EDVARD KARDELJ UNIVERSITY, LJUBLJANA
FACULTY OF ELECTRICAL AND COMPUTER ENGINEERING,
* INSTITUTE OF ONCOLOGY, LJUBLJANA
YUGOSLAVIA

LOW INTENSITY DIRECT CURRENT AS AN ANTITUMOR AGENT?

Miklavčič D, *Serša G, Magister S, Reberšek S, Vodovnik L.

Abstract – New cancer treatment modalities are in demand since many clinical cases do not respond to conventional approaches. In the past some successful applications of electricity for tumor growth retardation have been reported. In our preliminary study a fibrosarcoma (Sa–1) grown subcutaneously in A/J mice was used. Direct current (DC) with amplitude of 0.6 mA was delivered daily for 15 minutes through stainless steel electrodes, positive electrode being inserted into the tumor site and negative subcutaneously in the vicinity of the tumor. Tumor volume measurements in control and DC treated groups showed significant ($p<0.005$) retardation of tumor growth in treated animals. Histological examinations and evaluation of the extent of necrosis in excised tumors at the end of experiment were performed. No differences were found. The results also point to the necessity of further attempts of current level and related treatment time optimization for improvement of antitumor effect of direct current.

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Introduction – New cancer treatment modalities are in demand, since many clinical cases do not respond to the conventional approaches of surgery, radiotherapy, chemotherapy neither to some newer modalities like hyperthermia (1) and immunotherapy (2). The control of growth and differentiation in biologic systems are some of the most intriguing problems.

A casual case, reported by Eason in 1776 (3), pointed to the possibility of employing electricity in containment of tumors. The spirit of enthusiasm intiated therapeutic experimentation with electricity during the eighteenth century. Later on, the electronegativity of growing region with respect to a slower growing or nongrowing region in the same organism was observed. It was shown that a growing fetus or a growing uterine tumor would cause the uterus to be electronegative to the outer abdominal surface (4, 5). Moreover, Burr (4, 6) stated, that the pattern of the potential differences defined an electrodynamic field whose forces critically influence the growth and development in living organisms.

These findings encouraged researches to employ different external sources of electrical energy in order to slow or to stop the rapid tumor growth (7–12) by means of altering potential being inherent to it.

Humphrey and Seal (7) worked with sarcoma 180 in mice and in 1959 they reported encouraging results of the biologically acceptable low level electrical direct current (DC) therapy. They reported few examples of complete tumor regression in experimental animals which survived a year after tumor free. Recently some experiments concerning effects of low level DC current on tumor growth in vivo (8–11) have been reported. In clinical pratice similar approach of electrotherapy with very promising results have been applied by Nordenström (12).

The aim of our study was to answer the question whether low intensity direct current has an antitumor effect on murine fibrosarcoma.

Materials and Methods – Animals and Tumors: Inbred A/J mice were purchased from the Institute Ruđer Bošković, Zagreb, Yugoslavia. They were 8–12 weeks old at the start of the experiment. Mice were of the same sex, and were housed 5–7 per cage.

Animals were weighed every two days in order to follow their body condition. Animals in both groups lost their weight during the treatment for approximately 10%. No difference was found between DC treated and control group.

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Sarcoma (Sa–1), syngeneic to A/J mice was used. Tumors were generated by subcutaneous injection of $5x10^5$ viable tumor cells dorsolaterally. Single tumor cell suspension was obtained from ascitic fluid of the tumor bearing animals.

Treatment: Seven days after the inoculation, when tumors reached the average volume of 45 ± 17 mm^3 (average diameter of 4.4 mm), the treatment began. Tumors with average diameter of less than 3.5 mm were excluded from the experiment. Animals were randomly divided into four groups (A to D), each one consisting of six mice, marked individually. The treatment current was provided by a battery powered constant current source (manufactured in Laboratory of Biocybernetics, Faculty of Electrical and Computer Engineering, Ljubljana, Yugoslavia) and was set to the value of 0.6 mA which produced current density of less than 80 μA/mm^2 (calculated on geometrical electrode area). Four current sources were marked from A to D and each of them was used for the treatment of equally marked group. Current sources A and D were active while B and C were placebos, inactivated by independent technician. The investigators who performed the experiment, measurements and analyses ignored these data and they knew them only when the analyses were completed. Their hypothesis was compared then to the real situation.

The treatment began on the seventh day after inoculation and was continued for nine consecutive days. Experimental animals received 15 minutes of electrotherapy daily. Active electrode (anode) which was inserted directly into the tumor site, was positive, while negative electrode (cathode) was placed subcutaneously 25–30 mm caudally. All animals underwent apparently the same treatment, but two current sources were placebos and two were active. The proper performances of all current sources were checked every three days by a technician who was not involved otherwise in the experiment. The treatment was carried out in absence of anaesthesia.

Electrodes: Electrodes made of stainless steel were 20 mm long, 0.7 mm in diameter, with rounded tip. Anode was isolated except upper four millimeters whereas cathode was not. Sets of electrodes were changed after approximately every hour of operation because of erosion of the anode in order to keep the chosen current density as constant as possible, otherwise it would be changed due to variation of electrodes active area.

Collection of Experimental Data: On each day of treatment, day seven to 15 and the day 16, tumor volume was deduced (eq. 1) from three principal diameters (13) measured with vernier caliper by a third person. On the day 16 mice were sacrificed and autopsies were performed. The tumors were excised, histologically examined and the percentage of necrosis along greatest tumor diameter was evaluated (14).

$$V = \frac{\pi a b c}{6} \qquad \ldots 1$$

The treatment was started with 24 animals (12 in each group, control and DC treated). During the experiment four animals were excluded from further consideration because of the tumor exulceration. At the end of the experiment another two animals were taken out of consideration because of fast growing tumor satellites, induced at inoculation, that initiated problems in tumor diameter measurements. Taking into account all these, our analyses and all the following result are based on the control and DC treated group, consisting of eleven and seven animals, respectively.

Results – Antitumor Effect of DC Current Treatment: Effect of the DC current is presented on the tumor growth curves by average tumor volume with standard error of the mean for control and DC treated animals (Fig. 1). For each day the significance test (t-test) has been performed and the values of t-statistics are listed, as well. The average tumor volume in the DC treated animals was significantly smaller compared to those of control group with the

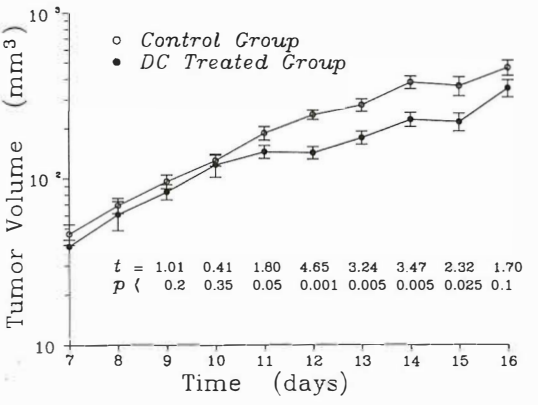

Fig. 1 – Tumor growth during the period when direct current treatment was applied; vertical bars: standard error of the mean.

probability of 95% ($p<0.05$) from day 11 to day 15 and on days 12 to 14 with the probability of more than 99.5% ($p<0.005$).

Histological Appearance: In all animals focal necrosis was visible on the surface of the tumor. Postmortem examination showed no electrical injury in tumor or surrounding tissue. Microscopic examination of the histological sections showed no difference between control and DC treated tumors with respect to inflammatory cells infiltration and tissue burns. The evaluation of the tumor necroses of the excised tumors at the end of the experiment, 49 ± 9% in DC treated group and 47 ± 8% (AM ± SD) in control group, did not show massive degenerative changes in the tumors of the DC treated group.

Discussion – The direct current level and the geometry of electrodes in our experiment was chosen to produce a current density of 75 $\mu A/mm^2$ and a charge density of 68 mAs/mm^2. These values were calculated on the basis of active geometrical electrode area and can be assumed considerably lower taking into account the »real« electrode area which can be even more than 1.4 times larger (15). Current and charge densities used can therefore be classified in the lower range of those used by other experimenters (7–11).

The antitumor effect of low level direct current applied can be observed. The results were obtained after very careful design and performance of the experiment and are comparable to the results obtained by Humphrey and Seal (7) with similar protocol. The applied 15 minutes per day therapy obviously yields antitumor affect, but is not sufficient for radical tumor treatment. Therefore we expect the prolongation of daily treatment to express better tumor growth retardation (7, 10).

Our further work on the same tumor model is concentrated on the optimization of DC current level and the related treatment time in order to achieve better antitumor effect.

The other branch of our research work is the interaction of DC current treatment and biological response modifiers (interferon – α, interleukin 2, tumor necrosis factor).

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Izvleček

PROTITUMORSKO DELOVANJE ŠIBKIH ENOSMERNIH ELEKTRIČNIH TOKOV?

Doslej znane zvrsti zdravljenja raka v mnogih primerih nimajo zadovoljivega učinka, zato še vedno iščemo nove načine terapije. V zgodovini je znanih nekaj poročil o uporabi enosmernega električnega toka za zdravljenje tumorjev. V naši preliminarni slepi študiji smo testirali vpliv enosmernega električnega toka (0.6 mA) na zavoro rasti eksperimentalnega fibrosarkoma (Sa–1), injiciranega v podkožju A/J miši. Ponavljajočo terapijo (15 minut dnevno), 9 zaporednih dni, smo izvajali z jeklenimi elektrodami, tako da je bila anoda (+) vstavljena v sredino tumorja, katoda (–) pa v podkožje v bližino tumorja. Meritve volumnov tumorja v skupini živali, zdravljenih z enosmernim tokom, kažejo, da je prišlo do statistično značilne upočasnitve rasti v primerjavi s kontrolno skupino ($p<0.005$). Terapija z enosmernim električnim tokom ni uplivala na obseg nekroze tumorjev na koncu terapije in ni povzročila vnetne reakcije v tumorju.

Rezultati kažejo, da električni tok lahko zavira rast eksperimentalnega tumorja, vendar je za radikalnejšo terapijo potrebna optimizacija terapevtskih parametrov in terapevtske sheme.

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Author's address: D. Miklavčič, el. eng., Faculty of Electrical and Computer Engineering, Tržaška 25, 61000 Ljubljana, Yugoslavia.

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