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Animal models for treatment of unresectable liver tumours: a histopathologic and ultra-structural study of cellular toxic changes after electrochemical treatment in rat and dog liver

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Abstract

Introduction: Electrochemical treatment (EChT) has been taken under serious consideration as being one of several techniques for local treatment of malignancies. The advantage of EChT is the minimal invasive approach and the absence of serious side effects. Macroscopic, histopathological and ultra-structural findings in liver following a four-electrode configuration (dog) and a two-electrode EChT design (dog and rat) were studied. *Materials and methods*: 30 female Sprague–Dawley rats and four female beagle dogs were studied with EChT using Platinum:Iridium electrodes and the delivered dose was 5, 10 or 90 C (As). After EChT, the animals were euthanized. *Results*: The distribution of the lesions was predictable, irrespective of dose and electrode configuration. Destruction volumes were found to fit into a logarithmic curve (dose–response). Histopathological examination confirmed a spherical (rat) and cylindrical/ellipsoidal (dog) lesion. The type of necrosis differed due to electrode polarity. Ultra-structural analysis showed distinct features of cell damage depending on the distance from the electrode. Histopathological and ultra-structural examination demonstrated that the liver tissue close to the border of the lesion displayed a normal morphology. *Conclusions*: The in vivo dose-planning model is reliable, even in species with larger tissue mass such as dogs. A multi-electrode EChT-design could obtain predictable lesions. The cellular toxicity following EChT is clearly identified and varies with the distance from the electrode and polarity. The distinct border between the lesion and normal tissue suggests that EChT in a clinical setting for the treatment of liver tumours can give a reliable destruction margin.

Keywords: Animal model; Unresectable liver tumour; Cellular toxic change; Electrochemical treatment

1. Introduction

Electrochemical treatment (EChT) of tumours has been regarded as a probable technique since the 18th century [1] and several reviews of EChT are available [2–4]. The electrolysis of tumours was re-introduced in a clinical setting by the Swedish professor Björn Nordenström. In the early 1980s, he treated mainly thoracic metastases of mammary tumours, which were considered as non-responding to conventional treatment [5,6]. The method has become wide spread in China and more than 15,000 patients have been treated for different malignant, as well as benign, tumours. These results have been reported at conferences and in the literature [4,7]. The reliability of the clinical and scientific reports has been questioned in the Western world. Recent studies in Europe and Australia, mainly performed on normal tissue in animals have shown promising results regarding the minimal invasive approach and the absence of serious side effects following EChT [8–16].

Besides the introduction and spread of EChT in China there has been an increasing interest of minimal invasive techniques for the treatment of malignant as well as benign

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tumours in the Western world. Equipment for stereotactic irradiation, percutaneous ethanol injection therapy (PEIT), cryotherapy, radio-frequency treatment, etc., have been developed and they have only to some extent been evaluated in controlled and randomised studies. These techniques are all commonly used today. Regarding the experience of EChT in China and recent reports, EChT is a technique that deserves further exploration.

In cases when the treatment electrodes are inert, such as platinum, metal dissolution is negligible. The main reactions at direct current electrolysis of tissue are decomposition of water and oxidation of chloride, at the anode, which produce chlorine and hydrogen ions. At the cathode the main reactions are the production of hydrogen and hydroxyl ions due to the hydrolysis of water. The presence of extreme local pH changes in tissue surrounding the electrodes, during and after EChT treatment, has been confirmed in many studies [5,15,17,18]. pH values as low as 1 have been detected in tissue adjacent the anode [17] while a pH as high as 13 has been measured near the cathode [19]. At these non-physiological conditions vital proteins become denatured and precipitate [5,19,20]. The extreme pH conditions in the vicinity of the electrodes have also been predicted in several theoretical studies [21–23].

Moreover, charged substances, dissolved or suspended in tissue, migrate in the electric field and accumulation of ions and charged tissue constituents are obtained at certain and different locations in the electric field. The electric field influences the ion exchange across the cell membranes. Hence, the transmembrane potential is altered and thereby the conditions, e.g. for many essential enzyme-regulated reactions [5,24].

Miklavèiè et al. [32] have also shown that the immunological response is of importance by performing electrotherapy in "field" configuration, where electrodes were placed subcutaneously outside of the tumour in a way that the tumour was situated in between the electrodes, in immunodeficient nude and immunocompetent mice. Electrotherapy was much more effective in immunocompetent mice based on the observed tumour growth retardation, thus demonstrating that antitumour effectiveness of electrotherapy greatly depended on host's immune response. Further experiments were conducted by combining electrotherapy with concomitant immunotherapy in order to potentiate the antitumour effect of electrotherapy. Immunotherapy consisted of local delivery of genetically engineered cells selected for IL-2 secretion. This combined treatment was much more effective than any of the treatments alone [25].

No publications are available where ultra-structural features of an EChT lesion are described. Consequently, as a part of a scientific evaluation of cellular toxicity and dose extrapolation between species, this animal study was conducted. Furthermore, the multi-electrode design for EChT was compared with a two-electrode setting.

2. Materials and methods

All experiments conformed fully to the decisions of the Swedish animal ethical committee (no C 112/96, rat and C 76/99, dog).

Thirty Sprague–Dawley rats were studied of which 27 were divided into three treatment groups, referred to as group A ($n=8$), B ($n=10$), C ($n=9$) and the remaining three served as controls. The dog group, referred to as group D, consisted of four healthy female beagles, bred for research purposes only, and were provided by the Faculty of Veterinary Medicine, Department of Small Animal Clinical Sciences, (SLU), Uppsala, Sweden. Each group was treated with a constant, direct current of 1 and 5 mA (rats) or 25 mA (dogs) for a specific duration of time, corresponding to a coulomb dose of 5 or 10 C (rats) and 90 C (dogs) (Table 1).

2.1. Electrochemical treatment

The liver treatment design for rat is essentially as described earlier with a few modifications [16]. Briefly, two identical spherical electrodes made of Pt:Ir (9:1), with a diameter of 1 mm, connected to a thinner, Teflon®-insulated pin, were used (Permascand, Sundsvall, Sweden), corresponding each to an active electrode area of 3.14 mm^2. The treatment was performed during general anaesthesia with midazolam (Dormicum®, Roche, Stockholm, Sweden) 1.2 mg/ml and fentanyl-fluanisone (Hypnorm®, Janssen Animal Health, Buckinghamshire, UK) diluted 1:4, 0.3 ml/100 g, injected intraperitoneally. The rats were

Table 1
The number of samples analysed for each treatment is stated (n)

Group	n	Current (mA)	Duration (min)	Coulomb dosage (C)	Current density (mA/mm^2)	Anodic (mm^3)	Cathodic (mm^3)
A	8	1	85	5	0.32	96 ± 31	154 ± 32
B	10	5	19	5	1.59	178 ± 60	249 ± 88
C	9	5	35	10	1.59	359 ± 75	433 ± 101
D	6	25	60	90	0.79	1569 ± 357	1660 ± 156

The current density of the electrode is presented as mA/mm^2. For the dog group (D) results were collected from both one and two-pair electrode configurations. The mean diameter was calculated for the spherical rat destruction, while the mean radius (r_m) and length of the cylindrical destruction were used in the dog study. The anodic and cathodic lesions are presented as volume (mm^3) $\pm$ 1 standard deviation, spherical volume ($\pi d_m^3/6$) and cylindrical ($\pi l r_m^2$).

placed in dorsal recumbency, under a heating lamp to avoid anaesthetic-induced hypothermia. A linea alba incision was made and the liver was everted. After blunt insertion of the electrodes, the anode in the left medial lobe and the cathode in the left lateral lobe, the liver was placed in situ in the abdominal cavity. The distance between the electrodes was 25–30 mm.

Direct current was passed through the electrodes with a constant-current power supply, consisting of a potentiostat (Wenking LT 73, Gerhard Bank Elektronik, Göttingen, Germany) coupled to an adjustable resistance. The current and voltage were continually monitored and data were recorded on a computer. Muscle twitching was avoided by using linear current ramps of 2 min duration at the beginning and end of each treatment. Control rats underwent an identical study protocol, with the exception that no current was passed through the electrodes. Duration of the treatment of the control rats corresponded to A (two rats due to the longest duration) and C (one rat), to assure that no adverse effects could be assigned to the electrodes alone, making the total number of animals used 30. All rats were euthanized immediately after the treatment.

In the dogs, general anaesthesia was generated by a symptomatic, IV induction with sodium thiopental (Sodium Pentothal®, Abbot Scandinavia, Solna, Sweden) 15–25 mg/kg and maintained by spontaneous inhalation of 2.5% isoflurane (Isoflo® vet, Shering-Plough AB, Stockholm, Sweden) in 100% Oxygen. Pre-anaesthetics and analgesics were given by subcutaneous injections of acepromazine (Plegicil® vet, Pharmacia and Upjohn Animal Health, Helsingborg, Sweden) 0.1 mg/kg and a NSAID preparation of carprofen (Rimadyl® vet, Orion Pharma, Animal Health, Sollentuna, Sweden) 4 mg/kg. The dogs were placed on a warming device, T/Pump® (Gaymar Industries, NY, USA) of circulating water to avoid hypothermia during anaesthesia.

Two or four identical string electrodes, made of Pt:Ir (9:1), with a diameter of 0.5 mm, Teflon®-insulated except for a 20-mm active electrode tip, were used (Permascand), corresponding each to an active electrode area of 31.4 mm^2. The liver was exposed using a linea alba excision and the electrodes were placed blunt into the liver through a polymethyl methacrylate plate that was fixed into a supporting gantry to avoid breathing divergency of the electrodes. In two of the dogs, two electrodes were placed 20 mm apart in the same liver lobe. In two other dogs, four electrodes (two pair) were placed as corners in a square with a 20-mm side in the same liver lobe, thus making the total number of EChT lesions six for anode and cathode, respectively. In this setting, the lesions should not become confluent and hence allowed an examination of untreated tissue in between the electrodes. The procedure, electrode placement and macroscopic lesions are shown in Fig. 1. The electrodes were coupled to DC power sources as described above. In the four-electrode design, each pair of electrodes was connected to a separate

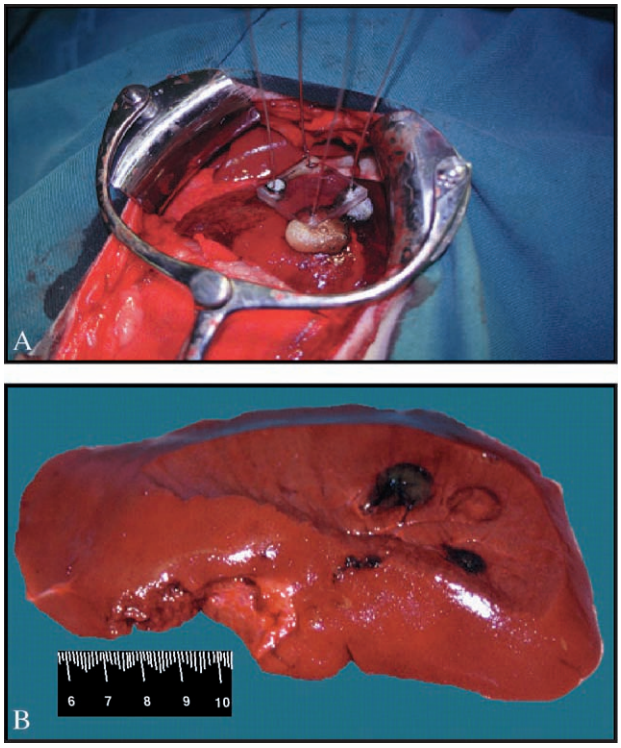

Fig. 1. Electrochemical treatment of liver tissue in dog indicating the four-electrode design and macroscopic lesion (A, B). A large linea alba incision had been made and the platinum electrodes are placed in a square pattern 2 cm apart. The two separate electrode pairs are controlled from one current supply each. (B) displays the macroscopic lesions immediately after the treatment. Note the black lesions of the anodes. No interference occurred, allowing studies on untreated liver tissue between the lesions.

power source to allow accurate control of the delivered dose. The current and voltage were monitored and recorded as for the rat model. Likewise, 2-min linear current ramps were used to avoid patient discomfort. The dogs were euthanized after the treatment with an IV injection of sodium-pentobarbital 2.5%. Body temperature was measured both prior to and after EChT.

2.2. Macroscopic and histopathological examination

The part of the liver with macroscopic visible destruction zones was resected *en bloc*, cloven and measured. The mean diameter (d_m) was measured for the spherical rat destruction, while the mean radius (r_m) and length (l) of the approximately cylindrical destruction were determined in the dog study. The lesions are presented as volume (mm^3) and the values for spherical volume ($\pi d_m^3/6$) and cylindrical ($\pi l r_m^2$) are indicated in Table 1. Representative slices of the lesions and normal liver in between the electrodes were fixed in 10% buffered formalin, processed, embedded in paraffin, sectioned and stained by haematoxylin and eosin. Formalin fixation of the lesions was performed for histopathological examination. In the four-electrode design, large-tissue sections were prepared to visualise all four

lesions in the same preparation. The tissue was left in formalin for 7 days to allow accurate fixation.

2.3. Ultra-structural examination

Samples were taken from areas at different distances from the electrodes, i.e. the centre (area 0), 2–4 mm from the centre (area 1), 4–6 mm from the centre (area 2) and macroscopically normal tissue (area 3) (Fig. 2). Small pieces were directly placed in 2% glutaraldehyde and 0.5% paraformaldehyde in 0.1 M sodium cacodylate buffer (caco) containing 0.1 M sucrose and 3 mM $CaCl_2$, pH 7.4 at room temperature. After fixation over the night at 4 °C specimens were rinsed in 0.15 M caco and postfixed in 2% OsO_4 in 0.07 M caco + 3 mM $CaCl_2$ for 2 h and dehydrated in ethanol into acetone and infiltrated in LX-112. Sections were cut and placed on formvar coated cuppergrids and contrasted with uranyl acetate and lead citrate. Sections were examined in a Leo 906 transmission electron microscope at 80 kV. Cells were chosen randomly at low magnification. In selected cells, micrographs were taken in the nuclear and the cytoplasm. The micrographs were printed to a final magnification of 18,400 and examined in a blind fashion.

2.3.1. Evaluation

The damages were graded by a 1–7 scale. From 1, where the cells displayed a normal ultra-structural morphology, to 7, where cells are necrotic. In between, the evaluation was based on the ultra-structure of mitochondria, granular endoplasmic reticulum (RER), golgi, peroxisomes, glycogen and nucleus chromatin.

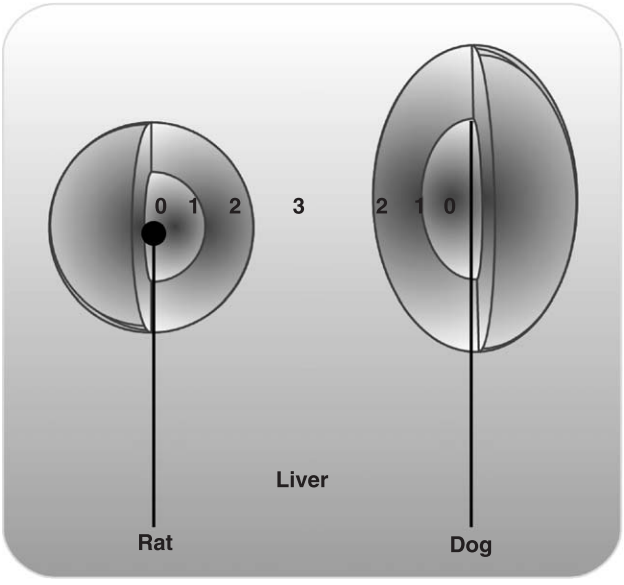

Fig. 2. Schematic of the tissue sampling for ultra-structural examination. Both in the rat (spherical) and dog (ellipsoid/cylindrical) lesions samples were collected, from the anode and cathode, respectively, at the centre of the lesion (0), in between the centre and border of destruction (1), at the border of destruction (2) and in normal tissue, situated between the treatment electrodes (3).

2.4. Blood sampling, body temperature, heart rate and saturation

An acid–base sample, collected in a heparinised syringe, was taken by cardiac puncture (rat) or from the cephalic vein (dog) prior to euthanasia and was analysed on a blood-gas detector (ABL 5®, Radiometer Copenhagen, France). Blood gas values were compared to reference values for each species.

In the dogs, blood samples were collected both prior to and directly after terminated EChT.

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltranspeptidase (γ-GT), bilirubin, alkaline phosphatase (ALP) and fibrinogen were determined (Konelab 30, KONE Instrument, Finland). Haematology parameters, such as haemoglobin (Hb), total white blood cell count (WBC) and thrombocytes were also analysed (Cell-Dyn 3500, Abbot Diagnostics, USA). Reference values were obtained from the Department of Clinical Chemistry, SLU, Uppsala.

Body temperature was measured before and after EChT in all animals. In the rats, heart rate, breathing frequency and colour of the mucous membranes were monitored manually and recorded every 10th minute during treatment. In the dogs, heart rate, arterial oxygen saturation, end-tidal CO_2 and respiratory rate were monitored continuously during treatment using a TuffSat® (Datex-Ohmeda, Louisville, KY, USA).

2.5. Statistics

Standard statistical methods were employed; using Student's *t*-tests (paired and two sample), comparing destruction volumes, differences between anodic and cathodic EChT and blood parameters before and after treatment. Values of $p < 0.05$ were considered indicative of statistical significance. Data are expressed as mean ± S.D. (standard deviation).

3. Results

All animals completed the study and none died due to the anaesthesia or the EChT. The heart rate remained stable and no animal suffered from arterial oxygen desaturation. There were no signs of cerebral irritation or respiratory distress. The body temperature remained normal (± 0.5 °C).

The electrolysis progressed similarly in both species. Due to space limitations, we have decided to visually present the histopathological findings for dog and ultra-structural for rat. In addition, focus has been put on the anodic lesion, especially for the ultra-structural evaluation, since it is frequently considered to be more toxic than the cathode (Fig. 2).

3.1. Macroscopic observations

When EChT started, gas formation was seen 60 s after the 2-min ramp from both electrode sites. A typical chlorine smell was developed after a few minutes from the anode. All bleedings induced at the placement of the electrodes were coagulated by the electrolysis within 2 min after the EChT onset. At the anode the lesion became black, as haemoglobin was transformed into haemin due to the acidified milieu. The anodic lesion became hard and firm, while the cathodic destruction was oedematous.

The calculated lesion/dose ratio (mm^3/C) was higher in rats both at anodic ($p < 0.0004$) and cathodic ($p < 0.001$) sites. The lesions were larger at the cathode, due to the oedema. The destruction volumes are shown in Table 1. The relationship between the lesion and dosage was logarithmically shaped for both electrodes (Fig. 3). A close parity between an estimated dose-planning curve described elsewhere and the dose–response data was confirmed [22,23].

3.2. Histopathology

Histopathological findings verified the macroscopic destruction (Fig. 4). At the anodic site, a marked dehydration of the tissue and hepatocytes with pycnotic nucleus and small or absent cytoplasm were observed. There was a sharp border between the lesion and surrounding, normal, liver tissue and, at this border of destruction, numerous micro vascular thrombi had occurred thus framing the lesion from normal tissue. The cathodic destruction was dominated by a striking oedema, due to electro-osmosis, that caused nuclear and cytoplasmic swelling with occasional disruption of the plasma membranes. The chromatin was condensed and an increased number of cytoplasmic vacuoles were present. Despite very few infarctions in the lesions there was a rather sharp border between the electrolytic necrosis and surrounding, apparently normal, liver tissue.

In the four-electrode configuration, the destruction did not expand to a confluent necrosis. The normal liver tissue directly adjacent to these lesions was considered to be completely normal based on macroscopic, histopathological and ultra-structural examination.

In the control rats, only some small bleedings along the electrode sites could be detected. All the studied liver parenchyma appeared to be normal both by macroscopic and histopathological examination.

3.3. Ultra-structural examination

Anode area 0, judged as grade 6, displayed severe changes (Fig. 5A). The mitochondrias and peroxisomes

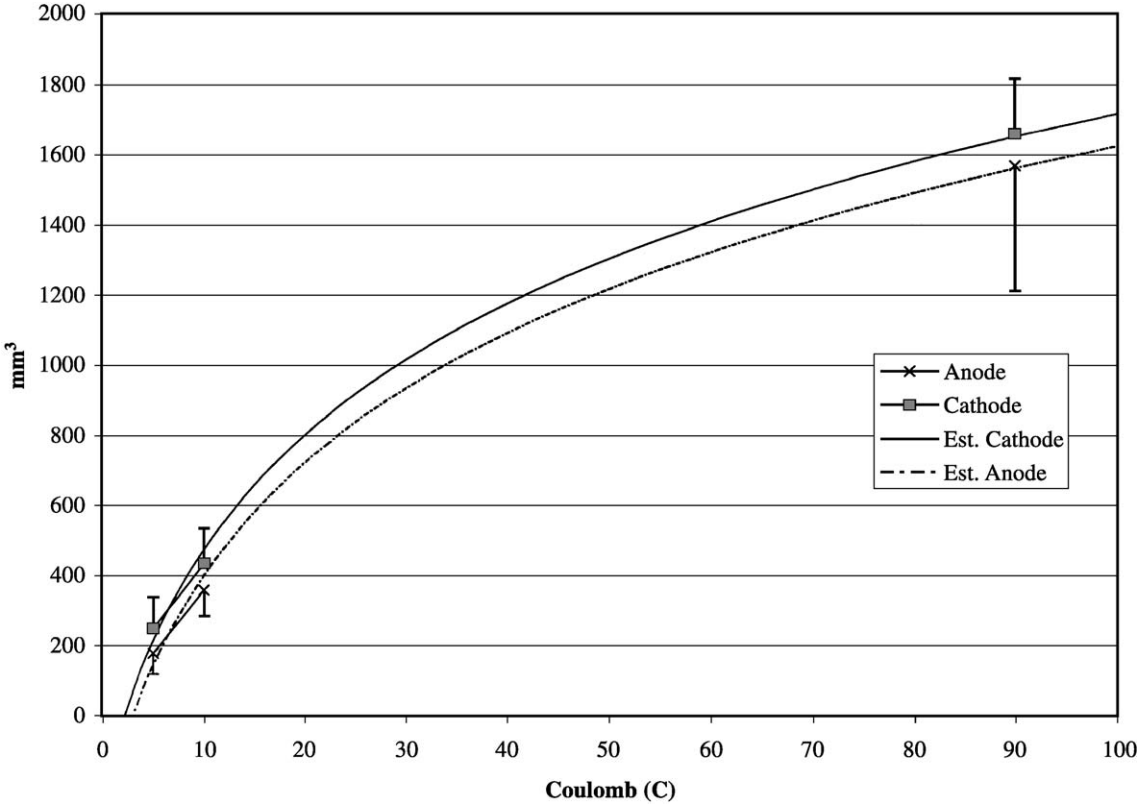

Fig. 3. The chart shows the macroscopically determined volume of destruction after treatment with 5 and 10 C in rat liver and 90 C in dog liver. It also shows the estimated (Est.) dose–response curve for both anodic and cathodic EChT. The relation is almost logarithmic for both treatments, suggesting that the destruction volume has harder to propagate, as it grows larger in size.

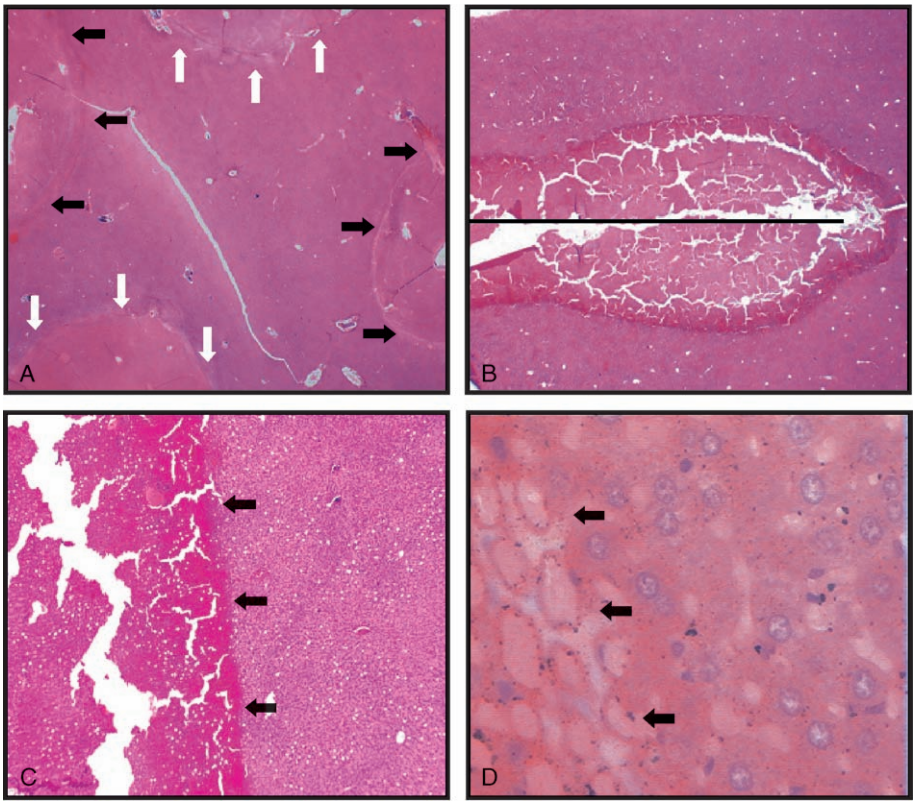

Fig. 4. Histopathological examination of the dog liver, using two pair of electrodes. (A) Four extensive and well-defined areas of coagulative necrosis are seen (magnification, 10.5 ×). All four electrode lesions are seen in this large tissue section of the liver. The anodic lesions (black arrows) are dehydrated, with pycnotic nuclei and a small rim of marginal infarctions at the border. The cathodic lesions (white arrows) are oedematous with cellular swelling and occasional disruption of the plasma membranes. No infarctions are revealed. Both anodic and cathodic lesions have a very sharp demarcation to normal tissue. (B) A longitudinal section of the anodic lesion with the central canal visualised with an animated electrode (magnification, 83 ×). (C) A higher magnification of the sharp border (black arrows) between the lesion and adjacent liver parenchyma at the anode (magnification, 212 ×). (D) At the cellular level it is only approximately a three-hepatocytes width (black arrows) that separates normal and destructed liver tissue at the anode (magnification, 420 ×).

displayed diffuse amorph morphology without outer membrane. The ribosomes on the RER were much electron-denser and a diffuser lumen compared to the controls. The cytoplasm showed a loose appearance and increased number of membrane bodies. Furthermore, the glycogen lost the characteristic morphology. The euchromatin in the nuclear showed a patchy redistribution in the nucleus and the heterochromatins were more loosely compared to controls. Anode area 1, judged as grade 3, displayed severe damages mostly in the mitochondria. The outer membranes of the mitochondria were well defined but most of the mitochondria matrixes were extracted. The peroxisomes were less affected but the matrixes were looser compared to controls. The RER were well defined and looked normal. Only minor changes were detected in the nuclear chromatin (Fig. 5B). Anode area 2, judged as grade 1, displayed an ultra-structural morphology that could not be distinguished from controls (Fig. 5C). The control and Anode area 3, judged as grade 1 displayed a normal ultra-structural morphology with dense mitochondria, well-defined RER and peroxisomes and glycogen. Furthermore, the nucleus displayed well-defined euchromatin and heterochromatin (Fig. 5D).

At the cathode no correlations, as for the anode, could be made between cellular damage and the distance from the treatment electrode (figure not shown). Cathode area 0, judged as grade 7, displayed severe changes. Cathode area 1, judged as grade 2, did not display as severe damage as Anode area 1. Cathode areas 2 and 3 were both judged as grade 1 and displayed a normal ultra-structural morphology with dense mitochondria and well-defined nucleus with normal euchromatin and heterochromatin.

3.4. Blood sample analyses

Venous blood gas results were similar, after termination of EChT in all animals studied, showing no significant change compared to normal reference values.

Liver enzymes in the dog (AST and ALT) tended to rise after EChT ($p < 0.12$ and 0.11, respectively). No other

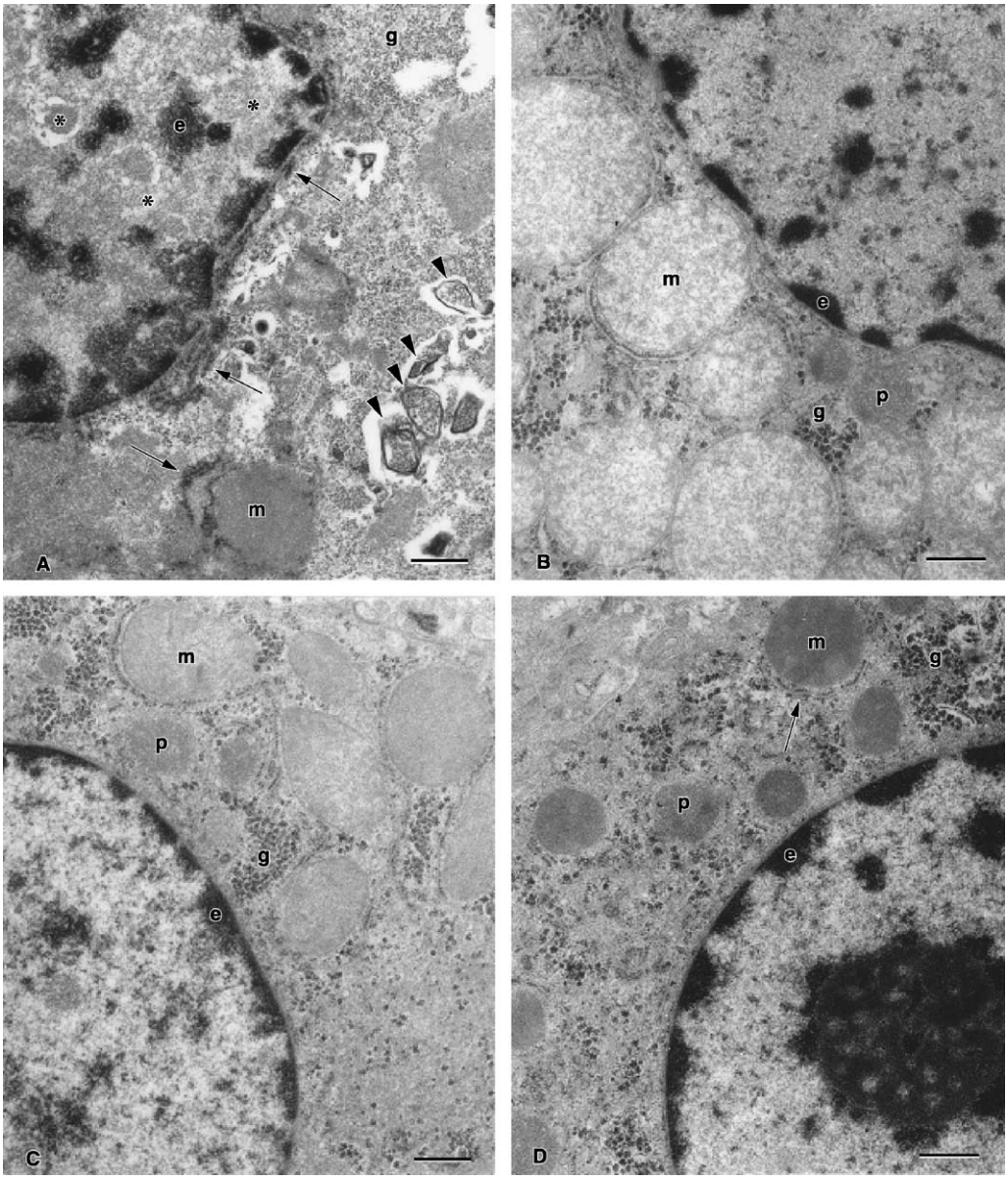

Fig. 5. Ultra-structural morphology in rat hepatocytes following anodic EChT (5 mA 10 C). Mitochondria (m), peroxisome (p), glycogen (g), euchromatin (e), RER (arrows). (A) Anode (area 0; centre of the lesion), displayed severe changes such as diffuse amorph morphology in mitochondrias without outer membrane. Ribosomes on the RER were much electron-denser with a diffuser lumen and increased number of membrane bodies in cytoplasm (arrowhead). The euchromatin in the nuclear showed a patchy redistribution in the nucleus and the heterochromatins were more loose (*). (B) Anode (area 1), showed severe damages in the mitochondria. The outer membranes were well defined but most of the mitochondria matrixes were extracted. The matrixes in peroxisomes were less affected but were looser. (C) Anode (area 2) displayed an ultra-structural morphology that could not be distinguished from controls. (D) The control displayed a normal ultra-structural morphology with dense mitochondria, well-defined RER, peroxisomes and glycogen. The nucleus displayed well-defined euchromatin and heterochromatin. Bars = 0.5 μm.

changes were discernible concerning the blood parameters tested.

4. Discussion

As in studies where new drugs are first tested on healthy patients and preceded by careful toxicological studies in vitro, the introduction of EChT in a clinical setting should be considered as no exception. While using healthy animals and one tissue at a time, more safe conclusions can be drawn, e.g. concerning dose-planning, tissue destruction and immunological response to EChT, before commencing clinical studies on inoculated tumours and eventually cancer patients. In this way the number of animals used are reduced due to a more optimised research strategy and the studies are based on a well established hypothesis and not only on empirical data.

Minimally invasive techniques for treatment of malignant and benign disorders have received increasing atten-

tion during the last decades and several of them have been established in clinical practice, such as laparoscopic and thorachoscopic surgery. Equipment for local tumour destruction such as cryotherapy, thermo-laser therapy, radio-frequency treatment, have also been established. The prognosis for liver malignancies is dismal if the tumour is considered to be unresectable and if the patient is not a candidate for liver transplantation. Despite the development of more efficacious chemotherapeutic regimes, cure of liver malignancies is rare. However, primary liver cancers, i.e. small hepatocellular carcinoma (HCC), which is quite common, has been found to be successfully treated with a local destructive method, percutaneous ethanol injection therapy (PEIT) [26]. Lately, the efficacy of radio-frequency treatment of such liver tumours has been reported to be comparable with PEIT [27–29]. Liver metastases following colorectal carcinomas (CRC) are common and the majority is considered as unresectable due to the spread in the liver or to the high age of the patient. Recently developed techniques for local destruction of tumours such as cryo-surgery, thermo-laser and radio-frequency have been considered as promising methods both as a single treatment but also as a combined regime together with liver surgery, adjuvant chemotherapy, etc. Fosh et al. [30] presented a very interesting, yet small study, on the combination of liver surgery and EChT in nine patients with hepatic deposits from colorectal carcinoma, previously deemed inoperable using currently accepted criteria. The group stated that electrolysis with resection might confer a disease-free and overall survival benefit.

The minimally invasive techniques developed are all rather expensive. Regarding the lower costs and the promising experiences of EChT in China, EChT is a technique worth further exploration. More than 15 000 treatments of different malignant and benign tumours have been preformed in China and the efficacy of EChT is reported to be high with absence of serious side effects [4–7]. In the current experimental study no animals suffered from any serious side effects, which is in agreement with previous studies [8,10,14]. The level of liver enzymes is reported to rise after EChT in liver tissue but was found to decrease to a normal level within one week [10,14]. The absence of a significant rise in liver enzymes in the present report is probably dependent on the study protocol employed and the tendency to increase could have been more prominent after 24 h.

When the electrolytic process has been in progress for some time, a marked dehydration occurs at the anode due to electro-osmosis. This dehydration leads to impairment in electrode contact and the electrolysis is hampered. Moreover, the impaired contact results in an acute rise in the voltage, which can be harmful to the patient. In order to overcome this problem, some authors have suggested the use of saline infusions into the anodic area [5]. However, that design can dilute the toxic species produced during electrolysis, which in turn may reduce the efficacy of the treatment. Increased re-hydration can also allow the buffering species, whose access is hindered to the anode by the dehydration and marginal infarctions, to re-enter the destructive area. In the cathode lesion, the dose–response curve is similar to that at the anode, with decreased efficacy over time. The tissue lesion is characterised by an oedema, which conceivably allows the buffering species to hamper the electrolysis to some extent. Therefore a multi-electrode set up is probably more efficient in terms of tissue/tumour destruction in order to use the maximal toxic capacity of each electrode. This setting is already in use in China when treating larger tumours with EChT [7].

The anodic lesion has been regarded as more toxic in former studies [10,31]. It is therefore important to assure that the two electrolytic destructions do not interfere with each other, thus creating a zone of neutralisation where the two fronts meet. This is important to consider when the treatment electrodes are constructed. The most efficient concept is conceivably to have a cluster of anodes inside the tumour, framed by cathodes at, or adjacent to, the tumour border. This configuration has been described earlier in small murine tumours by Miklavèiè et al. [32]. The single electrode model has its obvious limitations in a future clinical setting, when treating liver malignancies, due to the logarithmic dose–response curve.

The ultra-structural features of an EChT lesion has not been studied earlier. The current findings elucidate that the lesion is not homogenous from the centre to the macroscopically visible border of the destruction zone. Even in light microscopy, it is very hard to distinguish any prominent difference in the cellular morphology throughout the lesion. Electron microscopic analysis shows a successively milder toxic influence on the treated cells as the distance from the electrode centre increases at the anode. At the cathode, the distribution of toxic features was uneven through the investigated material and no correlation could be made with the distance from the treatment electrode. This is probably due to the oedematous environment that makes the “borders” less accurate and the toxic species might be more easily distributed in the lesion, but at the same time fails to build up a high concentration. In general, the cathodic lesions had a milder appearance compared to the anode. The current ultra structural examination is easier to interpret in normal liver tissue, than in a malignant tumour mass since malignancies carry spontaneous necrosis and degenerated chromatin as a “normal” feature. However, future studies ought to be focused on the electrolysis and its effect on tumour tissue.

The logarithmic pattern of the dose–response curve is very important to consider when interpreting earlier works in the EChT field. There are a wide variety of reports, in different species including human, regarding the rate of necrosis per coulomb depending on electrode type, positioning and current applied [18,33,34]. Robertson et al. [10]

suggested that the dose–response was almost linear in rat liver tissue. This is true for small lesions, but with increasing dose the destruction curve becomes more logarithmic. This phenomenon was explained by Berendson and Olsson [22]. As the destruction volume increases more buffering species are recruited at the border of the lesion and the pH-gradient becomes more and more diluted. Thus, the tissue damaging properties decline. In addition, at the anode, the impaired contact, increasing with higher degree of dehydration, disturbs the conductivity. Hence, it is of importance to perform studies in larger animals with a large liver tissue mass, as the current study, to achieve a more reliable dose-planning for electrochemical treatment in humans and companion animals. In studies where the animals are sacrificed days or weeks after the EChT it is very hazardous to estimate the original size of the lesions (due to secondary inflammatory changes). Despite extensive scanning magnetic resonance imaging (MR) [35,36] after EChT a dose-planning model can scarcely be validated through such experiments. Recently, thorough theoretical dose-planning models, both for anodic and cathodic destruction, has been published [23,37]. These theoretical models were adjusted to experimental models based on rats. The present study demonstrates that the previous dose-planning model is reliable in other species as well.

Different opinions regarding which electrode polarity that is the most toxic in the EChT lesions have been published. Some authors have shown that the cathode has the highest efficacy [32], while other reports variable results with respect to polarity [6]. Most of the reports, however, are consistent with our findings and claim that the anodic lesion is more prominent and toxic than the cathodic counterpart [10,18,38]. When determining the efficacy of EChT the acute size of the lesion is only one parameter to take into consideration. As done in this study, histopathological and ultra structural examinations show that cellular changes were more striking in the anodic lesions.

In the multi-electrode design we could obtain predictable lesions and the dose–response curve suggests a logarithmic relationship. The cellular toxicity in the lesion following EChT is clearly identified and it varies with the distance from the electrode for the anode. Finally, the distinct border between the lesion and apparently normal liver tissue suggests that EChT in a clinical setting for treatment of liver tumours can give a reliable destruction margin.

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References

[1] T. Cavallo, A complete treatise on electricity in theory and practice, Dilly, London, 1777.
[2] D.C. Schechter, Origins of electrotherapy: I., N. Y. State J. Med. 71 (1971) 997–1008.
[3] D.C. Schechter, Origins of electrotherapy: II., N. Y. State J. Med. 71 (1971) 1114–1124.
[4] E. Nilsson, H. von Euler, J. Berendson, A. Thörne, P. Wersäll, I. Näslund, A.-S. Lagerstedt, K. Narfström, J.M. Olsson, Review—electrochemical treatment of tumours, Bioelectrochemistry 51 (2000) 1–11.
[5] B.E. Nordenström, Biologically Closed Electric Circuits: Clinical, Experimental and Theoretical Evidence for an Additional Circulatory System, Nordic Medical Publications, Stockholm, 1983.
[6] B.E. Nordenström, Electrochemical treatment of cancer. I: variable response to anodic and cathodic fields, Am. J. Clin. Oncol. 12 (1989) 530–536.
[7] Y.L. Xin, Organisation and spread of electrochemical therapy (ECT) in China, Eur. J. Surg. Suppl. (1994) 25–29.
[8] D.P. Berry, A.R. Dennison, R. Ward, G.J. Maddern, Electrolytic ablation of colorectal liver metastases: 1-year histological patient follow-up, Dig. Surg. 17 (2000) 518–519.
[9] B.G. Fosh, J.G. Finch, A.A. Anthony, M. Texler, G.J. Maddern, Electrolytic ablation of the rat pancreas: a feasibility trial, BMC Gastroenterol. 1 (2001).
[10] G.S. Robertson, S.A. Wemyss-Holden, A.R. Dennison, P.M. Hall, P. Baxter, G.J. Maddern, Experimental study of electrolysis-induced hepatic necrosis, Br. J. Surg. 85 (1998) 1212–1216.
[11] S.A. Wemyss-Holden, G.S. Robertson, P.M. Hall, A.R. Dennison, G.J. Maddern, Electrolytic treatment of colorectal liver tumour deposits in a rat model: a technique with potential for patients with unresectable liver tumours, Dig. Dis. 18 (2000) 50–57.
[12] S.A. Wemyss-Holden, G.S. Robertson, A.R. Dennison, P.M. Hall, J.C. Fothergill, B. Jones, G.J. Maddern, Electrochemical lesions in the rat liver support its potential for treatment of liver tumors, J. Surg. Res. 93 (2000) 55–62.
[13] S.A. Wemyss-Holden, P.M. Hall, G.S. Robertson, A.R. Dennison, P.S. Vanderzon, G.J. Maddern, The safety of electrolytically induced hepatic necrosis in a pig model, Aust. N. Z. J. Surg. 70 (2000) 607–612.
[14] S.A. Wemyss-Holden, G.S. Robertson, A.R. Dennison, P.S. Vanderzon, P.M. Hall, G.J. Maddern, A new treatment for unresectable liver tumours: long-term studies of electrolytic lesions in the pig liver, Clin. Sci. 98 (2000) 561–567.
[15] H. von Euler, E. Nilsson, A.-S. Lagerstedt, J.M. Olsson, Development of a dose-planning method for electrochemical treatment of tumors. A study of mammary tissue in healthy female CD-rats, Electro. Magnetobiol. 18 (1999) 93–105.
[16] H. von Euler, E. Nilsson, J.M. Olsson, A.-S. Lagerstedt, Electrochemical treatment (EChT) effects in rat mammary and liver tissue. In vivo optimizing of a dose-planning model for EChT of tumours, Bioelectrochemistry 54 (2001) 117–124.
[17] D. Miklavcic, G. Sersa, M. Kryzanowski, S. Novakovic, F. Bobanovic, R. Golouh, L. Vodovnik, Tumor treatment by direct electric current-tumor temperature and pH, electrode material and configuration, Bioelectrochem. Bioenerg. 30 (1993) 209–220.
[18] L. Samuelsson, L. Jonsson, Electrolyte destruction of lung tissue. Electrochemical aspects, Acta Radiol. Diagn. Stockh. 21 (1980) 711–714.

[19] K. Li, Y. Xin, Y. Gu, B. Xu, D. Fan, B. Ni, Effects of direct current on dog liver: possible mechanisms for tumor electrochemical treatment, Bioelectromagnetics 18 (1997) 2–7.
[20] R. Lemberg, M. Legge, Hematin Compounds and Bile Pigments, Interscience Publ., New York, 1949.
[21] J. Berendson, D. Simonsson, Electrochemical aspects of treatment of tissue with direct current, Eur. J. Surg. Suppl. (1994) 111–115.
[22] J. Berendson, J.M. Olsson, Bioelectrochemical aspects of the treatment of tissue with direct current, Electro. Magnetobiol. 17 (1998) 1–16.
[23] E. Nilsson, J. Berendson, E. Fontes, Development of a dosage method for electrochemical treatment of tumours: a simplified mathematical model, Bioelectrochem. Bioenerg. 47 (1998) 11–18.
[24] L. Vodovnik, D. Miklavcic, G. Sersa, Modified cell proliferation due to electrical currents, Med. Biol. Eng. Comput. 30 (1992) 21–28.
[25] D. Miklavcic, D. An, J. Belehradek Jr., L.M. Mir, Host's immune response in electrotherapy of murine tumors by direct current, Eur. Cytokine Network 8 (1997) 275–279.
[26] T. Livraghi, Guidelines for treatment of liver cancer, Eur. J. Ultrasound 13 (2001) 167–176.
[27] T. Livraghi, S. Lazzaroni, F. Meloni, Radiofrequency thermal ablation of hepatocellular carcinoma, Eur. J. Ultrasound 13 (2001) 159–166.
[28] T. Livraghi, Treatment of hepatocellular carcinoma by percutaneous interventional methods, Eur. Radiol. 11 (2001) 2207–2219.
[29] S.N. Goldberg, Radiofrequency tumor ablation: principles and techniques, Eur. J. Ultrasound 13 (2001) 129–147.
[30] B.G. Fosh, J.G. Finch, M. Lea, C. Black, S. Wong, S. Wemyss-Holden, G.J. Maddern, Use of electrolysis as an adjunct to liver resection, Br. J. Surg. 89 (2002) 999–1002.
[31] B.E. Nordenström, Survey of mechanisms in electrochemical treatment (ECT) of cancer, Eur. J. Surg. Suppl. (1994) 93–109.
[32] D. Miklavcic, L. Vodovnik, F. Bobanovic, S. Rebersek, G. Sersa, S. Novakovic, R. Golouh, Local treatment of tumors by electric direct current, Electro. Magnetobiol. 11 (1992) 109–125.
[33] L. Samuelsson, L. Jonsson, Electrolytic destruction of tissue in the normal lung of the pig, Acta Radiol. Diagn. Stockh. 22 (1981) 9–14.
[34] Y. Xin, F. Xue, B. Ge, F. Zhao, B. Shi, W. Zhang, Electrochemical treatment of lung cancer, Bioelectromagnetics 18 (1997) 8–13.
[35] N.J. Dodd, J.V. Moore, T.V. Taylor, S. Zhao, Preliminary evaluation of low-level direct current theraphy using Magn Res Img Spect, Phys. Med. 9 (1993) 285–289.
[36] D.T. Griffin, N.J.F. Dodd, S. Zhao, B.R. Pullan, J.V. Moore, Low-level direct electrical current therapy for hepatic metastases: I. Preclinical studies on normal liver, Br. J. Can. 72 (1995) 31–34.
[37] E. Nilsson, E. Fontes, Mathematical modelling of physicochemical reactions and transport processes occurring around a platinum cathode during the electrochemical treatment of tumours, Bioelectrochemistry 53 (2001) 213–224.
[38] D.T. Griffin, N.J. Dodd, J.V. Moore, B.R. Pullan, T.V. Taylor, The effects of low-level direct current therapy on a preclinical mammary carcinoma: tumour regression and systemic biochemical sequelae, Br. J. Can. 69 (1994) 875–878.