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The Influence of Implantation Site on Tumor Growth and Blood Flow*

STUART W. YOUNG,† NORMAN K. HOLLENBERG and HERBERT L. ABRAMS‡

Department of Radiology, Harvard Medical School and Peter Bent Brigham Hospital, Boston, Massachusetts 02115, *U.S.A.*

Abstract—*A study was made of the differences in tumor growth and their relationship to tumor blood flow in several different tissues. A suspension of V2 carcinoma cells was implanted in the liver, spleen, kidney, testicle and ear of* 34 *New Zealand white rabbits. Two weeks after implantation, V2 carcinoma and surrounding normal host tissue blood flow was determined with radioactive microspheres. Total tumor weight was found to be related to the site of implantation: liver* (8.7 g), *spleen* (8.4 g), *kidney* (2.9 g), *testicle* (1.2 g) *and ear* (0.4 g). *The amount of gross caseous necrosis in the V2 carcinoma was also related to implantation site. The organ with the largest proportion of grossly non-necrotic tumor was the liver* (5% *necrotic*) *and that with the least was the spleen* (62% *necrotic*). *V2 blood flow also varied with implantation site, the highest being found in the kidney* (1.3 ml/g/min) *and the lowest in the ear* (0.14 ml/g/min). *Regression analysis revealed that tumor blood flow was directly related to resting blood flow in the surrounding host parenchyma. However, no relationship existed between tumor perfusion and either tumor size or the amount of central necrosis.*

INTRODUCTION

TUMOR growth depends upon the dynamic interaction of a transformed malignant cell and the tissue within which it resides, an interaction which has reasonably been compared to seed (metastatic malignant cells) falling on soil (organs subject to metastatic spread) with a range of fertility [1, 2]. Extensive attempts have also been made to relate the blood supply of malignant tumors to their growth characteristics [3–7]. For example, the malignant tumor's vasculature has been considered to be responsible for the development of central tumor necrosis [8]. During another study [9], we observed that implantation site influenced both tumor growth and central necrosis. Because tumor blood flow could account fot these observations, this study was designed to evaluate systematically differences in tumor growth and necrosis and their relationship to tumor blood flow in widely dispersed tissues.

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†Current address: Stuart W. Young, M.D., Department of Radiology, Stanford University Medical Center, Stanford CA 94305, U.S.A.

‡Reprint requests to Herbert L. Abrams.

MATERIALS AND METHODS

Animals and tumor preparation

Thirty-four New Zealand white rabbits weighing approximately 2.5 kg each were utilized. A suspension of V2 carcinoma cells was prepared from the subcutaneous tumors of carrier rabbits. After sacrifice with intravenous sodium thiopental, the tumors were removed, cleaned, and minced in sterile containers. A cell suspension was prepared by collagenase and viokase digestion as described in detail for this laboratory [10]. Viable tumor cells were defined by trypan blue dye exclusion, and the concentration of the suspension was adjusted with buffered balanced salt solution to contain approximately 10^6 viable tumor cells/0.1 ml of suspension. Test animals were anesthetized with intravenous sodium thiopental (30 mg/kg), a laparotomy was performed, and 0.1 ml of the suspension was injected into the liver (right and left medical lobule), spleen and kidneys. Additional injections were made into the testicles and ear. Bleeding around the injection sites was controlled with topical thrombin, and virtually all of the innoculum was retained within each injected organ.

Protocols

At approximately 2 weeks after tumor inoculation, blood flow determinations were performed immediately prior to sacrifice with potassium chloride. All V2 cell suspensions injection sites were inspected for tumor growth, and the well-encapsulated V2 carcinomas were carefully dissected from the surrounding parenchyma. Dissections were performed to eliminate all host tissue from the tumor specimen. A separate sample of normal host tissue was also taken from the organ of each tumor site. The necrotic caseous central portions of the V2 carcinoma were separated by gross dissection from the cohesive, nonfriable peripheral portions. After these two specimens were weighed and labelled "necrotic" and "viable" V2 carcinoma respectively, the combined weights of these two portions were recorded as total tumor weight. Appropriate tissue samples of the tumors from the various organs were fixed in formalin and stained with hemotoxylin and eosin to evaluate the adequacy of the V2 separation from surrounding normal parenchyma and the separation of gross central necrosis from peripheral intact "viable" tumor'

Blood flow measurements

Rabbits were anesthetized, and catheters were passed into the left ventricle and abdominal aorta just proximal to the bifurcation [4]. Blood pressures were monitored with Stratham P23 DC transducers on a Grass polygraph recorder. V2 carcinoma and host organ flows were determined after injecting 2,000,000 radioactive carbonized microspheres ($15 \pm 3\,\mu m$) into the left ventricle as previously described in detail from this laboratory [11]. After sacrifice all samples of the normal organ parenchyma, intact viable V2 carcinoma, and grossly necrotic V2 carcinoma were counted separately in a scintillation well counter.

Statistical analysis

Tumor blood flow and tumor weight data were tested for significance with the Wilcoxon rank sum test [12]. Correlation of V2 carcinoma and surrounding normal host blood flows were assessed by regression and chi square analysis [13].

RESULTS

Take rate and tumor weight

The take rate per implantation site was approximately 65%. There were no significant differences in take rate among injection sites (Table 1), but total tumor weight was related to the site of implantation. Liver tumors were largest (8.70 ± 1.38 g), followed by spleen (8.41 ± 2.01 g), kidney (2.87 ± 0.68 g), testicle (1.17 ± 0.38 g) and the ear (0.44 ± 0.2 g) (Table 1). Weights of tumors growing in paired organs were not significantly different and these data were pooled for analysis. The total weights of liver and splenic tumors were not significantly different ($P > 0.10$). Kidney tumors were significantly larger than testicular tumors ($P < 0.05$), and testicular tumors were significnatly larger than those in the ear ($P < 0.01$).

The amount of gross central caseous necrosis in the V2 carcinoma was also related to implantation site as shown in the regression analysis of total vs viable (grossly intact tumor rim) tumor weight for each organ (Table 1.) A grossly viable tumor with no caseous center would have a slope of 1.0 (line of equality), which was not observed in this study. The organ with the largest proportion of grossly viable tumor was the liver (slope = 0.95; Fig. 1) and that with the least was the spleen (slope = 0.38; Fig. 2). In addition the relationship of total weight to grossly viable tumor weight was consistent within each organ over a wide range of tumor weights (Table 1), suggesting that increased tumor mass did not directly correlate with a larger percentage of gross central caseation.

Blood flow

The V2 central necrotic tissue had no detectable blood flow. Blood flow in the intact viable rim of the V2 carcinomas varied with implantation site (Table 2). The highest flow was in the kidney (1.3 ± 0.30 ml/g/min) and the lowest was in the ear (0.14 ± 0.07 ml/g/min). Blood flow within the renal V2 carcinomas was significantly greater than the blood flow of tumors in any other organ tested ($P < 0.005$, chi square test). Blood flow differences among testicular, liver, and splenic V2 carcinomas were not significant, but the blood flow of ear tumors was significantly less than in other organ tumors ($P = 0.026$, Fisher exact test). The well-encapsulated tumors were easily dissected from the surrounding parenchyma and histologically relatively little or no identifiable host organ tissue remained within the tumor samples.

Regression analysis of tumor blood flow and blood flow in the surrounding host parenchyma revealed a significant relationship in

Table 1. V2 growth in five organs

	Liver	Spleen	Kidney	Testicle	Ear
Tumor takes per implant site	43/63	20/25	37/64	32/65	19/34
Percentage of takes	68%	80%	58%	49%	56%
Total tumor weight (g)*	8.70 ±1.8 (43)	8.41 ±2.0 (20)	2.87 ±0.68 (37)	1.17 ±0.38 (32)	0.44 ±0.20 (19)
Viable tumor weight (g)*	7.29 ±1.8 (43)	4.45 ±1.0 (20)	2.13 ±0.46 (37)	0.94 ±0.32 (32)	0.36 ±0.17 (19)
Total wt. (x) vs viable wt. (y) $y=mx+b$	$y=0.95x-0.95$ ±0.03 ±0.5	$y=0.38x+1.23$ ±0.1 ±0.9	$y=0.67x+0.22$ ±0.03 ±0.1	$y=0.65x-0.01$ ±0.05 ±0.1	$y=0.78x+0.04$ ±0.03 ±0.03
r	0.97	0.75	0.97	0.92	0.99
F	773	23.1	609	173	745
P	$\ll 0.01$	$\ll 0.01$	$\ll 0.01$	$\ll 0.01$	$\ll 0.01$

* = Mean ±S.E.M.

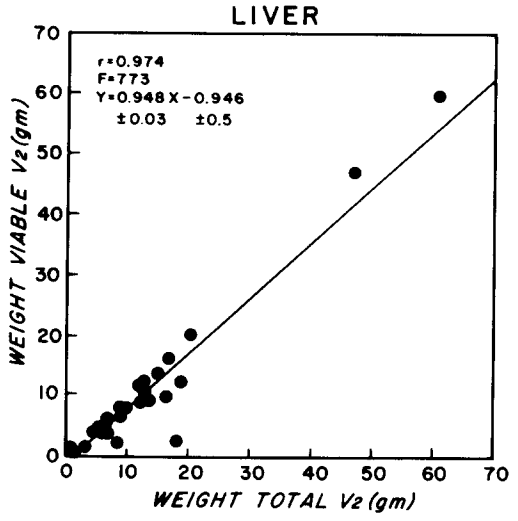

Fig. 1. Total vs viable weight (intact tumor rim) of hepatic V2 tumors.

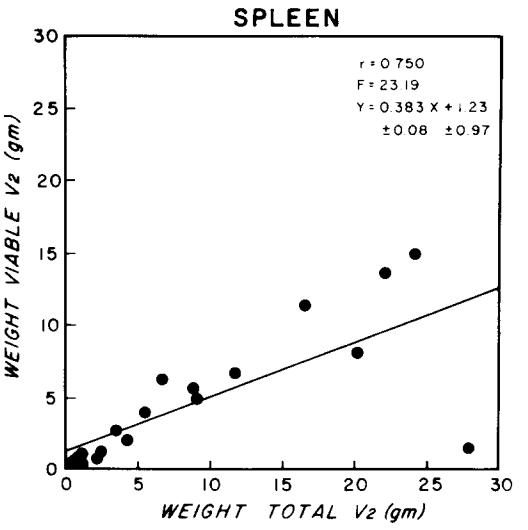

Fig. 2. Total vs viable weight (intact tumor rim) of splenic V2 tumors.

Table 2. Blood flow to host tissue and V2 carcinoma in six sites

Organ (No. of samples)	Blood flow (ml/gm/min)*	Normal deviation† (%)	$y=mx+b$‡	r
Ear (8)				
Normal	0.025±0.01	80	$y=6.8x-0.03$	0.85§
V2	0.14±0.07	140		
Spleen (12)				
Normal	1.12±0.3	98	$y=0.02x+0.2$	0.59§
V2	0.42±0.11	88		
Testicle (25)				
Normal	0.19±0.03	89	$y=1.5x+0.25$	0.51§
V2	0.55±0.10	89		
Kidney (24)				
Normal	2.96±0.34	54	$y=0.36x+0.26$	0.40¶
V2	1.3±0.30	115		
Liver (32)				
Normal	0.52±0.1	104	$y=0.17x+0.4$	0.25
V2	0.50±0.07	76		
All organs (101)			$y=0.32x+33$	0.53§

*S.E.M.
†S.D./mean value (%).
‡y is normal organ blood flow; x is V2 carcinoma blood flow in that organ
§$P<0.01$.
¶$P<0.05$.

four of the five organs studied (Table 2). Although the V2 carcinoma perfusion within each organ type varied substantially, flow was equally variable in the normal host parenchyma. Not only was the V2 carcinoma blood flow significantly higher in organs with a higher parenchymal blood flow, but within each organ the V2 carcinoma blood flow was related to the blood flow to the parenchyma of that organ at the time of evaluation.

No relationship could be established between tumor perfusion and either tumor size or the amount of gross central necrosis (Fig. 3).

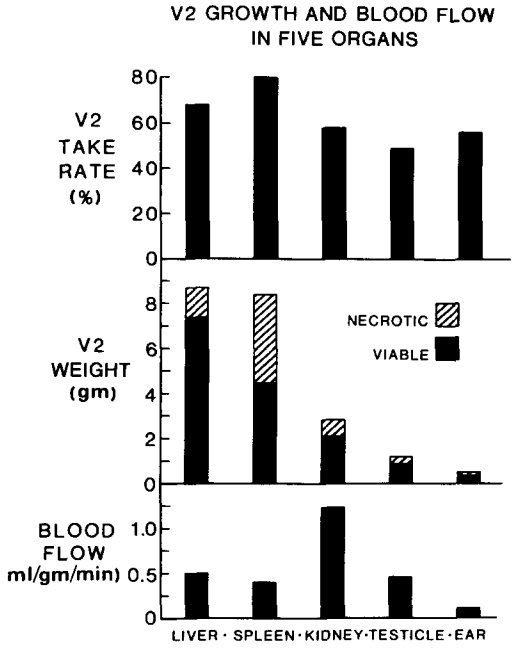

Fig. 3. V2 growth, blood flow and take rate in 5 rabbit organs.

Discussion

In this study the implantation site was found to influence tumor perfusion. These observations differ from those reported by Edlich *et al.* for the same tumor [3]. V2 carcinoma was implanted in skeletal muscle, kidney, and several gastrointestinal sites. Blood flow estinates of perfusion with ^{86}Rb revealed small but insignificant differences in tumor perfusion as a function of site. The different observations in these two studies are probably in part related to methodology. Edlich *et al.* apparently determined blood flow for the entire tumor. Perfusion decreases within central necrotic tissue (4,8), and V2 carcinomas of approximately 10–14 days of age, depending in part upon the organ in which they were implanted, have a variable amount of non-perfused necrotic tissue. In addition, rubidium extraction is sensitive to factors that operate at the level of transport processes for potassium, so that determinations of blood flow based on rubidium can differ substantially from total tissue blood flow [14].

The V2 implantation site influenced both the rate of tumor growth and its tendency to develop gross central caseation and necrosis. The V2 is an immunogenic tumor and regional differences in immunologic resistance to malignant tumor growth could account for some regional variability, but once the tumors had become vascularized, circulating host immunologic defenses would have equal access to malignant tissue regardless of its location. If only local immunologic factors were determining V2 growth, the exuberant splenic tumor growth would be difficult to explain. Mechanical tissue pressure might account for some differences in growth and perfusion [1, 2], especially for subcutaneous tumors in the ear, but organ size and capsular thickness did not correlate with tumor growth or perfusion in the other organs studied. Regional nutritional variation might partly account for the large tumors in the liver, but the primarily hepatic arterial blood supply would presumably decrease the effect of nutrients supplied by the portal vein. Regional differences in nutrition could not explain the differential V2 growth in spleen, testicle, kidney and ear.

The character of tumor growth has often been related to tumor neovasculature and blood flow. During the incipient stages of growth, before vascularization, the diffusion of oxygen and its consumption are balanced at about 100–150 μm from the closest capillary [15, 16] for all metabolically active tissues. Thus despite the autonomy of neoplastic cell replication, the growth of a solid tumor mass demands concomitant development of a vascular supply if the mass is to exceed a diameter of more than a few cells. When solid tumors are prevented from vascularizing, either *in vivo* or *in vitro* [3, 17], the tumor mass achieves a steady state at less than 1–2 mm. The steady state in these simplified models is maintained by cell proliferation in the outer zone balancing necrosis in the center of the spheroid.

Hardman made one of the first, and what is still one of the most incisive, surveys of the early literature dealing with tumor blood supply as a determinant of tumor growth [8]. He noted enlargement of the feeding vessels in normal areas and defined three major concentric zones in glial tumors which differed primarily in their blood supply. A narrow outer zone had the greatest cellularity and rate of cell multiplication (which Hardman attributed to the fact that it was closest to the arterial supply derived from the surrounding normal tissues). Cells in the middle zone were apparently viable but clearly had less mitotic activity. This zone had tortuous, often dilated and disordered small vessels and sinusoids. The inner zone was necrotic, which was attributed to ischemia. these findings clearly suggested a gradient of blood supply to Hardman.

Basically similar tumor architecture has been recognized in a number of tumor models [18–20]. Although each tumor has its own pattern, a concept has evolved in which the

blood supply serves not only as a determinant of overall growth but also of the tumor's internal pattern of growth and ultimately of its architecture. In other studies, however, little direct correlation has been found between the character of tumor growth and its blood flow [3, 21, 22].

In this study we attempted to relate tumor growth differences to local perfusion. However, neither tumor growth nor the amount of gross central caseation correlated with tumor blood flow. Blood flow in fact was related to implantation site and varied from tumor to tumor without regard to either overall size or the proportion of central necrosis. Survival of the vascular network of the invaded organ within a growing malignancy is known to occur [6] and may have a large influence on observed tumor blood flow. To some extent this may have influenced our results as well. But with the exception of the liver mean V2 and organ blood flow were significantly different and histologically little residual host organ tissue remained in the tumor specimens studied.

The variability in V2 carcinoma blood flow was related to the blood flow to the host organ not only from organ to organ but even within the same type of organs. Much the highest growth rate was found in the liver and spleen. Much smaller tumors were found in the kidney, the testicle, and the ear. Although the two regions with the lowest flow rate, the testicle and ear, supported a much slower rate of tumor growth, tumor growth did not parallel blood flow. The kidney, which has much the highest flow, had an intermediate growth rate. Similarly, the proportion of gross central caseation and necrosis in each site also varied strikingly.

The central necrotic tissue does not receive a detectable blood supply, as demonstrated with both microspheres and with iodoantipyrine radioautography. The absolute failure of perfusion in that zone is as likely to be a consequence of the necrosis as its cause. In addition, hypoxic and necrotic cells are present throughout all portions of growing tumors where blood flow can be demonstrated. Thus, in this study, not only was the rate of tumor growth not related to the perfusion rate, but the grossly necrotic fraction of the tumor was independent of blood flow.

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