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Reduced toxicity of a new formulation—peplomycin adsorbed on activated carbon particles—in mice

Akeo Hagiwara,[1] Toshio Takahashi,[1] Kiyoshi Sawai,[1] Satoshi Shoubayashi,[1] Chouhei Sakakura[1] and Shozo Muranishi[2]

[1]First Department of Surgery, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602, Japan. Fax: (+81) 75 211 7093. [2]Department of Biopharmaceutics, Kyoto College of Pharmacy, Misasagi-nakauchi-cho 5, Yamashina-ku, Kyoto 607, Japan.

We studied the acute toxicity and pathological effects of peplomycin adsorbed on fine activated carbon particles (PEP-CH) injected subcutaneously in mice. The 50% lethal dose value was 41.2 mg/kg in terms of peplomycin, which was 1.52 times that of the peplomycin aqueous solution (PEP-AQ) of 27.1 mg/kg. Deaths occurred from 5 to 31 days after administration of PEP-CH and from 5 to 22 days after administration of PEP-AQ solution. These figures are remarkably different from another report in which the mice given PEP-AQ died within 10 days.

Key words: Activated carbon, peplomycin, toxicity.

Introduction

A new drug-delivery formulation of peplomycin which is adsorbed on fine activated carbon particles (PEP-CH) was developed for chemotherapy against lymph node metastases. We have already reported that as compared with intramuscular peplomycin aqueous solution (PEP-AQ) in dogs, intramuscular PEP-CH selectively delivers a greater concentration of peplomycin to the regional lymph nodes for a longer period, and lower levels of peplomycin are found in the blood plasma and in other organs.[1] PEP-CH also had superior therapeutic effects on lymph node metastases of MH134 mouse ascites hepatoma cell line when compared with the same dose of PEP-AQ.[2] This paper describes the reduced toxicity of PEP-CH in mice.

Materials and Methods

Preparation of PEP-CH

PEP-CH is a suspension of activated carbon particles adsorbing peplomycin. Activated carbon M-1500 (1500 m^2/g in specific surface area and 20 nm in diameter), which was prepared in our laboratory, was used. Activated carbon particles (50 mg/ml) and 30 mg/ml polyvinylpyrrolidone (PVP K-30®, Nakarai Chemicals Co., Ltd, Kyoto, Japan) were added to saline and the mixture was kneaded with three rollers into a suspension of which the particle size was 150 nm on average.[3] Peplomycin, one of the bleomycin derivatives[4] (Pepleo Inj®, Nippon Kayaku Co., Ltd, Tokyo, Japan) at 25 mg/ml was dissolved in the activated carbon suspension and the resulting solution was shaken for 2 h so that the dissolved peplomycin would be adsorbed onto the activated carbon. PEP-CH, composed of 50 mg/ml activated carbon, 30 mg/ml polyvinylpyrrolidone and 25 mg/ml peplomycin, was diluted with saline to the required concentrations (5.5–12.0 mg/ml).

As PEP-AQ, peplomycin was dissolved in saline to the required concentrations (4.2–9.2 mg/ml) in aqueous solution. The activated carbon suspension without peplomycin, prepared as described, was another control drug.

Toxicity in mice

One hundred and sixty male mice (C_3H strain, 5 weeks old) were purchased from the Shimizu Laboratory Animal Center (Kyoto, Japan). 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 the end of the experiment. On day 0, after being acclimatized to breeding for 5 days, the mice (19–23 g in body weight) were divided into 16 groups of 10 mice each. Seven groups were given PEP-CH, another seven groups were given PEP-AQ, another group was given activated carbon suspension without peplomycin

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Correspondence to A Hagiwara

and the last group was given nothing. The drugs were given in 0.1 ml of volume subcutaneously at the left thorax with a 27 gauge needle. In the seven groups given PEP-CH, dosages ranging from 26 to 57 mg peplomycin/kg of body weight were given in seven dose steps, which were increased at a rate of 1.14/step. In the other seven groups given PEP-AQ, dosages ranging from 20 to 44 mg of peplomycin/kg of body weight were given in seven dose steps, increasing at the same rate. The group given activated carbon suspension without peplomycin was given a dosage of 114 mg of activated carbon/kg of body weight, which corresponded to the amount of activated carbon in the PEP-CH (peplomycin of 57 mg/kg dose).

The mice were observed daily for 36 days after administration, and the body weight change and the date of death were recorded. The surviving animals were sacrificed on day 36. The 50% lethal dose value (LD_{50}) was calculated for each dosage formulation using Litchfield–Wilcoxon's method. All animals were autopsied for macroscopic and microscopic changes in body tissues. The lungs, thymus, heart, liver, spleen, kidneys and testes were removed; the weights of these organs were recorded. These organs were prepared for microscopic examination with hematoxylin–eosin stain. Since the main toxic effect of bleomycin (of which peplomycin is a derivative) is pulmonary fibrosis, the lungs were prepared with Mallory–Azan stain to clearly detect such fibrosis.

Results

LD_{50} value

The LD_{50} value of each dosage formulation was as follows: PEP-CH = 41.2 mg/kg (38.0–44.3 mg/kg at 95% level of confidence) in terms of peplomycin and PEP-AQ = 27.1 mg/kg (25.0 to 29.2 mg/kg at 95% level of confidence).

There were no deaths in mice given activated carbon suspension without peplomycin.

Toxic symptoms, body weight change and date of deaths

The toxic symptoms in mice given PEP-CH were similar to those in mice given PEP-AQ. Doses close to the LD_{50} values for either formulation caused weakness beginning on day 0, lethargy and eyelid discharge begining on day 2, and dishevelment and emaciation increasing with time. In mice given high doses of peplomycin (more than 30 mg/kg for PEP-AQ or 50 mg/kg for PEP-CH), abnormal behavior such as round-running, rolling and ataxia were notable symptoms. The mice given high doses of peplomycin were dead within 10 days after administration. However, in groups given relatively low doses, some of the mice were dead on later days ranging from 12 to 31 days after PEP-CH administration or from 11 to 22 days after PEP-AQ administration (Tables 1 and 2).

Table 1. Mortality of mice given PEP-CH

Dose of peplomycin (mg/kg)	Mortality	Date of death (days after administration)
57.0	10/10	5, 5, 5, 6, 6, 6, 6, 6, 6, 6
50.0	10/10	7, 8, 9, 10, 10, 10, 10, 12, 16, 18
43.9	7/10	7, 7, 7, 7, 8, 14, 23
38.5	3/10	11, 26, 31
33.8	1/10	15
29.6	0/10	—
26.0	0/10	—

Table 2. Mortality of mice given PEP-AQ

Dose of peplomycin (mg/kg)	Mortality	Date of death (days after administration)
43.9	10/10	5, 6, 6, 6, 6, 6, 6, 7, 7, 7
38.5	10/10	5, 6, 6, 7, 7, 10, 10, 12, 13, 16
33.8	8/10	5, 6, 6, 6, 6, 12, 18, 22
29.6	9/10	8, 8, 10, 10, 11, 11, 11, 12, 16
26.0	4/10	6, 7, 12, 14
22.8	0/10	—
20.0	0/10	—

Body weight changes are shown in Figures 1–3. In the mice given PEP-CH at doses close to the

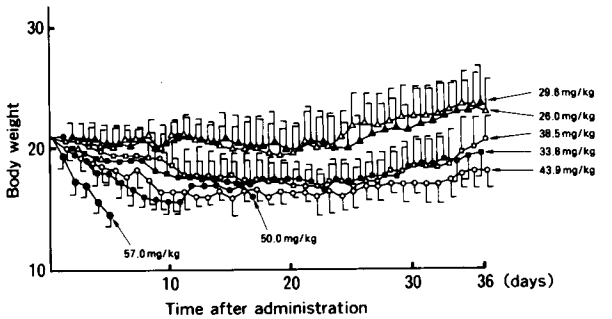

Figure 1. Body weight changes in mice given PEP-CH. In the mice given PEP-CH at peplomycin doses of 38.5 or 43.9 mg/kg, which were close to the LD_{50} value, body weight decreased for the first 21 or 25 days. Body weight began to increase on day 27, but had not returned to its pre-administration level by day 36.

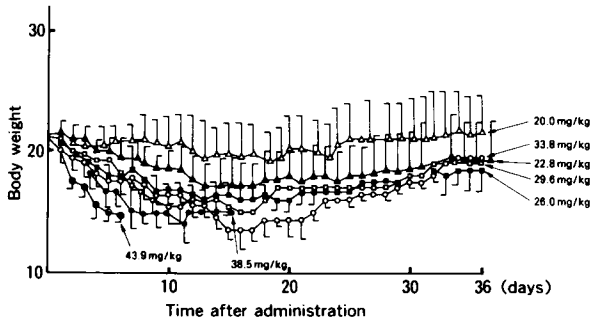

Figure 2. Body weight changes in mice given PEP-AQ. In mice given aqueous peplomycin solutions at doses of 26.0 or 29.6 mg/kg, which were close to the LD_{50} value, body weight decreased for the first 13 or 10 days. Body weight began to increase on day 21 or 17, but had not returned to its pre-administration level by day 36.

LD_{50} value, body weight decreased for the first 21–25 days and began to increase on day 27. The body weight that was lost was not fully recovered by day 36 (Figure 1). In mice given PEP-AQ at doses close to the LD_{50} value, the body weight decreased until days 10–13, and began to increase on days 17–21 and was not fully restored to the preadministration level by day 36 (Figure 2). Body weight increased similarly in the mice given activated carbon without peplomycin and those that were given nothing (Figure 3).

Autopsy findings

The autopsy findings were similar in mice given PEP-CH and those given PEP-AQ, except for the accumulation of activated carbon in the regional lymph nodes and injection site. The organ weight of mice surviving until day 36 is shown in Tables 3 and 4.

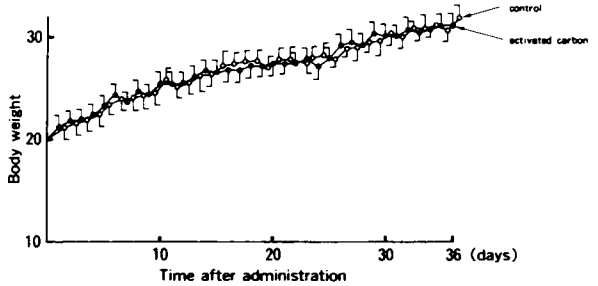

Figure 3. Body weight changes in mice given nothing and in mice given activated carbon only. The body weights were increased with time to a similar extent in each group.

Table 3. Organ weight change in surviving mice given PEP-CH

Organ[a]	Peplomycin dose (mg/kg)					
	Control[b]	26.0	29.6	33.8	38.5	43.9
Lung	0.27	0.20	0.23	0.18	0.22	0.23
(SD)	(0.03)	(0.03)	(0.03)	(0.04)	(0.03)	(0.01)
Heart	0.15	0.11	0.12	0.10	0.11	0.12
(SD)	(0.01)	(0.01)	(0.01)	(0.02)	(0.01)	(0.02)
Liver	1.82	1.30	1.43	1.20	1.43	1.37
(SD)	(0.19)	(0.17)	(0.29)	(0.13)	(0.22)	(0.19)
Kidney	0.29	0.18	0.19	0.15	0.18	0.18
(SD)	(0.03)	(0.04)	(0.03)	(0.02)	(0.03)	(0.03)
Spleen	0.09	0.06	0.06	0.05	0.06	0.05
(SD)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.01)
Thymus	0.04	0.04	0.04	0.02	0.02	0.02
(SD)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.00)
Testis	0.08	0.09	0.09	0.07	0.07	0.06
(SD)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.02)

[a] Mean organ weight (in g).
[b] Mice given activated carbon without peplomycin.

The weights of lung, heart, liver and kidney were lower in mice given peplomycin preparations than in the control mice. However, the weights did not decrease with the dose of the peplomycin preparations (Tables 3 and 4). The lungs looked slightly congestive, and the liver and the kidney were anemic. Microscopically, no fibrous changes were found in the lung, even in the specimens stained by the Mallory–Azan method, and there were no remarkable changes in the liver and the kidney.

Table 4. Organ weight change in surviving mice given PEP-AQ

Organ[a]	Peplomycin dose (mg/kg)					
	Control[b]	20.0	22.8	26.0	29.6	33.8
Lung	0.27	0.23	0.22	0.21	0.22	0.25
(SD)	(0.03)	(0.03)	(0.03)	(0.02)	—	—
Heart	0.15	0.11	0.09	0.10	0.11	0.13
(SD)	(0.01)	(0.02)	(0.01)	(0.03)	—	—
Liver	1.82	1.28	1.20	1.33	1.38	1.39
(SD)	(0.19)	(0.22)	(0.25)	(0.91)	—	—
Kidney	0.29	0.20	0.16	0.15	0.16	0.19
(SD)	(0.03)	(0.04)	(0.03)	(0.02)	—	—
Spleen	0.09	0.06	0.05	0.05	0.04	0.04
(SD)	(0.01)	(0.02)	(0.01)	(0.01)	—	—
Thymus	0.04	0.04	0.03	0.03	0.02	0.02
(SD)	(0.01)	(0.01)	(0.01)	(0.01)	—	—
Testis	0.08	0.09	0.06	0.06	0.05	0.06
(SD)	(0.01)	(0.01)	(0.01)	(0.01)	—	—

[a] Mean organ weight (in g).
[b] Mice given activated carbon without peplomycin.

Weights of other organs (spleen, thymus and testes) tended to decrease with the peplomycin dose in both dosage formulations (Tables 3 and 4). In the thymus and the spleen of the mice dying due to toxicity, severe lymphoid hypoplasia was seen on microscopic examination. However, in mice surviving up to day 36, these microscopic findings of lymphoid hypoplasia were improved. For the testes, there were no loosened structures of the spermatogenesis cells under microscopic examination.

Discussion

We have reported that PEP-CH delivers lower levels of peplomycin to whole body tissues (except for the injection site and the regional lymph nodes) than conventional PEP-AQ.[1] This drug distribution suggests that the systemic toxicity of PEP-CH will be reduced. The present examination shows that, compared with PEP-AQ, the lethal toxicity of PEP-CH is markedly reduced. Deaths of mice given PEP-CH occurred at a later date than those of mice given PEP-AQ. These results suggest that the toxicity of PEP-CH is extended over a longer period as compared with that of PEP-AQ. This difference is probably caused by PEP-CH slowly releasing peplomycin.

Itoh *et al.*[5] reported that the LD_{50} value of PEP-AQ was 88 mg/kg and that ICR mice were dead within 10 days after administration in an acute toxicity test. The LD_{50} value and the date of deaths in our present experiment are remarkably different from those reported by Itoh *et al.* In our experiment, the body weight loss was not recovered for longer than 10 days and the mice died later than 10 days. These prolonged body weight losses and late deaths induced by peplomycin suggest that the toxic effects of peplomycin last for longer periods of time than those reported by Itoh *et al.* who used mice of another strain. Previously, we reported that PEP-CH has remarkably reduced pulmonary fibrosis in ICR mice as compared with PEP-AQ.[6] Surprisingly, in the present experiment, neither PEP-CH nor PEP-AQ brought about definite pulmonary fibrosis as described by Itoh's report and our previous report. The above-mentioned differences between the present experiment and Itoh's experiment or our previous experiment may be due to the difference in the strain of mice.

Toxic symptoms and autopsy findings were similar for the two peplomycin dosage formulations. The activated carbon without peplomycin caused no toxic effects.

Conclusion

We conclude that (i) the lethal toxicity of PEP-CH is 65.7% that of PEP-AQ, (ii) PEP-AQ does not induce a new kind of toxicity, but the toxic effects are slightly prolonged and (iii) the activated carbon suspension, at these doses, has no toxic effects.

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