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VASCULAR ENDOTHELIUM AS THE VULNERABLE ELEMENT IN TUMOURS

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Abstract

The difference between blood vessels in tumours and normal tissues has been recognised for a long time, but little has been done to exploit this in cancer therapy. An overview is presented of the possible contribution of vascular mediated injury with several existing forms of treatment. Interventive radiology and, to some extent, hyperthermia are most obviously mediated through ischaemic cell death. The successful cure of many tumours with radiation, and the complete regressions seen with systemic chemotherapy may also be partially due to a vascular component of damage. The proliferation characteristics of endothelial cells in normal and tumour blood vessels are summarised. These have been derived from single injections or repeated administration of tritiated thymidine. A very large and consistent difference exists between the turnover times. The tumour endothelium is proliferating 20 to 2000 times faster than any normal tissue endothelium in the adult. The single exception is the placenta which has even more rapid proliferation than the tumour endothelium. The large difference in proliferation rates, coupled with the poor wall structure, lack of innervation and lack of collateral supply, make the blood vessels an attractive target for tumour therapy. Possible means of utilizing this are outlined.

Vascular supply has always been of interest to radiotherapists, but in general they have regarded any differences between normal and tumour vessels as being disadvantageous to therapy. Hypoxic radioresistance and failure to achieve complete distribution of chemotherapeutic drugs are both considered to be reasons for failure of current forms of therapy. Conversely, the susceptibility of normal blood vessels to radiation injury has been postulated as one of the mechanisms by which late undesirable radiation side effects are mediated (26, 31, 42).

Strangely, the possibility of enhancing *tumour* damage by an ischaemic mechanism has not been proposed as a realistic method of treating cancer until relatively recently. It now appears that hyperthermia is more effective in damaging tumours than normal tissues, at least partly because there is a specific sensitivity of tumour blood vessels, which can collapse after a mild heat treatment (1, 32, 33, 34). Similarly, interventive radiology has developed as a means of cancer therapy (4, 30). This involves the introduction of embolic materials, including cyanocrylates and autologous thrombin, via a catheter, into the main supplying arteries to a large tumour. This approach depends upon the ability to demonstrate and catheterize a critical arterial supply; hence tumours in kidney, ovary or liver are especially suitable for such treatment. A third vascular approach has been that of FOLKMAN and colleagues (13, 15, 16, 17). They have identified tumour angiogenesis factors (TAF) which are produced by tumours, and have sought methods of antagonising these factors and thereby preventing the further production of new blood vessels.

Recently a large difference between the proliferation kinetics of endothelial cells in normal and tumour blood vessels has been identified (factor of

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Table 1

Proportion of hypoxic cells in tumours

Carcinomas	Per cent	Sarcomas	Per cent
Adenocarcinoma MTG-B	21	CBA sarcoma F	>50
Squamous carcinoma D	18	Gardner lymphosarcoma	1
C_3H mammary carcinoma	7	C_3H sarcoma KHT	14
Carcinoma KHJJ	19	Rhabdomyosarcoma BA 1112	15
C_3H mammary carcinoma		Osteosarcoma C22LR	14
Female	1	Fibrosarcoma RIB5	17
Male	17	Fibrosarcoma KHT	12
Squamous carcinoma G		Sarcoma EMT6	35
Intradermal	<1	CBA sarcoma F	
Subcutaneous	>46	In situ	<10
Carcinoma DC	10–30	Excised	50
C_3H mammary carcinoma		Sarcoma S	<0.01
Small	0.2	Sarcoma S (fast)	1–30
Large	>20	Sarcoma FA	30–70
WHT carcinoma MT		Sarcoma BS	5–25
In situ	>80		
Excised	5		
Carcinoma RH	2–25		
Carcinoma NT	7–18		
Human tumours			
Human tumour nodules	12–20		
Human tumour nodules	1–80		

20–2 000), and it has been postulated that cytotoxic therapy can be directed at these proliferating cells in the tumour vasculature, with the intention of producing an avalanche of tumour cell deaths (5, 7). In this way poor vascularisation can be used as the Achilles heel of solid tumours.

Hypoxia in tumours

Radioresistance in tumours was first postulated in 1953 by GRAY et coll. (19) and the histologic basis for this was put forward by THOMLINSON & GRAY (41). The vasculature in tumours is inadequate to provide oxygen and nutrition to all the cells produced by cell division within the tumour. Thus at a critical distance from each tumour blood vessel, it was postulated that the oxygen tension would fall below 5 mmHg partial pressure and the cells would become 2.5 to 3.0 times more resistant to radiation (Fig. 1).

In this concept of diffusion hypoxia each vessel is surrounded by a cuff of viable, well oxygenated cells. At a distance of 90 to 140 μm the oxygen supply becomes depleted by metabolism, the cells become hypoxic and eventually die and form necrotic areas. Thus a corded structure is visible in many tumours, both in mice and in man (24, 41), and hypoxic cells can be demonstrated to exist (Table 1). In addition to this form of hypoxia there may be cyclic hypoxia of whole regions of the tumour as individual tumour blood vessels become occluded for a period of time before re-opening. This cyclic

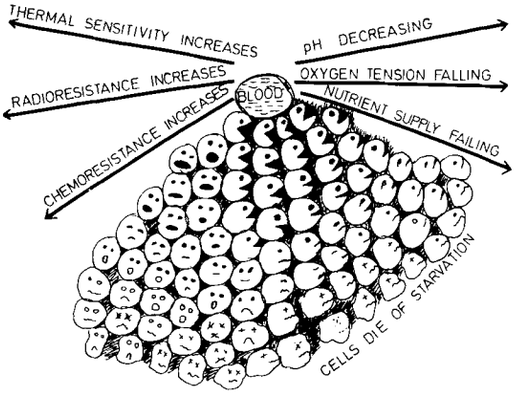

Fig. 1. Diagram to represent the failure of the nutrient supply with increasing distance from a blood vessel. The changes in oxygenation, proliferation and acidity influence the response to radiation, chemotherapy and hyperthermia.

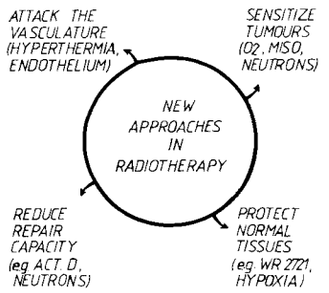

Fig. 2. Several new approaches in radiotherapy which seek to exploit differences between the tumour and normal tissues.

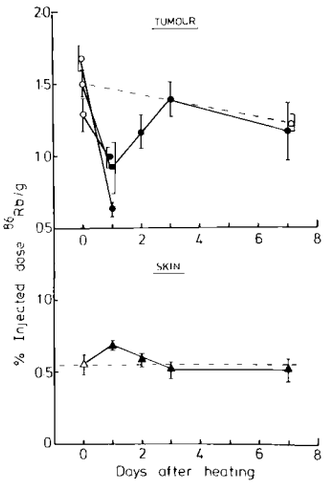

Fig. 3. Changes in blood flow (relative perfusion) in mouse tumours or in skin after heating for 1 hour at 42.8°C. A large decrease in perfusion is seen in tumours, which persists for several days. By contrast increased perfusion is seen in skin. From STEWART & BEGG (36).

Table 2

Advantage points in response to hyperthermia

	Thermal killing	Thermal sensitization
Hypoxia	No	No
Low pH	Yes	No
Phase of cell cycle	Yes	No
Poor heat dissipation in tumours	Yes	Yes
Collapse of blood supply	Yes	No

opening and closing of vessels may last for only a few seconds or for minutes or hours. During the period of closure *all* the tumour cells supplied by that vessel will be made temporarily radioresistant. If the closure persists for at least 12 hours, the cells will probably die an ischaemic death (8, 22).

Hyperbaric oxygen, electron affinic radiosensitizers and high LET radiations (e.g. neutrons) have all been proposed as methods for overcoming the tumour radioresistance resulting from hypoxia (Fig. 2). All three approaches have a strong radiobiologic rationale and are founded on a large body of experimental data, obtained both for cells in vitro and for tumours in vivo. The rationale for these approaches is that hypoxic cells exist in tumours but *not* in normal tissues, and therefore a *differential* increase in radiosensitivity will be obtained by overcoming the hypoxia. Many reviews have been written on this topic and do not need summarising again here (e.g. 10, 12, 18).

Alternative new approaches in radiotherapy (Fig. 2) include the use of specific normal tissue radioprotectors, attempts to manipulate the repair capacity of tumours more than normal tissues, or methods of attacking the tumour vasculature. This latter approach seems to be the basis for the effectiveness of heparin and corticosteroids as recently demonstrated by FOLKMAN et coll. (17) and is also at least part of the rationale for the effectiveness of hyperthermia (34).

Vascular approaches to cancer therapy

The use of hyperthermia in the treatment of cancer is even older than the use of ionising radiation (e.g. 44). It has long been recognised that mild hyperthermia (e.g. the fever accompanying erysipelis or that following injection of certain toxins) can lead to tumour regression (3, 28). In the last decade a great deal of laboratory work, together with many clinical investigations, have demonstrated that there is a sound biologic basis for treating cancer *locally* with hyperthermia, but that whole body hyperthermia is too toxic to be widely used. Interest has therefore focussed on methods of delivering heat locally to the desired tumour volume, i.e. by focussed or regional heating techniques (9, 21).

The biologic reasons for expecting a larger effect in tumours than in normal tissues almost all relate to the poor vasculature of tumours (Table 2). Cells which are at low pH or are deprived of nutrients are more sensitive to mild heat. In addition, tumour blood vessels are unable to respond to hyperthermia by increasing their blood flow. Therefore the tumours become hotter, because they are less able than normal tissues to dissipate the heat when it is applied locally. A further advantage results because

Table 3

Heat dose for vascular stasis in various tumours

Tumour	Animal	Temp./time	Reference*
Walker carcinoma 256	Rat	43.0°C/1 h	SONG et coll. (1980)
Walker carcinoma 256	Rat	43.0°C/1 h	GULLINO et coll. (1980)
Yoshida sarcoma	Rat	42.0°C/1 h	DICKSON & CALDERWOOD (1980)
BA-1112 rhabdomyosarcoma	Rat	42.5°C/40 min	EMAMI et coll. (1981)
BA-1112 rhabdomyosarcoma	Rat	40.0°C/1 h	ENDRICH et coll. (1979)
BA-1112 rhabdomyosarcoma	Rat	42.5°C/2 h	REINHOLD et coll. (1978)
DS-carcinoma	Rat	42.0°C/30 min	VAUPEL et coll. (1983)
13762A carcinoma	Rat	43.5°C/1 h	RAPPAPORT & SONG (1983)
SCK carcinoma	Mouse	40.5°C/30 min	SONG et coll. (1980)
Mammary carcinoma	Mouse	42.0°C/40 min	BICHER et coll. (1980)
Ependymoblastoma	Mouse	42.0°C/1 h	SUTTON (1980)
SAFA tumour	Mouse	42.5°C/1 h	STEWART & BEGG (1983)
Squamous carcinoma	Hamster	43.0°C/30 min	EDDY et coll. (1980)
VX2 carcinoma	Rabbit	42.5°C/40 min	DUDAR & JAIN (1983)

* For details of references see (34).

tumour blood vessels actually collapse after moderate heat treatments (Table 3). The perfusion through these tumour vessels remains poor for at least 24 hours, as is illustrated in Fig. 3. By contrast the blood flow in skin and other normal tissues transiently increases during and after hyperthermia. The decrease in blood flow in tumours probably leads to ischaemic and nutritional death of the many tumour cells which were dependent upon each vessel.

A very direct approach using vascular attack on tumours has been that of the interventive radiologists. Catheters have been introduced into the main supplying arteries of many different tumours and massive tumour regression has been observed. The substances injected are either damaging to the endothelium, e.g. alcohol, act as foci for clot formation, e.g. autologous thrombin, steel springs, dacron threads, or are directly occlusive, e.g. cyanoacrylatates, biodegradable microspheres (4, 30, 43, 45). The overall effect of all these approaches is to cause local vessel embolisation leading to ischaemic death of dependent tumour cells. Although originally devised as a means of staunching bleeding and of debulking tumours before surgery, it has been found to be an effective palliative treatment which can keep large tumours under control for many years if repeated at intervals.

FOLKMAN and his colleagues have, for more than a decade, investigated the substances produced by tumours to induce new blood vessel formation (13–17). Their initial objective was to isolate and purify the tumour angiogenesis factors (TAF) in

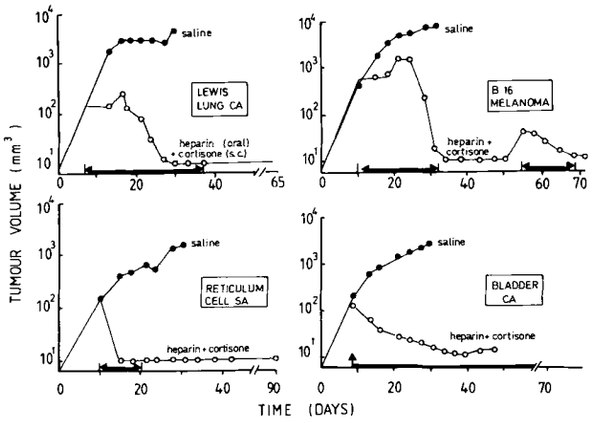

Fig. 4. Regression of tumours after treatment with heparin and cortisone, which inhibits angiogenesis. Data redrawn from FOLKMAN et coll. (17).

order to block them by immunologic or other means and hence prevent any further angiogenesis. A large body of work has been performed in this area and it is now clear that TAF is produced by most solid tumours, that it is a chemotactic agent which causes directional budding of capillaries and that the endothelial proliferation is secondary to endothelial cell migration (14). Heparin has been shown to be essential for angiogenesis and a heparin antagonist (protamine) has been shown to be effective in causing tumour regression in vivo (40). Recently, and unexpectedly, it has been shown that heparin itself, if given in combination with cortisone, is a very potent inhibitor of tumour growth (17). This is illustrated in Fig. 4 for four different tumour types. Prolonged

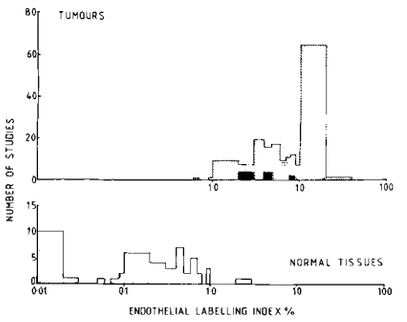

Fig. 5. Histograms showing the uptake of tritiated thymidine into endothelial cells of experimental tumour and normal tissues. There is hardly any overlap of the histograms and the median values differ by a factor of ~40. The black columns represent counts of endothelium in human tumours, which are similar to those in rodents.

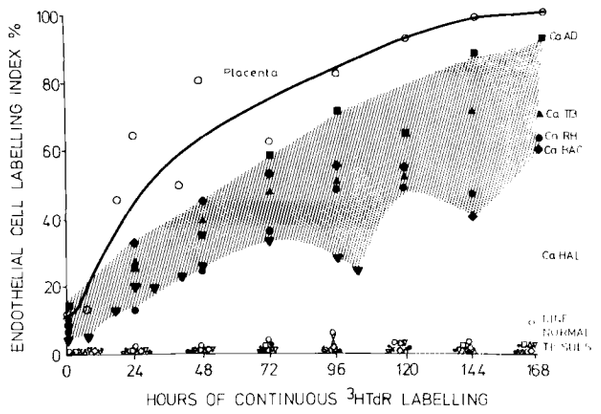

Fig. 6. Uptake of tritiated thymidine after repeated injections every 8 hours for a period of 7 days. The uptake is rapid in 5 different types of mouse tumour, and also in placenta (from 10–17 days gestation). The uptake is very slow in the adult normal tissues which have been studied (i.e. brain, muscle, jejunum, skin, heart, kidney, lung, liver and bladder) and is not related to the proliferation rate of the parenchymal cells. Data from HOBSON & DENEKAMP (25).

treatment with this combination can result in complete tumour cure if a particular brand of heparin, Panheparin, is used. (Unfortunately this has recently been withdrawn from the market.) The biochemical basis of this effect is not understood, but parallel experiments on angiogenesis in chick allantoic membranes indicate that it *is* mediated via the vasculature.

Endothelial cell proliferation studies

The quantitation of cell proliferation kinetics became possible in the 1950's when high resolution autoradiography, the recognition of the discrete phases of G_1, S, G_2 and M in the cell cycle, and the manufacture of a labelled specific precursor of DNA (tritiated thymidine) all occurred (27, 29, 39). Since then the proliferation kinetics of tumour cells in both experimental tumours and in human tumours have been widely studied (see 35 for review). However, until recently, little attention was paid to the stromal elements which were necessary to support the neoplasms. TANNOCK (38) and GUNDUZ (20) showed that the proliferation rate was high in the endothelium of vessels in mouse mammary tumours. We have recently analysed the vascular endothelial kinetics in many types of experimental tumour and have shown that tumour endothelium is almost always rapidly dividing, in marked contrast with the slowly proliferating endothelium in normal tissues (5, 7, 25). Fig. 5 illustrates this difference.

Most experimental tumours show endothelial labelling indices, after a single injection of ^{3}HTdR, in the range 10 to 20 per cent. Occasionally values as low as 1 to 2 per cent or as high as 32 per cent are observed. The median value from an analysis of approximately 200 tumours is 9.0 per cent. By contrast, the 1 hour labelling index in normal tissue endothelium is usually below 1 per cent, often being as low as 0.01 per cent (23). The median value from our own and other published studies is 0.22 per cent (7). Thus a large difference exists in the uptake of tritiated thymidine into the vessels of tumour and normal tissue endothelium. If a similar difference could be exploited in the uptake of an S-phase specific cytotoxic drug, this would clearly be an approach to tumour therapy worthy of further investigation. Indeed it may contribute to the effectiveness of chemotherapy on solid tumours as it is used at the present time.

Fig. 5 also shows labelling index values for a variety of human tumours which have been labelled by intra-arterial infusion of ^{3}HTdR or by incubation of biopsy materials with ^{3}HTdR in vitro. The material was provided for us by clinicians in several institutes (C. Nervi, Rome, T. Hoshino, San Francisco, J. Kummermehr, Munich). The labelling index values for these human tumours are similar to those found in mice and are grossly different from the normal tissue values.

We have recently extended the labelling studies in endothelium to include repeated injections of ^{3}HTdR every 8 hours over a period of 7 days (25). In this way all cells entering DNA synthesis should be labelled, and the turnover time of the endotheli-

Table 4

Differences between tumour and normal blood vessels

Normal vasculature	*Tumour vasculature*
Is 3-dimensionally adequate	Is 3-dimensionally inadequate
Is innervated	Leads to:
Has collateral potential	Nutritional deprivation
Has well-constructed walls	Hypoxic cells
Can increase blood flow in response to demand	Acidic cells
	Areas of necrosis
It is:	Vessels have:
Resistant to radiation	Poor wall structure
Can withstand 90% depletion of endothelium (arterioles)	No innervation
	Fewer capillaries
	More sinuses
Endothelial cells proliferate very slowly	No collateral potential
	Endothelial cells which proliferate rapidly

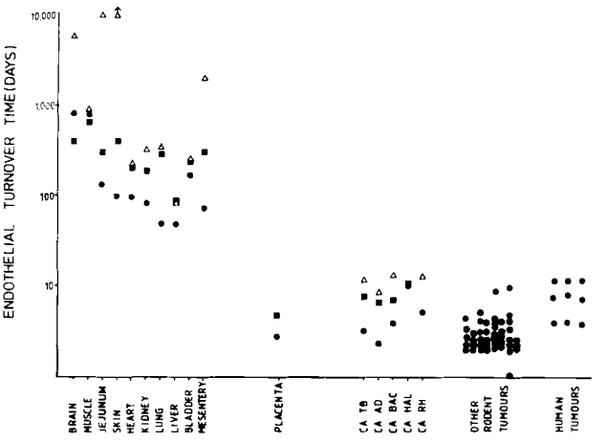

Fig. 7. Endothelial turnover times calculated from the tritiated thymidine labelling indices. Three estimates have been made when continuous labelling data were available. ○ from 1 hour L.I., ■ from 73 hour L.I., △ from 168 hour L.I. From HOBSON & DENEKAMP (25).

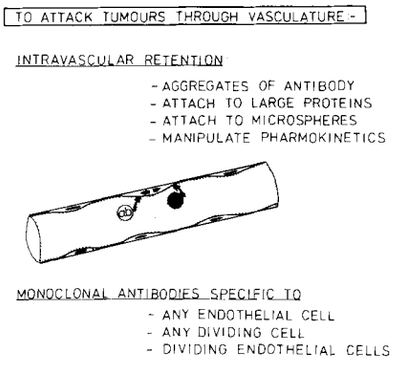

Fig. 8. Possible methods by which chemotherapy could be directed at the proliferating endothelial cells in tumour blood vessels, in order to produce an avalanche of tumour cell deaths.

um can be calculated as the time required to achieve 100 per cent labelled cells. Fig. 6 shows the data obtained for 5 experimental tumours, compared with 9 normal tissues and placenta. In the tumours 35 to 60 per cent of the endothelial cells were labelled within 2 to 3 days. In the normal tissues only 2 to 3 per cent were labelled in the same time period, regardless of whether it was a rapidly dividing tissue (e.g. jejunum) or a slowly dividing tissue (e.g. heart, lung, brain). The continuous labelling data confirm that the large difference between tumours and normal tissues is real, and is not simply an artefact resulting from diurnal variations in ^{3}HTdR uptake, or from the poor statistical accuracy of 1 hour labelling indices.

The uptake of ^{3}HTdR into the placenta, when labelling starts on the tenth day of gestation, is even greater than that in any of the experimental tumours (Fig. 6). This indicates that vascular endothelium, when stimulated to divide by a developing foetus, is capable of even more rapid proliferation than that which is elicited by tumour angiogenesis factors.

The 1 hour labelling indices and the continuous labelling data can both be used to estimate the proliferation rate of the endothelial cells in tumour and normal tissue vessels. Fig. 7 shows turnover times for vessels in normal tissues and tumours calculated from such data. This figure includes all our own data on 10 normal tissues and 5 tumour types studied with continuous labelling, 19 tumour types studied with single injections and the human tumour biopsies. The normal tissue values are 20–2 000 × longer than those in the tumour blood vessels. The proliferation rate in placenta is even faster than in many tumours. Compiled data are also shown for other studies of experimental tumours from the literature (20, 38). The turnover time of endothelial cells in human tumours is remarkably similar to that in mouse tumours, in spite of large differences in tumour size and doubling times in the two species.

It is clear from this analysis that a large and consistent difference in the proliferation kinetics of tumour and normal vasculature exists, which offers the potential for exploitation as a means of directing cytotoxic therapy at tumours whilst sparing normal vessels. Apart from the differences in proliferation rates, several other differences in the characteristics of tumour vessels exist which might make them more susceptible to cytotoxic attack (Table 4). We

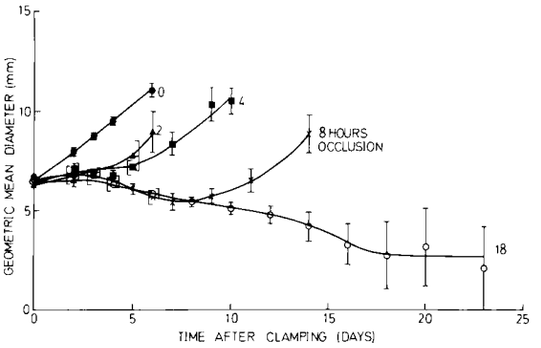

Fig. 9. Delay in tumour growth induced by the application of a clamp for 2, 4, 8 or 18 hours. A significant cell kill is achieved with 2–4 hours clamping. With occlusion for 15 hours or longer, some tumours were permanently controlled. From DENEKAMP et coll. (8).

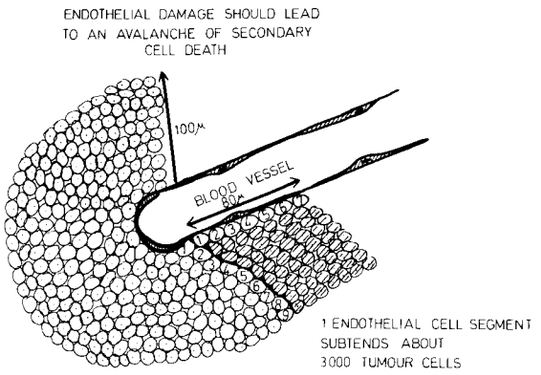

Fig. 10. Schematic illustration of how many tumour cells would be at risk if even a small segment of a capillary was occluded, so that their nutrient supply was completely lost. If an entire vessel was closed down an even greater avalanche of deaths should occur.

have proposed that these differences might be exploited using one or more of the approaches outlined in Fig. 8 (5, 6). Monoclonal antibodies may be developed against dividing endothelial cells, and these could be used to target conjugated cytotoxic drugs or radioisotopes to the tumour endothelium. Alternatively, it may be possible to manipulate the pharmacokinetics of existing chemotherapeutic agents (either by means of pH, lipophilicity or by attachment to large carrier molecules) so that they are not freely diffusible throughout the body, but are retained within the vascular network. In this way, the only proliferating cells that would be accessible to such drugs would be those immediately adjacent to the blood, i.e. the endothelium. In this way the drug dose and side effects on rapidly dividing parenchymal cells in bone marrow, intestine and hair follicles should be reduced.

Ischaemic death

Death due to nutrient failure has been postulated as the cause of necrotic foci, or cords of viable tissue in seas of necrosis, at least since 1955 (41). Measurements of the rate of production of tumour cells and the transit time across viable tumour cords allows an estimate to be made of the lifetime of naturally occurring nutrient-deprived hypoxic cells in tumours. If these cells exist as a single layer at the boundary between viable and necrotic tissue, their lifespan would only be 5 to 10 hours (22, 24). We have recently performed some crude clamping experiments on mouse tumours to test the cytotoxic effectiveness of vascular occlusion (8). Application of a clamp to the base of a subcutaneous tumour for only 2 to 4 hours produced significant delay in the growth of the tumour (Fig. 9). For clamping times of 15 hours or longer some tumours were permanently controlled, without the addition of any other form of therapy. The necessity for many hours of occlusion is in good agreement with the 5 to 10 hour estimate of the natural lifespan of hypoxic cells in tumours and also with data obtained from hypoxic incubation in vitro (2). These findings, together with the clinical success of interventive radiology (4, 30) encourage us to pursue the goal of aiming cytotoxic therapy at tumours indirectly, by some systemic approach to causing *tumour blood vessel* damage. Fig. 10 illustrates the large gain in cell kill that may be achieved in this way. Each endothelial cell subtends a segment of a tumour cord containing many thousands of tumour cells. Occlusion of small capillaries should therefore result in a huge avalanche of tumour cell deaths. It is anticipated that such an approach would be most effective if combined with localised radiotherapy or hyperthermia to large tumour deposits. It has the advantage, however, that it would also be effective on micrometastases, since any tumour nodule in excess of 250 to 500 μm diameter will be evoking a new blood supply, because nutrient diffusion will by then have become inadequate.

Conclusions

Several recent approaches to tumour therapy are based on the concept of occluding the blood supply

to tumours and thereby depriving them of a nutrient supply. Any such approach is only possible if a differential effect can be achieved on tumour blood vessels relative to normal vessels. The large difference in proliferation parameters between such vessels has been identified as a means by which such a differential effect can be achieved.

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