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The influence of degradable starch microspheres on liver uptake of 5-fluorouracil after hepatic artery injection in the rat

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The effect of degradable starch microspheres (DSM) on the distribution of 5-fluorouracil (5-FU) after hepatic artery injection was studied in normal rats. Carbon-14-labelled 5-FU was injected separately or together with DSM into the hepatic artery. Radioactivity was measured in liver tissue, bile, peripheral blood, and urine. When microspheres were added, the liver uptake of 5-FU was increased; its peak concentration in peripheral blood decreased, as did the early urinary excretion of radioactivity. The addition of degradable starch microspheres to hepatic artery injections of cytostatic drugs might be of value in increasing the drug concentration in tumour tissue and reducing systemic toxicity.

Intra-arterial injection of degradable starch microspheres (DSM) causes a transient reduction of regional blood flow (Arfors et al 1976). Their use with cytostatic drugs has been explored in liver malignancies. In the rat, concurrent injection of DSM decreased the toxicity of 5-fluorouracil given into the hepatic artery (Lindell et al 1978), presumably due to reduced systemic exposure. It may also be assumed that liver uptake of the drug is increased when it is given concurrently with DSM. Measurements of cytotoxic drugs in the general circulation after hepatic artery injections with and without DSM have been made in man (Dakhil et al 1982; Gyves et al 1983; Teder et al 1983). Liver uptake has only been measured for inulin (Lindell et al 1978), a hydrophilic, inert substance whose general properties do not resemble those of a cytostatic drug.

We therefore decided to measure liver uptake, biliary excretion, blood concentrations, and urinary excretion of 5-fluorouracil after hepatic artery injection with and without DSM in the rat.

Material and methods

Male Wistar rats (SPF, Möllegaard, Denmark) 250–300 g, were anesthetized with chloral hydrate i.p. (36 mg/100 g). Only one compartment (liver tissue, blood, urine or bile) was studied in each animal to minimize catheter problems and haemodynamic disturbances due to prolonged operation times.

Operation procedure. All animals had a left side nephrectomy and one femoral vein catheterized. For hepatic artery injections a tapered catheter was inserted retrogradely into the gastroduodenal artery (Leivestad & Malt 1973). Blood samples were obtained from the tail artery. Urine and bile was sampled from catheters in the right ureter and the common bile duct. Fluid losses were substituted by physiological saline 0·5 ml i.v. every 15 min.

5-Fluorouracil (5-fluoro-6[^{14}C]uracil) (Hoffman-La Roche Inc., Nutley, NJ), dissolved in water, 0·2 mg ml^{-1}, 20 μCi ml^{-1} (0·74 MBq ml^{-1}), was added to commercial 5-fluorouracil (Hoffman-La Roche) aqueous solution (31·25 mg ml^{-1}) in the proportions 1 : 9, giving 5-fluorouracil 28·15 mg ml^{-1} in the injected solution.

Degradable starch microspheres were crosslinked starch microspheres (Pharmacia AB, Uppsala, Sweden). These swell in water and are completely degraded by α-amylase (Lindberg et al 1983). Their diameters were 19 ± 3 μm in the swollen condition (batch Ph BR 42B 19B, 92789b). In-vitro, at an amylase concentration of 25 μkat litre^{-1}, half the microsphere mass is dissolved in 9 min. The microspheres were delivered dry and added to the 5-FU solution 1 h before the injections.

Drug injections. Into each animal 5-fluorouracil, 50·7 mg, 3·6 μCi (1·8 ml), was injected via a femoral vein, or via the hepatic artery alone, or mixed with degradable starch microspheres, 30 mg. All injections were made over 1 min. During the arterial injections retrograde flow into the common hepatic artery was prevented by a vessel loop.

Sampling. Blood was collected in 50 μl pipettes from the tail artery at the times in Table 1. Urine and bile were collected directly into glass counting vials every 10 min

Table 1. Radioactivity in liver as a percentage of the total activity injected. The liver vessels were irrigated with saline. Mean ± s.e.m.

Time after inj. (min)	Mode of injection: Hepatic artery 5-FU	Hepatic artery 5-FU + DSM	
5	7·52 ± 0·5 (n = 9)	10·87 ± 0·74 (n = 7)	$P < 0·01$
10	6·36 ± 0·15 (n = 8)	9·36 ± 0·3 (n = 8)	$P < 0·001$
30	9·88 ± 1·41 (n = 8)	8·77 ± 0·79 (n = 9)	n.s.

n.s. = not significant.

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for 90 and 120 min, respectively. Urine and bile production were estimated by weight.

Liver specimens were obtained 5, 10 and 30 min after injection. The animals were bled and the livers removed and flushed with saline, 10 ml, via the portal vein and hepatic artery. The irrigation fluid was measured for activity. The whole liver was homogenized and samples (1 g) taken for radioactivity measurement.

Measurement of radioactivity. The samples were processed as described by Lindell et al (1978) and then measured for activity in a liquid scintillation counter (LKB Wallac, LSC 81000, Turku, Finland). The counting efficiency was determined with internal standard and each sample count was corrected for quenching.

Statistical method. Student's *t*-test (two-tailed) was used for comparison of the means.

Results

The uptake of 5-FU in the liver is shown in Table 1. In the liver, significantly higher concentrations of radioactivity were found 5 and 10 min after hepatic artery injection of 5-FU together with the degradable microspheres. Thirty min after injection the liver radioactivities were the same in animals given 5-FU only or 5-FU mixed with degradable microspheres.

The radioactivity in the irrigation fluid, as a percentage of the total activity injected (± s.e.m.), was, 5 min after the injection, 1·40 ± 0·57 in the 5-FU only group, and 1·90 ± 0·19 in the 5-FU + DSM group. Ten min after injection, the corresponding values were 1·23 ± 0·59 and 1·30 ± 0·63 and 30 min after injection the values were 0·64 ± 0·19 and 0·77 ± 0·33. None of these differences were significant.

The blood concentrations (as total radioactivity) of 5-fluorouracil after the two modes of hepatic artery administration are given in Table 2. Coadministration of degradable microspheres decreases the peak concentration of circulating 5-FU by 33%. Three min after the end of the injection, this difference has decreased to 20%, after which time the differences are no longer statistically significant. It is also apparent from Table 1 that the peak 5-FU concentration after hepatic artery injection with microspheres is lower than after intravenous injection.

Table 2. Radioactivity per ml blood as a percentage of the total activity injected (mean ± s.e.m.) N = 8 in each group.

	Mode of injection					
Time after end of inj. (min)	(a) Hepatic artery 5-FU	(a) vs (b)	(b) Hepatic artery 5-FU + DSM	(b) vs (c)	(c) Femoral vein 5-FU	(c) vs (a)
0·25	1·93 ± 0·125	**	1·33 ± 0·141	*	1·98 ± 0·237	
0·5	1·95 ± 0·140	**	1·30 ± 0·165		1·68 ± 0·119	
0·75	1·68 ± 0·151		1·28 ± 0·131		1·43 ± 0·080	
1	1·60 ± 0·113	*	1·18 ± 0·128		1·23 ± 0·070	*
1·25	1·55 ± 0·124	**	1·08 ± 0·092		1·13 ± 0·053	**
1·5	1·45 ± 0·130	**	1·03 ± 0·088		1·10 ± 0·053	*
1·75	1·33 ± 0·113	*	1·03 ± 0·080		0·98 ± 0·045	*
2	1·25 ± 0·098	*	0·95 ± 0·050		0·93 ± 0·037	**
3	1·00 ± 0·065	*	0·80 ± 0·038		0·75 ± 0·033	**
5	0·75 ± 0·050		0·65 ± 0·050		0·58 ± 0·025	**
10	0·53 ± 0·037		0·58 ± 0·025	**	0·45 ± 0·033	
15	0·43 ± 0·032		0·44 ± 0·023		0·39 ± 0·022	
20	0·43 ± 0·052		0·44 ± 0·015	*	0·36 ± 0·023	
30	0·35 ± 0·020		0·40 ± 0·013	*	0·32 ± 0·027	
40	0·31 ± 0·017	**	0·37 ± 0·009	*	0·29 ± 0·025	

* $P < 0·05$. ** $P < 0·01$.

The urinary excretion of radioactivity (Table 3) was significantly lower for the first 30 min after intra-arterial injection of 5-FU with microspheres, compared with hepatic artery injection of 5-FU only. This difference had disappeared at the end of the observation period. When 5-FU was injected intravenously or into the hepatic artery without microspheres, the urinary excretions of radioactivity were similar. The diuresis was similar in the three treatment groups.

Table 3. Cumulative radioactivity in urine as a percentage of the total amount injected (mean ± s.e.m.). N = 6 in each group.

	Mode of injection			
Time after end of inj. (min)	(a) Hepatic artery 5-FU	(a) vs (b)	(b) Hepatic artery 5-FU + DSM	(c) Femoral vein 5-FU
0–10	5·11 ± 0·43	*	2·06 ± 0·76	4·20 ± 1·27
0–20	9·06 ± 0·67	*	4·85 ± 1·17	7·79 ± 1·64
0–30	11·81 ± 0·75	*	7·30 ± 1·42	10·60 ± 2·15
0–40	13·79 ± 0·97		9·57 ± 1·64	11·95 ± 2·41
0–50	15·45 ± 1·17		11·79 ± 1·86	13·69 ± 2·48
0–60	16·73 ± 1·40		13·43 ± 1·77	15·44 ± 2·68
0–70	18·06 ± 1·58		14·93 ± 1·74	16·94 ± 2·85
0–80	19·15 ± 1·71		16·45 ± 1·89	18·30 ± 3·14
0–90	20·03 ± 1·81		17·67 ± 1·94	19·89 ± 3·34

* $P < 0·05$.
(b) vs (c) and (c) vs (a) showed no significant differences.

The excretion of radioactivity in bile was low, amounting to 1% of the dose administered over 120 min irrespective of the mode of administration. The rate of bile production was similar in the three treatment groups (n = 6 in each group).

Discussion

Radiolabelled 5-FU was used to evaluate the effect of the degradable starch microspheres on the distribution of 5-fluorouracil. In measurements of the total carbon-14-radioactivity no distinction can be made between 5-FU and its metabolites. However, as the primary aim of this study was to measure the effect of the microspheres on the uptake of 5-FU in liver tissue, both unchanged 5-FU and its metabolites have been included. In the blood concentration curves, the differences between the three modes of administration are seen in the first few minutes when probably very little 5-FU metabolism has taken place. Plasma concentration curves similar to our blood concentration curves

have been obtained by direct photometric measurement on samples taken 2–60 min after intracarotid arterial injection of a similar 5-FU dose (187 mg kg^{-1}) in rats (Plumb & Gardner 1981). The photometric assay is also non-specific, but less so than the measurement of total radioactivity, since at least one major metabolite, carbon dioxide, does not contribute to the absorbance at 269 nm.

In the presence of microspheres, significantly higher radioactivities were found in the liver 5 and 10 min after injection (Table 1). Since the levels of radioactivity in the irrigation fluid did not differ significantly between animals given 5-FU only or 5-FU mixed with microspheres, it is probable that a substantial part of the radioactivity in the liver is extravascular. At 30 min after injection, the radioactivities in the liver did not differ between the two modes of hepatic artery administration of 5-FU. Here the effect of the microspheres is difficult to evaluate as the radioactivity probably originates from recirculating drug and metabolites.

Although the liver is the principal site of 5-FU catabolism (Chaudhuri et al 1959), we found the cumulative radioactivity in bile to be low, possibly due to elimination of catabolites mainly through lungs and kidneys (Mukherjee et al 1963). Within 90 min of administration, 17–20% of the injected radioactivity had been excreted through the kidneys. When 5-FU was injected together with microspheres, the excretion of radioactivity in urine was significantly decreased up to 30 min after injection, indicating that less 5-FU was available to the systemic circulation.

Depression of bone marrow activity appears to be related to the early plasma concentration of 5-FU (Cohen et al 1974). Lindell et al (1978) found decreased leucopenia and increased survival when 5-FU was injected with DSM into the hepatic artery. No drug measurements were made in that study, but they assumed that this decreased toxicity was due to an influence of the microspheres on the early plasma concentration of 5-FU. The suggested influence of the DSM on the early plasma concentration of 5-FU is confirmed in this study.

In conclusion, hepatic artery injection of 5-FU together with degradable starch microspheres increases the uptake of 5-FU in the liver, decreases the peak concentration of 5-FU in peripheral blood and significantly delays the renal excretion of 5-FU. In the treatment of liver malignancies, the addition of degradable starch microspheres to 5-FU or other cytostatic drugs for administration via the hepatic artery might be of value to increase the drug concentration in the liver tumour and to reduce systemic toxicity.

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