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Arterial Occlusion Reduces Tumour Cell Lodgement in the Rat Liver

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Abstract—*The lodgement of tumour cells (TCs) is a key event in the development of metastases in distant organs. Experimental and clinical studies have shown that ligation of the hepatic artery may reduce the mass of established metastases. In the present study the effect of hepatic artery or portal vein occlusion on the early phase of metastasis development, i.e. TC lodgement, was investigated. Occlusion of the hepatic artery immediately before intraportal TC infusion reduced TC lodgement, while a temporary occlusion of the portal vein directly after the TC infusion led to increased TC lodgement. It is speculated that the decrease in TC lodgement after arterial occlusion is due to local increase in blood flow, which might enhance the passage of the TCs through the liver, and to a decrease in pH causing an increased rate of TC destruction. The increased TC lodgement after portal vein occlusion, on the other hand, should mainly be due to flow reduction, promoting TC trapping in the liver microvasculature.*

INTRODUCTION

THE TRAPPING of circulating tumour cells (TCs) in the microvasculature and the subsequent early period of survival are generally referred to as TC lodgement. Since the process is a key event in the development of haematogenous metastases in distant organs [1] it would be of great importance to find methods that could reduce the lodgement of circulating TCs. In previous studies we have shown that thrombocytopenia and peripheral serotonin (5-HT) receptor blockade have a certain capacity to reduce TC lodgment in the lung and liver of experimental animals [2–4]. In our further studies on different factors that might influence the complex TC lodgement process, a possible influence of arterial occlusion on TC lodgement has been brought into focus. Hepatic artery occlusion has been used with variable results in experimental and clinical situations on primary and secondary liver tumours [5, 6].

In the present investigation, experimental studies were performed in rats in order to study the effect on TC lodgement of occlusion of the hepatic artery immediately prior to infusion into the portal vein. For comparison, TC lodgement was also studied after a temporary occlusion of the portal vein immediately after the TC infusion. These experiments were combined with studies *in vitro* in order to evaluate the response of the TCs to controlled acidosis, using normal rat leukocytes as a reference. In addition, experiments were performed to analyse whether arterial ischaemia could reduce metastasis formation.

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MATERIALS AND METHODS

Animals

Male hooded rats of the Lister strain, weighing 200–250 g, were used. Anaesthesia was induced by intraperitoneal injection of sodium pentobarbital (5 mg/100 g body wt) and diazepam (0.4 mg/100 g body wt).

Tumour cells

The tumour was a syngeneic methylcholantrene induced fibrosarcoma (received from the Chester Beatty Research Institute, Sutton, Surrey, U.K.). This tumour metastasizes spontaneously to lymph nodes and causes development of hepatic nodules when injected intraportally.

The TC suspensions were prepared as described by Ivarsson and Rudenstam [7]. The viability of the cells in the suspensions was always >85%, as estimated by nigrosin staining [8]. The TCs were labelled with [^{125}I]5-iodo-2-deoxyuridine and radioactivity measured by means of a well-type NaI (T1) detector [9]. The pulses from the detector were

analysed in a single-channel analyser. Each sample was counted for 1 min. Background signals, always few compared with ^{125}I pulses, were subtracted.

Leukocytes

Rat leukocytes were separated from whole blood according to a procedure developed by Braide [10]. Briefly, blood was drawn into heparinized test tubes, by aortic puncture, and then diluted 1 + 1 in PBS (phosphate buffered saline), with NaCl added to an osmolarity of 400 mOsm. The diluted blood was layered on top of a two-step Percoll® (Pharmacia Fine Chemicals, Sweden) gradient of 400 mOsm with densities 1.085 and 1.100 kg/l. After centrifugation at 800 ***g*** for 60 min, the leukocytes were harvested.

Experimental procedure:

In vivo. The liver was exposed by a midline abdominal incision and the left hepatic artery was dissected free and cauterized. Half a ml of TC suspension containing 10^6 cells/ml was then slowly injected into a mesenteric vein. Six rats were killed 5 min after the TC injection and 14 rats after 3 h; the rats were killed by rapid exsanguination through the aorta. The livers were then removed and divided between the occluded left lobe and the right lobes. A standardized procedure for this division had previously been established in preliminary experiments by ink injection after clamping the blood supply to the left lobe (Fig. 1). This was performed to ensure that the different liver specimens were correctly separated [11]. The samples were washed in tap water, weighed and stored in glass tubes for radioactivity measurements. The amount of isotope in the different parts of the livers was determined as counts per min and gram liver tissue.

In 12 animals the portal vein was clamped for 5 min immediately after the TC infusion. TC lodgement in the liver was analysed directly after the clamping in six animals and 3 h later in six animals. Twelve animals were used as controls, i.e. without clamping; six animals were analysed 5 min after TC injection and six animals 3 h later.

In vitro. To test the pH sensitivity of the TCs the following experiment was undertaken. TCs were suspended at room temperature in Parker's 199 solution of two different pH levels, 7.3 and 6.7, the low value corresponding to pH values found in ischaemic livers [12]. Samples were collected from each suspension after 5 min and then at 10 min intervals. The TCs were stained with nigrosin [8] and the number of viable cells were counted in a Bürker chamber. The final counts were made after incubation for 90 min.

The leukocytes were also suspended in Parker's solution of pH 7.3 and 6.7 and analysed in the same way as the TCs.

Metastasis experiments. Unlabelled TCs (5×10^5) were injected into the portal vein of five rats, after ligation of the arterial supply to the left lobes. Four weeks later, the rats were killed and examined macroscopically for liver metastases.

RESULTS

In vivo

Following 5 min of arterial ischaemia the TC counts/min × g liver tissue did not differ significantly between the normal and arterially occluded liver lobes. Three hours later, however, there was a significant difference between the ischaemic left lobes and the normally perfused right lobes, the left lobes having, in average, 48% lower cell counts than the right lobes (Table 1).

Immediately after portal clamping for 5 min, the TC lodgement was significantly higher than in the controls. After 3 h the number of TCs lodged in the liver was still significantly higher in the clamped group than in the controls (Table 2).

In vitro

At the start of the experiments the viability of the TCs was 90% and 84% at pH 7.3 and 6.7, respectively. The number of viable TCs in the medium with pH 6.7 decreased continuously during the 90 min incubation period and at the end of the period only 35% of the TCs were still viable. The viability among the TCs incubated at the physiological pH was then 68%, i.e. almost twice as high (Fig. 2).

The leukocyte viability was 92% and 84% at pH 7.3 and 6.7, respectively, at the start of the experiment. The number of viable leukocytes incubated at pH 6.7 decreased continuously during 90 min, but at a slower rate than the TCs. After 90 min 57% of the leukocytes were viable at pH 6.7 and 70% at pH 7.3 (Fig. 2).

In the metastasis experiments there were great numbers of macroscopically visible metastases in all of the normally perfused liver lobes, whereas in the arterially ligated left lobes only a single metastasis was seen in two of the rats.

DISCUSSION

In comparison with the effect of vascular occlusion on established metastases, information about the effects of vascular occlusion on the first step in the metastatic process, i.e. the lodgement phase, is scarce. To our knowledge, the only study comparable with the present one is that by Fisher *et*

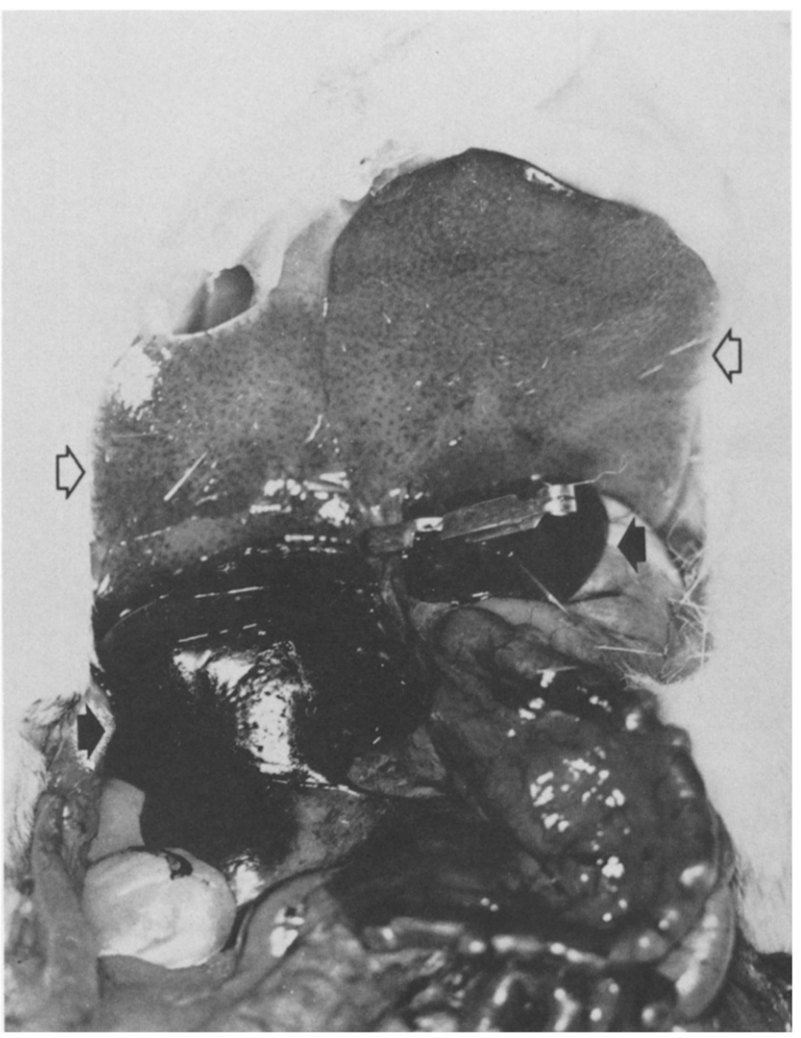

Fig. 1. ***Photograph*** *of rat liver after ink injection with the left hepatic artery occluded. Open arrows depict the ischaemic left lobes and the black arrows the normally perfused right lobes.*

Table 1. *Number of lodged TCs/g liver tissue* 5 min *and* 3 h *after intraportal injection of* 5×10^5 *TCs; hepatic artery occlusion immediately before the TC injection*

	5 min ($n = 6$)	3 h ($n = 14$)
Right lobes	7.202 (6.512–8.045)	2.237 (1.812–2.650)
Left lobes	6.334 (6.047–6.628)	1.094* (851–1.496)

*Significant difference from right lobes ($P < 0.01$; Wilcoxon). The range is given in parentheses.

Table 2. *Number of lodged TCs in the liver* 5 min *and* 3 h *after intraportal injection of* 5×10^5 *TCs; portal vein occlusion (PVO) for* 5 min *after the TC injection*

	5 min ($n = 6$)	3 h ($n = 6$)
Controls	61.928 (56.347–65.216)	15.789 (12.851–18.082)
PVO	110.589* (92.931–140.646)	21.185* (17.835–26.362)

*Significant difference from controls ($P < 0.01$; Wilcoxon). The range is given in parentheses.

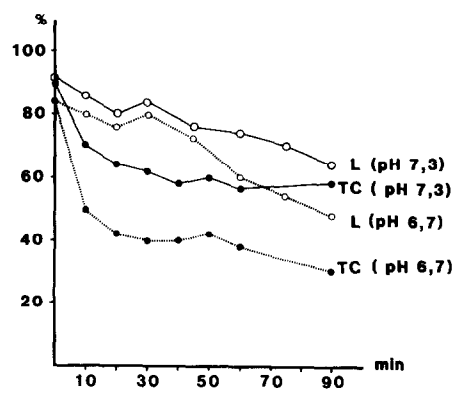

Fig. 2. *Diagram showing the decay, over* 90 min, *in the percentage of viable TCs and leukocytes in Parker's solution of two different pH values.*

al. [13], who found an enhanced metastasis formation after ligation of the hepatic artery, performed 3 days prior to portal TC infusion or 10 min after the infusion. These findings disagree with the present results, which showed that hepatic artery occlusion significantly reduced both TC lodgement and metastasis formation. Concerning the experiments by Fisher *et al.*, where ligation of the hepatic artery was performed 3 days before TC injection, it may be speculated that the increased metastasis formation was due to microvascular endothelial cell damage caused by the arterial occlusion. Damage of the endothelium is known to enhance extravasation of TCs and metastasis formation [14]. In the present study, with injection of TCs immediately after hepatic artery ligation, there should have been no major endothelial cell damage during the lodgement phase. On the other hand, it cannot be precluded that some local acidosis had developed already at this time, causing an increased rate of destruction of TCs. As shown in this study, the TCs were significantly less resistant to acidosis than normal leukocytes, the TCs showing a much more rapid decrease in viability during the first 10 min of exposure to a low pH.

An additional way of explaining the present results is that fewer TCs became lodged when infused in close connection with arterial occlusion, because of rheological alterations in the liver microcirculation. It has been shown that occlusion of the hepatic artery leads to a decrease in the flow resistance in the portal system and, indirectly, to an increased flow [15]. An increased flow, i.e. increased shearing forces in the portal sinusoids and veins, should reduce the tendency of intraportally injected TCs to become lodged in the liver. This hypothesis is supported by the present findings of a significant increase in the number of lodged TCs after a temporary flow reduction obtained by portal occlusion.

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