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Hydralazine-enhanced Selective Heating of Transmissible Venereal Tumor Implants in Dogs*

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Abstract—*This study was designed to test the hypothesis that vasodilator drugs can enhance selective heating of solid tumors by producing a favorable redistribution of blood flow between tumor and normal tissues. Subcutaneous transmissible venereal tumor implants were heated by inductive diathermy using Helmholtz coils in 8 dogs. The temperature rise in tumor and adjacent muscle was measured before and after giving hydralazine* (0.5 mg/kg *i.v.*). *Blood flow to the tumors and underlying muscle was measured with radioactive tracer microspheres. Before hydralazine treatment mean muscle blood flow was about one-third tumor blood flow* (0.11 ± 0.02 *vs* 0.28 ± 0.09 ml/min/g), *and tumor and normal muscle temperatures were not significantly different* (40.0 ± 0.6 vs 39.7 ± 0.1°C). *After hydralazine tumor blood flow decreased and muscle blood flow increased in every dog, and selective heating of the tumors became possible. Muscle blood flow averaged* 0.67 ± 0.13 ml/min/g, 17 *times greater than tumor blood flow, which decreased to* 0.04 ± 0.02 ml/min/g. *Core tumor temperature was* 48.0 ± 0.9 *vs* 38.5 ± 0.5°C *for underlying muscle. Blood pressure was maintained at* 80 ± 5.7 mmHg. *These results demonstrate that adjuvant treatment with vasodilators is a promising technique to increase the temperature difference between tumors and surrounding normal tissues during local heat therapy.*

INTRODUCTION

LOCAL hyperthermia therapy for cancer involves the application of microwaves, radiofrequency current or high intensity ultrasound to warm only the tumor-bearing region while core body temperature is maintained near 37°C. Such local heat treatment is now being tested clinically at several institutions with encouraging, but not uniformly successful, results [1–7]. The goal of local heat therapy is to raise the temperature of the tumor mass to a lethal level [8–10] without damaging surrounding normal tissue. Ideally, the temperature of the entire tumor would be raised to cytotoxic levels which drop abruptly at the tumor edge to levels not damaging to normal tissues.

In practice, the tissue temperatures actually attained depend on the balance between the rate of heat deposition and the rate of heat removal from the tissues [10, 11]. The results of animal experiments [12] and computer modeling [10, 11] clearly show that blood perfusion is the primary mechanism for heat removal from tissue volumes greater than a few cubic centimeters. Hence, many authorities now believe that the relatively low blood flow found in certain tumors as compared to surrounding normal tissue explains the selective heating of tumor tissue often observed during local hyperthermia [1–3, 10, 13–15]. Presumably, such blood flow differences are the result of functional differences between tumor and normal microvasculature.

Tumor vessels are typically enlarged, elongated capillaries without smooth muscle which are maximally dilated at all times [16–18]. As a result, tumor vasculature presents a fixed resistance to blood flow. In contrast, normal arterioles in adjacent tissues maintain a high degree of autoregulatory control over local

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blood flow and are capable of responding to heat and metabolic alterations, as well as to vasoactive drugs.

We hypothesized, therefore, that this difference in the responsiveness of tumor and normal vasculature can be manipulated to produce selective temperature elevation in a tumor during local heat therapy [19, 20]. Specifically, pretreatment of a patient with a direct-acting vasodilator drug would be expected to lower vascular resistance in normal tissues, increasing perfusion in them while simultaneously shunting blood flow away from the tumor. Such effects have, in fact, been described in experimental animals for the vasodilators isoproterenol, chlorpromazine, amyl nitrate [21] and prostaglandin E_2 [22]; and in human patients for the vasodilator prostaglandin E_1 [23].

Accordingly, we performed the following study to determine the effect of the direct-acting vasodilator, hydralazine, on the distribution of blood flow and the temperature rise in tumor and adjacent normal tissue during local heat therapy. We studied the canine transmissible venereal tumor because its size and geometry approximates that of human tumors.

MATERIALS AND METHODS

Transplantation of the canine transmissible venereal tumor (TVT)

Serial transplantation was performed by subcutaneous injection of single-cell suspension prepared by trypsinization of primary tumors according to the methods of Epstein and Bennett [24] and Cohen [25]. Each dog received an inoculation ($2–4 \times 10^8$ cells) in the medial aspect of the upper hindlimb. At the time of the experiment, tumor weight ranged from 22 to 100 grams (mean, 55 g) after a growing period of 41–110 days (mean, 56 days).

Animal preparation and thermometry

Eight tumor-bearing mongrel dogs weighing 7.0–19.5 kg were used. Anesthesia was induced with thiopental sodium and maintained by methoxyfluorane, nitrous oxide and oxygen inhalation. Dogs were placed in dorsal recumbency on a V-shaped animal board. Catheters were placed in the left ventricle for injection of radioactive tracer microspheres to measure blood flow; in the right brachial artery for withdrawal of reference blood samples; in the left brachial artery for monitoring blood pressure; and in a brachial vein for drug and fluid administration.

Hollow, open-ended plastic cannulae (1 mm diameter) were inserted into the tumor center and into normal muscle underlying the tumor. These cannulae permitted repeated insertion and withdrawal of rapidly responding (0.1 sec rise time) hypodermic-style thermistors (Yellow Springs Instruments Co., Inc., Yellow Springs, OH, U.S.A.) for monitoring tissue temperature. These temperatures were recorded on a 2-channel rectilinear strip-chart recorder (Houston Instruments, Houston, TX, U.S.A.). A flexible temperature probe was placed in the esophagus, at the level of the heart, to monitor core body temperature. Arterial blood pressure and core body temperature were recorded continuously by a graphic recorder.

Microsphere technique

The use of radioactively labeled tracer microspheres to measure regional blood flow is well-established [22, 26, 27]. In this study tracer microspheres (3M Co., Minneapolis, MN, U.S.A.) with mean diameter of $15 \pm 0.9\ \mu m$ were used. Microspheres containing four different γ-emitting labels ([^{141}Ce], [^{85}Sr], [^{95}Nb] and [^{46}Sc]) were employed, allowing four blood flow measurements to be made during the experiment. The microspheres were suspended in 10% dextran. For each trial a 2.0 ml aliquot of well-mixed suspension containing approximately 10^6 microspheres was injected into the left ventricle and the catheter was flushed with 10 ml of 0.9% saline. This catheter had multiple side-holes to promote homogeneous mixing of the microsphere suspension with blood. A reference blood sample was collected from the right brachial artery into a motor-driven syringe at a constant rate of 7.3 ml/min, starting 10 sec before the injection of microspheres and continuing for a total of 120 sec. This sample provided a known blood flow into a 'reference organ' for the purpose of calibrating regional blood flow in tissue samples.

To determine tissue perfusion at the end of each experiment, the heated tumor was excised, weighed and placed in plastic vials for determining radioactivity. Several samples of underlying muscle tissue were also taken. Measurement of the radioactivity, in counts per minute (counts/min), due to each of the four radionuclides in each tissue sample and in the four reference blood samples was performed by a Beckman 8000 γ-spectrophotometer. The blood flow in each tissue sample was calculated according to the following proportion:

$$\frac{\text{Tissue flow (ml/min)}}{\text{Tissue activity (counts/min)}} = \frac{\text{Reference organ flow (ml/min)}}{\text{Reference organ activity (counts/min)}}.$$

The blood flow values determined for each sample were divided by the weight of that sample to provide a value for tissue perfusion expressed in ml/min/g. Total flow (cardiac output) was also determined by the microsphere technique using the relationship:

$$\frac{\text{Total flow (ml/min)}}{\text{Total activity injected (counts/min)}} = \frac{\text{Reference organ flow (ml/min)}}{\text{Reference organ activity (counts/min)}}.$$

Experimental design

We selected the vasodilator, hydralazine, for this study because (1) it is direct-acting, having selective action on arterioles; (2) it has a relatively long duration of action; and (3) it is clinically safe and widely used in human medicine [28, 29]. To study the effect of hydralazine on the distribution of blood flow between normal and tumor tissue and to relate the flow distribution to heating, blood flow and temperature of tumor and underlying normal tissue were measured under four different conditions. First, [^{141}Ce]-labeled microspheres were injected (Trial 1) to measure the control level of blood flow after blood pressure, core temperature and tissue temperature had stabilized following surgery (about 30 min).

The tumor-bearing region of one hindlimb was then heated by passing 13.56 MHz radiofrequency current, generated by a Birtcher diathermy unit (Birtcher Corporation; El Monte, California, USA), through a set of Helmholtz coils which encompassed the region. The arrangement of the coils and tumor-bearing limb is depicted in Fig. 1. The coils were positioned such that the sites of temperature measurement in tumor and normal tissue were exposed to approximately the same field strength. To avoid interactions between the thermistors and the induced magnetic field we employed a pulsed heating technique. Several heating episodes lasting 1–5 min each were used to bring the temperature of the normal tissue to a steady-state target level of approximately 40°C. Tissue temperatures were only monitored during the short period (approximately 1 min) between heating episodes when the radiofrequency current was turned off. The thermistors were removed from the cannulae during radiofrequency heating to prevent current concentration near the tip of such a conductive implant, which may have caused tissue damage as well as self-heating of, and possible damage to, the thermistors [30]. [^{85}Sr]-labeled microspheres were injected (Trial 2) when the target temperature had been maintained for at least 10 min to determine the effect of heating alone on the distribution of perfusion.

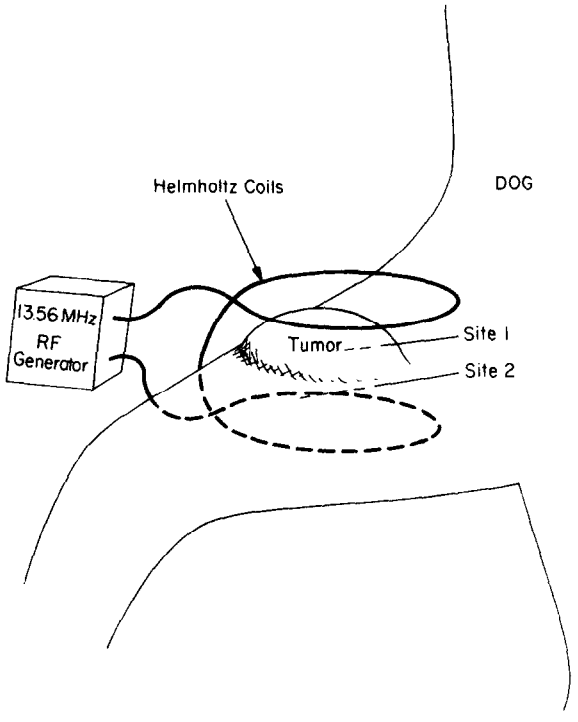

Fig. 1. Schematic diagram depicting the orientation of the Helmholtz coils with respect to the tumor-bearing limb. Sites 1 and 2 indicate the positions of cannulae in the tumor and underlying muscle for insertion of hypodermic-style thermistors.

As the temperatures of the tumor and surrounding normal tissue returned to baseline values, hydralazine was injected intravenously (0.5 mg per kg body weight). Arterial blood pressure was monitored to determine the onset of drug effect (tachycardia and increased pulse pressure). When the effect of the drug on pulsatile blood pressure had stabilized (approximately 15 min), [^{95}Nb]-labeled microspheres were injected (Trial 3) to determine the change in distribution of perfusion due to hydralazine alone.

Finally, the tumor-bearing region was heated as before in the presence of hydralazine effect. The position of the Helmholtz coils and the sites of temperature measurement remained unchanged. A steady-state temperature of approximately 40°C for the normal tissue again served as a target. After the target temperature had been maintained for at least 10 min, [^{46}Sc]-labeled microspheres were injected (Trial 4).

At the end of the experiment, dogs were

killed by an injection of saturated KCl (approximately 2 ml) into the left ventricle and tissue samples were excised for weighing and determination of radioactivity. The Mann–Whitney statistics were calculated for the blood flow and temperature data for each trial to test the null hypotheses that the blood flow rates and temperatures of tumor and underlying muscle were the same.

RESULTS

Redistribution of blood flow

Figure 2 presents the mean blood flow data for tumor and underlying muscle samples obtained under the four test conditions previously described. Under normothermic control conditions (Trial 1) the TVT had a significantly greater level of perfusion than the adjacent muscle ($u = 8.0$; $P \leq 0.01$). After heating alone (Trial 2), tumor and muscle blood flows were not significantly different. The addition of hydralazine under normothermic conditions (Trial 3) greatly increased tissue perfusion in the muscle samples to a level 5.4 times the control value. At the same time perfusion of the tumor tissue dropped to about one-half of the control value. These changes were slightly enhanced by heating. The final observations, after heating (Trial 4), revealed that perfusion of the normal tissue, rather than being about one-third that of the tumor tissue (control), had increased to 17 times that of the tumor ($u = 1.0$; $P \leq 0.01$).

Temperature data

The complete temperature–time curves for a typical experiment are shown in Fig. 3. Temperatures attained after local heating for tumor and underlying muscle in all 8 experiments are presented in Table 1. When the temperature of the normal tissue was raised to a steady-state target level of approximately 40°C before giving hydralazine, the temperature measured in the tumor was never higher than 42.5°C and never more than 2.5°C higher than normal tissue temperature. In 4 of the 8 dogs it was impossible to raise the temperature of the tumor to a level higher than the normal tissue before hydralazine treatment. When, after the injection of hydralazine, the temperature of the normal tissue was again raised to a target level of about 40°C, tumor temperature rose as high as 51.6°C. Although the positions of the Helmholtz coils remained the same, temperature differences between tumor and normal tissue averaging 9.5°C (range, 4.5–12.6°C) were obtained after hydralazine (Table 1). In every animal, hydralazine treatment permitted dramatic selective heating of the tumor.

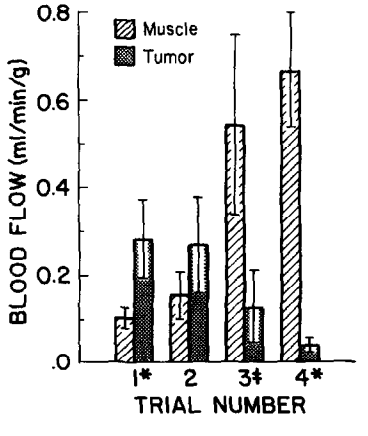

*Fig. 2. Mean blood flow data for tumor and underlying normal muscle obtained during four different test conditions: normothermic, no drug (Trial 1); hyperthermic, no drug (Trial 2); normothermic, hydralazine (Trial 3); and hyperthermic, hydralazine (Trial 4). *Significant at $P \leq 0.01$; ‡significant at $P \leq 0.05$.*

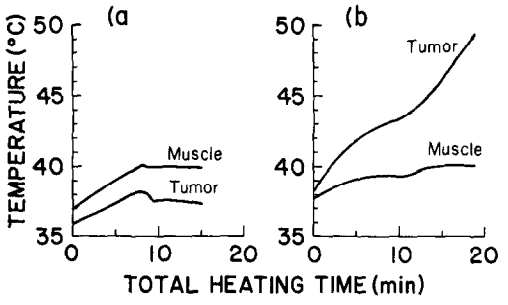

Fig. 3. (A) Intratumor and underlying normal muscle temperatures attained without hydralazine (Trial 2). (B) Intratumor and underlying normal muscle temperatures attained after hydralazine treatment (Trial 4).

Hemodynamic data

Table 2 presents cardiac output, blood pressure and total peripheral resistance values

Table 1. Final temperatures (°C) attained after local heating before and after hydralazine treatment

Experiment No.	Before hydralazine (Trial 2) Normal muscle	Tumor	After hydralazine (Trial 4) Normal muscle	Tumor
1	39.6	39.0	39.5	45.0
2	40.0	37.6	40.0	49.2
3	39.4	40.2	38.9	49.2
4	39.3	41.0	37.6	49.4
5	39.9	41.7	40.0	51.6
6	39.9	39.6	38.5	45.0
7	39.5	38.4	36.5	44.8
8	40.0	42.5	36.9	49.5
Mean	39.7	40.0	38.5	48.0*
±S.E.	0.1	0.6	0.5	0.9

*$P \leq 0.01$.

Table 2. Hemodynamic data (mean ± S.E.)

Trial No.	Conditions	Mean arterial pressure (mmHg)	Cardiac output (l/min)	Total peripheral resistance (mmHg/l/min)
1	Normothermia, no drug	113.1 ± 4.4	4.75 ± 1.0	32.0 ± 6.5
2	Hyperthermia, no drug	111.3 ± 4.1	3.52 ± 0.6	41.4 ± 8.4
3	Normothermia, hydralazine	87.5 ± 8.0	6.15 ± 1.4	18.8 ± 3.5
4	Hyperthermia, hydralazine	80.6 ± 5.7	5.93 ± 1.1	17.1 ± 3.3

obtained at the times of microsphere injection. At the doses of hydralazine employed, total peripheral resistance dropped to approximately one-half the control value, mean blood pressure was significantly less than control, but cardiac output increased by about 50%. Although mean pressure decreased, perfusion was not compromised after treatment with hydralazine.

DISCUSSION

Pretreatment of TVT-bearing dogs with the direct-acting vasodilator, hydralazine, produced a highly favorable redistribution of blood flow between normal and tumor tissue, making possible selective heating of the tumors to therapeutic temperatures. Although the present protocol is limited in that only one tumor model was studied, this particular model provided a rigorous test of the specific therapeutic effect of hydralazine. Prior to hydralazine treatment normal muscle blood flow was no greater, and usually much less, than tumor blood flow, making selective tumor heating impossible. After hydralazine treatment normal muscle blood flow averaged 17 times that in the tumor and an average temperature difference of 9.5°C was observed.

Recently, we noted that Dickson and Calderwood's [8] compendium of single-treatment thermal death times for various human and animal tumors could be summarized using the simple expression,

$$t = \left[\frac{2.5}{T-42}\right]^2,$$

where T = tumor temperature (°C) and t = required exposure time for tumor necrosis (hr) [10]. According to this relationship, for the intratumor temperatures achieved in this study irreversible damage could be expected in as little as 4.1 min (at 51.6°C) to 42 min (at 45°C). At the same time, the increased perfusion of surrounding normal tissue produced by hydralazine kept normal tissue temperature well below 42°C.

Selective tumor heating of the magnitude achieved in this study has rarely been described. However, when comparable heating is achieved clinically the results are encouraging. Storm reported that in more than three-quarters of the human tumors in which temperatures of 42–50°C were achieved by radiofrequency heating, minimal normal tissue injury was sustained while moderate to marked necrosis occurred in successfully heated tumors [1]. LeVeen *et al.* showed that radiofrequency therapy producing a mean intratumor temperature of 48.4°C in seven patients consistently caused necrosis of cancerous tissue with little destruction of normal tissue [3]. It is likely, therefore, that treatment with a vasodilator drug prior to local hyperthermia therapy will increase the number of tumors which can be selectively heated while decreasing the incidence of normal tissue damage.

The therapeutic effect of hydralazine observed in this study was obtained at doses which did not depress blood pressure unacceptably. Although the drug decreased peripheral resistance by about one-half, blood pressure was maintained at reasonable levels by the reactive increase in cardiac output (Table 2). These effects are expected for a direct-acting vasodilator and appear to be within clinically reasonable limits.

Ideally, cancer therapy should destroy neoplastic cells without injuring nearby normal cells, just as modern antibiotics destroy bacteria without damaging the host. Un-

fortunately, the chemical differences between cancer cells and normal cells are relatively subtle. However, we believe there are crucial anatomic and functional differences between tumor blood vessels and normal blood vessels which make the whole tumor, as opposed to individual tumor cells, selectively vulnerable to local hyperthermia. The results of this study show that it is possible to treat a tumor as a specific 'organ' which responds to a drug in a qualitatively different manner than normal organs. For the TVT system, the difference in response to hydralazine was unequivocal: blood flow to normal tissue increased; blood flow to tumor tissue decreased. This functional difference is rationally related to well-reported anatomic differences between blood vessels in tumor and normal tissues; specifically, the lack of smooth muscle in the walls of tumor vessels [16, 17, 18]. This selective response was easily exploited to produce selective heating of the tumor. As local hyperthermia techniques improve, adjuvant treatment with vasodilators is likely to increase the intratumor temperatures which can be safely attained and decrease the severity of thermal damage to surrounding normal tissue.

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