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Cisplatin microcrystals suspended in oil—toxicity in mice

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A new dosage format, cisplatin microcrystals suspended in oil (CDDP-oil), was developed for the treatment of peritoneal carcinomatoses. We studied the acute toxicity of CDDP-oil injected intraperitoneally in mice. The 50% lethal dose was 30.3 mg/kg (27.1–33.7 mg/kg at the 95% confidence level), which was 1.79 times that of a cisplatin aqueous solution (CDDP-sol) of 16.9 mg/kg (16.1–17.8 mg/kg at the 95% confidence level). There were no significant differences in the duration of the toxic effects and the toxic symptoms between these two dosage forms. However, the severity of weight loss in the group given CDDP-oil was less than the group given CDDP-sol.

Key words: Cisplatin, intraperitoneal chemotherapy, oil suspension, toxicity.

Introduction

Intraperitoneal chemotherapy for peritoneal carcinomatosis cannot be fully achieved by the use of drugs in aqueous solution because small water-soluble molecules, such as commonly-used anticancer drugs, are rapidly absorbed into the blood stream through the blood capillary walls when injected intraperitoneally.[1] To overcome this difficulty, we have developed a new useful dosage format for cisplatin, which is a widely used anticancer drug. This format consists of cisplatin microcrystals suspended in oil (CDDP-oil). This dosage format is retained in the peritoneal cavity for a longer period and is gradually absorbed through the lymph capillary walls into the regional lymphatic system rather than into the blood stream.[1] Therefore, cisplatin is released over a longer period at the site where the oil-containing format is retained and is delivered into the blood stream at a lower concentration. These characteristics led us to postulate that CDDP-oil would be useful for intraperitoneal chemotherapy and ought to reduce the toxicity of cisplatin when compared with a cisplatin aqueous solution. Thus, we examined the toxicity of CDDP-oil in mice and report the reduced toxicity of this new dosage format in this paper.

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Materials and methods

Preparation of dosage forms

The anticancer drug cisplatin, which is one of the platinium compounds discovered by Rosenberg,[2] was kindly donated by Nippon Kayaku Co. (Tokyo, Japan). The cisplatin microcrystals were less than 500 μm in length as determined by scanning electron microscopy and were very slightly soluble in oil according to our preliminary examination. The new dosage format was made from cisplatin microcrystals suspended in a 4:1 mixture of sesame oil (sesame oil®; Nakaraitesque Co., Kyoto, Japan) to lipiodol (Lipiodol Ultra Fluid®, Kodama Yakuhin Co., Tokyo, Japan). Cisplatin microcrystals were suspended in the oil mixture with a magnetic stirrer for 6 h under sterile conditions, and were prepared to yield the required concentrations of cisplatin suspension in oil. As a control, a cisplatin aqueous solution (Landa Inj.®, Nippon Kayaku Co.) was diluted with normal saline to give the concentrations required. Another control drug was the same oil mixture without cisplatin. All drugs were used within 1 h after their preparation.

Drug administration protocol

Two hundred and ten CDF1 male mice (4 weeks old, weighing 25 g on average) were purchased from the Shimizu Laboratory Animals Center (Kyoto, Japan). The mice were divided into 30 groups of seven mice each: 18 groups received CDDP-oil (CDDP-oil groups), 10 groups received CDDP-sol (CDDP-sol groups), one group received

the oil mixture without cisplatin (oil group) and the last group received nothing (control group). The mice were kept under standard conditions (specific pathogen free, room temperature 22°C, relative humidity 60%, day–night cycle 12 h, fed on standard mouse bait and tap water freely) for 7 days before the drug administration until 21 days after the administration.

On 0 day, when the mice averaged 25 g, the drugs were given intraperitoneally using a 20 gauge needle. In the 18 groups receiving CDDP-oil, doses from 13.4 to 70.7 mg cisplatin/kg body weight were given in 18 dose increments, increasing at a ratio of 1.1 per step. In the 10 groups receiving CDDP-sol, doses from 12.6 to 22.6 mg cisplatin/kg body weight were given in 10 dose increments, increasing at a ratio of 1.067 per step. The oil group received 1 ml of the oil mixture.

The mice were observed daily for 21 days after the administration, and the day of death, body weight change and toxic symptoms of each dosage were recorded. The surviving animals were sacrificed on day 22. The 50% lethal dose value (LD_{50}) was calculated using probit analysis for each dosage format.

Results

LD_{50} value

The LD_{50} value for CDDP-oil was 30.3 mg/kg in terms of cisplatin (27.1–33.7 at the 95% confidence level). The LD_{50} value for CDDP-sol was 16.9 mg/kg (16.1–17.8 at the 95% confidence level). The LD_{50} value for CDDP-oil was 1.79 times that of CDDP-sol. There were no deaths in the oil and control groups.

Toxic symptoms and body weight loss

The toxic symptoms in mice given CDDP-oil were similar to those in mice given CDDP-sol. Doses close to the LD_{50} value for either format brought about dishevelment, lethargy, weakness, diarrhea and/or bloody feces, eye lid discharge that was sometimes bloody, and paling of the ears. However, these symptoms improved 6–16 days after administration in the survivors. In the groups given CDDP-oil, almost all of the deaths were observed between day 1 and day 7, and only one mouse given a low dose (16.9 mg/kg) died on day 12 (Table 1). In the groups given CDDP-sol, almost all of the deaths were observed between day 5 and day 7, and only one mouse given a low dose (14.3 mg/kg) died on day 10 (Table 2).

Table 1. Mortality of mice given CDDP-oil

Dose of cisplatin (mg/kg)	Mortality	Day of death
13.4	0/7	—
15.4	0/7	—
16.9	1/7	12
18.6	1/7	5
20.5	2/7	5, 6
22.5	1/7	6
24.8	2/7	1, 2
27.3	2/7	2, 5
30.0	5/7	1, 4, 5, 7, 7
33.0	5/7	1, 1, 6, 6, 7
36.3	4/7	5, 5, 6, 6
39.9	6/7	1, 1, 4, 5, 5, 5
43.9	6/7	1, 1, 2, 5, 5, 6
48.3	6/7	1, 1, 1, 1, 2, 2
53.1	6/7	1, 1, 1, 2, 5, 6
58.5	7/7	1, 1, 1, 2, 2, 6, 7
64.3	7/7	1, 1, 2, 3, 4, 5, 7
70.7	7/7	1, 1, 4, 4, 5, 5, 5

Table 2. Mortality of mice given CDDP-sol

Dose of cisplatin (mg/kg)	Mortality	Day of death
12.6	0/7	—
13.4	0/7	—
14.3	1/7	10
15.3	0/7	—
16.3	6/7	5, 6, 6, 6, 7, 7
17.4	2/7	6, 6
18.6	5/7	5, 5, 5, 5, 7
19.8	6/7	5, 5, 5, 6, 7, 7
21.2	7/7	5, 6, 6, 6, 6, 6, 6
22.6	7/7	5, 5, 5, 6, 6, 6, 6

Figures 1–3 show the body weight changes of mice in each group. In mice given CDDP-oil at doses close to the LD_{50} value, the body weight reached its minimum (about 18–19 g) on day 5 and then began to increase from day 6–7. The body weight had fully recovered to its pre-administration level by day 16 (Figure 1). In mice given CDDP-sol at the doses close to the LD_{50} value, the body weight decreased to its minimum (about 16–17 g) at day 5. This level was lower than the minimum of the CDDP-oil group. The weight of the mice in the CDDP-sol group also began to increase on day

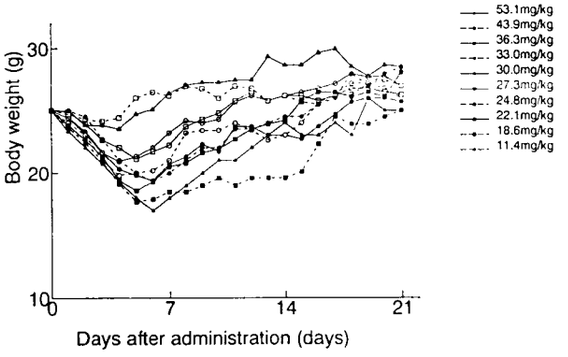

Figure 1. Body weight changes in mice given CDDP-oil (some groups were omitted). In mice given CDDP-oil at doses close to the LD_{50} value, the body weight reached its minimum (about 18–19 g) on day 5. The body weight then began to increase from 6–7 and recovered fully to the pre-administration level by day 16.

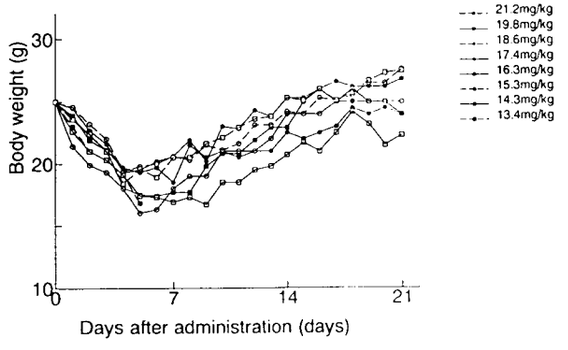

Figure 2. Body weight changes in mice given CDDP-sol. In mice given CDDP-sol at doses close to the LD_{50} value, the body weight decreased to its minimum (about 16–17 g) on day 5. This degree of weight loss was greater than that observed in the CDDP-oil group. The weight then began to increase on day 6 and was restored to its pre-administration level by day 16.

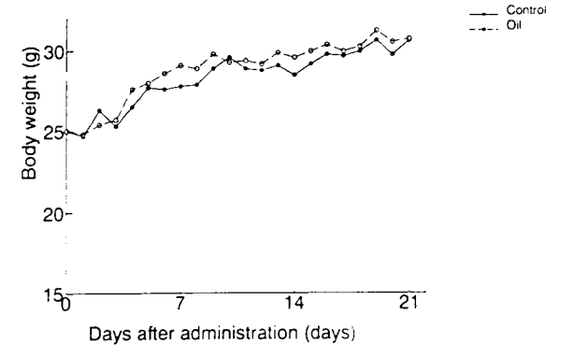

Figure 3. Body weight changes in mice given the oil mixture without cisplatin (oil group) or no treatment (control group). There were no significant differences in body weight change between the two groups.

6 and was restored to its pre-administration level by day 16 (Figure 2). In the oil and control groups, there were neither toxic symptoms nor any appreciable body weight losses (Figure 3).

Discussion

Effective anticancer chemotherapy requires that the anticancer drug be delivered selectively to the lesion at a sufficiently high concentration over a long period and be at a low concentration in other body regions. However, many anticancer drugs are composed of small water-soluble molecules, which readily diffuse through the blood capillary walls at the injection site into the general circulation.[1] Therefore, it is difficult to maintain a high local concentration over a long period in the peritoneal cavity, especially when the drugs are administered intraperitoneally in the form of aqueous solutions. On the other hand, oil is retained intraperitoneally and is usually absorbed through the lymph capillary walls into the regional lymphatic system.[1] Utilizing these properties, we developed a new dosage format consisting of cisplatin microcrystals suspended in oil. Our previous investigation revealed that intraperitoneally administered CDDP-oil maintained its cisplatin activity at higher levels over a longer period in the intraperitoneal tissues and delivered a lower concentration of cisplatin to the general circulation than CDDP-sol. These facts led us to hypothesize that this format of CDDP-oil reduces systemic cisplatin toxicity when injected intraperitoneally to treat peritoneal carcinomatosis.

In the present experiments, the lethal toxicity of CDDP-oil was observed to be 55.8% of the cisplatin aqueous solution toxicity. The LD_{50} value of the cisplatin solution reported here is similar to that in Schaeppi *et al.*'s report.[3] The severity of weight loss in mice given CDDP-oil was also less than the cisplatin aqueous solution; however, the weights of both groups recovered to their pre-administration levels at a similar date. Therefore, it would appear that the toxic effects of CDDP-oil were reduced but prolonged slightly as compared with CDDP-sol. This prolongation is likely to be caused by CDDP-oil being focally retained and slowly releasing cisplatin. Toxic symptoms were similar between the two cisplatin dosage formats and were similar to those in Kochiba and Sleight's reports[4] on cisplatin solution toxicity. The control oil mixture without cisplatin brought about no toxic effects.

From these facts, we concluded that the lethal toxicity of the CDDP-oil is 55.8% of the CDDP-sol. Although the toxicity effects were slightly prolonged, there was a reduction in their severity and no additional toxic symptoms were induced by the change of dosage format.

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