount of cisplatin-loaded solid dispersion was 3·67 ± 0·41 mg h $mL^{-1}$. By using the mean AUC values, the mean absolute bioavailability of cisplatin from the solid dispersion was estimated to be 31·4%. The mean residence times (MRT) after intravenous infusion and after oral administration of the solid dispersion were 3·89 ± 0·05 h and 6·13 ± 0·43 h, respectively. The MRT obtained after oral administration of the cisplatin-loaded solid-dispersion preparation is longer than that after intravenous infusion. It is, therefore, also suggested that the oral preparation has sustained-release characteristics. The mean terminal elimination half-life of cisplatin from the solid-dispersion preparation was 60·6 ± 11·2 h. As this value is longer than that obtained after intravenous infusion in rats, flip-flop phenomena are thought to occur after oral administration of the solid-dispersion preparation.

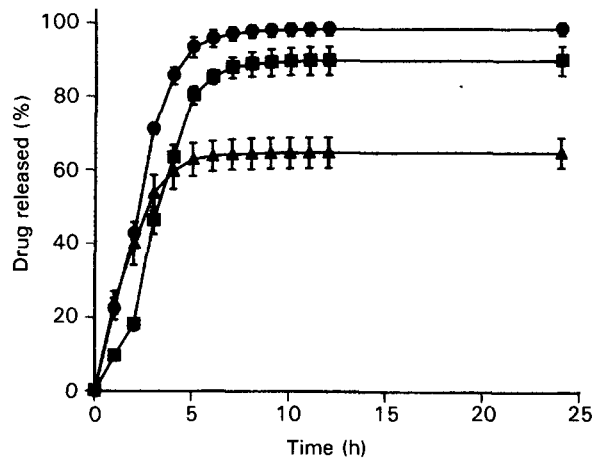


FIG. 1. Dissolution profiles of cisplatin from solid dispersions prepared from different weight ratios of cisplatin, ethylcellulose and stearic acid: ■, 1 : 10 : 10; ●, 1 : 10 : 5; ▲, 1 : 10 : 2. Each point represents the mean ± s.e.m. from three experiments.

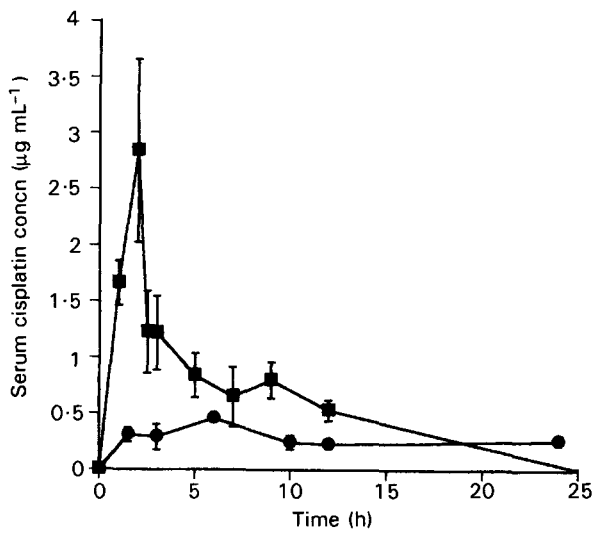


FIG. 2. Plots of serum cisplatin level against time during and after intravenous infusion (10 mg $kg^{-1}$, ■) and after oral administration (10 mg $kg^{-1}$, ●) to rats. Each point represents the mean ± s.e.m. from five experiments.

Table 1. Pharmacokinetic parameters of cisplatin after intravenous infusion or oral administration (10 mg $kg^{-1}$) to rats.

| | Intravenous infusion (solution) | Oral administration (solid dispersion) |
|---|---|---|
| Maximum concentration ($\mu g\ mL^{-1}$) | 2·84 ± 0·82 | 0·46 ± 0·03 |
| Time to reach maximum concentration (h) | 2·00 | 6·00 |
| Area under serum concentration–time curve ($\mu g\ h\ mL^{-1}$) | 11·7 ± 2·62 | 3·67 ± 0·41 |
| Bioavailability (%) | 100 | 31·4 |
| Mean residence time (h) | 3·89 ± 0·05 | 6·13 ± 0·43 |
| Half-life (h) | 10·0 ± 5·78 | 60·6 ± 11·2 |

Each value represents the mean ± s.e.m. of results from five experiments.

Table 2. Pharmacokinetic parameters of cisplatin after oral administration (30 mg $kg^{-1}$) to mice.

| | Physical mixture | Solid dispersion |
|---|---|---|
| Maximum concentration ($\mu g\ mL^{-1}$) | 1·21 | 1·24 |
| Time to reach maximum concentration (h) | 1·00 | 1·00 |
| Area under serum concentration–time curve ($\mu g\ h\ mL^{-1}$) | 5·26 | 11·6 |
| Mean residence time (h) | 6·40 | 10·2 |
| Half-life (h) | 6·87 | 63·5 |

Each value represents the mean of results from five experiments.

The same study was performed using mice, for which the dose of cisplatin administered was 30 mg $kg^{-1}$. Fig. 3 shows the effect of the preparation on the profiles of mean serum cisplatin levels against time after oral administration in mice. The mean peak levels (1·21 ± 0·25 mg $mL^{-1}$ and 1·24 ± 0·16 mg $mL^{-1}$) appeared 1 h after oral administration both of physical mixture and of solid-dispersion preparations. The pharmacokinetic parameters of cisplatin are presented in Table 2. As shown in this table, the AUCs and MRTs of the physical mixture and solid dispersion were 5·26 mg h $mL^{-1}$ and 11·59 mg h $mL^{-1}$ and 6·40 h and 10·16 h, respectively. Because a longer mean $t_{\frac{1}{2}}$, 63·47 h, was obtained after oral administration of the cisplatin-loaded solid-dispersion preparation, flip-flop phenomena are thought to occur in mice as well as in rats. From these pharmacokinetic parameters it is, therefore, suggested that cisplatin was released from the solid-dispersion preparation in a sustained-release fashion.

Morphological studies were performed to determine the structural differences between the solid dispersion and the physical mixture. The results are shown in Fig. 4. In the physical mixture the three components exist in their authentic forms and each component has a small diameter of about 10–50 $\mu m$. In the solid dispersion, however, the particle sizes were larger, with diameters of approximately 200 $\mu m$.

The next study was of the effect, in mice, of cisplatin dose (10 mg $kg^{-1}$, 20 mg $kg^{-1}$ and 30 mg $kg^{-1}$) on the profiles of mean serum cisplatin levels against time after oral administration of the solid-dispersion preparation cisplatin–ethylcellulose–stearic acid 1 : 10 : 5. The results are shown in Fig. 5. The mean peak cisplatin levels (0·43 ± 0·09 mg $mL^{-1}$, 0·99 ± 0·21 mg $mL^{-1}$ and 1·24 ± 0·16 mg $mL^{-1}$) appeared 1 h after administration of three different doses of cisplatin solid-dispersion preparations (10 mg $kg^{-1}$, 20 mg $kg^{-1}$ and 30 mg $kg^{-1}$). Table 3 shows the effect of dose (10 mg $kg^{-1}$, 20 mg $kg^{-1}$ and 30 mg $kg^{-1}$) on the pharmacokinetic parameters of cisplatin after oral administration to mice. Because of the small size of mice, it was impossible to obtain consecutive blood samples after drug administration and so only one cisplatin serum concentration was obtained from each animal. Pharmacokinetic analysis was performed using the mean serum cisplatin concentration at each AUC values were 6·81 mg h $mL^{-1}$, 8·94 mg h $mL^{-1}$ and 11·59 mg h $mL^{-1}$ for doses of 10 mg $kg^{-1}$, 20 mg $kg^{-1}$ and 30 mg $kg^{-1}$, respectively, and each MRT value was approximately 10 h.

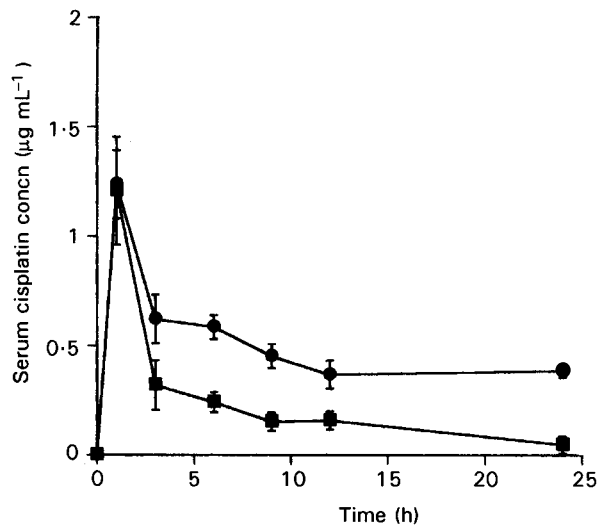


FIG. 3. Effect of formulation (■, physical mixture; ●, solid dispersion) on profiles of serum cisplatin level against time after oral administration, 30 mg $kg^{-1}$, to mice. Each point represents the mean ± s.e.m. from five experiments.

## Discussion

Cisplatin is a valuable and potent antitumour drug with demonstrated clinical activity in the treatment of a broad spectrum of solid tumours, including small-cell lung cancer (Loehrer & Einhorn 1984). Combination chemotherapy with cisplatin and vinblastine has recently been shown to have a synergistic effect in clinical studies (Nakano et al 1996). In that study cisplatin was administered by a parenteral route; from the stand point of

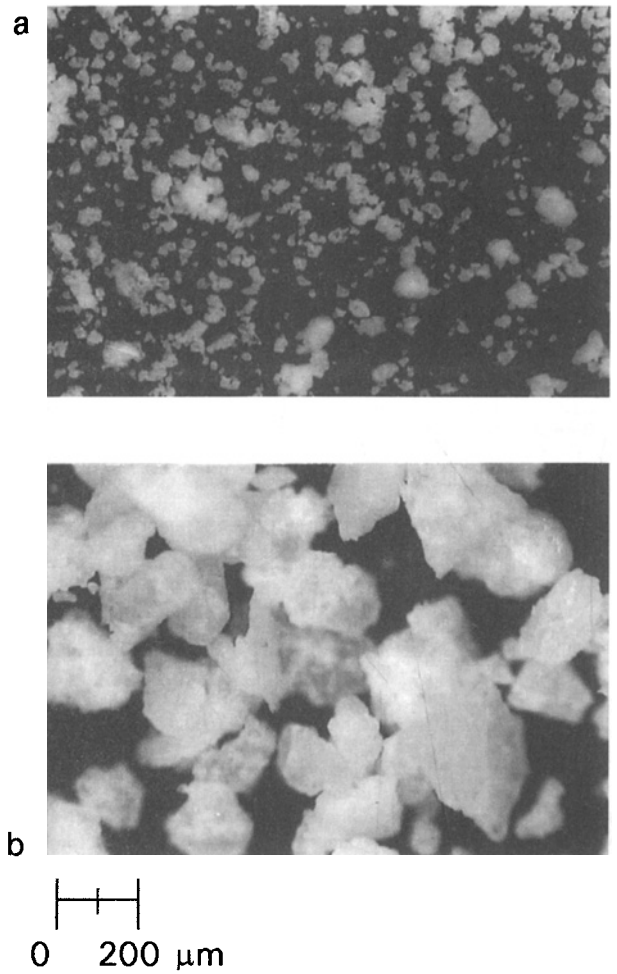


FIG. 4. Photomicrographs of the test cisplatin preparations: a, physical mixture (cisplatin–ethylcellulose–stearic acid 1 : 10 : 5); b, solid dispersion (cisplatin–ethylcellulose–stearic acid 1 : 10 : 5).

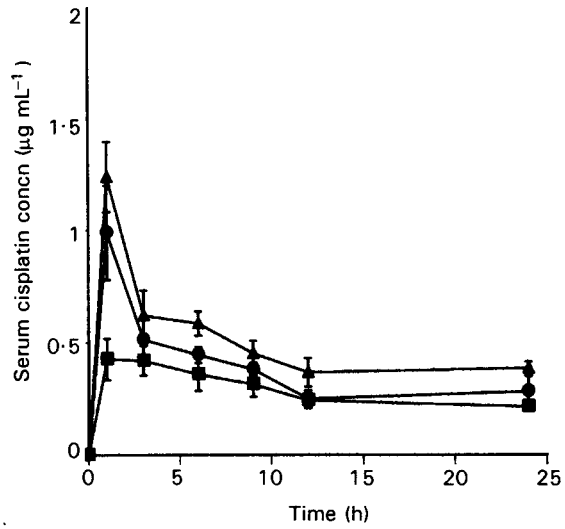


FIG. 5. Effect of dose (■, 10 mg kg$^{-1}$; ●, 20 mg kg$^{-1}$; ▲, 30 mg kg$^{-1}$) on profile of serum cisplatin level against time after oral administration to mice. Each point represents the mean ± s.e.m. from five experiments.

clinical therapy, however, non-parenteral and especially oral therapy is preferable because the quality of life of the cancer patients will be increased. Previously, a sustained-release microporous capsule of cisplatin was prepared and evaluated (Houjou et al 1996). In that study, the bioavailability of cisplatin was measured to be about 15% after oral administration to rabbits (20 mg rabbit$^{-1}$). In the study presented here, however, a higher mean bioavailability value (31·42%) was obtained in rats. Dissolution studies were performed with the microporous cisplatin capsule, certainly almost 100% of the formulated amount of cisplatin was released within 24 h, although these in-vitro dissolution studies were performed in the presence of more test fluid than is likely present in-vivo. In-vivo the water content of the large intestinal cavity is considerably lower than that found in the small intestine, because water is re-absorbed in the large intestine. After transfer of the microporous cisplatin capsule into the large intestine, therefore, the release rate of cisplatin is likely to be considerably reduced. This might account for the lower bioavailability (15%) obtained in that study. In the studies presented here a microparticulate cisplatin solid dispersion was used. Because the mean diameter of the solid-dispersion preparation was 200 μm, the dissolution of cisplatin is thought to occur even though the water content in the environment is low. The bioavailability of cisplatin from the solid-dispersion preparation was not determined in mice because such studies would require the use of too many animals. Comparison of the profiles of serum cisplatin concentration against time for both rats and mice, however (Figs 2 and 4) suggests that the bioavailability of cisplatin in mice is of the same order as that determined in rats.

According to the clinical results of our study with lung cancer patients, the minimum effective concentration of cisplatin is approximately 0·3 mg mL$^{-1}$ (Ike et al 1996). As reported by van der Vijgh (1986), Tosetti (1988) and Perera (1992), cisplatin is highly bound to proteins in serum and it has been pointed out that the unbound cisplatin is effective against tumour cells (Guchelaar et al 1994). In this study, therefore, the concentration of unbound cisplatin in mice serum samples was measured by a

Table 3. Effects of dose on pharmacokinetic parameters of cisplatin after oral administration of solid dispersion to mice.

| | Dose | | |
|---|---|---|---|
| | 10 mg kg$^{-1}$ | 20 mg kg$^{-1}$ | 30 mg kg$^{-1}$ |
| Maximum concentration (μg mL$^{-1}$) | 0·43 | 0·99 | 1·24 |
| Time to reach maximum concentration (h) | 1·00 | 1·00 | 1·00 |
| Area under serum concentration–time curve (μg h mL$^{-1}$) | 6·81 | 8·94 | 11·6 |
| Mean residence time (h) | 10·5 | 9·87 | 10·2 |
| Half-life (h) | 64·4 | 51·8 | 63·5 |

Each value represents the mean of results from five experiments.

centrifugation method using a centrifuging filtration device (Amicon, Centrifree micropartition devices, No. 4104). The unbound cisplatin concentration was measured to be 0·10 mg $mL^{-1}$ when the total serum cisplatin concentration was 0·30 mg $mL^{-1}$. By dividing the unbound cisplatin concentration by the total concentration, the unbound fraction of cisplatin in serum was estimated to be 0·33. Comparing the value of free fraction (0·30) reported in the study in man (Ike et al 1996), there is no significant difference between the two species. Our new oral sustained-release cisplatin solid-dispersion system is, therefore, thought to be effective in mice, although experimental animal study must be performed.

The safety of the cisplatin solid-dispersion system was also considered in these studies. A water-insoluble microporous capsule was initially used in our cisplatin studies to avoid direct contact of cisplatin with the intestinal tract as such direct contact with cisplatin could promote side-effects, particularly necrosis. In addition to gastrointestinal necrosis, nephrotoxicity, emesis and neurotoxicity are reported as side effects of cisplatin (Comis 1994). Pathological examinations were performed to confirm the safety of the oral cisplatin solid-dispersion system. The entire gastrointestinal tracts were removed from the rats and mice at the end of the experiments and were stained with haematoxyline-eosin after fixation in formalin. Pathological examinations were performed in the laboratory of the Department of Thoracic Surgery, Chest Disease Research Institute of Kyoto University. We did not detect any changes in the gastrointestinal preparations from the stomach to the anus in either rats or mice. In this study, therefore, there were no observable side effects, particularly in the gastrointestinal tract. The body weights of the animals were recorded for 2 weeks after the administration of cisplatin preparations and no weight loss was observed in either rats or mice. These results support the safety of our new oral sustained-release cisplatin solid-dispersion preparation. The superiority of the solid-dispersion system as compared with the microporous capsule lies in its applicability to small animals. Experimental therapy with anti-tumour drugs is performed with mice, because most of the proliferated tumour cells are confirmed to survive in this animal. As we are planning to perform a therapeutic experiment with mice infected with several kinds of tumour cell (P815, M5076 and others), these results provide useful information necessary for performing these experimental therapeutic studies in mice.

In conclusion, a new oral sustained-release cisplatin solid-dispersion system was prepared and both in-vitro dissolution studies and in-vivo availability studies were performed. After the oral administration of a cisplatin solid-dispersion preparation comprising cisplatin–ethylcellulose–stearic acid, 1 : 10 : 5, with a cisplatin dose of 10 mg $kg^{-1}$, serum cisplatin concentrations were maintained at levels greater than 0·30 mg $mL^{-1}$ for 24 h in mice. As the unbound cisplatin fraction in mouse serum was not significantly different that in serum samples from man, this new oral sustained-release cisplatin preparation will contribute to the chemotherapy of lung-cancer patients.

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