Effects of Several Vasoactive Drugs on the Vascular Resistance of MT-W9B Tumors in W/Fu Rats

Randy Jirtle, Kelly H. Clifton, and John H. G. Rankin

Radiobiology Laboratories, Wisconsin Clinical Cancer Center, and Departments of Human Oncology and Radiology [R. J., K. H. C.] and Physiology and Gynecology-Obstetrics [J. H. G. R.], University of Wisconsin Medical School, Madison 53706, and Wisconsin Perinatal Center, Madison General Hospital [J. H. G. R.], Madison, Wisconsin 53715

ABSTRACT

These experiments were designed to study the effects of vasoactive drugs on normal and malignant tissue in W/Fu rats. The increase in resistance to tumor blood flow elicited by a bolus injection of 10 µg of norepinephrine was greater than that elicited in the surrounding mammary gland tissue. A 10-fold increase in the resistance to tumor blood flow in one animal was sustained for 30 min by the infusion of norepinephrine at the rate of 1.39 µg/min, whereas a smaller initial increase in mammary gland vascular resistance decreased with time. In contrast, the increase in resistance to tumor blood flow caused by a bolus injection of angiotensin II was less than that observed in the mammary gland tissue. A 20-fold increase in mammary gland vascular resistance could be maintained for at least 5 min by infusion of angiotensin II at the rate of 1.39 µg/min. In comparison, such treatment caused only a 3-fold increase in the resistance to tumor blood flow. A bolus injection of 1 µg of isoproterenol decreased the vascular resistance in all normal tissues studied, but the resistance to blood flow in the tumor remained unchanged. The results of these experiments indicate that there may be methods whereby the tumor blood flow can be manipulated for therapeutic purposes and to assist radiographic visualization of tumors.

INTRODUCTION

The literature concerning the response of tumor vasculature to vasoactive drugs is often contradictory. For example, it has been reported that tumors respond poorly to epinephrine when compared to responses of the surrounding tissue (1, 22), that the arterioles supplying a tumor respond to epinephrine in a manner similar to that of normal somatic vessels distant from the tumor (11), and that changes in tumor blood flow secondary to epinephrine infusion are more pronounced than those in the surrounding normal tissue (7).

Much of this conflict arises because of variations in tumor-host systems, anesthetic techniques, the state of the preparation, and the techniques used for measuring blood flow. We have recently shown that radioactive microspheres can be used to measure blood flow to the V2 carcinoma in the rabbit (16). In these studies we have found that the increase in resistance to tumor blood flow after the injection of norepinephrine was greater than that of the surrounding normal tissue. The rabbit preparation suffered from the disadvantage that the foreign tumor material produced an immune reaction in the surrounding tissue which confused the results. We have determined that a superior preparation can be obtained with the use of the inbred W/Fu rat carrying the MT-W9B mammary tumor. We have recently demonstrated that it is possible to measure tumor blood flow twice in an unanesthetized rat with 2 injections of microspheres containing different radioactive labels (10). In the experiments reported here, we have used that preparation to examine the effects of various vasoactive drugs on the resistance to blood flow in normal and malignant tissues.

MATERIALS AND METHODS

Animal and Tumor System. Female isogeneic W/Fu rats weighing approximately 240 g were housed 2 to a suspended cage in a temperature-controlled room with 12 hr of light daily. Food and water were given ad libitum.

Tumor suspensions were prepared for transplantation with a Snell cytosieve as previously described (5) and were adjusted to a 33% volume of centrifugally packed cellular material from an MT-W9B mammary adenocarcinoma (11). Inocula of 0.10 ml were injected into both axillary mammary glands and the right inguinal mammary gland.

Surgical Procedure. The animals were anesthetized with ether, and catheters were surgically placed into both the left ventricle of the heart and the left femoral artery when tumors reached approximately 1 g. The animals were returned to cages and allowed to recover for approximately 3 hr before the experiments were performed. Full details of these procedures are described elsewhere (10).

Microsphere Technique. Approximately 70,000 radioactively labeled microspheres were slowly flushed into the left ventricle of the heart with 0.6 ml of 0.9% NaCl solution. A Harvard withdrawal pump was used to withdraw simultaneously a femoral arterial blood sample for 1 min at a rate of 0.51 ml/min. For reasons previously described, all tissue blood flows were estimated with spheres 25 µm in diameter (10). The spheres were labeled with either 125I, 46Sc, 85Sr, 141Ce (Minnesota Mining and Manufacturing Co., St. Paul, Minn.), 69Mn, or 109Cd (New England Nuclear, Boston, Mass.).

Approximately 15 min later, 0.1 ml of various vasoactive drugs of differing concentrations was flushed into the left ventricle of the heart with 0.5 ml of 0.9% NaCl solution. After 0.75 min, 25-µm microspheres, labeled with an isotope with a characteristic γ spectrum that differed from that of the first, were flushed into the left ventricle of the heart.
with 0.6 ml of 0.9% NaCl solution. An integrated arterial blood sample was again withdrawn. In 1 series of experiments, the second batch of microspheres was injected either 0.75, 2, or 5 min after a bolus injection of 10 μg of norepinephrine. In another series of experiments, either norepinephrine or angiotensin II was infused into the right jugular vein at a rate of 1.39 μg/min for various lengths of time before the second batch of labeled microspheres was injected.

The animals were sacrificed by the injection of 0.10 ml of euthanasia solution (Vet Labs, Lenexa, Kans.) into the left ventricle of the heart. The skin from the right inguinal region, the right inguinal mammary gland, the lower hind leg muscle, and the tumors were dissected free of surrounding extraneous tissue and were individually placed into glass counting vials. For each tissue sample the 2 isotope activities and the corresponding number of spheres were determined by appropriate data reduction of the output from a 3-channel NaI well counter equipped with pulse height analyzers (17).

The rationale for our use of microspheres to measure tumor blood flow is as follows. The microsphere method is the only method that permits the simultaneous measurement of blood flows to many regions in the same animal (12, 18). Buckberg et al. (4) have evaluated the method and have reported on the potential sources of error. The method has been used in rats by many investigators (10, 13, 14, 21). Nishiyama et al. (14), using microspheres, measured the regional distribution of cardiac output in unanesthetized rats. McDevitt and Nies (13) have also reported that microspheres can be used to estimate the cardiac output and distribution in rats, provided that the blood samples contain more than 400 spheres. They reported that the injection caused no hemodynamic changes and that the results agree with published estimates calculated by other techniques. Tsuchiya et al. (21) have used microspheres in unanesthetized rats and reported that there were no hemodynamic alterations and that the reproducibility of 3 separate injections to each rat was excellent. We have previously used microspheres to measure the blood flow to the V2 carcinoma in rabbits (16), and we have performed extensive experiments on the use of this technique for the measurement of mammary tumor blood flow in unanesthetized rats (10). We conclude that microspheres can be used to measure regional blood flows in unanesthetized rats.

Resistance Ratio Calculations and Statistical Analysis. The tissue blood flows were calculated by the equation

$$F_T = (WR/N_a) \times N_T$$

where $$F_T$$ is the tissue blood flow (ml/min), $$WR$$ is the withdrawal rate of the integrated arterial blood sample, $$N_a$$ is the number of spheres in the withdrawn blood sample, and $$N_T$$ is the number of spheres in the sample tissue. The systemic arterial blood pressure was measured with a Statham pressure transducer and recorded on chart paper. Since the pressure could not be monitored when blood was being withdrawn from the femoral artery, the average arterial blood pressure during this time was estimated by linear interpolation. On the assumption that the mean venous pressure is equal to zero, the tissue vascular resistance ($$mm Hg/ml/min/g$$) could be calculated by the equation

$$R_T = Pa/F_T$$

where $$Pa$$ is the average systemic arterial blood pressure ($$mm Hg$$).

The tissue blood flows and systemic arterial pressures were estimated before and after the injection of vasoactive drugs, which allowed calculation of the resistance to blood flow in the various tissues at these times. The ratio of the tissue blood flow resistance after the injection of a vasoactive drug to that before injection (i.e., resistance ratio = treatment/control) yields an estimate of the fractional change in tissue vascular resistance caused by the drug. Therefore, if 0.9% NaCl solution is injected rather than vasoactive drugs, theoretically the resistance ratio should be 1.0. However, with this animal system, the resistance ratios, under this condition, for skin, muscle, mammary gland, and tumors were previously determined to be 1.61, 1.24, 1.60, and 1.90, respectively (10).

The statistical procedure, ANOVA, was used to compare 2 or more independent sample means, and the paired t test was used to compare both the tumor blood flows and resistance ratios to the corresponding values in the various normal tissues. A $$\chi^2$$ test was used to determine whether the distribution of the resistance ratios was adequately described by a normal probability density function (3).

Drugs. Norepinephrine (Levophed bitartrate, Winthrop Laboratories, New York, N. Y.), angiotensin II (Bachem, Inc., Marina del Rey, Calif.) and isoproterenol (Isuprel hydrochloride; Winthrop Laboratories) were all diluted to the appropriate concentration in 0.9% NaCl solution and were always stored in the frozen state to minimize deterioration.

RESULTS

Frequency Distribution of the Resistance Ratio Data. A $$\chi^2$$ test demonstrated that the distribution of the resistance ratios (i.e., treatment/control) could not be described adequately by a normal probability density function ($$p < 0.001$$). The blood flow and the resistance ratio distributions could, however, be normalized with a natural logarithmic (ln) transformation of the data and all parametric statistical tests (e.g., ANOVA, and t tests) were therefore performed after ln transformations.

Effect of Norepinephrine. The ratio of vascular resistances (i.e., treatment/control) as a function of the injected dose of norepinephrine for normal and malignant tissues is shown in Chart 1. A bolus injection of 5 μg of norepinephrine produced a maximum response in the mammary gland, the muscle, and the skin. In the tumor, more than 5 μg of norepinephrine were required to maximize the resistance ratio ($$p < 0.05$$). The average resistance ratios for the malignant tissue, after bolus injections of 10 or 20 μg of norepinephrine, were significantly larger than those of the normal surrounding mammary gland tissue ($$p < 0.01$$).

The increase in vascular resistance after a bolus injection of 10 μg of norepinephrine was maintained longer in the malignant tissue than in the surrounding normal mammary gland tissue (i.e., 2 min, $$p < 0.001$$). After 5 min the resist-
Effects of Drugs on Rat Tumor Vascular Resistance

When norepinephrine was infused into the jugular vein at a rate of 1.39 μg/min, the resistance ratio for the tumor tissue growing in the mammary glands was significantly greater than the control value of 1.90 (95% confidence interval, 1.14 to 3.18) and was significantly larger than that of all normal tissues studied for the entire 30-min infusion period (Chart 3a). The vasculature of the mammary gland partially escaped from the effect of norepinephrine in 30 min of infusion (p < 0.01) but did not return completely to the control value. In contrast, the resistance ratio of the tumors growing in mammary gland tissue did not significantly change during the 30 min of infusion. During norepinephrine infusion the relative blood flow to the tumor was initially 4 times that of the surrounding tissue (Chart 3b). The blood flows to the tumor and surrounding tissues were almost identical after 15 min of infusion. Compared to skin and mammary gland, muscle tissue, although sensitive to bolus injections of norepinephrine, was relatively unresponsive to its infusion at the rate used.

Effect of Angiotensin II. In contrast to the results obtained with norepinephrine, we found that a bolus injection of angiotensin II elicited a smaller resistance ratio increase in the tumor than in the mammary gland (Chart 4). Similar results were obtained when angiotensin II was infused at a rate of 1.39 μg/min (Chart 5a). Short-term constant infusion studies also demonstrated that the vasculatures of the
mammary gland and of the skin were unable to escape significantly from the constricting effect of the drug for at least 5 min. During the infusion the blood flow to the skin and mammary gland dropped approximately 93%, whereas that to the tumor decreased only 35% (Chart 5b). Muscle tissue did not respond significantly to either bolus injections (20 µg) or constant infusions of angiotensin II. However, the relative blood flow increased during the infusion (Chart 5) because the systemic arterial pressure increased from 91 ± 5 mm Hg. The magnitude of the changes in resistance to tissue blood flows could be estimated. The microsphere technique, however, does not allow one to determine the position in the vascular network (i.e., preexisting normal tissue vasculature and/or newly formed tumor vasculature) where the change in resistance to tumor blood flow occurred. We have thus avoided the term “response of tumor vasculature” throughout. The results of these studies indicate that the change in the resistance to tumor blood flow caused by the administration of norepinephrine, angiotensin II, or isoproterenol was of a different magnitude than that of the surrounding normal tissue. These differences may be exploitable to improve the treatment and diagnosis of certain tumors.

Bolus injections of norepinephrine at all doses studied caused an increase in the resistance to blood flow in both normal and malignant tissue. The resistance ratio for normal tissue was maximal after 5 µg, but malignant tissue required more than 10 µg. The maximal response observed in the malignant tissue was, however, significantly larger than that of all normal tissues studied including the mammary gland, the normal tissue from which the tumor vasculature was derived (2) (Chart 1). Edlich et al. (7) observed similar results when they compared the vascular response of the amelanotic melanoma to that of the surrounding skin and muscle after a systemic administration of norepinephrine.

We also observed that the resistance ratio remained elevated for a longer period of time in the tumor tissue than in the surrounding normal tissue, which implied that the malignant tissue did not possess the same ability to escape from the effect of norepinephrine. This conclusion was confirmed when norepinephrine was infused at a constant rate of 1.39 µg/min. The resistance ratio of the tumor tissue did not decrease significantly for 30 min, whereas the resistance ratio of the mammary gland tissue decreased toward control.

A tumor weighing approximately 1 g was found to have a relative blood flow (ml/min/g) about 330% greater than that of mammary gland tissue. After 15 min of constant infusion, both the mammary gland tissue and the overlying skin had partially escaped from the effect of the norepinephrine, whereas the malignant tissue had not. As a consequence the relative tumor blood flow was only 10% greater than that of the mammary gland.

These results may have considerable practical importance. For example, Steckel et al. (20) have proposed the use of catecholamines in conjunction with radiotherapy to protect the normal tissue at risk (e.g., intestine and kidney). Our results indicate that, if the tumor to be treated is growing in the normal tissue at risk, the infusion of norepinephrine will equally protect the malignant and surrounding normal tissue since their blood flows will be reduced to a similar level. If the tumor is not in the normal tissue at risk it would be preferable to infuse the vasoactive drugs at a rate such that an insignificant dose is delivered to the tumor upon its recirculation.

Dickson et al. (6) have shown that the temperature in the Yoshida tumor heated in a radiofrequency field, was 2–3°

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**DISCUSSION**

The microsphere technique enabled us to measure tissue blood flows in conscious, minimally disturbed rats before and after an exogenous administration of a vasoactive drug.

**Table 1**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. of observations</th>
<th>Resistance ratio (treatment/control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>14</td>
<td>0.363 (0.231–0.573)</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>14</td>
<td>1.139 (0.874–1.483)</td>
</tr>
<tr>
<td>Skin</td>
<td>14</td>
<td>1.245 (0.959–1.616)</td>
</tr>
<tr>
<td>Tumor</td>
<td>21</td>
<td>1.751 (1.436–2.135)</td>
</tr>
</tbody>
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* Numbers in parentheses, 95% confidence interval.

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Since the average systemic arterial blood pressure was also monitored, the magnitude of the changes in resistance to tissue blood flows could be estimated. The microsphere technique, however, does not allow one to determine the position in the vascular network (i.e., preexisting normal tissue vasculature and/or newly formed tumor vasculature) where the change in resistance to tumor blood flow occurred. We have thus avoided the term “response of tumor vasculature” throughout. The results of these studies indicate that the change in the resistance to tumor blood flow caused by the administration of norepinephrine, angiotensin II, or isoproterenol was of a different magnitude than that of the surrounding normal tissue. These differences may be exploitable to improve the treatment and diagnosis of certain tumors.

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Dickson et al. (6) have shown that the temperature in the Yoshida tumor heated in a radiofrequency field, was 2–3°
lower than that in the surrounding muscle. They concluded that the blood flow to the tumor was greater than that to the muscle. We have shown that such a condition exists when the MT-W9B tumor is growing in the mammary gland. If such a phenomenon is shown to hold more generally, the effectiveness of hyperthermia treatment would be severely compromised. However, the constant infusion of norepinephrine significantly lowers the difference between the blood flow to the malignant tissue and the blood flow to the surrounding normal tissue. This presumably would reduce temperature differences during hyperthermia therapy. In addition, since tumor blood flow can be significantly decreased for extended periods of time, the intratumoral pH would presumably decrease to a level that is even lower than that which normally occurs (9), thus increasing the sensitivity of the malignant cell to hyperthermia treatment (15).

When angiotensin II was injected into tumor-bearing rats, the results were opposite to those resulting from norepinephrine. The increase in the resistance ratio for tumors at all doses was significantly less than that for the mammary gland tissue and skin. When infused at a rate of 1.39 μg/min, angiotensin II reduced the blood flow to the tumor by 35%, whereas the flow to the mammary gland and overlying skin was decreased approximately 93%. Therefore, after the initiation of angiotensin II infusion, the blood flow to the tumor is 3400% rather than 330% greater than that to the surrounding normal mammary tissue. By the constant infusion of angiotensin II, this condition can be maintained for at least 5 min.

Recently, it has been shown that the therapeutic results achieved by cesium therapy of breast carcinomas compare favorably with other modalities of treatment, and mutilating surgery was avoided in 80% of those patients who survived 5 years (19). If human mammary gland tissue responds to angiotensin II like rat mammary tissue, our results imply that local infusion of angiotensin II may further improve the radiation treatment of mammary carcinomas by protecting the normal mammary gland tissue from radiation damage. Ekelund et al. (8) have demonstrated that angiotensin II improved radiographic visualization of bone and soft tissue tumors more than did norepinephrine and tolazoline. Our results suggest that this probably is due to the fact that, unlike most normal tissues, the resistance to tumor blood flow is not greatly increased with angiotensin II. We suggest that, since the mammary gland vasculature becomes more sensitive to angiotensin II, the radiographic resolution of mammary gland tumors may also be enhanced by a prior local injection of this drug.

When isoproterenol, a vasodilator, was injected into tumor-bearing animals, the resistance to blood flow decreased in all normal tissues studied. The vasculature of skeletal muscle was particularly sensitive to its action, and the blood flow almost doubled after a bolus injection of 1 μg. The resistance to tumor blood flow, however, was unchanged; but since the systemic arterial pressure decreased, the tumor blood flow also decreased. These results provide an explanation of why β-receptor agonists do not usually enhance the radiographic visualization of tumors surrounded by skeletal muscle (8). In addition, they imply that tumor vasculature is insensitive to this β-receptor agonist and/or is maximally dilated.

The microsphere technique has enabled us to study simultaneously the response of normal and malignant tissue to vasoactive drugs. The response of the tumor relative to that of the surrounding normal tissue is dependent upon the drug injected, its concentration, and the time after its administration. The reason(s) for these differences are not currently known; nevertheless the results indicate that the appropriate use of vasoactive drugs might well improve both the therapy and radiographic visualization of tumors.

ACKNOWLEDGMENT

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