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A new dosage form comprising a suspension of activated carbon particles adsorbing aclarubicin: toxicity in mice

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A new dosage form (ACR-CH), a suspension of small activated carbon particles adsorbing aclarubicin, was studied for its toxicity and histopathological effects on organs in mice. The 50% lethal subcutaneous dose of ACR-CH was 83.5 mg/kg, a value 2.42 times that (34.5 mg/kg) of the aclarubicin aqueous solution. The duration of the toxic effects of ACR-CH was prolonged compared with that of the aclarubicin aqueous solution. On autopsy there was no remarkable difference in macroscopic and microscopic examinations between the two dosage forms.

Key words: Aclarubicin, activated carbon, drug delivery system, subcutaneous administration, toxicity.

Introduction

A new dosage form (ACR-CH), a suspension of activated carbon particles adsorbing aclarubicin, was developed for pre- and intra-operative chemotherapy for lymph-nodal metastases of breast cancer. Experiments in rats revealed that ACR-CH delivered a large amount of aclarubicin selectively to the regional lymph nodes while the levels to the whole body were low, when compared with those of the aclarubicin aqueous solution.[1] These results lead us to postulate that ACR-CH should show reduced toxicity. This paper describes the reduced toxicity of ACR-CH in mice.

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Materials and methods

Preparation of dosage forms

Fifty mg/ml of activated carbon (Activated Carbon Mitsubishi #1500AA^R, Mitsubishi Chemicals Co. Ltd, Tokyo) 20 nm in diameter and 1480 m^2/g in specific surface area, and 20 mg/ml of polyvinylpyrrolidone (polyvinylpyrrolidone K-30^R, Nakarai Chemicals Co. Ltd, Kyoto) were mixed in saline and kneaded with three rollers to turn the carbon particles into a suspension[1,2] in which the average size of particles was 157 nm.[3] The activated carbon suspension was sealed in a glass tube and sterilized at 120°C for 10 min. Twenty mg/ml of aclarubicin (Aclacynon^R, Sanraku Co., Tokyo), one of the anthracyclines,[4] was added to the carbon suspension and the mixture was shaken at 120 cycles per minute (cpm) for 1 h at 37°C so that the adsorption was at equilibrium. The mixture was then diluted with saline into two concentrations of ACR-CH. The first, for doses of aclarubicin of 75 mg/kg or less, comprised 5 mg/ml of aclarubicin, 12.5 mg/ml of activated carbon and 5 mg/ml of polyvinylpyrrolidone in saline. The second, for doses of 97.5 mg/kg or more, comprised 10 mg/ml of aclarubicin, 25 mg/ml of activated carbon

and 10 mg/ml of polyvinylpyrrolidone in saline. An aqueous solution of 5 mg/ml of aclarubicin in saline (ACR-sol) was prepared as a control drug. The drugs were used within 6 h after preparation.

Drug administration

Ninety-six CDF1 female mice (19 to 20 g in body weight, 4 weeks old, purchased from Shimizu Laboratory Animal Center, Shizuoka, Japan) were divided into 16 groups of six mice: seven groups for ACR-CH administration (ACR-CH groups), eight groups for ACR-sol administration (ACR-sol groups) and one group (activated carbon group) for administration of carbon suspension without aclarubicin. 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) from 7 days before drug administration until 15 days after administration.

Drugs were injected subcutaneously into the back with a 25-gauge needle on day 0. In the seven ACR-CH groups, ACR-CH was given at a dose ratio of 1.3:1 from 34.1 to 164.8 mg of aclarubicin/kg. In the eight ACR-sol groups, ACR-sol was given at eight doses in a dose ratio of 1.15:1 from 20.7 to 55.1 mg of aclarubicin/kg. A suspension of activated carbon at 400 mg/kg without aclarubicin was given to the activated carbon group.

The mice were checked daily for 14 days after administration to determine the day of death and body weight changes. Terminally ill animals were sacrificed and regarded as dead animals. The 50% lethal dose value (the LD_{50} value) was calculated using Litchfield-Wilcoxon's method for each dosage form.

Any survivors on day 15 were sacrificed and all animals were autopsied for macroscopic and microscopic changes in body tissues. The injection site, heart, lung, liver, thymus, stomach, intestines, spleen, kidney and testis were taken for tissue samples which were prepared with hematoxylin-eosin stain for microscopic examinations.

Results

The LD_{50} value

The LD_{50} value of ACR-CH was 83.5 mg/kg of aclarubicin (72.6 to 96.0 at 95% level of confidence). The LD_{50} value of ACR-sol was 34.5 mg/kg of aclarubicin (31.0 to 38.4 at 95% level of confidence).

Table 1. LD_{50} value of ACR-CH and ACR-sol (95% level of confidence)

	LD_{50} value (mg aclarubicin/kg)
ACR-CH[b]	83.5 (72.6–96.0)
ACR-sol[c]	34.5 (31.0–38.4)
Activated carbon	≫400 mg/kg[a]

[a] In terms of activated carbon.
[b] Aclarubicin adsorbed on activated carbon particles.
[c] Aclarubicin aqueous solution.

The LD_{50} value of ACR-CH was 2.42 times that of ACR-sol. There were no deaths in the mice given activated carbon suspension without aclarubicin (Table 1).

Toxic symptoms and body weight change

Toxic symptoms in mice given ACR-CH were similar to those of mice given ACR-sol. Doses close to the LD_{50} values of both forms brought about

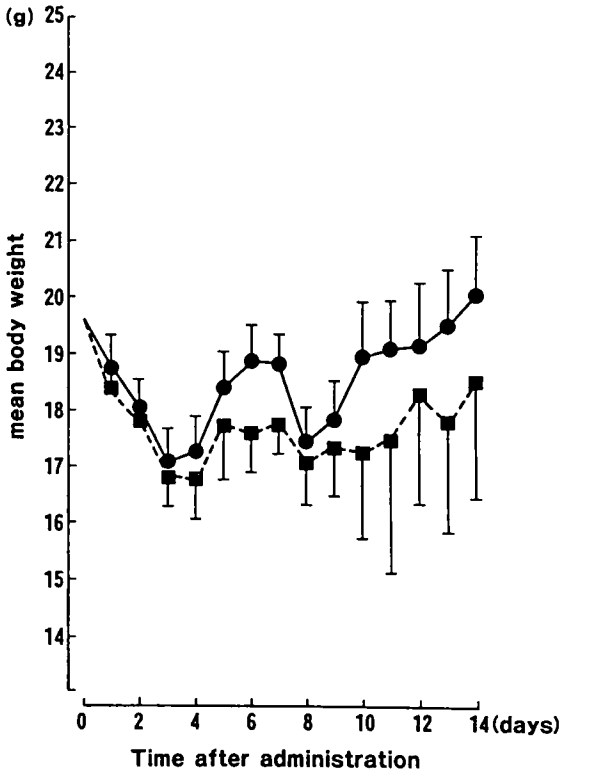

Figure 1. Body weight change of mice given ACR-CH. In the mice given ACR-CH at a moderate dose (57.4 mg/kg, which was 69% of the LD_{50} value, ●) or at a large dose (75.0 mg/kg, which correspond to 90% of the LD_{50} value, ■), the body weight loss was not recovered until 13 or more days after the administration.

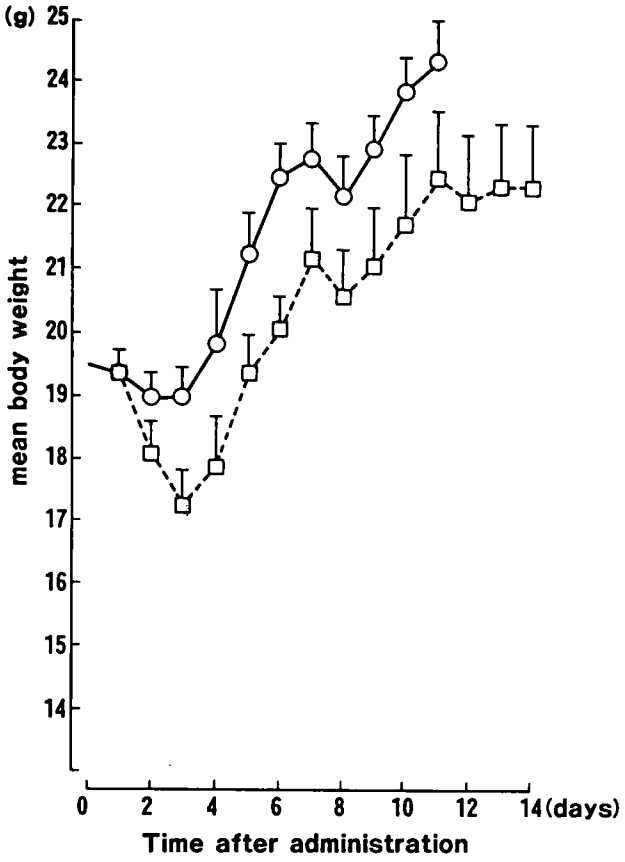

Figure 2. Body weight change of mice given ACR-sol. In the mice given ACR-sol at a moderate dose (20.7 mg/kg, which was 60% of the LD_{50} value, ○) or at a large dose (31.5 mg/kg, which was 91% of the LD_{50} value, □), the body weight loss was recovered within 4 to 6 days after administration.

dishevelment, lethargy, weakness, diarrhea sometimes with muco-bloody feces, eyelid discharge and ulceration of the injection site. In the ACR-sol groups, deaths occurred from day 2 to day 8. In the ACR-CH groups, mice died from day 1 to day 10. There were no deaths more than 10 days after injection.

Body weight changes are shown in Figures 1 and 2. In the mice given a moderate dose of ACR-CH (57.4 mg aclarubicin/kg, which was 69% of the LD_{50} value), body weight loss was prolonged to day 13. In those mice receiving a large dose of ACR-CH (75.0 mg aclarubicin/kg, which corresponded to 90% of the LD_{50} value), the weight loss was not recovered during the observation period. In the ACR-sol group, however, the mice given a moderate dose (20.7 mg/kg, 60% of the LD_{50} value) and a large dose (31.5 mg/kg, 91% of the LD_{50} value) of aclarubicin recovered body weight loss within 4 to 6 days.

These results indicate that the toxicities of ACR-CH tended to be prolonged. No toxic symptoms were seen in the activated carbon group.

Autopsy findings

In high doses (greater than the LD_{50} values) of both dosage forms, a remarkable atrophy of the thymus, congestion and moderate atrophy of the spleen, and congestion and mucosal bleeding of the intestines were seen macroscopically. Microscopically, atrophy, especially disappearance or scattered appearance of lymphocytes, of the thymus (Figure 3), and congestion with relatively mild decrease of lymphocytes number in the spleen (Figure 4), and congestion of the kidney and lung, and degeneration and congestion of the intestinal mucosa (Figure 5) were found. Thus macroscopic and microscopic findings were similar for the two dosage forms. Severe necrotic and inflammatory changes were seen in the subcutaneous tissues and muscle tissues contiguous to the injection site (Figure 6). These changes at the injection site were severe and widespread in the ACR-CH groups, when compared with those in the ACR-sol groups.

Discussion

We have already reported[1] that most of the injected ACR-CH is retained at the injection site and delivered to the lymphatic system, where the ACR-CH releases aclarubicin slowly over a long period, with low levels of aclarubicin being distributed to other body tissues. Since aclarubicin's dose-limiting factors are its heart toxicity and bone marrow suppression,[5,6] it is thought that the drug-distribution properties of ACR-CH will reduce the aclarubicin's systemic toxicities.

Indeed, toxicity experiments in mice showed that ACR-CH's lethal toxicity was reduced to 41.3% of that of ACR-sol. Day of death and body weight changes indicated that the toxic effects of ACR-CH were prolonged, as compared with those of ACR-sol. The elongation of toxic effects may be caused by ACR-CH's being retained locally and slowly releasing aclarubicin.[1] Except for the injection site, autopsy of the animals receiving the

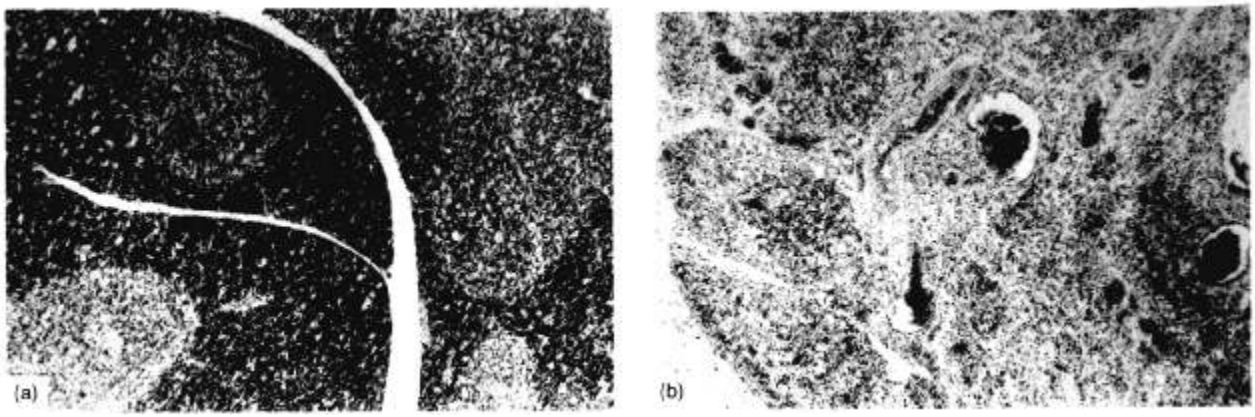

Figure 3. Microscopic view of the thymus (× 32). (a) The thymus of a mouse given a low dose (40% of the LD_{50} value) of aclarubicin. The thymus seems almost normal. (b) The thymus of a mouse given a large dose (90% of the LD_{50} value) of aclarubicin. Remarkable congestion and disappearance or scattered appearance of lymphocytes are seen.

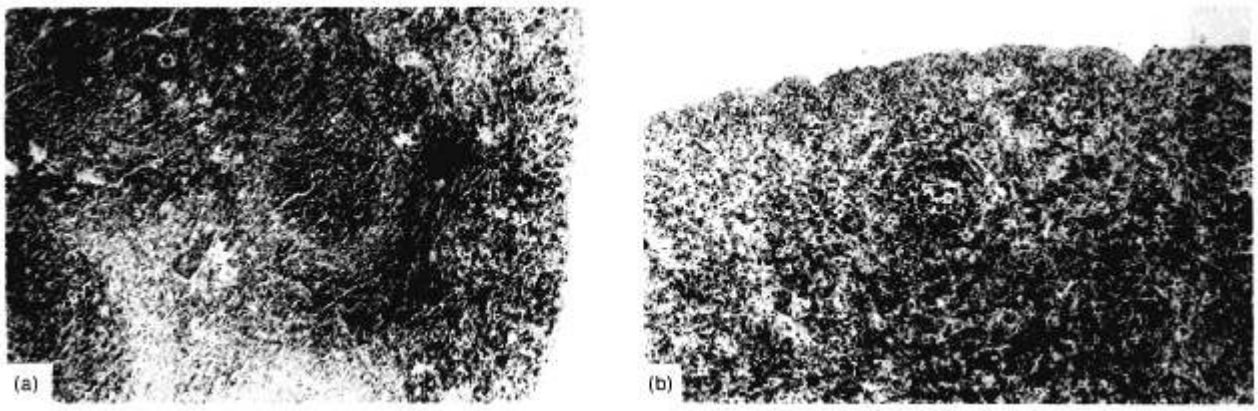

Figure 4. Microscopic view of the spleen (× 32). (a) The spleen of a mouse given a moderate dose (69% of the LD_{50} value) of aclarubicin. Congestion is seen. Lymphocyte number is almost normal, however. (b) The spleen of a mouse given a large dose (90% of the LD_{50} value) of aclarubicin. The decreasing of lymphocytes number is mild as compared with the thymus.

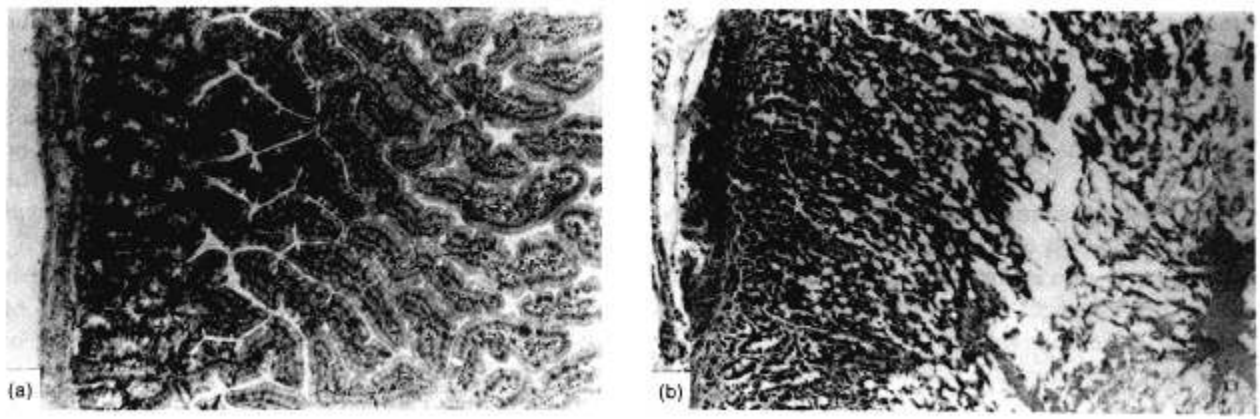

Figure 5. Microscopic view of the intestine (× 32). (a) The intestine of a mouse given a low dose (40% of the LD_{50} value) of aclarubicin. The mucosal layer is normal. (b) The intestine of a mouse given a large dose (90% of the LD_{50} value) of aclarubicin. The mucosal epithelium turns into degeneration.

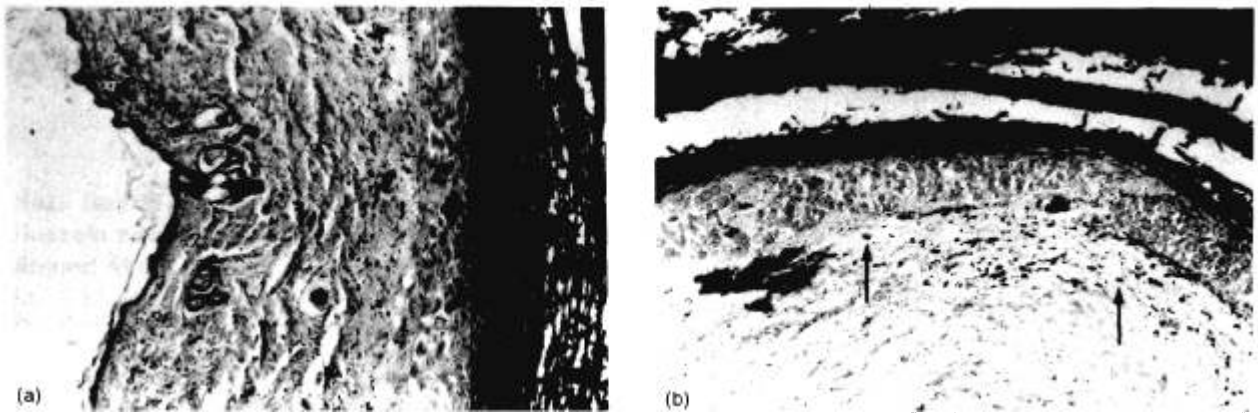

Figure 6. Microscopic view of the injection site (× 32). (a) The skin and subcutaneous tissues contiguous to the injection site. The full thickness of the skin and subcutaneous tissues turns into severe degeneration. The remaining carbon is seen as a black layer (arrow). (b) The subcutaneous tissue and muscle layer contiguous to the injection site. Severe degeneration is induced in the muscle layer and the subcutaneous tissues. Macrophages phagocyting the carbon particles are seen (arrow).

two dosage forms gave similar macro- and microscopic findings. Histological changes in the regional lymph nodes were not examined in the present experiment, because the back, which served as the drug injection site, had no definite regional lymph nodes.

ACR-CH's lethal toxicity was markedly reduced and there were no additional toxic effects induced by the change into the ACR-CH dosage form.

ACR-CH caused severe necrotic changes at the injection site. In the clinical use of ACR-CH for breast cancer, however, ACR-CH is injected pre- and intra-operatively into and around the primary cancer lesion which is then resected during the surgery. In this way the severe inflammatory and necrotic effects at the injection site seen in the mice will cause no clinical problem. We conclude that the lethal toxicity of ACR-CH was reduced to 41.3% of that of ACR-sol. The effects of toxicities were slightly prolonged without an increase in severity and no additional toxic effects were induced by the change of dosage form.

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