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Electrochemical Modification of Tumor Growth in Mice[1]

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We evaluated the effect of direct electrical current on large tumors in mice. Lewis lung carcinoma greater than 1 cm in the shortest dimension was treated percutaneously with 20 mA for 15 mm. Separate groups were given one or more than one (two or three) percutaneous electrical treatments (PET). A third group was given sham electrical treatment, and a fourth group had surgical excision of the tumor. Animals in both PET groups survived longer and had smaller primary tumors at death compared with the sham group. PET did not alter the systemic course of the disease, judged by lung and spleen weights and by histological observation of the extent of metastatic burden in the lung. Surgery resulted in long-term survival of 17%, and an increase in average survival time compared with both PET and sham treatment. PET produced rapid and polarity-dependent alterations in physiological solutions *in vitro*, and it is likely that similar electrochemical processes mediated the observed reduction in tumor growth. PET is potentially useful as an adjuvant modality because it reduces local tumor mass but does not alter the extent of metastasis.

INTRODUCTION

Chemotherapeutic agents and immunomodulators are effective in the Lewis lung carcinoma model as judged by several endpoints including retardation of the rate of tumor growth. For example, cvclophosphamide on Days 12 and 18 following tumor implantation [1] and daily treatment with levan beginning on Day 1 [2] each resulted in reduced tumor size. Combined cyclophosphamide and hyperthermia produced greater inhibition compared with the effects produced when they were administered separately [3]. A similar additive effect was seen with *P. granulosum* KP-45 and hyperthermia [4].

Although the effects of drugs and hyperthermia can he additive in animal tumor models, the combined techniques have thus far produced only marginal clinical benefits. One factor limiting the efficacy of' hyperthermia is the inherent difficulty in producing a localized and focused effect, particularly when the tumor lies deep to the skin as in the lung or brain. Electrochemical tissue destruction is another physical process potentially capable of joint use with drugs. Well-localized tissue destruction can be achieved by the passage of direct current (DC) through the target tissue, and percutaneous electrical treatment (PET) of tumors has been studied in animal models [5-8] and patients [9].

Because the animal studies involved relatively small tumors, the effect of PET on large tumors (1 cm in diameter) remains unknown. The use of PET in patients seems promising [9], but several important questions have not been considered including those related to dose, polarity, mechanism of cell death, and impact on the metastatic character of the tumor. Our previous work [7] has been extended to consider these questions, and we report here our results using large tumors.

METHODS AND MATERIALS

Female C57 black mice (,Jackson Laboratory, Bar Harbor, ME) were used in all experiments. Lewis lung carcinoma (LLC) (Animal and Human Tumor Bank, Worcester, MA) was maintained in the mice by serial subcutaneous transfer. LLC is a rapidly growing carcinoma that metastasizes to the lung within a few days of implantation and typically results in death of the animal in fewer than 20 days. Under general anesthesia (intraperitoneal Rompun (8 mg/kg) and ketamine (40 mg/ kg), a 5-mm skin incision was made over the lumbar spine, and a cannula was passed under the skin. About 25 mg of tumor obtained from a donor that had been implanted 12-14 days previously was passed through the cannula and deposited subcutaneously at the base of the neck. Removal of the tumor from the donor and implantation of the host required no more than 60 mm, during which time the tumor was immersed in Hank's solution. The skin incision was closed with one or two interrupted sutures (6-0 vicryl). All mice were housed individually and fed and watered *ad libitum*; individual food and water consumption were not measured, but total intake was similar in all groups.

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Growth of the tumor was monitored by daily caliper measurements, and the DC was administered when the shortest tumor dimension was greater than 1 cm (10-13 days following implant). Under general anesthesia, two platinum electrodes (0.3 mm in diameter, 10 mm long) (E2, Grass Instruments, Quincy, MA) were passed parallel to one another (6 mm apart) into the tumor in a frontal plane at right angles to the longest axis of the tumor. The current was supplied by a constant-current source (Model 224, Keithley, Cleveland, OH), and both the current and voltage were monitored continuously throughout the treatment with autoranging multimeters (Model 175, Keithley).

Optimal treatment conditions were defined in an initial series of studies. Separate groups of mice were exposed to 5 mA (for 5 or 30 mm) or 20 mA (for 5, 10, 15, or 30 mm). The mice were killed 2-3 days after exposure and the tumor was bisected in the plane of the electrodes. The width of the gross necrotic zone around each electrode was measured to the nearest millimeter. On the basis of these studies, 20 mA for 15 mm was used in all subsequent studies.

Mice with tumors larger than 1 cm were randomized to four groups. One group received a single percutaneous electrical treatment (PET-1), and a second group received 2 or 3 electrical treatments (PET-2); subsequent treatments were given 1-3 days after the preceding treatment. In a third group, the primary tumor was removed surgically, and the skin was closed with 6-0 vicryl. In the two PET groups, the voltage varied automatically (8-10 V) to maintain the current at its preset value of 20 mA. Application of the current sometimes resulted in significant cardiac arrhythmias; consequently, 1 mg of xylocaine was given intraperitoneally 30 mm prior to each PET exposure. The animals in the fourth group served as controls for the PET; they were anesthetized, injected with xylocaine, and electrodes were placed in the tumor, but no current was administered (each control animal received one sham treatment). If a mouse died within 12 hr of treatment, its death was considered to be a result of the stress of treatment (one to five mice, depending on the group).

Each animal placed on study was followed until death or until it was classified as a survivor (90 days following treatment, at which time it was killed). The primary tumor was removed within 12 hr of death; viable tumor was dissected from grossly necrotic regions, and the wet wt of the viable tumor was recorded. The wet wts of the lungs were determined to evaluate the possible effect of PET on metastatic spread of the tumor. A rapid and progressive increase in spleen weight occurs in mice bearing LLC [10]; spleen wet wts were therefore recorded.

A separate group of mice was used to study the histological effects of PET. Twenty-four hours following administration of 20 mA the mice were killed, and representative sections of the tumor were removed from the region of the anode and cathode, fixed in formalin embedded in wax, sectioned at 10 m, and stained with hematoxylin and eosin. Similar histological procedures were used to prepare lungs recovered from five randomly chosen mice in each study group following their death from the tumor.

To observe the effects of DC currents on the pH of unstirred solutions in vitro, 5-20 mA were passed through normal saline, Hank's solution, and tissue culture medium containing 10% fetal calf serum (Dulbecco's) using platinum electrodes. Regions of different pH were visualized by the addition of pH-sensitive dyes to the solution (sodium salts of cresol red, bromo phenol blue, bromocresol purple, and thymol blue).

We hypothesized that one PET would destroy tumor tissue and hence result in a smaller primary tumor at death, when compared with no treatment. We also hypothesized that multiple PET would produce further reductions in primary tumor burden. The data were evaluated using Student's two-tailed unpaired t test at a significance level of 5%.

RESULTS

Results of the various treatments are shown in Table 1. The control mice survived an average of 18.8 days (measured from the time they would have been treated if they received PET) and exhibited an average tumor weight at death of 5.6 g. The animals in both PET treatment groups survived longer and exhibited a smaller primary tumor burden at death, compared with the controls. Lung and spleen weights were not affected by PET (Table 1).

Four of the 24 animals in the Surgery group survived the disease. They were killed after 90 days, and no evidence of either primary or metastatic disease (lungs) was found. The average survival time of the remaining animals in the Surgery group was almost twice that of the control animals. This relatively long survival time facilitated regrowth of the primary tumor to a size that was statistically indistinguishable from that of the tumor burden at death in the control animals. The relatively long survival time resulted in a significant increase in lung metastatic burden (Table 1).

Small multi-focal metastases were observed in the lungs of all tumor-bearing mice following death from the disease. The tumor occupied 4-8% of the area of a typical microscopic field, irrespective of the treatment received.

Tumor tissue biopsied from the electrodes was completely necrotic, but the form of necrosis differed between electrodes. Cells from the region of the anode had died prior to sacrifice of the animals, but exhibited distinct nuclei and plasma membranes that were not disrupted. In contrast, the cathodal cells were disrupted, and the typical cellular features could not be identified.

In saline, pH changes occurred immediately upon initiation of current; the pH of the solution adjacent to the

TABLE I

Results of Single (PET- 1) and Multiple (PET-2) Electrical Treatment of the Primary Tumor in C57 Female Mice Bearing Subcutaneous Lewis Lung Carcinoma

Treatment group	No. of mice	Time implant to treatment (days)	Average survival time (days)	Survivors (%)	Tumor weight (g)	Lung weight (mg)	Spleen weight (mg)
Control	34	11.2 ± 0.3	18.8 ± 1.1	0	5.6 ± 0.6	218 ± 13	238 ± 19
PET-1	31	11.2 ± 0.4	24.0 ± 1.2*	0	3.6 ± 0.4*	221 ± 18	256 ± 17
PET-2	48	12.3 ± 0.3	24.4 ± 1.0*	0	3.9 ± 0.3*	257 ± 19	213 ± 15
Surgery	24	11.4 ± 0.3	36.9 ± 1.8*	17	3.9 ± 0.7	391 ± 46*	171 ± 19*

Note. The average value and standard error of the mean are listed. The indicated statistically significant differences were determined with respect to the corresponding values in the control group.

* $P < 0.025$.

cathode and anode became about 12 and 2, respectively, within 2 mm. The spatial characteristics of the pH gradient were dependent on the magnitude and duration of the current; when the solution was vigorously stirred during passage of the current, no gradients were observed. pH changes also occurred in buffered solutions and in solutions containing dissolved proteins, but they occurred more slowly compared with the changes in saline observed under similar conditions.

DISCUSSION

PET-1 reduced the primary tumor burden and lengthened average survival time, with no concomitant increase in the spread of the disease to the lungs as determined by the gross weight of the lungs and spleen (Table 1) and by histological observation of the lungs. We previously showed that the portion of the primary tumor not killed by PET grew at the same rate as untreated tumor [7]. Thus, taken together, our studies showed that PET significantly lessened tumor burden, but did not increase metastasis or growth rate in the portion of the tumor that survived treatment. Since PET was not administered until 11-12 days after the tumor was implanted, it was quite unlikely that the local treatment would reduce metastatic spread; even amputation of a tumor-hearing limb after this period does not reduce metastatic growth [11, 12].

Average survival time, tumor weight, and lung and spleen weight observed in the PET-2 group were essentially identical to those seen in the PET-1 group. That is, additional electrical treatments did not produce corresponding changes in measured endpoints, and our second hypothesis was therefore not supported by the results. A significant portion of the tumor was destroyed following the initial PET, but the external dimensions of the tumor were not appreciably altered because there was no biological mechanism (within the time frame of the experiment) for removal of necrotic tumor. Many of the subsequent PET were actually administered to necrotic tissue, and consequently no benefit of multiple treatments could he demonstrated.

The electrodes were about 1 cm from the heart, and consequently their electric field interacted with that of the heart to produce arrhythmia; use of xylocaine permitted most animals to survive treatment. Arrhythmias are not expected on theoretical grounds in man because the volume conductor (the heart) is much larger. Indeed, in a series of 26 patients treated with DC currents up to 80 mA no arrhythmias were seen [9].

Among the expected oxidation/reduction reactions when DC current is passed through aqueous solutions [13] is the evolution of hydrogen at the cathode, resulting in a localized increase of OH-. Other electrochemical processes drive the anode acidic as a consequence of the evolution of oxygen. Detailed measurements of these processes were not made, but the reactions were observed to produce shifts in pH more quickly in saline, compared with buffered or protein solutions. The electrode reactions in tumor tissue undoubtedly proceed at different rates and involve other processes, possibly including the production of chlorine [14]. The tumor-destroying character of the DC currents was likely due to the pH changes and the toxic effect of the electrochemical species generated at the electrodes. The observation that DC-induced tumor necrosis was polarity specific-coagulative and ischemic at the anode and cathode, respectively-is further evidence that the mechanism of tumor destruction was electrochemical in nature.

Nordenstrom [9] used 80 mA to treat inoperable lung tumors; the anode was placed in the tumor and the cathode was placed in a vascular channel so that the constant flow of blood would dilute the cathodal electrochemical species; he specifically avoided the use of the cathode in the tumor because of fears that it would promote metastatic spread of the disease. We observed tissue destruction at both the anode and cathode, but no effect on metastastic spread of the tumor. Thus, placing both electrodes in the tumor is more effective than the one-tumor electrode procedure.

In summary, relatively weak percutaneous DC currents destroyed a significant amount of tumor tissue in large tumors (>1 cm in diameter) and prolonged survival, Reduction of' the primary tumor burden did not alter the extent of metastasis or splenomegaly. Cures were possible (Table 1), but PET produced no cures. PET may he useful as an adjuvant treatment in connection with either chemotherapy or radiation because it reduces local tumor mass but does not increase the speed of the disease. However, the ability of PET to produce tumor destruction that is additive to that produced by standard treatment has not been demonstrated,

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