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Toxicity of a new dosage format, cisplatin incorporated in lactic acid oligomer microspheres, in mice

Akeo Hagiwara,[CA] Toshio Takahashi, Tsunetoshi Sasabe, Michitoshi Ito, Masaki Lee, Chouhei Sakakura, Satoshi Shobayashi, Shozo Muranishi and Shigeru Tashima

A Hagiwara, T Takahashi, T Sasabe, M Ito, M Lee, C Sakakura and S Shobayashi are at the First Department of Surgery, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji Kamigyo-ku, Kyoto 602, Japan. Fax: 075-211-7093. S Muranishi and S Tashima are at the Department of Biopharmaceutics, Kyoto College of Pharmacy, Kyoto 607, Japan.

A new drug delivery system, cisplatin incorporated into lactic acid oligomer microspheres (CDDP-MS), was developed for the treatment of peritoneal carcinomatoses. We studied the acute toxicity and pathological effects of CDDP-MS injected intraperitoneally in mice. The 50% lethal dose was 23.8 mg/kg (21.3–26.7 mg/kg at 95% level of confidence), which was 1.76 times that of the cisplatin aqueous solution of 13.5 mg/kg (11.9–15.3 mg/kg at 95% level of confidence). The duration for the restoration of the body weight loss was prolonged when CDDP-MS at doses close to the 50% lethal dose was administered, as compared with the cisplatin aqueous solution at doses close to its 50% lethal dose. On autopsy there were no macroscopic or microscopic differences between the two dosage forms.

Key words: Cisplatin, lactic acid oligomer microspheres, toxicity.

Introduction

A new drug delivery system, lactic acid oligomer microspheres incorporating cisplatin (CDDP-MS), was developed for intraperitoneal chemotherapy of peritoneal carcinomatoses. We have reported that, in rats, intraperitoneal CDDP-MS delivered a greater concentration of cisplatin selectively to the intraperitoneal tissues for a longer period, and lower levels of cisplatin were found in the blood plasma and in the extraperitoneal organs than were caused by conventional intraperitoneal cisplatin aqueous solution.[1] CDDP-MS also prolonged the survival time of mice with peritoneal carcinomatosis induced by M5076 ovarian cancer better than the same dose of cisplatin in the form of an aqueous solution.[2] This paper furthers this line of research and describes CDDP-MS's reduced toxicity in mice.

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[CA] Corresponding Author

Materials and methods

Preparation of drug

Poly-*d,l*-lactide, with an average molecular weight of 9600 as measured by gel-permeation chromatography using standard polystyrenes,[3] was supplied by The Research Center for Medical Polymers and Biomaterials, Kyoto University, Kyoto, Japan. Cisplatin was donated by Nippon Kayaku Co. (Tokyo, Japan).

The new dosage format of lactic acid oligomer microspheres incorporating the cisplatin was prepared using an oil-in-oil emulsion method.[3] Cisplatinum at 10 mg/ml and poly-*d,l*-lactide at 90 mg/ml were dissolved in dimethylfolmamide. The resulting solution and 10 volumes of castor oil were mixed. The mixture was then emulsified by agitation at 250 r.p.m. at room temperature. The emulsion was stirred at 45°C for 24 h to evaporate

the dimethylfolmamide, so that the remaining poly-*d,l*-lactide emulsion containing cisplatin formed into microspheres. The cisplatin microspheres were dried under vacuum for 2 days at room temperature to completely remove the dimethylfolmamide. The microspheres were then sieved and the fraction which was 50–150 μm in diameter was used for the study. CDDP-MS was administered in a suspension in normal saline. As a control, an aqueous solution of cisplatinum for injection (Landa Inj^{R}, Nippon Kayaku Co.) was diluted with normal saline to give the required concentrations of cisplatin in aqueous solution.

Animal experiment

A total of 154 mice (BDF_1, 4 weeks old, weighing 19 g) were purchased from the Shimizu Laboratory Animal Center (Shizuoka, Japan). The mice were divided into 22 groups each composed of seven mice. Eleven groups were given CDDP-MS, a further nine groups received cisplatin solution, another group was given empty microspheres and the last group was given nothing. The mice were maintained under standard conditions (specific pathogen free, room temperature of 22°C, relative humidity of 60%, day–night cycle of 12 h), and were allowed free access to standard mouse chow and tap water from 5 days before drug administration until 22 days after administration.

On day 0 after acclimatizing breeding for 5 days, when the mice were 20 g in body weight on average, drugs were given intraperitoneally in 1 ml of normal saline with a 20 gauge needle. In the 11 groups receiving CDDP-MS, doses from 16.3 to 31.1 mg cisplatin/kg body weight were given in 11 increasing dose steps, increasing at a ratio of 1.066 per step. In the nine groups receiving the cisplatin solution, doses from 8.47 to 24.1 mg cisplatin/kg body weight were given in nine increasing dose steps, increasing at a ratio of 1.14 (equal to 1.066^2) per step. The control group received empty microspheres without cisplatin, at a dosage of 330 mg/kg, which corresponded to more than the amount of microspheres contained in the CDDP-MS at cisplatin of 33.5 mg/kg dose.

The mice were observed daily for 21 days after the administration, and the body weight change and the day of death were recorded. The surviving animals were sacrificed on day 22. The 50% lethal dose value (LD_{50}) was calculated using Litchfield–Wilcoxon's method for each dosage format. All animals were autopsied for macroscopic and microscopic changes in the body tissues. The heart, liver, spleen and kidney were removed, and the weights of these organs were recorded. These organs as well as the lungs, adrenal gland, thymus, stomach, intestines, lymph nodes, back muscle and testis were all removed for tissue samples, which were prepared with hematoxylin–eosin stain for microscopic examinations.

Results

LD_{50} value

The LD_{50} value for CDDP-MS was 23.8 mg/kg (21.3–26.7 mg/kg at 95% level of confidence). The LD_{50} value for cisplatin solution was 13.5 mg/kg (11.9–15.3 mg/kg at 95% level of confidence). There were no deaths in mice given empty microspheres.

Toxic symptoms and body weight change

The toxic symptoms in mice given CDDP-MS were similar to those of mice given the cisplatin solution. Doses close to the LD_{50} values of either format brought about dishevelment, shivering, lethargy, weakness, diarrhea, eye-lid discharge which was sometimes bloody and paleness of the ears. These symptoms improved 8–11 days after administration in the survivors. In the groups given CDDP-MS, almost all of the deaths were seen between day 4 and day 7, and only one mouse given a low dose (19.8 mg/kg) died on day 9 (Table 1). In the groups

Table 1. Mortality of mice given CDDP-MS

Dose of cisplatin (mg/kg)	Mortality	Day of death
16.3	0/7	—
17.4	0/7	—
18.6	0/7	—
19.8	1/7	9
21.1	3/7	6, 7, 7
22.6	3/7	6, 6, 7
24.1	5/7	4, 5, 5, 5, 6
25.8	5/7	5, 5, 6, 6, 7
27.1	6/7	4, 4, 4, 4, 4, 5
29.4	7/7	4, 5, 5, 5, 5, 5, 5
31.1	7/7	4, 4, 5, 5, 5, 5, 5

Table 2. Mortality of mice given aqueous cisplatin solution

Dose of cisplatin (mg/kg)	Mortality	Day of death
8.47	0/7	—
9.65	0/7	—
11.0	1/7	6
12.5	3/7	5, 5, 5
14.3	5/7	4, 5, 5, 5, 6
16.3	5/7	3, 4, 4, 5, 5
18.6	7/7	4, 4, 4, 5, 5, 5, 5
21.2	7/7	3, 4, 4, 4, 5, 5, 5
24.1	7/7	3, 4, 4, 5, 5, 5, 5

given the cisplatin solution, deaths were seen from day 3 to day 6 (Table 2).

Body weight changes are shown in Figures 1–3. In the mice given CDDP-MS at doses close to the LD_{50} value, body weight reached its minimum value on days 4 and 5, and began to improve from day 9–11. The lost body weight was not fully recovered until day 21 (Figure 1). In mice given cisplatin solution at doses close to the LD_{50} value, the body weight decreased up to day 4, and began to increase from day 5, and was restored to its pre-administration level on day 16 (Figure 2). Intraperitoneal empty microspheres induced neither toxic symptoms nor body weight loss (Figure 3).

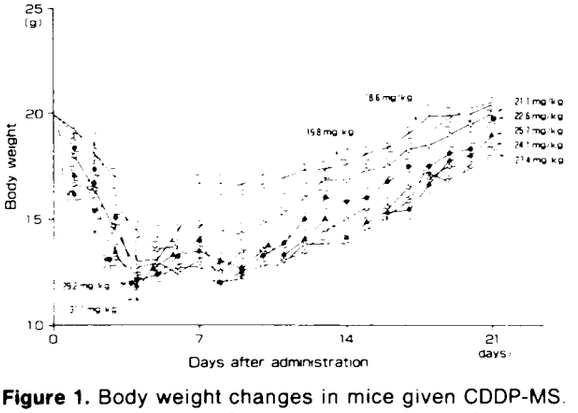

Figure 1. Body weight changes in mice given CDDP-MS. In mice given CDDP-MS at doses close to the LD_{50} value, body weight decreased for the first 4–5 days. The mice began to recover the lost weight from day 9–11; however the pre-administration level was not reached until day 21.

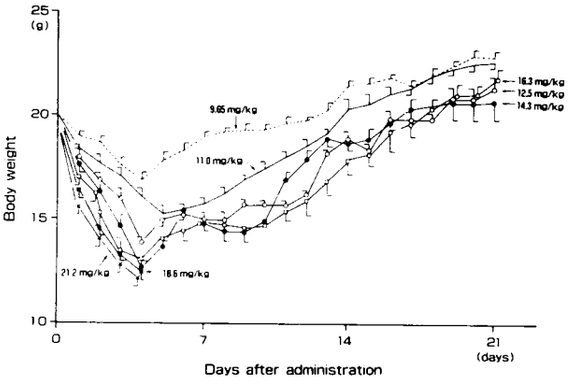

Figure 2. Body weight changes in mice given cisplatin solution. In mice given aqueous cisplatin solutions at doses close to the LD_{50} value, the body weight decreased for the first 4 days. The weight then began to increase from day 5 and was restored to its pre-administration level on day 16.

Autopsy findings

The autopsy findings were similar in mice given CDDP-MS and aqueous cisplatin solution, except for intraperitoneal adhesion, which was seen in one of seven mice given the maximal dose of CDDP-MS (cisplatin at 31.1 mg/kg). In normal mice (control) and the 68 mice surviving up to day 22, there were no pathological findings. In the mice which died of toxicity, the following pathological changes were seen.

Liver. In the mice dying due to toxicity, liver weight was lower than in mice surviving up to day 22 and

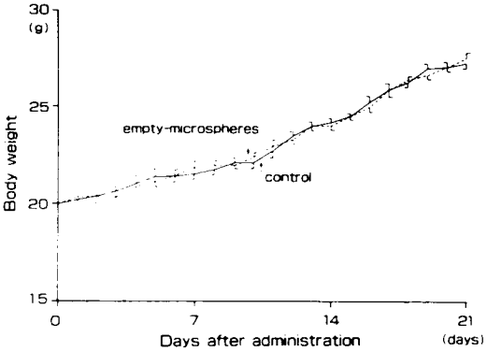

Figure 3. Body weight changes in mice given empty microspheres or nothing (control). There was no difference of the body weight change between the two groups.

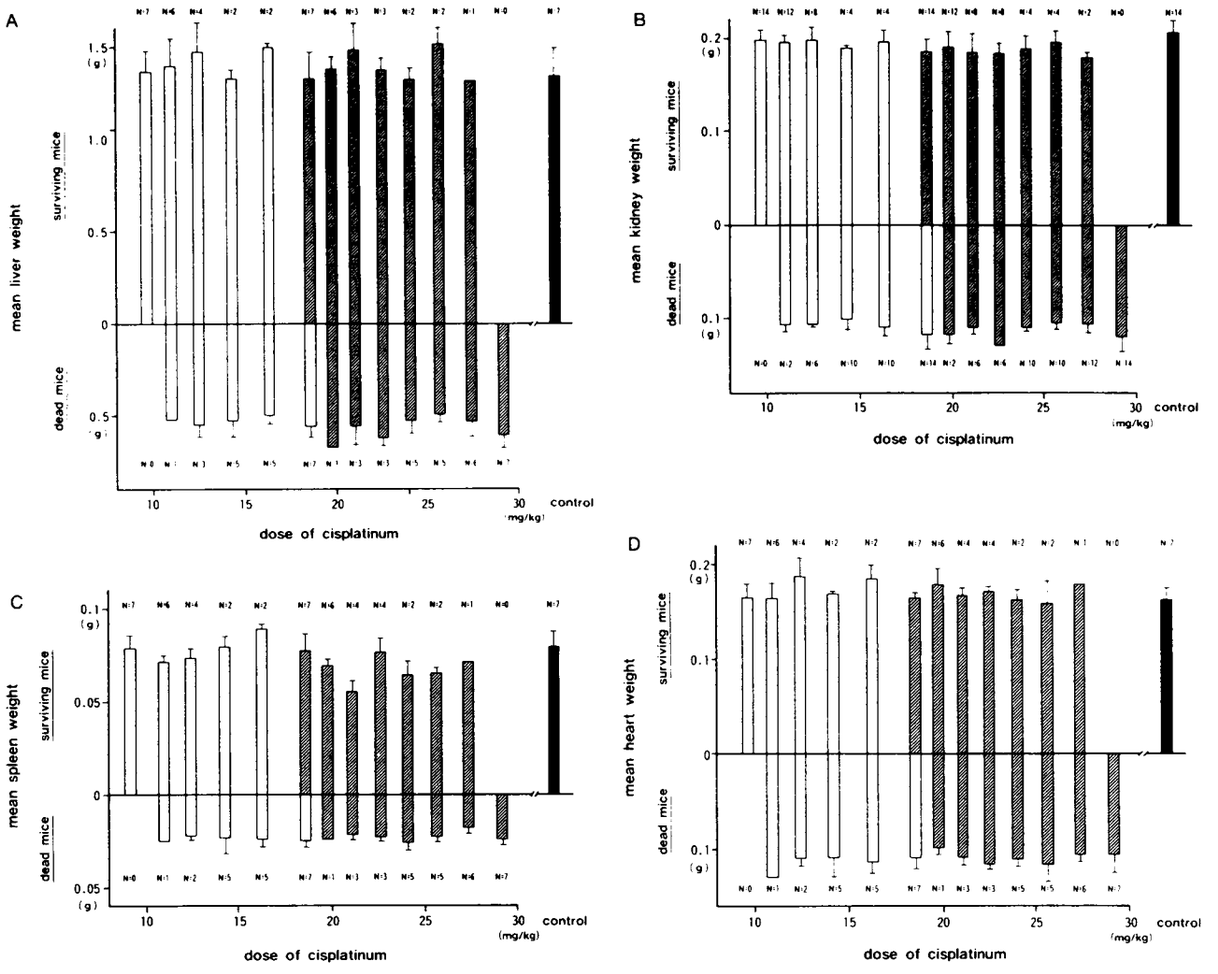

Figure 4. Organ weight changes induced by drug toxicity. The weights of specific organs had decreased in mice dying due to toxicity. The weights were restored to normal in mice surviving to day 22. There were no differences in organ weights between the mice given the two dosage formats. White columns indicate the organ weight of mice given cisplatin aqueous solution, striped columns indicate that of mice given CDDP-MS and black columns indicate that of normal mice.

was lower than in the control with normal mice (Figure 4A). Macroscopically, a slight congestion was seen. Microscopic examination revealed neither vacuolation nor degeneration of the liver parenchymal cells.

Kidney. In the mice dying due to toxicity, the kidney became atrophied (Figure 4B) and looked pale. Microscopically, there were hyaline-like casts in the lumen of lossened tubules, with scattered vacuolations and peeling off of the cuboidal epithelial cells, sometimes accompanied by interstitial hemorrhage (Figure 5).

Spleen. In the spleen, remarkable atrophy and weight loss were seen in the mice dying due to toxicity (Figure 4C). Microscopically, atrophy of the white pulp and disappearance of the lymphocytes were noted. In mice surviving to day 22, these findings were improved (Figure 6).

Stomach and intestines. The gastric mucosa was swollen and degenerating, especially in the foveolar epithelium. Inflammatory cells were infiltrating into the swollen muscle layer (Figure 7). Thickening of the muscle layer, necrosis, severe degeneration

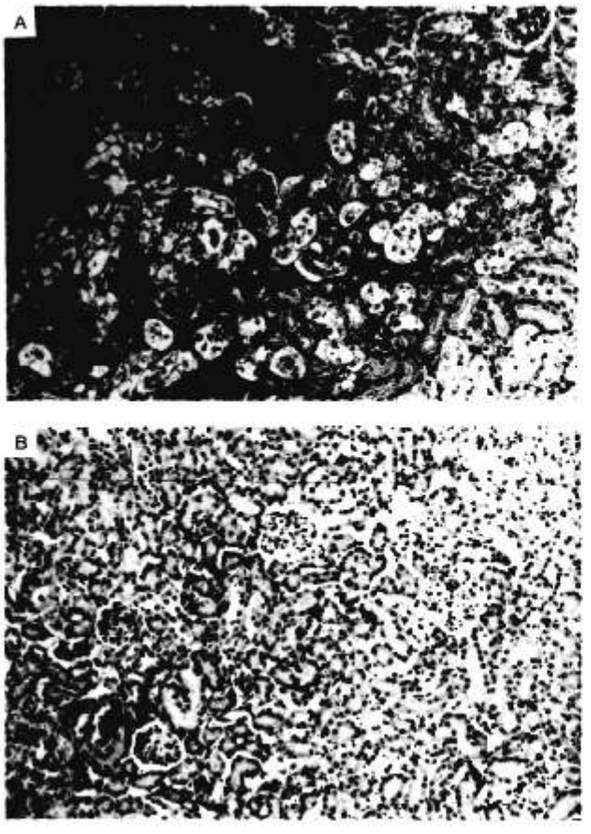

Figure 5. Microscopic view (×64) of the kidney. (A) Vacuolation and peeling off of the tubular epithelium and hyaline-like casts were seen in the mice dying due to toxicity. (B) These changes were not seen in the survivors.

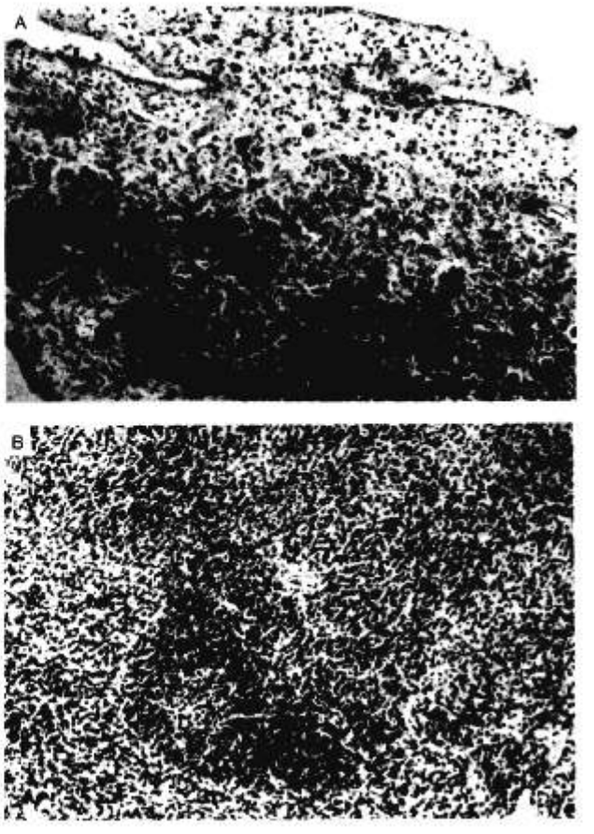

Figure 6. Microscopic view (×64) of the spleen. (A) Spleens of mice dying due to toxicity. Lymphocyte number decreased remarkably, especially in the white pulp region. (B) These changes were improved in the survivors.

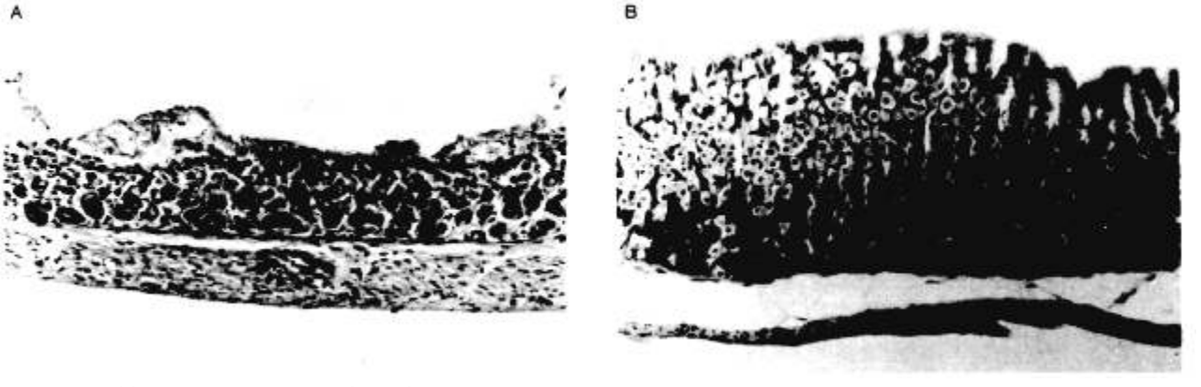

Figure 7. Microscopic view (×64) of the glandular stomach. (A) In the mice dying due to toxicity of a moderate dose of CDDP-MS, the foveolar layer degenerated, while the gastric glandular layer was only loose. The muscle layer became loose and thick with infiltration of inflammatory cells. (B) In the survivors, the findings were almost normal.

Figure 8. Microscopic view (×25) of the small intestines. (A) In the mice dying due to toxicity, peeling off and degeneration of the epithelium with vacuolation of the epithelial cells were seen. The inflammatory cells were infiltrating into the thickening muscle layer. (B) In the survivors, there were no such changes except for the changes of the muscle layer.

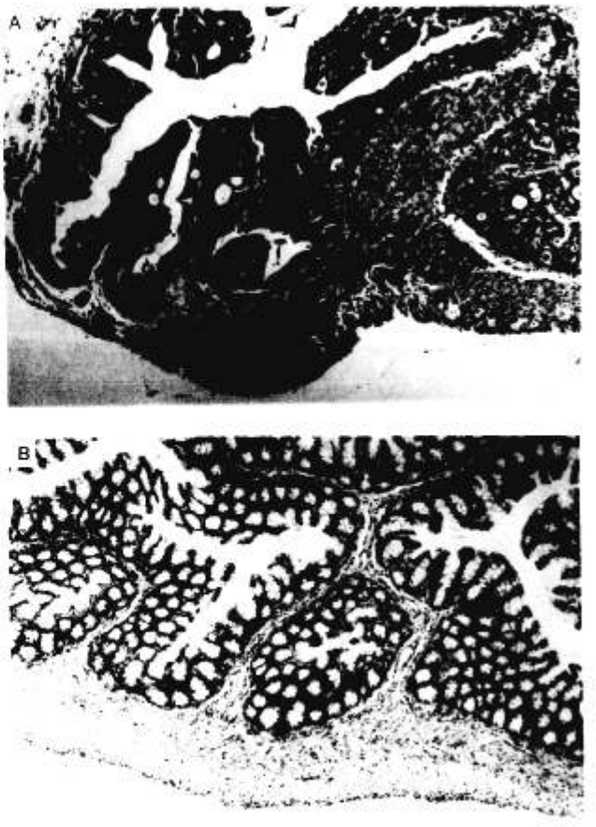

Figure 9. Microscopic view (×25) of the large intestine. See legend to Figure 8 for description.

accompanied by swelling, vacuolation and sloughing of the epithelium were wide-spread on the intestinal wall (Figures 8 and 9).

Thymus. In the mice dying due to toxicity, severe atrophy of the thymus, which sometimes had a scar-like appearance, was seen. Microscopically, atrophic changes in the cortex with lymphoid hypoplasia were noted (Figure 10).

Lung and heart. Macroscopic and microscopic examinations showed no particular changes, except for weight losses of the lungs and heart (Figure 4D), and intraalveolar bleeding in the lungs (Figure 11) in six of the 72 mice.

Adrenal gland. Microscopically, severe atrophy of the cortex was seen in the mice dying due to toxicity (Figure 12).

Lymph nodes. In the mice dying due to toxicity, the lymphocytes appeared scattered and the lymphoblasts in the lymph nodules had disappeared. In the survivors, the germinal centers were remarkable and the changes seen in the dead mice were improved (Figure 13).

Testis. In the 72 mice dying due to toxicity, the tissue became loose. In the survivors this change was improved.

Back muscles. There were no changes induced by the toxicity.

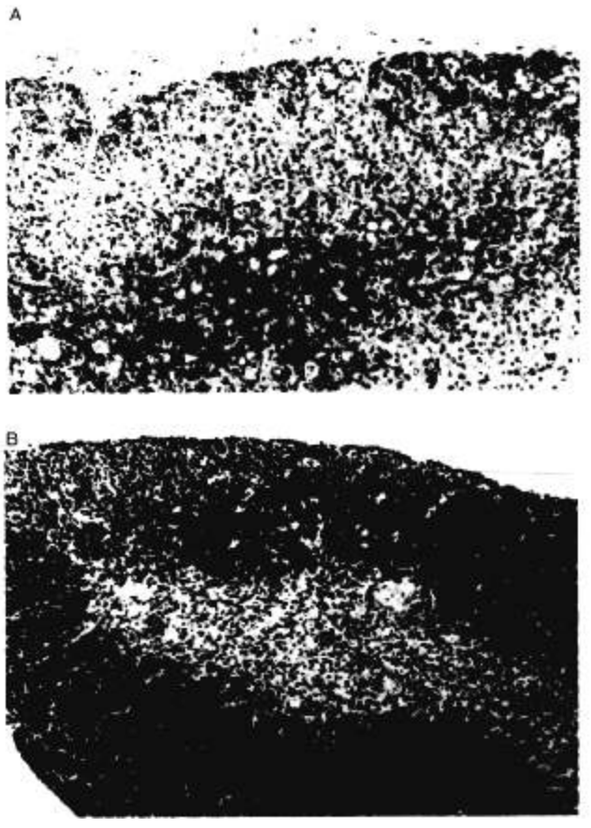

Figure 10. Microscopic (× 64) view of the thymus. (A) The thymus became atrophied and lymphocytes disappeared in mice dying due to toxicity. (B) These changes were not seen in the survivors.

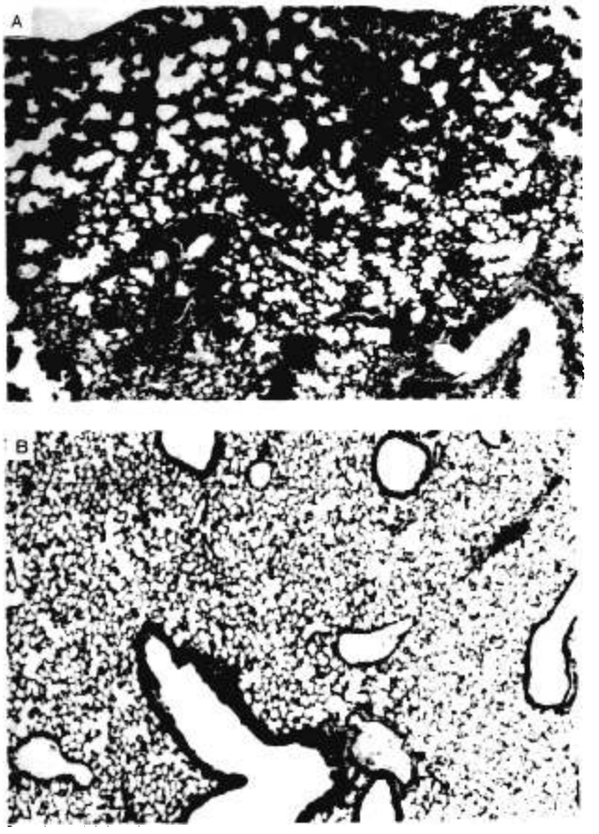

Figure 11. Microscopic view (× 64) of the lungs. (A) In some mice dying due to toxicity, intra-alveolar bleeding and pneumonia were seen. (B) The findings seen above were not noted in the survivors.

In the mice given empty microspheres, there were no pathological changes.

Discussion

It has been reported that intraperitoneal CDDP-MS delivers lower levels of cisplatin to whole body tissues (except for the intraperitoneal tissues) than conventional aqueous cisplatin solution.[1] This drug distribution suggests that CDDP-MS's systemic toxicity will be reduced. Our present examination shows that the lethal toxicity of CDDP-MS is reduced to 56.7% of that of the cisplatin solution. The LD_{50} value of the cisplatin solution reported here is similar to that in Schaeppi's report.[4] The prolonged body weight loss in the mice given CDDP-MS suggests that its toxic effects are prolonged slightly as compared with the cisplatin solution. This phenomenon is likely caused by CDDP-MS being locally retained and slowly releasing cisplatin.[1] Toxic symptoms and autopsy findings were similar for the two cisplatin dosage formats, and were also similar to those in Kociba's report[5] on the cisplatin solution. The empty microspheres comprised of a lactic acid oligomer, which was degradable *in vivo*, brought about no toxic effects.

These facts lead us to conclude that (i) the lethal toxicity of CDDP-MS is 56.7% that of the cisplatinum solution, (ii) a new kind of toxicity is not induced by the change in a new dosage format; however, the toxic effects are slightly prolonged, and (iii) the lactic acid oligomer microspheres in CDDP-MS induce no toxic effects.

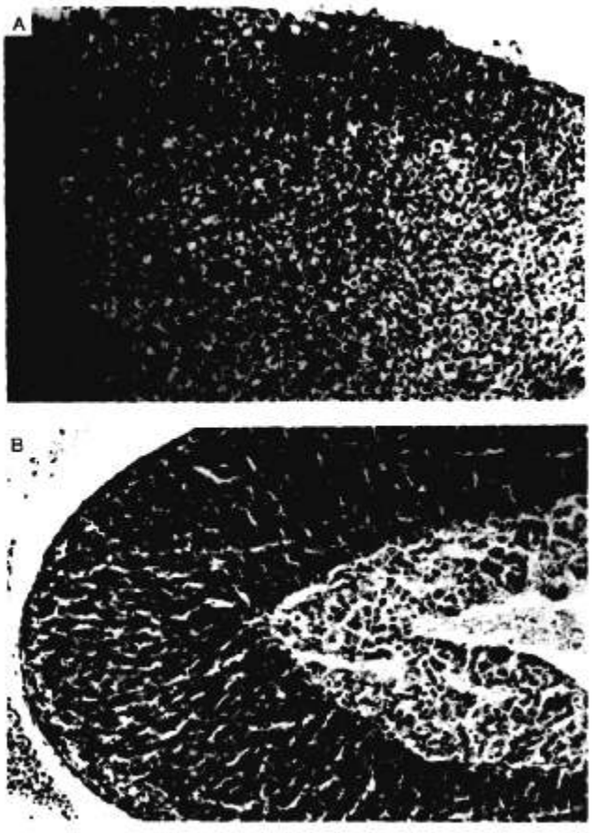

Figure 12. Microscopic view (×64) of the adrenal gland. (A) Severe atrophy of the cortex was noted in mice dying due to toxicity. (B) The changes were improved in the survivors.

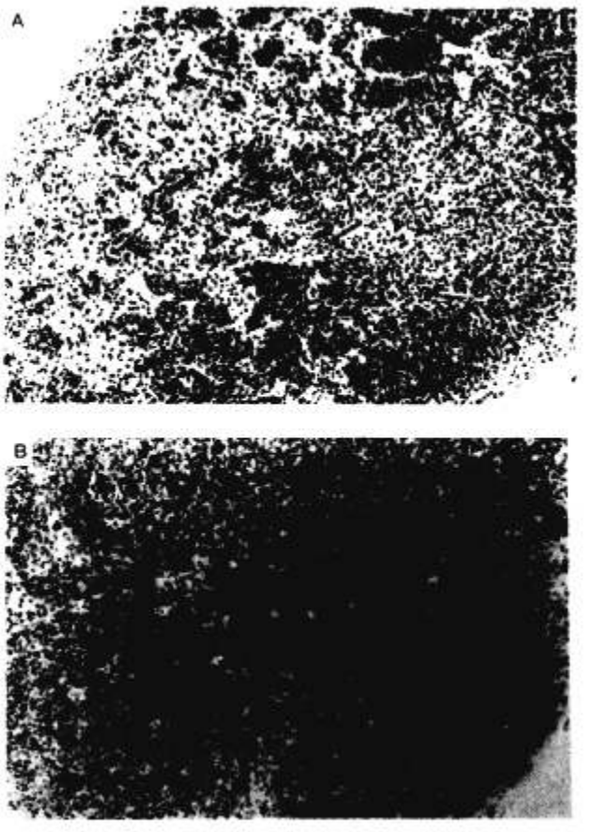

Figure 13. Microscopic view (×64) of the mesenteric lymph nodes. (A) Scattered lymphocytes and a profound disappearance of the lymphoblasts were seen in mice dying due to toxicity. (B) Lymphocyte number was not decreased. The germinal centers reappeared again in the lymphatic nodules with cell debris.

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