ELECTROCHEMICAL THERAPY APPLIED TO TREAT DOG'S CANCEROUS MAMMARY GLANDS AND MODEL FOR LYMPHOCYTES-TUMOR CELLS POPULATION UNDER ELECTROCHEMICAL THERAPY

M. Telló*, L. Oliveira***, R.T. Oliveira**, R. Zanella*, G.A.D. Dias*, A. Cardona*; C.C.F. Silva**

*Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil
**Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil
***Veterinary Teaching Hospital, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

tello@ee.pucrs.br

Abstract: This paper describes the results attained by our research group from the clinical applications of Electrochemical therapy (EChT in short) in dogs presenting mammary tumors. Sixteen dogs presenting complex carcinoma, adenocarcinoma and carcinoma were separated in four groups. Group 1 received 5 to 9 mA during 60 min, Group 2 received 10 to 15 mA during 90 min, Group 3 received 20 to 30 mA during 90 min and Group 4 received 40 to 50 mA during 90 min. The results of treatment was: CR (Complete Response) 75,0%, PR (Partial Response) 12,50% and NC (No Change) 12,50%. The total effective rate (CR + PR) was 87,50%. No pain was verified after EChT.

Introduction

The electrochemical therapy (EChT) of tumors implies that tumor area is treated with a continuous current (Direct current) or constant voltage through two or more electrodes placed inside the tumor. Reports on the anti-tumor effect of direct current are dated from the end of the 19th century. However, Prof. Björn Erik Wilheim Nordenström [1] is considered to be a pioneer in the treatment of tumors using direct current and combination therapies in patients. Prof. Nordenström, in the late of 1970s, started to treat primary lung cancers using EChT. Direct current flowing through electrodes in the tumor starts an electrolytic process, where positively charged ions (H^+, Na^+, K^+, etc) migrate to the cathode and negatively charged ions (Cl^-, etc) migrate to the anode. Electrochemical products of electrolysis are formed. Toxicity of these products has a destructive effect over the tumor. Due to these processes the area around the cathode becomes strongly alkaline (around pH 13) and around the anode becomes very acid (around pH 2) [1]. Necrosis are induced, micro thromboses that blocks the blood flow to the tumor occur [2] and immune response is stimulated. The prevalent mechanism of anti-tumor action of EChT is not yet completely understood. Fig. 1 shows an application session of EChT in a mammary gland of a dog that has cancer.

Figure 1: EChT session.

The response of EChT together chemotherapeutic drugs to treat cancer are been evaluated. Specifically, the canine coetaneous mast cell tumor are been treated with continuous electric current and prednisone (EChT + Prednisone). Mast cell tumors are one of the most common skin tumors in dogs, with variable response to surgery and chemotherapy. In our experiments four dogs presenting mast cell tumors grade I were separated in two groups: group 1 received EChT alone and group 2 received EChT + Prednisone. Complete response of 50 % was obtained in group 1 and 100% in group 2.

Materials and Methods

Animals

EChT experiments using dogs were approved by the Ethical Committee of Veterinary Teaching Hospital of Federal University of Rio Grande do Sul.

The animals had to have recurrent or progressive disease, have previously undergone another treatment, or the owner refused other standard treatment, such as surgery or systemic chemotherapy are accepted to EChT.

Each owners of dogs written informed consent before the beginning of treatment.

Six adult female mongrel dogs and ten pet female dogs were used for this experiment. The mongrel dogs were provided by the Municipal Kennel and were housed individually at the Veterinary Teaching Hospital of Federal University of Rio Grande do Sul. The pet female dogs after the treatment session were kept in the hospital for about 2 to 4 hours and then come back to your homes. After that, they were examined weekly in

ISSN: 1727-1983

order to evaluate the treatment effects and possible side effects.

Table 1 shows the general characteristics of the four groups used to evaluate the EChT response.

Table 1: EChT groups.

Group	Applied Direct Current Value (mA)	Treatment session time (min)
1	5.0 – 9.0	60
2	10.0 – 15.0	90
3	20.0 – 30.0	90
4	40.0 – 50.0	90

The dogs were placed under general anesthesia for application of direct current.

Equipment

Electronic device was used to apply the Direct current into the target volume (tumor area). Basically, the apparatus consists of an injection Direct current system which the desired value of current is previously adjusted. Indeed, the device is galvanically isolated at to uncouple the animal under treatment from the power supply. Electronic device is connected to two or more platinum electrodes. When the Direct current flows into the tumor the electrical parameters of the tumor changes. During a session of EChT in the occurrence of the tumor electrical parameters variation, the electronic device keeps the previously adjusted value of the Direct current. The current available values of the Direct current are in the range of 0.0 mA to 100.0 mA.

Electrochemical therapy procedure

Continuous current was applied in the range of 60 to 90 minutes, through two or more electrodes (the number of electrodes depends on the tumor size) inserted 2.0 to 3.0 cm apart in a cancerous mammary glands of dogs.

Mammary glands of dogs 1, 2 and 3 were treated using stainless steel electrodes. Platinum electrodes (90% Pt + 10%Rh) were used in all other dogs.

All dogs presenting mammary tumors were clinically examined, location and size of tumors were recorded and the mammary masses were submitted to aspirative needle biopsies. Thoracic radiographs were taken. The dogs were evaluated clinically after the treatment by visualization, palpation and size of the gland and measurement of body temperature.

The neoplastic glands were treated in two sessions weekly. Positive electrodes (anode) were introduced at the center of the lesion and negative electrodes (cathode) 2.0 to 3.0 cm away. Treatment was performed in two mammary glands of dogs number 1 and 2.

Follow-up physical examinations and evaluation of lesions were conducted before each session of treatment. Any event occurring at the mammary gland during or immediately after the treatment was recorded. Aspirative fine needle biopsies were taken after each four applications of EChT or with 15 days intervals after the final of the treatment.

Treatment was stopped when cytopathological exams of the treated glands showed normal gland cells or absence of neoplastic cells.

Specific characteristics of the EChT procedure are shown in Table 2.

Table 2: Specific characteristics of the EChT.

Group	Number of Electrodes	Tumor Volume (cm^3)*	Tumor
1	2	45	Complex Carcinoma
	4	67	Adenocarcinoma
	2	10	Complex Carcinoma
	4	135	Complex Carcinoma
2	4	65	Complex Carcinoma
	4	24	Complex Carcinoma
	4	35	Complex Carcinoma
	4	20	Complex Carcinoma
3	2	1	Carcinoma
	3	2	Complex Carcinoma
	4	1,6	Complex Carcinoma
	3	8	Complex Carcinoma
4	4	14	Complex Carcinoma
	4	16	Complex Carcinoma
	2	8	Carcinoma
	2	12	Complex Carcinoma

*The volume was evaluated using the expression [3]: $V = \pi abc/6$, a, b and c are three orthogonal diameters.

Results

All dogs tolerated well the sessions of anesthesia and treatment. Anesthetic recoveries were uneventful. The dogs were submitted to general anesthesia because of the excited temperament of the dogs.

All treated mammary glands, after an initial increase of the mass presumably due to edema, foci of necrosis and liquefaction of the neoplasm occurred. Decrease of tumour mass occurred gradually, caused by inflammation, macrophage cells and necrosis induced in the tumor by the continuous current treatment. Destruction of tumour cells was confirmed through cytopathological exams at the end of the treatment [4].

Figure 2 shows the obtained results.

Three dogs were sacrificed. Necropsies were done in these 03 dogs to evaluate the possibility of systemic effects caused by EChT. No alterations were found in adjacent tissues of treated region, as well as in distant organs.

The EChT results were:
Groups 2, 3 and 4 CR 100%
Group 1 PR 50% and NC 50%

The next item shows a proposal model to describe the dynamics of cancer cells under the influence of EChT. This model is not new, and is an adaptation of the model used in reference [5].

ISSN: 1727-1983

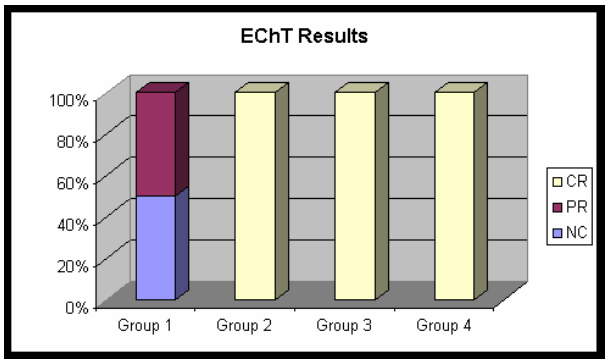

Figure 2: EChT results.

Model lymphocytes-tumour cells under EChT

In this model, we try to describe the dynamics of the cancer cells and three select elements of the immune system, the NK, LAK and T helper cells into the anatomic region of the tumor. The components are identified by their concentration, without taking into account their three-dimensional distribution. Here, the number of cancer, NK, LAK and T helper cells are denoted, respectively, by T(t), K(t), L(t) and H(t). In this model we are not considering the existence of metastasis or interactions with primary tumor. With this intent, the following equations were considered from reference [5]:

$$\frac{dT}{dt}(t) = aT(t) - bT(t)K(t) - cT(t)L(t)\,, \qquad (1)$$

$$\frac{dK}{dt}(t) = d - eK(t) - f\,H(t)K(t) + gT(t)\,, \qquad (2)$$

$$\frac{dL}{dt}(t) = -eL(t) + f\,H(t)K(t) \qquad (3)$$

and

$$\frac{dH}{dt}(t) = h - kH(t) + m\,H(t)\left[T(t) - nT(t)^2\right], \qquad (4)$$

where:

- aT(t) represents the cancer cells proliferation into the anatomic region of the tumor;
- -bT(t)K(t) and -cT(t)L(t) represents the cancer cells inhibition, under the actuation of the NK and LAK cells, respectively, into the anatomic region of the tumor;
- d represents the entrance of the NK cells into the blood flow;
- -eK(t) and -eL(t) represents the apoptosis of the NK ans LK cells, respectively, into the anatomic region of the tumor;
- f H(t)K(t) represents the NK cells transformation into LAK cells into the anatomic region of the tumor;
- gT(t) represents the increasing of NK cells under the presence of the cancer cells into the anatomic region of the tumor;
- h and -kH(t) represents, respectively, the bone marrow arising and the apoptosis of T helper cells into the anatomic region of the tumor;
- mH(t)(T(t) – n $T(t)^2$) represents the T helper cells proliferation, under cancer cells presence into the anatomic region of the tumor.

To solve this system of nonlinear stiff differential equations we use the IMSL DIVPAG subroutine. This subroutine is used to solve an initial-value problem for differential equations using either the implicit Adams-Moulton method (up to order twelve) or the backward differentiation formulas BDF (up to order five, called Gear's Stiff method).

To show the efficiency of this methodology to solve eqs. (1-4), we consider a problem described by eqs. (1-4) and initial conditions T(0) = 1000, H(0) = 700, K(0) = 500 and L(0) = 1 cells. The parameters chosen are: a = 0.35, b = 0.00001, c = 0.001, d = 0.68, d = 0.0014, f = 0.1, g = 0.002, h = 0.38, k = 0.00055, m = 0.0005 and n = 0.0004. The results for the T(t), K(t), L(t) and H(t) number of cells are presented in Figure 3.

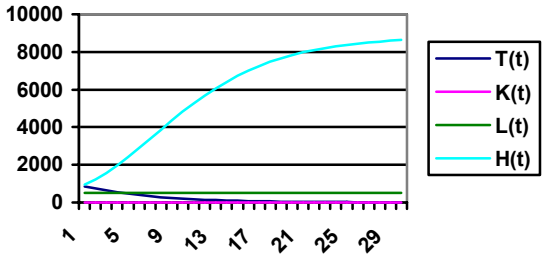

Figure 3: The results for the T(t), K(t), L(t) and H(t)

The most parameters used in the simulation must be measured (it isn't a easy task and it is very expensive). Nevertheless, a mathematical tool is available to analyze (in the future) the dynamic behavior of immune system under the influence of DC Current.

Figures

Next figures 4 – 19 shows EChT of some animals treated using direct current.

ISSN: 1727-1983

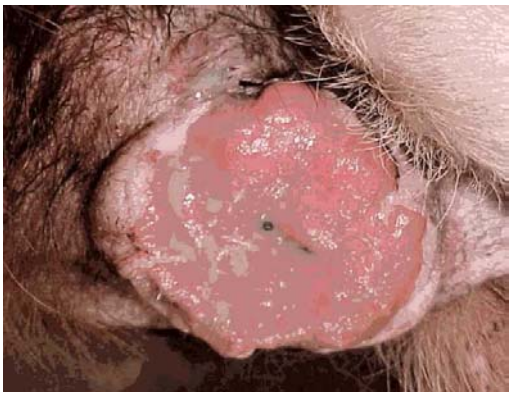

Figure 4: Dog 1-tumor before EChT.

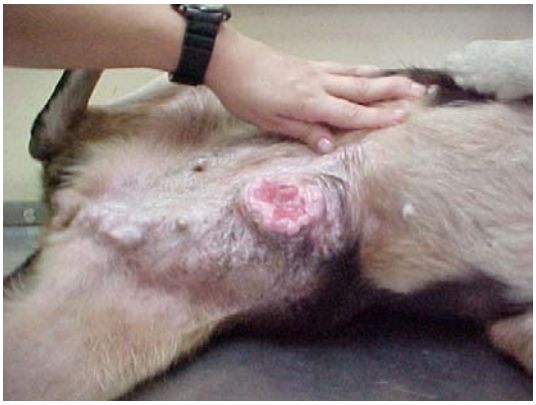

Figure 5: Dog 1- end of EChT (CR).

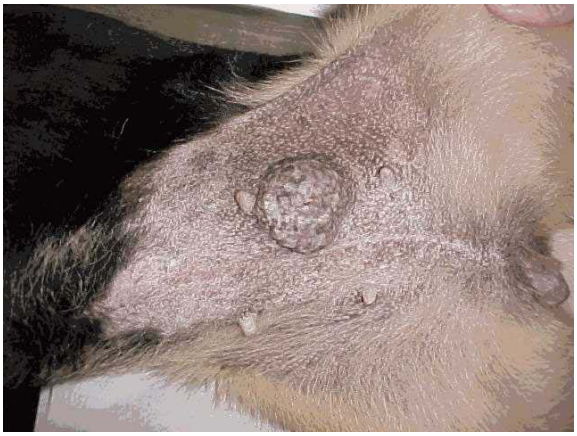

Figure 6: Dog 3-tumor before EChT.

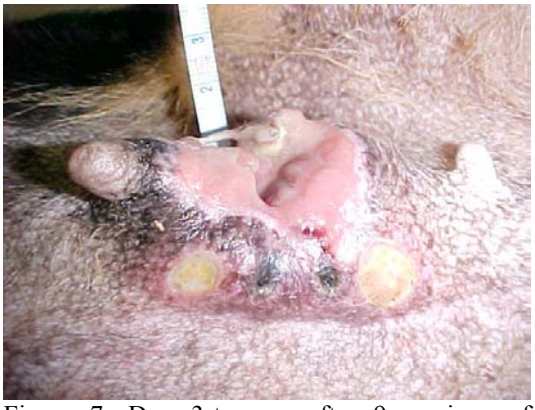

Figure 7: Dog 3-tumour after 9 sessions of treatment (31 days after the 1st DC current application).

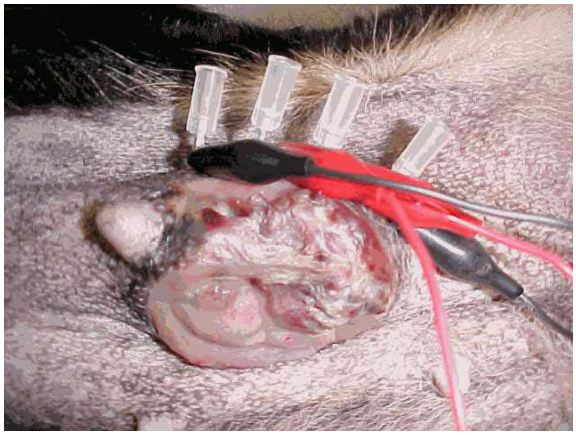

Figure 8: Dog 3-electrode dispositions (two anodes (center) and two cathodes (periphery) of tumour).

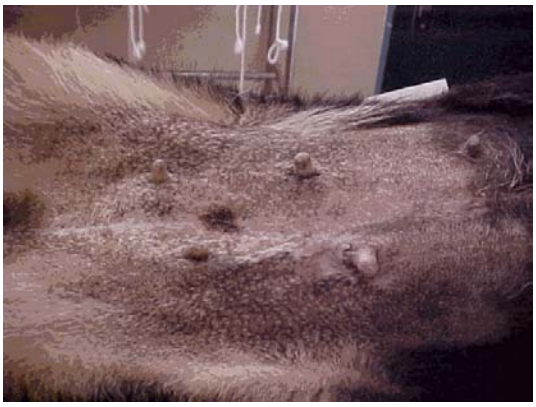

Figure 9: Dog 3-end of EChT (CR).

Figure 10: Dog 4-tumor before EChT.

Figure 11: Dog 4-tumor after 7th session of EChT (34 days after the 1st DC current application).

ISSN: 1727-1983

Figure 12: Dog 4-treated area 50 days after 1st DC current application (CR).

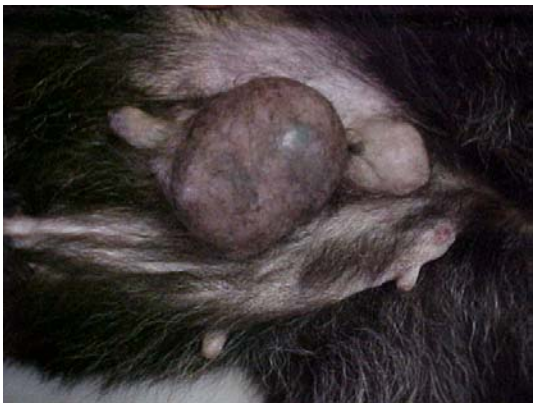

Figure 13: Dog 6-Tumor before EChT.

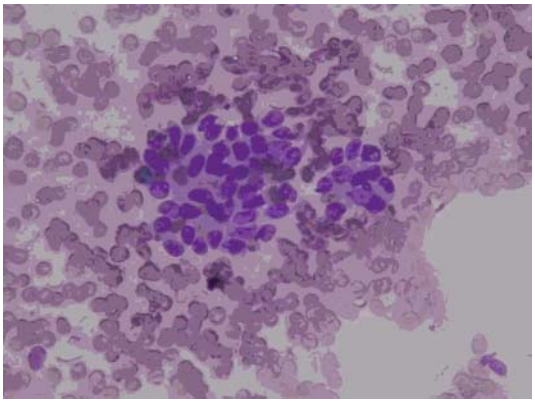

Figure 14: Dog 4 – cancer cells before EChT.

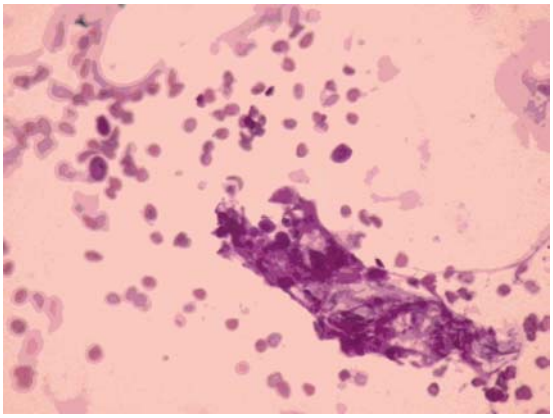

Figure 15: Dog 4 – Cellular debris and macrophage cells after EChT.

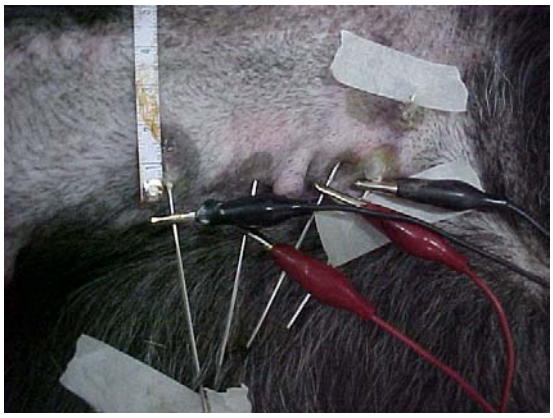

Figure 16: Dog 6-necrotic area formation.

Figure 17: Dog 6-CR after EChT.

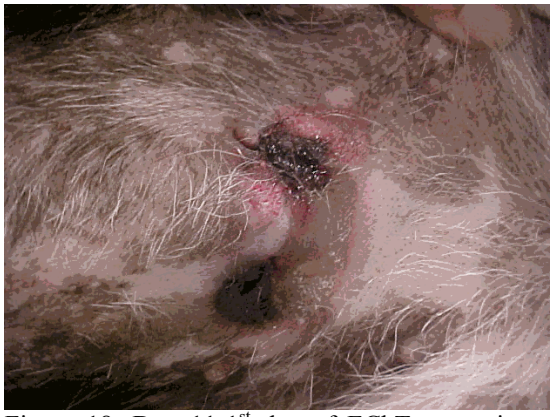

Figure 18: Dog 11-1st day of EChT: necrotic area formation.

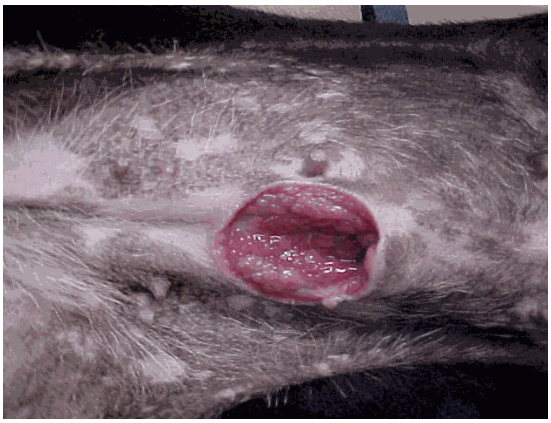

Figure 19: Dog1 1-After 8 days of EChT.

ISSN: 1727-1983

Conclusions

Our results suggest that EChT is safe and effective process for destroying neoplastic cells. Up to now the treatment results were: 75.0 % Complete Response (CR), 12.5 % Partial Response (PR), 87.5 % CR + PR and 12.5 % Non-Change (NC). However, many doubts about EChT persist. EChT appear to provide: clinical effectiveness, low cost, improve the quality of life for the patient, ability to administer repeat treatments, low side effects, compatibility with other cancer therapies, high benefit/cost ratio for the patient, EChT does not depend upon the cell cycle for therapeutic efficacy and pores in the cancer cell membrane are open under the electric field exposure enhancing the killing effect by influx of anticancer drugs.

Indeed, to solve the system that describes the cells dynamic under EChT (system of nonlinear stiff differential equations) we use the IMSL DIVPAG subroutine. This subroutine is used to solve an initial-value problem for differential equations using either the implicit Adams-Moulton method (up to order twelve) or the backward differentiation formulas BDF (up to order five, called Gear's Stiff method). The parameters used in the simulations must be measured in the future

References

[1] NORDENSTRÖM, B. E. W. (1983): 'Biologically Closed Electric Circuits – Clinical, Experimental and Theoretical Evidence for an Additional Circulatory System', (Nordic Medical Publications, Sweden).

[2] TELLÓ, M., DIAS, G. A. D, CARDONA, A. V., RAIZER A. (2001): 'Tumor Compression Due Application of DC Current', IEEE Transactions on Magnetics, **37**, pp. 3753-3756.

[3] MIKLAVCIC, D. et alli (1993): 'Tumor Treatment by Direct Current – tumor temperature and pH electrode material and configuration', Bioelectrochemistry and Bioenergetics, pp. 209-220.

[4] TELLÓ, M. et alli. (2004): 'O Uso da Corrente Elétrica no Tratamento do Câncer' (in Portuguese), (EDIPUCRS, Porto Alegre, Brazil) First Edition.

[5] SZYMANSKA, Z. (2003): 'Analysis of Immunotherapy Models in the Context of Cancer Dynamics', Int. J. Appl. Math. Comput. Sci., **13**, pp. 407–418.

ISSN: 1727-1983