On the prevention of haematogenous tumour metastasis to liver and lung

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Summary. Vascular endothelium is lined by a layer of alpha, 2, macroglobulin (AMG). This protide is believed to regulate the rate of extravasation of tumor cells. Experiments are described which show that increasing the deposition of AMG (enhancing its production and blocking its lysis) reduces the incidence of tumour metastases to liver and lung.

The number of circulating tumour emboli is no indication of the extent of secondary growth. The majority of tumour cells undergo intravascular destruction[2,3]. The establishment of secondary tumour depends on the number of cancer cells which transmigrate the vascular wall and implant in extravascular sites. The rate of extravasation is determined by the permeability of the vessel wall – permeability in turn being regulated by the amount of alpha, 2, macroglobulin (AMG) deposited on vascular endothel[4,5]. Thus a thick AMG layer would retain the tumour cells in the vascular tree whilst a thin or defective layer would allow their escape into the extravascular compartment.

The amount of AMG deposited is the balance between its production and its destruction. AMG is produced i.a. by mononuclear cells[6] and lysed by proteases. Thus, in order to enhance the deposition of AMG on vascular endothel – and so to contain the tumour cells within the vascular tree – it is necessary to step up its production and at the same time to block its lysis. The present experiments show that these measures, singly or combined, can impair the development of haematogenous tumour metastases to liver and lung.

Materials and methods. Animals: Outbred female rats of Charles River strain weighing 150 g were obtained from Messrs Lewenstein, Yokneam (Israel).

Tumour: A tumour which appeared at the site of i.m. testosterone injections, was used. Old male rats were given 2 2.5-mg injections of testosterone propionate (Organon) into the right thigh muscle. In 4 out of 10 animals so treated, there appeared a huge thigh tumour which spread to the lung and to the axial lymph nodes. This tumour was transplantable to homologuous recipients and retained its tendency to metastasize. Histologically it is an undifferentiated sarcoma. It can be maintained by serial passage.

Chemicals: As proteinase inhibitor, apronitin (Trasylol, Bayer) was used. This is a purified, concentrated, naturally occurring enzyme inhibitor from bovine lung, 1.0 ml corresponding to 10,000 kallikrein inactivator units. It was used in undiluted form. The immunostimulant employed was Freund's complete adjuvant (Difco) (FCA). The proteinase used was trypsin (Difco) prepared as 0.2% solution in saline.

Preparation and administration of tumour suspension: 1 volume of tumour was suspended in 1 volume of saline and passed through a fine-meshed nylon sieve. Amounts of 0.5 ml of this suspension were injected by i.m. or i.p. route. For certain experiments (perfusion of limb) amounts of 1.0 ml of tumour suspension were injected into the right thigh muscle. For i.v. injection, this suspension was allowed to stand for 15 min at room temperature for the larger fragments to settle to the bottom of the test-tube; the supernatant was aspirated via a tuberculin needle and injected in amounts of 0.1–0.2 ml into the femoral or portal vein.

For another series of experiments, the tumour suspensions were centrifuged, the supernatant decanted and the cells resuspended in an equal volume of saline or Trasylol. These suspensions were incubated for 45 min prior to inoculation.

Experimental procedure: The animals were divided into 12 groups.

Group A received i.v. injections of 0.1 ml of tumour cells in saline.

Group B received i.v. injections of 0.1 ml of tumour cells in Trasylol.

Group C received i.v. injections of 0.1 ml of tumour cells in Trasylol, followed by i.m. injections of 4.0 ml of trypsin solution on day 0, 1, 2 and 3 after i.v. tumor. Experimental and control animals were sacrificed on the 11th day after tumour administration and the lungs examined for the presence of tumour deposits and weighed.

Group D received i.p. inoculations of tumour suspended in saline.

Trasylol. The animals were sacrificed on the 11th day and the abdomen examined for the presence of tumour. For the

Table 1. Incidence of pulmonary and peritoneal tumours in animals receiving i.v. or i.p. inoculations of tumour suspensions. Tumour incubated in saline or in Trasylol in vitro or perfused with Trasylol in vivo

Group	Route of injection	Incidence	Weight of lung (g) Range	Average
Lung				
A Tumour/saline vitro	i.v.	30/30	1.3–8.2	3.5±1.9
B Tumour/Trasylol vitro	i.v.	4/32	1.3–1.7	1.2±0.2
C Tumour/Trasylol vitro	i.v.			
+ trypsin	i.m.	5/7	1.8–2.2	2.0
Peritoneum				
D Tumour/saline vitro	i.p.	12/15		
E Tumour/Trasylol vitro	i.p.	13/14		
Lung				
F Tumour perfused with Trasylol vivo	i.v.	14/26	0.8–1.5	1.2
G Tumour perfused with Trasylol vivo	i.v.			
+ trypsin to host	i.m.	8/10	1.0–9.2	3.0±0.5
Peritoneum				
H Tumour perfused with Trasylol vivo	i.p.	9/10		

Average weight of normal lung of rat weighing 150 g: 1.3±0.1 g.

perfusion experiments, amounts of 1.0 ml of tumour suspension were injected into the right thigh muscle. The animals were laparotomized 11 days later and 10.0 ml of Trasylol was infused via the right hypogastric artery. The animals were sacrificed 1 h later, the tumour excised and processed in the usual manner.

Group F received i.v. injections of perfused tumour.

Group G received i.v. injections of perfused tumour followed by administration of trypsin as above.

Group H received perfused tumour i.p. These experiments were terminated as the previous groups.

Group I received intraportal injections of tumour in saline (0.2 ml).

Group J received 0.2 ml of FCA into the spleen and, 4 days later, 0.2 ml of tumour in saline into the portal vein.

Group K received intraportal injection of tumour in Trasylol.

Group L received 0.2 ml of FCA into the spleen and, 4 days later, 0.2 ml of tumour in Trasylol into the portal vein.

The size of the liver tumours was scored thus: Less than $\frac{1}{3}$ of the liver +, $\frac{1}{2}$ of the organ + + and the whole liver replaced by tumour + + +. The experiment was terminated 11 days after the intraportal injection.

Results. Table I shows that i.v. inoculation of tumour suspended in saline produces large lung tumours whilst infusion of tumour cells incubated in Trasylol yielded few and small pulmonary growths (groups A and B). However, when the recipients of tumour in Trasylol were treated with trypsin, they developed pulmonary tumours similar to those of the controls (group C). There was no difference in the incidence of peritoneal tumours in the recipients of i.p. tumour/saline or tumour/Trasylol (groups D and E).

The technique of perfusion was not entirely satisfactory. Preliminary experiments with dye injections had shown that, in order to achieve adequate perfusion, it was necessary to inject the dye under some slight pressure. However, by this procedure there was leakage from the injection site. But even under these circumstances, there was significant inhibition of pulmonary tumour in animals receiving i.v. inoculations of "perfused tumour" (group F). The tumours that did grow were few in number and did not significantly increase the weight of the lungs. The concomitant administration of trypsin again led to massive pulmonary tumour growth - 1 lung reaching a weight of 9.2 g (group G). Perfused tumour readily grew on i.p. inoculation (group H).

Liver weight appeared to be an unreliable guide as to the amount of tumour infiltration into that organ. Tumour volume was therefore assessed as indicated before. Table II shows that tumour injected into the portal vein took and grew readily (group I) whilst intralienal stimulation somewhat reduced tumour take (group J). Tumour suspended in Trasylol also grew on intraportal inoculation but to a lesser extent (group K) whilst a combination of intrasplenic FCA and exposure of tumour cells to Trasylol led to a significant inhibition of hepatic tumour growth (group L). These experiments carried a high mortality as FCA-treated animals tended to bleed profusely from the puncture site in the portal vein.

Table 2. Incidence and extent of hepatic tumour in animals receiving intraportal injections of tumour incubated in saline or in Trasylol and stimulated with intrasplenic inoculations of FCA

Group	I Tumour/ saline	J Tumor/ saline +FCA	K Tumour/ Trasylol	L Tumour/ Trasylol +FCA
Score	12/13 + + +	4/7 + +	4/11 +	2/13 +

High postoperative mortality due to bleeding from the puncture site. Each group originally consisted of 15 animals.

Discussion. Monocytic infiltration in a primary tumour reduces the incidence of haematogenous metastases. It does not affect the size of the primary[7]. The presence of a large peripheral tumour has been shown to inhibit the establishment of pulmonary metastases[8]. This phenomenon has been ascribed to the protective action of a humoral agent (not an antibody) produced by lymphocytes[9].

It is therefore suggested that the protective substance is an AMG released by tumour associated monocytes. This protide diffuses into the tumour effluent and coats the endothel of vessels draining tumour, usually the pulmonary arteries. Such vessels are thus rendered impervious to cellular elements[10].

Some tumours are either deficient in monocytes and/or produce excess amounts of proteases. These are the tumours that readily disseminate to the lungs - as is the case with our experimental tumour. Inactivation of such proteases by exposing the tumour cells to proteinase inhibitors converts these tumours into their non-metastasizing variant, i.e. into a tumour incapable of setting up haematogenous metastases (table 1). Such treatment does not destroy the tumour cells: Trasylol-treated cells grew on i.p. inoculation, they also grew in the lungs of recipients treated with supplementary protease (trypsin).

The technical difficulties of tumour perfusion in vivo in rats have already been alluded to. These are unlikely to occur in larger species. Yet, even in present experimental conditions, there was significant inhibition of tumour growth after i.v. injection of perfused tumour - a state of affairs that could be reversed by administration of trypsin to the recipient.

Trasylol-treated tumour cells grew to some extent on intraportal injection. It is believed that the AMG coating of terminal portal venules or of hepatic sinusoids is deficient, and that tumour cells can extravasate even if their proteases are blocked. Measures to increase AMG production and deposition were therefore adopted. These consisted of stimulation of splenic monocytes, the assumption being that the AMG so released would reach the liver via the splenic vein. It is of course also possible that small amounts of FCA travelled to the liver and stimulated the monocytes in situ. Whatever the precise mechanism, present experiments show that the combination of intrasplenic inoculation of FCA together with the exposure of tumour cells to Trasylol prior to intraportal infusion, greatly reduced the incidence of liver tumours via the portal route.

It is therefore conceivable that perfusion of the tumour bed with proteinase inhibitor, together with intrasplenic injection of an immunostimulant, might inhibit the growth of secondary tumour in liver and lung.

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