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The Value of Various Forms of Regional Administration of Cisplatin in the Treatment of Liver Metastases in the Rat

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Abstract—*Tumour growth was induced exclusively in the livers of* 40 F_1 *WagRij/BN rats (eight groups of five rats each) by intraportal inoculation of a squamous cell carcinoma. Four groups were treated by infusion of cisplatin (to which the tumour was sensitive) for a total of three doses of* 3 mg/kg *body weight at* 5*-day intervals and one group served as control. Treatment was given systemically, via the hepatic artery, via the portal vein and the hepatic artery simultaneously, and via the portal vein. The untreated animals had a mean survival time of* 119 days, *as opposed to* 211, 249, 192 *and* 239 days *for the treated animals. The animals treated via the hepatic artery lived significantly longer on average* ($P = 0.035$) *than those treated systemically or via the portal vein. The combination of intraportal and intra-arterial infusion had no advantage over infusion via the hepatic artery alone. The route of administration does not appear to influence the general toxicity of cisplatin.*

INTRODUCTION

SYSTEMIC administration of chemotherapy for treatment of liver metastases has not yet achieved the desired results. Regional administration has also been tried, but clinical results to date have not yielded any decisive answers. We evaluated the various forms of administration by means of an animal model which satisfied the following criteria: (1) the tumour growth should be limited to the liver; (2) the untreated animal should survive for at least a month in order to be able to receive adequate therapy and for evaluation of results; and (3) the liver metastases should be induced as naturally as possible.

MATERIALS AND METHODS

Liver metastases

Liver metastases were induced in 80 male F_1 WagRij/BN rats under ether anesthesia by injecting a non-antigenic squamous cell carcinoma suspension (20 million cells in 0.5 ml) into a mesenteric vein. Two weeks after inoculation relaparotomy was performed to 'manipulate' the livers in order to trigger 'dormant cells'. According to the study of Fisher and Fisher [1] liver metastases were demonstrated in 75% of rats on second relaparotomy 3 weeks after inoculation. The visible subcapsular liver tumours varied in size from about 1 to 1.5 mm in diameter and can therefore be classified as micrometastases [2].

Cytostatic agent

Cisplatin (*cis*-diamminedichloroplatinum) 3–4.5 mg/kg body weight had a clearly observable effect on the tumour ($P < 0.001$). A dose-effect study resulted in a dosage scheme in which excessive regression of tumour growth and toxicity could be avoided. This was performed with cisplatin 3 mg/kg body weight given three times at 5-day intervals as a 3-hr infusion (Fig. 1).

Treatment

To assess the most effective administration route, the following treatments were simultaneously administered at random to eight groups of five rats each, 3 weeks after inoculation with the tumour cell suspension: (1) infusion of cisplatin via the jugular vein; (2) infusion of cisplatin via the hepatic artery; (3) infusion of 0.9% NaCl via the jugular vein; (4) infusion of cisplatin via the

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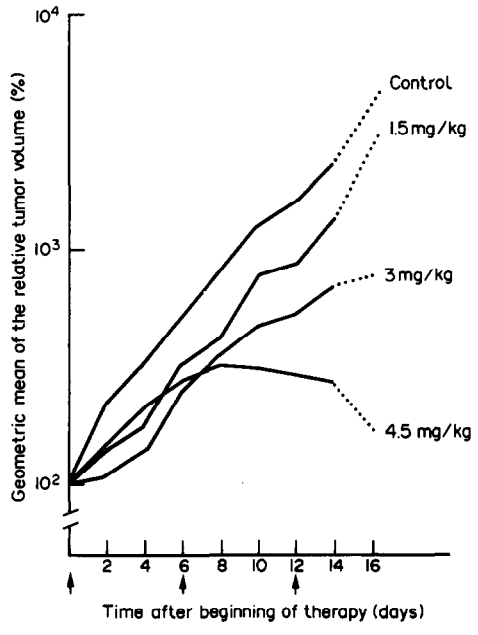


Fig. 1. Change of the relative tumour volumes with time in four groups of five rats during treatment with cisplatin given as an intravenous bolus every 5 days *for a total of three doses.*

portal vein; or (5) infusion of cisplatin via the hepatic artery and the portal vein.

For infusion of the jugular vein in silicone a rubber cannula (diameter 1 mm) was used. For cannulation of the portal vein and the hepatic artery, polyethylene cannulae (PE 10) were introduced at laparotomy into the gastroduodenal artery and the pyloric vein respectively and fixed in position [3]. For this purpose an operating microscope (magnification, × 30–45) and a set of microsurgical instruments were used. All cannulae were tunnelled subcutaneously to the neck region where a flexible, swivel-mounted stainless steel cannula was attached. The infusion system was so constructed that it did not impede the movements of the rat and no torsion or leakage of the infusion line could occur. Braun Unita I pumps were used for the infusion, which was set at a rate of 0.5 ml/hr. Heparin (40 units/24 hr) was also given.

Table 1. Mean total body weight (g), *liver weight* (g) *and ratio of liver weight/total body weight at post-mortem examination of the rats in five treatment groups*

Treatment group	Mean total body weight	Mean liver weight	
1	318.13	21.63	0.067
2	338.13	25.75	0.076
3	369.38	28.75	0.078
4	296.25	20.63	0.070
5	315.63	24.05	0.078

$$\text{Normal value ratio: } \frac{\text{liver weight}}{\text{total body weight}} = 0.026.$$

After the treatment, lasting 17 days in all, the rats were placed in separate cages until death. From the time of inoculation until death the rats were weighed twice a week. At post-mortem examination all organs were inspected for metastases, and the liver was carefully dissected out and weighed.

RESULTS

Tumour growth

In total eight uncomplicated experiments on 40 rats were performed. At post-mortem no extra-hepatic metastases were found, although in nearly all cases almost the entire liver was taken up by tumour (Fig. 2). In the treated and the untreated animals the increase in liver weight due to tumour growth was very similar: the ratio of liver weight/total body weight was three times that of the constant ratio determined on healthy rats (Table 1). In conclusion it can be said that all rats died in the same stage of their disease. However, the treated group lived nearly twice as long as the untreated group. This can be explained by tumour growth inhibition due to cytostatic infusion.

Table 2. Survival (in days) *after tumour cell inoculation*

		Treatment group 1	2	3	4	5	$\bar{x}_2$
Experiment:	I	191	269	117	168	309	234
	II	173	330	108	167	264	234
	III	315	315	86	267	150	262
	IV	252	251	133	172	225	225
	V	201	273	130	260	292	257
	VI	254	215	115	240	215	231
	VII	137	184	152	86	250	164
	VIII	161	154	113	177	205	174
		$\bar{x}_1$ 211	249	119	192	239	
	S.D.	±59	±61		±60	±51	

S.D.: standard deviation; $\bar{x}$: mean survival per treatment.

Fig. 2. Rat liver with tumour; post-mortem specimen, saggital section. 1. Liver tissue. 2. Tumour tissue.

Survival

The untreated animals (control group 3) had a mean survival time of 119 days and the cisplatin-treated animal groups survived on average 211 (group 1), 249 (group 2), 192 (group 4) and 239 days (group 5), respectively (Table 2). The control group lived significantly shorter ($P < 0.001$) than the cisplatin-treated animals according to an analysis of variance for randomised block design. In groups 2 and 5, which received the infusion via the hepatic artery, the difference was most pronounced; both groups lived twice as long as the control group. Comparison of the treatment groups reveals that the differences between them are not statistically significant ($P > 0.1$). Nevertheless a significant difference ($P = 0.035$) was seen in favour of the intra-arterial treatment, arranging the treatment groups on their actual disposition (Tables 3 and 4). This arrangement compares the treatment 'with' intra-arterial infusion (i.e. hepatic artery infusion: infusion via the portal vein and the hepatic artery) and 'without' intra-arterial infusion (i.e. systemic infusion: portal vein infusion). Treatment 'with' intraportal infusion (i.e. intraportal infusion: infusion via the portal vein and hepatic artery) was compared with treatment 'without' intra-portal infusion (i.e. systemic infusion: hepatic artery infusion).

Combination of intraportal and intra-arterial infusion (group 5) offered no advantage over intra-arterial infusion alone (group 2). Treatment via the portal vein resulted in the shortest survival time, even shorter than systemic treatment.

Toxicity

The changes in body weight give reliable information about the degree of toxicity to which the rats were exposed. The degree of toxicity was assessed over a limited time period by the differences in the weights noted directly before and after a cytostatic treatment cycle (Table 5). The degree of toxicity over a longer period is given by the body weights at inoculation and at post-mortem examination (Table 6).

As regards both short-term (Table 7) and long-term (Table 8) toxicity, there was no significant difference seen in average weight changes in the groups that were treated 'without' and those treated 'with' intra-arterial infusion ($P > 0.1$), using an analysis of variance for a randomised block design.

Table 3. Arrangement of four treatment groups according to the method of treatment; a randomised block design

systemic infusion; group 1	hepatic artery infusion group 2	➧	without intraportal inf.; group 1 + 2
portal vein infusion; group 4	hepatic artery & portal vein inf.; group 5	➧	with intraportal inf.; group 4 + 5
▼ without intra-arterial inf. group 1 + 4	▼ with intra-arterial inf. group 2 + 5		

Table 4. Analysis of variance for the mean survival data according to the method of treatment

211 ± 59; group 1	249 ± 61; group 2	➧	without intraportal inf.; groups 1 + 2 230 ± 60	➧ $P > 0.10$
192 ± 60; group 4	239 ± 51; group 5	➧	with intraportal inf.; groups 4 + 5 215 ± 56	
▼ without intra-art. inf.; groups 1 + 4 201 ± 60	▼ with intra-art. inf.; groups 2 + 5 244 + 56			
▼ $P = 0.035$				

Mean survival times (in days) ± S.D.

Table 5. Mean total body weight of the rats over a limited time period, directly before and after† the cytostatic treatment cycles*

Treatment group	Mean T.B.W.*	Mean T.B.W.†	Difference ± S.D.
1	341.25	314.37	-26.88 ± 9.61
2	344.37	316.87	-27.50 ± 18.13
3	358.75	378.75	+20.00
4	340.00	313.62	-26.25 ± 13.82
5	343.12	315.62	-27.50 ± 12.82

Table 6. Mean total body weight of the rats over a longer period at inoculation and at post-mortem examination†*

Treatment group	Mean T.B.W.*	Mean T.B.W.†	Difference ± S.D.
1	295.62	318.12	22.50 ± 77.1
2	305.62	338.12	32.50 ± 42.2
3	308.75	369.37	60.62
4	297.50	296.25	-1.25 ± 76.6
5	307.50	355.62	48.12 ± 76.6

Table 7. Comparison of the groups treated 'with' and 'without' intraportal and intra-arterial infusion; a randomised block design

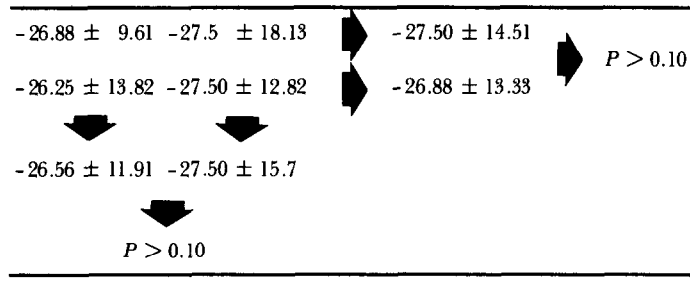
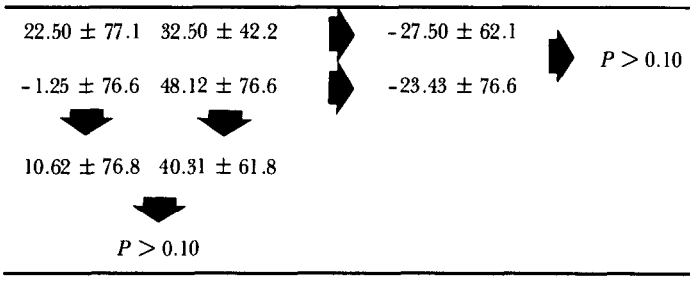


Table 8. Comparison of the groups treated 'with' and 'without' intraportal and intra-arterial infusion; a randomised block design



DISCUSSION

Although surgical treatment of liver metastases is seldom possible, this is the only method that results in long-term survival [4-7]. With chemotherapy this is not yet the case. It is hoped to achieve better results by continuous regional infusion of the liver. In theory regional treatment has advantages over systemic therapy: the cytostatic drug reaches the liver in higher concentrations while the systemic toxicity is less, which means that administration may be continued for long periods. Nevertheless the results in man are still poor. A survival of longer than 1 yr after commencement of the treatment is seldom seen [8-11].

The superiority of the intra-arterial administration of cytostatic drugs has by no means been established, since few adequate comparative studies have been performed. In the prospective study of Grage *et al.* [12], which is the only one of any significance in this regard, no differences in objective improvement and median survival time between intravenous and intra-arterial administration were seen. However no control group was used in their study.

In the short-term controlled clinical trials will be unable to select the most successful form of local infusion. In an experimental animal model, where control and standardization is possible, a short-term answer can be achieved.

Building upon the results of other investigators [2, 13-17], we have been successful in developing a liver metastases model in a strain of inbred rats which has not been hampered by extra-hepatic tumour growth. At the same time an infusion system through which long periods of treatment can be given without complications was developed. In this model infusion of cisplatin via the hepatic artery is more successful than other regional infusion methods or systemic infusion, success being measured in terms of a significantly longer survival time. In the clinical study of Grage *et al.* [12] such a result could not be demonstrated, while in the study of Cotino [16] no clear conclusions could be drawn on this matter.

Contrary to the assumption that only macrometastases [2] can be more successfully treated by infusion via the hepatic artery alone, in our study this was also true for micrometastases. The assumption that micrometastases can be more successfully treated with simultaneous infusion of the portal vein and the hepatic artery finds no support in our experiments.

Although with regard to the intraportal treatment of micrometastases the preliminary results of Taylor *et al.* [18] appeared promising, the results of our investigation and that of Ackerman *et al.* [2] do not support the assumption that infusion via the portal vein has any advantage in the case of micrometastases. Treatment by intraportal infusion of macrometastases would appear to have just as little purpose, because these derive 80-100% of their blood supply from the hepatic artery.

In conclusion, it may be said that the most important clinical consequence of our study is that the best results in cytotoxic treatment of liver metastases can be expected from infusion of the hepatic artery.

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