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Cisplatin microcrystals suspended in oil: pathological study of acute toxicity in mice

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The pathological changes brought about by the i.p. administration of a new dosage format, cisplatin microcrystals suspended in oil (CDDP–Oil), was examined in mice. CDDP–Oil decreased the absolute weight and the relative weight (organ weight/body weight) of the kidney, liver and spleen in the mice receiving the dosage form. However, the severity of the reduction of the organ weight in the CDDP–Oil administration groups was not different from that in the cisplatin aqueous solution (CDDP–Sol) administration groups. Histologically, severe degeneration and atrophy were recognized in the kidney, large intestine, small intestine, thymus, spleen, bone marrow and lymph nodes in the CDDP–Oil administration groups as well as CDDP–Sol administration groups. However, there were no additional changes in the macroscopic and microscopic findings in the CDDP–Oil groups. From these results, we concluded that this dosage form did not change the toxicity of cisplatin in terms of pathological effects.

Key words: Cisplatin, oil suspension, acute toxicity.

Introduction

We developed a new dosage format, cisplatin microcrystals suspended in oil (CDDP–Oil), which was found to be very useful for i.p. chemotherapy because of its retention in the peritoneal cavity and slow release of cisplatin over a long period. In our previous report we examined the acute toxicity of CDDP–Oil for i.p. administration in mice. The 50% lethal dose of CDDP–Oil was 30.3 mg/kg (27.1–33.7 mg/kg at 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).[1] In this study, we examined the systemic pathological changes in acute toxicity of CDDP–Oil after i.p. administration in mice in comparison with those for CDDP–Sol.

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Materials and methods

Preparation of dosage forms

Cisplatin microcrystals were kindly donated by Nippon Kayaku (Tokyo, Japan). CDDP–Oil was made from cisplatin microcrystals suspended in a 4:1 mixture of sesame oil (Sesame oil®; Nakaraitesque, Kyoto, Japan) to lipiodol (Lipiodol Ultra Fluid®; Kodama Yakuhin, Tokyo, Japan). Cisplatin microcrystals were suspended in the oil mixture with a magnetic stirrer for 6 h in 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) was diluted with normal saline to give the concentration required. As another control, the same oil mixture without cisplatin was also prepared. All drugs were used within 1 h of preparation.

Drug administration protocol

Two hundred and ten CDF1 male mice (4 weeks old, weighing 25 g on average) were purchased from 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 (no treatment group). The mice were kept under standard conditions in a specific pathogen-free environment, room temperature of 22°C, relative humidity 60%, day–night cycle 12 h, with standard mouse chows and tap water available freely for 7 days before the drug administration until 21 days after the administration.

On day 0, the drugs were given i.p. using a 20-gauge needle. In the 18 groups receiving CDDP–Oil, doses from 13.4 to 70.7 (13.4, 15.4, 16.9, 18.6, 20.5, 22.5, 24.8, 27.3, 30, 33, 36.3, 39.9, 43.9, 53.1, 58.5,

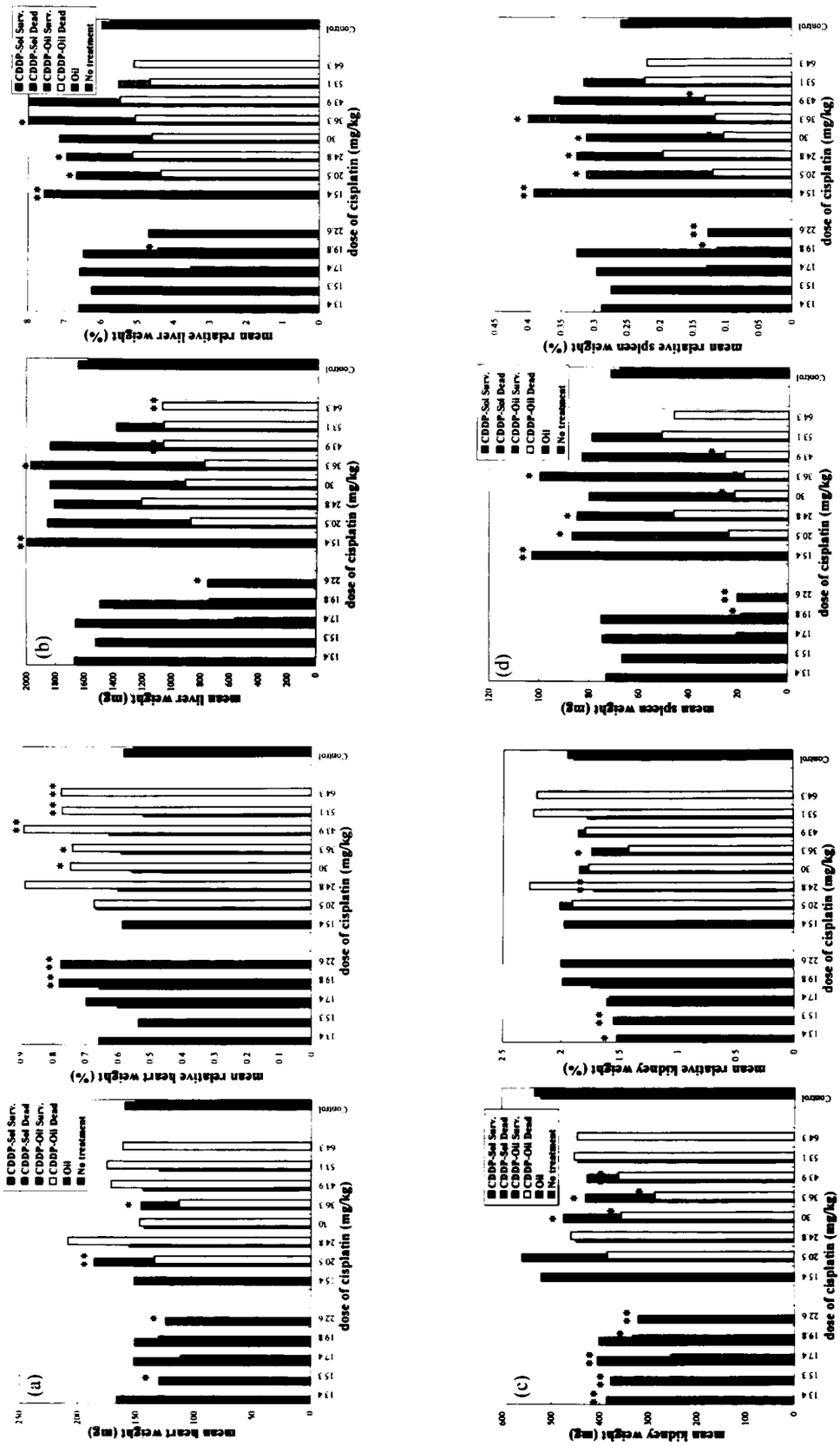


Figure 1. Changes in the absolute and relative organ weights induced by drug toxicity (a, heart; b, liver; c, kidney; d, spleen). Generally, the weight of the liver, kidney and spleen were decreased in mice that died of toxicity, whereas those weight changes were restored to normal in the mice that survived to day 22. There were no differences in those four organs weights between the mice given the two dosage forms. $^{*}p < 0.05$, $^{**}p < 0.01$ (compared to no treatment group by Mann–Whitney analysis).

64.3 and 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 (12.6, 13.4, 14.3, 15.3, 16.3, 17.4, 18.5, 19.8, 21.2 and 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.

Observation by autopsy and measurement of organ weight

The mice were observed daily for 21 days after the administration, and the day of death and the body weight were recorded. The surviving animals were sacrificed on day 22. All animals were autopsied and observed for macroscopic changes in the body tissues. The heart, liver, spleen, kidneys, lungs, stomach, small and large intestines, adrenal glands, thymus, lymph nodes, testis, and bone marrow were all removed for tissue samples, which were prepared with hematoxylin & eosin stain for microscopic examination. Next, the weights of heart, liver, spleen and kidney of the mice in all groups were recorded. The absolute organ weights and the relative organ weights (organ weight/body weight) in the CDDP–Oil group, CDDP–Sol group and Oil group were then compared statistically with values in the no treatment group by the Mann–Whitney method.

Results

Organ weight changes induced by drug toxicity

There was no difference in the absolute and relative organ weights of heart, liver, kidney and spleen between the Oil group and the no treatment group. The changes of organ weights observed in the administration groups were as follows.

Heart. There was no remarkable difference in absolute heart weight between mice that survived up to day 22 and mice that died due to toxicity, compared with the two control groups. There was no difference between the CDDP–Oil groups and the CDDP–Sol groups. However, the relative heart weight of the mice that died due to toxicity was increased in both the CDDP–Oil groups and the CDDP–Sol groups, because of the decreased body weight of the mice (Figure 1A).

Liver. The absolute and relative weights of the liver in the mice that died of toxicity were much lower than values in the two control groups. The weight changes in the mice that survived to day 22 were restored to the level of the control groups. However, there were no remarkable differences between the CDDP–Oil groups and the CDDP–Sol groups. Similar findings were also noted in the relative liver weight (Figure 1B).

Kidney. Generally, the absolute kidney weight was decreased in all administration groups. However, the reduction in the mice that died due to toxicity was greater than in the surviving mice. There was no remarkable difference between the CDDP–Oil groups and the CDDP–Sol groups (Figure 1C).

Spleen. The absolute and relative weights of the spleen in the mice that died of toxicity were much lower than in the control groups. However, the changes were restored in the surviving mice. There were no apparent differences between the CDDP–Oil groups and the CDDP–Sol groups (Figure 1D).

Macroscopic and microscopic findings

The autopsy findings were very similar in mice given CDDP–Oil and CDDP–Sol. There were no additional abnormal findings in the CDDP–Oil groups compared with the CDDP–Sol groups. In the mice that survived to day 22 in the administration groups and the two control (oil and no treatment) groups, there were no pathological findings. In the mice that died due to toxicity, the following pathological changes were seen.

Kidney. In the mice that died of toxicity, the kidney became atrophied and looked pale. Microscopically, there was vacuolation and sloughing of the renal tubular epithelium with hyaline-like casts in the lumen of the loosened tubules (Figure 2A). However, these changes were not seen in the surviving mice (Figure 2B).

Liver. In the mice that died of toxicity, the liver was atrophied and congested. Microscopically, there was vacuolation and degeneration in some areas of parenchymal cells (Figure 3A). However, there were no abnormal findings in the survivors (Figure 3B).

Stomach and intestines. Among the mice that died due to toxicity, the gastric mucosa was swollen,

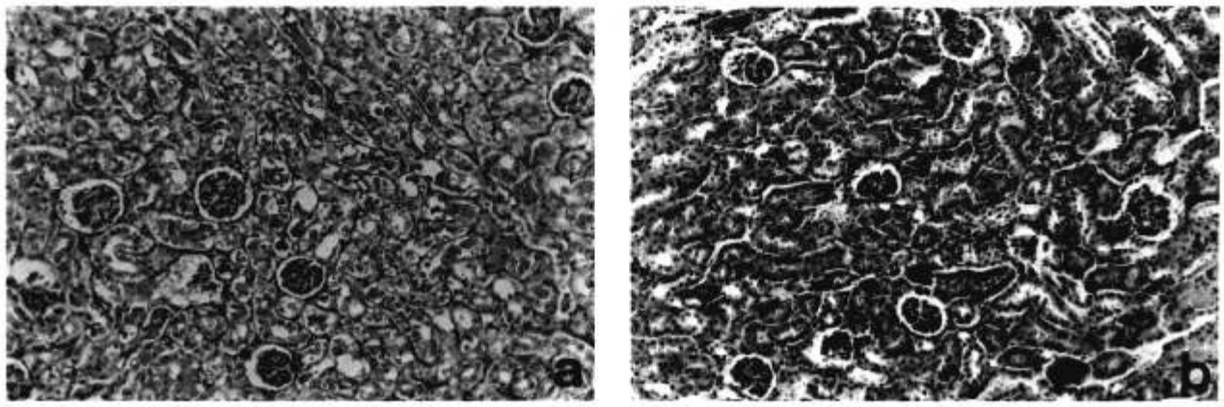


Figure 2. Microscopic view of the kidney. (× 50). (a) Vacuolation and sloughing of the tubuler epithelium with hyaline-like casts in the lumen of the loosened tubules were seen in the mice that died due to toxicity. (b) These changes were not seen in the surviving mice.

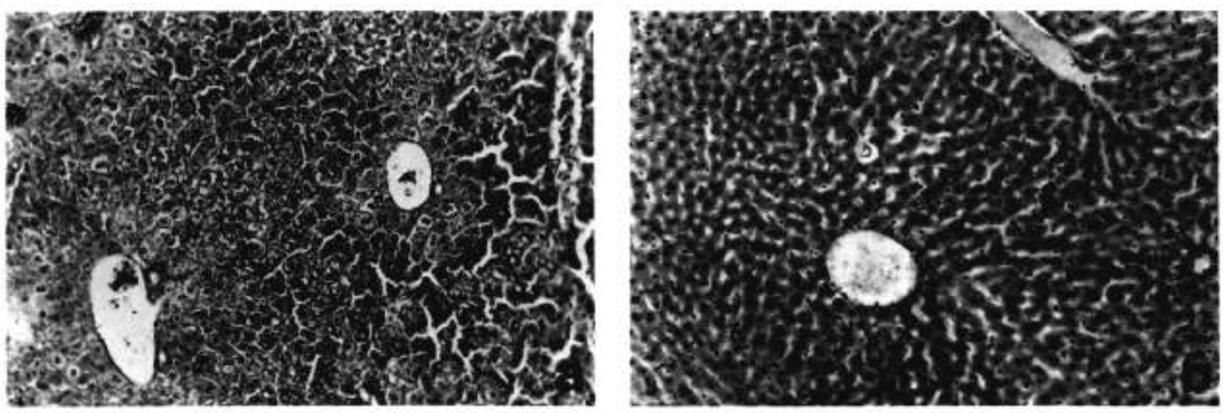


Figure 3. Microscopic view of the liver (× 50). (a) In the mice that died due to toxicity, there was vacuolation and degeneration in the some areas of parenchymal cells. (b) There were no abnormal findings in the survivors.

degenerating and atrophied in the foveolar epithelium (Figure 4A). The epithelium of the small and large intestine was also swelling, vacuolated and sloughing in the extensive range of intestinal wall (Figures 5A and 6A). Those changes were not seen in the survivors (Figures 4B, 5B and 6B).

Lung and heart. Macroscopically and microscopically, no pathological changes were seen except for intra-alveolar bleeding in several animals that died due to toxicity (Figure 7A). However, no abnormality such as, for instance, pulmonary embolism due to oil was seen (Figure 7B).

Spleen. In the spleen, remarkable atrophy was seen in the mice that died due to toxicity. Microscopically, atrophy of the white pulp and disappearance of lymphocytes were observed remarkably (Figure 8A). In mice that survived to day 22, these findings were not observed (Figure 8B).

Thymus. Severe atrophy of the thymus was seen in the mice that died due to toxicity. In several mice, there was a scar-like appearance. Microscopically, atrophic changes with lymphoid hypoplasia in the cortex were noted (Figure 9A). These changes were not seen in the survivors (Figure 9B).

Lymph nodes. In the mice that died of toxicity, systemic lymph nodes including the intra-abdominal lymph nodes showed severe atrophy. Microscopically, the disappearance of lymphocytes and lymphoblasts was remarkable (Figure 10A). In the

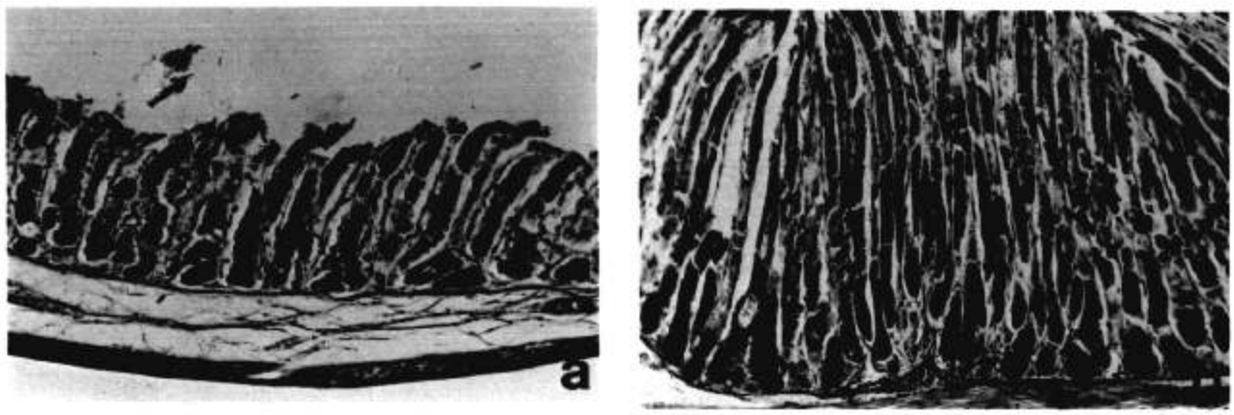


Figure 4. Microscopic view of the glandular stomach (× 50). (a) In the mice that died of toxicity, the foveolar layer was swollen, degenerated and atrophied. (b) These changes were not seen in the survivor.

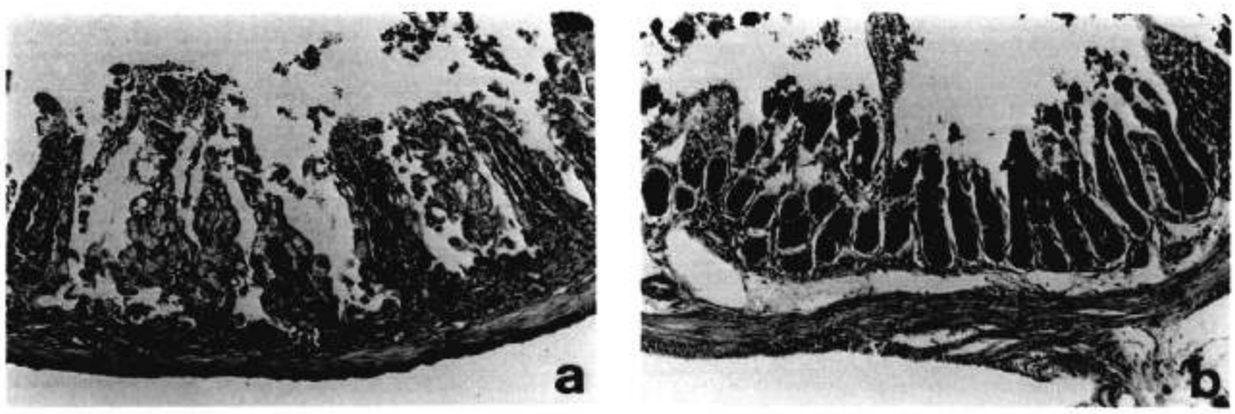


Figure 5. Microscopic view of the small intestine (× 50). (a) In the mice that died due to toxicity, intestinal epithel was swelling, vacuolated and sloughing in the intestinal wall. (b) In the survivor, these changes were not seen.

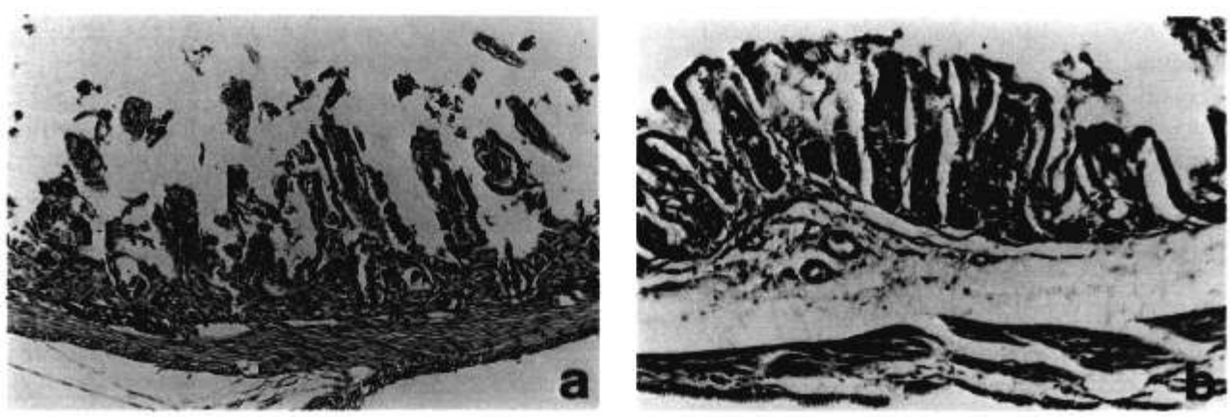


Figure 6. Microscopic view of the large intestine (× 50). See Figure 5 legend.

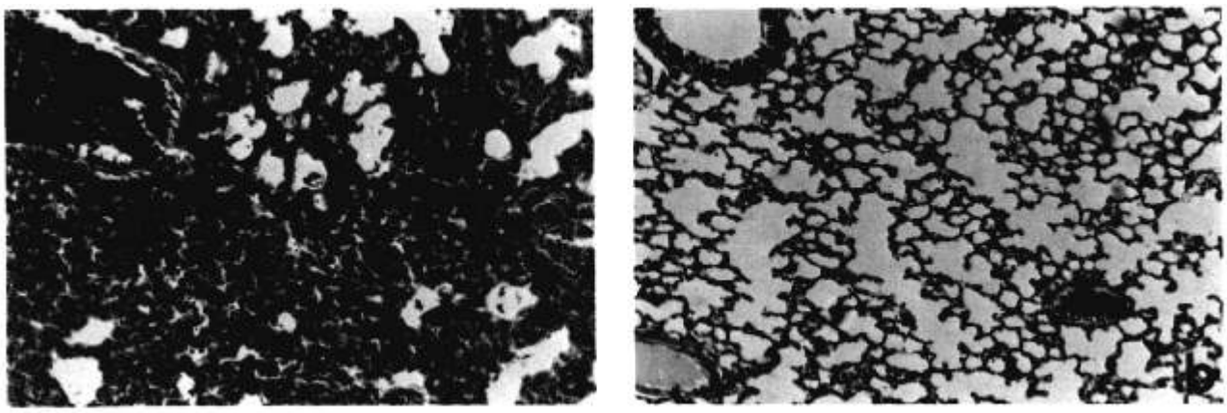


Figure 7. Microscopic view of the lung (× 50). (a) In the several mice that died due to toxicity, intra-alveolar bleeding was seen. (b) These abnormalities were not seen in the survivors.

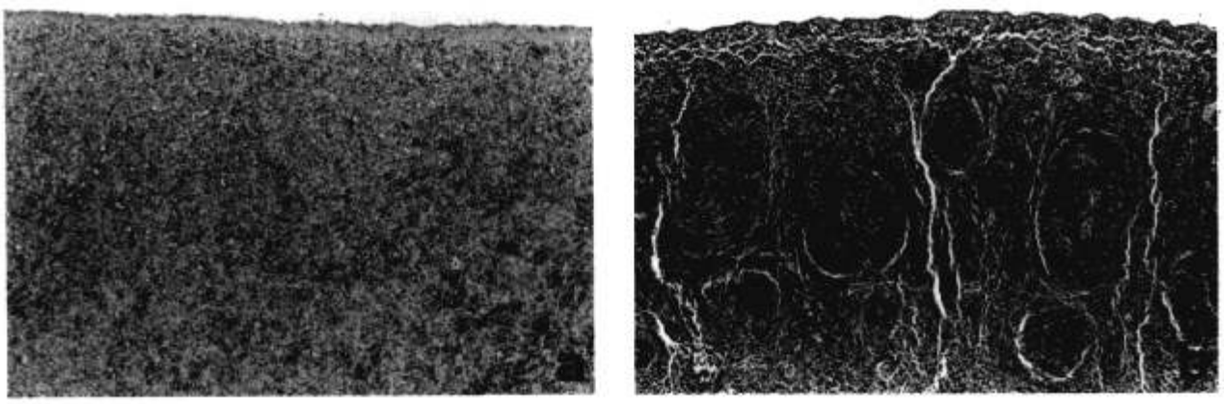


Figure 8. Microscopic view of the spleen (× 20). (a) In the mice that died of toxicity, remarkable atrophy of the white pulp and disappearance of lymphocytes were seen. (b) In the mice that survived to day 22, these findings were not observed.

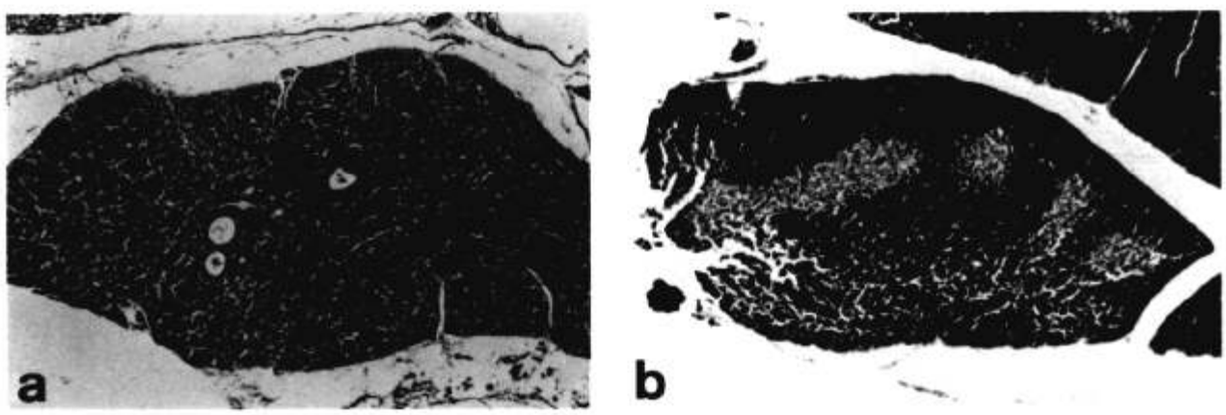
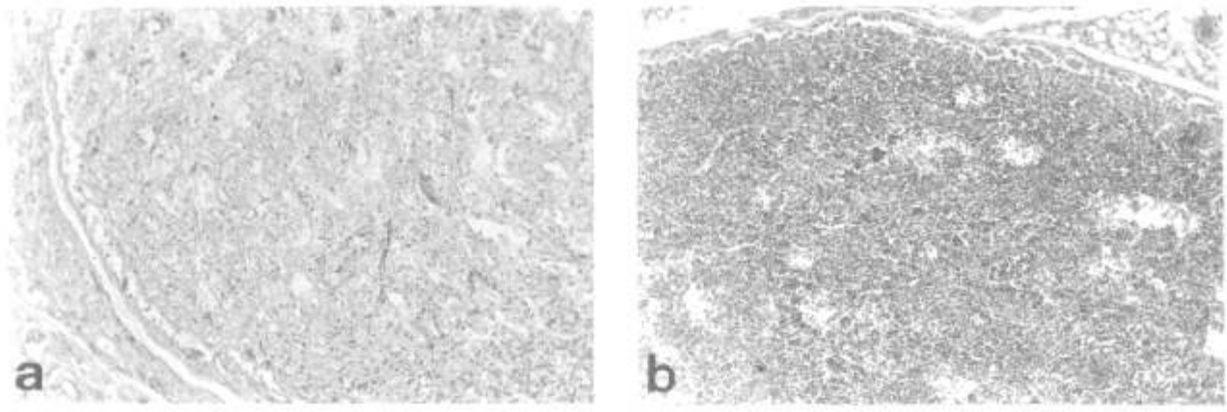


Figure 9. Microscopic view of the thymus (× 20). (a) In the mice that died of toxicity, severe atrophic changes with lymphoid hypoplasia were noted, especially in the cortex. (b) These changes were not seen in the survivors.



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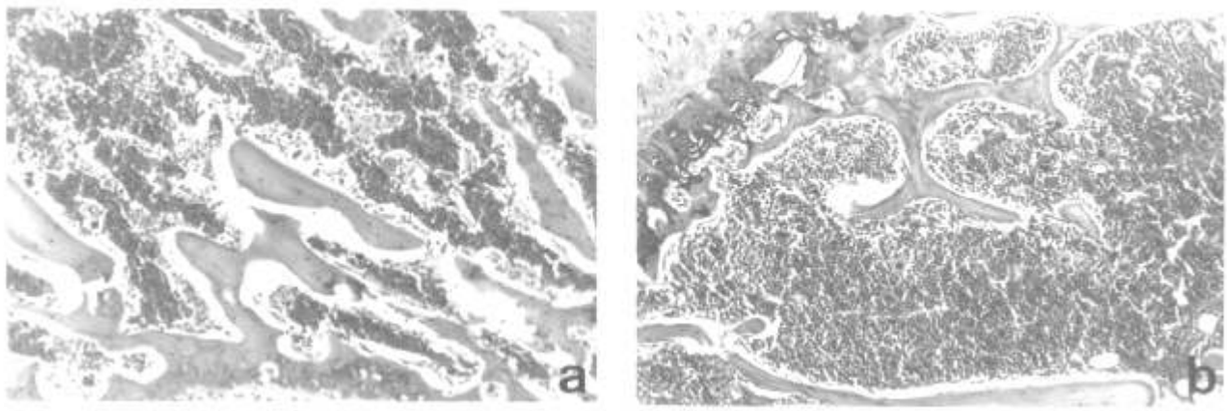

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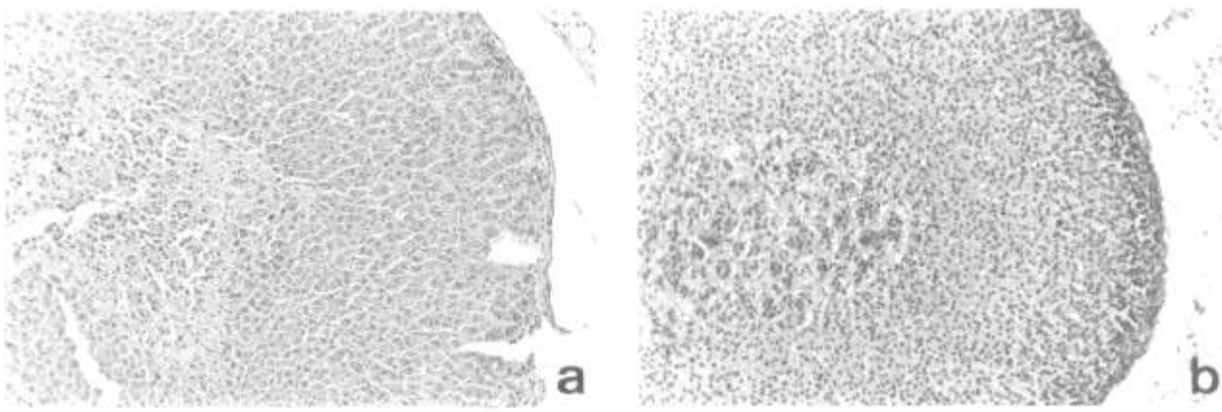

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survivors, lymphocyte numbers were not decreased and the germinal centers reappeared in the lymph nodes (Figure 10B).

Bone marrow. In the dead mice, bone marrow in the femoral bone was atrophied and hypocytic. Microscopically, remarkable hypoplastic changes were seen in stem cells. Only red blood cells were seen (Figure 11A). In the survivors, these changes were not observed (Figure 11B).

Adrenal glands. In the dead mice, severe degeneration and atrophy of the cortex was seen (Figure 12A). In the survivors, these changes were not be seen (Figure 12B).

Testis. In the mice that died of toxicity, the testis were severe atrophied. Microscopically, the tissue appeared looked loose due to hypocytic change. In the survivors, this change was improved.

Discussion

Cisplatin is one of the most widely used chemotherapeutic agents. The major organs affected by cisplatin toxicity are kidney, gastro-intestinal tracts, bone marrow, lymphoid tissues and ear.[2–6] In particular, nephrotoxicity is the most important dose-limiting factor for the clinical use of cisplatin.[3,7,8] In an attempt to reduce the systemic toxicity of cisplatin, we developed a new dosage format, cisplatin microcrystals suspended in oil (CDDP–Oil). Oil is retained in the peritoneal cavity on i.p. injection and is absorbed through lymph capillaries into the regional lymphatic system.[10] These properties allowed CDDP–Oil to maintain cisplatin activity at a high level for a long period in the i.p. tissue and a low level of cisplatin was delivered to the circulating blood compared with cisplatin aqueous solution (CDDP–Sol) (manuscript in preparation). Therefore, CDDP–Oil is suggested to be useful for i.p. chemotherapy.

In our previous report, the 50% lethal dose of CDDP–Oil was reduced to 1.78 times that of CDDP–Sol.[1] In this study, continuing our investigation of CDDP–Oil, we observed systemic pathological changes after the i.p. injection of CDDP–Oil in mice and compared the effects with those of CDDP–Sol. Autopsy findings and microscopic examination showed that the organs affected by CDDP–Oil were mainly kidney, gastro-intestinal tracts, bone marrow, lymphoid tissues such as spleen, thymus and lymph nodes as well as CDDP–Sol.[3,4,5,9] There were no additional abnormal macroscopic and microscopic changes by changing dosage form. According to the comparison of organ weight changes between the two dosage forms, both CDDP–Oil and CDDP–Sol reduced the weight of the kidney, liver and spleen to the same degree and did not affect the weight of the heart in the mice that died due to toxicity. Whereas, in the mice that survived to day 22, these organ weight changes were virtually restored.

From these results, we concluded that this new dosage format, CDDP–Oil, did not increase the toxicity of cisplatin. This dosage form was thought to be useful for i.p. chemotherapy.

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