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Short report

Therapeutic effects of 5-fluorouracil microspheres on peritoneal carcinomatosis induced by Colon 26 or B-16 melanoma in mice

Akeo Hagiwara, Chouhei Sakakura, Morio Shirasu, Junya Yamasaki, Tsuyoshi Togawa, Toshio Takahashi, Shozo Muranishi,[1] Suong-hyu Hyon[2] and Yoshihito Ikada[2]

First Department of Surgery, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602, Japan. Fax: (+81) 75 251 552; Tel: (+81) 75 251 5527. [1]Department of Biopharmaceutics, Kyoto College of Pharmacy, [2]Research Center for Medical Polymers, Kyoto University, Shogoin, Kawara-cho 53, Sakyo-ku, Kyoto 602, Japan.

The delivery formulation 5-FU-MS [5-fluorouracil (5-FU) incorporated in microspheres composed of a poly(glycolide-co-lactide) matrix] slowly releases 5-FU over 3 weeks. 5-FU-MS delivers higher concentrations of the drug to the i.p. tissues for a longer period of time with lower blood plasma concentrations than does an aqueous 5-FU solution and reduces toxicity. In this study, we evaluated the therapeutic effects of 5-FU-MS on peritoneal carcinomatosis in mice. Four days after an i.p. inoculation with Colon 26 or B-16 PC melanoma, 5-FU at 200 mg/kg was administered i.p. as 5-FU-MS or as an aqueous solution of 5-FU. 5-FU-MS extended the survival of mice bearing Colon 26 or B-16 PC melanoma significantly better than the equivalent dose of aqueous 5-FU solution. [© 1998 Rapid Science Ltd.]

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Introduction

Peritoneal carcinomatosis is one of the most common modes of recurrent disease in digestive as well as ovarian cancers. Intraperitoneal anticancer drugs in an aqueous solution are common treatments for peritoneal carcinomatosis. However, they are not always effective because it is difficult to maintain drug concentrations at efficacious levels for long periods of time in aqueous solutions in the peritoneal cavity. Small, water-soluble molecules, such as aqueous 5-fluorouracil (5-FU) solution, are rapidly absorbed into systemic circulation through blood capillaries located in the subperitoneum.[1] In contrast, corpuscular particles such as microspheres are gradually absorbed and retained in the peritoneal cavity for long periods.[1] Utilizing the difference in absorption through the peritoneum between aqueous solutions and corpuscular particles, we developed a new formulation (5-FU-MS) which consists of microspheres containing 5-FU. An animal experiment has been reported in which i.p. 5-FU-MS delivers concentrated 5-FU for a longer time to the i.p. tissues, while lower concentrations of the drug are seen in the rest of the body.[2] In the present study, we examined the therapeutic effects on peritoneal carcinomatoses induced by two kinds of transplantable cancers in mice.

Correspondence to A Hagiwara

Materials and methods

Drug preparation

Poly(glycolide-co-lactide) (Biodegmer^{R}; Biomaterials Universe, Kyoto, Japan; an average molecular weight of 14 000) was used to synthesize the microspheres which serve as the drug carrier. 5-FU (a gift from Kyowa Hakko Kogyo, Tokyo, Japan) was used as the anticancer drug.

5-FU-MS, consisting of 5-FU incorporated in microspheres of poly(glycolide-co-lactide) matrix, was prepared using a water-in-oil emulsion method. A mixture of 10 mg/ml 5-FU and 90 mg/ml poly(glycolide-co-lactide) was dissolved in 97% of acetic acid. The resulting solution was emulsified in 10 volumes of

liquid paraffin by stirring at 250 r.p.m. for 2 days at 30 C. The emulsion was formed into microspheres containing 5-FU using an evaporation method. The microspheres were vacuum dried for 2 days and sieved. The fraction with an average diameter of 24 μm was used for the study. A suspension of 5-FU-MS in saline with 0.01% Tween 80 to keep the microspheres well dispersed was administered. As a control, an aqueous solution of 5-FU for clinical use (5-FU Kyowa®; Kyowa Hakko Kogyo) was diluted with saline.

Therapeutic examination

One hundred male BALB/c mice, aged 5 weeks and maintained under standard conditions, were purchased from Shimizu Laboratory Animal Center (Kyoto, Japan). Colon 26 (a kind gift from Shionogi Pharmaceutical, Osaka, Japan), which induces peritoneal carcinomatosis when inoculated i.p., was used as the experimental tumor and maintained i.p. in BALB/c carrier mice. Ascites fluid containing free Colon 26 cells was taken from the mice. Colon 26 cells were suspended in Hanks' solution. The cell viability was greater than 95%, as determined by the Trypan blue exclusion test. The 100 mice received an i.p. inoculation with 10^6 cells/mouse of free Colon 26 cells on day 0.

The drugs were given on day 4, because a preliminary experiment showed that i.p. inoculated Colon 26 cells had established peritoneal metastases 4 days after the inoculation. The mice were divided into five equal groups of 20 mice each. Group 1 received i.p. administration of a suspension of 5-FU-MS at a 5-FU dose of 200 mg/kg (the 5-FU-MS group). Group 2 received i.p. aqueous 5-FU solution at the same 5-FU dose (the 5-FU solution group). Group 3 received empty microspheres and aqueous 5-FU solution (the empty-MS+5-FU solution group) i.p. Group 4 received only the empty microspheres (the empty-MS group) i.p. Group 5 received no treatment (control group). Since the 5-FU-MS group (Group 1) receiving 200 mg/kg in terms of 5-FU also received 1.8 g/kg in terms of the MS matrix, the empty-MS group (Group 4) was given 1.8 g/kg of empty-MS suspended in saline. In the empty-MS+5-FU solution group (Group 3), 200 mg/kg of the 5-FU solution plus 1.8 g/kg of the empty-MS were given. Dead mice underwent autopsy, and were examined macroscopically and microscopically to determine whether the cause of death was drug toxicity or cancer. The survivors were sacrificed on day 150 and examined for the remaining cancer tissues microscopically.

The therapeutic effect on the survival times was compared by the log-rank test between the various formulations. When the probability value (p) was less than 0.05, the difference was defined to be statistically significant.

A similar therapeutic study was carried out using BDF1 male mice who were inoculated i.p. with B-16 PC melanoma cells at 10^6 cells/mouse. The mice received humane care according to the institutional guidelines for the use of animals in research, testing and education.

Results and discussion

The therapeutic results against Colon 26 (Table 1) show that 5-FU-MS (Group 1) extended the survival of mice bearing Colon 26 significantly better ($p < 0.001$) than the same dose of aqueous 5-FU (Group 2). The median survival was 32 days (T/C% of 142%) with 5-FU-MS and 27 days (T/C% of 120%) with 5-FU solution. 5-FU solution plus empty-MS extended the median survival to 26.5 days (T/C% of 118%) (Group 3), which was not significantly different in the therapeutic effect from the 5-FU solution group (Group 2). The median survival time in the empty-MS group (Group 4) was 22

Table 1. Therapeutic effects of 5-FU solution and 5-FU-MS against Colon 26

Group	Treatment	Median survival (days [range])	T/C%[a]	No. of survivors[b]	No. of toxic deaths[c]
1	5-FU-MS	32 [25 – > 150]	142	1	0
2	5-FU solution	27 [25 – 35]	120	0	0
3	empty-MS+5-FU solution	26.5 [25 – 33]	118	0	0
4	empty-MS	22 [20 – 25]	98	0	0
5	control	22.5 [20 – 25]	100	0	0

[a]Median survival day in the treatment group/median survival day in the control group.
[b]Number of mice surviving for 150 days after cancer cells inoculation.
[c]Number of mice dead from drug toxicity, as determined by autopsy.

Table 2. Therapeutic effects of 5-FU solution and 5-FU-MS against B-16 PC melanoma

Group	Treatment	Median survival (days [range])	T/C%[a]	No. of survivors[b]	No. of toxic deaths[c]
1	5-FU-MS	32 [27−>150]	139	2	0
2	5-FU solution	29.5 [11−37]	128	0	2
3	empty-MS+5-FU solution	29 [11−35]	126	0	2
4	empty-MS	24 [20−27]	104	0	0
5	control	23 [20−26]	100	0	0

[a]Median survival day in the treatment group/median survival day in the control group.
[b]Number of mice surviving for 150 days after cancer cells inoculation.
[c]Number of mice dead from drug toxicity, as determined by autopsy.

days (T/C% of 98%), which was not significantly different from that in the control group (Group 5).

Similar therapeutic results were noted against peritoneal carcinomatosis induced by B-16 PC melanoma (Table 2). 5-FU-MS (Group 1) extended the survival of mice bearing B-16 PC melanoma significantly better ($p < 0.05$) than the same dose of aqueous 5-FU (Group 2). There was no difference in the therapeutic effect between the 5-FU solution plus empty-MS (Group 3) and the 5-FU solution (Group 2). The median survival time in the empty-MS group (Group 4) was 24 days (T/C% of 104%), which was not significantly different from that in the control group (Group 5).

Autopsy determined that the mice who survived for 150 days were cancer-free microscopically. Thus, i.p. 5-FU-MS had superior therapeutic effects on peritoneal carcinomatoses induced by two kinds of transplantable cancers of Colon 26 and B-16 PC melanoma, as compared to the same dose of the 5-FU solution.

References

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