ABSTRACT

We have investigated the effect of 5-hydroxytryptamine (5-HT) on SMT-2A mammary adenocarcinoma blood flow during localized microwave hyperthermia treatment. Tissue blood flow in isogenic female W/Fu rats was estimated using 25-μm-diameter 113Sn-labeled microspheres. An intraarterial injection of 5-HT (1 mg/kg) into either conscious or anesthetized (Nembutal, 20 mg/kg) animals resulted in a 53% reduction in tumor blood flow, while that of the surrounding skeletal muscle remained unchanged. Because of the selective reduction in tumor perfusion, the blood flow of the normal and malignant tissue was equal after 5-HT injection. This blood flow equivalence remained unaltered after 45 min of heating at 42°C. Consequently, the temperature in the tumor was not significantly different from that in the surrounding normal tissue. In contrast, when the tissues were heated at 42°C without 5-HT, the tumor blood flow was significantly greater than that in the surrounding musculature, resulting in the tumor being 1°C lower than the muscle temperature. An intratumoral injection of 5-HT (0.25 mg) reduced the tumor blood flow by 92%, and the blood flow of the surrounding muscle was reduced by 57%. These tissue blood flows were not significantly altered by heating at 44°C for 45 min, and the tumor temperature was 0.7°C greater than that in the muscle. When heating at 44°C was performed without 5-HT injection, the tissue temperatures were equal. Thus, both an intraarterial and an intratumoral injection of 5-HT prior to hyperthermia treatment significantly improved the temperature differential between the neoplastic and surrounding normal tissue. Of additional interest was the observation that an intratumoral injection of 0.15 M NaCl also resulted in a preferential increase in the tumor temperature.

INTRODUCTION

A nonuniform vascular density and distribution of blood flow impair the ability to effectively treat tumors with all presently available nonsurgical treatment modalities, including hyperthermia. Tumors can be preferentially heated if the delivery of energy is physically restricted to only the malignant tissue, but in practice, this is often a difficult situation to achieve. However, since heat in tissues is primarily dissipated by blood perfusion, selectively reducing tumor blood flow with vasoactive agents would also result in the differential heating of tumors. To alter tissue vascular resistance, a wide spectrum of naturally occurring chemicals which act on vascular smooth muscle cells can be used, and a particularly interesting drug is 5-HT. This vasoactive agent is widely distributed in nature and possesses a variety of pharmacological actions, and although it is not commonly utilized as a therapeutic agent, there are several reported instances where it has been used experimentally in the treatment of cancer. The i.p. injection of 5-HT causes a rapid and more profound fall of oxygen tension in tumors than in the normal tissues (1, 2), and repeated injections of 5-HT have resulted in the inhibition of tumor growth (3, 4). The s.c. injection of 5-HT at a distance from the Walker 256 carcinoma has been shown to significantly improve the efficacy of tumor treatment with hypertonic glucose solutions injected i.t. (5, 6). Also, Creile (4) demonstrated that the injection of 5-HT i.t. increased the susceptibility of tumors to heat-induced damage. These investigations indicate the therapeutic value of 5-HT, and the oxygen studies by Cater et al. (1, 2) imply that it may preferentially reduce tumor blood flow when injected systemically or directly into the tumor mass. The purpose of this study was to investigate the effects of 5-HT on tumor microcirculation and to determine whether this vasoactive agent would be of use as an adjuvant to the hyperthermia treatment of neoplasms.

MATERIALS AND METHODS

Animal and Tumor System. Isogenic female W/Fu rats were housed in a temperature-controlled room with a 12-h light-dark cycle, and they were fed standard laboratory rat chow and water ad libitum. A cell suspension of the viable portion of the SMT-2A mammary adenocarcinoma was prepared and injected into the right soleus muscle according to a previously published procedure (7, 8). Four weeks later, when the average tumor weight was approximately 2 g, the tumors located in the hind leg musculature were heated with microwaves at a frequency of 915 MHz.

Surgical Technique. Estimation of tissue blood flow with microspheres requires the cannulation of both the femoral artery and left ventricle of the heart. The catheters used in this procedure were prepared in the laboratory by fusing a PE 10 catheter with a PE 50 catheter. Animals bearing tumors of the desired size were anesthetized with ether prior to the cannulation procedure. After the vascular insertion of the PE 10 portion, both catheters were filled with heparinized (25 units/ml) isotonic saline (0.15 M NaCl) and subsequent to sealing were externalized at the dorsal aspect of the neck. All animals were returned to individual cages and allowed 3 h to recover from the effects of surgery and ether anesthesia before further experimentation.

Microwave Heating of Tissues. It was necessary to anesthetize the animals for the hyperthermia experiments to facilitate both the heating and monitoring of tissue temperatures with implantable thermistor probes. For anesthetization, Nembutal (20 mg/kg) was slowly infused into the arterial circulation with 0.5 ml of isotonic saline. The anesthetized animal was placed on a styrofoam board covered with a heating pad to control the body temperature when necessary. Body temperature was recorded via a rectal probe. The tumor-bearing leg was prepared by clipping the hair before inserting the thermistor needle probes. Previous studies (unpublished) have shown that, under the experimental conditions used in these investigations, the temperature distribution within a
the skin surface (i.e., in the outer viable rim of the tumor). The tumor-bearing leg was positioned between 2 plastic bags containing 37°C water before the direct-contact microwave applicator was placed into position over it. Microwave heating was performed at 915 MHz with a direct-contact applicator (9 sq cm) oriented such that the 2 thermistor probes were perpendicular to the microwave electric field and equidistant from the applicator edges. Utilizing a pulsed microwave generation system previously described, localized heating with automatic feedback was controlled to ±0.1°C (9).

**Drug Studies.** 5-HT (serotonin:creatinine:sulfate complex) was purchased from Calbiochem and diluted in 0.15 M NaCl. In one group of experiments, the SMT-2A tumor-bearing female W/Fu rats were infused i.a. with 1 mg of 5-HT per kg 10 min after anesthetization. After an additional 10 min, the tumor-bearing leg was heated at 42°C for either 10 or 45 min. Studies were also performed, where the 5-HT was injected i.t. prior to hyperthermia treatment. To facilitate the localized injection of 5-HT into the tumor mass, the animals were first anesthetized with Nembutal (20 mg/kg). After the animal was unconscious for 10 min, either 0.1 ml of isotonic saline or 5-HT in isotonic saline (2.5 mg/ml) was injected into the tumor. After the local injection, the 25-gauge needle was left in place for approximately 10 min to reduce leakage of the injectant. At the end of this time, the needle was removed, and when subsequent heating was to be performed, a thermistor needle probe was positioned into approximately the same location. An additional thermistor probe was located in the normal musculature surrounding the tumor. Tissue blood flow was measured at the end of these heating periods by the microsphere technique described below.

**Blood Flow Measurements.** The procedure for tissue blood flow measurement, as utilized in our laboratory, has been previously described in detail (8). Briefly, during the microwave heating of the tissues (10 or 45 min after initiation), approximately 90,000 113Sn-labeled microspheres 25 μm in diameter were flushed into the left ventricle of the heart with 0.5 ml of heparinized isotonic saline. The blood sample, withdrawn at a rate of 0.51 ml/min from the femoral artery during and for 1 min after the microspheres were infused, served as a reference sample for calculating the blood flow in the tissue of interest. Within 1 min after the microsphere injection, the animal was killed with Nembutal, and the tissues of interest were dissected from the animal. The radioactivity in the grossly viable portion of the tumor and in various normal tissues which were removed from the animal was determined with a NaI well counter and converted into the number of spheres by the use of a microsphere standard prepared according to a previously published procedure (10).

Tissue blood flow was calculated by using the formula

\[ FT = (WR/NB) \times (NT) \]

where \( FT \) is the tissue blood flow (ml/min/g), \( WR \) is the rate of the arterial blood sample withdrawal (ml/min), \( NB \) is the number of microspheres in the withdrawn blood sample, and \( NT \) is the average number of microspheres per g of tissue. The systemic arterial blood pressure was measured with a Millar pressure transducer (Millar Instruments, Houston, TX). On the assumption that the mean venous pressure is approximately equal to zero, the tissue vascular resistance (\( RT \) mm Hg/ml/min/g) was calculated by the equation

\[ RT = Pa/FT \]

where \( Pa \) is the average systemic arterial blood pressure.

**Statistical Analysis.** The unpaired \( t \) test assuming equal variances and the one-way analysis of variance were used to compare sample means. All blood flow data were normalized with a natural log transformation prior to utilizing these parametric statistical tests (8).

**RESULTS**

**Systemically Infused 5-HT into Conscious and Anesthetized Animals.** To determine the effects of 5-HT on the microcirculation of normal and malignant tissues, 1 mg of 5-HT per kg was infused i.a. into a group of SMT-2A tumor-bearing conscious rats, and 30 min later, tissue blood flow was measured. The arterial pressure averaged 101 ± 3 mm Hg (\( n = 8 \)), which was not significantly different (\( P > 0.1 \)) from the control animals (101 ± 5 mm Hg, \( n = 13 \)). Chart 1 shows that a systemic injection of 5-HT into conscious animals significantly (\( P = 0.02 \)) increased the tumor vascular resistance and reduced the tumor blood flow by 53% (\( P < 0.02 \)). In contrast, the vascular resistance and blood flow of the surrounding muscle were insignificantly different (\( P > 0.1 \)) from those in the control animals.

Since in these hyperthermia studies we utilized animals anesthetized with Nembutal, the effect of systemically infused 5-HT on malignant and normal tissue blood flow in anesthetized animals was also investigated. In these experiments, 5-HT was infused i.a. 10 min after the anesthetization, and 30 min later, the tissue blood flow was measured. Anesthetization with Nembutal did not significantly alter the blood flow of the muscle or the SMT-2A tumor nor the arterial pressure. The arterial pressure in the anesthetized animals, however, was significantly (\( P = 0.02 \)) reduced from 101 ± 3 mm Hg (\( n = 20 \)) to 88 ± 6 mm Hg (\( n = 12 \)) by the infusion of 5-HT. Nevertheless, the 5-HT-induced vascular responses in the tumor and muscle were found to be similar to those obtained in the conscious animals (Chart 1). Thus, 5-HT delivered systemically caused a selective increase in tumor vascular resistance and a concomitant reduction in blood flow, regardless of whether the animals were conscious or anesthetized.

**Systemically Infused 5-HT in Conjunction with Localized Hyperthermia.** Because of the selective effects of systemically
injected 5-HT on the tumor vasculature, its effectiveness in improving the localized heating of tumors with microwaves was investigated. In these studies, 1 mg of 5-HT per kg was infused i.a. 10 min after anesthetization, and 10 min later, the hind leg musculature surrounding the SMT-2A tumor was heated at 42°C for 10 or 45 min. The vascular resistance and blood flow after 5-HT injection versus time of heating are shown in Charts 2B and 3B, respectively. For comparison, the results are also shown when hyperthermia treatment was performed without a prior systemic injection of 5-HT (Charts 2A and 3A). The initial tumor vascular response observed when hyperthermia was combined with 5-HT administration was a transient but significant (P < 0.05) increase in the vascular resistance with a concomitant decrease in the blood flow; this initial heat-induced vasoconstriction occurred regardless of whether 5-HT was administered. After 45 min of heating, the tumor vascular resistance in the 5-HT-treated animals had decreased below that observed after 10 min of heating, but the blood flow was not significantly different from that in the unheated tumors. Additionally, because of the equivalency of the normal and malignant tissue blood flow (P > 0.1) after 45 min of heating, the tumor temperature (41.7 ± 0.2°C) was found not to be significantly different (P > 0.1) from the controlling temperature in the surrounding musculature (42°C). This is in contrast to what was observed when 5-HT was not given prior to heating. Under this experimental condition, though the blood flow of the muscle increased after 45 min of heating (Chart 3A), it was still significantly lower than that of the tumor, and consequently, the tumor temperature was approximately 1°C lower than, rather than equal to, the temperature in the muscle (11). Thus, the systemic infusion of 1 mg of 5-HT per kg 10 min prior to heat treatment significantly improved the temperature differential between the malignant and normal tissue when compared to that obtained without the use of 5-HT. In an attempt to further improve the temperature differential between the malignant and normal tissue, 5-HT was injected i.t.

Injection of 5-HT i.t. in Conjunction with Localized Hyperthermia. Chart 4 (unheated) shows that an i.t. injection of 5-HT (0.25 mg in 0.1 ml of isotonic saline) significantly increased the vascular resistance and reduced the blood flow of the unheated tumor and musculature. This was a direct effect of 5-HT on the tumor vasculature, rather than an indirect effect, resulting from a rise in the i.t. pressure, since an injection of an equivalent volume of isotonic saline into the tumor had no significant effect on the vascular resistance or the blood flow in either the malignant or normal tissue when compared to the unheated control values.

After heating the muscle at 44°C for 45 min, the normal and malignant tissue blood flow increased to equal levels when 5-HT was not injected prior to heat treatment (Chart 4, heated control). As previously reported (11), under these circumstances, the tumor temperature is also not significantly different (P > 0.1) from 44°C. However, an i.t. injection of 5-HT prior to hyperthermia treatment completely inhibited the normally observed heat-induced vasodilation (11) to the extent that the blood flow of both the normal and malignant tissues after 45 min at 44°C was not significantly different (P > 0.1) than that in the unheated 5-HT-injected tissues. Under these experimental conditions, the tumor temperature was 0.7°C higher than that in the muscle, a temperature significantly greater (P < 0.001) than the 44°C in the surrounding normal tissue. Interestingly, the injection of 0.15
The purpose of this study was to determine the effectiveness of the vasoactive agent, 5-HT, in selectively reducing tumor blood flow during microwave hyperthermia treatment. The results of this investigation unequivocally demonstrate that 5-HT injected systemically into conscious or anesthetized rats resulted in a marked preferential reduction in tumor blood flow. Additionally, the use of 5-HT during hyperthermia treatment was shown to significantly improve the temperature differential between the malignant and surrounding normal tissue.

The blood flow of tumors is characterized by its nonhomogeneous nature, but the perfusion rate in the rapidly expanding grossly viable regions is often, though not always, greater than that of normal tissues (for review, see Refs. 11 and 12). Since blood flow significantly influences the ability to heat tissues, hyperthermia treatment of neoplasms would be enhanced if the blood flow significantly influences the ability to heat tissues, hyperthermia treatment of neoplasms would be enhanced if the tumor vasculature is normally close to maximally dilated. The vasodilating agent, hydralazine, has been utilized for this purpose (13–15). Its use in tumor-bearing animals was shown to significantly increase the blood flow in the normal tissue relative to that in the tumor, and as a consequence, the temperature differential between the malignant and normal tissue during hyperthermia treatment was improved (13, 15). However, though hydralazine is used clinically to treat hypertension, its usefulness as an adjuvant to hyperthermia treatment may be limited by the reported high incidence of untoward side effects (16).

Vasoconstrictive agents would also be potentially useful as adjuvants to hyperthermia treatment, if they preferentially reduced malignant tissue blood flow. We specifically chose to study the vasoactive agent 5-HT because previous studies have implied that this drug may have a selective effect on the tumor microcirculation (1–6). To quantify the effect of 5-HT on tissue vascular resistance and blood flow, we utilized the radiolabeled microsphere technique. The microspheres used were 25 µm in diameter, since we have previously shown that there is minimal shunting of this size sphere through the sinusoid vessels of the SMT-2A tumor, even when it is heated to temperatures up to 44°C (11). The animals in the hyperthermia studies were anesthetized with Nembutal to facilitate the localized heating and monitoring of tissue temperature. Although anesthetics are known to often alter the responsiveness of the vasculature to vasoactive agents (17), the dose of Nembutal used in this study was not found to significantly change the tumor vasculature response to the systemic injection of 5-HT from that observed in conscious rats.

When 5-HT was infused i.a. into either conscious or anesthetized rats, the blood flow of the tumor was reduced by approximately 50%, whereas that of the surrounding normal vasculature remained unchanged. Cater ef al. (1) observed that an i.p. injection of 5 mg of 5-HT per kg produced a more rapid and greater fall in the oxygen pressure in the tumor than in skeletal muscle and bone marrow. Subsequently placing these animals in a hyperbaric oxygen chamber did not significantly improve the oxygen pressure levels in the tumor (2). Thus, they concluded that their data implied 5-HT selectively reduced tumor blood flow.

Our measurement of tumor blood flow after 5-HT injection substantiates this conclusion.

The mechanisms for this preferential effect of 5-HT on tumor vasculature are presently unknown. However, since tumor vasculature is close to maximally dilated under normal conditions (18), its ability to autoregulate its blood flow is compromised when the arterial pressure is reduced. As a consequence, a significant reduction in arterial blood pressure after 5-HT injection would result in a disproportionate reduction in tumor blood flow relative to that in the skeletal muscle. This cannot be the mechanism for the 5-HT effect on tumor blood flow, since in both the conscious and anesthetized rats, an i.a. injection of 5-HT resulted in a significant and selective vasoconstriction in the tumor; the vascular tone in the muscle was not significantly altered. Our conclusion is consistent with that of Cater ef al. (2).

There is considerable variability in the vascular response to 5-HT (19) which has been found to be dependent upon the animal species, drug dose, vascular bed, and route of drug administration (20–24). However, it has been shown in the dog forelimb, hindlimb, kidney, and mesentery that the local injection of 5-HT increases vascular resistance when the initial resistance is low.
and vice versa (22). If these results with normal tissues can be
generalized to encompass tumor vasculature, 5-HT would be
expected to produce a proportionately greater vasoconstrictive
response in neoplastic tissue than in the surrounding normal
musculature in those tumors where the tumor vascular resist-
ance is substantially lower (e.g., SMT-2A mammary adenocar-
cinomas).

The vasculature of a locally invading malignant tumor is com-
prised of both incorporated normal vessels with their resistive
elements and newly formed embryonic vessels, which are devoid
of both smooth muscle and innervation (25). Majno et al. (26)
have suggested, based upon their electron microscopy research,
that individual endothelial cells contract when exposed to 5-HT.
This would produce a bulging into the capillary lumen, thereby
increasing the vascular resistance and decreasing tumor blood
flow. A direct effect of 5-HT on microvascular endothelium, if it
exists, may be more pronounced in the tumor vasculature be-
cause of its embryonic nature. Endothelial cell contraction may
not only increase the vascular resistance because of an intralu-
minal bulging, but it could also result in the formation of endothe-
lial gaps with ensuing vascular leakage and edema formation.
Tumors have been shown to have a higher hydraulic pressure
than that of normal tissues (27) and therefore would be suscepti-
table to edema-induced collapse of the microcirculation. Although
dema formation after 5-HT injection occurs in rat tissues, it is
not as prevalent in the dog (24). Thus, 5-HT shows species
variability with regard to edema formation, and if this is the
principal mechanism via which 5-HT reduces tumor blood flow
in rats, direct extrapolation of these results to other animal
species would not be appropriate.

Irrespective of the mechanisms for the preferential and dra-
matic reduction in tumor blood flow following 5-HT adminis-
tration, they support the idea that 5-HT may be a very useful
adjuvant to clinical hyperthermia treatment. Crile (4) demon-
strated that the effectiveness of tumor heating increased with
the dosage of 5-HT and that the local injection of 5-HT increased
tumor cell survival of heat when given prior to, rather than
after, the heat treatment. The reason for this increased thermal
damage is clarified by our results. An i.t. injection of even 1 mg
of 5-HT per kg significantly reduces the blood flow of both the
tumor and surrounding musculature, and after 45 min of heating
at 44°C (muscle temperature), the blood flow of both tissues still
had not increased. This is in contrast to the significant increase
in normal and malignant tissue blood flow during heat treatment
without 5-HT. In the hyperthermia experiments performed by
Crile, the tumors were heated for either 15 or 20 min in a water
bath controlled at 44°C; neither the normal nor the malignant
issue temperature was recorded. Tissues in a hot water bath
are heated primarily by conduction. Since the ability to heat
tissues under these experimental conditions is directly depend-
ent upon blood flow, a significant reduction in this parameter
will greatly enhance the tissue temperature. The local injection of 5-
HT prior to heating thus results in an elevation in the malignant
issue temperature to a value which is closer to the ambient
water bath temperature than occurs without 5-HT. Thus, the
cytotoxic effects of the heat treatment are enhanced.

Another interesting result of our studies was that a local
injection of 0.15 M sodium chloride alone also significantly
improved the temperature differential between the tumor and the
surrounding musculature after 45 min of microwave heating at
44°C. This does not result from a reduced tumor blood flow
secondary to an increased i.t. pressure from the locally injected
saline, since the blood flow in unheated tissues after sodium
chloride injection was identical to that in the un.injected tissues.
In a microwave field, the energy absorbed is proportional to the
issue conductivity. The total tissue conductivity is the sum of
the conductivity due to dispersive processes and the ionic con-
ductivity (28). In biological tissues, the ions in the aqueous
environment give rise to the ionic conductivity of the tissue. An
i.t. injection of a saline solution may significantly increase the
ionic conductivity of the tumor, resulting in an enhanced absorp-
tion of microwave energy and, consequently, an elevated tem-
perature. Not only does an injection of saline potentially improve
the absorption of microwave energy, it also results in the tumor
blood flow being significantly lower than that in the surrounding
muscle after 45 min of heating at 44°C. If the ionic conductivity
is an important factor in determining the ultimate temperature
differential between malignant and normal tissue, the tempera-
ture differential should be even greater after saline injection than
after an i.t. injection of 5-HT, since in the latter instance, the
blood flows were found to be equal after 45 min of heating. This
hypothesis concurs with our experimental results, since the
temperature differential after saline injection (1.1°C) is signifi-
cantly greater than the 0.7°C observed after 5-HT injection.
Thus, we suggest that, when microwaves are utilized for heating
neoplasms, i.t. injections of simple ionic solutions may be useful
in improving both the uniformity of the temperature within the
tumor and the overall temperature differential between the mali-
nant and surrounding normal tissue. This hypothesis is presently
being tested.

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5-HT AND HYPERTHERMIA TREATMENT

Effect of 5-Hydroxytryptamine on Tissue Blood Flow and Microwave Heating of Rat Tumors

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