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Combined Intermittent Dearterialization and Intraperitoneal 5-Fluorouracil Administration for Liver Tumours in the Rat

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Abstract—*Arterial occlusive therapy in the palliation of liver cancers has gone a long way since the first attempt at hepatic artery ligation. While efforts in permanent hepatic dearterialization have been frustrating in the face of fast developing collaterals, temporary inhibition of hepatic arterial blood flow appears to offer definite advantages. The effect of a single transient hepatic arterial occlusion with and without the addition of intraperitoneal 5FU was tested in Wistar–Furth rats bearing liver tumours. No advantage was observed in terms of tumour growth inhibition unless toxic doses of 5FU were used. A 5-day course of repeated treatment using intermittent dearterialization combined with intraperitoneal 5FU infusion was next tested and was found to be an efficient approach in reducing tumour growth rates. We prefer the intraperitoneal rather than the intraportal route for the infusion of oncolytic drugs because it avoids the problem of portal thrombosis and at the same time deals with any concomitant extrahepatic disease.*

INTRODUCTION

THE TREATMENT of unresectable hepatic malignancies has often been fraught with dismay. Primary liver cancers are well known for their rapid fatal outcome and hepatic metastases from gastrointestinal cancers are an extremely common clinical problem bringing many distressing symptoms. While medical attitudes generally incline towards a leave-alone policy, we prefer to search for an appropriate palliative measure for this group of patients who, more often than not, have been abandoned as being hopeless.

Various studies on human and animal models have shown that both primary and metastatic liver cancers derive their blood supply mainly from the hepatic artery [1, 2]. This has provided the theoretical basis for the use of induced arterial ischaemia in the treatment of liver malignancies. Ligation of the hepatic artery for liver cancer was performed more than two decades ago [3, 4]. However, the rapid development of collateral circulation has been a major impediment to success, nor has the more extensive operation of liver dearterialization shown any better results. Temporary and repeated dearterialization has subsequently been introduced to reduce the formation of collateral circulation [5–7]. Improved understanding of the biology of free oxygen radical production in liver ischaemia has prompted us to explore the use 'pulse ischaemia' in the killing of cancer cells [8]. Advances in technology have been timely. Implantable occluding devices are now available [9], and have made possible the repeated application of intermittent arterial ischaemia for extended periods of time in patients with liver malignancies. In addition, access to the peritoneal cavity has become extremely convenient with the use of subcutaneous injection ports. We chose the intraperitoneal route for the infusion of 5-fluorouracil (5FU) to patients because it is a safe way of delivering high concentrations of drug to the peritoneal cavity and portal circulation [10–12]. The peripheral portions of 'large' liver tumours are known to have a portal blood supply [13, 14]. While peritoneally infused cytotoxic drugs can have access to this portion of the tumour, they also deal with any extrahepatic peritoneal metastases at the same time. As clinical trials are under way, this therapeutic concept is being tested in an animal tumour

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system. In the folllowing experiments, the responses of a single hepatic arterial occlusion (HAO), with and without a dose of 5FU were studied as a background to the effect of a 5-day course of combined therapy.

MATERIALS AND METHODS

Animals

Thirty-six inbred Wistar–Furth rats (Anticimex, Stockholm, Sweden) weighing 250–350 g were used. They were fed a standard laboratory diet (R3 Astra-Ewos, Sweden) and tap water *ad libitum*.

Tumour

An *N*-methyl-*N*-nitrosoguanidine-induced adenocarcinoma of the rat colon was used [15]. The tumour was propagated by weekly intraperitoneal passage and tumours with generation numbers 38–39 and 47–49 were used in the following experiments. Tumour cell suspensions were prepared using a trypsin buffer solution and vital counts were made in a haemocytometer after adding trypan blue. In each rat a 0.1 ml suspension of 1×10^6 viable cells was injected subcapsularly into the left lateral lobe of the liver via a small midline laparotomy.

Hepatic artery occlusion

Transient arterial ischaemia was obtained by placing a vascular clip on the proper hepatic artery after dividing all accessory blood vessels to the liver and removing the clip after 1 h. The efficiency of this ischaemia technique and its reversibility has been established by coeliac angiography in a previous study [16].

Anaesthesia

Ether anaesthesia was used in all experiments.

Experiment 1

Five days after tumour inoculation, all rats were subjected to a laparotomy and the tumour diameters were measured with vernier callipers. The tumour volumes were estimated according to the formula: $V = a \times b^2/2$ where a is the largest and b is the smallest diameter [17].

Next, the liver was freed from its peritoneal attachments and the hepato-oesophageal branch of the oesophageal artery which comes from the left gastric artery was divided. The rats were then randomly allocated into five groups: a sham-operated control ($n = 5$), and four others each subjected to either: a single HAO ($n = 5$), a single intraperitoneal injection of 5FU at 15 mg/kg ($n = 5$), a combination of a single HAO and intraperitoneal 5FU at 15 mg/kg ($n = 4$) or a combination of a single HAO and intraperitoneal 5FU at 150 mg/kg ($n = 3$). In the last two groups the 5FU was injected 24 h after the HAO.

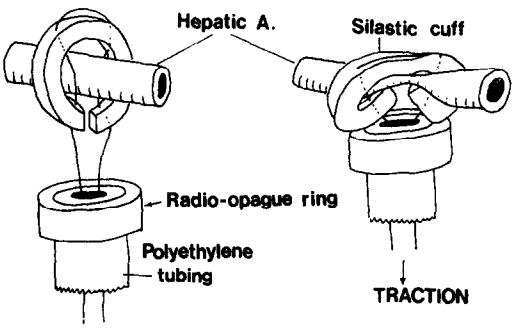

Fig. 1. The hepatic artery occluder used in the rat.

On the 9th day after tumour inoculation, a repeat laparotomy was done on all rats and the tumour diameters were again measured.

Experiment 2

Fourteen rats were used. They were similarly operated at 5–7 days after tumour transplantation by which time the liver tumours were approximately 0.2 cm^3 in size. Next, the gastroduodenal artery was ligated and a vascular occluder implanted around the common hepatic artery. Details of this vascular occluder model were described in a previous study [18]. Basically it consists of a short Silastic cuff (Cat. No.: 602–205 Dow Corning) with a Prolene 4'0' suture placed through its wall in a purse-string fashion (Fig. 1). The cuff is split and then positioned round the common hepatic artery while the two ends of the Prolene suture are threaded through a polyethylene tube (PE 100) which is tunneled subcutaneously and brought out through the tail of the rat. Intermittent occlusion of the hepatic artery can be effected by traction on the Prolene suture at the tail of the animal. For the purpose of cytotoxic infusion a Silastic tube (Cat. No.: 602–135 Dow Corning) was left intraperitoneally and similarly exteriorized via the tail of the animal. Daily 1 h occlusions of the hepatic artery were delivered to rats in the treatment group for the first five post-operative days. Simultaneous intraperitoneal infusion of 5FU (Roche) at a dosage of 15 mg/kg and diluted to 1 mg/ml in normal saline was given with the help of an infusion pump (Injectomat 50, Fresenius, F.R.G.). Control rats underwent sham operation with intraperitoneal normal saline infusions only. All rats were heparinized with 400 U/kg/day by the intraperitoneal route. On the 6th post-operative day, coeliac angiography was performed on all rats using a fine angiographic catheter (20755 tubing, 0.6 × 0.3 mm OD/ID, Surgimed, Denmark) which was inserted into the aorta via a femoral arteriotomy. Angiographic assessment of the patency of the hepatic artery is necessary since previous studies showed that a significant fraction of the rats

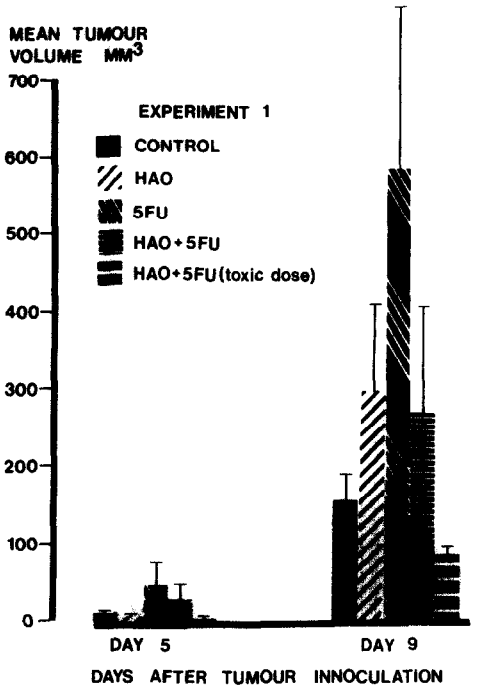

Fig. 2. Single occlusion and single dose 5FU therapy in rat liver tumours. Vertical bars indicate standard error.

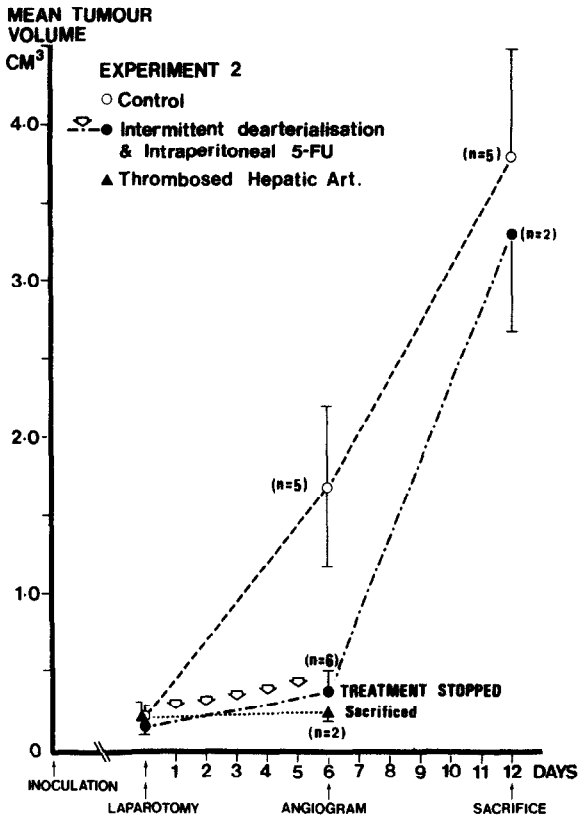

Fig. 3. Retarded growth rates resulting from a 5 day course of therapy with intermittent dearterialization and intraperitoneal 5FU. Vertical bars indicate standard error.

developed permanent obliteration of the vessel with the repeated use of this occluder [18]. Rats with patent hepatic arteries were kept alive after the angiographic procedure. All antitumour therapy was then stopped and a further evaluation of tumour sizes performed on the 12th day. Results were expressed as means ± S.E.M. Statistical tests were not carried out in view of the small number of animals.

RESULTS

Single treatment (Fig. 2)

Neither a single HAO nor a single therapeutic dose of 5FU had any tumour growth inhibitory effect. No advantage in the treatment was observed even when the two were combined. Paradoxically, the growth rates appeared to be enhanced. When a toxic dose of 150 mg/kg was employed in conjunction with HAO, diarrhoea developed in all the animals and the liver tumour growth was retarded.

Repeated combination therapy (Fig. 3)

Of the eight rats allocated to the treatment group, all were alive and well at Day 6, but only six rats successfully completed the course of intermittent dearterialization and intraperitoneal 5FU therapy without hepatic artery thrombosis. Their livers showed reduced tumour volumes compared with control rats. The remaining two rats were found on angiography to have permanently occluded hepatic arteries, and at laparotomy to have similarly retarded tumour growth. As our interest revolves around intermittent arterial occlusive treatment, these two rats were sacrificed. Of the six rats allocated to the control group one had a tumour transplantation failure and was therefore excluded from the study. Four rats, all from the treatment group, died immediately after the angiographic procedure from radiological contrast toxicity. Treatment was terminated in the remaining two rats and this allowed acceleration of tumour growth again to reach sizes comparable with controls at Day 12. By then carcinomatosis peritonei was noted in one rat in the control group. Histological examination of the treated tumours showed necrosis of the central three-fourths with dilated vessels and fibrin thrombi. The necrotic changes were reaching the surface of the liver in some cases. However, non-specific central tumour necrosis of varying degrees was also a consistent histological feature in all rats in the control group and those treated with a single 1 h arterial occlusion.

DISCUSSION

Rational approaches to chemotherapy of solid cancers are generally based on growth kinetic principles. It is known that the 'growth fraction' of viable tumour cells decreases during tumour growth and this slowing is exponential in nature resulting in the generation of a Gompertz growth curve. It follows that the sensitivity of the viable cell population

to antimetabolite drug therapy will necessarily be reduced in parallel to tumour growth [19]. It is the aim of our treatment regime to reduce the tumour mass by repeated ischaemia with the expectation that the growth fraction of the surviving cells will consequently rise and render these cells vulnerable to destruction by concurrent 5FU therapy.

Accelerated growth of solid tumours subjected to a single dose of irradiation is a well recognized phenomenon. This has been explained on the basis of an increased growth fraction together with a shortened cell-cycle time in the surviving cells that have been stimulated to proliferate. The results of experiment 1 can be interpreted on a similar basis since single sub-curative doses of cytotoxic drugs and ischaemia were used. The use of a toxic dose of 5FU resulted in a difference in tumour response with its inevitable toxicity. One possible approach to reducing toxicity would be to divide this high dose into several repeated treatments.

In experiment 2, no signs of toxicity from 5FU were present in those rats treated by intermittent arterial occlusion and standard doses of repeated intraperitoneal chemotherapy. Permanent hepatic artery obliteration, although a necessary hazard of this therapy in rats, has fortunately not been observed in humans undergoing clinical trials to date.

There is a growing body of evidence that intermittent arterial ischaemia is an effective way of inducing tumour necrosis. Free oxygen radicals are readily produced in liver ischaemia [8] and hepatic necrosis readily develops after transient arterial occlusion in animal experiments [20].

Recent studies have shown that temporary hepatic dearterialization may synchronize the pool of cycling tumour cells [21]. With this in mind, and as part of our effort in substantiating the value of combined ischaemia and 5FU therapy, we will be investigating towards defining the optimal timing of the chemotherapy in relation to the ischaemia.

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