Differences in lodgement of tumour cells in muscle and liver

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Differences in the lodgement of circulating tumour cells in various organs are considered an important factor in metastatic organ selection. The present vital microscopic studies show that the pattern of intravascular arrest of tumour cells in muscle after intra-arterial injection is similar to that observed earlier, in the liver, after intraportal injection. However, parallel isotope studies on the lodgement process (at 5 min and 3 h after injection) showed that the tumour cells trapped in the muscle microvasculature were destroyed at a higher rate than in the liver. Tumour cells kept in test tubes, and thus not being subjected to the shearing forces of the circulation, had a higher survival rate than cells trapped in the muscle. The results indicate that stronger retardation forces acting on the tumour cells in muscle (arterial dissemination) than in the liver (venous dissemination) may be one mechanism behind the increased tumour cell destruction in muscle.

Introduction

Cancer metastases usually develop in various organs according to a fixed pattern. Metastases rarely develop in muscle, whereas they are common in lungs and liver. The mechanisms behind this organ selection pattern are not completely understood although many explanations have been proposed [6, 10, 11].

The organ selection for metastasis formation could be due to differences in the number of circulating tumour cells being arrested in the microvasculature of different organs and/or to a difference in the destruction of arrested cells. A number of different mechanisms have been suggested for intravascular killing of arrested tumour cells, including interactions with different defending cells such as lymphocytes, neutrophils and monocytes, as well as with complement, antibodies or cytotoxic factors. Mechanical factors have also been proposed to play a role in tumour cell destruction [15].

Theoretically, it is less likely that tumour cells, which may be very rigid [3], should become trapped in the microvessels when circulating at arterial perfusion pressure than when circulating at the lower perfusion pressures of the portal system or the pulmonary artery. Another theory recently proposed by Weiss and Dimitrov [15] is that circulating tumour cells are damaged by haemodynamic forces, implying that many more tumour cells are destroyed when distributed arterially, e.g. to a muscle capillary bed, than when delivered at low driving pressure to the microvasculature of the lungs or the liver.

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In earlier studies we have used vital microscopy and isotope techniques to study the arrest and lodgement of intraportally injected tumour cells in the rat liver [3, 14]. In the present investigation the same methods have been employed to obtain corresponding data on tumour cell arrest and lodgement in muscle after intra-arterial tumour cell injection.

Material and methods

Animals
Male hooded rats of the Lister strain, weighing about 200 g, were used. Anaesthesia was induced by intraperitoneal injection of sodium pentobarbital (5 mg/100 g b.wt.) and diazepam (0.4 mg/100 g b.wt.). The rats were then loaded with ethidium bromide (0.1 mg/ml saline), 2 ml/kg per hour intravenously. Ethidium bromide, which is excluded by normal cell membranes but penetrates into damaged cells, then giving a red fluorescence [4], was used as an indicator of tumour cell death in the vital microscopic experiments.

Tumour cells
The tumour used was a syngeneic methylcholanthrene-induced fibrosarcoma (received from the Chester Beatty Research Institute, Sutton, Surrey, England). This tumour metastasizes to lymph nodes and causes development of hepatic nodules when injected intraportally. Tumour cell suspensions were prepared as described by Ivarsson and Rudenstam [8]. The viability of the cells in the suspensions was about 90 per cent as estimated by nigrosin staining [9]. For the lodgement experiments the tumour cells were labelled with $^{125}$I-5-iodo-2-deoxyuridine and radioactivity was measured according to Skolnik et al. [13].

The tumour cells used in the vital microscopic studies were labelled with fluorescein isothiocyanate (FITC, Sigma Chemical Co., St Louis, USA) [7]. Two millilitres of FITC (0.1 mg/ml) were added to 10 ml of the tumour cell suspension which was incubated for 1 h at room temperature and then washed once at 250 g for 5 min. This staining procedure, which made the tumour cells fluoresce in green, was used to enable their detection in the blood stream. Before i.v. infusion the tumour cells were resuspended in Parker’s medium and samples were taken for viability tests. The staining and additional washing procedure reduced the proportion of viable cells (see above) to 70 ± 5 per cent.

Lodgement experiments
Half a millilitre of tumour cell suspension, containing $10^6$ tumour cells/ml, was injected by retrograde infusion through the epigastric artery into the femoral artery of each leg. One group of animals ($n = 5$) was killed by exsanguination 5 min after the tumour cell injection. The legs were amputated just proximal to the epigastric–femoral artery junction. The skin was removed and all leg muscles were separated from the bones.

A second group of animals ($n = 4$) was killed 3 h after tumour cell injection, followed by the same procedure as described above.

The radioactivity in the muscle and bone tissue was compared with the amount of radioactivity of the injected number of tumour cells. Since injections of tumour cells were given selectively, each leg was regarded as a separate entity in the analyses.