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Tumour treatment by direct electric current: electrode material deposition

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Abstract

Direct current electrotherapy was demonstrated to be an effective and relatively inexpensive local treatment of murine solid subcutaneous tumours. The insufficient knowledge of the mechanisms involved hinders its more extensive use in clinical oncology. Attempts were made to establish the correlation between the effectiveness of electrotherapy and deposition of the electrode material in the tumours. Electrotherapy was performed as a 1 h single-shot treatment in different electrode (gold) configurations, one electrode inserted in the tumour being the anode and its pair subcutaneously in the vicinity of the tumour being the cathode, and vice versa, and with both electrodes placed subcutaneously in the tumour vicinity. Tumour growth and the amount of electrode material in tumours were determined at different times after single-shot electrotherapy with gold needle electrodes. Different electrode configurations were employed with respect to tumours at the same current level and different current levels were used in the same electrode configuration. The amount of gold deposited in the tumour was observed with respect to tumour response to electrotherapy. Tumour growth after 0.6 mA direct current electrotherapy was significantly retarded with respect to controls regardless of the electrode configuration, with no significant differences among them. The amount of gold per tumour, however, varied significantly with the electrode configuration. In the experiments in which both electrodes were placed subcutaneously outside the tumour, increasing current intensities produced a "dose"-dependent tumour growth response to electrotherapy which was not related to the amount of gold found in the tumours. The results obtained in this study showed that the electrode material deposition (gold) and possibly consecutive metal toxicity were not the major mechanism involved.

Keywords: Tumour treatment; Direct electric current; Electrode material deposition

1. Introduction

Direct electric current has been reported as having antitumour activity in different tumour models [1–8] and in human clinical trials [1,9,10]. Its efficacy varied due to the different currents used (μA to tens of mA) [3,11,12] duration of electrotherapy (15 min per day to continuous treatment) [13,14] and was additionally influenced by different tumour models, electrode materials and geometry employed. In a previous study we used currents ranging from 0.1 to 1.0 mA delivered via needle electrodes made of different metals (Pt, Pt–Ir, Au, Ag, Ti, stainless steel) in various configurations [15]. The measurements of tumour bioelectric potential [13] temperature [15] and pH [15] did not provide satisfactory answers about the mechanisms involved.

One of the physico-chemical reactions associated with direct current flow in the tissue is also metal anode dissolution. Metal anions dissolved from the positive electrode diffuse into the surrounding tissue where they can react with inorganic and organic species and potentially form toxic products [16]. In high concentrations metals by themselves are known to have toxic properties. Also, the way in which metals were found to have an antitumour effect [17] led us to investigate the amount of electrode material deposited in a tumour after the direct current electrotherapy of tumours. In order to determine whether metal deposited from electrodes during electrotherapy has an antitumour effect, we sought to establish a correlation between metal deposited in tumours and antitumour effectiveness using different currents and electrode

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configurations. Electrotherapy was performed with different electrode configurations and current levels. The electrodes were made of gold wire and the results for the amounts of gold deposited in tumours were compared with growth of the tumours following the electrotherapy with the same electrode configurations and current levels.

2. Experimental

2.1. Animals and tumours

Female and male mice of A/J strain were purchased from the Rudjer Bosković Institute, Zagreb, Croatia. Animals were maintained at constant room temperature (24°C) with a natural day/night light cycle in a conventional mouse colony. For the experiments, mice in good condition, without signs of fungal or other infection, 8–12 weeks old were used. Fibrosarcoma Sa-1 cells for tumour transplantation were obtained from the ascitic form of the tumour. Subcutaneous solid tumours were initiated by injection, dorsolaterally in the animals, of 5×10^5 viable fibrosarcoma Sa-1 cells syngeneic to A/J mice. Animals inoculated with tumour cells were maintained in plastic cages until the tumours reached approximately 50 mm^3 in volume. Subsequently they were marked individually and randomly divided into smaller groups subjected to a specific experimental protocol. Animals receiving the same treatment (experimental groups) were maintained together in smaller plastic cages (5–8 per cage) and were fed ad libitum.

2.2. Electrodes and electrotherapy

Electrodes were inserted as follows: (i) one electrode with its tip in the tumour and the other subcutaneously (s.c.) in the vicinity of the tumour 5–8 mm distant from tumour edge or (ii) both electrodes s.c. in the vicinity of the tumour 5–8 mm distant from tumour edges. The former was considered as cathodic electrotherapy when the electrode inserted directly in the tumour was negative and anodic when the polarity was reversed. The latter electrode configuration was named "field" electrotherapy. In the experiments three controls were also designed: (iii) pure, no electrodes being inserted: (iv) electrode control, which corresponded to electrode configuration (i); and (v) field control, which corresponded to configuration (ii). In the controls no current flowed, but the electrodes were inserted for 1 h as specified.

Electrotherapy was performed as a single-shot treatment of 1 h duration with 0.6 mA as cathodic, anodic and field electrotherapy after the tumours had reached 30–40 mm^3 in volume. In the field electrotherapy configuration, in addition to 0.6 mA currents of 0.1, 0.2 and 1.0 mA were also employed. The current and voltage were monitored constantly during the electrotherapy.

2.3. Tumour response to electrotherapy assessment

A tumour's response to the electrotherapy was determined by measuring three main tumour diameters every day after treatment by means of a vernier calliper. The tumour volume was estimated using the equation $V = \pi abc/6$, where a, b and c are three measured tumour diameters. For each day the mean tumour volume with the corresponding standard deviation was calculated in each experimental group. Mean volumes and standard deviations were presented as tumour growth curves. For each tumour the doubling time (DT) was estimated. The mean tumour doubling time was calculated along with the corresponding standard deviation for each specific experimental group as mean $\pm$ s.d. (n). The difference between experimental groups was statistically tested by means of the Mann–Whitney rank-sum test employing Bonferoni's correction when multiple comparisons were done.

2.4. Determination of gold deposited in tumours

Tumours were excised immediately after the end of 1 h of electrotherapy, i.e. at 0 h, or 1, 4, 8, 24, 48 or 96 h after the end of a single-shot electrotherapy. The total amounts of gold in the tumours were determined by instrumental neutron activation analysis (INAA) [18] measuring the induced activity of the ^{198}Au isotope. The main nuclear characteristics of this isotope are presented in Table 1. Lyophilized, carefully weighed samples and corresponding standards (20 μg Au ml^{-1}) were sealed into plastic tubes and irradiated simultaneously in the pneumatic system of the TRIGA Mark II

Table 1
Nuclear characteristics of gold important for instrumental neutron activation analysis [19]

Reaction	Abundance of nuclide/%	Half-life of product/days	Cross-section/barn	Major γ-lines/keV (absolute abundance/%)
$^{197}Au(n, \gamma)^{198}Au$	100	2.693	98.9	411.8 (95.5); 675.9 (0.8); 1087.7 (0.16)

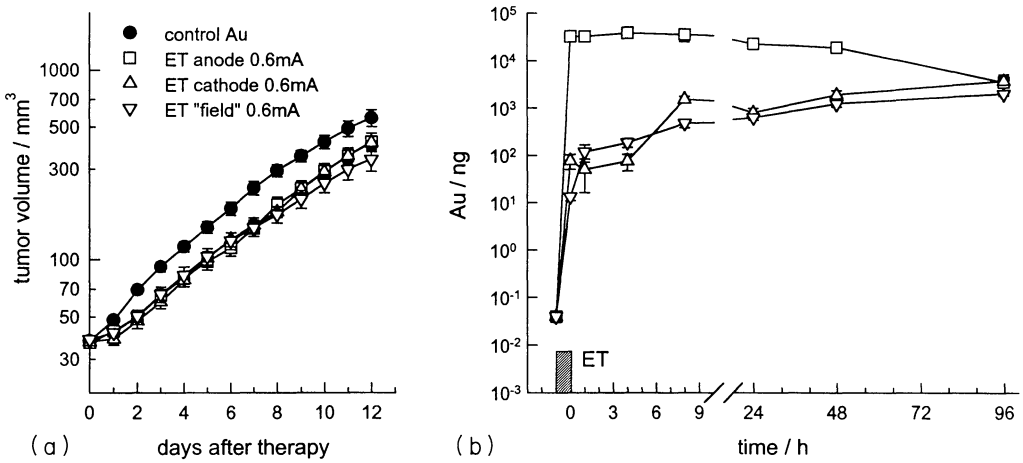

Fig. 1. Tumour growth following the electrotherapy with 0.6 mA direct current in different electrode configurations on day 0 is presented in comparison with control growth (a) mean values for 9–11 mice; vertical bars, standard error of the mean). Tumour growth was retarded irrespective of electrode configuration, i.e. one electrode inserted into the tumour being the anode and the other subcutaneously in the vicinity of the tumour being the cathode or vice versa, or both electrodes placed subcutaneously outside of the tumour ("field" electrotherapy). In all electrode configurations employed using a 0.6 mA direct current, gold deposited from the electrodes was determined in tumours by INAA at different times after the electrotherapy was performed ((b) mean values for 5–8 tumours; vertical bars, standard deviation).

reactor in Ljubljana, at a neutron flux of 4×10^{12} n cm^{-2} s^{-1}. On the basis of our preliminary experiments, the time of irradiation was 20–60 min depending on the position of the electrode in the tumour during the treatment, i.e. depending on the expected level of the amount of gold in the tumour. Irradiated samples were cooled for a few days and then transferred into glass measuring vials. The intensity of gamma lines was measured on a planar HP-Ge coaxial detector, covered by a 0.5 mm thick cadmium plate to stop the β-particles and therefore to lower the spectral background. The intensity of the ^{198}Au gamma line at 411.8 keV was measured until appropriate counting statistics were reached; any appreciable interference was indicated near the gamma line. The amount of gold in the tumour was given in ng as mean ± s.d. (n).

3. Results

A single-shot electrotherapy with a 0.6 mA direct current for 60 min after the tumours reached ca. 40 mm^3 induced statistically significant tumour growth delay, irrespective of the electrode configuration, when compared with controls (Fig. 1(a)). All three controls i.e. (iii) "pure" control, having no electrode inserted,

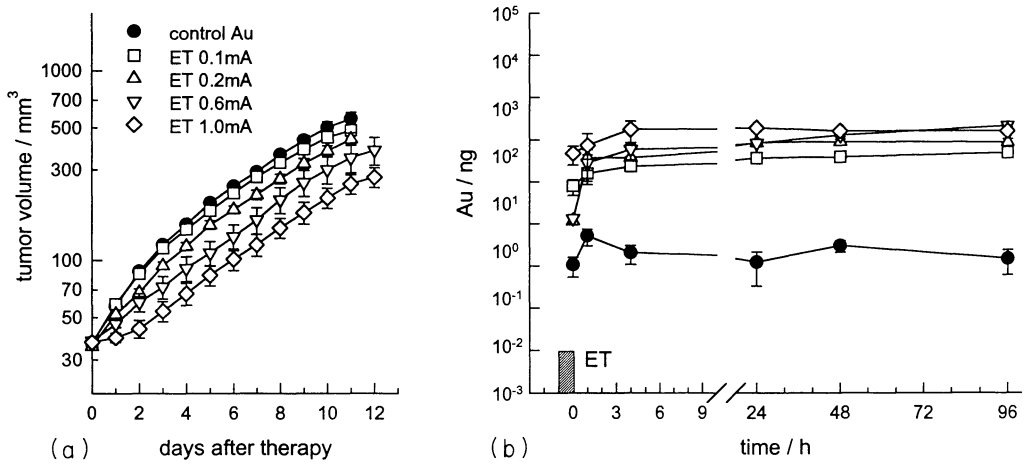

Fig. 2. Tumour growth following electrotherapy on day 0 with increasing values of direct current in "field" electrode configurations (i.e. both electrodes placed subcutaneously outside the tumour) is presented in comparison with control growth ((a) mean values for 9–11 mice; vertical bar, standard error of the mean). Gold deposited from the electrodes employed was determined in tumours by INAA at different times at all currents used and in controls (b) mean values for 5–8 tumours; vertical bars, standard deviation).

Table 2
Electrode metal deposited in tumour in different controls with associated tumour doubling time (DT), given as mean ± s.d. (n)

	Au/ng	DT/days
Control (pure)	0.04 ± 0.02 (5)	2.1 ± 0.6 (10)
Control (field)	1.07 ± 1.58 (9)	2.2 ± 0.5 (9)
Control (electrode)	7.71 ± 7.31 (5)	2.3 ± 0.3 (9)

(iv) electrode control, having one electrode inserted in the tumour and the counterpart subcutaneously in the vicinity of the tumour, and (v) "field" control, having both electrodes inserted subcutaneously in the vicinity of the tumour, grew at identical rates but had different associated tumour metal contents (Table 2). In Fig. 1(a) pooled controls are shown. The induced tumour growth delay was also estimated by calculating the mean tumour doubling time (DT/days) for each experimental group. The mean DT in anodic, cathodic and "field" electrotherapy was 3.8 ± 0.9 (11), 3.9 ± 0.8 (9) and 4.1 ± 1.7 (10), respectively, and compared with 2.2 ± 0.5 (28) pooled control by means of Mann–Whitney rank-sum test yielded a significant difference at the $p < 0.001$ level in all three electrode configurations.

Determination of gold content in tumours at different times after the end of 1 h of electrotherapy of 0.6 mA in all three electrode configurations revealed that the tumour gold content followed different patterns in each electrode configuration/polarity (Fig. 1(b)). The amount of gold in the tumours became comparable (of the same order of magnitude) only after 96 h.

In further studies, the dose response of tumours to "field" electrotherapy was obtained (Fig. 2(a)). The control here and in further studies was "field" control (v). As previously, the tumour gold content was determined at different times after 1 h of "field" electrotherapy (Fig. 2(b)). From these results it was evident that a plateau of gold accumulated in tumours was reached within 4 h after the completion of electrotherapy. The content in the tumours at 4, 24, 48 and 96 h after electrotherapy was averaged for each current level. The effectiveness of "field" electrotherapy at different currents was then plotted against the plateau tumour gold content (Fig. 3). The dose dependence of the tumour response to the current level was not accompanied proportionally by the levels of tumour gold content at corresponding currents (Figs. 2 and 3).

4. Discussion

Single-shot electrotherapy of 1 h duration with a 0.6 mA direct current delivered via gold needle electrodes retarded tumour growth when compared with controls irrespective of the electrode configuration and current polarity. Cathodic, anodic and "field" electrotherapy induced virtually the same tumour response (Fig. 1(a)). These results, although obtained with the use of gold electrodes, are in good accord with our previous results obtained using Pt–Ir alloy as the electrode material [15]. In the experiment where a 0.1–1.0 mA direct current was delivered in the "field" electrode configuration a clear dose-, i.e. current level-dependent tumour response was obtained (Fig. 2(a)). With all electrode configurations and currents used the deposition of electrode material in tumours was determined by means of INAA in order to reveal possible metal toxicity as an underlying mechanism of the observed tumour growth delay.

Tumour growth delay after 1 h of cathodic, anodic and "field" electrotherapy at 0.6 mA was virtually the same (Fig. 1(a)), with a few orders of magnitude difference in metal content immediately after electrotherapy (Fig. 1(b)). The content of gold in tumours after 4 days (96 h) became comparable. However, the tumour response dynamics observed in the tumour growth curves indicate that the antitumour effect (induced growth delay) was achieved earlier (Fig. 1). The amount of gold determined in the tumours following the "field" electrotherapy with different current levels were of the same order of magnitude (Fig. 2(b)) although the effectiveness of electrotherapy was dose dependent (Fig. 1(a)). Moreover, electrotherapy with a 0.1 mA direct current produced no antitumour effect but was accompanied by an extreme elevation of the tumour gold content with respect to controls which was comparable to the tumour gold content after application of 0.2, 0.6 and 1.0 mA (Fig. 2). The gold concentration in tumour in the "field" configuration reached a plateau within 4 h after field electrotherapy (Fig. 2(b)). Therefore, the

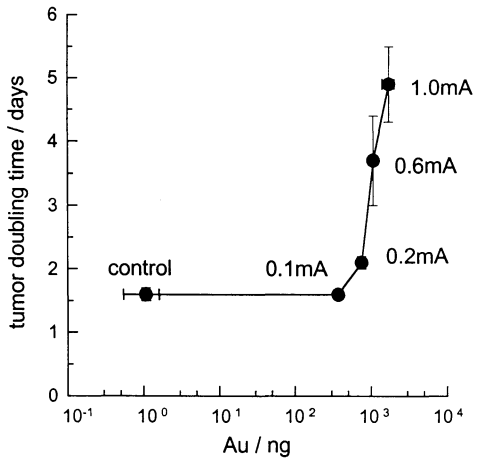

Fig. 3. Tumour response to single-shot electrotherapy with direct current of different values in the "field" configuration is presented as tumour doubling time with respect to the plateau amount of gold deposited in tumours at corresponding current values. Horizontal and vertical bars represent standard deviations (the graph was constructed from data presented in Fig. 2).

measured values at 4, 24, 48 and 96 h were averaged and related to the observed tumour growth delay represented by tumour doubling times at currents of 0.1, 0.2, 0.6 and 1.0 mA (Fig. 3). From this comparison it was observed that a significant increase in tumour doubling time failed to be associated with a significant increase in tumour gold concentration. Also, a range of three orders of magnitude in deposited gold (from control to 0.1 mA current) produced no difference in tumour growth, i.e. an increased gold concentration alone induced no tumour growth delay. A dose-dependent tumour response with respect to current employed was observed, however (Fig 2b). The tumour growth in all three controls (iii, iv and v) represented by tumour doubling time was identical with different gold concentrations (Table 2).

From the results presented in this paper and our previous results where a similar tumour growth delay was induced by the use of different electrode materials [15], we conclude that within the parameters of electrotherapy employed in our experiments the role of gold deposition, even though it might have played a role in the antitumour effect observed, was not crucial for the observed antitumour effect.

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