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Selective delivery of 5-fluorouracil (5-FU) to i.p. tissues using 5-FU microspheres in rats

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A new formulation has been developed for the delivery of 5-fluorouracil (5-FU) in treating peritoneal carcinomatosis. The new formulation (5-FU-MS) involves the incorporation of 5-FU into microspheres composed of a poly(glycolide-co-lactide) matrix. The incorporated 5-FU is released slowly over a 3 week period. We investigated the drug distribution and pharmacokinetics of 5-FU in rats receiving an i.p. injection of 5-FU-MS or aqueous 5-FU solution. The concentration of 5-FU was higher in the i.p. tissues (omentum and mesentery) and lower in the extraperitoneal tissues (blood plasma, lung and heart) in rats given 5-FU-MS than in rats given the aqueous 5-FU solution. Pharmacokinetic analysis showed that the area under the curve (AUC) was significantly greater in the omentum and the mesentery than in other tissues of rats given 5-FU-MS. There was no significant difference in the AUC in the tissues of rats given the aqueous 5-FU solution.

Key words: Animal experiments, drug distribution, 5-fluorouracil, i.p. chemotherapy, microspheres.

Introduction

Peritoneal carcinomatosis is a common manifestation of postoperative recurrent disease in patients with digestive and ovarian cancers. Patients with such cancers who developed peritoneal carcinomatosis are often treated with anticancer drugs in an aqueous solution administered i.p.[1] However, small, water-soluble molecules, such as 5-fluorouracil (5-FU) in aqueous solution, are rapidly absorbed through the capillaries into the systemic circulation,[2] so that it is difficult to maintain a high i.p. concentration of the drug for a long period. However, corpuscular particles, such as microspheres, are retained in the peritoneal cavity for a long period.[2] The new formulation of 5-FU (5-FU-MS), consisting of microspheres incorporating 5-FU, has been developed to enhance the therapeutic effects of 5-FU on peritoneal carcinomatosis[3] and to reduce the drug's systemic toxicity.[4] This paper reports the drug distribution and the pharmacokinetics of i.p. administered 5-FU-MS in rats.

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Materials and methods

Drug preparation

Poly(glycolide-co-lactide) (Biodegmer®, Biomaterials Universe, Kyoto, Japan; average molecular weight of 14 000) is a biodegradable substance used to control drug release.[5] The drug 5-FU was a gift from Kyowa Hakko Kogyo (Tokyo, Japan).

The new formulation (5-FU-MS) is composed of 5-FU incorporated in microspheres of a poly(glycolide-co-lactide) matrix. It was prepared using a water-in-oil emulsion method, as follows: 10 mg/ml 5-FU and 90 mg/ml poly(glycolide-co-lactide) were dissolved in a 97% solution of acetic acid. The resulting solution was emulsified in 10 volumes of liquid paraffin by stirring at 250 r.p.m. at 30°C for 2 days. The emulsion was made into microspheres containing 5-FU using an evaporation method. The microspheres were dried in a vacuum for 2 days and then filtered through a sieve. We used microspheres with an average diameter of 24 μm. We administered 5-FU-MS in a saline suspension with 0.01% Tween 80, which was added to maintain the dispersion of the microspheres. Control rats received an aqueous solution of 5-FU (5-FU Kyowa®, Kyowa Hakko Kogyo) diluted with saline containing 0.01% Tween 80 to yield the required concentration.

5-FU-MS is designed to release the incorporated 5-FU slowly: 70% of the incorporated 5-FU is

released during the first 7 days and the remaining 30% of the drug is released over a 2 week period.

Drug distribution

Fifty male rats (Wistar strain, weighing 150 g, Shimizu Laboratory Animal Center, Kyoto, Japan) were bred under standard conditions (specific pathogen-free, room temperature of 22°C, relative humidity of 60%, day–night cycle of 12 h). The rats were divided into two equal groups—the 5-FU-MS group and the 5-FU solution group.

The rats were anesthetized with s.c. injections of pentobarbital sodium to relax the abdominal muscles. Rats received 150 mg/kg 5-FU (equal to 40 ml/kg) i.p. either in the form of 5-FU-MS ($n = 25$) or in the form of the aqueous 5-FU solution ($n = 25$). Five rats from each group were sacrificed 1, 6 or 24 h or 4 or 16 days after drug administration. Blood was obtained through a heart puncture and centrifuged at 3000 r.p.m. for 5 min to separate plasma. The supernatants (plasma) were stored at −100°C for assay of the 5-FU concentration. The omentum and the mesentery, which are the implantation sites of the majority of i.p. seeded malignant cells,[6] were resected to obtain i.p. tissue samples. Extraperitoneal tissue samples were obtained from the heart and the lung. Samples of the kidney, which secretes 5-FU into the urine, were obtained to determine the 5-FU concentration. Samples of the liver and the spleen, which contain the reticuloendothelial tissues, were obtained to examine the entrapment of 5-FU-MS particles in the reticuloendothelial system. The samples were stored at −100°C. Tissue samples were minced in a 3-fold volume of distilled water and homogenized to obtain suspensions of tissue fractions. The tissue fractions and microsphere particles were removed by centrifugation at 6000 r.p.m. for 5 min. The concentration of 5-FU in the supernatant was determined by high performance liquid chromatography[7] (LC-6A System®, Shimazu, Kyoto, Japan) with absorption spectroscopy at a wavelength of 264 nm.[8] The assay limit was 10 ng per milliliter of plasma or gram of tissue.

If the 5-FU concentration was below the assay limit in more than two samples out of five samples obtained at the same time point, the 5-FU concentration was defined as 'not detectable'. Difference between the two dosage formulations were examined by analysis of variance, when the 5-FU concentrations in both formulations were 'detectable'.

Pharmacokinetics were examined with moment analysis with the non-linear least-squares program for microcomputers.[9] The area under the curve (AUC) and the mean residence time (MRT) were calculated for each tissues. ΔMRT in blood plasma [= (MRT in blood plasma in the 5-FU-MS group) − (MRT in blood plasma in the 5-FU solution group)] was calculated, because ΔMRT is considered to indicate the mean period of time for which 5-FU-MS releases free 5-FU in the peritoneal cavity.

The animals received humane care according to the institutional guidelines for animal welfare.

Statistical methods

When the $p < 0.05$, the difference was considered to be statistically significant.

Results

Drug concentration

Intraperitoneal tissues. The concentration of 5-FU in the omentum was significantly greater at 1 h, 6 h, and 4 days in the 5-FU-MS group than in the 5-FU solution group, and remained elevated in the 5-FU-MS group throughout the 16 day observation period (Table 1). The 5-FU concentration in the mesentery was lower in the 5-FU-MS group than in the 5-FU solution group at 1 h after administration, but increased in the 5-FU-MS group at 6 h and remained elevated for 16 days (Table 2). The concentration of 5-FU in the mesentery decreased rapidly in the 5-FU solution group.

Blood plasma. The concentration of 5-FU in plasma was significantly higher in the 5-FU solution group at 1 and 6 h, but decreased rapidly and was not detectable 4 days after administration (Table 3).

Extraperitoneal tissues. The 5-FU concentration in the heart was significantly lower at 1 and 24 h in the 5-FU-MS group than in the 5-FU solution group (Table 4). The 5-FU concentration in the 5-FU-MS group decreased slowly and was detectable up to day 16, whereas the concentration in the 5-FU solution group decreased rapidly and was undetectable on day 16.

The concentration of 5-FU in the lung was significantly lower in the 5-FU-MS group for the first 24 h than in the 5-FU solution group. The concentration of 5-FU in the lung decreased slowly in the 5-FU-MS group, but decreased rapidly in the 5-FU solution group (Table 5).

Table 1. 5-FU concentration in the omentum

Time after administration	5-FU concentration mean value in μg/g [95% confidence interval]		*p* value in difference
	5-FU-MS group	5-FU solution group	
1 h	551 [304 to 798]	14.2 [−233 to 261]	$p < 0.01$
6 h	693 [326 to 1060]	2.1 [−364 to 369]	$p < 0.025$
24 h	363 [−166 to 893]	1.3 [−228 to 231]	NS[a]
4 days	153 [21.6 to 285]	0.21 [−22.4 to 22.8]	$p < 0.05$
16 days	17.3 [3.06 to 31.6]	0.015 [−14.3 to 14.3]	NS[a]

[a]NS, not significant.

Table 2. 5-FU concentration in the mesentery

Time after administration	5-FU concentration mean value in μg/g [95% confidence interval]		*p* value in difference
	5-FU-MS group	5-FU solution group	
1 h	12.7 [3.14 to 22.4]	30.3 [20.7 to 39.9]	$p < 0.025$
6 h	261.5 [8.6 to 514.4]	2.25 [−2.8 to 7.3]	$p < 0.05$
24 h	61.0 [21.1 to 101]	1.08 [−17.0 to 19.1]	$p < 0.05$
4 days	22.2 [7.08 to 37.4]	0.297 [−14.8 to 15.4]	$p < 0.05$
16 days	1.70 [0.349 to 3.04]	ND[a] —	—

[a]ND, not detectable.

Table 3. 5-FU concentration in the blood plasma

Time after administration	5-FU concentration mean value in μg/g [95% confidence interval]		*p* value in difference
	5-FU-MS group	5-FU solution group	
1 h	1.57 [−9.39 to 12.5]	50.0 [39.0 to 61.0]	$p < 0.005$
6 h	0.028 [−1.7 to 1.7]	4.20 [1.9 to 6.5]	$p < 0.05$
24 h	0.020 [−0.020 to 0.060]	0.055 [0.015 to 0.096]	NS[a]
4 days	0.013 [0.000 to 0.026]	ND[b] —	—
16 days	ND[b] —	ND[b] —	—

[a]NS, not significant.
[b]ND, not detectable.

Table 4. 5-FU concentration in the heart

Time after administration	5-FU concentration mean value in μg/g [95% confidence interval]		*p* value in difference
	5-FU-MS group	5-FU solution group	
1 h	1.81 [−2.93 to 6.56]	28.7 [24.0 to 33.8]	$p < 0.005$
6 h	0.41 [−1.62 to 2.45]	3.00 [0.97 to 5.04]	NS[a]
24 h	0.053 [−0.072 to 0.178]	0.389 [0.264 to 0.514]	$p < 0.005$
4 days	0.0932 [0.0085 to 0.178]	0.0955 [0.0008 to 0.190]	NS[a]
16 days	0.0176 [0.0065 to 0.0287]	ND[b] —	—

[a]NS, not significant.
[b]ND, not detectable.

Table 5. 5-FU concentration in the lung

Time after administration	5-FU concentration mean value in μg/g [95% confidence interval]		*p* value in difference
	5-FU-MS group	5-FU solution group	
1 h	2.80 [−7.05 to 12.7]	37.9 [28.0 to 47.7]	$p < 0.005$
6 h	1.25 [−2.92 to 5.42]	10.7 [6.57 to 14.91]	$p < 0.01$
24 h	0.261 [−11.2 to 11.7]	4.56 [34.2 to 57.1]	$p < 0.005$
4 days	0.138 [−0.411 to 0.687]	0.839 [0.290 to 1.39]	NS[a]
16 days	0.252 [−0.068 to 0.571]	0.0135 [−0.344 to 0.371]	NS[a]

[a]NS, not significant.

Kidney. The 5-FU concentration in the kidney was significantly lower in the 5-FU-MS group at 1 h than in the 5-FU solution group and remained lower in the 5-FU-MS group for 4 days (Table 6).

Liver and spleen. The 5-FU concentration in the liver was lower in the 5-FU-MS group than in the 5-FU solution group for the first 4 days; the difference was significant at 6 h (Table 7). The 5-FU concentration was undetectable on day 16 in the 5-FU solution group.

The 5-FU concentration in the spleen was higher in the 5-FU solution for the first 24 h; the difference was significant at 1 and 6 h (Table 8). The decline of 5-FU concentration from day 4 to 16 was steeper in the 5-FU solution group than in the 5-FU-MS group.

Pharmacokinetic analysis

In the 5-FU-MS group, the areas under the curve (AUCs) were significantly greater in the omentum and the mesentery than in other tissues (Table 9). In the 5-FU solution group, the AUCs were similar in all tissue groups. The AUCs in the omentum and the mesentery tended to be greater in the 5-FU-MS group than in the 5-FU solution group.

Table 6. 5-FU concentration in the kidney

Time after administration	5-FU concentration mean value in μg/g [95% confidence interval]		*p* value in difference
	5-FU-MS group	5-FU solution group	
1 h	6.56 [−22.1 to 35.2]	109 [80.2 to 138]	$p < 0.005$
6 h	7.44 [−4.95 to 19.8]	16.6 [4.18 to 28.9]	NS[a]
24 h	0.296 [−0.101 to 0.694]	0.820 [0.423 to 1.22]	NS[a]
4 days	0.156 [−0.107 to 0.418]	0.353 [0.060 to 0.646]	NS[a]
16 days	0.098 [0.015 to 0.181]	0.005 [−0.88 to 0.98]	NS[a]

[a]NS, not significant.

Table 7. 5-FU concentration in the liver

Time after administration	5-FU concentration mean value in μg/g [95% confidence interval]		*p* value in difference
	5-FU-MS group	5-FU solution group	
1 h	5.51 [−16.4 to 27.4]	19.9 [−2.02 to 41.8]	NS[a]
6 h	0.658 [-0.101 to 1.42]	2.60 [1.84 to 3.36]	$p < 0.005$
24 h	0.219 [−0.251 to 0.690]	0.589 [0.119 to 1.06]	NS[a]
4 days	0.216 [−0.038 to 0.470]	0.042 [−0.212 to 0.297]	NS[a]
16 days	0.317 [0.207 to 0.427]	ND[b] —	—

[a]NS, not significant.
[b]ND, not detectable.

Table 8. 5-FU concentration in the spleen

Time after administration	5-FU concentration mean value in μg/g [95% confidence interval]		*p* value in difference
	5-FU-MS group	5-FU solution group	
1 h	1.33 [−8.98 to 11.6]	42.3 [32.0 to 52.6]	$p < 0.005$
6 h	6.56 [−1.58 to 14.7]	20.2 [12.0 to 28.3]	$p < 0.05$
24 h	3.32 [1.45 to 5.19]	5.04 [3.17 to 6.91]	NS[a]
4 days	2.74 [0.319 to 5.17]	0.685 [−2.06 to 3.36]	NS[a]
16 days	0.397 [−0.049 to 0.842]	0.0075 [−0.490 to 0.505]	NS[a]

[a]NS, not significant.

Table 9. AUC and MRT

	5-FU-MS group		5-FU solution group	
	AUC[a] (10^2 μg/g·h)	MRT[b] (h)	AUC (10^2 μg/g·h)	MRT (h)
Omentum	583	89.6	1.67	41.6
Mesentery	102	63.2	2.19	30.5
Plasma	0.11	164	1.76	5.78
Heart	0.35	122	1.55	15.2
Lung	1.97	548	24.7	26.9
Kidney	1.96	183	6.19	14.4
Liver	1.18	182	1.27	13.0
Spleen	8.46	130	7.04	26.7
	$p < 0.025$		NS[c]	

[a]Area under the curve.
[b]Mean residence time.
[c]Not significant.

The MRT was significantly greater in the 5-FU-MS group than in the 5-FU solution group for all samples. ΔMRT was calculated as 158 h (6.6 days).

Discussion

Studies have shown that 5-FU is highly effective in treating many types of cancers.[11,12] Water-soluble small molecules, such as 5-FU in aqueous solution, are readily absorbed into the circulation and do not maintain a high concentration over the long term. The anticancer activity of 5-FU is more dependent on the duration of drug exposure than on the drug concentration.[13] Therefore, a bolus injection of an aqueous 5-FU solution is not necessarily effective against peritoneal carcinomatosis.

Intraperitoneal injection of 5-FU in a great volume (2 l) of solution[14,15] prolongs the retention time of 5-FU and enhances the drug's therapeutic effects in the peritoneal cavity. Intraperitoneal administration of 5-FU has been found to be more effective than i.v. administration for the treatment of peritoneal carcinomatosis.[1] However, even when administered in a great volume of solution, i.p. 5-FU is absorbed relatively quickly into the systemic circulation,[10] resulting in a steep decline in the concentration in i.p. tissues. Continuous i.p. infusion of 5-FU using an implantable device[16] distributes a high concentration of 5-FU to the i.p. cavity for a prolonged period of time.

In the present study, we administered 5-FU in a volume of 40 ml/kg (equal to 2 l/50 kg) to study the drug distribution when administered in a great volume of solution or suspension. The present result showed that 5-FU-MS delivered a high concentration of 5-FU over a prolonged time to the omentum and the mesentery. Both the AUCs and the MRTs in these tissues were greater than in the rats given in an aqueous solution.

Small particles, such as microspheres, are gradually and selectively absorbed through milky spots,[17] which are located mainly on the omentum and the mesentery.[6] These milky spots are the sites of implantation of i.p. seeded malignant cells.[6] Thus i.p. 5-FU-MS selectively targets malignant cells implanted in the peritoneum. The present results are in agreement with the results of a preliminary study showing that 5-FU-MS was superior to 5-FU solution for the treatment of peritoneal carcinomatosis in mice.[3]

The 5-FU concentrations in extraperitoneal tissues and plasma were smaller in the 5-FU-MS group than in the 5-FU solution group in the present study. The MRTs in these extraperitoneal tissues were much greater in the 5-FU-MS group than in the 5-FU solution group, although the AUCs were rather smaller. Those findings indicate that 5-FU-MS delivered much lower concentrations of 5-FU to extraperitoneal tissues. This pharmacokinetic property of 5-FU-MS, which was also observed in a previous study in mice, should reduce systemic toxicity.[4] The ΔMRT, the mean duration of release of the incorporated 5-FU from 5-FU-MS, was 6.6 days, somewhat less than the releasing duration obtained in a preliminary experiment *in vitro*. In the preliminary experiment, 5-FU-MS released 70% of the incorporated 5-FU into a buffer solution and saline during the first 7 days and the remainder of the drug over the next 2 weeks.

Conclusion

In conclusion, 5-FU-MS delivered greater concentrations of 5-FU for a longer period of time and selectively targeted i.p. tissues as compared to the aqueous 5-FU solution.

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