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# New formulation of 5-fluorouracil in microspheres reduces toxicity in mice

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**A new dosage formulation of 5-fluorouracil incorporated in microspheres (5-FU-MS) was developed for the treatment of peritoneal carcinomatosis. We studied the acute toxicity and side effects of i.p. 5-FU-MS in mice. The 50% lethal dose value for 5-FU-MS was 535.4 mg/kg of 5-FU, which was 2.22 times that of the aqueous 5-FU solution. Deaths occurred 12–17 days after the administration of 5-FU-MS, but within 11 days after the administration of aqueous 5-FU. Thus, lethal toxicity appeared later with 5-FU-MS than with aqueous 5-FU. There were no differences in pathologic findings on autopsy between mice given the two dosage formulations.**

*Key words*: 5-Fluorouracil, intraperitoneal chemotherapy, microspheres, toxicity.

## Introduction

The i.p. administration of 5-fluorouracil (5-FU) is one effective form of i.p. chemotherapy.[1] The i.p. continuous infusion of aqueous 5-FU solution using totally implantable devices selectively delivers high concentrations of 5-FU to the peritoneal cavity for long periods of time.[2] We have developed 5-FU-MS, which is composed of 5-FU incorporated into microspheres of a lactide/glycolide copolymer, as another drug-delivery formulation for i.p. chemotherapy. Preliminary experiments in rats revealed that, as compared to i.p. aqueous 5-FU, i.p. 5-FU-MS selectively delivers greater concentrations of 5-FU to the i.p. tissues for longer periods of time, and lower levels of 5-FU are found in the rest of the body and in the blood plasma.[3] 5-FU-MS has superior therapeutic effects on peritoneal carcinomatosis induced by the mouse B-16 melanoma cell line, when compared to the same dose of aqueous 5-FU solution.[1] In the present study, we examined the lethal dose, histologic changes in organ tissues, body weight changes and intoxication symptoms of i.p. 5-FU-MS and aqueous 5-FU solution in mice.

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## Materials and methods

### Preparation of 5-FU-MS

We used a lactide/glycolide copolymer[4] (PLGA; Biodegmer®, Biomaterials Universe, Kyoto, Japan), which is composed of lactide and glycolide at a molar ratio of 4:1 and has a molecular weight of 14 000, as a biodegradable microspheres matrix. 5-FU (50 mg) and PLGA (450 mg) were dissolved in 97% acetic acid. The resulting solution was emulsified in liquid paraffin. The emulsion was stirred at 250 r.p.m. at 30°C for 48 h, to form microspheres containing 5-FU by evaporation. The microspheres were rinsed with hexane, dried under a vacuum and passed through a sieve. Microspheres with an average diameter of 24 $\mu$m were used for this study. Microspheres were stored dry at −10°C. 5-FU-MS is designed to release 70% of the incorporated 5-FU during the first 7 days and all of the 5-FU over 3 weeks in saline or phosphate buffered solution *in vitro*. 5-FU-MS was suspended in physiological saline with 0.01% Tween 80 to keep the microspheres well dispersed. The microsphere suspension was administered within 1 h after preparation.

### Toxicity in mice

One hundred twenty-six male mice ($BDF_1H$ 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 food and tap water from 5 days before drug administration until the end of the experiment. On day 0, after being acclimatized to the living conditions for 5 days, the mice (20–22 g in body weight) were divided into 18 groups of seven mice each. Eight groups were given 5-FU-MS, eight groups were given aqueous 5-FU, one group was given a suspension of microspheres without 5-FU (empty microspheres) and the last group was given nothing. The drugs were given in a volume of 1 ml i.p. using a 20 gauge needle. In the eight groups given 5-FU-MS, doses ranging from 223.3 to 800.0 mg/kg of 5-FU were given in eight dose levels, which were increased at a rate of 1.2-fold/level. In the eight groups given aqueous 5-FU, doses ranging from 134.0 to 480.0 mg/kg of 5-FU were given in eight dose levels, increasing at the same rate. The dose of the empty microsphere suspension was 7200 mg of microspheres/kg of body weight, which corresponded to the amount of microspheres in 5-FU-MS at a 5-FU dose of 800 mg/kg.

The mice were observed daily for 21 days after the drug administration, and body weight changes and date of death were recorded. The surviving animals were sacrificed on day 22. The 10, 50 and 90% lethal dose values ($LD_{10}$, $LD_{50}$ and $LD_{90}$ values) were calculated for each dosage formulation, using the probit method.[5] All animals were autopsied for macroscopic and microscopic changes in their body tissues. The lungs, heart, liver, spleen, kidneys, adrenal glands, thymus, intestines and bone marrow were removed. The weights of the lungs, heart, liver and spleen were recorded. The organs were prepared for microscopic examination with hematoxylin–eosin stain.

## Results

### Lethal dose values

The $LD_{10}$, $LD_{50}$ and $LD_{90}$ values for 5-FU-MS were 382.8, 535.4 (472.4–611.1: 95% level confidence interval) and 748.8 mg/kg in terms of 5-FU, respectively (Table 1). The $LD_{10}$, $LD_{50}$ and $LD_{90}$ values for aqueous 5-FU were 179.4, 241.6 (215.4–270.8: 95% level confidence interval) and 325.5 mg/kg, respectively (Table 1). The lethal toxicity of 5-FU-MS was less than half that of the aqueous 5-FU solution.

**Table 1.** Lethal dose values of the 5-FU formulations

| Dose values | Aqueous 5-FU (mg/kg) | 5-FU-MS (mg/kg) |
|---|---|---|
| $LD_{10}$ | 179.4 | 382.8 |
| $LD_{50}$ | 241.6 | 535.4 |
| (95% confidence level) | (215.4–270.8) | (472.4–611.1) |
| $LD_{90}$ | 325.5 | 748.8 |

There were no deaths in mice given empty microspheres.

### Intoxication symptoms, body weight changes and date of death

The intoxication symptoms in mice given 5-FU-MS were similar to those in mice given aqueous 5-FU. Doses close to the $LD_{50}$ values for either formulation caused weakness beginning on day 0 or 1, lethargy beginning on day 2 or 3, and dishevelment and emaciation that increased over time.

In the mice given aqueous 5-FU, all the deaths were seen within 11 days (9 days in median) of administration of the drug (Table 2). However, in the groups given 5-FU-MS, the mice died later, 12–17 days (14 days in median) after administration of the drug (Table 3). The dates of deaths were different

**Table 2.** Mortality of mice given aqueous 5-FU

| Dose of 5-FU (mg/kg) | Mortality rate | Date of death (days after drug administration) |
|---|---|---|
| 134.0 | 0/7 | — |
| 160.8 | 0/7 | — |
| 192.9 | 1/7 | 11 |
| 231.5 | 4/7 | 9, 10, 11, 11 |
| 277.8 | 5/7 | 8, 10, 11, 11, 11 |
| 333.3 | 6/7 | 8, 8, 9, 9, 9, 10 |
| 400.0 | 7/7 | 7, 7, 8, 8, 9, 10, 10 |
| 480.0 | 7/7 | 8, 8, 8, 9, 10, 11, 11 |

**Table 3.** Mortality of mice given 5-FU-MS

| Dose of 5-FU (mg/kg) | Mortality rate | Date of death (days after drug administration) |
|---|---|---|
| 223.3 | 0/7 | — |
| 267.9 | 0/7 | — |
| 321.5 | 0/7 | — |
| 385.8 | 1/7 | 14 |
| 463.0 | 2/7 | 14, 14 |
| 555.6 | 4/7 | 13, 13, 14, 16 |
| 666.7 | 5/7 | 13, 13, 14, 14, 16 |
| 800.0 | 7/7 | 12, 12, 14, 14, 16, 17, 17 |

significantly ($p \ll 0.01$) between the two drug formulations.

In the mice given 5-FU-MS at doses close to the $LD_{50}$ value, weight loss continued for the first 13–15 days and recovered to pre-administration levels on days 14–16 (Figure 1). In mice given aqueous 5-FU at doses close to the $LD_{50}$ value, the weight loss also continued until day 13 or 14, and recovered to pre-administration levels on day 14 or 15 (Figure 2). Body weights increased similarly in the mice given empty microspheres and those given nothing (Figure 3).

## Autopsy findings

The macroscopic and microscopic autopsy findings were similar in mice given 5-FU-MS and those given aqueous 5-FU, except for the accumulation of microspheres on the peritoneum in mice given 5-FU-MS. The lungs appeared slightly congested, and the liver and kidneys were anemic. The adrenal glands, thymus and spleen were atrophic. Severe hypoplasia was seen in these organs and in the bone marrow of mice dying from toxicity. The intestinal mucosa of dead mice showed severe erosion, sometimes combined with hemorrhagic changes. Microscopically, the erosion and hemorrhage were accompanied by necrotic or degenerative changes, atrophy of the mucosal layer, dilatation of the glandular lumens, infiltration by inflammatory cells and interstitial hemorrhage. However, in mice surviving up to day 22, these microscopic findings were improved.

The weights of the lung, heart, liver and kidney were lower in dead mice than in the survivors (Tables 4–7). There were no significant differences between the two dosage formulations. In the dead mice, the spleen was more atrophic than the other organs. This trend was more marked in dead mice given aqueous 5-FU than in those given 5-FU-MS.

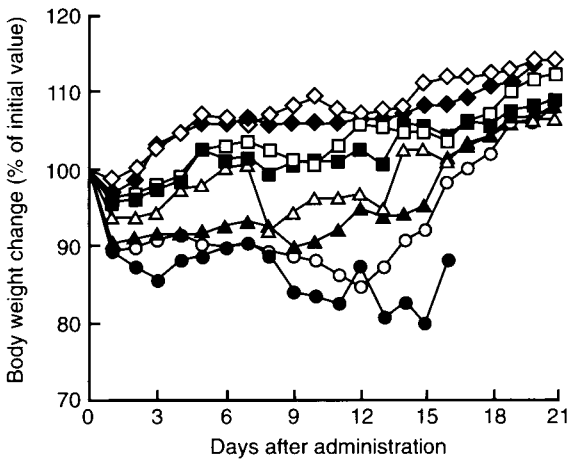


**Figure 1.** Body weight changes in mice given 5-FU-MS. In the mice given 5-FU-MS at 5-FU doses of 463.0 or 555.6 mg/kg, which were close to the $LD_{50}$ value, weight loss continued for the first 13–15 days. Body weights returned to pre-administration levels on day 14 or 16. ●, 800 mg/kg; ○, 666.7 mg/kg; ▲, 555.6 mg/kg; △, 463.0 mg/kg; ■, 385.8 mg/kg; □, 321.5 mg/kg; ◆, 267.9 mg/kg; ◇, 223.3 mg/kg.

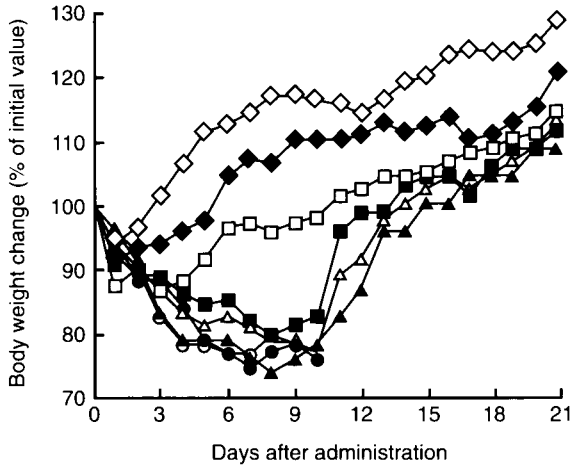


**Figure 2.** Body weight changes in mice given aqueous 5-FU. In mice given aqueous 5-FU at doses of 231.5 or 277.8 mg/kg, which were close to the $LD_{50}$ value, body weight loss continued for the first 13–14 days, and recovered to pre-administration levels on day 14 or 15. ●, 480.0 mg/kg; ○, 400.0 mg/kg; ▲, 333.3 mg/kg; △, 277.8 mg/kg; ■, 231.5 mg/kg; □, 192.9 mg/kg; ◆, 160.8 mg/kg; ◇, 134.0 mg/kg.

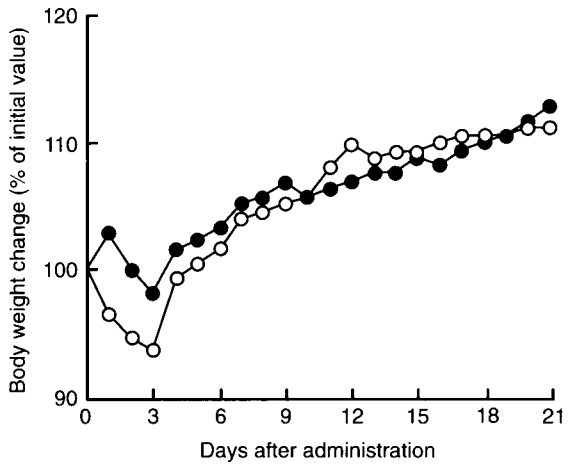


**Figure 3.** Body weight changes in untreated mice and in mice given microspheres without 5-FU. The body weights increased over time to a similar extent in each group. ●, Mice given nothing; ○, mice given empty microspheres.

**Table 4.** Organ weight changes in surviving mice given 5-FU-MS

| 5-FU dose | Weight of organ [g (mean)] (SD) | | | |
|---|---|---|---|---|
| | Heart | Liver | Spleen | Kidney |
| 0 | 0.188 (0.012) | 1.80 (0.120) | 0.141 (0.015) | 0.440 (0.022) |
| 223.3 | 0.172 (0.020) | 1.80 (0.020) | 0.126 (0.009) | 0.447 (0.021) |
| 267.9 | 0.175 (0.015) | 1.72 (0.180) | 0.126 (0.010) | 0.457 (0.018) |
| 321.5 | 0.158 (0.085) | 1.50 (0.168) | 0.121 (0.012) | 0.396 (0.052) |
| 385.8 | 0.157 (0.085) | 1.47 (0.171) | 0.119 (0.013) | 0.379 (0.040) |
| 463.0 | 0.150 (0.015) | 1.63 (0.013) | 0.151 (0.017) | 0.242 (0.083) |
| 555.6 | 0.139 (0.003) | 1.85 (0.160) | 0.265 (0.104) | 0.398 (0.023) |

**Table 5.** Organ weight changes in dead mice given 5-FU-MS

| 5-FU dose | Weight of organ [g (mean)] (SD) | | | |
|---|---|---|---|---|
| | Heart | Liver | Spleen | Kidney |
| 385.8 | 0.128 (—)[a] | 1.33 (—)[a] | 0.11 (—)[a] | 0.33 (—)[a] |
| 463.0 | 0.157 (0.002) | 1.40 (0.065) | 0.127 (0.004) | 0.341 (0.005) |
| 555.6 | 0.154 (0.006) | 1.11 (0.334) | 0.104 (0.032) | 0.333 (0.041) |
| 666.7 | 0.138 (0.013) | 1.31 (0.297) | 0.112 (0.017) | 0.345 (0.060) |
| 800.0 | 0.120 (0.019) | 1.09 (0.164) | 0.09 (0.014) | 0.277 (0.021) |

[a]Number of sample = one.

In mice given empty microspheres, there were none of the pathologic changes described above, except for the i.p. accumulation of the empty microspheres.

## Discussion

We have reported previously that 5-FU-MS delivers lower levels of 5-FU to body tissues, except for i.p. tissues, than does a conventional aqueous 5-FU solution.[1] This drug distribution suggests that the systemic toxicity of 5-FU-MS will be reduced.

The present study reveals that the lethal dose of 5-FU-MS is increased markedly, as compared to that of aqueous 5-FU. The deaths of mice given 5-FU-MS occurred at later dates than those of mice given the aqueous 5-FU. This result indicates that the toxicity of 5-FU-MS is extended over a longer period, as compared to that of aqueous 5-FU. This difference is probably due to the slow release of 5-FU from the microspheres.

5-FU has activity against wide spectrum of cancers.[6] Both in clinical treatment[7,8] and animal experiments,[9,10] the toxicity of 5-FU is characterized by bone marrow suppression, damage to lymphoid

**Table 6.** Organ weight changes in surviving mice given aqueous 5-FU

| 5-FU dose | Weight of organ [g (mean)] (SD) | | | |
|---|---|---|---|---|
| | Heart | Liver | Spleen | Kidney |
| 0 | 0.188 (0.012) | 1.80 (0.12) | 0.141 (0.015) | 0.440 (0.022) |
| 134.0 | 0.208 (0.010) | 1.94 (0.122) | 0.143 (0.039) | 0.468 (0.041) |
| 160.8 | 0.211 (0.017) | 1.83 (0.109) | 0.141 (0.032) | 0.432 (0.036) |
| 195.9 | 0.203 (0.029) | 1.88 (0.118) | 0.156 (0.049) | 0.427 (0.026) |
| 231.5 | 0.180 (0.015) | 2.03 (0.146) | 0.162 (0.019) | 0.440 (0.015) |
| 277.8 | 0.174 (0.010) | 2.02 (0.183) | 0.205 (0.035) | 0.450 (0.040) |
| 333.3 | 0.153 (—)[a] | 2.26 (—)[a] | 0.225 (—)[a] | 0.428 (—)[a] |

[a]Number of sample = one.

**Table 7.** Organ weight changes in dead mice given aqueous 5-FU

| 5-FU dose | Weight of organ [g (mean)] (SD) | | | |
|---|---|---|---|---|
| | Heart | Liver | Spleen | Kidney |
| 192.9 | 0.14 (—)[a] | 0.98 (—)[a] | 0.105 (—)[a] | 0.297 (—)[a] |
| 231.5 | 0.128 (0.010) | 1.018 (0.167) | 0.093 (0.018) | 0.307 (0.032) |
| 277.8 | 0.113 (0.007) | 1.022 (0.193) | 0.075 (0.010) | 0.288 (0.018) |
| 333.3 | 0.124 (0.021) | 0.982 (0.263) | 0.075 (0.021) | 0.298 (0.027) |
| 400.0 | 0.115 (0.019) | 0.867 (0.272) | 0.054 (0.023) | 0.286 (0.029) |
| 480.0 | 0.118 (0.009) | 0.703 (0.152) | 0.059 (0.007) | 0.266 (0.028) |

[a]Number of sample = one.

tissues and toxic effects on the mucosa of the digestive tract. The autopsy and histologic findings in the present study show that the two 5-FU dosage formulations induced similar toxic changes in the digestive tract, lymphoid tissues and bone marrow. There were no additional histologic changes in mice given 5-FU-MS, except for the accumulation of microspheres in the peritoneal cavity. Intraperitoneal 5-FU-MS caused similar intoxication symptoms to aqueous 5-FU. These results suggest that the toxic side effects of 5-FU-MS and aqueous 5-FU solution are identical, and that no additional toxicity is induced by the change in the dosage form.

In the present study, the acute toxic effects on laboratory data, such as hematologic analysis and chemical analysis of the blood and urine, were not examined. Although further studies with such analyses must be performed, the present results suggest that (i) the lethal dose of 5-FU-MS is more than twice that of aqueous 5-FU, (ii) there is no evidence of new toxicities, but the toxic effects of 5-FU are slightly prolonged with the change in dosage form, and (iii) empty microspheres, at these doses, induce no toxicity.

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