Effect of Vasoactive Drugs on Tissue Blood Flow in the Hamster Melanoma

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Summary

Previous quantitative estimates of blood flow of transplanted tumors have been lower than surrounding tissue. Using a modification of the Fick principle with antipyrine-131, tissue blood flow was estimated in the hamster amelanotic melanoma implanted in subcutaneous tissue over the femoral triangle. Mean tumor blood flow was higher than adjacent tissue, decreased with tumor age, and responded uniquely to vasoactive drugs.

Introduction

The blood supply of tumors has been the subject of few investigations (1, 4-7, 12-14, 18, 25). The technics used in these studies usually did not permit quantitative measurements of blood flow through neoplastic tissue. The first quantitative measurements of blood flow in tumor were carried out by Gullino and Grantham (13). They compared the venous return from isolated organs replaced by tumor with the distribution of 4K and 9Rb in s.c. implanted rat and mouse tumors. Using these technics, they reported that the level of tumor blood flow was independent of tumor size, histologic type, or site of implantation and was consistently lower than surrounding normal tissue. The uptake of 9RbCl in s.c. implanted hepatomas was 15-fold lower than normal liver. In addition, the blood flow changes in response to cold, ganglionectomy, acetyl-ß-methylcholine, and adrenaline were similar in tumor and uninvolved tissue (14).

A low level of tumor perfusion was also demonstrated by our group (21) investigating the distribution of antipyrine-131 in the s.c. transplanted mammary carcinomas of the mouse. Tissue blood flow in this tumor was found to be equivalent to the blood flow of overlying skin and 0.5 that of adjacent gastrocnemius muscle.

It was the purpose of this investigation to report quantitative blood flow determinations in a transplantable hamster melanoma whose blood flow was higher than adjacent tissues. In addition, the present study was conducted to evaluate the changes in tissue perfusion in this tumor in response to different vasoactive drugs.

Materials and Methods

The hamster amelanotic melanoma (Amel 3) was employed in this study. It was prepared for transplantation by the mince suspension technic described by Fortner et al. (10) and injected s.c. in the femoral triangle of 6- to 8-week-old male and female Syrian golden hamsters.

Each animal was anesthetized with i.p. pentobarbital sodium (1 mg/10 gm body weight). Through a cannula inserted into the jugular vein, approximately 3 mc antipyrine-131 diluted in normal saline were infused at 0.68 ml/min for 2 min by a Harvard microinfusion pump. At the end of this period, the limb was amputated terminating the effective blood supply to the implanted tumor and its surrounding tissue. A final arterial sample was obtained from the severed femoral artery at the time of amputation.

Individual samples of skin, gluteus muscle, and tumor were carefully dissected from the amputated limb. These were blotted and weighed in stoppered bottles along with the arterial sample. The number of cpm/gm of 131I was then determined for each of these samples using a well-type scintillation counter.

Antipyrine, labeled with 131I, was used as the reference substance for determining local tissue perfusion. The parent compound, antipyrine, is a lipid-soluble molecule whose blood-tissue permeability is so large that its clearance is limited by the rate of blood flow (20). The flow-limited nature of antipyrine, established by comparing the distribution of this compound with deuterium oxide, provides the theoretical basis for estimating regional blood flow from blood and tissue concentrations of this substance (15).

The calculation of tissue blood flow employs a modification of the Fick principle used in blood flow studies. This principle states that the rate of uptake of a test substance is equal to the rate of inflow of that substance via the arteries minus the rate of outflow via the veins.

Stated symbolically, this is:

\[ \frac{dA}{dt} = FaCa - FvCv \]

where \( F \) equals flow in ml/min, \( A \) equals tissue concentration in cpm/gm of antipyrine-131, \( t \) equals time in min, \( Ca \) equal arterial concentrations in cpm/ml, and \( Cv \) equals venous concentration in cpm/ml.

This equation is based on the assumptions that the reference substance is conserved (8) and that the arterial inflow equals the venous outflow. Since \( Fa = Fv \), the equation is expressed as follows:

\[ \frac{dA}{dt} = F (Ca - Cv) \]

or

\[ F = \frac{A}{\int Ca dt - \int Cv dt} \]

1 Antipyrine-131I was purchased from Abbott Laboratories; arterenol from Winthrop Laboratories; adrenaline from Burroughs Wellcome & Co., Inc.; vasopressin from Parke, Davis & Co.
For technical reasons, it is difficult to obtain arterial and venous samples in small laboratory animals for each tissue studied. Therefore, certain approximations for these values are made. These estimations are concerned with the time curves for the antipyrine-131 I concentration in tissues, and venous and arterial blood (18).

When a tissue is perfused by a substance such as antipyrine which is distributed according to a flow-limited process and which has a tissue-to-blood coefficient of 1 (16, 17), the concentration of the substance in its volume of distribution in venous blood will at all times equal the concentration of the substance in its volume of distribution in the tissue. Therefore ∫Cov dt may be estimated by (Axt)/2. Since the rise in concentration of antipyrine-131 I in the skin, muscle, and amelanotic tumor during a continuous infusion of the reference substance has been demonstrated to be linear with time, this assumption is valid for this system.3

In previous tissue blood flow determinations (3, 19) employing antipyrine administered by bolus injection, the arterial sample used has been collected by continuous sampling. Since continuous arterial collection is difficult in hamsters, an approximation of the mean arterial concentration was made. This estimate was justified by work in our laboratory which showed that during a continuous infusion of antipyrine-131 I in hamsters, the arterial concentration of the reference substance rose linearly with time. Thus, the mean arterial concentration (Cà) has been estimated by dividing the final arterial concentration by 2, or Cà = Caf/2. The final equation takes the following form:

\[ F = \frac{A}{(Caf/2 - A/2)t} \]

Tissue blood flow determinations were performed on hamsters divided into 4 experimental groups. In the 1st group, blood flow determinations on 20 hamsters established control values of tissue blood flow at 2 different periods of tumor implantation. Equal groups of animals were evaluated on the 15th–18th and 25th days after implantation.

The remaining 3 experimental groups were comprised of hamsters with amelanotic melanoma implanted 15-18 days prior to the blood flow determinations. Tissue blood flow measurements were performed after the administration of vasoactive drugs. In the 2nd and 3rd groups, varying doses of arterenol2 and adrenaline2 were administered in the 2-min antipyrine infusion. In the last group, varying concentrations of vasopresine3 diluted to 0.5 ml normal saline were infused for 5 min prior to the 2-min flow determination.

Results

**Tissue Blood Flow of s.c. Transplanted Tumors with Respect to Tumor Age (Table 1)**

In the 1st experimental group, tissue blood flow studies were performed on 20 hamsters divided according to their tumor implantation date. The mean tissue blood flow in the 15- to 18-day-old tumors was 10-fold greater than the mean muscle flow and 5-fold greater than the mean skin flow. However, similar measurements performed on hamsters 25 days after implantation revealed that mean tumor blood flow was reduced 43% from the tissue flow levels reported for the 15- to 18-day-old tumor. Furthermore, there was an elevation in the mean skin and muscle flow associated with this reduction in mean tumor flow.

This alteration in tumor blood flow with respect to tumor age was correlated with progressive tumor necrosis. Examination of these large tumors revealed grossly necrotic areas with viable tissue present usually at the periphery. Microscopic sections of the necrotic portion of the tumor demonstrated large areas of cellular debris with intact neoplastic cells noted only around the vessels (22).

Elevation of the skin and muscle flow surrounding the 25-day-old tumor was most likely a manifestation of tumor metastases or local inflammation. Although gross examination of these tissues revealed no obvious neoplastic invasion, serial histologic sections demonstrated sheets of tumor cells or leukocytes interposed between muscle bundles or scattered throughout the subcutaneous tissue.

**Effect of Adrenaline on the Distribution of Tissue Blood Flow**

An examination of the changes in the distribution of tissue blood flow after the infusion of i.v. adrenaline demonstrated alterations in tumor blood flow which were quite distinct from those noted in the adjacent muscle and skin. After the administration of 0.5 and 1 µg/min adrenaline, there was a reduction in the mean tumor flow associated with an elevation in mean skeletal muscle flow. However, these changes were most marked following 1 µg/min of this drug. A comparison of tissue blood flow in control animals with those receiving this dosage revealed a 186% elevation in mean skeletal muscle flow and a 87% reduction in the mean tumor flow. No significant alterations in mean skin flow was noted after these low doses of adrenaline.

Reduced perfusion in all tissues was noted following higher doses of adrenaline, but the changes were most marked in the tumor. After infusing 4 µg/min adrenaline a 90% reduction in mean tumor flow was noted as compared to a 70% and 17% reduction in mean skin and muscle flow respectively.

**Effect of Arterenol on the Distribution of Tissue Blood Flow**

An evaluation of the effect of arterenol on tissue blood flow suggests that the tumor vasculature was more sensitive to this effect.
TABLE 2
EFFECT OF ADRENALINE ON DISTRIBUTION OF TISSUE BLOOD FLOW IN HAMSTERS WITH MELANOMA

<table>
<thead>
<tr>
<th>EPINEPHRINE (µg/min)</th>
<th>NO. OF HAMSTERS</th>
<th>DAYS FROM IMPLANTATION</th>
<th>MEAN TISSUE FLOW IN ml/min/gm OF TISSUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Skin</td>
</tr>
<tr>
<td>0.5</td>
<td>5</td>
<td>15-18</td>
<td>0.112 ± 0.041</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>15-18</td>
<td>0.106 ± 0.029</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>15-18</td>
<td>0.048 ± 0.012</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>15-18</td>
<td>0.028 ± 0.002</td>
</tr>
</tbody>
</table>

* S.D.

TABLE 3
EFFECT OF ARTERENOL ON DISTRIBUTION OF TISSUE BLOOD FLOW IN HAMSTERS WITH MELANOMA

<table>
<thead>
<tr>
<th>NOREPINEPHRINE (µg/min)</th>
<th>NO. OF HAMSTERS</th>
<th>DAYS FROM IMPLANTATION</th>
<th>MEAN TISSUE FLOW IN ml/min/gm OF TISSUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Skin</td>
</tr>
<tr>
<td>1.25</td>
<td>6</td>
<td>15-18</td>
<td>0.093 ± 0.029</td>
</tr>
<tr>
<td>2.50</td>
<td>5</td>
<td>15-18</td>
<td>0.075 ± 0.029</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>15-18</td>
<td>0.057 ± 0.019</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>15-18</td>
<td>0.019 ± 0.006</td>
</tr>
</tbody>
</table>

* S.D.

Effect of Vasopressin on the Distribution of Tissue Blood Flow (Tables 4 and 5)

In contrast to the blood flow changes in tumor following catecholamine infusion, the alterations in tissue perfusion in the melanoma in response to vasopressin were similar to those in adjacent skin and muscle. After the administration of progressively increasing doses of vasopressin, marked reduction in mean tissue blood flow was noted without significant changes in the muscle to tumor or muscle to skin ratios.

After the injection of 0.2 units of vasopressin, over a 5-min period prior to flow determinations, a reduction in mean tissue blood flow was noted in all tissues studied, but tumor flow was 8-fold greater than muscle. When 1.6 units were infused, the mean tumor flow was still 6 times higher than skin and 9 times higher than muscle.

Discussion

The increased vascularity of many types of neoplasms in man has been demonstrated by various methods including arteriography (4), capillary permeability to fluorescein (6), skin temperature over superficial neoplasms (5), and the oxygen content of venous outflow (7). Yet, quantitative estimates of transplantable tumor blood flow have revealed that tumor perfusion was consistently lower than adjacent tissue (13, 21).

The results of tissue blood flow determinations of the s.c. hamster melanoma are the 1st quantitative evidence suggesting that tumor flow may be higher than the surrounding tissue. The uptake of the blood flow indicator by the melanoma 15 days after transplantation was 5-10 times greater than adjacent skin and muscle respectively. Recent studies in our laboratory have compared the uptake of antipyrine-131I with 4Cr-labeled ceramic microspheres in this tumor. These 2 entirely different blood flow techniques have demonstrated remarkably similar levels of uptake of the 2 isotopes in skin, muscle, and tumor. Thus, the elevated uptake of the reference agent in tumor was most likely a reflection of tissue blood flow rather than a result of stagnation or pooling of blood (11). However, with advancing age and progressive necrosis the uptake of antipyrine-131I by tumor was markedly reduced to approximately ½ the uptake level of the smaller tumors.
Effect of Vasoactive Drugs on Tissue Blood Flow

In addition to its unique blood flow characteristics, the tissue flow in the hamster melanoma demonstrated a response to vaso-active drugs distinct from that of normal tissue. The changes in blood flow in skin and muscle induced by adrenaline infusions were consistent with the results of other reports (9, 23, 26). Elevation in skeletal muscle flow was noted following 1 µg/min adrenaline, while higher doses of this drug resulted in a marked reduction in muscle perfusion. These alterations can be accounted for by the transient or sustained dilatation of skeletal muscle vessels occurring after low doses of this drug and marked vasoconstriction following higher doses (9). In addition, the reduction in muscle flow following arterenol and vasopressin as well as the depressed skin perfusion noted after each of these drugs reiterates the direct vasoconstrictive action of these agents on the vasculature of skin and muscle (23, 26).

In contrast, changes in tumor blood flow secondary to catecholamine infusion were more pronounced than those in the adjacent skin and muscle. Administration of low doses of adrenaline and arternenol was associated with a marked reduction in tumor flow without any perceptible change in skin flow. With higher doses of these drugs, perfusion to all tissues was markedly reduced, but these changes were substantially greater in the neoplastic tissue. Although similar reductions in tumor blood flow were noted following vasoopressin infusion, there were no significant alterations in the tissue flow ratios.

These findings differ from those obtained by Gullino (14) in studies on the blood flow of ovarian and renal tissue implants of rat sarcoma and carcinoma. By cannulating the venous outflow of isolated organs replaced with tumor, Gullino found that the tumor vasculature seemed to behave like that of the host organ. The i.v. administration of adrenaline resulted in a proportional reduction of flow in these “tissue-isolated tumors” and normal organs, while acetyl-β-methylcholine infusion produced an enhancement of flow in both of these tissues.

In attempting to evaluate these conflicting reports, it is important to critically examine the experimental methods. In the present study, the technic of tissue blood flow measurement excludes arteriovenous shunting, while the venous outflow collection is the sum of both tissue perfusion and flow through arteriovenous shunts. Since a significant amount of arterial blood in tumors has been reported to be shunted directly into the venous system (24), venous outflow measurements probably include a large quantity of blood that has not passed through the capillary bed. Therefore, if these vasoactive drugs altered the distribution of flow between the arteriovenous shunts and tissue, this change in perfusion would not be evident from venous blood collection measurements.

The lack of agreement between these investigations might also be attributed to the differing structural characteristics of the vasculature of these tumors. Algire (1) studied the tumor vasculature of carcinoma, sarcoma, and melanoma of the mouse through a transparent membrane inserted in a skin flap. The vascular system of the carcinoma and sarcoma was characterized by randomly sinusoidal channels of large diameter without evidence of differentiation into arterioles and venules. In contrast, examination of the malignant melanoma revealed a better organized blood vessel arrangement with thinner capillaries and evidence of differentiation of some tumor vessels into arterioles and venules (2).

In view of these conflicts, extensive anatomic and physiologic investigations of the tumor vasculature will be necessary to interpret these differences. Solutions to this problem can only come after careful hemodynamic examinations are made of many different types of tumors during a similar phase of growth.

Acknowledgment

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References


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