Cancer Letters, 69 (1993) 81–84
Elsevier Scientific Publishers Ireland Ltd.

Potentiation of bleomycin antitumor effectiveness by electrotherapy

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(Received 30 November 1992)
(Accepted 15 December 1992)

Summary

Electrotherapy was investigated for its ability to increase the responsiveness of murine tumors to bleomycin treatment. Mice bearing fibrosarcoma were treated with 250 μg bleomycin and then with 0.6 mA direct current (DC) for 60 min. Antitumor effects of single treatments were moderate with bleomycin, but significant with electrotherapy. Combined treatment with bleomycin followed by electrotherapy was more effective than either treatment alone. Tumor growth delay of the animals after combined treatment was greater than the summation of tumor growth delays after single treatments. The results of our study indicate that bleomycin and electrotherapy treatments interact, with electrotherapy potentiating the effectiveness of bleomycin treatment.

Keywords: bleomycin; electrotherapy; fibrosarcoma experimental

Introduction

Electrotherapy with direct current (DC) has proved to be an effective antitumor agent on several animal models [3,4,6,19] as well as in clinics [8,14,22]. The mechanisms of its antitumor action remain unclear, although biochemical reactions in the vicinity of the electrodes were recognised as one [10]. Besides, possible influences of electric current on tumor cells were suggested [1,5,21]. In order to avoid mechanical and biochemical intrusion into the tumor, an attempt was made to subject the tumor to electric current by electrodes, inserted subcutaneously in the vicinity of the tumor. In this electrode configuration virtually the same antitumor effect was achieved as with electrodes inserted directly into the tumor [10]. Nevertheless, with all different electrode configurations partial control of the tumor growth was achieved, however the tumors eventually regrew from the remaining viable tumor cells [9]. In order to potentiate the efficacy of electrotherapy, some attempts were made, by combining electrotherapy with systemic chemotherapy [7,15] or immunotherapy [17,18].

Bleomycin is used in the treatment of many malignancies [20]. It is an antitumor agent which is generally believed to exert its chemotherapeutic effect through damage to cellular DNA, by single and double strand breaks [2]. However, plasma membrane seems to limit the passage of the drug into the cell and therefore impedes its efficacy [16]. Some attempts were made to increase drug uptake by transient membrane electroporation [11,12] and by drug targeting using direct current [7,23]. Bleomycin is positively charged and therefore attracted by the negative electrode [23]. Increasing

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Printed and Published in Ireland

drug uptake by electric pulses and drug targeting by direct current are some ways of facilitating bleomycin chemotherapy. No previous information was available until now about interaction of bleomycin with non-invasive electrotherapy, when electrodes are not inserted into the tumor.

The aim of our study was, to determine antitumor effectiveness of electrotherapy, where electrodes were placed outside the tumor in combination with bleomycin. The interaction of the two treatments was determined by tumor growth delay of subcutaneous fibrosarcoma tumors in mice.

Materials and Methods

Animals

Female A/J mice were purchased from Rudjer Bošković Institute, Zagreb, Croatia. Animals were maintained at constant room temperature (24°C) at a natural day/night cycle, in a standard animal colony. Mice in good condition, without signs of fungal or other infections (8–10 weeks old) were used in the experiments. Each experimental group consisted of 7–11 mice.

Tumors

Fibrosarcoma SA-1 cells, syngeneic to A/J mice, were obtained from the ascitic form of the tumor. Solid subcutaneous tumors, dorsolateral in animals, were initiated by injection of 5×10^5 viable SA-1 cells. After the tumors reached 30–40 mm^3 in volume, animals were randomly divided into experimental groups on day D0. On each consecutive day the tumor volume was calculated from orthogonal tumor diameters measured by vernier calliper gauge. Tumor doubling time (DT) was determined for individual tumors and tumor growth delay (GD) from the mean DT of experimental groups [18]. The non-parametric Mann-Whitney Rank-Sum test was employed for comparison of tumor volumes. Student's *t*-test was employed for statistical evaluation of results presented by means of growth delay after the *F*-test was fulfilled.

Bleomycin therapy protocol

Bleomycin was purchased from Mack, Illertissen, Germany. It was dissolved in physiological saline (250 μg/0.5 ml concentration) and injected into the lateral tail vein of the animals. In the combined treatment, animals were treated with bleomycin 60 min before electrotherapy.

Electrotherapy

The DC source was designed and manufactured at the Faculty of Electrical and Computer Engineering, Ljubljana, Slovenia. Current and voltage were continuously monitored during electrotherapy with 0.6 mA DC of 1-h duration. Current was delivered through Pt/Ir (90/10%) alloy needle electrodes (1.0 mm diameter, 22.0 mm long) with rounded tips and inserted subcutaneously 5–10 mm from the margin of the tumor on the two opposite sites. The control group was treated in the same way as the experimental group, except that no current flowed.

Results

Antitumor effect of bleomycin

Systemic intravenous treatment of animals with 250 μg bleomycin had moderate, statistically signi-

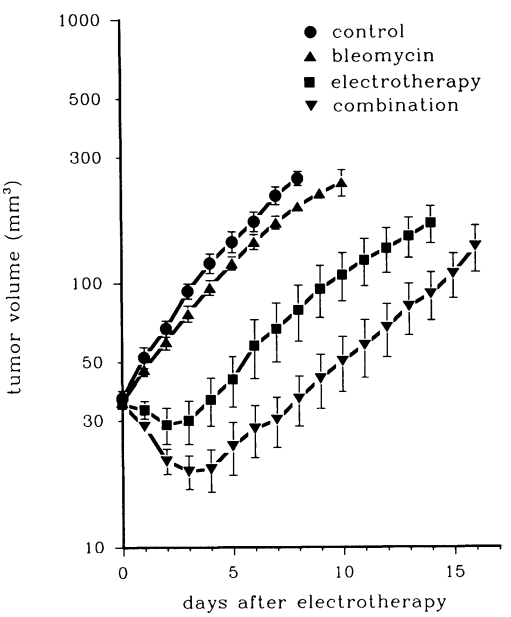

Fig. 1. Growth curves of SA-1 tumors after bleomycin (250 μg) or/and electrotherapy treatment with 0.6 mA direct current treatment for 1 h. Electrodes were placed subcutaneously 5–10 mm from the margins of the tumor.

ficant antitumor effect on SA-1 subcutaneous tumors starting 4 days after the treatment ($P < 0.05$) with tumor growth delay 0.5 ± 0.2 days ($\nu = 12$; ν, degrees of freedom) (Fig. 1).

Antitumor effect of electrotherapy

Treatment of SA-1 tumors with 0.6 mA DC for 1 h had statistically significant antitumor effect ($P < 0.001$), starting the first day after the treatment (Fig. 1). Tumors regressed for the first 4 days, but thereafter outgrew again and their growth rate was the same as in the control group. Growth delay of the electrotherapy-treated group was 6.7 ± 1.3 days ($\nu = 15$).

Antitumor effect of combined electrotherapy and bleomycin treatment

When combining electrotherapy and bleomycin treatments, the tumors regressed quicker than after electrotherapy alone and outgrew again only after 7 days. Thereafter their growth rate was the same as in the electrotherapy treated group (Fig. 1). Tumors were statistically significantly smaller compared to the electrotherapy group starting 8 days after the treatment ($P < 0.05$). Also tumor growth delay was statistically significantly greater than growth delay achieved in the electrotherapy group (10.8 ± 1.9 days, $\nu = 16$, $P < 0.05$).

Discussion

This study shows that electrotherapy with electrodes placed subcutaneously outside of the treated tumor and bleomycin treatment interact, with electrotherapy potentiating the effectiveness of bleomycin treatment.

Electrotherapy alone proved to be effective in controlling local tumor growth. Several electrode configurations and current levels were employed [9,19], their effectiveness being moderate to good. In our previous study we demonstrated that electrotherapy with a cathodic electrode inserted into the tumor, and electrotherapy with electrodes placed outside the tumor, 5–10 mm from the tumor margin, have apparently the same antitumor effect [10].

Nannmark et al. [13] reported increased capillary permeability to macromolecules by direct current. They demonstrated that current densities similar to those in our study, induced macromolecular leakage on the capillary level. The observed interaction of electrotherapy and bleomycin in treatment of sarcoma tumors may be attributed to increased permeability of capillaries and therefore increased drug accumulation in the tumors.

Since bleomycin is positively charged, it can be accumulated around the cathode and therefore higher concentrations of drug attracted into the tumor [23]. In our study, however electrodes were outside of the tumor and nevertheless we demonstrated interaction of the two treatments in antitumor effectiveness.

The other mechanism could be transient electroporation of the cell membrane leading to better effectiveness of the drug at lower concentrations. This mechanism was demonstrated by use of electric pulses [11,12]. With direct currents this possibility has not been demonstrated and seems not plausible at the electric field level applied in our study.

The results of our study indicate that combined treatment of electrotherapy and bleomycin interact so that electrotherapy potentiates effectiveness of bleomycin in vivo. Observed potentiation of bleomycin effectiveness by local electrotherapy is interesting, since substantial antitumor effect can be achieved with administration of small amounts of the drug, which limits its side effects.

Acknowledgement

This research was supported by The Ministry of Science and Technology of the Republic of Slovenia.

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