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Uptake of 5-fluorouracil (5-FU) in peritoneal metastases in relation to the route of drug administration and tumour debulking surgery: an autoradiographic study in the rat

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Abstract

Patients with peritoneal metastases from colorectal cancer have a poor prognosis. Aggressive treatment by debulking surgery and intraperitoneal (i.p.) chemotherapy has been suggested as an alternative therapy. However, the drug penetrance into the tumour in relation to the administration route and surgical reduction of the tumour is not well known. We compared locoregional administration with intravenous (i.v.) injection. Thirty-four in-bred rats with peritoneal metastases were randomly allocated into eight groups and injected with ^{14}C-labelled 5-fluorouracil (5-FU) either through the i.v. or i.p. route, with or without a preceding tumour debulking, and were sacrificed after 2 or 8 h. Tumour radioactivity was visualised by autoradiography and quantified by a computer-based image analysis. After 8 h, 19 debulked and i.p.-injected tumours had a higher drug uptake, 63.2±28 (mean±standard deviation (SD)) kBq/g than 62 native i.p.-injected tumours (32.8±14) or 22 debulked and i.v.-injected tumours (18.5±18, $P=0.002$). After 8 h, 9 small tumours (<median 571 pixels) which underwent i.p. injection and tumour reduction had a higher drug uptake (77.4±26) than 29 non-debulked and i.p.-injected (35.1±17) or eight debulked and i.v. injected tumours (23.0±16, $P=0.004$). For larger tumours (⩾median 571 pixels), 16 debulked and i.p.-injected tumours had a higher radioactivity (drug uptake) (150.7±63) at 2 h than 49 i.p.-injected native tumours (48.5±59) or 11 reduced and i.v.-injected tumours (19.9±13, $P=0.03$). At 8 h, 10 debulked and i.p.-injected tumours had a higher drug uptake (50.3±24) than 33 native and i.p.-injected (30.8±10) or 14 debulked and i.v.-injected tumours (16.0±19, $P=0.001$). These results indicate that a debulking procedure and locoregional treatment of peritoneal metastases is associated with an increased level of 5-FU in the tumours.

Keywords: Peritoneal metastases; Tumour reduction; Intraperitoneal chemotherapy

1. Introduction

Colorectal cancer with peritoneal or local metastases usually has a poor prognosis [1–4] and treatment remains a challenging problem. Intravenous (i.v.) 5-fluorouracil (5-FU) alone or in combination with other drugs is commonly used in order to achieve a regression of the tumour and a prolonged survival [5–9]. Resistance to cytotoxic drugs is one important obstacle to successful treatment [10–12]. Furthermore, a poor vascular supply [13] and high osmotic pressure [14] might prevent the efficient uptake of chemotherapeutic agents into tumour tissues. In order to obtain the maximum concentration of the chemotherapy agent in the target organ, intraperitoneal (i.p.) administration has been suggested as an alternative route of administration [15–17]. In addition, tumour-debulking surgery preceding i.p. chemotherapy has been proposed to improve the therapeutic efficacy [17–19]. However, drug penetrance into the tumour following different routes of administration and reduction of the tumour by surgery is not well known.

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The primary aim of this study was to assess the uptake of 5-FU following locoregional administration compared with i.v. injection in a model of peritoneal metastases. We also wanted to test whether cytoreductive surgery might further improve the uptake of 5-FU into the tumour.

2. Materials and methods

2.1. Animals

Forty-six in-bred female Wistar rats (Möllegaards Ltd., Denmark), weighing 150–240 g (mean 201 g) were used. They had free access to a standard laboratory diet and water. A 10-day period of acclimatisation preceded the first surgical procedure. The regional Ethics Committee for Animal Research, Uppsala, Sweden approved all of the experiments.

2.2. Tumour cells

The experimental tumour was a colonic adenocarcinoma of rat origin [20]. The tumour cells were grown as a monolayer culture in a nutrient mixture composed of Ham's F-10 (Flow laboratories Swedish AB, Stockholm, Sweden) supplemented with 10% fetal calf serum, 2mM L-glutamine, penicillin (20 U/ml) and streptomycin (20 μg/ml). Tumour aggregates were achieved mechanically with a scraper (Cell Lifter, Costar Corporation Cambridge, MA, USA).

2.3. Surgical procedures

The animals were anaesthetised with 36 mg/ml of chloral hydrate in a mixture of sodium pentobarbital and manganese oxide in distilled water administered i.p. at a dose of 3.3 ml/kg of body weight and the abdomen was opened by a 1.5 cm midline incision. A suspension containing 1.0×10^7 viable tumour cells was applied by injection into the peritoneum. After inoculation, the muscle layer and skin were closed with 4.0 Ethilon sutures. Three weeks later, a second laparatomy was performed. Thirty-four animals which had macroscopic tumour growth in the abdomen were subjected to further experiments, while 12 rats (nine rats died immediately after the second laparatomy and three animals had no macroscopic tumour) were excluded from further analysis. The animals were randomly allocated to either i.v. or i.p. 5-FU injection and to a tumour-debulking procedure or not. The standardised debulking procedure consisted of excision of half of the tumour by means of a division of the tumours that were located in the right half of the abdomen. To minimise blood loss, tumours in the left half of the abdomen were left *in situ*. Tumours in i.v.- and i.p.-injected rats were denoted i.v. and i.p., respectively, and those subjected to the debulking procedure were denoted i.v._{TR} and i.p._{TR}. In the i.v. group, cannulation of the right femoral vein with a plastic catheter was performed as previously described in Ref. [21]. In the i.p. group, a 1.2-mm cannula was inserted through the abdominal wall as a guide to introduce an i.p. plastic catheter (Polyethylene Tubing, PP 380, Swevet AB, Stockholm, Sweden). After introducing the catheter, the needle was withdrawn and the abdominal wall was closed. A dose of 23 μCi 5-fluoro[2-^{14}C]uracil, (ICN Pharmaceuticals, Inc., Irvine, California, USA, specific activity 8.9 mCi/mmol radiochemical purity 93–98%) was dissolved in 2 ml 0.9% NaCl (37 °C). The injected volume of 2000-μl (i.p.) or 300 μl (i.v.) was administered over 30 seconds immediately after closure of the abdominal wall. The animals were killed in a CO_2 chamber 2 or 8 h after the labelled 5-FU administration.

2.4. Autoradiography and distribution analysis

After sacrifice, the rats were immediately frozen in ethanol, cooled with dry ice to −78 °C for 10 min. The frozen rats were then mounted in an aqueous gel of carboxymethyl cellulose, which was rapidly frozen around the animals. Sagittal whole-body sections, 20 or 60 μm thick, were attached onto a tape (No. 810, Minnesota Mining & Manufacturing Co., USA). The sectioning was performed at −20 °C with a cryomicrotome (PMV Co., Stockholm, Sweden) as previously detailed in Refs. [22,23]. The sections were freeze-dried and apposed to X-ray film (Agfa Structurix D7, Agfa-Geavert, Belgium) for exposure. For subsequent quantification of autoradiograms, carbon-14 standard staircases (Autoradiographic ^{14}C-Micro-Scales, Amersham, UK) were co-exposed with the sections. An objective evaluation of the sections was performed by computer-based image analysis [24]. Briefly, autoradiogram films were transilluminated, captured by a video camera, and stored in a computer. Regions of interest were analysed from the digitised picture presented on the image display system. Uptake was expressed as density and the area as pixels. The radioactivity distributions within the metastases, i.e. density in the periphery vs. the central portion of the tumour were studied. Furthermore, the relationship between tumour area and 5-FU uptake was assessed in native tumours, i.e. in the i.v. and i.p. groups. The animals and the sections were coded and the analysis was done in a blinded manner.

2.5. Statistical methods

Results are expressed as means ± standard deviations (SD). *A two-factor repeated measures analysis of variance* was performed to determine if the mean drug uptake in the tumours differed between the groups. In

Table 1
Number of rats and peritoneal metastases (in parentheses) according to the route of administration, tumour debulking and time for sacrifice

	2 h	8 h
i.v.	4 (43)	4 (68)
i.p.	6 (98)	4 (62)
$i.v._{TR}$	4 (26)	4 (22)
$i.p._{TR}$	4 (22)	4 (19)

i.v., Intravenous injection; i.p.; intraperitoneal injection; $i.v._{TR}$, intravenous injection after debulking procedure; $i.p._{TR}$, intraperitoneal injection after debulking procedure.

Table 2
Radioactivity concentration in peritoneal metastases (kBq/g tissue) in relation to the route of administration, tumour debulking and time for sacrifice. Numbers are means with standard deviations (SD) in parentheses

	2 h	8 h
i.v.	72.5 (94)	22.1 (13)
i.p.	82.2 (113)	32.8 (14)
$i.v._{TR}$	26.1 (20)	18.5 (18)
$i.p._{TR}$	157.7 (81)	63.2 (28)

i.v., Intravenous injection; i.p.; intraperitoneal injection; $i.v._{TR}$, intravenous injection after debulking procedure; $i.p._{TR}$, intraperitoneal injection after debulking procedure.

this analysis, the group and rat were treated as 'between' factors and the tumour as a 'within' factor. *Tukey's Studentized Range Test* [25] was used to conduct multiple comparisons among the four groups. Furthermore, the same analyses as above were performed after stratification by tumour size. The Spearman rank correlation test was used to analyse drug uptake in relation to the tumour area. A two-tailed P value of less than 0.05 was considered to be statistically significant. Where appropriate, a *Bonferroni* correction was used to maintain an overall type I error rate of 0.05.

3. Results

3.1. Metastatic growth

A total of 491 peritoneal metastases were observed. Left-sided non-debulked tumours were excluded in animals which were subjected to debulking and this left 360 tumours eligible for the present analysis (Table 1). The metastases were evenly distributed in the peritoneal cavity. The distribution of the tumours in the cryosections corresponded to the macroscopic findings.

3.2. Overall metastatic uptake

The average radioactivity concentration was compared among the four different groups (i.v., i.p., $i.v._{TR}$, $i.p._{TR}$) and at two different sacrifice times (2 and 8 h). After 8 h, the mean radioactivity concentration differed among the four groups, where the $i.p._{TR}$ group had a higher uptake than the i.p. or $i.v._{TR}$ groups ($P = 0.002$, Table 2, Fig. 1). The 95% Confidence Intervals (CIs) for the mean differences, $i.p._{TR}$–i.p. and $i.p._{TR}$–$i.v_{TR}$ were [10.6–50.2] and [21.0–68.3], respectively. However, at 2 h, no significant difference was found between the groups ($P = 0.61$). A slightly higher radioactivity concentration in the tumour periphery than in tumour centre was found in all groups, except in the i.v. group at 8 h (Table 3).

3.3. Uptake in relation to the tumour size

There was an inverse relationship between the tumour area (pixels) and 5-FU uptake in i.v.-treated ($r = -0.18$, $P = 0.02$) as well as i.p.-treated tumours ($r = -0.18$, $P = 0.01$, Fig. 2). The analysis was also performed separately in small tumours (< 571 pixels) and large tumours

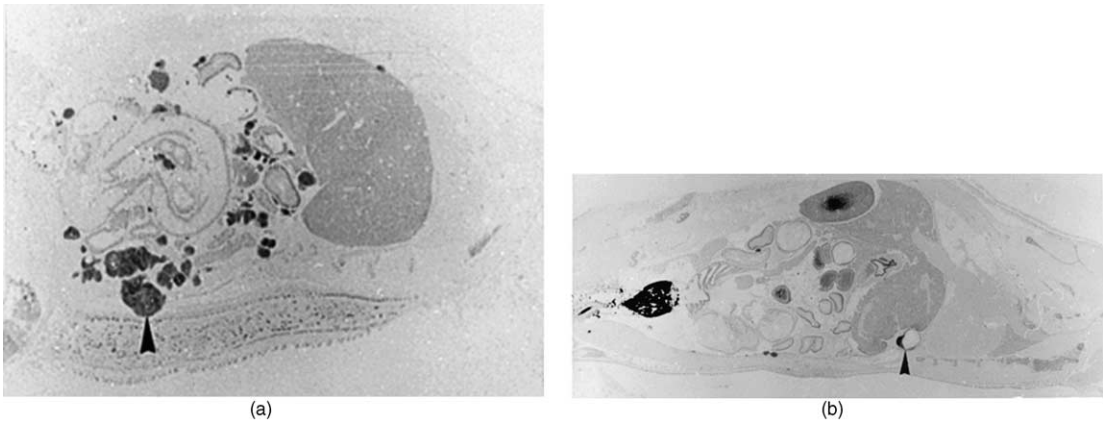

Fig. 1. Autoradiographs of whole body section of rats showing uptake of ^{14}C-labelled 5-fluorouracil (5-FU) in peritoneal metastases (arrows). A rat treated with intraperitoneal (i.p.) 5-FU and debulking ($i.p._{TR}$) is seen to the left and a rat treated with intravenous (i.v.) 5-FU and debulking ($i.v._{TR}$) to the right.

Table 3
Radioactivity concentration (kBq/g tissue) in the tumour centre and tumour periphery according to the route of administration, tumour debulking and time for sacrifice. Numbers are means with SD in parentheses

	2 h		8 h	
	Centre	Periphery	Centre	Periphery
i.v.	60.8 (86)	66.4 (85)	20.6 (14)	18.5 (10)
i.p.	66.9 (112)	76.2 (93)	25.3 (16)	29.7 (15)
i.v.$_{TR}$	19.2 (18)	24.1 (16)	14.4 (16)	18.1 (14)
i.p.$_{TR}$	86.5 (91)	165.5 (64)	43.1 (30)	63.0 (33)

i.v., Intravenous injection; i.p.; intraperitoneal injection; i.v.$_{TR}$, intravenous injection after debulking procedure; i.p.$_{TR}$, intraperitoneal injection after debulking procedure.

Table 4
Average metastatic radioactivity (kBq/g tissue) according to the route of administration, tumour debulking and time for sacrifice after stratification by median tumour area (571 pixels). Numbers are means with SD in parentheses

	Small tumours (<571 pixels)				Large tumours (⩾571 pixels)			
	n	2 h	*n*	8 h	*n*	2 h	*n*	8 h
i.v.	30	86.4 (107)	34	24.8 (17)	13	40.4 (38)	34	19.4 (7)
i.p.	49	115.9 (141)	29	35.1 (17)	49	48.5 (59)	33	30.8 (10)
i.v.$_{TR}$	15	30.7 (24)	8	23.0 (16)	11	19.9 (13)	14	16.0 (19)
i.p.$_{TR}$	6	176.1 (121)	9	77.4 (26)	16	150.7 (63)	10	50.3 (24)

n, number.

(⩾571 pixels), where 571 pixels was the median tumour size. In small tumours, the differences among the four groups after 8 h in favour of i.p.$_{TR}$ in comparison with i.p. or i.v.$_{TR}$ were still observed ($P=0.004$, Table 4), with 95% CIs for the differences i.p.$_{TR}$–i.p. [12.5–72.2] and i.p.$_{TR}$–i.v.$_{TR}$ [16.4–92.5]. However, at 2 h no clear differences were observed ($P=0.74$).

In large tumours, i.p.$_{TR}$-treated tumours had a higher uptake after 2 h than i.p.- or i.v.$_{TR}$-treated ($P=0.03$, Table 4). The 95% CIs for i.p.$_{TR}$–i.p. and i.p.$_{TR}$–i.v.$_{TR}$ were [23.3–181.2] and [23.5–238.2], respectively. After 8 h, large tumours in the i.p.$_{TR}$ group had a higher uptake ($P=0.001$, Table 4), in comparison with i.p. or i.v.$_{TR}$, with 95% CIs for the mean differences i.p.$_{TR}$–i.p.: [5.6–33.5] and i.p.$_{TR}$–i.v.$_{TR}$ [18.3–50.4]. Furthermore, in large tumours after 8 h, the uptake was higher in the i.p. than i.v.-treated tumours, 95% CI for the mean difference i.p.–i.v. [1.9–20.8].

4. Discussion

This study was designed to assess the influence of tumour debulking and the mode of administration on 5-FU uptake in peritoneal metastases. 5-FU-containing regimens have been shown to improve survival in patients with metastatic colorectal cancer [5,7] and uptake of 5-FU is thus of therapeutic importance. An interesting finding in this study was that debulked and i.p.-treated tumours overall showed a higher uptake at 8 h compared with native and i.p.-treated tumours and also compared with i.v.-treated metastases. It is

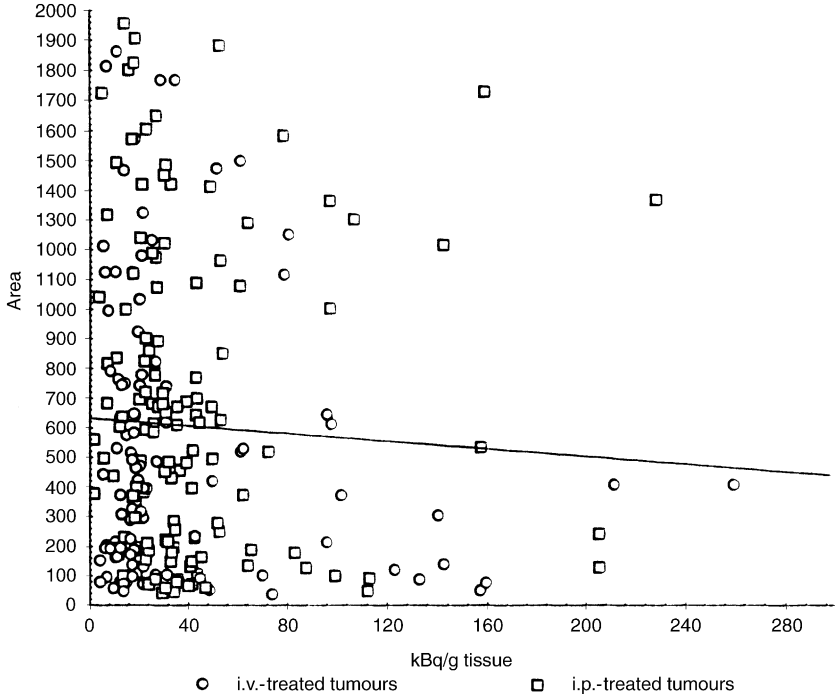

Fig. 2. Relationship between radioactivity concentration and tumour area. Circles = i.v.-treated and squares = i.p.-treated.

reasonable to conclude that drug uptake is improved by regional drug delivery if the tumour surfaces are exposed to the chemotherapy agent. The most striking difference in concentration was observed at 8 h when debulked and i.p.-treated tumours showed a three-fold increased 5-FU concentration compared with i.v.- and i.v.$_{TR}$-treated groups and a two-fold increase relative to the i.p.-treated tumours. This difference could also be seen at 2 h, although a statistically significant difference was observed only for the larger tumours. The drug concentration in the i.v.$_{TR}$-treated tumours was markedly lower at 2 h which may be due to a more rapid clearance of the drug caused by increased bleeding from the tumours.

The strong advantage for i.p. administration that we observed has potentially important clinical applications. Patients with tumour dissemination restricted to the abdominal cavity may preferably be treated with i.p. chemotherapy since i.v. treatment will invariably fail to reach therapeutic drug concentrations. Furthermore, non-radical tumour excision may also be indicated in order to improve locoregional drug delivery. It may thus be worthwhile to excise bulky peritoneal tumour masses also in patients with extensive peritoneal carcinomatosis.

5-FU cytotoxicity is dependent on conversion to the active metabolite, 5-fluoro-deoxyuridine-monophosphate, which is a potent inhibitor of the target enzyme thymidylate synthetase [26]. 5-fluoro-uridine-diphosphate and 5-fluoro-uridine-triphosphate are also produced and these compounds are cytotoxic, presumably due to their incorporation into RNA. The major catabolites of 5-FU are fluoro-ureidoproprionic acid and fluoro-β-alanine [27]. However, a previous study has demonstrated that the radioactivity measured represents 5-FU as well as drug anabolites and catabolites [28]. By 2 h, the metabolites 5-fluoro-uridine and 5-fluoro-uridine-monophosphate predominate, constituting approximately 40% and 20%, respectively, of the 5-FU-originated radioactivity. By 8 h, the active metabolite, 5-fluoro-deoxyuridine-monophosphate, is believed to represent approximately half of the total radioactivity. Catabolites constituted less than 10% of the radioactivity at all time-points and this supports the fact that the total amount of radioactivity is a relevant end-point since it represents potentially cytotoxic prodrug or active metabolites [28]. Tracer amounts of radioactivity were used in the present study and no cytotoxic effect can be anticipated by the ^{14}C-5-FU. The route of tumour spread and implantation in the present model resembles the human situation in association with surgery for colorectal cancer, i.e. tumour cells are implanted on de-epithelised peritoneal surfaces. This may be advantageous when studying drug uptake that is dependent on the tumour blood supply, as well as drug diffusion. The present experimental system is thus probably a satisfactory model for studying drug uptake.

Clinically, the prognosis of the patient with peritoneal dissemination of their colorectal cancer is poor when treating with systemic chemotherapy and most patients with peritoneal metastases die within 6–12 months [5,7,29]. However, there have been favourable reports following i.p. chemotherapy alone or in combination with cytoreduction [17–19,30–32]. A previous study on the uptake of cisplatin with a molecular weight (mol. wt.) of 300.6 kDa and carboplatin with a mol. wt. of 317.3 kDa in rat peritoneal tumours, showed a favourable uptake for cisplatin [33]. 5-FU, which has a lower mol. wt. (130.1 kDa) could be ideal for use in the i.p. setting. However, the influence of the mol. wt. of drugs on their tissue diffusion has been shown to be limited [34]. Another aspect to consider is that the drug may need to diffuse and cross membranes, which have different solubilities for the drug [35]. The finding of the present study supports this hypothesis, i.e. when membranes are mechanically disintegrated and 5-FU is given i.p., the uptake is higher. An increased drug accumulation due to local diffusion is also indicated by the lower radioactivity concentration in the tumour centre compared with the tumour periphery (except for the i.v. group at 8 h), which is consistent with previous findings in Ref. [36].

Another way to enhance peritoneal drug uptake has been through hyperthermia (41–42 °C), possibly through an increase in cell membrane permeability, alteration of active drug transport, a change in cell metabolism and decrease in interstitial fluid pressure [37–39]. Additionally, synergism between heat and chemotherapy can increase the anti-tumour effect [40]. Furthermore, heated i.p. chemotherapy had been applied clinically in combination with tumour debulking surgery [30–32].

In conclusion, this autoradiographic study in the rat showed that the uptake of 5-FU and its metabolic products in peritoneal metastases were higher when the tumours underwent debulking and the drug was administered i.p.

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