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Hepatic Arterial Infusion (HAI) Chemotherapy Proves to be Advantageous in the Treatment of Experimental Liver Tumors

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Abstract—*To evaluate the best locoregional approach in the treatment of hepatic tumors we investigated four different treatment modalities. Sixty female Sprague–Dawley rats were randomized to the following treatment groups: (1) untreated control; (2) hepatic artery ligation; (3) hepatic artery ligation plus portal 5-FU chemotherapy; (4) arterial 5-FU chemotherapy; (5) portal 5-FU chemotherapy. All animals received Novikoff hepatoma cells inoculated in the median liver lobe. After tumor inoculation animals were treated according to their randomization and surgery was performed for repeated tumor size measurements. Through hepatic artery ligation, hepatic artery ligation plus portal 5-FU infusion as well as through arterial 5-FU infusion a significant tumor growth inhibition compared to the untreated control could be achieved. The portal infusion group even showed an accelerated tumor growth; however, this effect was not significant. We conclude that the intraarterial application in terms of clinical feasibility and duration of response should be the preferred approach in locoregional therapy of liver malignancies.*

INTRODUCTION

LOCOREGIONAL CHEMOTHERAPY is gaining increasing attention in the treatment of liver tumors [1]. The direct approach to the tumor with a theoretically increased concentration of the chemotherapeutic agent at the tumor site as well as the utilization of the metabolic capacity of the liver (first-pass effect) and the thereby decreased systemic toxicity for some drugs are the major advantages of this treatment modality. Still it is not clear whether the arterial approach is advantageous compared with the portal administration of cytostatics in the locoregional therapy of liver malignancies.

In clinical as well as experimental studies it could be proven that primary and secondary neoplasms of the liver derive their blood supply almost exclusively from the hepatic artery. On the other hand, particularly the vital peripheral areas of the tumors as well as micrometastases in the liver which are caused by tumor spread via the portal vein gain nutrients mainly through the portal vein system [2]. It has been shown that interruption or diminishing of the arterial liver blood supply can influence the growth of liver tumors [3]. Cytotoxic effects of an intraarterial as well as an intraportal chemotherapy have also been described [4].

The aim of our study was therefore to examine the efficacy of the different kinds of locoregional treatment modalities in a standardized tumor model.

MATERIALS AND METHODS

Animals

Fifty to 80-day-old female Sprague–Dawley rats were used as experimental animals (Zentralinstitut für Versuchstierkunde, Hannover, F.R.G.). The animals were kept under normal conditions (room temperature 22 ± 2°C, relative humidity 55 ± 10%, day–night rhythm of 12 hr).

All surgical procedures were carried out under ether anesthesia.

Tumor model

For this study we used a transplantable rat liver tumor, the Novikoff hepatoma [5]. By injecting a single cell suspension of Novikoff hepatoma cells into the peritoneal cavity the solid tumor can be transformed into an ascitic form. Injecting Novikoff ascites cells subcapsular in the liver results in solid tumor growth. Novikoff hepatoma cells can be stored in liquid nitrogen without decreased transplantability. All animals received 5×10^5 Novikoff

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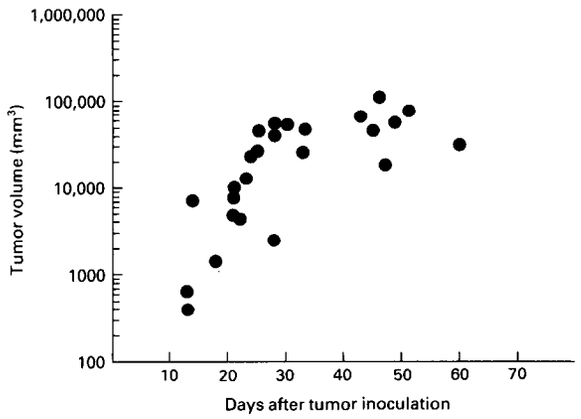

Fig. 1. Dependence of tumor size from time of implantation

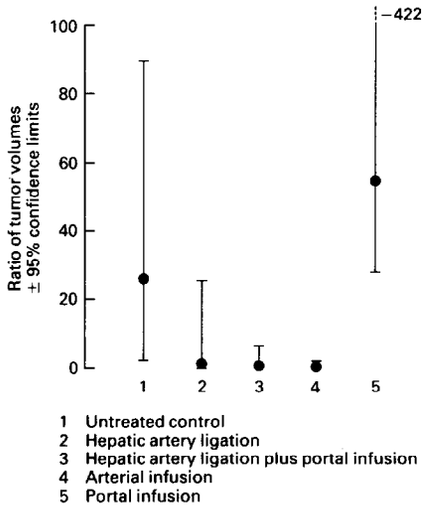

Fig. 3. Effect of different treatment modalities on tumor growth

ascites cells subcapsular into the central liver lobe. To avoid a peritoneal contamination with tumor cells the injection site was closed with Histoacryl® (B. Braun, Melsungen, F.R.G.). Intrahepatically inoculated Novikoff cells produce a spheroid nodule which can be measured at the time of surgery. For the calculation of tumor volumes both the vertical diameters (a,b) of the tumor were measured and volumes (V) were estimated according to the equation

$$V = (a \times b^2)^{-2}.$$

Figure 1 shows the dependence of tumor size from the time of implantation in untreated rats.

Treatment groups

All tumor inoculated animals were randomized to the following five groups with 12 animals each:

1. Untreated control group
2. Ligation of the hepatic artery
3. Ligation of the hepatic artery in combination with portal 5-fluorouracil chemotherapy
4. Arterial 5-fluorouracil chemotherapy
5. Portal 5-fluorouracil chemotherapy.

Study design

On day 0 all animals were randomized to the different groups. After randomization all animals received Novikoff hepatoma cells intrahepatically as described above. On day 7 after tumor transplantation all animals underwent laparotomy. At the time of surgery the tumor diameters were measured and catheters for the administration of chemotherapy were implanted, or hepatic artery ligation was carried out. On the following day continuous infusion chemotherapy was started over a period of 5 consecutive days in groups 3–5. On day 21 after tumor transplantation all animals underwent again a laparotomy for tumor size measurement.

Chemotherapy and drug administration

Continuous chemotherapy was performed by an external infusion pump (Fig. 2). The application technique was described by us earlier. 5-Fluorouracil was used in a dosage of 30 mg/kg/day over a period of 5 consecutive days. So the overall dosage in all chemotherapy groups was 1.220 mmol/kg. The drug was dissolved in saline solution with 25 I.E. heparin/ml.

Hepatic artery ligation

When ligation of the hepatic artery was planned we first identified the common hepatic artery arising from the aorta and preparation of the bifurcation of the gastroduodenal and the proper hepatic artery was performed. After identification of the vessel a double ligation with 6-0 silk followed.

Statistical analysis

In the different groups the relation between tumor size on day 7 and 21 after tumor inoculation were calculated. For statistical evaluation of the different treatment modalities compared to the untreated control group the Wilcoxon rank sum test was used.

RESULTS

Eleven animals were not eligible for evaluation for different reasons. Four animals of the untreated control group died from tumor progression before day 21 of the experiment. In the ligation group, one animal died postoperatively, two other animals showed anatomic variations of the hepatic artery. In groups 4 and 5 there were two animals in each group that showed no hepatic tumor at the time of laparotomy for catheter implantation.

The treatment results are summarized in Table 1 and Fig. 3. All animals of the untreated control group showed an increase in the tumor size during the observation period. The relation between tumor size on day 7 and day 21 of the experiment was in

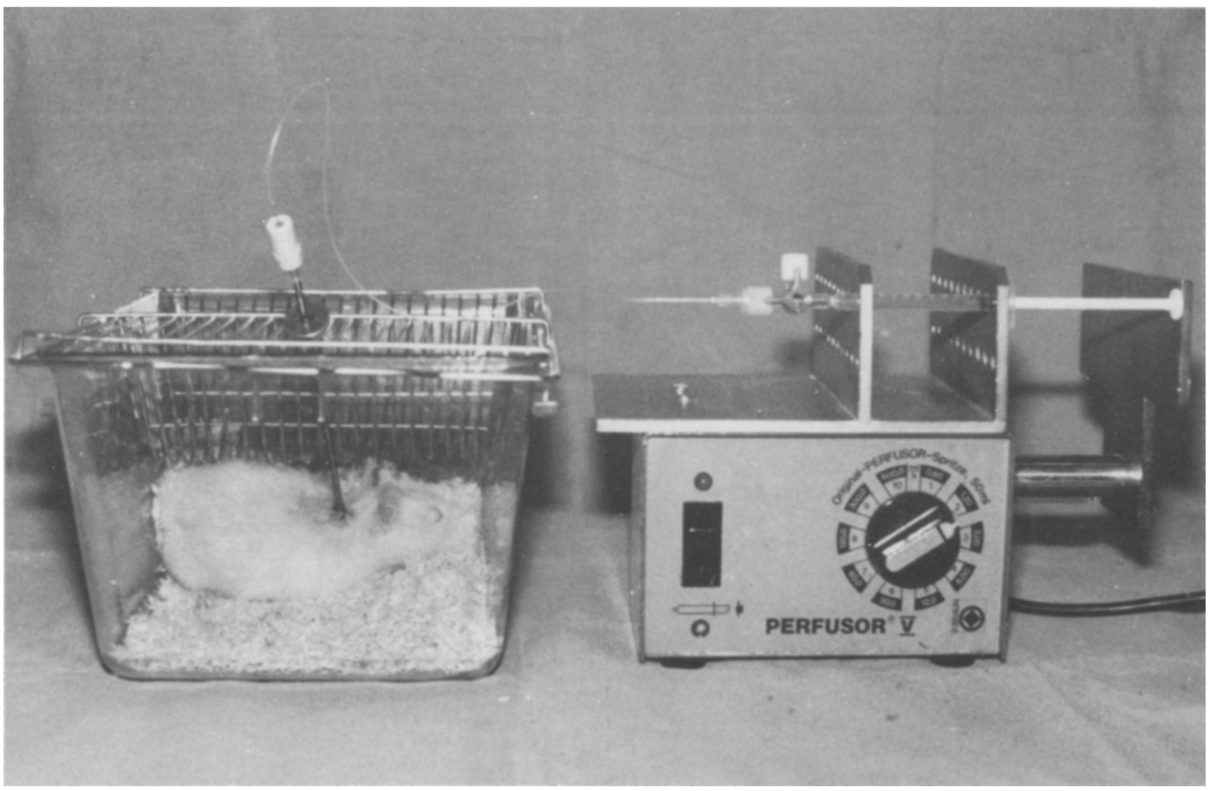

Fig. 2. Unrestrained rat during infusion with an external infusion pump

Table 1. *Ratio of pre- and post-therapeutic tumor volumes: table of individual values*

	Untreated control	Hepatic artery ligation	Hepatic artery ligation + portal 5-FU	Arterial 5-FU	Portal 5-FU
	13.92	1.14	96.00	1.07	142.50
	2.34	25.00	6.25	1.23	2344.00
	36.00	5.72	0.20	0.00	54.25
	86.80	0.08	0.02	1.95	422.00
	80.00	0.00	0.05	0.45	55.55
	15.60	250.00	20.80	1.65	51.84
	89.30	0.00	0.16	0.24	21.22
	14.22	2.37	5.10	0.30	78.42
		0.00	0.00	0.18	41.66
			1.66	9.18	28.00
			0.00		
			0.19		
n	8	9	12	10	10
Median	25.8	1.14	0.19	0.76	54.9
Confidence interval	2.34–89.3	0.0–25.0	0.02–6.25	0.18–1.95	28.0–422.0

median times 25.8 ranging from 2.34 to 89.3. In the portal infusion group there was even an accelerated tumor growth compared to the untreated control. The median increase of tumor volume was 54.9. However, that effect was not statistically significant compared to the untreated control group.

Following ligation of the hepatic artery four out of nine animals developed a more than 50% reduction of tumor size compared to pretherapeutic tumor volumes, three out of nine showed at least a tumor growth inhibition whereas two animals did not respond to treatment at all (Table 1). The median relative tumor size reduction after ligation was 1.14. Statistical comparison with the control group revealed a significant difference ($P = 0.036$).

The combination of hepatic artery ligation and portal infusion of 5-FU gave similar results as hepatic artery ligation alone. From 12 animals seven showed a more than 50% reduction in tumor size.

The tumor growth inhibition of hepatic artery ligation combined with portal infusion of 5-FU was highly significant compared to the untreated control but this treatment was not superior to hepatic artery ligation alone. Animals receiving intraarterial 5-FU infusion therapy also showed an effective inhibition of tumor growth. Only one out of 10 animals showed no response to therapy. The median ratio of pre- and post-therapeutic tumor volumes was 0.76. The difference between intraarterial 5-FU infusion therapy and the untreated control was highly significant ($P = 0.0001$).

In summary, hepatic artery ligation, hepatic artery ligation combined with portal 5-FU infusion as well as intraarterial 5-FU infusion showed significant tumor growth inhibition compared to an untreated control group. There was no significant difference between these three different treatment modalities.

DISCUSSION

The aim of our study was to investigate the influence of different locoregional treatments on tumor growth. In earlier studies using the Novikoff hepatoma model, we could demonstrate an increased tumor growth inhibition by intraarterial infusion chemotherapy compared to systemic application of fluorinated pyrimidines [6]. A significant reduction of tumor growth through intraarterial 5-FU chemotherapy could be reproduced in this study. Tumor remission in this experimental model was also achieved by hepatic artery ligation. This is in accordance with other experimental and clinical data reported in the literature [3]. The major problem of hepatic artery ligation is the formation of collateral circulation to the tumor. As a result the duration of response to hepatic artery ligation is usually short [7].

In our hands, a combination of hepatic artery ligation with portal 5-FU chemotherapy could not improve the tumor growth inhibiting effect of hepatic artery ligation alone. Considering that hepatic artery ligation plus portal 5-FU chemotherapy bears the risk of portal thrombosis combined with impaired arterial blood supply to the liver, this treatment can cause acute liver necrosis in the patients [4]. It is remarkable that an increase of tumor volume could be seen in the group with intraportal 5-FU infusion alone. At present one can only speculate on the reason for this effect. Perhaps

cytotoxic effects on the hepatocytes directly located near the tumor tissue facilitates tumor invasion [8]. Considering the blood supply of normal liver tissue and liver tumors, it is well known that liver tumors derive their blood supply mainly from the hepatic artery whereas normal liver tissue is preferentially supplied by the portal vein [2]. Therefore a portal application of cytotoxic drugs may cause even more damage to normal liver tissue than to the tumor cells. It has to be left open whether the criteria of treatment success chosen in our study are sufficient to assess the value of the different locoregional treatment modalities in general. In particular, the effect of a sequential application of the chemotherapy has to be left unconsidered because repeated cycles were impossible for technical reasons in our model. Furthermore it remains open, whether the results obtained with 5-FU are transferrable to other cytotoxic agents. Whether inhibition of tumor growth through locoregional chemotherapy results in an increase of life span of the animals is not clear and cannot be answered by this study. On the other hand, tumor remission is one important prerequisite to achieve prolonged survival. Our animal experiments may give some advice which kind of locoregional 5-FU chemotherapy approach is of most advantage.

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