Electrochemical Treatment of Tumours

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To my beloved family,
Katarina and Paul!

It is not a matter of
the size of the dog in the fight,
but the size of the fight in the dog.
An old American saying

Abstract

von Euler, H., 2002. *Electrochemical treatment of tumours.* Doctor's dissertation. ISSN 1401-6257, ISBN 91-576-6386-6.

By electrochemical treatment (EChT) neoplastic tissue is supplied with a continuous direct current through two or more electrodes placed in or near the tumour. The EChT has been taken under serious consideration as being one of several techniques for local treatment of malignancies. The advantage of the therapy is the minimal invasive approach and few serious side effects. EChT has not yet been universally accepted due to the absence of essential preclinical studies and controlled clinical trials. Uncertainties regarding the destruction mechanism of EChT also hinder the development of an optimised and reliable methodology for serving as a complement in the oncologic treatments used in the Western world.

This thesis investigates the correlation between the pH profile in the tissue surrounding the electrodes and the macroscopic, histopathological and ultrastructural tissue destruction obtained after EChT. Experiments designed to display both the normal, as well as tumour tissue response, is described. A separation between the polarities of the electrodes has been assured to allow specific examinations of the different reactions. To verify the relation between cell damage and pH an *in vitro* model was conducted where tumour cells were exposed to a pH gradient. To investigate if the same destructive mechanisms could be observed in tumour tissue *in vivo* an animal model was performed with the same cell line. The *in vivo* experiments also served as a control and calibration of a mathematical dose-planning model.

To examine the change of pH in tissue extensive *in situ* pH-measurements were performed with a micro-combination glass electrode.

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 the macroscopically detectable lesions. 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 tissue close to the border of the lesions displayed a normal morphology.

In the tumour model *in vivo*, significant changes in proliferation rate were seen both in the cathode and anode reaction. Apoptosis were induced in the anodic treatment suggesting that secondary cell destruction was caused by necrosis with cathodic EChT and apoptosis or necrosis at the anode. The findings agree with the results from the *in vitro* experiment.

Keywords: apoptosis, cancer, direct current, dose planning, electrotherapy, *in vitro*, *in vivo*, liver, mammary tumour.

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Contents

Appendix

Papers I-V

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I. von Euler, H., Nilsson, E., Lagerstedt, A-S. and Olsson, J.M. 1999. Development of a dose-planning method for electrochemical treatment of tumors. A study of mammary tissue in healthy female CD-rats. *Electro- and Magnetobiology,* 18, 93-105.

II. von Euler, H., Nilsson, E., Olsson, J.M. and Lagerstedt, A-S. 2001. Electrochemical treatment (EChT) effects in rat mammary and liver tissue. In vivo optimizing of a dose-planning model for EChT of tumours. *Bioelectrochemistry*, 54, 117-24.

III. von Euler, H., Söderstedt, A., Thörne, A., Olsson, J.M. and Yongqing, G. 2002. Cellular toxicity on the R3230AC rat mammary tumour cell line by a pH-gradient. An in vitro model for investigation of the tumour destructive properties of electrochemical treatment (EChT) of tumours. *Bioelectrochemistry*. Accepted.

IV. von Euler, H., Olsson, J.M., Hultenby, K., Thörne, A. and Lagerstedt, A-S. 2002. Animal models for treatment of unresectable liver tumours:A histopathologic and ultra-structural study of cellular toxic changes after electrochemical treatment (EChT) in rat and dog liver. Submitted.

V. von Euler, H., Ståhle, K., Thörne, A. and Yongqing, G. 2002. Cell proliferation and apoptosis in rat mammary cancer after electrochemical treatment (EChT). Submitted.

Abbreviations and definitions

A	Ampere
BrdU	5-Bromo-2'-deoxyuridine
C	Coulomb (Ampere second; As)
CFN	Centrala Försöksdjursnämnden or The Swedish National Board for Laboratory Animals
CD	A Sprague Dawley rat
CPP32	Caspase-3
EChT	Electrochemical treatment
g	gravitational constant
i.p.	Intraperitoneal
Ir	Iridium
mA	Milliampere
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5,-diphenyl tetrazolium bromide
PCM	Phase contrast microscope
Pt	Platinum
RER	Granular endoplasmic reticulum
Rhodium	Rh
rpm	revolutions per minute
RPMI 1640	A cell culture media
V	Volt

Introduction

In Electrochemical Treatment (EChT) of tumours electrolysis is used to destroy tumour tissue. Several contributory factors seem to be involved in the tissue destruction. Their respective roles in producing the anti-tumour effects are not fully understood. Mans fascination of the power of electricity and its use in oncology spans from the late 18th century. A short overview of landmarks in the use of different electrical applications is shown in table 1. Reports of the use of EChT can be found as far back in history as the mid 19th century (Crussel, 1847). Although it is hard to critically review these experiments a few attempts have been made to describe the use of EChT (Schechter, 1979; Watson, 1991; Nordenström, 1994b; Nilsson, von Euler *et al.*, 2000). The modern history of electrochemical treatment started 1959, when Humphrey and Seal reported encouraging results with sarcoma tumours in mice (Humphrey & Seal, 1959). After this a number of trials were conducted in which animal tumour models and *in vitro* experiments would serve as a base for the introduction of EChT in the clinical setting.

Table 1. Electrotherapeutic approaches to tumour destruction

Treatment mode	**Treatment principle**	**References**
Static electricity	Surface electrification by anodyne or counter-phlogistic actions.	(Cavallo, 1777)
Electrocautery	Electrothermic coagulation using multiple metallic hooks or wire electrodes.	(Duchenne, 1872)
Electrolysis or electrochemical treatment	Electrolytic destruction of tissue after application of platinum electrodes into the tumour.	(Crussel, 1847)
Electroatrophy	Application of abrupt alternating current across needles, which included both poles within the tumour.	(Inglis-Parsons, 1893)
Cataphoresis	Amalgamation of gold electrodes with salts of mercury and by electrical diffusion impregnating the tumour with tumouricidal doses of mercury.	(Massey, 1898; 1914; 1924)
Electrostatic treatment	An electrode inserted into an organ is electrostatically charged in relation to ground. A field of the same polarity as the electrode is created.	(Nordenström, 1983)
Electrophoretic chemotherapy	Electrophoretic transportation of electrically charged chemotherapeutic drugs through the tumour tissue or electric attraction of the charged drug into the tumour tissue.	(Nordenström, Eksborg *et al.*, 1990)
Electropermeabilization	Very short (100 μsec) intense (1300 V/cm) pulses administered through two external electrodes located on each side of the treated nodule, used in combination with chemotherapy.	(Mir, Belehradek *et al.*, 1991)

The electrolysis

When two or more electrodes are inserted into the tissue a series of electrochemical reactions are taking place. When a practically insoluble electrode material such as platinum is used, the main electrode reactions are decomposition of water along with oxidation and reduction of substances dissolved in the tissue. At the anode the evolution of oxygen as well as acidification and formation of chlorine occur:

$$2H_2O \rightleftarrows O_2 + 4H^+ + 4e^- \quad (1)$$

$$2Cl^- \rightleftarrows Cl_2 + 2e^- \quad (2)$$

In addition, chlorine might react with water causing further acidification:

$$Cl_2 + H_2O \rightleftarrows HClO + H^+ + Cl^- \quad (3)$$

Experimental results as well as theoretical estimations have indicated that the spreading of hydrogen ions in tissue surrounding the anode is considerably larger than the spreading of chlorine (Berendson & Olsson, 1998). At the cathode, water is decomposed into hydrogen and hydroxyl ions:

$$2H_2O + 2e^- \rightleftarrows H_2 + 2OH^- \quad (4)$$

Due to the formation of acidic and basic heamatin, the tissue surrounding the anode and cathode becomes dark brown (Lemberg & Legge, 1949). The acidic heamatin contains methaemoglobin whilst the dark area around the cathodic lesion consists of haemochromogens (Samuelsson & Jonsson, 1980; 1981). The spreading of chlorine causes a grey coloured zone close to the anode, which is considerably smaller than the heamatin zone (Samuelsson & Jonsson, 1981).

The delivered charge (dose) is usually expressed in Coulomb (C). Coulomb is a unit of electrical charge equal to the amount of charge transferred by a current of 1 ampere in 1 second (As). Different strategies have been applied for treatments; some researchers have used constant voltage and a variable current, while others have kept the current constant to let the voltage vary instead. For both methods the Coulomb dose can be determined. A schematic of the EChT treatment and electrolysis is shown in figure 1.

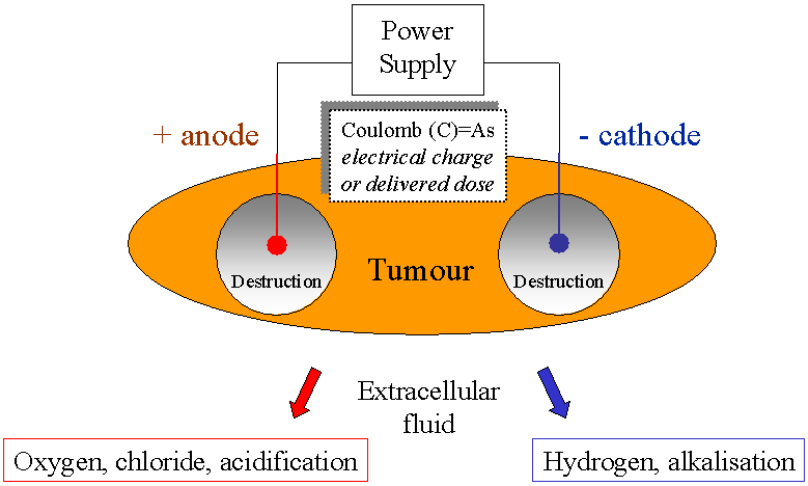

Figure 1. A schematic on the principle for electrochemical treatment of tumours.

The Swedish experience

One of the pioneers in Sweden was the professor in radiology Björn Nordenström who in the mid eighties published a series of reports on EChT of human tumours (Nordenström, 1983; 1989). He treated lung metastases (carcinomas) and a few primary lung tumours. Many of the patients were, for various reasons, unsuitable for surgical, radiotherapeutic, or chemotherapeutic treatment. The average delivered coulomb (C) dosage was 80 C/cm of tumour diameter. Regression was obtained in 12 out of 26 tumours and no signs of progression were detected after a 2–5-year follow-up period in these cases. There was no reported lethal outcome of the therapy, although tumours with a diameter larger than 3 cm did not always respond well to the treatment. One woman had a big metastasis (4 cm) and three other between 1-2 cm in diameter, one situated in the right and two in the left lung. After EChT the tumours disappeared and she is still alive, more than 20 years after the treatment. Among the side effects, Nordenström noticed slight fever, one postoperative pneumothorax and local pain during EChT.

Professor Nordenström also used combination therapies between EChT and chemotherapy. In a couple of reports Nordenström and colleagues investigated the additive effect of Doxorubicin with EChT (Eksborg, Nordenström *et al.*, 1990; Nordenström, Eksborg *et al.*, 1990). Some positive responses could be detected in a group of 14 patients with large lung neoplasms, incurable with conventional cancer therapy (Nordenström, Eksborg *et al.*, 1990).

Despite the promising results of EChT the technique gained a low interest among colleagues in Sweden and the rest of the Western world.

The Chinese experience

In the republic of China EChT is seen as a good complement in the treatment of tumours since it is considered inexpensive compared to other traditional techniques and reliable. The results of the Chinese experience have been presented in numerous conferences and reports (Matsushima, Takahashi *et al.*, 1994; Xin, 1994; 1998). At first, the reports were not taken seriously in the Western world due to the poor quality. This has changed and recently many papers have been published from groups in Europe, USA and Australia that use different variants of the techniques described by the Chinese (Robertson, Wemyss-Holden *et al.*, 1998; Turler, Schaefer *et al.*, 2000; Wemyss-Holden, Hall *et al.*, 2000; Wemyss-Holden, Robertson *et al.*, 2000). Up to the present day, more than 15 000 treatments of human tumours have been preformed in China. A summary of the results can be found in a review from 2000, by Nilsson and co-workers (Nilsson, von Euler *et al.*, 2000).

The destructive mechanisms

Electric field and Chlorine

Many suggestions have been made on which should be the main cause of tissue destruction after electrochemical treatment. Some authors claim that the electric field has an important impact on cell death or tumour tissue remodelling (Vodovnik, Miklavčič *et al.*, 1992; Nordenström, 1994a; Vijh, 1999). The electric field causes a flux of interstitial water, electro-osmosis, from the anode towards the cathode, since the water molecules act like a dipole. Consequently, the tissue surrounding the anode dehydrates while oedema is obtained around the cathode (Nordenström, 1983; Vijh, 1999). 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 (Nordenström, 1983; Vodovnik, Miklavčič *et al.*, 1992).

Others have considered chlorine (Cl) as being the most toxic factor (Samuelsson & Jonsson, 1980; Samuelsson, Olin *et al.*, 1980).

The impact of pH

With little doubt though, most reports have elucidated the extreme pH-gradient that occurs at EChT. At the anode, as low pH as 1 has been measured (Nordenström, 1983; Miklavčič, Serša *et al.*, 1993). While at the cathode the prominent alkalisation yields pH levels as high as 13 (Nordenström, 1983; Li, Xin *et al.*, 1997). At these extreme pH values, the tissue proteins become denaturated and the cell structure collapses and the cell eventually dies (Lemberg & Legge, 1949; Nordenström, 1983; Li, Xin *et al.*, 1997).

The pH changes during EChT have also been predicted through many theoretical calculations. Berendson *et al.* studied the spreading of hydrogen ions and molecular chlorine around spherical and planar platinum anodes (Berendson & Simonsson, 1994; Berendson & Olsson, 1998). Nilsson *et al.* have investigated the formation and spreading of potential toxic species around both the anode and cathode, preferably with a spherical electrode configuration (Nilsson, Fontes *et al.*, 1998; 1999; Nilsson & Fontes, 2001).

Dissolved ions

The above are correct in an environment were the treatment electrodes are inert. If the electrode is made from a soluble material such as Copper (Cu), Rhodium (Rh) or brass (Zn-Cu alloy) the electrolysis causes metal ions to dissolve in the tissue (Samuelsson, Jonsson *et al.*, 1991). In that case, the metal ions can have toxic capacity by themselves.

Miklavčič, Serša and co-workers, have shown both the importance of the pH change in tissue during EChT as well as the formation of metal ions during electrolysis when using other materials than Platinum (Pt) (Miklavčič, Fajgelj *et al.*, 1994). In their experiments they also showed that the intralesional temperature change is marginal and hence, most likely, do not influence the cell survival (Miklavčič, Serša *et al.*, 1993).

Aims of the study

The aim of this study was to investigate how electrochemical treatment affects normal and tumour tissue. Moreover, interest was focused on whether the electrode polarity mattered in terms of destruction type and treatment efficacy. In addition to the work in different tissues and cell cultures, the thesis includes parts of an earlier described dose-planning model. The results from this thesis enable an investigation of the validity of the mathematical models, as well as of their applicability for dose planning. This was done:

- by studying the correlation between the pH profile in the tissue surrounding the electrodes and the macroscopic, histopathological and ultrastructural tissue destruction obtained directly after terminated EChT.

- by studying the reactions with a single electrode configuration. In all experiments a separation between the polarities of the electrodes have been assured to allow specific examinations of the different reactions occurring around the anode and the cathode, respectively.

- by studying the cellular response to unphysiologic pH. To verify the relation between cell damage and pH an *in vitro* model was conducted where tumour cells were exposed to a pH gradient.

- by studying the EChT effect in tumour tissue. To investigate if the same destructive mechanisms could be observed in tumour tissue *in vivo* an animal model was preformed with the same cell line that had formerly been used *in vitro*.

- by studying the accuracy of an earlier described theoretical model. The *in vivo* experiments also served as a control and calibration of a mathematical dose-planning model.

Materials and methods

Ethics

Every step has been taken to follow the guidelines from the The Swedish National Board for Laboratory Animals (CFN) to Reduce, Refine and Replace experiments with animals. Some animal experiments have been *Replaced* by performing the pH exposure to rat mammary cancer cells *in vitro* (Paper III) and hereby *Reduced* the number of animals used in the tumour inoculation model (Paper V) to a minimum, since we already knew what damages to expect and only wanted to verify that the same mechanisms are occurring *in vivo*. The experiments were also *Refined* by using a lot of time to create the protocols and conduct as many terminal experiments possible, where the animals were treated under general anaesthesia and euthanised without first waking up. Due to this, the amount of pain and discomfort during the trial has been minimised.

Treatment with EChT in adult female CD rat mammary and liver tissue complies with the decision of the Swedish animal ethical committee C 112/96. The treatment of liver tissue in dog complies with decision nr C 76/99 while the tumour model in rats complies with the code S 84/00.

The methodology and results will only be described briefly since it has already been thoroughly penetrated in the papers included in the thesis.

Normal rat mammary tissue (Paper I)

To study the effect of electrochemical treatment on normal tissue, mammary gland in 52 adult female rats weighing 270-350 g, were treated (1-5 mA, 10-85 min) under general anaesthesia using midazolam (Dormicum®, Roche, Stockholm) 1.2 mg/ml and fentanyl-fluanisone (Hypnorm®, Janssen Animal Health, Buckinghamshire, UK) diluted 1:4, which was injected *i.p.* 0.3 ml/100 g (Flecknell, 1993), in a terminal (acute) experiment. A heating lamp was placed 20 cm above the animal to decrease hypothermia. Constant direct current was used in order to investigate the influence of current level, treatment time and coulomb dosage on tissue destruction. The electrodes used were Platinum:Iridium (Pt:Ir) (9:1) with spherical tips. Extensive *in situ* pH-measurements were performed with a micro-combination glass electrode (MI-413, Microelectrodes Inc., USA) encased in an 18-gauge stainless steel needle. The tip of the needle had a diameter of 1.3 mm. The electrode was designed for measurements in the pH range of 0-14. The equipment used for EChT is shown in Figure 2.

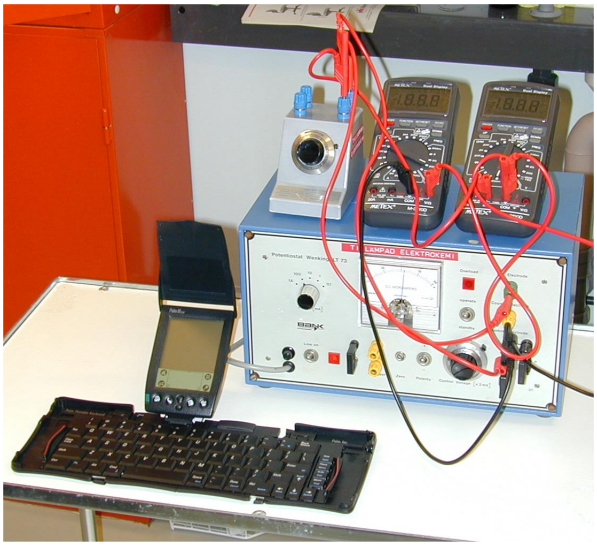

Figure 2. The equipment used for EChT. In the background the potentiostat is seen. In the foreground a Palm pilot IIIXe is attached to a portable keyboard.

Macroscopic examination of treated mammary tissue revealed an almost spherical necrosis with heavy dark colour due to the heamatin produced during EChT. Histological examination showed that the direct current induced two different types of necrosis depending on the polarity of the electrode. Firstly, at the anode, a coagulative necrosis with pycnotic nuclei, intravascular thrombosis and extravasation of blood cells was obtained. Although it appeared that the histological architecture was still rather evident, the tissue was suffering from severe dehydration. On the other hand, at the cathode, a prominent oedema with large, vacuolarly degenerated nuclei (hypoosmosis) and lysed erythrocytes appeared. It was harder to follow the original structure of the tissue. Consequently, the border between oedematous, damaged tissue and intact tissue was not as clear as in the anodic section.

Steep pH profiles, with pH values switching from unphysiological to neutral status within only a few millimetres, were obtained in tissue surrounding the electrodes. Significant differences $P<0.05$ (paired t-tests), in lesion sizes and pH profiles were obtained when using a wide range of currents while holding the coulomb dosage constant. The pH at the border of the anodic and cathodic lesions correlated well to certain values, 4.5 to 5.5 at the anode and between 9 to 10 at the cathode, irrespective of current or coulomb dosages delivered. This indicates that in a clinical situation pH values known to cause total tissue destruction could be used as markers to evaluate the efficacy of treatment.

Comparison between EChT in normal rat mammary and liver tissue (Paper II)

This study compared results from treatment of liver and mammary tissue focusing on destruction and pH changes in the tissue close to the treatment electrodes. Subsequently, data were compared with a dose-planning model.
Mammary or liver tissue in 50 adult female Sprague Dawley rats (CD) was given EChT with a constant, direct current (1-5 mA, 18-85 min). The electrodes used were Pt:Ir (9:1) with spherical tips. *In situ* pH measurements were taken with a micro-combination glass electrode as earlier described in Paper I. All rats tolerated the treatment well. The animals were euthanased after EChT without first waking up.

Spherical lesions were produced in both liver and mammary tissue. No significant difference (paired t-tests) was detected when comparing the size of the lesions in the two kinds of tissue.

Similar pH profiles were obtained in tissue surrounding the electrodes, with pH values changing from unphysiological to neutral status within the space of a few millimetres. One clear difference was that in all treatment groups, the liver tissue proved to be significantly more acidic close to the anode, and more alkaline close to the cathode than did mammary tissue. The pH at the border of the macroscopic destruction zone, regardless of tissue type or coulomb dosage, correlated well with specific values already described in Paper I.

The mathematical dose-planning simulations indicated that in tissue surrounding the anode, the pH profile continued to change, due to diffusion and chemical reaction, with time after the current had been cut. At the lower amperage (1 mA), the experimental pH profiles showed a very good agreement with those of simulated pH, obtained immediately after current shutdown. The simulated pH profile was approximately halfway between the profiles obtained in mammary tissue and liver tissue. If the diffusion-reaction process following treatment was taken into account, the model considerably overestimated the pH in tissue surrounding the anode. By contrast, at the higher amperage (5 mA), the comparison between measured and predicted pH profiles showed the correlation to be closest where the diffusion-reaction process was included in the simulation.

The analogous destruction patterns in mammary and liver tissue support the hypothesis that EChT has similar results in at least these two different types of tissue. This implies that the destructive pattern caused by the treatment may be the same also in tumours.

Cellular toxicity on a rat mammary tumour cell line exposed to a pH-gradient (Paper III)

In EChT pH alteration in tissue is the major cause to cell damage. The aim of this study was to evaluate the cellular toxicity of a pH gradient on the R3230AC mammary tumour cell line, clone-D (kindly provided by Prof. G. Bussolati,

University of Turin, Italy) *in vitro*. The results could be used to interpret the cell damaging effects seen in electrochemical treatment of tumours.
Tumour cells were treated for 10, 20 or 30 min respectively with pH 3.5, 5, 7, 9, 10 and 11. The following methods for detecting cellular damage were applied: viability assay 3-(4,5-dimethylthiazol-2-yl)-2,5,-diphenyl tetrazolium bromide (MTT), morphological observation in phase contrast microscope (PCM) and light microscope, nucleotide analogue incorporation (BrdU; 5-Bromo-2'-deoxyuridine), caspase-3 activity measurement and detection of DNA fragmentation by agarose gel electrophoresis.

In the MTT-assay, it was found that the pH was toxic to cells in a pH-dependent and time-dependent manner. The effects were most prominent by pH 3.5, 10 and 11 for 30 min ($p<0.01$). Morphologically, cells in pH 3.5 and 5 had shrunk, were round and had condensed chromatin, whereas a prominent cell swelling and nuclear expansion were seen in the pH 9 and 10-treated cells. Gross cytolysis was found in pH 11. The BrdU incorporation assay indicated that proliferation rate is inhibited markedly both with decreasing and increasing pH. Significant rise in the caspase-3 activity was found in all acidic treatment groups ($p<0,05$) except for pH 5 and 20 min, still caspase-3 activity were 54% higher than normal in this group. The fold-increase in CPP32 activity was determined by comparing the results with the negative control, pH 7 (value 1). No significant differences between normal control and the alkaline group could be detected. Treatment of the cells with pH 3.5 for 30 min created a pattern characteristic of internucleosomal fragmentation, DNA ladder (Figure 3). Treatment of the cells with other pH and duration of exposure did not cause marked DNA fragmentation.

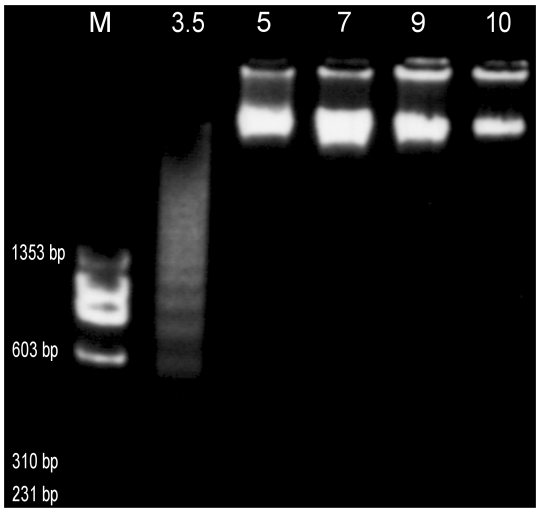

Figure 3. Detection of DNA fragmentation by agarose gel electrophoresis (1.5% agarose gel for 240 min at 65 V). pH-values are marked above each lane, exposure time; 30 min. M=DNA fragment size marker. Treatment of the cells with pH 3.5 and 30 min created a pattern characteristic of internucleosomal fragmentation, DNA ladder (apoptosis).

The pH changes inhibit proliferation and viability. The pathway of killing tumour cells in low pH has at least two directions; apoptosis and cell necrosis whereas alkaline pH results only in cell necrosis. The study suggests that low pH environment can induce apoptosis in unphysiological condition comparable with tissue pH at EChT. In addition, it seems that R3230AC mammary tumour cells are more tolerant to high pH than to acidic changes. This supports the theory that anodic EChT should be more efficient than cathodic.

Electrochemical treatment in normal rat and dog liver (Paper IV)

The purpose of this study was to examine whether an *in vivo* dose-planning model developed for rat liver is reliable in species with larger liver tissue mass *i.e.* dogs and eventually humans. Tissue lesions were compared between a four-electrode arrangement (dog) and a two-electrode EChT design (dog and rat). Macroscopic, histopathological and ultrastructural findings following EChT were determined.

Thirty (30) female Sprague Dawley rats and 4 female beagle dogs bred for research purposes only (provided by the Faculty of Veterinary Medicine, Department of Small Animal Clinical Sciences (SLU), Uppsala, Sweden), were studied with EChT during general anaesthesia. Platinum:Iridium (9:1) electrodes, 0.5 mm in diameter with either spherical- (rat) or string- (dog) configuration were used and the delivered dose was 5, 10 or 90 Coulombs (As). Haematological values, liver enzymes (only in dogs) and acid-base balance, were determined in blood before and after EChT. The animals were euthanised directly after termination of EChT, without being allowed to wake up. The spherical (rat) or cylindrical (dog) volume of the lesions was determined and samples were taken for histopathological and ultra-structural investigations.

The distribution of the lesions was predictable irrespectively of doses and electrode designs. Destruction volumes, calculated according to the macroscopic measurements, were found to fit into a logarithmic curve (dose-response). Histopathological examination confirmed the spherical and cylindrical/ellipsoidal area of tissue destruction but the type of necrosis was different at the anode and cathode, respectively (Figure 4).

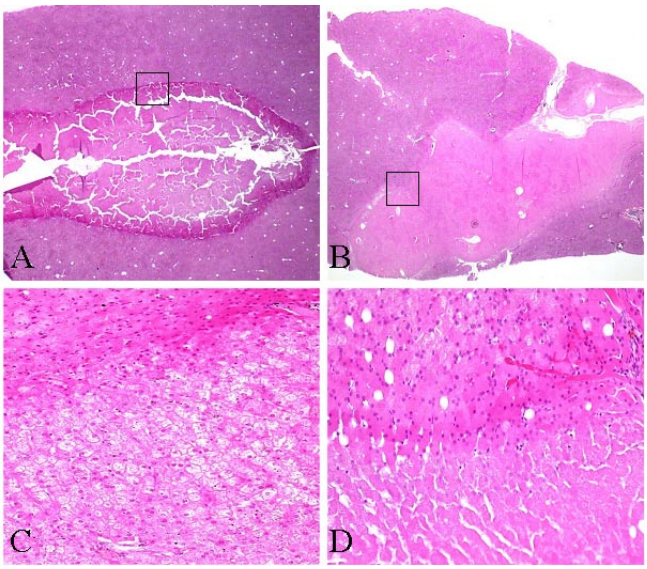

Figure 4. Histopathological examination of the normal dog liver, using two pair of electrodes. A) An extensive and well-defined area of coagulative necrosis is seen (magnification 10.5X). All four electrode lesions are seen in this large tissue section of the liver. The anodic lesion is dehydrated, with pycnotic nuclei and a small rim of marginal infarctions at the border. B) The cathodic lesions (same magnification as A) 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. C) A higher magnification (420 X) of the border of destruction close to the anode. D) Showing the cathodic counterpart to C.

Ultra-structural analysis showed distinct features of cell damage depending on the distance from the electrode (Figure 5a and b). Histopathological and ultra structural examination demonstrated that the liver tissue close to the border of the lesion displayed a normal morphology.

The *in vivo* dose-planning model developed for rat liver is reliable in species with larger tissue mass such as dogs. A multi-electrode EChT-design could obtain predictable lesions and the dose-response curve suggests a logarithmic relationship. Moreover, the cellular toxicity following EChT is clearly identified and it varies with the distance from the electrode and electrode polarity. Furthermore, 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.

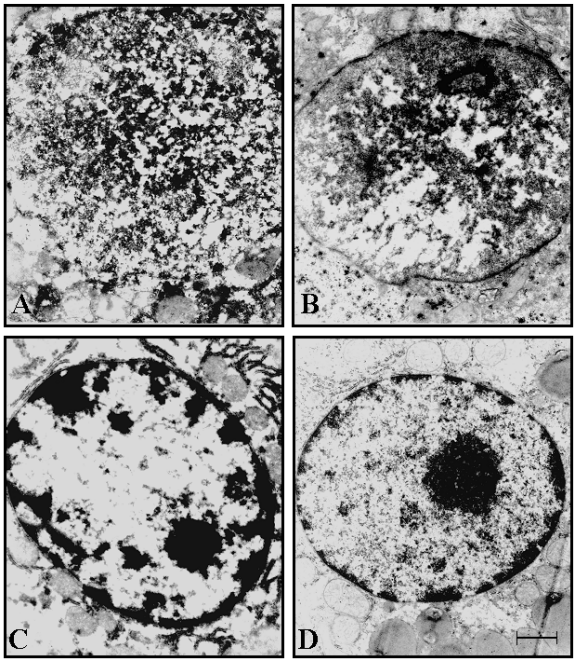

Fig. a

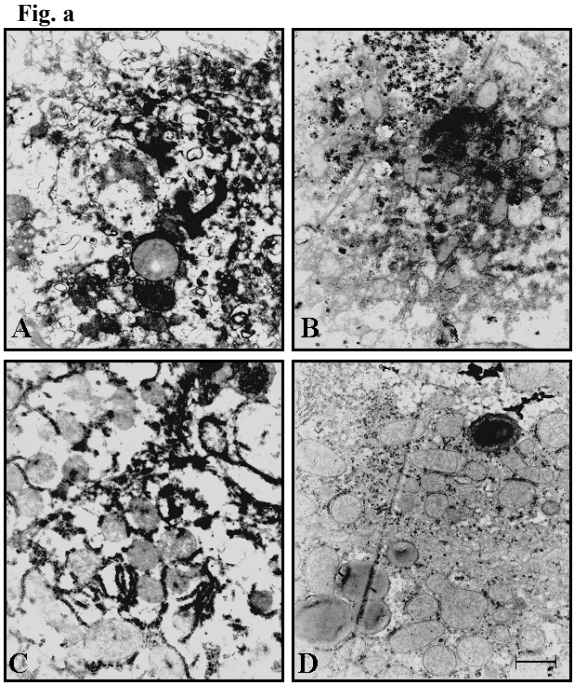

Fig. b

Figure 5a and b. Ultra-structural morphology in rat hepatocytes (nucleus a and cytoplasm b) following anodic EChT (5mA 10C), depending on the distance from the electrode. A shows a nucleus totally disrupted. B displayed severe damages mostly in the mitochondria (cytoplasm). 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 granular endoplasmic reticulum (RER) was well defined and looked normal. Only minor changes were detected in the nuclear chromatin. C) The liver tissue from the border of the destruction had an ultra-structural morphology that barely could be distinguished from controls. 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). The control (Fig 5aD and 5bD), displayed a normal ultra-structural morphology with dense mitochondria, distinct RER, peroxisomes and glycogen. Furthermore, the nucleus displayed natural euchromatin and hetero-chromatin. Bars=1 μm.

Cell proliferation and apoptosis in rat mammary cancer after Electrochemical Treatment (Paper V)

In vivo studies were conducted to evaluate the toxic changes and effectiveness of EChT on an animal tumour model.

Tumours were induced in twenty-eight female Fischer 344 rats weighing approximately 200 g by injecting $0.5x10^6$ cells from the R3230AC rat mammary tumour cell line clone D subcutaneously into the left and right region 1 cm caudal to the scapula. When the tumour volumes reached approximately 400 mm^3, the animals were anaesthetised as earlier described in *e.g.* Paper I. EChT was conducted by inserting a thread platinum electrode into the tumour. The delivered doses were 10 or 20 Coulomb (C) with a constant current of 10 mA. The positive and negative control groups were subjected to the same conditions but without EChT. The negative control was injected with cell media (RPMI 1640). The rats were kept for 0, 7 or 14 days post treatment. Three hours prior to euthanasia an *i.p.* injection of Bromodioxyuridine (BrdU) 100 mg/kg were given. The rats were euthanased by administering 3 ml Na-pentobarbital 2.5% *i.p.*, The lesions were extirpated and samples were collected for histopathological, and immunohistochemical examination.
Histopathological changes resembled the lesions described earlier for normal mammary tissue in the rat.

Significant changes in proliferation rate, as well as macroscopic measurable growth retardation, were seen both in the cathode and anode reaction, compared to untreated control. Apoptosis were induced in the anodic treatment (Figure 6) suggesting that cell destruction were caused by necrosis with cathodic EChT and apoptosis together with necrosis at the anode. The findings agree with the earlier result from Paper III, where an *in vitro* pH gradient exposure was applied for the same cell line as used in the inoculation model.

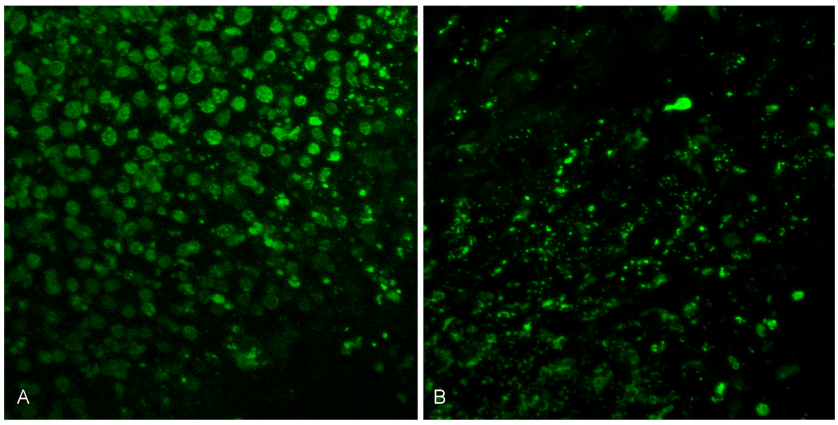

Figure 6. Immuno fluorescence microscopic examination of apoptosis in a rat mammary tumour using *TUNEL*. A) Tumour cells at the border of destruction, anode; 20 C, 10 mA (objective 64 X). The nuclei show typical signs of apoptosis, with good morphology. B) The border of destruction around the cathode (same magnification as in A). At the cathode, the morphology is poor and the tumour tissue has gone into complete necrosis.

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 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.

We have chosen the CD-rat as an animal model for normal tissue to study the destruction mechanism with EChT. The change to Fischer 344 rats in the inoculation model was because the tumour cell line used was derived from this strain.

In Paper I, mammary tissue in adult female rats was treated with constant direct current in order to investigate the influence of current level, treatment time and coulomb dosage on tissue destruction. The electrodes used were Pt:Ir (9:1) with spherical tips. This geometry generated a practical tool for comparing destructive effects with a proposed three-dimensional destruction in the dose-planning model earlier described (Nilsson, Fontes *et al.*, 1999). Extensive *in situ* pH-measurements were performed with a micro-combination glass electrode. Macroscopic examination of treated mammary tissue revealed an almost spherical necrosis with heavy dark colour due to the heamatin produced during EChT. All of these inputs helped in refining the dose-planning model.
The theoretical modelling has been presented earlier in a thesis of Nilsson (Nilsson, 2000). Hence, the present thesis has focused on the destructive properties and destruction mechanisms of EChT *in vivo* and *in vitro*.

The mechanisms for cell destruction of a tumour differ between *e.g.* radiotherapy and electrolytic treatment. The limitations of radiotherapy to effectively achieve tumour control in the centre of a gross tumour is mainly due to the fact that a large portion of the cells might be hypoxic because of impaired blood supply and a high intratumoral pressure. A good oxygenation of the tissue will result in development of more oxygen radicals and more DNA-damage during radiotherapy (Riley, 1994). In addition, peripheral parts of the tumour might periodically become hypoxic by shunting of the blood (Zywietz, Reeker *et al.*, 1997).

Samuelsson *et al.* reported 1991 encouraging data on the combination of radiotherapy and EChT in tumour tissue (Samuelsson, Jonsson *et al.*, 1991). They studied the effect of EChT (25 mA, 20 min) and subsequent radiotherapy, both in experimental tumours (malignant mesothelioma and colon carcinoma) in rats. In another paper Samuelsson *et al.* used the combination therapy on lung tumours in six pigs and in a single human lung tumour patient (Samuelsson, Lamm et al., 1985). In the former study, current was supplied through two wire electrodes,

either copper or platinum, placed about 10 mm apart in the centre of the tumour. The combined treatment resulted in tumour growth inhibition and in 75% of the cases the tumours disappeared. In the group that received only radiotherapy, 75% of the tumours remained. The authors hypothesised that the inflammatory reaction around the electrolytic lesion lead to increased blood flow and higher oxygenation of the tumour, and thereby made the tumour more radiosensitive.

Electrolysis can occur independently of the oxygenation status, since the cell death is 100% in tissue when the pH <2 (Paper I). In peripheral areas outside the gross tumour volume where single- or groups of tumour cells have invaded the normal stroma, radiation can selectively kill cancer cells while the bulk of normal cells will be preserved. The electrolytic technique will not be selective as far as we know in such an area, destroying tumour and normal cells with the same mechanisms, unless the electrical field can effectively kill tumour cells. This question needs to be investigated. Another important question to address is if it is possible to enhance the intended effect of ionising radiation in the target area by modulating the electric potential using a single electrode inserted into the tumour.

The main advantage with electrolytic tumour destruction is that energy can be deposited into deeply situated targets without affecting tissues in the periphery of the target area. This can be achieved simply by isolating the part of the electrode that passes through the normal tissue. Even if using the most sophisticated conformal planning techniques for radiotherapy, ionisation of normal tissue surrounding the tumour will always take place to a certain degree.

As described earlier, combination treatments with different chemotherapy regimes have been tried (Nordenström, 1983; Eksborg, Nordenström *et al.*, 1990; Serša, Novakovic *et al.*, 1993). It seems that EChT can enhance tumour size reduction when used together *e.g.* with doxorubicin or bleomycin.

The largest impact on tumour treatment with electric current and cytotoxic compounds is however the Electrochemotheraphy (Mir, Belehradek *et al.*, 1991; Mir, 1994; Serša, Cemazar *et al.*, 1995). Electrochemotherapy consists of intravenous (or intratumourally) administration of a low dose of an antineoplastic *e.g.* bleomycin, followed by local delivery of electric pulses on tumour nodules by means of two electrodes located at each side of the nodule. Hence, it is not the electric field itself that damages the cells but the electric pulses cause an electropermeabilization which enhances the transport of the drug over the cell membrane and concentrates the drug in the tumours. This technique has recently been reported to be clinically used on horses with a special type of skin tumours called sarcoids (Rols, Tamzali *et al.*, 2002).

The tissue destruction with EChT, in large part, follows Faraday's Law I; In any electrolytic process the amount of chemical change produced is proportional of the total amount of electrical charge passed through the cell. In Paper I the induced tissue damage in the low current treatment (1 mA, 5 C) was smaller than in the groups with higher current (2.5; 5 mA and 5 or 10 C) but not proportional suggesting that *in vivo* application of electric current may be interacted and

impaired by *e.g.* the buffering systems. This implies that current and time, as well as the more commonly used coulomb dosage, should be used to a greater extent as dose parameters in EChT.

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, several authors have suggested the use of saline infusions into the anodic area (Nordenström, 1983). 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 at the anode by the dehydration and marginal infarctions, to re-enter the destructive area. In Paper IV we showed that the single electrode model has its obvious limitations in a future clinical setting, when treating malignancies, due to the logarithmic dose-response curve (Figure 7). In the cathode lesion the dose-response curve is similar to that at the anode, which decreased efficacy over time. The tissue lesion is characterised by an oedema, which conceivably allows the buffering species to hamper the electrolysis. 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.

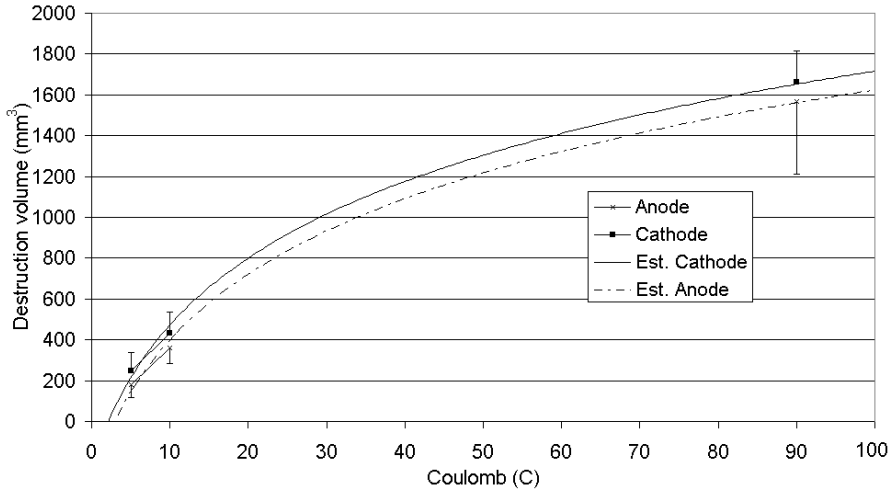

Figure 7. The chart shows the macroscopically determined volume of destruction after treatment with 5 and 10 C in rat liver and 90 C in dog liver. It also shows the estimated (Est.) dose-response curve for both anodic and cathodic EChT. The relation is almost logarithmic for both treatments, suggesting that the destruction volume has harder to propagate, as it grows larger in size.

In Paper II and IV we showed that the behaviour of electrochemical reaction *in vivo* is essentially the same disregarding different tissue (mammary or liver tissue) and different species and destruction size (liver destruction in rat versus dog). This is important when a comparison should be made between the many different trials that have been reported in the field of EChT. The differences between electrode materials used and the variations in combination therapies used are of course not applicable in this aspect.

Paper IV also described that the ultra-structural features of an EChT lesion are 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.

In Paper III and V the influence of pH changes on the R3230AC rat mammary tumour cell line was investigated. The *in vitro* experiment showed that the extracellular pH change inhibits proliferation and viability. The pathway of killing tumour cells in low pH had at least two directions; apoptosis and cell necrosis (mainly apoptosis), whereas high pH resulted in cell necrosis. This entails that EChT, with its ability to create a fiercely toxic pH gradient, is an effective way of treating tumours. The following tumour inoculation experiment confirmed the findings in Paper III. Hence the anodic treatment with induction of apoptosis must be due to intratumoral acidification since no increase of apoptosis could be detected with the cathodic treatment. Paper V also shows that EChT gives a significant decrease in tumour proliferation *in vivo*.

The development of different EChT treatment modalities is gaining increasing interest in the Western world. Basic investigations of the cellular behaviour in both normal and neoplastic tissue are good complements to the large-scale models and long terms studies of the safety effects of electrochemical treatment that recently have been published. To, in the future, anticipate the introduction of EChT as a complement in the treatment of tumours in humans as well as companion animals now appears realistic.

References

Berendson, J. and Olsson, J.M. 1998. Bioelectrochemical aspects of the treatment of tissue with direct current. *Electro- and Magnetobiology 17(1)*, 1-16.

Berendson, J. and Simonsson, D. 1994. Electrochemical aspects of treatment of tissue with direct current. *European journal of surgery (Suppl 574)*, 111-115.

Cavallo, T. 1777. *A complete treatise on electricity in theory and practice*. London, Dilly.

Crussel, G. 1847. Die Electrilytishen Heilanstalt in Moscow. *Med. Zeitung Russlands 4*, 2041.

Duchenne, G. 1872. *De l'Electrisation Localisée*. Paris, Baillière.

Eksborg, S., Nordenstrom, B.E. and Beving, H. 1990. Electrochemical treatment of cancer. III: Plasma pharmacokinetics of adriamycin after intraneoplastic administration. *American journal of clinical oncology 13(2)*, 164-166.

Flecknell, P.A. 1993. Anaesthesia of animals for biomedical research. *British journal of anaesthesia 71(6)*, 885-94.

Humphrey, C.E. and Seal, E.H. 1959. Biophysical Approach toward Tumor Regression in Mice. *Science 130*, 388-390.

Inglis-Parsons, J. 1893. *The Healing of Rodent Cancer by Electricity*. London, Bale.

Lemberg, R. and Legge, M. 1949. *Hematin compounds and bile pigments*. New York, Interscience Publ. Inc.

Li, K., Xin, Y., Gu, Y., Xu, B., Fan, D. and Ni, B. 1997. Effects of direct current on dog liver: possible mechanisms for tumor electrochemical treatment. *Bioelectromagnetics 18(1)*, 2-7.

Massey, G.B. 1898. *Conservative Gynecology and Electro-Therapeutics*. Philadelphia, F.A. Davis Co.

Massey, G.B. 1914. Ionization treatment of cancer. End-result of twenty years' work. A summary of 300 cases. *American journal of surgery 28*, 329.

Massey, G.B. 1924. *Practical Electrotherapeutics and Diathermy*. New York, Macmillan.

Matsushima, Y., Takahashi, E., Hagiwara, K., Konaka, C., Miura, H., Kato, H. and Koshiishi, Y. 1994. Clinical and experimental studies of anti-tumoural effects of electrochemical therapy (ECT) alone or in combination with chemotherapy. *European journal of surgery (Suppl 574)*, 59-67.

Miklavčič, D., Fajgelj, A. and Serša, G. 1994. Tumour treatment by direct electric current: Electrode material deposition. *Bioelectrochemistry and Bioenergetics 35*, 93-97.

Miklavčič, D., Serša, G., Kryzanowski, M., Novakovic, S., Bobanovic, F., Golouh, R. and Vodovnik, L. 1993. Tumor treatment by direct electric current - tumor temperature and pH, electrode material and configuration. *Bioelectrochemistry and Bioenergetics 30*, 209-220.

Mir, L.M. 1994. Antitumor electro-chemotherapy. *Bull. cancer Paris 81(9)*, 740-748.

Mir, L.M., Belehradek, M., Domenge, C., Orlowski, S., Poddevin, B., Belehradek, J., Jr., Schwaab, G., Luboinski, B. and Paoletti, C. 1991. Electrochemotherapy, a new antitumor treatment: first clinical trial. *Comptes rendus de l'Academie des sciences. Serie III 313(13)*, 613-618.

Nilsson, E. 2000. *Modelling of the Electrochemical Treatment of Tumours. Department of Chemical Engineering and Technology, Applied Electrochemistry. Stockholm, Royal Institute of Technology, Doctor's thesis.* 1-44. ISSN 1104-3466.

Nilsson, E. and Fontes, E. 2001. Mathematical modelling of physicochemical reactions and transport processes occurring around a platinum cathode during the electrochemical treatment of tumours. *Bioelectrochemistry 53,* 213-224.

Nilsson, E., Fontes, E. and Berendson, J. 1998. Electrochemical Treatment of Tumours: A Simplified Mathematical Model, Part I. *Bioelectrochemistry and Bioenergetics 47,* 11-18.

Nilsson, E., Fontes, E. and Berendson, J. 1999. Electrochemical treatment of tumours: a simplified mathematical model. *Journal of electroanalytical chemistry. 460*, 88-99.

Nilsson, E., von Euler, H., Berendson, J., Thörne, A., Wersäll, P., Näslund, I., Lagerstedt, A.-S., Narfström, K. and Olsson, J.M. 2000. Review - Electrochemical Treatment of Tumours. *Bioelectrochemistry 51*, 1-11.

Nordenström, B.E. 1983. *Biologically Closed Electric Circuits: Clinical, experimental and theoretical evidence for an additional circulatory system*. Stockholm, Nordic Medical Publications.

Nordenström, B.E. 1989. Electrochemical treatment of cancer. I: Variable response to anodic and cathodic fields. *American journal of clinical oncology 12(6)*, 530-6.

Nordenström, B.E. 1994a. Electrostatic field interference with cellular and tissue function, leading to dissolution of metastases that enhances the effect of chemotherapy. *European Journal of Cancer (Suppl 574),* 121-135.

Nordenström, B.E. 1994b. Survey of mechanisms in electrochemical treatment (ECT) of cancer. *European Journal of Cancer (Suppl 574),* 93-109.

Nordenström, B.E., Eksborg, S. and Beving, H. 1990. Electrochemical treatment of cancer. II: Effect of electrophoretic influence on adriamycin. *American journal of clinical oncology 13(1)*, 75-88.

Riley, P.A. 1994. Free radicals in biology: oxidative stress and the effects of ionizing radiation. *International journal of radiation biology 65,* 27-33.

Robertson, G.S., Wemyss-Holden, S.A., Dennison, A.R., Hall, P.M., Baxter, P. and Maddern, G.J. 1998. Experimental study of electrolysis-induced hepatic necrosis. *The British journal of surgery 85(9),* 1212-6.

Rols, M.P., Tamzali, Y. and Teissie, J. 2002. Electrochemotherapy of horses. A preliminary clinical report. *Bioelectrochemistry 55,* 101-105.

Samuelsson, L. and Jonsson, L. 1980. Electrolyte destruction of lung tissue. Electrochemical aspects. *Acta radiologica 21(6),* 711-714.

Samuelsson, L. and Jonsson, L. 1981. Electrolytic destruction of tissue in the normal lung of the pig. *Acta radiologica 22(1)*, 9-14.

Samuelsson, L., Jonsson, L., Lamm, I. L., Linden, C.J. and Ewers, S.B. 1991. Electrolysis with different electrode materials and combined with irradiation for treatment of experimental rat tumors. *Acta radiologica 32(2)*, 178-181.

Samuelsson, L., Lamm, I. L., Mercke, C.E., Stahl, E. and Jonsson, L. 1985. Electrolytic tissue destruction and external beam irradiation of the lung. An experimental and clinical investigation. *Acta radiologica 26(5),* 521-524.

Samuelsson, L., Olin, T. and Berg, N.O. 1980. Electrolytic destruction of lung tissue in the rabbit. *Acta radiologica 21(4)*, 447-454.

Schechter, D.C. 1979. Flashbacks: containment of tumors through electricity. *Pacing and clinical electrophysiology 2(1),* 100-114.

Serša, G., Cemazar, M. and Miklavčič, D. 1995. Antitumor effectiveness of electrochemotherapy with cis-diamminedichloroplatinum(II) in mice. *Cancer research 55(15),* 3450-3451.

Serša, G., Novakovic, S. and Miklavčič, D. 1993. Potentiation of bleomycin antitumor effectiveness by electrotherapy. *Cancer letters 69(2),* 81-84.

Turler, A., Schaefer, H., Schaefer, N., Maintz, D., Wagner, M., Qiao, J.C. and Hoelscher, A.H. 2000. Local treatment of hepatic metastases with low-level direct electric current: experimental results. *Scandinavian journal of gastroenterology 35(3),* 322-328.

Watson, B.W. 1991. The treatment of tumours with direct electric current. *Medical science research 19,* 103-105.

Wemyss-Holden, S.A., Hall, P.M., Robertson, G.S., Dennison, A.R., Vanderzon, P.S. and Maddern, G.J. 2000. The safety of electrolytically induced hepatic necrosis in a pig model. *The Australian and New Zealand journal of surgery 70(8),* 607-612.

Wemyss-Holden, S.A., Robertson, G.S., Dennison, A.R., Vanderzon, P.S., Hall, P.M. and Maddern, G.J. 2000. A new treatment for unresectable liver tumours: long-term studies of electrolytic lesions in the pig liver. *Clinical science 98(5),* 561-567.

Vijh, A.K. 1999. Electrochemical Treatment of Tumors (ECT): Electroosmotic Dewatering (EOD) as the Primary Mechanism. *Drying technology 17(3)*, 585-596.

Vodovnik, L., Miklavčič, D. and Serša, G. 1992. Modified cell proliferation due to electrical currents. *Medical and biological engineering 30(4),* 21-28.

Xin, Y.L. 1994. Organisation and spread of electrochemical therapy (ECT) in China. *European journal of surgery (Suppl 574),* 25-29.

Xin, Y.L. 1998. The clinical advance in application of EChT within the past ten years. *Preprints from the 2nd international symposium on electrochemical treatment of cancer, 27-30 Sep., Beijing, China,* 81-92.

Zywietz, F., Reeker, W. and Kochs, E. 1997. Changes in tumor oxygenation during a combined treatment with fractionated irradiation and hyperthermia: an experimental study. *International journal of radiation oncology, biology, physics 37(1),* 155-162.

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Conclusions

- In the centre of the electrodes, total tissue destruction occurred. However the ultra-structural features of an EChT 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.

- At the anode, a marked dehydration and acidification occurred with maintained tissue architecture. At the cathode, oedema and alkalisation were the dominating features. Moreover, the tissue lost the structural constitution.

- The *in vitro* experiment showed that the extracellular pH change inhibits proliferation and viability. The pathway of killing tumour cells in low pH had at least two directions; apoptosis and cell necrosis (mainly apoptosis), whereas high pH resulted in cell necrosis.

- *In vivo* the induction of apoptosis was concentrated to the border of destruction at the anode, probably due to the acidification that occurs. This is also supported by the *in vitro* study (Paper III). The cell proliferation rate was reduced significantly in the whole tumour, both for the anodic and cathodic treatment, suggesting that secondary mechanisms such as the inflammatory response, tumour ischemic anoxia and radical formations also impaired the tumour progression after EChT. Hence, the treatment had a significant effect on tumour cell's proliferation far from the evidently destroyed area.

- The mathematical model predicted an acidic zone, around the anode, of about the same radius as that measured in mammary and liver tissue in Paper II. At the higher amperage (5 mA), the simulated pH profile agreed rather well with those observed experimentally, while the model considerably underestimated the acidity at the lower amperage (1 mA). The effect of a specified coulomb dose is not always consistent. By decreasing the treatment current, the buffer systems of the body can, to a larger extent, counteract the spreading of toxic species around the electrodes. Thus, the number of applied coulombs corresponds to the amount of reaction products formed at the electrodes but does not directly describe their distribution in tissue. The multi-electrode EChT-design used in Paper IV could obtain predictable lesions even in large destructions.

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