JOURNAL OF SURGICAL RESEARCH **41,** 198-201 (1986)

Electrical Treatment of Lewis Lung Carcinoma in Mice[1]

ANDREW A. MARINO, PH.D.,*,[2] DON MORRIS, M.D.,†,‡ AND THOMAS ARNOLD†

*Departments of *Orthopaedic Surgery and †Surgery, Louisiana State University Medical Center, P.O. Box 33932, and ‡Veterans Administration Medical Center, Shreveport, Louisiana 71130-3932*

Submitted for publication March 7, 1985

Direct electrical current of sufficient magnitude and duration can destroy tissue. This capability may be clinically useful in some cases involving inoperable metastatic lesions. In principle, a tumor could be treated with direct current administered via a percutaneous electrode insulated along its entire length except for the portion actually inserted into the tumor. An animal model was developed to study the effect of direct electrical current on tumor growth. The growth of implanted Lewis lung carcinoma in mice was inhibited following the administration of 2 mA for 1 hr, 1-3 treatments. The effect occurred in both small and large tumors. The results suggest that the electrical technique is potentially useful for treating some tumors. © 1986 Academic Press, Inc.

INTRODUCTION

Inoperable metastatic lesions are a difficult clinical problem. Microwave hyperthermia may prove to be a useful treatment modality, at least for superficial lesions [1]. Another possible approach involves the use of direct electric current delivered through electrodes in physical contact with tissue. Some success with this technique has been reported in animal [3, 8] and human [7] studies.

The effectiveness of hyperthermia treatment depends on a differential response to heat by tumor cells as compared to normal cells [4]. In contrast, sufficiently strong electrode-delivered currents can destroy any tissue, and they have no demonstrated specific effect on tumor cells. By controlling factors such as current magnitude, duration, and electrode geometry, it may be possible to destroy tumors with minimal concomitant damage to normal tissue. The aim of the present study was to develop an animal model to study the effect of direct electric current on tumor growth.

METHODS

Lewis lung carcinoma (Animal and Human Tumor Bank, Worcester Massachusetts) was implanted on the back of C57/DL/6 mice (Jackson Laboratory, Bar Harbor, Me.). Under light ether anesthesia, the host mouse was shaved from the middle of the back to the base of the neck and a 5-mm incision was made at the level of the midback. Forceps were inserted into the incision just under the skin to make a subcutaneous pouch for the tumor implant which consisted of a small portion (25-35 mg) cut from the periphery of a tumor that had been implanted in a donor mouse 15-20 days previously. Removal of the tumor from the donor and subsequent implantation in the host required no more than 60 mm, during which time the tumor was immersed in Hank's solution. The implant was placed approximately 1.5 cm caudal to the base of the skull, and the incision was closed with 1 or 2 interrupted sutures (6-0 vicryl). All mice were housed individually, and fed and watered *ad libidum.*

The electrical circuitry used to administer direct current (dc) is depicted in Fig. 1. The current and voltage were monitored continuously throughout each treatment course. Since the variable resistor was much larger than the effective resistance between the transcutaneous electrodes, the electrical events at the treatment site did not influence the total current. The voltage was adjusted to produce a total current of 2 mA, which typically occurred at an electrode potential difference of

[1]Supported in part by a grant from the Edward P. Stiles Trust Fund.

[2]To whom correspondence should be addressed.

0022-4804/86 $1.50

about 3 V. In one experiment alternating current (ac) was employed; circuitry similar to that shown in Fig. 1 was used to pass 2 mA at 60 Hz.

During treatment the mice were constrained in a specially designed apparatus that restricted their movement and permitted direct access to the tumor site. For treatment, 2 platinum electrodes (E2, Grass Instruments, Quincy, Massachusetts) were passed parallel to one another through the tumor at right angles to the long axis of the body (Fig. 1). The tumor was roughly ellipsoidal, and the placement of the electrodes corresponded to the approximate location of the foci. The current was increased slowly from 0 to 2 mA to permit habituation. All treatments lasted 1 hr, caused no apparent pain or discomfort, and required no anesthesia. The mice did not exhibit any local or systemic toxicity that could be attributed to the treatment.

In the first study, the mice were treated one or more times beginning on the third day after implantation. One group received one dc treatment *(n = 5)*. Another group *(n = 21)* received two additional dc treatments whenever the tumor — which had been diminished in volume because of the previous treatment — again became palpable. A third group *(n = 5)* received one ac treatment, and a fourth group *(n = 26)* served as the control. Periodically, beginning on the third day following implantation, the mice were weighed and the tumor length and width was measured with calipers. A tumor volume was calculated from the length and width measurements under the assumption that the tumor was a prolate spheroid. For convenience, the data are expressed as the radius of the sphere having an equal volume.

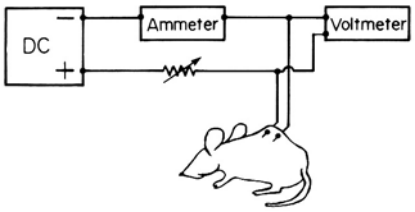

FIG. 1. Electrical circuitry for administration of dc currents to mice with implanted tumors.

In the second study, mice *(n = 25)* were treated on Day 12 following implantation, and the change in the equivalent tumor radius on Day 16 was measured and compared to the change that occurred in the control mice during the same time period.

The control mice were treated the same as those in the treatment group with regard to tumor implantation, mechanical restraint, and insertion of platinum electrodes: the absence of the electrical current was the only salient difference between the two groups. Each group consisted of roughly equal numbers of males and females of comparable age (2-4 months).

The implant site and lungs of all animals that died during the study were examined to ascertain the presence of primary tumor and metastatic lesions. The latter was established by profusing the trachea with India ink [10]. Animals that lived 50 days beyond implantation were defined to be survivors.

RESULTS

Initial treatment 3 days postimplant. The results using dc are shown in Table 1 and Fig. 2. The average equivalent tumor radius in the 1-treatment group decreased below the detection level by Day 7, but tumor growth resumed on Day 9 and continued at a rate comparable to that of the control group. In animals that received 3 treatments there was essentially no measurable tumor for 17 days following implantation; thereafter, tumor growth resumed at a rate comparable to that of the control group (Fig. 2).

Of the 21 animals that received 3 treatments, 6 survived and 3 had no primary tumor when death occurred from lung metastases. Thus, there was primary tumor regression in 43% of the animals (9 of 21) compared to 11.5% in the control group (3 of 26): this difference was significantly different ($P < 0.05$) as determined by the chi-square test. The lung metastases were too numerous to count, and it therefore was not possible to determine whether different numbers of metastases oc-

TABLE 1
EFFECTS OF dc TREATMENT IN MICE IMPLANTED WITH LEWIS LUNG CARCINOMA[a]

Animal group	Survival time (days)	Survival %	Primary tumor regression %	Equivalent tumor radius at death (mm)
1 treatment (n = 5)	32.5 ± 4.4 (n = 4)	20% (1/5)	20% (1/5)	14.0+2.0 (n = 4)
3 treatments (n = 21)	31.1 ± 5.2 (n = 15)	28.6% (6/21)	43%* (9/21)	10.8 ± 6.4** (n = 15)
Control (n = 26)	32.9 +4.4 (n = 23)	11.5% (3/26)	11.5% (3/26)	14.8 +2.2 (n = 23)

[a]All experimental animals were treated on the third day following implantation. Animals in the 3-treatment group received 2 additional treatments as needed when the primary tumor again became palpable. Primary tumor regression was recorded for the survivors and for mice that had no primary tumor when death occurred (from lung metastases).

* $P < 0.05$, $\chi 2$, 2 X 2, continuity correction.

** $P < 0.05$, t test.

curred in the treatment groups as compared to the control group.

A single treatment using ac (n = 5) had no effect on tumor size during the time interval studied (3-12 days postimplant). At 12 days postimplant, the average equivalent tumor ra dii of the treatment and control groups were 6.8 ± 1.4 and 6.3 ± 2.1 mm, respectively (statistically insignificant difference as determined by the unpaired t test).

Treatment at 12 days postimplant. In mice treated with dc on Day 12, there was no net tumor growth on Day 16; in the same period the equivalent tumor radius in the control mice increased by 30% (Table 2). The differ ence between the two groups in the rate of tumor growth was statistically significant $P < 0.01$, unpaired t test).

No differences in body weight among any of the treatment or control groups were seen. Generally, the mice gained 5-10 g during the period of study, and exhibited a 1- to 2-g weight loss during the 48-hr period immedi ately preceding death.

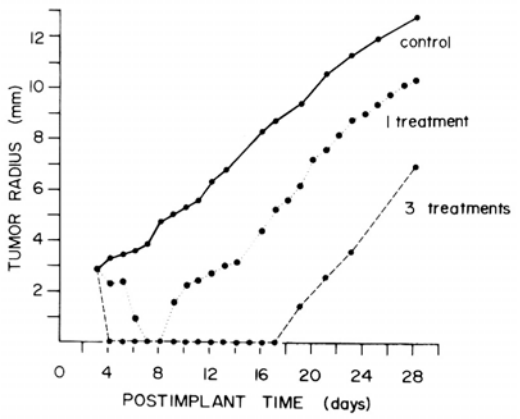

FIG. 2. Tumor volume in mice following electrical treatment. Treatment was begun on the third day following implantation. One group (n = 5) received 1 treatment. A second group (n = 21) received 2 additional treatments.

DISCUSSION

Direct electrical current (2 mA for 1 hr) delivered via transcutaneous platinum electrodes

TABLE 2
EFFECT OF 1 dc TREATMENT ADMINISTERED ON THE 12TH DAY AFTER IMPLANTATION

	Equivalent tumor radius (mm)		
	Day 12	Day 16	Day 16 – Day 12
Control (n = 26)	6.3 ± 2.1	8.2 ± 2.7	1.9 ± 0.9*
Treatment (n = 25)	7.1 ± 1.8	7.0 ± 4.0	–0.1 ± 2.5

* $P < 0.01$, t test.

markedly retarded the growth of 3-mm-radius tumors, and increased the tumor regression rate (Fig. 2, Table 1). The same treatment also retarded the growth of 7-mm-radius tumors (the volume was 38 times greater than that of the smaller tumor) (Table 2). Joule heating (2 mA X 3 v = 6 mW) was too small to account for the effect on growth. Metallic ions also were not involved because platinum is known to be inert under the conditions studied. It therefore seems likely than the observed effects were due to electrochemical changes.

Electrolysis of aqueous solutions between inert electrodes begins about 1.7 V [5], and results in the liberation of hydrogen and ox ygen gases at the cathode and anode, respectively. Although no net pH change occurs, the reactions produce local pH changes in which the cathode area becomes basic and the anode area becomes acidic [2, 6]. The electrochem ical reactions in the tissue are undoubtedly more complex than those that occur in aqueous solution, but evolution of hydrogen and oxygen probably are still the dominant processes. The concomitant pH changes — to the extent that they overcome the body's buff ering capacity — may be responsible for the observed effects on tumor growth. The absence of an effect on tumor growth with ac supports this view: in this case, the electrochemical events at each electrode are identical and they do not produce a pH gradient. Other possible mechanisms underlying the observed effects involve the current density — a maximum of about 20 mA/cm2 which occurred at the electrode-tissue interface — or the total charge passed through the tissue (7.2 coulombs per treatment bout).

Whatever the actual physical basis for the effect on tumor growth, the net effect of the dc was to destroy tumor tissue, not to mod ulate cell mitotic rate. In 43% of the mice that received 3 treatments, the primary tumor was completely destroyed (Table 1), and in the remaining animals, when measurable growth resumed, it occurred at a rate of about 0.5 mm/day which was essentially the same as that seen in both the control and 1-treatment group (Figure 2).

The electrical parameters used were chosen arbitrarily, and it seems likely that other combinations of current magnitude and duration, and electrode configuration, will be more effective in destroying the tumor. It is also possible that a useful approach could be fashioned from the combined action of electricity and immunomodulators [9].

SUMMARY

Direct electrical current (2 mA for 1 hr, 1 3 treatments) inhibited the growth of im planted Lewis lung carcinoma in mice in both small and large tumors. The results suggest that the electrical technique is potentially use ful for treating inoperable metastatic lesions.

REFERENCES

1. Baker, H. W., Snedecor, P. A., Goss, J. C., Galen, W. P., Gallucci, J. J., Horowitz, I. J., and Dugan, K. Regional hyperthermia for cancer. *Am. J. Surgery* **143:** 586, 1982.
2. Glasstone, S. *Electrochemistry*. Princeton, N.J.: van Nostrand, 1942.
3. Humphrey, C. H., and Seal, E. C. Biophysical approach toward tumor regression in mice. *Science* **130:** 388, 1959.
4. Kase, K., and Hahn, G. M. Differential heat response of normal and transformed human cells in tissue culture. *Nature (London)* **255:** 228, 1975.
5. Lingane, J. L. *Electroanalytical Chemistry.* New York: Interscience, 1953.
6. Marino, A. A., and Becker, R. O. The effect of electric current on rat tail tendon collagen in solution. *CaIc. Tiss. Res.* **4:** 330, 1970.
7. Nordenstrom, B. Biologically closed electric circuits: Activation of a vascular interstitial closed electric circuit for treatment of inoperable cancers. J. *Bioelectricity* **3:** 137, 1984.
8. Schauble, M. D., Habal, M. B., and Gullick, H. D. Inhibition of experimental tumor growth in hamsters by small direct currents. *Arch. Pathol. Lab. Med.* **101:** 294, 1977.
9. Szmigielski, S., Zaboklicki, S., Gil, J., Jeljaszewicz, J., and Pulverer, G. Inhibition of Lewis lung carcinoma in mice by local microwave hyperthermia combined with immunomodulating propionibacterium granulosum KP-45. *Cancer Immunol. Immunother.* **16:** 151, 1984.
10. Wexler, H. Accurate identification of experimental pulmonary metastases. J. *Nat. Cancer Inst.* **36:** 641, 1966.