THE EFFECTS OF ELECTROCHEMICAL THERAPY ON COLON-25 TUMORS IN BALB-C MICE

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The purpose of the research was to treat immunodeficient mice, implanted with colon-25 tumors, with continuous and interrupted electrochemical therapy (ECT). ECT involves the placement of two electrodes, an anode near the center of the tumor and a cathode into the tumor periphery. A constant voltage is applied across the electrodes for a given period of time. The data showed that the interrupted and continuous ECT resulted in a decrease in mean tumor growth as compared to that of the sham controls. The histology of both ECT groups showed an increase presence of large vacuoles, randomly distributed tumor cells as well as the presence of "crevicing" in the medullary tissue. The differential leukocyte counts showed a distinct neutrophilia and lymphopenia in all groups at day 20 post tumor implantation. The results from the experimental groups appeared to support the findings of previous investigators.

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CHAPTER I

INTRODUCTION

For a number of years, medical practice in Asia and Europe has seen a growth in the use of less invasive therapeutic approaches or the incorporation of "alternative medicine" for the treatment and prevention of diseases and chronic disorders. These practices are less accepted by western practitioners (Laken and Cosovic, 1995). Many of these alternative procedures emanate from ancient practices, often techniques that were employed by indigenous peoples (Micozzi, 1995). For example, it is now widely recognized in modern medical science that there are a multitude of plants that were known among ancient peoples to have medicinal properties, and there are untold numbers of additional plant species whose extracts may have beneficial medicinal value (Duke, 1995). Likewise, there are undoubtedly a number of manipulative/mechanical procedures that have been in use over the centuries or have gone unexplored that deserve scrutiny using accepted scientific investigative procedures. An example of a more invasive mechanical procedure is acupuncture, which has been applied in Asian cultures for treatment of pulmonary diseases, tumors, tuberculosis, as well as a number of other diseases and disorders, but has only recently become more acceptable to western practitioners (Jobst, 1995). More recently, research employing the scientific method that incorporates testable hypotheses into its design has been applied to studies of the effectiveness of acupuncture in the treatment of a variety of disorders (Jobst, 1995). With the inception of the National Institutes of Health Office of Alternative Medicine in 1992, the realization that alternative medical procedures deserve scientific validation has extended to not only the field of medical research but also to the training of medical

practitioners (Laken and Cosovic, 1995). It is just such an approach that the research described herein takes to determine the efficacy of the application of electrical current to tumors and the potential therapeutic benefits of such a procedure.

Cancer has claimed the lives of millions of people all over the world. This year about 552,200 Americans are expected to die of cancer, more than 1,500 people a day (American Cancer Society, 2000). Cancer is the second leading cause of death in the US, exceeded only by heart disease (American Cancer Society, 2000). In 2000, about 1, 220, 100 new cancer cases are expected to be diagnosed and approximately, since 1990, 13 million new cases have been diagnosed (American Cancer Society, 2000). Ongoing research for new and better techniques in treating this deadly disease has led several investigators to report that locally applied direct current (DC), now called electrochemical treatment (ECT), destroys a variety of human and animal tumors *in vitro* and *in vivo* (Taylor et al., 1994). ECT involves the placement of two electrodes, an anode near the center of the tumor and a cathode into the tumor periphery. A constant voltage is applied across the electrodes for a given period of time.

In 1983, Nordentstrom was the first in recent years to utilize ECT for treating human tumors. Electrochemical therapy has been used in China since 1987 resulting in impressive clinical results (Xin, 1994). Since 1987, 4,081 cases with malignant tumors have been treated with ECT (Xin, 1994). Xin, (1994), reported the most impressive clinical data where 2516 cases from 66 hospitals were treated with ECT. Of the 2,516 cases, 2,124 patients were followed up for 1 to 5 years. The survival rates were 84.3% for year 1, 79.1% for year 2, 63.5% for 3 years, 57.8% for 4 years, and 46.6% for 5 years (Xin, 1994). However, Xin ,(1994), modified the methods introduced by, Nordenstrom, (1983), by inserting platinum

anodes near the center of a tumor, and the cathodes were applied in the periphery (Chou et al., 1995).

Various effects of ECT on tissues have been reported. The direct current, when applied to the cancer tissue, severely disturbs the living microenvironment of the cell (Chou et al., 1995). Due to electrophoresis, electrolysis, and electro-osmosis in the tumor tissues, the cells near the electrodes do not survive (Chou et al., 1995). The pH levels at the anode decrease to 2.0 and increase at the cathode to 13.0 causing the release of chlorine and hydrogen ions (Chou et al., 1995). It is believed that these strong acidic and basic conditions at the electrodes cause cancer cell death by denaturing cell proteins, enzymes and membranes (Chou et al., 1995). The worldwide experience with the effects of the application of ECT to various cancers goes back some 15 years with more than 5,000 patients been treated, predominantly in China (Xin 1997). The types of tumors that have been treated include: esophageal, lung, liver, breast, skin, thyroid, parotid, melanoma, oral cavity and prostrate cancers (Xin, 1997). Almost all of the work done on humans has involved patients that received other standard treatment prior to and/or following ECT. Relatively little work has been done on laboratory animals or humans under controlled laboratory conditions, where potential changes in tumors could be compared to those in control animals receiving no treatment.

The purpose of the research was to treat immunodeficient mice implanted with colon-25 tumors with ECT under such controlled conditions. The specific aims were (1) to measure the effects of ECT on the growth rate of tumor, (2) changes in histology of the tumors, and (3) to compare the effects of ECT administered continuously and at 10-sec intervals on the growth rate of implanted tumors. Based on the results of this research, we hoped to demonstrate that ECT administered under controlled laboratory conditions will show some of

the same beneficial results on tumor growth as seen with clinical data, as well as, to establish any differences in the therapeutic results between an interrupted and continuous current.

CHAPTER II

METHODS

Forty male and 40 female Balb-c mice, ranging in weight from (17-31g) were selectively bred at the University of North Texas Animal Care Facility and used in this study. Balb-c mice were chosen for this study because of their reduced immune system in order to facilitate the growth of the tumor. Each mouse was implanted with 2 mm x 2 mm medullary section (brei) of a human Colon-25 tumor from a donor mouse. The brei consists of the medullary section of the tumor sliced up in a petri dish and mixed with a Ringer's solution to insure cell viability during implantation (Fig. 1). This particular tumor was maintained in the laboratory for approximately 4 yr The 2 mm x 2 mm sections were then injected epidermaly between the sacral crest and the rib cage posteriorly using a 13-gauge hypodermic needle (Fig. 1). The mice were maintained at the University of North Texas Animal Care Facility and were subjected to LD 12:12 and were fed a steady diet of Teklab™ rodent pellets.

The electrodes used in the study were fabricated from platinum wires, Epoxylie 6001-M electrode insulator fluid, Devcon™ 5 Minute Epoxy glue, capillary tubing and acrylic. The platinum wires were coated with insulator fluid and allowed to dry. The insulation fluid, at the electrode tips, was removed with a scalpel under a dissecting microscope to create 0.5 mm length tips. Holes, measuring 1 cm apart, were drilled into the acrylic plate. Capillary tubes were placed into the holes, and the electrode wires were placed inside. Capillary tubes and electrode wires were secured to the acrylic plate with the epoxy glue (Fig. 2).

Electrical current was supplied by Heath Kit™ Regulated High Voltage Power Supply. Current and amperes were measured by two Keithley Instruments ™ 602 Electrometers. The electrodes were secured to a micromanipulator to insure constant steady

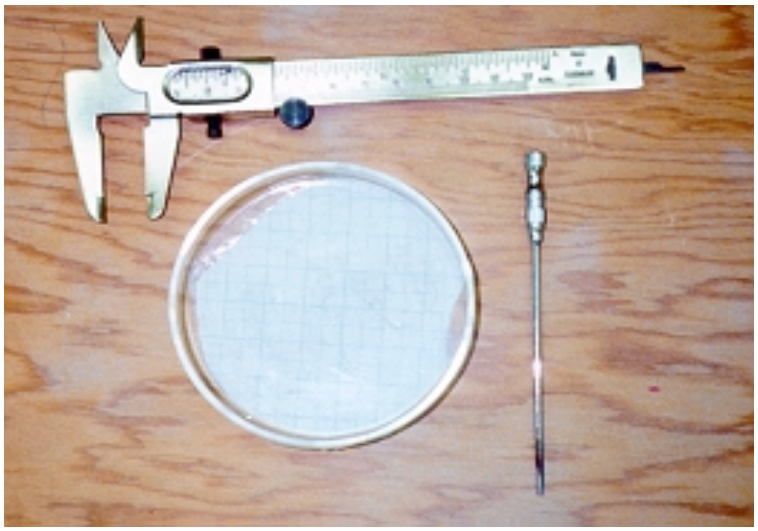


Figure 1. Petri Dish, 13 Gauge Hypodermic Needle, and Calipers

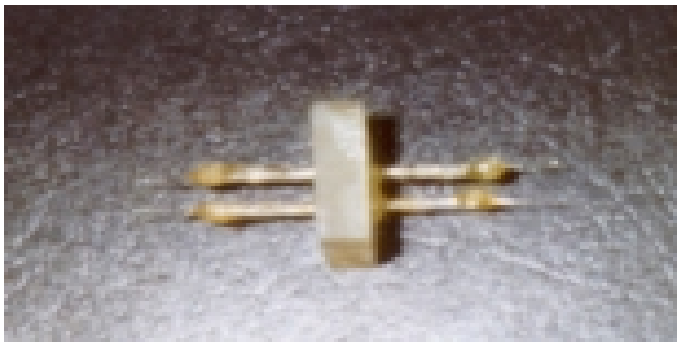


Figure 2. Fabricated Electrodes

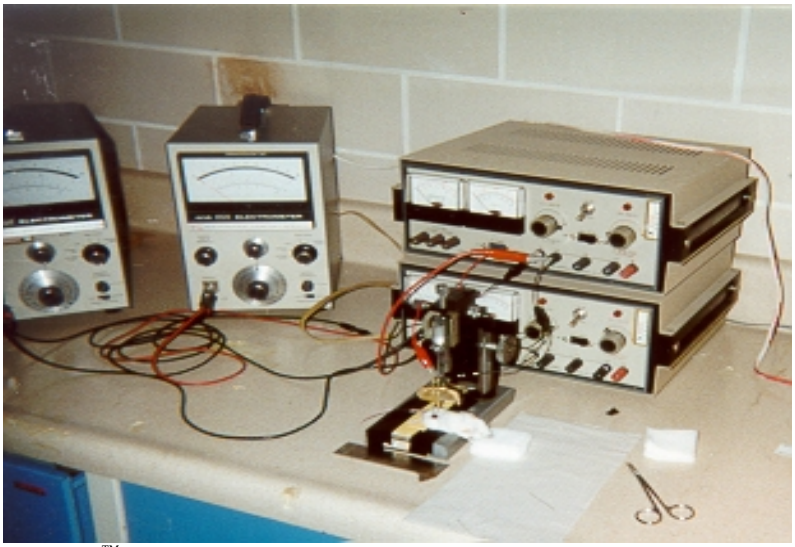


Figure 3. Heath Kit™ Regulated High Voltage Power Supply and Micromanipulator

electrode movement into and out of the tumor (Fig. 3). The electrodes were applied into the tumor by inserting the anode into the center of the tumor and the cathode, 1 cm away from the anode, into the periphery.

The animals were divided into control and experimental groups as follows:

I. Control groups

Group A: control animals with tumor only (10 males and 10 females)

Group B: sham control animals with electrodes implanted on day 10 post tumor implantation for 10 min. but with no current (10 males and 10 females)

II. Experimental animal groups

Group A: continuous current ECT (electrochemical therapy) for 10 min. at day 10 post tumor implantation (10 males and 10 females)

Group B: interrupted current ECT for 10 min. at 10-sec Intervals at day 10 post tumor implantation (10 males and 10 females)

The measurements and observations taken from each animal were: (1) change in tumor size measured on the outside of the skin to the nearest mm (length x width = $cm^{2)}$ using digital calipers (Fig 1), (2) changes in blood differential counts from blood collected from the caudal vein using capillary tubes on day 5 and day 20 post tumor implantation using Diff – Quick ™differential stains, and (3) changes in tumor histology. Instantaneous growth rates reflecting relative increases in tumor size over the period of application of ECT were calculated after Brody (1945) as

$$k = \ln M_2 - \ln M_1 / t_2 - t_1$$

where M_2 and M_1 are mean tumor sizes at times t_2 and t_1, respectively.

Sham control and experimental animals were anesthetized with ketamine diluted in distilled water (1 ml/10 ml of water) given intrapertoneally. ECT experiments were performed approximately on day 10 post tumor implantation, when the tumor reached 1 cm in length.

X-rays were taken of the mice at day 9 post tumor implantation, just prior to ECT, as well as on day 20 post tumor implantation prior to euthanasia, tumor removal, and blood collection. Photographs of tumors were taken with a 35 mm camera, *in situ* and following removal in order to record and measure overt features such as size, shape, color, weight, and vascularization.

Tumors were measured on a daily basis following day 5 post tumor implantation. On day 20 post tumor implantation, the tumors were removed, weighed and placed in a 2% formalin solution. Each tumor was embedded in paraffin and sectioned into 16-20 μm sections using an 820 Spencer™ microtome (Buffalo, New York). The sections were then stained using Harris Hamatoxylin 3 and eosin. Photomicrographs were taken, at 40 X magnification, using a Zeiss™ microscope fitted with a 35 mm Olympus camera.

Statistical analysis of the data was performed using a 2-tailed Students' t-test. The Institutional Animal Care and Use Committee at the University of North Texas approved this study.

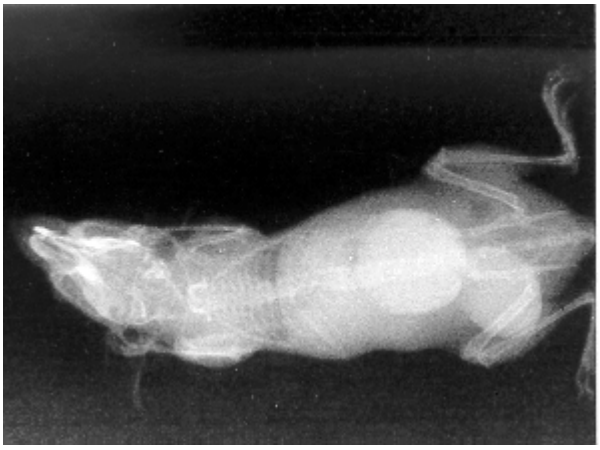
CHAPTER III

RESULTS

The results from the experimental groups appear to support the findings of previous investigators. As with the previous investigations, the experimental tumor sizes for the continuous and interrupted ECT groups showed a significant decrease when compared to the sham control group. The data obtained in the study are presented in the form of figures depicting the rate of growth of each tumor as reflected by changes in daily tumor size. Photographs and x-rays were also presented to show a typical encapsulated tumor *in situ* and a typical excised tumor. Histological changes observed with microscopy demonstrated changes in both cortical and medullary regions of control and ECT treated tumors

A typical tumor grown *in situ* shows overall size of the tumor, approximately half the size of the animal (Fig. 4). The tumor was solid, well defined and relatively symmetrical. During early tumor growth stages, growth of tumors progressed posteriorly toward the tail, but as the tumor continued to increase in size it progressed anteriorly toward the head, sometimes expanding through the abdominal wall and entering the abdominal cavity. Excised tumors from both control and experimental groups at day 20 post tumor implantation showed solid, well-defined forms (Fig 5).

There were also no significant differences in tumor growth between male and female animals in any of the groups and, therefore data from males and females were combined. Data comparing the size of tumors in control and sham control groups show no statistically significant differences in tumor sizes between the two control groups (Fig. 6). Instantaneous



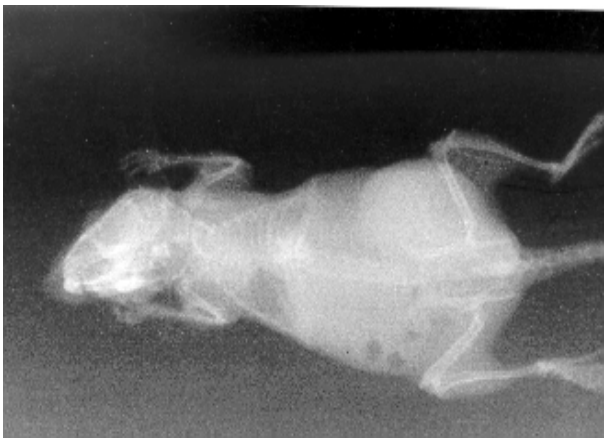


Figure 4. X-rays of Colon-25 tumors *in situ* from Balb-c mice

Figure 5. Excised tumors from control and experimental groups at day 20

growth rates (k) for sham control and control groups were 0.186 and 0.193, respectively, between days 5 and 10 and 0.085 and 0.096, respectively, between days 11 and 20. The mean size at day 15 post tumor implantation for the control groups was 2.26 cm^2, and that for the sham control groups was 2.32 cm^2. At day 20 post tumor implantation, the mean size for control groups was 3.21 cm^2, while that for the sham control was 3.46 cm^2. The data presented in Table 1 further confirmed the findings with the percent change between the control and sham control groups equaling 7.33%, however this difference was not significant. The standard error of the mean for the control group was .62 and was .86 for the sham control group

Interrupted ECT resulted in a decrease in mean tumor growth as compared to that of the sham controls (Fig. 7). Mean tumor sizes for the Interrupted ECT at day 12 post tumor implantation was 1.33 cm^2, at day 15 it was 1.74 cm^2, at day 17 it was 2.04 cm^2 and at day 20 it was 2.35 cm^2 as compared to 1.66 cm^2, 2.32 cm^2, 2.70 cm^2 and 3.46 cm^2 for the sham control at the same time periods, respectively (Table 2). A comparison of the instantaneous growth rates calculated for the interrupted ECT experimental group and sham control group between days 5 and 10, prior to treatment, indicates comparable rates of growth (k) of 0.223 and 0.186, respectively. Post-treatment rates for the same groups were 0.074 and 0.085, respectively (Fig. 7), a 13% difference in the growth rate of treated tumors compared to that of sham controls.

Mean tumor size of the continuous ECT is decreased, as well, when compared to that of the sham controls (Fig. 8). Mean tumor size for the continuous ECT at day12 post tumor implantation was 0.99 cm^2, at day 15 it was 1.12 cm^2, at day 17 it was 1.41 cm^2 and at day 20 it was 1.82 cm^2 as compared to mean sizes of tumors of the sham controls (Table 2). These differences were shown to be statistically significant (Table 2), and both the interrupted and

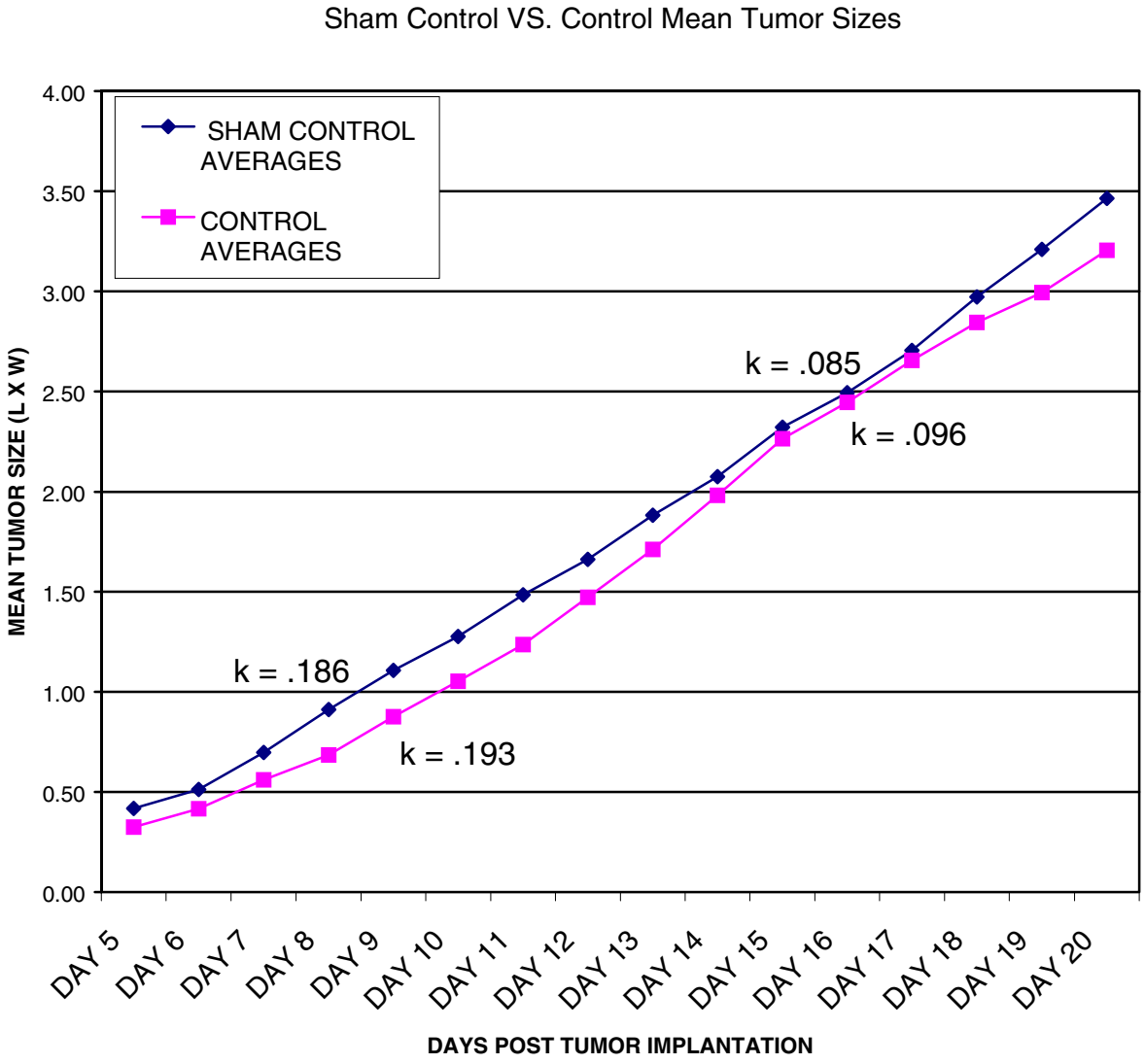


Figure 6. Summary comparing sham control and control mean tumor sizes

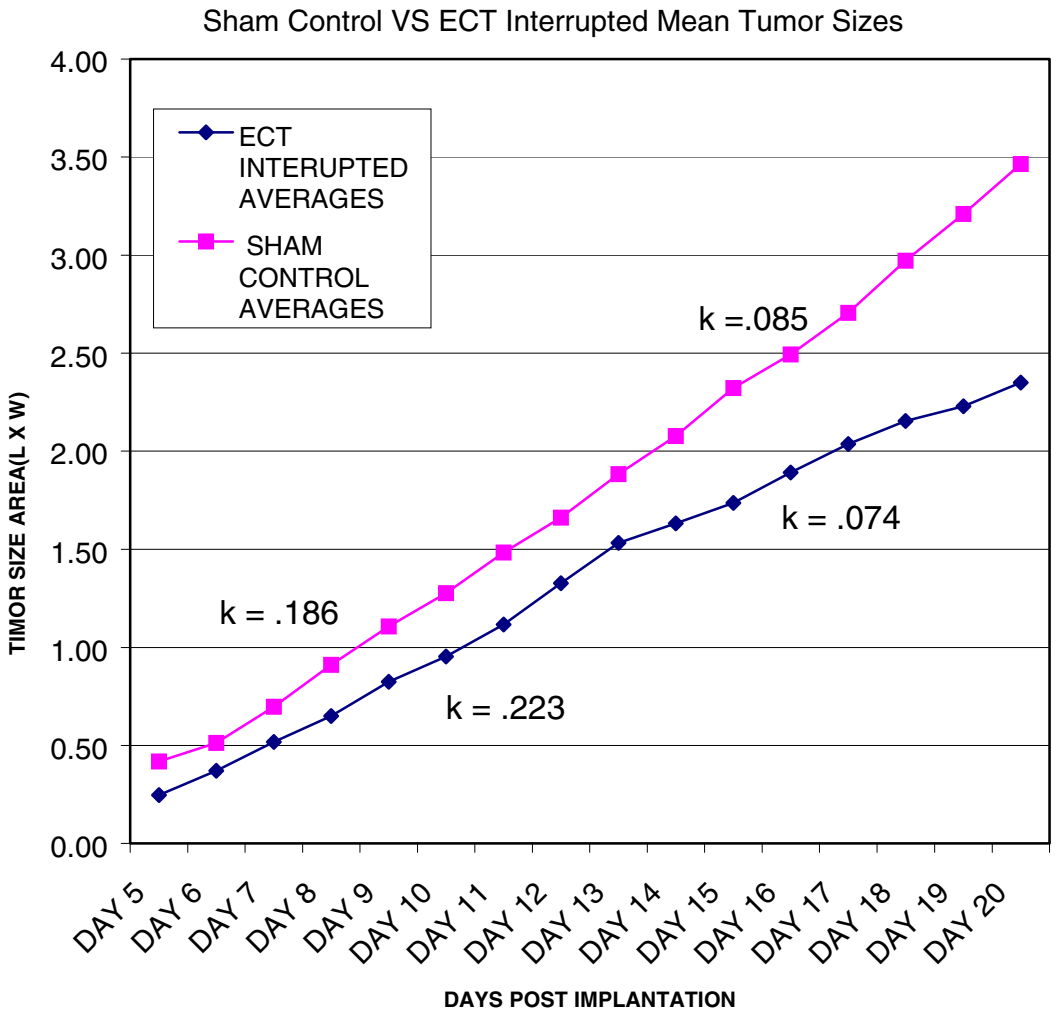


Figure 7 Effects of interrupted ECT on mean tumor size in Balb-c mice

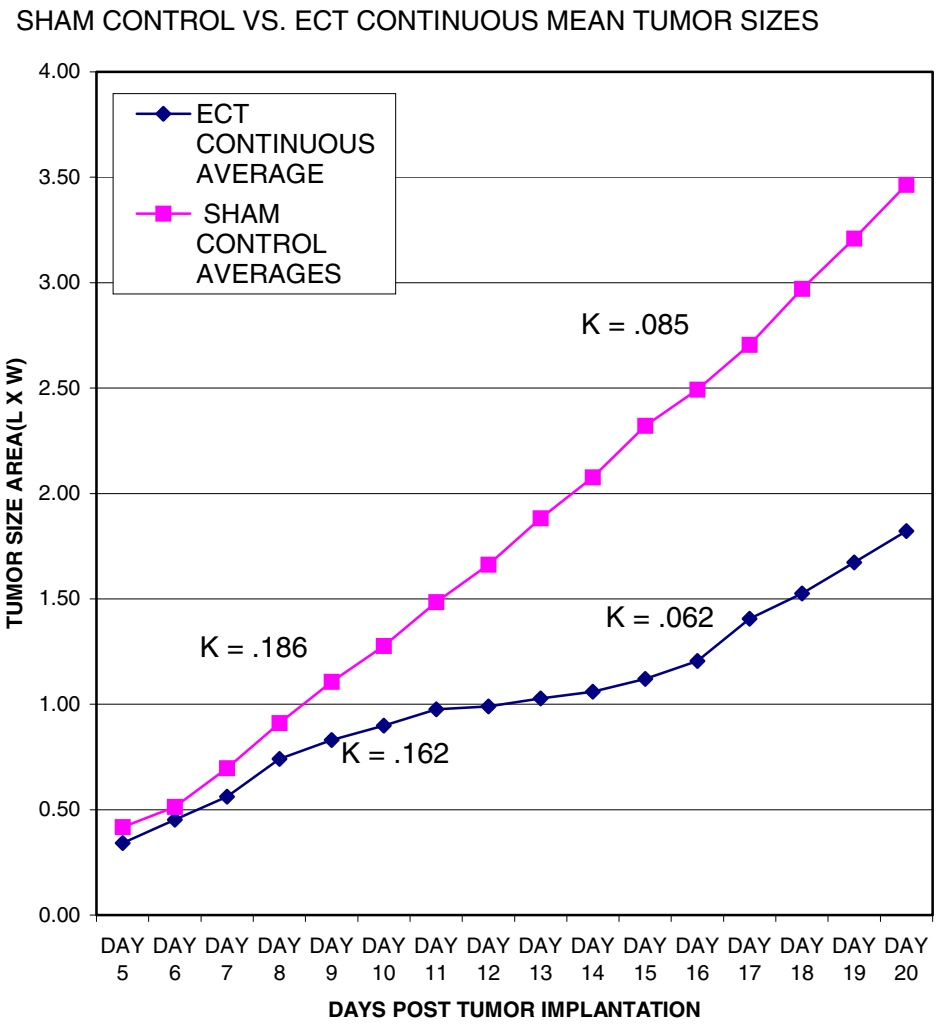


Figure 8. Effects of continuous ECT on mean tumor sizes

continuous ECT experimental groups had a statistically significant changes in mean tumor sizes. A comparison between instantaneous growth rates (k) for continuous ECT experimental group and the sham control indicates comparable rates of growth, 0.162 and 0.186, respectively, between days 5 and 10 prior to treatment (Fig. 8). Post-treatment rates of growth for continuous ECT experimental group and the sham control were 0.062 and 0.085, respectively (Fig. 9), a 27% difference in growth rate of treated tumors compared to that of sham controls.

The data indicate that both experimental procedures, interrupted and continuous, retarded the overall growth of the tumor producing with a percent change difference in tumor size of –31.7% for interrupted ECT and –47.1% for continuous ECT. Both differences in average tumor sizes and instantaneous rates of growth reflected the therapeutic affect of interrupted and continuous applications of ECT.

There was no correlation between the initial sizes of the tumors, before the ECT was applied at day 10 post-tumor implantation, and the overall growth rate of the tumors. A CORREL, correlation coefficient test, was run on the continuous and interrupted ECT groups. The results showed no significance between any of the mice in either group. In fact there was an inverse correlation between tumor size and growth rate. The larger initial tumor grew at a slower rate.

Histological sections of untreated tumors revealed typical tissue morphology with both the cortex and medulla evident (Fig. 10). The cortex was composed of well defined tissue layers interspersed by larger tumor cells with interstitial channels. Separation between cortex and medulla was evident, where the medullary portion displayed a homogeneous dispersion of darkly-stained tumor cells. Both sham and control tumor cells appear to be evenly distributed throughout the tissue without deterioration or vacuole formation.

Histology of a continuous ECT treated tumor shows a distinct change in the medullary portion (Fig. 10). This was presented by an increased presence of several large vacuoles, as well as, randomly distributed tumor cells. Also shown in the medulla is the presence of "crevicing". Interrupted ECT treated tumors also displayed large vacuoles in the medulla with the presence of "crevicing" (Fig. 10).

n	GROUPS	LENGTH X WIDTH cm^2		SEM	[%Δ]	Mean tumor weights (g)
20	CONTROL	3.20		0.62	0%	1.45
20	SHAM CONTROL	3.44		0.86	7.33%	1.45

$$\%\Delta = \frac{T - C}{C} \times 100$$

T = SHAM CONTOL

C= CONTROL

Statistical significance determined by a two tailed T test

Table 1. Summary comparing mean tumor sizes between the two control groups in Balb-c mice at day 20

n	GROUPS	LENGTH X WIDTH cm^2	Mean tumor weights (g)	SEM	[%Δ]	
20	SHAM CONTROL	3.44	1.45	0.86	0%	
20	CONTINUOUS ECT	1.82	1.36	0.48	-47.09%	***
20	INTERRUPTED ECT	2.35	1.34	0.52	-31.69%	***

$$\%\Delta = \frac{T - C}{C} \times 100$$

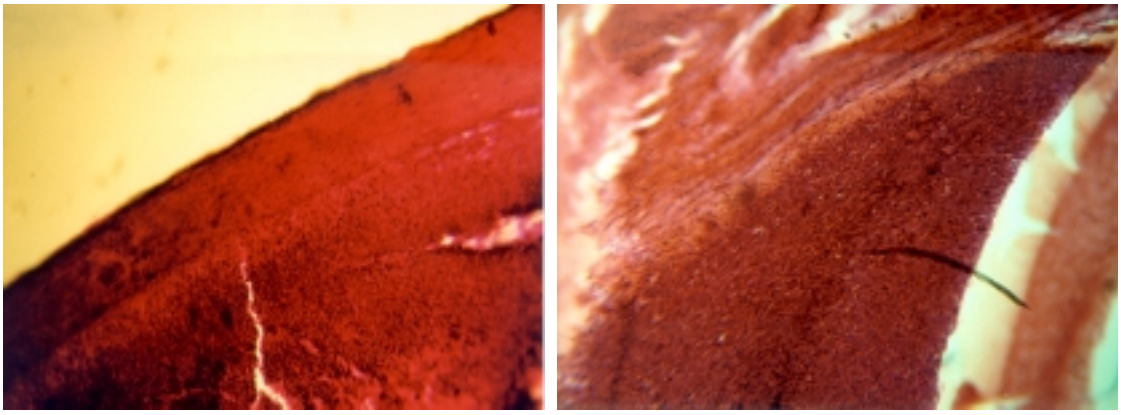
T = TEST GROUPS

C= SHAM CONTROL

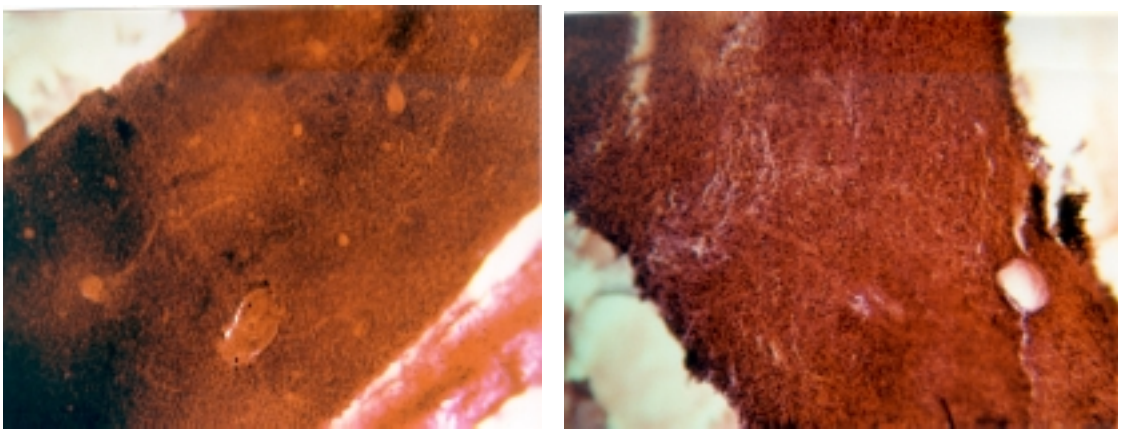
Statistical significance determined by a two tailed T test

*** Statistically significant @ p<.001

Table 2. Summary of the effects of continuous and interrupted ECT on mean tumor sizes in Balb-c mice on day 20



A. Control Tumor B. Sham Control



C. Continuous ECT D. Interrupted ECT

Figure 9. Histology sections of colon-25 tumors in Balb-c mice at day 20

CONTROL GROUP VS. NORMAL UNTREATED CONTROL

	T5 MEAN		SEM	T20 MEAN		SEM	%Δ	
MONOCYTE	2		1.7	1		1.80	-32%	*
LYMPHOCYTE	47		17.1	25		30.29	-47%	***
NEUTROPHIL	50		18.2	73		31.35	45%	***
EOSINOPHIL	1		0.9	1		0.68	-10%	
BASOPHIL	0		0.85	0		0.68	-33%	***

SHAM CONTROL GROUP VS CONTROL GROUP

	T5 MEAN		SEM	T20 MEAN		SEM	%Δ	
MONOCYTE	2		2.19	1		1.74	-49%	**
LYMPHOCYTE	53		14.05	17		31.84	-67%	***
NEUTROPHIL	43		14.30	80		32.99	87%	***
EOSINOPHIL	1		0.95	1		0.78	-13%	
BASOPHIL	0		0.81	0		0.78	0%	

SHAM CONTROL VS. CONTINUOUS ECT

	T5 MEAN		SEM	T20 MEAN		SEM	%Δ	
MONOCYTE	4		2.96	1		1.39	-67%	**
LYMPHOCYTE	53		9.32	19		7.60	-65%	**
NEUTROPHIL	41		7.63	79		8.85	95%	**
EOSINOPHIL	1		0.82	2		0.88	11%	
BASOPHIL	0		0.55	0		0.64	-56%	

SHAM CONTROL VS. INTERUPTED ECT

	T5 MEAN		SEM	T20 MEAN		SEM	%Δ	
MONOCYTE	3		2.75	1		1.30	-53%	
LYMPHOCYTE	53		11.12	20		9.16	-62%	**
NEUTROPHIL	42		9.81	77		10.08	82%	**
EOSINOPHIL	1		0.93	1		0.83	-4%	
BASOPHIL	0		0.53	0		0.62	0%	

$$\% \quad \frac{\textbf{T20 - T5}}{\textbf{T5}} \quad \textbf{X 100}$$

T5 = count at day 5
T20 = count at day 20

*p<.02, **p.002, ***p<.001, two-tailed t-test

Table 3 Summary of the effects of ECT on mean WBC differential counts in Balb-c mice implanted with Colon-25 tumors.

The effects of ECT on the differential leukocyte counts are summarized in Table 3. Data depict the change in 100 cell counts from day 5 and day 20 post tumor implantation. When compared to the differential leukocyte counts in mice without tumors, a distinct neutrophilia was found at day 20, as well as, a distinct lympopenia in all control and experimental groups. Control group mean lymphocyte and neutrophil counts on day 5 were 47 and 50 respectively, while at day 20 the mean counts changed to 25 and 73, respectively. The sham control mean counts at day 5 for lymphocytes was 53 and for neutrophils was 43, with values of 17 and 80, respectively, at day 20. These effects were also present with the experimental groups, where interrupted ECT treated groups had mean lymphocyte and neutrophil counts of 53 and 41 at day 5, respectively, and 19 and 79 at day 20, respectively. Continuous ECT treated group counts at day 5 were 53 and 42, respectively, and 20 and 77 at day 20, respectively. ECT, both interrupted and continuous, caused no statistically significant changes from the control groups in terms of the neutrophilia and lymphopenia.

CHAPTER IV

DISCUSSION

It was difficult to compare this *in vivo* study with most of the ECT studies that predominate the literature. Most of the previous research is performed on humans using ECT in conjunction with chemotherapy, radiation and surgery, with no controls. Very few studies are conducted on animals under controlled laboratory conditions. The few controlled laboratory studies that were performed on animals reached similar conclusions. Bohao et al, (1994), showed that ECT administered to mice with transplanted SRS solid tumors resulted in at least partial regression or disappearance and increased the survival time for the animals. Hai-Young and Gan-Zhong, (1994), studied the effect of ECT on immune functions of normal and tumor-bearing mice, and showed that ECT enhanced both cellular immune functions of T and B-lymphocytes and non-specific immune functions of the phagocytic system . Samuelsson (1994), combined ECT with radiotherapy in rats showing a substantial necrosis in tumor tissue.

The study was modeled after a study of Heiberg's (1991), who studied the effects of different voltages and dosages of ECT on overall effectiveness in nude mice with subcutaneous human colon cancer. Heiberg's (1991), work showed that a 7.5 v current was the most effective voltage, causing the greatest tumor reduction. Based on Heiberg's (1991), results, the current study attempted to expand on them following the previous protocol as much as possible as well as testing whether an interrupted current would have the same or greater effect than does a continuous current. There were no statistically significant

differences between the two treatments. The mean tumor size for the continuous ECT group was 1.82 cm^2 whole that for the interrupted group was 2.35 cm^2.

What possible mechanisms are involved with the anti-tumor actions of ECT? Tumors require an increase in blood vessel vasculature (angiogenesis) in order to grow larger than 1-2 mm^3 (Folkman, 1990). In addition to blood supply, tumors require optimal pH and cannot survive at a pH lower than 6.0 (Dobrowsky et al. 1991). According to Nordenstrom (1994), ECT adversely affects tumors due to several factors. First, since white blood cells, the primary tumor-fighters of the body, carry negative electric charges, placing a positive electrode directly into a tumor will attract more of the white blood cells to the tumor itself (Nordenstrom, 1994). Second, although cancer cells multiply faster than do normal cells, they are more vulnerable, the electric field created by ECT should create changes in the tumor cell environment, of which one would be a chemical reaction around the electrode. This reaction would be a build-up of Hydrogen ions (Nordenstrom, 1994). Third, around the outside of the tumor, the acidic reaction would kill some of the red blood cells or at least damage their hemoglobin, preventing delivery of oxygen to the tumor (Nordenstrom, 1994). Forth, the positive electric field should move water out of the tumor, thus shrinking it and causing the surrounding tissue to swell. This will increase pressure on the blood vessels, thereby blocking the flow of blood to the tumor (Nordenstrom, 1994). Finally, the chemical reaction at the electrodes would produce a pocket of gas, which could create a high-pressure cavity that might actually break the tumor mechanically from the inside out (Nordenstrom, 1994).

Young-Xi et al. (1997)hypothesized other therapeutic mechanisms for the impact of ECT on growth of tumors. They proposed that changes were induced to the peripheral environment of the tumor tissue, hence altering the potential of the cell membrane through

ionization and making positive and negative ions move to the electrodes. They also suggested that sodium and potassium ions move toward the cathode, carrying away a large quantity of water ,resulting in desiccated and contracted cells. Furthermore, the strong acid causes proteins to condense and precipitate, resulting in a serious disorder of electrolytes,and acidity, as well as, denaturation and necrosis of the tumor cells.

According to Douwes et al. (1997), ECT effectiveness is attributed to the sensitivity of tumor cells to changes in their microenvironment over that of normal cells. Under the application of ECT, the ionic composition of tissue fluid around cancer cells and the water content of tissues are altered considerably by electrophoresis and electroosmosis between the positive and negative electrodes, leading to the destruction of exposed tumor tissue.

The actual mechanism of the necrosis of the tumor and its overall regression from ECT treatment still remains elusive. But it is widely accepted that the disruption in the tumor microenvironment resulting in tumor necrosis and regression is a direct result from the movement of a current between the electrodes. The present study confirmed this by the appearance of large vacuoles and "crevicing" seen in the histological sections of the experimental groups.

This study produced a noticeable increase in neutrophil count (neutrophilia) concomitant to a drop in the lymphocyte count (lymphopenia) at day 20 post tumor implantation. These effects were observed in previously unpublished experiments carried out in this laboratory (Lott, personal comments). It has also been observed in other studies using Balb-c mice with mammary adenocarcinoma (Musiani et al. 1996). These increases in neutrophil counts might be attributed to ECT's role in increasing white blood cell recruitment and production at the site of the tumor.

Several areas of concern arose during this study. These centered around technical problems including (1) the use of ketamine as an anesthesia in mice, (2) the use of fabricated electrodes, (3) quantitation of the implants used, and (4) maintaining a constant current during the experimental procedure.

There was no published information found that presented the dosages needed to completely anesthetized a 25-g mouse for 10 min. The small size of the mice made the threat of over dosage a problem. During the trials of finding the proper dilutions and dosages to use safely, several mice died. The final dilution that was used (1 ml of ketamine/ 10 ml of water) still caused problems, because the effects of the drug differed with each mouse. The same dose that would completely anesthetize a specific mouse either would not have any effect on another or result in death of a mouse of the same weight. Gas anesthesia might have had better results, because it is well suited for smaller mammals.

In regards to the use of fabricated electrodes, it was hard to be completely sure if the electrodes were constructed properly to ensure the proper transfer of current to the tumors. The previous studies in the literature purchased and used prefabricated electrodes in order to insure their proper function (Heiberg, 1991). Due to the high cost of prefabricated electrodes, this study was resigned to using those fabricated by the investigator.

In order to address the problem of maintaining a constant current throughout the ECT experimentation, previous studies found in the literature used a computer controlled power-supply that continuously monitored the current, making adjustments when needed (Heiberg, 1991). The high cost of the computer prevented using a continuously controlled current. The power-supply used allowed only for the voltage to be controlled and remain constant. The current changed with the change in the tumor tissue resistance during ECT experiments. As the current was applied, ionic changes due to the release of hydrogen ions and chlorine ions

caused the resistance of the tissue to change, ultimately altering the current traveling from electrode to electrode across the tumor.

To accurately measure the amount of tumor implant given to each mouse, a grid was fixed under a petri dish so that 2 x 2-mm sections could be measured (Fig. 1). The same 13 gauge hypodermic needle was used each time to inject the tumor into the mice in order to also insure a constant amount of tumor implant. This is a very important aspect of the research, because size differences in implants could significantly alter the size and rate of tumor growth.

In summary, the data in this study show that: (1) ECT treatments, both continuous and interrupted, reduce the overall growth and size of implanted colon-25 tumors in Blab-c mice, (2) these ECT treatments produce a profound neutrophilia combined with a significant lymphopenia at day 20 post tumor implantation, and (3) ECT treatments cause histological changes to the tumor in the medulla increasing the number of vacuoles and appearance of "crevicing".

The therapeutic effects of ECT in treating all different forms of cancer can not be ignored. There have been to many significant positive results, especially from China in the past 15 years, where methods of alternative medicine have had positive results. It is crucial that more research be done into the feasibility of methods such as ECT being a useful adjuncts in certain cancer therapy protocols.

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