Mechanisms of Synergism between Arteriolar Embolization and Hyperthermia in a Rabbit V-2 Model of Solitary Hepatic Metastasis


ABSTRACT

In a V-2 model of solitary hepatic metastasis, residual tumor was histologically identified in the treatment field in only three of 14 (21%) animals subjected to microsphere embolization of tumor arterioles plus focal (43°C, 40 min) hyperthermia compared with seven of ten (70%) subjected to focal (43°C, 40 min) hyperthermia alone (P ≤ 0.05), five of seven (71%) (P ≤ 0.05) treated by occlusion plus shams heating, and five of five (100%) (P ≤ 0.01) sham-treated controls. Prior occlusion tended to reduce the radiofrequency power required for heat up and steady-state temperature maintenance of tumors (P ≤ 0.09 and P ≤ 0.06, respectively) and reduced the cooling rate after heating compared to unoccluded tumors (P ≤ 0.02) but did not affect mean time to temperature, maximum and minimum temperatures measured at the tumor-normal tissue interface, and animal core temperature compared with that of the hyperthermia alone treatment group. In ten other animals with hepatic V-2 tumors of comparable size subjected to focal hyperthermia plus or minus arteriolar embolization, temperatures were continuously monitored at four additional intratumor sites in a fixed geometric orientation around the heating probe. No significant differences were noted in maximum and minimum temperatures in comparably oriented probes over a 40-min heating period between the hyperthermia and the occlusion-hyperthermia treatment groups. In five other animals with solitary V-2 hepatic implants, comparable microembolization plus or minus focal tumor heating to 43°C, 40 min, did not significantly reduce tumor interstitial pH compared with pretreatment values. This study reproduces previously observed synergism between arteriolar embolization and hyperthermia but suggests the mechanism may be unrelated to observable differences in intratumor pH and thermal profile and may result from other mechanisms, perhaps by mimicking the angioocclusive effects of hyperthermia itself.

INTRODUCTION

In theory, vascular occlusion should lower intratumor pH and reduce convective heat loss in tumors. These effects suggest potential for synergism between occlusion and hyperthermia (1). In fact, several investigators have recently demonstrated synergistic effects between tourniquet occlusion and regional heating of tumors in animal hindlimb models (2-4).

In a previous attempt to develop a model more applicable to human cancer therapy, percutaneous catheterization and selective embolization of tumor arterioles with 20- to 80-μm Dextran-M microspheres were substituted for simple tourniquet occlusion and combined with highly selective heating of tumors produced by a hybrid radiofrequency device developed in our laboratory (5). This study suggested that the combination of arteriolar occlusion and focal hyperthermia was also synergistic (5). The mechanism of synergism, however, was unclear. In this study design, we elected to regulate one parameter of arterial occlusion, whole tumor blood flow, and two parameters of hyperthermic treatment, minimum intratumor temperature and total heating time (5). Vagaries in arterial blood supply of hindlimb tumors led to regional differences in intratumor arteriolar embolization prior to heating with resultant differences in rate of temperature rise in different regions of the tumor. This produced observed differences in intratumor maximal temperatures, temperature gradients, and heating rates between occluded and unoccluded tumors (5). The significance of these various factors in determining the outcome of hyperthermic therapy is disputed. A number of investigators have reported that minimum intratumor temperature and total heating time, rather than maximum intratumor temperature and intratumor temperature gradient, are the principal determinants of the adequacy of hyperthermic treatment (6, 7). Other investigators, however, have reported correlations between response to hyperthermia and maximum intratumor temperature and thermal gradient (8).

In the present study we hoped to further reduce variability in thermal parameters between hyperthermia and occlusion-hyperthermia treatment groups by controlling the minimum intratumor temperature, time to temperature, and heating time and by minimizing thermal gradients between opposite edges of the tumor. Standardization of these thermal variables should further clarify mechanisms underlying synergism between arteriolar occlusion and hyperthermia. The model chosen was a rabbit V-2 tumor model of solitary hepatic metastasis, since it was felt that in this site the possibility of asymmetric embolization of tumor feeder arteries would be less although the potential for collaterals from the portal vein was recognized (9). In addition, we planned to study the effects of arteriolar embolization plus or minus hyperthermia on tumor interstitial pH as another potential mechanism for synergism between occlusion and hyperthermia (10).

MATERIALS AND METHODS

V-2 Tumor. The V-2 tumor originated from a transformed skin papilloma induced by the Shope papilloma virus in a male Dutch belted rabbit (11) and has been adapted to grow in other rabbit strains as well. The tumors generally do not grow well in tissue culture and are maintained by serial animal passage (11). Compared with many normal tissues V-2 tumors are hypervascular (11), and they are among the more heat-resistant animal tumors (12). Two variants, the London strain and the Oxford strain, have been characterized with respect to their heat sensitivity. In the London strain a 50% cure rate is achieved with thermal doses in the range of 43°C for 180 to 240 min. In the Oxford strain, a 75% cure rate is achieved after 47°C for 30 min (equivalent to 43°C for 480 min) (12). The tumor utilized in these experiments was obtained from the laboratory of Dr. Arthur E. Bogden of the Breast Cancer Task force and since that time has been maintained through serial animal passage in New Zealand white rabbits and from frozen stock. Because variation in growth characteristics can occur in initial animal passages after being brought up from frozen stock, all tumors utilized in these studies were carried through four serial animal
passages prior to use in occlusion-hyperthermia experiments. Characteristically, tumors thus adapted grow rapidly at the inoculation site and metastasize at about 2 to 3 wk principally to the lungs and regional lymph nodes (11, 13). Untreated animals typically die of pulmonary metastases 6 to 8 wk after tumor inoculation (11, 13).

Tumor Inoculation. Male New Zealand white rabbits weighing ≤30 kg were utilized for these studies. Two wk prior to treatment, sedated (Nembutal, 25 mg/kg), locally anesthetized (Xylocaine) animals were explored through a small midline laparotomy incision using sterile technique. The left hepatic lobe was mobilized into the wound, and 0.2 ml of minced tumor fragments were injected just above the inferior hepatic margin. During injection considerable care was taken to avoid either injection into hepatic vessels or wide infiltration into hepatic parenchyma, which produces multicentric intralobar V-2 implants rather than solitary tumors. Initial tumor volume was determined at subsequent laparotomy according to the formula

$$V = \frac{ab^2}{6}$$

where a is the long and b the short diameter (5).

Selective Peripheral Occlusion. Selective occlusion of tumor vessels was accomplished in a manner similar to that of Adams et al. (14) and as described in previous publications from our laboratory (15). Two wk after inoculation of tumors in the left hepatic lobe, rabbits were again sedated with Nembutal (25 mg/kg) administered through an ear vein. A butterfly catheter was left in place during subsequent manipulations in case further sedation was necessary. For invasive procedures, sedation was supplemented by local infiltration with Xylocaine. A 3 French polyethylene catheter (Cook Inc., Bloomington, IN) molded into a "J" configuration was inserted into the femoral artery by a cutdown technique and advanced into the celiac axis under fluoroscopic guidance. Celiac arteriograms at ×2 magnification are made in the anterior-posterior projection by injecting 1 ml of Renographin-76 per sec for 3 s and by taking one film per sec for 10 s, the first film without delay. In this way the major blood supply of the hepatic V-2 tumor was identified. The catheter was then advanced into the proper hepatic artery, and by taking one film per sec for 3 s, the length of the exposed tip and is <3.0 cm (17, 18). Rate of heating in the vicinity of the probe shows a linear relationship with current flow through the probe as measured with a directional coupler (18). For production of larger hot spots, a combination of a single invasive antenna, external antennas, and the other elements of the system is utilized (18). Subsequent studies have demonstrated that many of these patterns can also be produced in dielectrically complex animal phantoms (19) and in living animals in both superficial and deep locations (13, 19).

To improve the tuning characteristics of this multielement system we recently interfaced the system with a Hewlett Packard 3577A Network Analyzer and a Hewlett Packard 35677A S-parameter test set (Hewlett Packard, Inc., Palo Alto, CA) using precision couplers and field detectors. This allows better characterization and optimization of field characteristics and of power transfer between the system elements over a frequency spectrum. This has markedly reduced incident radio-frequency power levels needed to produce heating and improved control of heating in tumors. During heating net incident radiofrequency power applied to the rings and capacitor plates was recorded at regular intervals.

Production of Focal Hot Spots in the Liver. Utilizing the system described above, focal hot spots were produced in V-2 tumors growing in the left hepatic lobe as follows. Sedated, locally anesthetized rabbits which had been inoculated with tumors 2 wk previously were then positioned in the supine position on a small plastic operating room table, and the abdomen was opened through a midline incision. If the left hepatic lobe containing the tumor lay directly on the stomach a small dry gauze pack was wedged between the two organs to minimize conductive heat transfer from the heated area of the liver to the stomach. The left lobe containing the tumor was exposed, and the invasive heating probe with exposed tip length equal to one-half the long diameter of the tumor was then inserted into the tumor midplane along the long axis. Two nonfield-perturbing Vitek temperature probes were then inserted into the periphery of the tumor on either side of the tunable antenna probe. Generally the perpendicular distance between the heating probe and the temperature probes was on the order of 1 to 1.5 cm which was roughly one-half the long tumor diameter. Based on heating patterns obtained in prior phantom and animal studies (13, 17) the temperature in probes positioned in this manner should reflect minimum intratumor temperatures in the approximate position of the tumor-normal liver interface. To confirm this premise after acquisition of the CliniTherm Sentry 1200 thermometry system, four additional intratumor temperature points were monitored during radiofrequency heating at fixed distances of 0.5 and 1.0 cm on both sides of the tuned probe antenna in ten other animals subjected to focal hyperthermia plus or minus arteriolar occlusion. The geometric relationship between the temperature probes and heating probe was maintained with an external plastic jig through which the probes were inserted. After positioning the heating probes and temperature probes, the animal was placed...
between the rings and capacitor plates of the radiofrequency heating device, and net radiofrequency power in the range of 10 to 60 W was applied. Power was adjusted to bring tumors to minimum temperatures of 43°C at both of the outermost temperature probes in approximately 15 min and to minimize intratumor temperature gradients. The system was tuned to maximize current flowing through the invasive probe. Temperatures in the nonfield-perturbing Vitek or CliniTherm probes were recorded at 1-min intervals during radiofrequency heating. When minimum target temperatures were reached in the two outermost temperatures probes, timed heating was begun and continued for 40 min. At the conclusion of heating, a Yellow Springs Instrument probe was inserted into the rectum to measure core temperature. Hepatic temperature probes were left in place after radiofrequency power was turned off, and initial cooling curves were obtained for 1 to 2 min, after which the temperature probes, heating probe, and sterile pack were removed, and the abdominal incision was closed with 3-0 catgut.

Experimental Design. To assess potential synergism between selective occlusion and hyperthermia several control groups were included. In 14 animals tumors were both selectively occluded and focally heated to minimum temperatures of 43°C for 40 min (occlusion-hyperthermia). In five animals tumors were sham heated (probes were inserted into tumors, and animals were placed within the radiofrequency applicator for 40 min with no power applied). In seven animals tumors were selectively occluded and sham heated (occlusion alone). Finally, in ten animals tumors were focally heated to minimum temperatures of 43°C for 40 min without prior occlusion (hyperthermia alone). Animals were sacrificed by Nembutal overdose at 28 days posttreatment and necropsied, and hematoxylin-eosin slides were prepared of the primary tumor site, any sites of metastatic disease, and other relevant tissues. Histological sections were analyzed by a veterinary pathologist in a single blinded fashion who had no knowledge of the treatment group; then individual readings were grouped by mode of treatment for analysis of trends. Data were analyzed by Fisher's exact test and by the unpaired t test.

Intratumor Temperature Gradient Studies. To determine if occlusion affected intratumor thermal gradients at sites other than the tumor-normal tissue interface, four additional intratumor sites at fixed distances of 0.5 and 1.0 cm on both sides of the tuned probe antenna were monitored during radiofrequency heating in ten animals with hepatic V-2 tumors of comparable size subjected to focal hyperthermia plus or minus arteriolar occlusion. Maximum and minimum temperatures recorded in probes at each probe position over a 40-min timed heating interval were then compared between occluded and nonoccluded tumors using the Student t test.

pH Effects of Arteriolar Embolization. To determine effects of arteriolar embolization plus or minus hyperthermia on tumor interstitial pH, needle pH electrodes (Microelectrodes, Inc., Londonderry, NH) connected to a Corning Model 10 pH meter (distributed by Scientific Products Division of American Hospital Supply) were utilized. These pH electrodes have an outer diameter of 0.8 mm, a range of 1 to 13, and an equilibration time of approximately 2 min. The electrodes are sheathed in a hypodermic needle to facilitate insertion. The instrument was calibrated using reference buffers at pH 7.0 and pH 10.0 before and after use in animals. In three sedated, locally anesthetized rabbits bearing hepatic V-2 implants of three different sizes (0.8 cm³, 2.3 cm³, and 24 cm³) a laparotomy incision was made, the reference electrode was placed in contact with the peritoneum, and using the needle pH probe, multiple points were sampled throughout the tumor volume to determine possible positional effects on tumor interstitial pH. In five sedated, anesthetized animals bearing hepatic V-2 implants of similar size to those utilized in occlusion-hyperthermia experiments, a laparotomy incision was made, the reference electrode was placed in direct contact with the peritoneum, and the interstitial probe was inserted into several locations throughout the tumor to sample pH variation as a function of probe position. Next the probe was positioned in a location that gave a representative intratumor pH near the periphery of the hepatic V-2 tumor. Arteriolar occlusion was performed as previously described, and pH measurements were recorded at regular intervals for up to 30 min.

In four of these five animals, pH probes were removed, and focal (43°C, 40 min) radiofrequency heating was performed; pH probes were then reinserted, and intratumor pH measurements were obtained in four separate positions throughout the tumor periphery.

RESULTS

Initial tumor volumes (4.0 ± 3.0 ml (occlusion-hyperthermia), 3.5 ± 3.0 ml (hyperthermia alone), 6.0 ± 2.7 ml (occlusion alone), and 6.1 ± 4.9 ml (sham treatment)) did not differ significantly in various treated and control groups. Because tumors were not accessible to palpation, serial tumor volumes could not be measured.

Gross findings at necropsy at 28 days posttreatment proved an unreliable guide to local tumor persistence, since in some instances considerable fibrosis (which was hard to distinguish grossly from tumor) was present in the treated area. This is illustrated by the gross appearance (Fig. 1) of the left hepatic lobe in two animals in which tumors were treated by occlusion-hyperthermia; one lobe shows only capsular fibrosis, and the other is diffusely fibrotic, yet both were histologically negative for tumor (Fig. 1). Distant metastases were observed in 7 of 14 (occlusion-hyperthermia) animals, 10 of 10 (hyperthermia alone) (P < 0.01 by Fisher's exact test), 6 of 7 (occlusion alone) (not significant), and 5 of 5 sham (not significant)-treated groups.

In the treated area, histological evidence of residual tumor was observed infrequently in the occlusion-hyperthermia treatment group, 3 of 14, compared with hyperthermia alone, 7 of 10 (P < 0.05, Fisher's exact test), occlusion alone, 5 of 7 (P < 0.05), and sham treatment, 5 of 5 (P < 0.01) groups.

Because these data suggested synergism between arteriolar occlusion and focal hyperthermia in the treatment region compared to other control groups, we went on to analyze various factors such as percentage of flow reduction, heat up time, total heating time, intratumor temperature distribution, and intratumor pH before and after occlusion which might be responsible for this synergism.

Flow Reduction after Occlusion. Visually estimated flow reduction did not differ significantly between occlusion alone (78 ± 18%) and occlusion hyperthermia treatment groups (83 ± 14%).

Radiofrequency Power Requirements in Occluded versus Unoccluded Tumors. To determine whether arteriolar occlusion reduced convective heat loss sufficiently to reduce radiofrequency power requirements during tumor heating, we calculated (a) mean maximum (net) radiofrequency power applied to the rings and capacitor plates during heat up to 43°C and (b) mean (net) steady-state power required for temperature maintenance. Maximum net radiofrequency power during heat up averaged 38 ± 22 W in (hyperthermia alone) versus 27 ± 16 W in (occlusion-hyperthermia)-treated groups (P < 0.09). Net steady-state power averaged 30 ± 18 W (hyperthermia alone) versus 21 ± 11 W (occlusion-hyperthermia) (P < 0.06) which suggests reduction in power requirements for comparable heating of occluded tumors.

Reduced convective heat loss should reduce the rate of cooling in occluded compared with unoccluded tumors. Initial cooling rates for occlusion-hyperthermia-treated tumors were significantly lower than hyperthermia alone-treated tumors (0.5 ± 0.4°C/min versus 0.8 ± 0.5°C/min) (P < 0.02).

Thermal Profiles of Occlusion-Hyperthermia and Hyperthermia-treated Tumors. Thermal characteristics that have been reported to affect hyperthermic treatment of cancer are: heat up time; total heating time; maximum and minimum intratumor temperature; and intratumor thermal gradient (6-8, 20, 21).
Fig. 1. A, appearance of a V-2 tumor implant in the left lobe of a rabbit liver after occlusion and immediately prior to 43°C hyperthermia for 40 min. B, same animal at necropsy 4 wk later. No gross tumor is apparent, but shrinkage of the involved region and capsular fibrosis are apparent. In C, in other animals, liver tumors treated by occlusion-hyperthermia appeared densely fibrotic; however, in both B and C there was no histologically identifiable tumor. D, microscopic section (H & E, ×25) of area of liver seen in B 4 wk after occlusion-hyperthermia treatment of the V-2 tumor seen in A and B. No residual tumor is histologically apparent, but perportal fibrosis and increased prominence of interlobular septae are evident. E, microscopic section (H & E, ×100) of same area seen grossly in C 4 wk after occlusion-hyperthermia of a solitary V-2 tumor. Well-preserved liver is seen on the left side separated from an area of necrotic tissue in which no viable tumor is observed.
Heat up time, defined as time to reach minimum intratumor temperatures of 43°C in both probes at the tumor periphery, was measured and did not differ significantly for occlusion-hyperthermia (13 ± 10 min) and hyperthermia alone (14 ± 6 min) treatment groups, although as previously noted radiofrequency power requirements were higher in unoccluded tumors.

Similarly, maximum recorded intratumor temperature in either of two probes at the tumor periphery during the 40-min heating period [48.1 ± 1.5°C (hyperthermia alone) versus 47.3 ± 2.8°C (occlusion-hyperthermia)], concurrent minimum temperature on the second probe [44.5 ± 1.6°C (hyperthermia alone) versus 43.0 ± 1.6°C (occlusion-hyperthermia)], temperature differential (Tmax - Tmin) [3.3 ± 2.5 (hyperthermia alone) and 4.7 ± 2.6 (occlusion-hyperthermia)], and core temperature [36.6 ± 0.7 (occlusion-hyperthermia) versus 37.4 ± 0.6 (hyperthermia alone)] did not differ significantly between the two groups.

In ten animals additional intratumor temperature points were plotted during a timed 40-min radiofrequency heating interval at four points at fixed distances of 0.5 and 1.0 cm on both sides of the tuned probe antenna. In occluded tumors, mean temperature minima/maxima of 48.1 ± 3.7/50.2 ± 3.3 and 46.0 ± 0.73/49.3 ± 4.6°C were recorded on the innermost (0.5 cm) probes and of 43.3 ± 0.62/44.2 ± 0.51 and 45.3 ± 3.3/46.7 ± 3.4°C on the outermost (1.0 cm) probes. In unoccluded tumors corresponding values were 46.5 ± 2.6/50.7 ± 2.7 and 47.6 ± 2.8/50.0 ± 4.0 (innermost probes) and 43.3 ± 0.8/44.3 ± 1.5 and 43.8 ± 1.3/46.5 ± 4.9 (outermost probes). These data confirm a relatively symmetrical intratumor thermal gradient which is maximal around the tuned probe antenna and demonstrate that there is no significant difference in mean maximal or minimal temperature at comparable measurement points in occluded versus unoccluded heated tumors.

pH Variation in Hepatic V-2 Implants and Effect of Arteriolar Occlusion plus or minus Hyperthermia. In three rabbits bearing V-2 hepatic implants from 0.8- to 24-cm³ size, mean interstitial pH varied relatively little throughout the tumor (7.17 ± 0.25, 7.17 ± 0.14, 7.19 ± 0.15).

Because hepatic metastases may have a dual (portal venous-hepatic artery) blood supply (2) and may develop collateral circulation after hepatic artery occlusion (9), we serially measured inter- and intratumor pH variance in untreated tumors in the other report, a larger fall in pH (0.5 ± 0.2 units) was observed in small C3H mouse mammary adenocarcinomas with a mean pretreatment pH of 6.75; however, in larger necrotic tumors of the same strain with a mean pretreatment pH of 7.21, significant pH changes were not observed posthyperthermia (21). The tumors in the present study were relatively large at the time of therapy (≥3.5 ml) with a mean pH of 7.17, and partial necrosis is commonly observed histologically in these tumors (5). These findings suggest that larger necrotic tumors may be less prone to pH changes after occlusion plus or minus hyperthermia than smaller tumors.

The lack of significant changes in pH and intratumor temperature profile with arteriolar occlusion plus or minus hyperthermia suggests that other mechanisms may be responsible for the synergism observed in this model. It has been demonstrated by other investigators that hyperthermia itself, particularly at temperatures in the 45–50°C range, produces vascular occlusion (22–24). Some investigators have suggested that these occlusive effects are an integral part of the effect of heat on tumors (23, 24). Demonstration of synergism between arteriolar occlusion and hyperthermia which is relatively independent of changes in pH and thermal profile adds additional weight to this speculation.

These studies further suggest that the occlusive properties of other types of embolic material be studied for potential synergism with hyperthermia and that pharmacological manipulation to reduce pH may further enhance synergism between arteriolar occlusion and hyperthermia. These are the subjects of ongoing investigations in our laboratory.

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ARTERIOlar OCCLUSION HYPERThERMIA

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