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Blood Flow Distribution Within Transplantable Tumours in the Mouse

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Abstract—*Tumour blood flow was estimated by fractional distribution of rubidium in two allografts (B16 melanoma and Lewis lung tumour) and two xenografts (Glioma 522, a human grade IV astrocytoma and Mel-mo, a human melanoma), in order to investigate the influence of certain tumour characteristics on tumour perfusion.*

In all four tumours perfusion decreased with increase in tumour weight. The rubidium extraction in Mel-mo was markedly lower than that of the other three tumours; this tumour was the most necrotic. Necrosis was patchy in Glioma 522 and Mel-mo, but predominantly central in B16 and Lewis lung tumour. However, all tumour nodules examined showed a similar pattern of rubidium extraction: high at the rim with a rapid fall towards the centre. It appeared that while overall blood flow may be related to the extent of necrosis, blood flow distribution within tumour nodules did not correlate closely its pattern.

INTRODUCTION

THE DISTRIBUTION of blood flow within tumours is of great relevance to cancer treatment: if drugs are unable to reach viable cells within tumours by passive diffusion from vascular capillaries, then cure of the tumour by chemotherapy would seem to be an unattainable goal. As Denekamp has remarked [1], if blood flow within tumours is inadequate to achieve therapeutic drug levels, then alternative delivery systems or different approaches to therapy would seem to be necessary.

Blood flow has been assessed both in experimental and in human tumours by means of dye dilution techniques [2, 3], by use of a wide variety of radiolabelled tracers [4–6] and by several physical methods [7–10]. Results obtained by the use of these various techniques have been contradictory more often than complementary.

Similarly, several workers have investigated the distribution of blood flow within tumours by dye uptake [11–13] or by using radiolabelled compounds and they too have obtained conflicting results. Some authors have failed to find any variation in blood flow within tumours [14], while others have demonstrated blood flow to be less within the centre of tumours than at the periphery [3, 8, 15].

The aim of this study was to examine whether total tumour blood flow varies with tumour type, weight or extent of necrosis and also to examine the distribution of blood within transplantable murine and xenografted human tumours, using the technique of fractional distribution of rubidium.

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METHODS

Cells (2×10^6) of B16 or Lewis lung tumour, Glioma 522 (a human grade IV astrocytoma) or Mel-mo (a human melanoma), were injected subcutaneously into the flanks of adult female C57 Black (B16 and Lewis lung tumour) or immune deprived [16] male CBA/Ca mice (Glioma 522 and Mel-mo). Subsequently, tumours were passaged when they were approximately 1 cm in diameter. Tumours were used in these experiments between second and fourth passages and at a variable time after implantation in order to study blood flow characteristics over a range of tumour sizes.

Tumour blood flow was determined by fractional distribution of rubidium [17, 18]. Each animal was injected via a lateral tail vein with 0.1 ml/10 g body weight of a rubidium chloride solution 1 mCi/ml (Amersham International), which had been diluted with phosphate buffered saline to an activity of approximately 20 μCi/ml. After 45 s, the animal received a lethal dose of 0.25 ml cold saturated potassium chloride solution intravenously. The mice were dissected and the tumours and organs blotted free of blood and weighed. Weighed samples of skin and of liver were taken and the other organs were divided into smaller portions where necessary so that they could be placed in small polycarbonate

vials. The entire mouse along with aliquots of blood and of the diluted rubidium solution, as well as 'blanks' (empty polycarbonate vials) to monitor background activity, were counted in a calibrated Hewlett-Packard Auto-gamma spectrometer. The blood flow through each organ and tumour, expressed as percentage distribution of cardiac output per gram of tissue (%FDFCO/g), was calculated from organ weights and counts, using a microcomputer, based upon the equation: counts per minute (sample) × 100/counts per minute (injected dose) × sample weight. %FDCO/g was used as an estimate of blood flow, since the cardiac output of the mouse cannot easily and accurately be measured, precluding the calculation of blood flow in absolute units [7, 19].

In order to study blood flow distribution, after weighing the whole tumour, the central strip of the equatorial slice was divided into segments, each of which was counted separately [15]. In these experiments, counts per gram of tissue were calculated and expressed as a percentage of the greatest value obtained in that tumour. This allowed comparison of tumours of differing weight and type.

Percentage tumour necrosis was estimated using the technique of point counting [20]. A series of B16 and Mel-mo tumours were weighed and an equatorial slice, which was taken to be representative of the tumour as a whole [21], was fixed in buffered formol saline, processed and sections stained with haematoxylin and eosin. Sections were examined at ×200 magnification using a 25 point random array graticule; the number of coincidences with areas of tumour necrosis were recorded and the percentage necrosis calculated: percentage necrosis = coincidences/point counted × 100%. The relative standard error was given by the formula: $RSE = \sqrt{(1 - p)}/\sqrt{n}$, where p is the probability of hitting an area of necrosis and n is the number of coincidences. Thus, in order to obtain a relative standard error in the order of 5% with a percentage tumour necrosis of approximately 10%, 3600 points or 150 high power fields must be counted [22]. In these experiments, 200 high power fields were examined in each tumour.

RESULTS

In all four tumours studied, blood flow (%FDCO/g) decreased as tumours increased in size. In each instance there was a log-linear relationship between tumour weight (mg) and blood flow with a high coefficient of correlation (Fig. 1).

Over the size range of tumours studied blood flow was much lower in Mel-mo tumours than in B16, Lewis lung tumour or Glioma 522, which all had a similar blood flow comparing tumours of equivalent weight.

However, in all four tumour lines blood flow distribution within individual tumour nodules was similar, diminishing away from the edge of the tumour towards the centre (Fig. 2). At the centre of tumours, rubidium uptake was as low as 25% (or less in very large tumours) of that at the periphery. This pattern of central decrease in isotope extraction was seen in each tumour studied, from the smallest (82 mg) to the largest (4522 mg), although the difference was most marked in the largest nodules.

Percentage tumour necrosis was estimated for a series of B16 tumours which weighed between 80 and 3612 mg and for Mel-mo tumours weighing between 37 and 2050 mg. Tumour necrosis was greater in Mel-mo than in B16 tumours of similar weight; in both tumours percentage necrosis increased in a linear fashion with the logarithm of tumour weight (Fig. 3). In Mel-mo necrosis was distributed throughout the nodules and sometimes involved the tumour periphery; Glioma 522 also exhibited patchy necrosis, but this appeared less severe than in Mel-mo and did not involve the tumour periphery. Necrosis was predominantly centrally located within B16 and Lewis lung tumour nodules which, with Glioma 522, all possessed a viable rim of tissue.

DISCUSSION

Tumours elicit an angiogenic response from the surrounding tissues [23] and may invade along the framework afforded by these new vessels [24, 25]. Thus the peripheral zones of tumours are likely to be well vascularized. However, a proportion of the capillary network within a tumour may not be in functional continuity with the general circulation [26] and under some circumstances may undergo regression [27] giving rise to a perfusion difference between the outer edge and the interior of tumours. Tumour blood flow has been investigated by several groups of workers, but there has been no overall agreement concerning the variation in blood flow with tumour characteristics such as weight [4, 7], site of tumour, or histology [7, 13]; the relationship between blood flow and necrosis has not been previously examined. Little has been published concerning blood flow distribution within tumours and there is little agreement between those working in the field. Kjartansson [14], using fractional distribution of rubidium, failed to find any variability in blood flow within methylcholanthrene-induced tumours in the rat; whereas Rogers [13], Edlich and Tookenay [28] and Davidson *et al.* [15] did find heterogeneity of rubidium uptake within hamster tumours and VX2 carcinomas in the rabbit, respectively.

In the experiments reported in this paper, blood flow per unit weight decreased as tumours increased in size and within each tumour blood flow

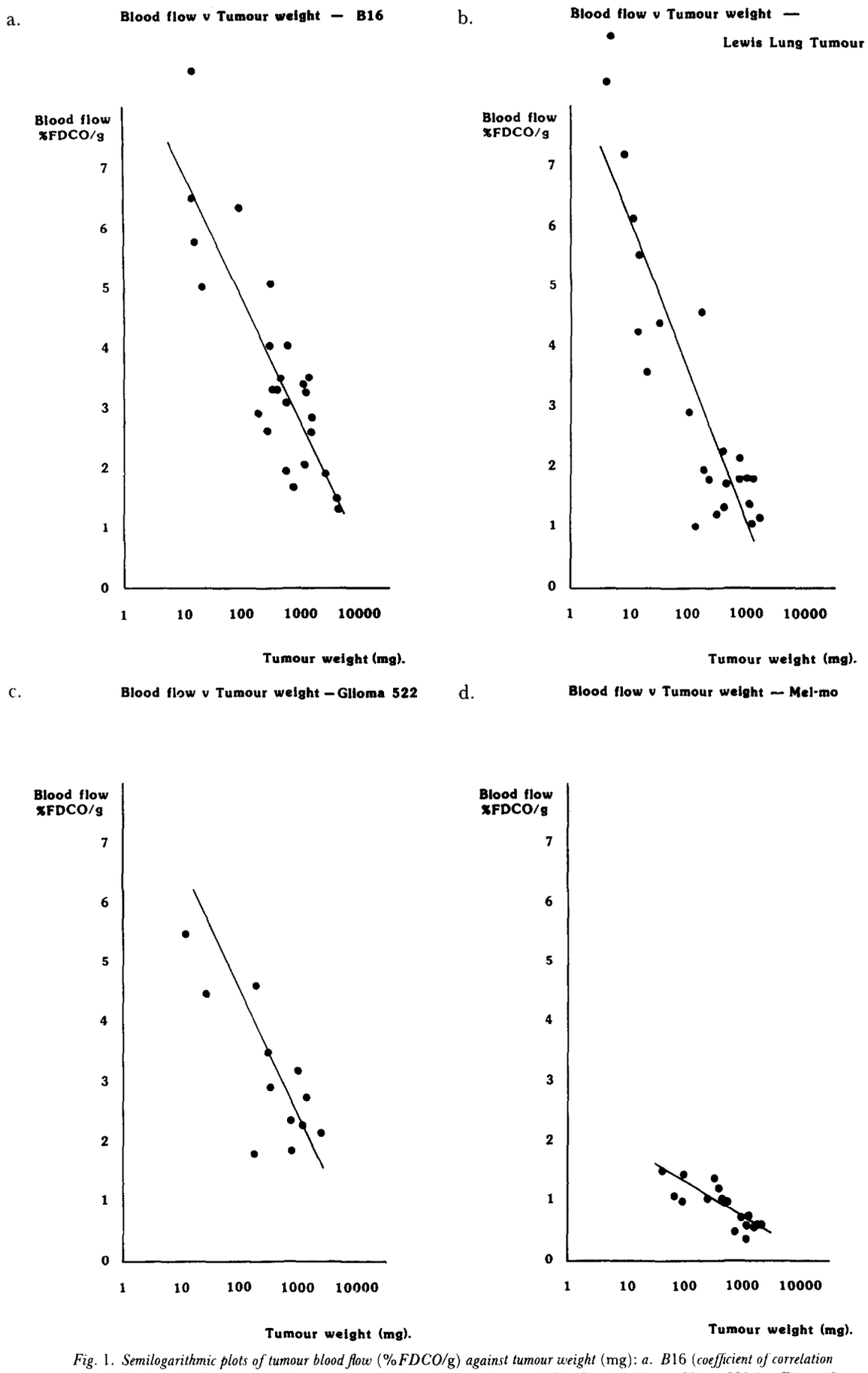

Fig. 1. *Semilogarithmic plots of tumour blood flow* (%*FDCO*/g) *against tumour weight* (mg): *a.* B16 (*coefficient of correlation* $= -0.85$; $P < 0.001$), *b. Lewis lung tumour* (*coefficient of correlation* $= -0.88$; $P < 0.001$), *c. Glioma* 522 (*coefficient of correlation* $= -0.75$; $P = 0.005$), *d. Mel-mo* (*coefficient of correlation* $= -0.77$; $P < 0.001$).

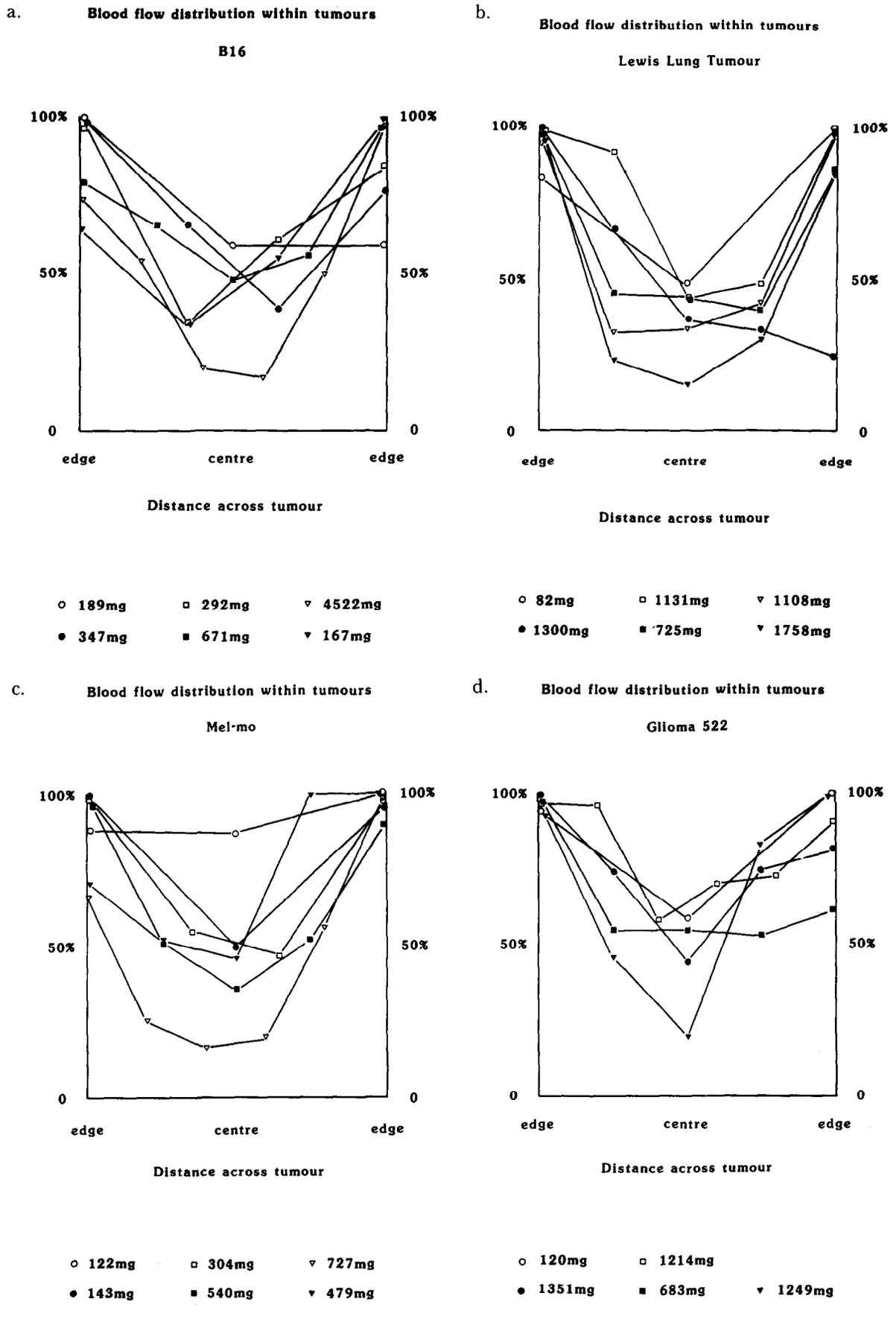

Fig. 2. Line diagrams representing ^{86}Rb uptake within tumour nodules: a. B16; b. Lewis lung tumour; c. Mel-mo; d. Glioma 522. E = edge of tumour nodule; C = centre of nodule.

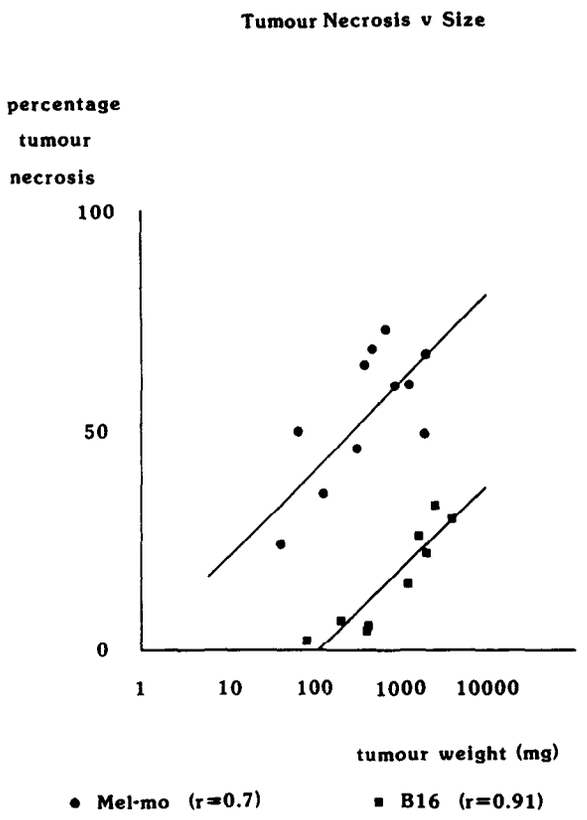

Fig. 3. Semilogarithmic plots of percentage tumour necrosis against tumour weight (mg).

diminished from the periphery towards the centre of the nodule, which would seem to imply that these experimental tumours are well perfused only at the periphery and the remainder is under perfused. Percentage tumour necrosis increased with the logarithm of tumour weight while blood flow (%FDCO/g) decreased. Thus, it may be inferred that there was an inverse relationship between tumour necrosis and blood flow as measured by fractional distribution of rubidium (said to reflect the size of the functioning capillary bed [17]). This inverse relationship between the functioning tumour capillaries and extent of necrosis has been supported by results obtained using intravital staining techniques [29]. The functioning capillary networks of B16 and Mel-mo tumours have been studied using the fluorescent dye H33342; percentage vascular volume decreased with the logarithm of tumour weight while percentage tumour necrosis increased, supporting the validity of the technique of fractional distribution of rubidium as a means of assessing the size of the functioning capillary bed.

Blood flow as measured by rubidium extraction decreased from the edge towards the centre of nodules both in tumours with central necrosis (B16 and Lewis lung tumours) and in those with patchy necrosis throughout the nodule (Glioma and Mel-mo). The same pattern of rubidium extraction was seen in tumours of all sizes, many of which showed no histological evidence of necrosis. In Mel-mo, which exhibited the lowest blood flow, necrosis included the edge of the tumour, an apparently well-vascularized zone, whereas B16, Lewis lung and Glioma 522 all included a viable rim. Thus, the relationships between perfusion and necrosis are not clear-cut. These results may imply that, whilst percentage necrosis is related to overall blood flow, other factors influence the its distribution within a tumour. Using H33342 it could be seen that more vessels were present at the periphery of nodules than in central zones [29]. Complementary results have been obtained previously using various dyes to qualitatively examine blood flow distribution [3, 12].

The existence of perfusion gradients within small tumours without obvious necrosis may indicate that chemotherapeutic agents are unlikely to produce complete remission of solid tumours even at an early stage in their development, unless the tumour cells are particularly sensitive to the administered drugs. Surgical removal of macroscopic tumours may aid the action of chemotherapeutic drugs both by reducing the volume of tumour through which the agents must diffuse and secondly by inducing angiogenesis in and around remaining tumour which may lead to increased drug delivery (angiogenesis may also result in increased tumour growth before the drugs can be administered for fear of interfering with wound healing).

It has been suggested that chemotherapeutic drugs may owe part of their effect to damage of endothelial cells with subsequent necrosis of tumour supplied by the damaged vessels, but this has not yet been confirmed in an experimental or clinical situation.

In conclusion, it would seem that treatment strategies which rely upon diffusion of drugs to tumour cells are unlikely to be successful alone and that alternative strategies are likely to be more rewarding, for example targeting chemotherapy to tumour blood vessels.

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