

Review

Electrochemical treatment of tumours

Eva Nilsson^{a,*}, Henrik von Euler^b, Jaak Berendson^a, Anders Thörne^c, Peter Wersäll^d,
Ingemar Näslund^d, Anne-Sofie Lagerstedt^b, Kristina Narfström^b, Jerker M. Olsson^e

^a Department of Chemical Engineering and Technology, Applied Electrochemistry, Royal Institute of Technology (KTH), SE-100 44 Stockholm, Sweden

^b Department of Small Animal Clinical Sciences, Swedish University of Agricultural Sciences (SLU), SE-750 07 Uppsala, Sweden

^c Department of Surgery, Huddinge University Hospital, Karolinska Institutet, SE-141 86 Huddinge, Sweden

^d Department of Oncology (Radiumhemmet), Karolinska Hospital, SE-171 76 Stockholm, Sweden

^e Department of Immunology, Microbiology, Pathology and Infection Diseases, Division of Pathology, Huddinge University Hospital, Karolinska Institutet, SE-141 86 Huddinge, Sweden

Received 23 September 1999; received in revised form 20 October 1999; accepted 28 October 1999

Abstract

The electrochemical treatment (EChT) of tumours implies that tumour tissue is treated with a continuous direct current through two or more electrodes placed in or near the tumour. The treatment offers considerable promise of a safe, simple and relatively noninvasive anti-tumour therapy for treatment of localised malignant as well as benign tumours. Although more than 10 000 patients have been treated in China during the past 10 years, EChT has not yet been universally accepted. The reason for this is the lack of essential preclinical studies and controlled clinical trials. Uncertainties regarding the destruction mechanism of EChT also hinder the development of an optimised and reliable dose-planning methodology. This article reviews the collected Chinese and occidental experiences of the electrochemical treatment of tumours, alone and in combination with other therapies. The current knowledge of the destruction mechanism underlying EChT is presented along with different approaches towards a dose planning methodology. In addition, we discuss our view of different important parameters that have to be accounted for, if clinical trials are to be initiated outside of China. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Cancer; Direct current; Dose planning; Electrochemical treatment (EChT); Tumour

1. Introduction

The electrochemical treatment (EChT) of tumours implies that tumour tissue is treated with a continuous direct current through two or more electrodes placed inside the tumour or in its close vicinity. Reports on the anti-tumour effect of low-level direct current date back to the end of the 19th century [1]. However, the interest of its underlying mechanism and possible therapeutic use waned until 1959, when Humphrey and Seal reported encouraging results with sarcoma tumours in mice [2]. Following this, a number of workers applied EChT in both animal tumour models and small-scale clinical studies. A short review on the application of direct current in the treatment of tumours was presented in 1991 [3]. Since then, many interesting studies have been published, including a vast amount

of clinical data from China. It is now appropriate to once again review the subject.

This article intends to give an overview of the experiences of the last three decades of electrochemical treatment of tumours and combination therapies. The present knowledge of the destruction mechanism underlying EChT is reviewed, along with different approaches towards a reliable dose planning. Finally, we discuss our view of different important parameters that have to be accounted for, if clinical trials are to be initiated also outside of China.

2. Experiences from the electrochemical treatment of tumours

2.1. Pioneering clinical studies by Nordenström

Björn Nordenström, a Swedish professor in radiology, is considered to be a pioneer in the treatment of tumours with direct electric current and combination therapies in

* Corresponding author. Tel.: +46-8-790-65-06; fax: +46-8-10-80-87.
E-mail address: eva@ket.kth.se (E. Nilsson).

patients. In the late 1970s, Nordenström started to treat primary lung cancers by applying current between two platinum wire electrodes. The anode was placed centrally in the tumour and the cathode approximately twice the tumour diameter away from the anode. The applied voltage was about 10 V. The patients were treated under local anaesthesia, and they were seldom uncomfortable during the treatment. A preliminary trial on five patients was published in 1978 [4], and in his book [5], written in 1983, Nordenström reported the results from the treatment of 26 lung tumours in 20 patients. Many of these patients were, for various reasons, unsuitable for surgical, radiotherapeutic, or chemotherapeutic treatment. The average delivered coulomb dosage (current multiplied by time) was 80 C/cm of tumour diameter. Regression was obtained in 12 out of 26 tumours and no signs of regrowth were detected after a 2–5-year follow-up period. There was no reported lethal outcome of the therapy, although tumours with a diameter larger than 3 cm did not respond well to the treatment. Among the side effects, Nordenström noticed slight fever and local pain during the treatment.

Nordenström thoroughly discussed possible destruction mechanisms behind EChT, such as extreme pH changes, electro-osmotic transport of water and the effect of current on transmembranous ionic transport [5,6]. He also pointed out that most tumours possess an excess of fixed electronegative charges on their surfaces and therefore only the anode should be placed inside the tumour so as to prevent the spreading of micrometastases during treatment. However, in a later work [7], Nordenström obtained positive results when he placed the cathode in the tumour.

Nordenström et al. [8] and Ekborg et al. [9], investigated the effects of a combined, simultaneous therapy of EChT and the chemotherapeutic agent, adriamycin. The purpose was to influence the distribution of the electropositively charged adriamycin by means of the electric field. When infused into the tumour via an anodic electrode, the electropositively charged adriamycin could, by electrophoresis, be dispersed uniformly into the surrounding tissue. Alternatively, adriamycin could be concentrated within the tumour region when applied intravenously and when the cathode was placed in the tumour. Using these techniques, the authors hoped to minimise the systemic side effects of chemotherapy. Beneficial effects were obtained in a preliminary study of 14 patients with large lung cancers, which were incurable by conventional methods [8].

One of the latest articles by Nordenström and his colleagues reports positive results from the electrochemical treatment of a patient with breast cancer [10]. Mammography, repeated at 6 monthly intervals in the 2 years after treatment, could not discern any trace of tumour remnants. The authors also proposed a combination therapy between an extensive electrochemical pretreatment and a restricted surgical operation. Ideally, the electrochemical treatment would kill undetected tumour foci and hence, post-operative recurrence would be avoided.

2.2. *Clinical trials in China*

An EChT project, based on the experiences of Nordenström and supported by the Chinese government, was initiated in China in 1987. Experimental and clinical data were rapidly gathered for 2 years, and in 1989, the electrochemical treatment of tumours was approved by a committee of experts, organised by the Ministry of Public Health in China, to be used throughout the entire country [11,12]. Professor Xin Yu-Ling and co-workers at The China–Japan Friendship Hospital in Beijing were assigned to organise postgraduate courses, and up to 1998, more than 2000 physicians have been trained in EChT.

The clinical experience of EChT in China was internationally presented at The First Conference of the International Association for Biologically Closed Electric Circuits in Medicine and Biology, held in 1993 in Stockholm, Sweden. A large amount of clinical material, covering more than 2000 cases of various tumour types, both benign as well as malignant, and treated during the period between 1987 and 1992, was discussed at this conference and subsequently published [13]. During this period, Xin and his collaborators modified Nordenström's electrode placement methodology. Instead of placing an anode in the tumour and a cathode far away from the tumour, they inserted a number of anodes into the centre of the tumour and the same number of cathodes just outside the tumour periphery.

The number of publications found in international journals, concerning the clinical results from China, are currently quite limited. However, a recent report [14] suggests that EChT provides a safe, simple, and effective complementary treatment for patients with lung neoplasms, who are neither suitable for surgery nor responsive to chemoradiotherapy. Of the 386 patients treated for lung cancer, the short-term (6 months) effectiveness was approximately 70% (complete and partial response) while the survival rates were approximately 85, 60 and 30% after 1, 3 and 5 years, respectively. Furthermore, tumours larger than 8 cm in diameter had poorer prognoses than those with a diameter measuring 4–8 cm. In this study, Xin et al. had again modified their electrode placement technique and both anodes and cathodes were now placed inside the tumours, with anodes in the centre and cathodes in the periphery. This modification did not only protect the normal tissue from destruction, but was also shown to enhance the therapeutic effect. It was stated that the tumour tissue-killing diameter around each electrode was about 3 cm, and thus, the distance between electrodes should not exceed 3 cm. Typical treatment parameters were 6–8 V, 40–80 mA and a coulomb dosage of 100 C/cm of tumour diameter.

A second international symposium in 1998 on the electrochemical treatment of cancer was held in Beijing, China, and it was reported that the treatment had been established in 1260 hospitals throughout China. Furthermore, more

Table 1

Short-term (6 months) efficacy of EChT. Objective remissions in 3802 cases of superficial malignant tumours treated with EChT in China, 1987–1997 [12]. Responses were determined by direct measurement. CR = complete response, PR = partial response, NC = no change, PD = progressive disease

	All <i>n</i>	CR		PR		NC		PD		CR + PR	
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Skin cancer	958	611	64	347	36	0	0	0	0	958	100
Malignant melanoma	227	79	35	27	12	27	12	94	41	106	47
Jaw and facial tumours	361	135	37	145	40	57	16	24	7	280	78
Oral (tongue or lip) cancer	138	46	33	75	54	11	8	6	4	121	88
Rhabdomyosarcoma	133	27	20	49	37	38	29	19	14	76	57
Chest–abdominal wall metastases	172	44	26	81	47	29	17	18	10	125	73
Vulva (penis or vagina) cancer	237	91	38	111	47	21	9	14	6	202	85
Breast cancer	644	180	28	296	46	64	10	104	16	476	74
Thyroid carcinoma	250	89	36	110	44	36	14	15	6	199	80
Parotid carcinoma	84	28	33	46	55	6	7	4	5	74	88
Other superficial tumours	598	154	26	280	47	99	17	65	11	434	73
Total	3802	1484	39	1567	41	388	10	363	10	3051	80

than 10 000 patients, with various kind of tumours, have been treated during the previous 10 years, indicating a tremendous clinical experience of the method [12]. For patients with neoplasms, who were neither suitable for curative surgery nor responsive to chemo- or radiotherapy, EChT was considered as an effective complementary treatment. In the cases of lung, liver, and esophageal cancer, the presented results indicated substantially lower mortality rates compared to the available statistics in occidental countries [15]. The 5-year survival rate for lung cancer treated with EChT was 39%, while the western figure is 14%. The corresponding values for cancer in the liver are 15% and 5%. Moreover, patients with noncurative esophageal cancer, treated with EChT, demonstrated a 5-year survival rate of 13%, which is similar to corresponding data reflecting the survival rate in all patients suffering from this neoplasm in western countries. However, randomised and controlled clinical trials are yet to be performed in China, and the characterisation and follow-up rate of patients, as well as the classification of the tumours, are only presented sparingly in the available reports [12].

Table 2

Short-term (6 months) efficacy of EChT

Objective remissions in 3840 cases of visceral malignant tumours treated with EChT in China, 1987–1997 [12]. Responses were determined by X-ray, ultrasonography, or computertomography. CR = complete response, PR = partial response, NC = no change, PD = progressive disease

	All <i>n</i>	CR		PR		NC		PD		CR + PR	
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Esophageal cancer	1595	348	22	766	48	319	20	162	10	1114	70
Lung cancer	1113	412	37	445	40	155	14	101	9	857	77
Liver cancer	961	240	25	427	44	210	22	84	9	667	69
Laryngeal cancer	21	9	43	9	43	2	10	1	5	18	86
Prostate cancer	20	8	40	7	35	3	15	2	10	15	75
Other visceral tumours	130	39	30	51	39	20	15	20	15	90	69
Total	3840	1056	28	1705	44	709	19	370	10	2761	72

These circumstances must be taken into consideration when comparing the efficacy of EChT to that of conventional anti-tumour therapies in occidental countries.

According to the data collected from 156 of the hospitals, 8240 patients, up until 1997, were treated with EChT, where 7642 of them suffered from malignant tumours and the remaining 598 had benign tumours. About half of the cancer patients (3802) had superficial malignant tumours (Table 1), while the remaining (3840) suffered from visceral neoplasms (Table 2). The short-term efficacy (complete and partial response) determined by direct measurement was 47–100% for the superficial neoplasms. Skin cancer, oral cancer, and parotid carcinoma demonstrated the highest response rates, while malignant melanoma responded markedly less. The effectiveness for treatment of visceral cancer, measured by X-ray, ultrasonography or computertomography, was approximately 70%, where laryngeal carcinoma tended to respond better to EChT than other visceral tumours. The corresponding values for patients suffering benign tumours was 76–99% (Table 3), and data showed that patients with haemangioma and thyroid tumours were cured to a great extent from their diseases. The results indicate that the short-term therapeutic effectiveness are more favourable for benign tumours and superficial cancers, such as those originating from

Table 3

Short-term (6 months) efficacy of EChT. Objective remissions in 598 cases of benign tumours treated with EChT in China, 1987–1997 [12]. Responses were determined by direct measurements, ultrasonography, or computertomography. CR = complete response, PR = partial response, NC = no change, PD = progressive disease

	All <i>n</i>	CR		PR		NC		PD		CR + PR	
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Hemangioma	296	202	68	90	30	4	1	0	0	292	99
Thyroid tumours	192	127	66	60	31	5	3	0	0	187	97
Prostate hypertrophy	110	51	46	33	30	23	21	3	3	84	76
Total	598	380	64	183	31	32	5	3	1	563	94

Table 4

Long-term efficiency of EChT determined as the 1–5-years survival rate in 3802 cases of superficial malignant tumours treated in China, 1987–1997 [12]

	All <i>n</i>	1-Year		2-Year		3-Year		4-Year		5-Year	
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Skin cancer	958	958	100	958	100	890	93	890	93	767	80
Malignant melanoma	227	194	85	157	69	91	40	19	8	0	0
Jaw and facial tumours	361	336	93	295	82	258	71	184	51	139	39
Oral (tongue or lip) cancer	138	129	93	124	90	111	80	99	72	85	62
Rhabdomyosarcoma	133	121	91	88	66	51	38	9	7	0	0
Chest–abdominal wall metastases	172	140	81	130	76	115	67	102	59	74	43
Vulva (penis or vagina) cancer	237	223	94	209	88	188	79	188	79	104	44
Breast cancer	644	618	96	568	88	404	63	404	63	323	50
Thyroid carcinoma	250	242	97	232	93	192	77	191	77	133	53
Parotid carcinoma	84	78	93	76	90	67	80	59	70	45	54
Other superficial tumours	598	501	84	428	72	338	57	327	55	245	41
Total	3802	3540	93	3265	86	2705	71	2472	65	1915	50

skin, oral cavity, thyroid and parotid gland. Electrochemical therapy was, in general, less effective on visceral neoplasms compared to superficial cancers, with the exception of melanoma and fibrosarcoma. The effectiveness of EChT on both malignant and benign tumours decreased with increasing tumour size, but this relationship was less pronounced in benign tumours.

The long-term survival rate of superficial and visceral malignant tumours, as well as benign tumours, is shown in Tables 4–6. Among patients with superficial neoplasms and benign tumours, the survival figures are virtually in agreement with the short-term results. The 5-year survival rate of superficial malignant tumours varied from 0% to 80% (Table 4), and malignant melanoma and rhabdomyosarcoma had dismal prognoses. Moreover, 60–80% of patients suffering from skin cancer and oral cancer survived for more than 5 years. The survival rate after 5 years of visceral malignant tumours varied from 13% to 67% (Table 5). The patient prognosis was worst for esophageal and liver cancer sufferers (approximately 15%), but the figures were less dismal for lung cancer patients (39%) and markedly better for patients suffering from laryngeal cancer (67%). The long-term results for patients

suffering from benign tumours are presented in Table 6, and as expected, the 5-year survival rate was high, in accordance with the short-term results (Table 3).

The electrode placement technique had once again been modified by Xin [12]. Anodes and cathodes were placed alternately, 2 cm apart, throughout the tumour volume. Typical electrical treatment parameters were 6–8 V and 50–80 mA. Different coulomb dosages were given depending on the type of tumour. For example, solid malignant tumours were given a dosage of 80–100 C/cm of tumour diameter, while benign hemangioma, which is rich in electrolytes, was treated with 30–40 C/cm of tumour diameter.

In addition to the results shown by the figures, a number of tumour diagnoses and macro- and microscopic photographic documentation of a great number of cases, before and after treatment, clearly demonstrated that destruction or reduction of malignant as well as benign tumours was achievable in patients. It was concluded from the conference, that patients with malignant tumours of less than 8 to 10 cm diameter who were unsuitable for surgery or were nonresponsive to radio/chemotherapy, and along with those with benign tumours, may benefit from EChT. However, it was clearly pointed out that EChT was a local therapy, and that it should be combined

Table 5

Long-term efficiency of EChT determined as the 1–5-years survival rate in 3840 cases of visceral malignant tumours treated in China, 1987–1997 [12]

	All <i>n</i>	1-Year		2-Year		3-Year		4-Year		5-Year	
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Esophageal cancer	1595	1285	81	969	61	483	30	233	15	205	13
Lung cancer	1113	1063	96	933	84	746	67	600	54	432	39
Liver cancer	961	771	80	577	60	209	22	184	19	145	15
Laryngeal cancer	21	21	100	21	100	19	90	17	81	14	67
Prostate cancer	20	20	100	19	95	18	90	16	80	10	50
Other visceral tumours	130	124	95	99	76	99	76	71	55	31	24
Total	3840	3284	86	2618	68	1574	41	1121	29	837	22

Table 6

Long-term efficiency of EChT determined as the 1–5-years survival rate in 598 cases of benign tumours treated in China, 1987–1997 [12]

	All <i>n</i>	1-Year		2-Year		3-Year		4-Year		5-Year	
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Haemangioma	296	296	100	296	100	296	100	296	100	296	100
Thyroid tumours	192	192	100	192	100	192	100	190	99	190	99
Prostate hypertrophy	110	110	100	108	98	91	83	88	80	78	71
Total	598	598	100	596	100	579	97	574	96	564	94

with surgery, radio-, chemo- and/or immunotherapy. Furthermore, EChT was contraindicated in late stage cancer patients.

2.3. Occidental studies in animals and patients

Several research groups in Europe, North America and Japan have applied EChT in many different *in vivo* tumour models and in a few clinical case studies, in parallel with Nordenström's work and the clinical activities in China. Several different approaches for the application of current have been employed, including different electrode shapes, materials and configurations as well as different current intensities and treatment times.

Schauble et al. [16] evaluated the effect of three different current levels (3, 0.5 and 0.001 mA, 1 h/day for 4 consecutive days) on the growth of melanoma tumours in hamsters. A stainless steel point electrode was placed in the tumour, as either anode or cathode, and a planar wire mesh electrode was applied to the skin of the chest. Tumour growth was inhibited and metastases were reduced. The effects were most pronounced with the anodic treatment by which the two higher currents produced necrosis.

Habal [17] treated hepatoma tumours in rats with a small direct current (0.4–0.6 μ A, 10–24 days) via an implanted power source [18]. A point-shaped platinum anode was placed in the centre of the tumour and a plate stainless steel cathode was placed on the surface of the power unit, which was in turn attached to normal tissue. The power sources were implanted after different times following the tumour implant, and it was found that tumour growth retardation only occurred when the treatments were started at an early stage. Moreover, tumour growth was enhanced when the treatment was started at an early stage and was then discontinued.

Samuelsson et al. tested EChT as a possible method for the destruction of tumours, especially lung metastases. One study [19] compared the results from the electrolytic treatment of colon adenocarcinoma tumours in rats with those obtained by surgery and came to the conclusion that the efficiency of the treatments were approximately equal. Current (10–40 mA, 1 h, one to three treatments) was applied by two torpedo-shaped platinum electrodes, with an anode placed in the centre of the tumour and a cathode positioned at the tumour surface. In one of the trials, more than 50% of the animals treated with either EChT or surgery were devoid of tumours when killed 7 months after inoculation. The EChT treatment did not seem to affect the number of metastases.

A subsequent article by the same research team reported a 60–80% tumour mass reduction from treatments (80 mA, 2–4 h) of five lung tumours in four patients [20]. In these treatments, the current was applied by two or three platinum electrodes, all placed in the tumour. Samuelsson et al. also studied the effect of EChT with subsequent radio-

therapy, both in experimental colon carcinoma tumours in rats [21] and in a lung tumour patient [22]. In the former study, current (25 mA, 20 min) 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 leads to increased blood flow and higher oxygenation of the tumour, and thereby made the tumour more radiosensitive.

David et al. [23] treated hamster melanoma tumours with different currents (0.1–2.4 mA) for 1 h/day in 5 consecutive days. A needle electrode made of either stainless steel or platinum–iridium alloy was placed in the tumour and served as either the anode or cathode. The counter electrode, made of aluminum foil, was applied to the skin of either the abdomen or left side of the hamster. Tumour growth reduction was obtained in all treatment groups, irrespective of electrode material or polarity.

Marino et al. [24] investigated the effect of direct current (2 mA, 60 min, one to three treatments) on the growth of implanted lung carcinoma in mice. Two platinum wire electrodes were placed parallel to one another into the tumour. Morris et al. [25] made a similar study, but used larger tumours and current (20 mA, 15 min, one to three treatments). These two studies showed that the EChT treatment was able to lessen tumour burden. Moreover, the treatment did not increase the presence of metastasis or the growth rate in the unaffected fraction of the tumour.

Heiberg et al. [26] applied different constant voltages (2.5–12.5 V) and coulomb dosages (30–50 C/cm³ tumour) on colon adenocarcinoma in mice, by means of two gold needles placed parallel to each other within the tumour. All the treated tumours showed significant volume reduction.

Miklavčič et al. [27–29,31] and Serša and Miklavčič [30], in a series of papers, studied the effect of direct current (0.2–1.8 mA) on fibrosarcoma and melanoma tumours in mice. Their studies involved both single (30–90 min) and repetitive treatments, as well as different electrode configurations and materials (Pt, Pt–Ir, Au, Ag, Ti, and stainless steel). Some of the experiments were run with one electrode placed in the tumour and a second placed subcutaneously in healthy tissue. Another electrode configuration, the so-called “field configuration”, involved two electrodes placed in healthy tissue on opposite sides of the tumour. Tumour growth retardation was obtained in all experiments, irrespective of electrode material and configuration. The anti-tumour effect depended proportionally on the current level, and cathodic treatments exhibited a better effect than anodic treatments [28,30]. The best effect was achieved in a study on melanoma tumours using a multiple-array electrode (three Pt–Ir cath-

odes placed in the tumour and two anodes placed subcutaneously in healthy tissue) [28]. Here, tumours showed regression with a cure rate of 40% after 4 months.

The same research team has also published papers describing EChT in combination with other anti-tumour therapies. Serša et al. [32] studied the effect of EChT followed by systemic bleomycin treatment on fibrosarcoma tumours in mice. Current was passed between two platinum–iridium needle electrodes placed outside of the tumour. The combined treatment was found to be more effective than either treatment acting alone. Other works concern the effects of EChT in combination with different immunomodulators, such as tumour necrosis factor (TNF- α) [33], interferon- α (IFN- α) [34,35] and interleukin-2 (IL-2) [36]. These combination therapies indicated potential beneficial effects in fibrosarcoma and melanoma tumours in mice. In a recent study, Miklavčič et al. [37] investigated the immune response in mice after electrochemical treatment, where the electrodes were placed on opposite sides of a sarcoma tumour. EChT was found to be less efficient in immunodeficient mice than in immunocompetent mice, and the authors concluded that the effectiveness of low-level direct current strongly depends on the host's immune response.

Recently, Miklavčič et al. [38] investigated the effect of EChT (0.6 mA, 1 h) on the perfusion and tumour growth of fibrosarcoma tumours (LPB and SA-1) in mice. The current was applied by two-needle platinum–iridium electrodes, placed on opposite sides of the tumour. Tumour growth retardation was obtained in both tumour models. An almost complete absence of staining with Patent Blue dye, in the SA-1 tumours, suggested that tumour growth delay resulted from prolonged vascular occlusion. However, the LPB tumours decreased only slightly in tumour staining, thus indicating a less effect of vascular damage. Jarm et al. [39] reported a continuation of the perfusion studies on SA-1 tumours, using a rubidium extraction technique. Tumour perfusion was found to decrease by more than 50% following treatment, and it was not until three days later that partial reperfusion occurred. The dynamics of these perfusion changes correlated well with the reported tumour growth data. The authors concluded, for this particular tumour model, that vascular occlusion could be the main anti-tumour mechanism.

Plesničar et al. [40] treated 12 melanoma skin tumours in five patients, where a steel needle cathode was inserted into the tumour and three or four self-adhesive plate anodes were placed on the skin, 30–40 mm from the edges of the lesion. All of the patients, with one exception, received electric treatment (1 mA, 30 min) in combination with systemic chemo- and/or immunotherapy. The treatments resulted in tumour mass reduction in all of the evaluable lesions (10 out of 12), but there were no observed complete responses.

A number of papers have been published in Japanese language journals that describe the anti-tumour effect of

EChT in animals and humans, either as a single type of therapy [41] or in combination with chemo- or radiotherapy [42–46]. In one article [47], published in *The European Journal of Surgery*, Matsushima et al. reviewed their clinical experience (27 tumours in 26 patients) with EChT alone, and in combination with systemic chemotherapy (mitomycin, adriamycin, peplomycin, vincristine, bleomycin, vindesine-sulfate and cyclophosphamide). The current (10–40 mA, 40–90 min) was supplied by two platinum electrodes placed in the tumour. More than 70% of the tumours showed regression, and in two of the cases, where only EChT was used, complete regression was observed.

Griffin et al. [48] evaluated the effect of different currents (1–5 mA, 30–90 min) on inoculated mammary carcinomas in mice. The animals were placed on a copper plate electrode, while a gold wire was placed inside the tumour and used as either the anode or cathode. The results showed that the volume of tumour destruction, obtained at a given coulomb dosage, was significantly greater due to anodic than cathodic treatment. Furthermore, the study revealed a linear relationship between the volume of regression induced in the tumour, and the delivered coulomb dosage.

Chou et al. [49] treated implanted fibrosarcoma tumours in mice and rats with a constant voltage load (≤ 10 V) for different durations (1–2 h) while using various electrode configurations. Two to five platinum wire electrodes were inserted into the mice tumours whereas four to seven electrodes were used in the rat tumour treatments. All of the treated tumours showed regression, although in many of the animals the tumours recurred. After multiple treatments, the best mouse tumour cure rate was 59%, after 3 months, and was 75% for the rats after 6 months.

3. Destruction mechanisms

Many studies have been dedicated to the investigation of the underlying destruction mechanisms of EChT. Several contributory factors seem to be involved in tissue destruction, although their respective roles are not yet fully understood. Early in 1959, Humphrey and Seal [2] hypothesised that direct current therapy would change the tumour's inherent negative bioelectrical potential (voltage difference between tumour and healthy tissue) and thereby causes an anti-tumour effect. This hypothesis was later tested and rejected by Miklavčič et al. [27]. Temperature effects due to current flow were once suggested as a possible destruction mechanism, but this has now been ruled out by both experimental measurements [23,26,29] and theoretical calculations [23,27].

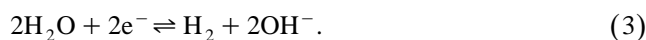
Most people involved in recent research agrees that the primary tissue destruction, obtained in the electrodes' close vicinity is caused by the toxic species produced in the electrochemical reactions during electrolysis. The electro-

chemical reactions occurring in the electrolysis of aqueous saline solutions are well documented, and the current yield of the different reactions strongly depends on the choice of electrode material and operating conditions [50]. If the anode material is electrochemically soluble (e.g., copper), the major part of the anodic current will consist of metal dissolution. A small amount of the anodic current is transferred by the oxidation and reduction of certain species already dissolved in the tissue. Some dissolvable metals (e.g., silver) form a nonconducting compound on the anode surface, which may cause current fluctuations. In cases where the anode material is made of a metal that can be passivated, such as platinum, metal dissolution is negligible. Passivity is caused by the formation of a thin electron-conducting oxide film that acts as a barrier to the anodic metal dissolution reaction. The main reactions, in these cases, are decomposition of water and oxidation of chloride:



There are also several anode materials, including gold and stainless steel, which do not belong to any of the categories mentioned above. Gold is only passive within a very narrow potential interval, while the metal dissolution reaction substantially increases at potentials above and below this potential interval [51]. Stainless steel behaves as a passive metal when no current is applied, while it dissolves due to local surface attacks when an anodic current is applied.

The cathode material is protected against electrochemical dissolution by the applied cathodic current, and the major electrochemical reaction in all cases is the decomposition of water into molecular hydrogen and hydroxyl ions:



Species produced at the anode and cathode are mainly transported to the surrounding tissue by diffusion due to concentration gradients, and by migration (charged species) due to the potential gradient. At the anode, the reaction products, which can be locally destructive, are different metal ions, hydrogen ions and various species containing oxygen and chlorine. Hydroxyl ions and molecular hydrogen are the destructive reaction products at the cathode. The electrochemical reaction products may also react with organic and inorganic tissue constituents, to potentially form new toxic products. Chlorine, for example, reacts with water to form hypochlorous acid, chloride, and hydrogen ions. Other types of secondary reactions may include an increase of intermediate toxic radicals, to which tumour cells are assumed to be more sensitive to than normal cells are [52].

The presence of extreme local pH changes in tissue surrounding the electrodes, during and after EChT treatment has been confirmed in many studies [5,29,53–55].

The pH values down to 1 have been detected in tissue adjacent to the anode [29], while a pH as high as 13 has been measured near the cathode [53]. At these unphysiological conditions, vital proteins become denatured and precipitate [5,53,56]. The extreme pH conditions in the vicinity of the electrodes have also been predicted in several theoretical studies [57–61]. Furthermore, in a recent study on mammary tissue in rats, von Euler et al. [54] found a correlation between pH and the size of anodic and cathodic lesions produced by platinum–iridium electrodes.

Several authors have identified chlorine [5,53,62] and hydrogen [5,53] evolving from the anode and cathode site, respectively. Samuelsson and Jönsson [63] measured the content of organically bound chlorine in anodic lesions, produced around platinum electrodes in the lungs of pigs, and found it to be significantly elevated compared with healthy tissue. They concluded that chlorine, through its oxidative properties, is the main agent responsible for the tissue destruction obtained in EChT treatments. Their conclusions were contradicted by the works of Berendson and Simonsson [57], and Berendson and Olsson [58], which presented several estimations of the spreading of chlorine and hydrogen ions in tissue surrounding a platinum electrode. The calculations indicated that the acidified zone around the anode is significantly larger than the chlorinated zone and thereby determines the extent of tissue destruction.

It has been suggested that the extreme pH condition in the vicinity of the electrodes causes electrocoagulation and thereby causing the shutdown of blood flow to the tumour [64,65]. This mechanism could possibly explain the anti-tumour effect obtained in EChT studies where the electrodes are placed outside the tumour. Miklavčič et al. [38] and Jarm et al. [39] tested this hypothesis and obtained promising results on fibrosarcoma tumours in mice. Tumour blood perfusion was measured following treatment with two-needle platinum–iridium electrodes, placed on opposite sides of the tumour. In one experiment [39], the relative tissue perfusion of the tumours was found to decrease by more than 50%.

The production of toxic electrolytic products may not fully explain the anti-tumour effects obtained in EChT studies. A series of *in vitro* studies with direct current, on both malignant and normal cells, has showed that the electric field itself influences both survival and proliferation of the cells [36,66–70]. Batista et al. [66,67] and Serša et al. [36] carried out *in vitro* studies with currents in the mA range. Their experiments resulted in an inhibition of cell proliferation. Other authors report both stimulating and suppressing effects on cell proliferation, in studies with currents in the order of μA [69,70].

The mechanism by which the electric field affects cell growth and survival has not yet been elucidated but is most probably a complex process. The electric field causes a flux of interstitial water (electro-osmosis) from the anode towards the cathode, and consequently, the tissue sur-

rounding the anode dehydrates while oedema is obtained around the cathode [5,71]. 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 and thereby the conditions for many essential enzyme-regulated reactions [5]. The possibility of direct current in inducing an immune response or enhancing the toxicity of immune cells has also been discussed [37].

4. Dose planning

A dose planning methodology is a prerequisite for reproducible and predictable treatment results. When Nordenström first started his clinical work, few guidelines existed to an optimal choice of treatment parameters and an arbitrary dose of 100 C/cm of tumour diameter was chosen [5]. Xin adopted this preliminary dose unit and based on their clinical experiences, later adapted the coulomb dose depending on the type of tumour (30–100 C/cm) [12].

In order to develop an optimised dose planning methodology, several authors have systematically investigated the dose–response relationship between the applied current, treatment time, coulomb dosage and tissue destruction. Samuelsson et al. [55] treated normal lung and liver tissue, in rabbits, with different coulomb dosages (2.5–40 C) and found a linear relationship between the volume of tissue destruction around the anode, and coulomb dosage. Griffin et al. treated both mouse mammary carcinomas [48] and normal rat livers [64] with different currents (1–5 mA) and treatment times. The relationship between the volume of primary tissue destruction and coulomb dosage (2–27 C) around both anode and cathode was also found to be linear in this case. Moreover, no differences could be detected between the biological effects obtained at lower and higher currents at a given coulomb dosage. Robertson et al. [72] made a similar study (1–5 mA, 1–5 C), in normal rat livers, and came to the same conclusions.

While the above data supports the hypothesis that coulomb dosage is a reliable dose parameter, other workers have reported the opposite. Von Euler et al. [54] treated normal mammary tissue in rats and obtained significant differences in lesion sizes when using currents of 1 and 5 mA at constant coulomb dosage (5 C), Yen et al. [68] studied the growth retardation and survival of human KB cells after electrochemical treatment and found that the cytotoxicity of EChT is related not only to coulomb dosage, but also to the way by which the coulomb dosage was delivered. Treatments with low current and longer treatment times resulted in lower cell viability, compared to treatments with higher current and shorter treatment time. Xin et al. [14] reported analogous results from clinical treatments of lung tumours.

Another approach towards a reliable dose planning methodology is the use of physicochemical simulation models. Such models are based on a set of physicochemical differential equations that describe the transport and reaction processes occurring at the electrodes during EChT. By solving these equations, concentration profiles of substances dissolved in tissue, and the potential profile within the tissue itself, can be simulated as a function of time. Hence, together with knowledge of the underlying destruction mechanism, the model can be used to predict the tumour destruction produced through EChT.

Several physicochemical simulation models of EChT have been proposed in the literature. Cvirn et al. [59] calculated the spreading of the alkaline zone around a spherical cathode while Berendson and Simonsson [57] and Berendson and Olsson [58] studied the spreading of hydrogen ions and molecular chlorine around spherical and planar platinum anodes. In both works, the authors stated that the calculations must be considered as preliminary estimations, since the influence of the potential field on electrode kinetics and the spreading of ions were neglected. In two recently published articles [60,61], Nilsson et al. developed a mathematical model of the processes occurring around a spherical platinum anode. Tissue was treated as a sodium chloride solution containing a bicarbonate buffer system, and in contrast to the other models, it included both electrode kinetics and transport due to migration in the electric field. A promising correlation was obtained when comparing their simulated pH profiles with those, and lesion sizes, found experimentally [54,55].

5. Discussion

A common method of characterising different EChT treatments, as has been mentioned above, is the amount of charge (in coulombs) that has been transferred between the electrodes. It is our opinion that other parameters, such as current density, time, electrode geometry and electrode configuration, also play important roles in the effect of the treatment.

The current density at the surface of a passivated metal, acting as an anode, determines the yield of the electrochemical reactions, i.e., oxygen evolution and chlorine formation. Tissue adjacent to the anode at low current densities becomes saturated with chlorine, and chlorine produced subsequently is lost to the surroundings [73]. Secondary reactions of chlorine with tissue play important roles as hydrogen ion generators, and the contribution of these reactions to the acidification of tissue, around the anode, increases with decreasing current density [73].

The current density also determines the electric field strength and thereby influences the transport of ionic species around the electrodes. The fact that the spreading of hydrogen ions occurs in an electric field implies that a higher spreading rate occurs compared to the situation

where a dilute solution of hydrochloric acid is infused into tissue, in the absence of an electric field. In the latter case, the transport rate of hydrogen ions is determined by the effective diffusion coefficient of hydrochloric acid, while in EChT, transport is determined by the migration rate of the hydrogen ion. A similar reasoning is valid for the transport of hydroxyl ions from the cathode, although this transport is lower than that of hydrogen ions.

The applied voltage is carefully noted as a treatment parameter in some of the published studies. It is important to observe that the thermodynamic potential difference between the anode and the cathode, the so-called Nernst's potential difference, is about 2 V due to the anodic reactions, oxygen and chlorine formation, and the hydrogen evolution reaction at the cathode. In addition to this potential difference, one must consider the total electrochemical polarisation at the electrodes and the ohmic losses in the tissue. Both these losses depend strongly on the geometrical shape and the configuration of the electrodes, which implies that the voltage between the anodes and cathodes is individual for every treatment set-up.

The transferred charge during treatment, given in coulombs, is a measure of the amount of chemical reaction products formed at the electrodes. However, the effect of a specified coulomb dose is not always consistent. For example, if the applied current is sufficiently low, buffering systems in the tissue may counteract the pH changes at the electrodes, and consequently, no acidification or alkalinisation is obtained. Thus, the number of coulombs corresponds to the amount of reaction products formed at the electrodes during a treatment but does not directly describe their distribution in the tumour.

The destructive reaction products, formed at the electrodes during the electrochemical treatment, give rise to both immediate cell death and long-term effects to the surrounding tissues. The extreme pH conditions in the vicinity of the electrodes causes an instant coagulative necrosis, while secondary changes such as decreased perfusion of tumours cause hypoxia and a diminished nutritional level. In addition, it has been demonstrated that the electric field itself influences both the survival and proliferation of the cells. It is of great interest to distinguish if there is a difference in these responses between tumour cells and normal cells. To deal with this and other related topics, further *in vitro* studies must be done. An *in vitro* environment enables a unique possibility to study the cells' response to different treatments, and also to isolate single factors created during EChT, which could be harmful to the cells. Through initiating histopathological and molecular biological studies, one may discern between injuries on cell membranes and cell nuclei, and possibly decide if apoptosis has occurred.

According to the Chinese clinical experience, EChT exhibited more promising effects on superficial than visceral tumours. A possible explanation to these experiences is that the buffer capacity of a superficial tumour is lower

compared to that of a visceral tumour. The reaction products in a superficial tumour, formed at the electrodes, would be confined by the presence of an outer boundary surface, which would enhance the toxic effect of the treatment.

Several authors have suggested the benefit of combining EChT with other established treatment methods. Electrochemical treatment in combination with chemotherapy [32] or radiotherapy [44] was shown to be more effective than either treatment acting alone. Moreover, immunologically related compounds, such as TNF- α , IFN- α and IL-2, used in combination with EChT were found to enhance the effect of the electrochemical treatment [33–36]. TNF- α gave massive necrosis after treatment, whereas the effectiveness of IL-2 differed significantly depending on the tumour strain. It was also found that the efficiency of EChT was less in immunodeficient mice compared to immunocompetent mice, thus indicating that the effectiveness of the treatment strongly depends on the host's immune response [37].

We propose two other combination therapies in addition to those suggested in the literature. EChT could be used to pretreat inaccessible parts of a tumour, or decrease the volume of a large tumour, before the remainder is removed by surgery. EChT may also serve as a complement to radiotherapy in the treatment of large tumours. The limitations of radiotherapy in effectively achieving tumour control in the centre of a large tumour is mainly due to the fact that a large portion of the cells might be hypoxic because of an impaired blood supply and high intratumoural pressure. A good oxygenation of the tissue will result in the development of more oxygen radicals and further DNA damage during radiotherapy [74]. In addition, peripheral parts of the tumour might periodically become hypoxic by the shunting of blood [75]. Hence, a possible combination therapy could involve an initial stage using EChT, placing the anode in the centre of the tumour, followed by radiotherapy. In this case, EChT would act both as an independent therapy and as an efficient oxygenating pretreatment to radiotherapy. In order to avoid the chlorinating part of the anodic process, an anode with a surface coating that promotes oxygen evolution (e.g., iridium oxide) could be used. Moreover, if the tumour is situated in a very sensitive organ, one may consider placing the cathode in a main vein instead of in normal tissue close to the tumour.

The clinical experience of EChT in China indicates that this method is a safe and simple anti-tumour therapy for treatment of localised malignant as well as benign tumours. Although more than 10 000 patients have been treated in China during the past 10 years, EChT has not yet been universally accepted. There is a need for further essential preclinical studies and reliably controlled clinical trials for the method to be applied outside of China. In addition, further systematic investigations of the destruction mechanisms behind EChT should be done. Some

promising dose-planning models have been reported in the literature and these physicochemical simulation models may, in the relatively near future, be used in the occidental hospitals as a basis for systematic planning of EChT. The highest priority should be given to molecular biological studies and controlled clinical trials in order to compare the suitability and efficiency of EChT with fully established anti-tumour therapies.

Acknowledgements

This research group is grateful for the financial support given to the research program by the Swedish Cancer Society. The continued work will be dedicated to the commemoration of our dear colleague, the late professor Daniel Simonsson, who was the scientific leader of this group, but has recently passed away after a longer period of illness. Philip Byrne is acknowledged for the linguistic revision of the manuscript.

References

- [1] F.H. Martin, Electrolysis in gynaecology; with a report of three cases of fibroid tumour successfully treated by the method. *J. Am. Med. Assoc.*, 7 (1886) 61–68; 85–90.
- [2] C.E. Humphrey, E.H. Seal, Biophysical approach toward tumour regression in mice, *Science* 130 (1959) 388–390.
- [3] B.W. Watson, The treatment of tumours with direct electric current, *Med. Sci. Res.* 19 (1991) 103–105.
- [4] B. Nordenström, Preliminary clinical trials of electrophoretic ionization in the treatment of malignant tumours, *IRCS Med. Sc.* 6 (1978) 537.
- [5] B.E.W. Nordenström, Biologically Closed Electrical Circuits: Clinical, Experimental and Theoretical Evidence for an Additional Circulatory System, Nordic Medical Publications, Stockholm, 1983.
- [6] B. Nordenström, Biologically closed electric circuits: activation of vascular interstitial closed electric circuits for treatment of inoperable cancers, *J. Bioelectr.* 3 (1984) 137–153.
- [7] B.E.W. Nordenström, Electrochemical treatment of cancer. Part I: Variable response to anodic and cathodic fields, *Am. J. Clin. Oncol.* 12 (1989) 530–536.
- [8] B.E.W. Nordenström, S. Ekborg, H. Beving, Electrochemical treatment of cancer. Part II: Effect of electrophoretic influence on adriamycin, *Am. J. Clin. Oncol.* 13 (1990) 75–88.
- [9] S. Ekborg, B.E.W. Nordenström, H. Beving, Electrochemical treatment of cancer. Part III: Plasma pharmacokinetics of adriamycin after intraneoplastic administration, *Am. J. Clin. Oncol.* 13 (1990) 164–166.
- [10] E. Azavedo, G. Svane, B. Nordenström, Radiological evidence of response to electrochemical treatment of breast cancer, *Clin. Radiol.* 43 (1991) 84–87.
- [11] Y.L. Xin, Organisation and spread of electrochemical therapy (ECT) in China, *Eur. J. Surg. Suppl.* 574 (1994) 25–30.
- [12] Y.L. Xin, The clinical advance in application of EChT within the past ten years. Preprints from the 2nd International Symposium on Electrochemical Treatment of Cancer, Beijing, 27–30 Sept. 1998, p. 81–92.
- [13] Y.L. Xin, Advances in the treatment of malignant tumours by electrochemical therapy (ECT), *Eur. J. Surg. Suppl.* 574 (1994) 31–35.
- [14] Y.L. Xin, F. Xue, B. Ge, F. Zhao, B. Shi, W. Zhang, Electrochemical treatment of lung cancer, *Bioelectromagnetics* 18 (1997) 8–13.
- [15] L.A.G. Ries, C.L. Kosary, B.F. Hankey, B.A. Miller, L. Clegg, B.K. Edwards, SEER Cancer Statistics Review, 1973–1996, National Cancer Institute, Bethesda, MD, USA, 1999.
- [16] M.K. Schauble, M.B. Habal, H.D. Gullick, Inhibition of experimental tumor growth in hamsters by small direct currents, *Arch. Pathol. Lab. Med.* 101 (1977) 294–297.
- [17] M.B. Habal, Effect of applied dc currents on experimental tumor growth in rats, *J. Biomed. Mater. Res.* 14 (1980) 789–801.
- [18] M.B. Habal, M.K. Schauble, Clinical device note: an implantable DC power unit for control of experimental tumor growth in hamsters, *Med. Instrum.* 7 (1973) 305–306.
- [19] L. Samuelsson, Electrolysis and surgery in experimental tumours in the rat, *Acta Radiol.* 22 (1981) 129–131.
- [20] L. Samuelsson, L. Jönsson, E. Ståhl, Percutaneous treatment of pulmonary tumors by electrolysis, *Radiologe* 23 (1983) 284–287.
- [21] L. Samuelsson, L. Jönsson, I.-L. Lamm, C.-J. Lindén, S.-B. Ewers, Electrolysis with different electrode materials and combined with irradiation for treatment of experimental rat tumours, *Acta Radiol.* 32 (1990) 178–181.
- [22] L. Samuelsson, I.-L. Lamm, C.E. Mercke, E. Ståhl, L. Jönsson, Electrolytic tissue destruction and external beam irradiation of the lung, *Acta Radiol.* 26 (1985) 521–524.
- [23] S.L. David, D.R. Absolom, C.R. Smith, J. Gams, M.A. Herbert, Effect of low level direct current on in vivo tumor growth in hamsters, *Cancer Res.* 45 (1985) 5625–5631.
- [24] A.A. Marino, D. Morris, T. Arnold, Electrical treatment of Lewis lung carcinoma in mice, *J. Surg. Res.* 41 (1986) 198–201.
- [25] D.M. Morris, A.A. Marino, E. Gonzalez, Electrochemical modification of tumor growth in mice, *J. Surg. Res.* 53 (1992) 306–309.
- [26] E. Heiberg, W.J. Nalesnik, C. Janney, Effects of varying potential and electrolytic dosage in direct current treatment of tumors, *Acta Radiol.* 32 (1991) 174–177.
- [27] D. Miklavčič, G. Serša, S. Novaković, S. Reberšek, Tumor bioelectric potential and its possible exploitation for tumor growth retardation, *J. Bioelectr.* 9 (1990) 133–149.
- [28] D. Miklavčič, L. Vodovnik, F. Bobanović, S. Reberšek, G. Serša, S. Novaković, R. Golouh, Local treatment of murine tumors by electric direct current, *Electro-Magnetobiol.* 11 (1992) 109–125.
- [29] D. Miklavčič, G. Serša, M. Kryžanowski, S. Novaković, F. Bobanović, R. Golouh, L. Vodovnik, Tumor treatment by direct electric current — tumor temperature and pH, electrode material and configuration, *Bioelectrochem.* Bioenerg. 30 (1993) 209–220.
- [30] G. Serša, D. Miklavčič, The feasibility of low level direct current electrotherapy for regional cancer treatment, *Reg. Cancer Treat.* 1 (1993) 31–35.
- [31] D. Miklavčič, A. Fajgelj, G. Serša, Tumour treatment by direct electric current: electrode material deposition, *Bioelectrochem. Bioenerg.* 35 (1994) 93–97.
- [32] G. Serša, S. Novaković, D. Miklavčič, Potentiation of bleomycin anti-tumor effectiveness by electrotherapy, *Cancer Lett.* 69 (1993) 81–84.
- [33] G. Serša, R. Golouh, D. Miklavčič, Anti-tumor effect of tumor necrosis factor combined with electrotherapy on mouse sarcoma, *Anti-Cancer Drugs* 5 (1994) 69–74.
- [34] G. Serša, D. Miklavčič, Inhibition of SA-1 tumor growth in mice by human leukocyte interferon alpha combined with low level direct current, *Mol. Biother.* 2 (1990) 165–168.
- [35] G. Serša, D. Miklavčič, Combined treatment of murine SA-1 tumors by human leukocyte interferon alpha and electrotherapy, *Radiol. Oncol.* 27 (1993) 280–285.
- [36] G. Serša, D. Miklavčič, U. Batista, S. Novaković, F. Bobanović, L. Vodovnik, Anti-tumor effect of electrotherapy alone or in combination with interleukin-2 in mice with sarcoma and melanoma tumors, *Anti-Cancer Drugs* 3 (1992) 253–260.
- [37] D. Miklavčič, D. An, J. Belehradec Jr., L.M. Mir, Host's immune

- response in electrotherapy of murine tumors by direct current, *Eur. Cytokine Network* 8 (1997) 275–279.
- [38] D. Miklavčič, T. Jarm, M. Čemažar, G. Serša, D.J. An, J. Belehradec Jr., L.M. Mir, Tumor treatment by direct electric current. Tumour perfusion changes, *Bioelectrochem. Bioenerg.* 43 (1997) 253–256.
- [39] T. Jarm, M. Čemažar, G. Serša, D. Miklavčič, Blood perfusion in a murine fibrosarcoma tumor model after direct current electrotherapy: a study with ^{86}Rb extraction technique, *Electro-Magnetobiol.* 17 (1998) 273–282.
- [40] A. Plesničar, G. Serša, L. Vodovnik, J. Jancar, L. Zaletel-Kragelj, S. Plesničar, Electric treatment of human melanoma skin lesions with low level direct electric current: an assessment of clinical experience following a preliminary study in five patients, *Eur. J. Surg. Suppl.* 574 (1994) 45–49.
- [41] H. Ito, N. Shigematsu, T. Nakayama, I. Nishiguchi, Y. Ando, S. Hashimoto, The experimental study of anti-tumor activity of direct current, *Nippon Gan Chiryō Gakkaiishi* 23 (1988) 696–702.
- [42] H. Ito, T. Nakayama, N. Shigematsu, I. Nishiguchi, S. Hashimoto, Increased anti-tumor activity of chemotherapeutic agents combined with direct current against murine transplanted tumors, *Gan to Kagaku Ryōho* 14 (1987) 1854–1859.
- [43] T. Nakayama, H. Ito, S. Hashimoto, Anti-tumor activities of direct current (DC) therapy combined with fractionated radiation or chemotherapy, *Nippon Igaku Hoshasen Gakkai Zasshi* 48 (1988) 1269–1275.
- [44] H. Ito, S. Hashimoto, Experimental study of the anti-tumor activity of direct current — an effective adjuvant therapy in irradiation, *Gan to Kagaku Ryōho* 16 (1989) 1405–1411.
- [45] Y. Matsushima, R. Amemiya, J.S. Liu, E. Tajika, H. Takakura, K. Oho, S. Hara, Direct current therapy with chemotherapy for the local control of lung cancer, *Nippon Gan Chiryō Gakkaiishi* 24 (1989) 2341–2348.
- [46] Y. Matsushima, J.S. Liu, E. Tajika, K. Nagai, Y. Koshiishi, K. Oho, Y. Hayata, Direct current therapy for local control of malignant tumors, *Nippon Geka Gakkai Zasshi* 91 (1990) 23–28.
- [47] Y. Matsushima, E. Takahashi, K. Hagiwara, C. Konaka, H. Miura, H. Kato, Y. Koshiishi, Clinical and experimental studies of anti-tumoural effects of electrochemical therapy (ECT) alone or in combination with chemotherapy, *Eur. J. Surg. Suppl.* 574 (1994) 59–67.
- [48] D.T. Griffin, N.J.F. 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. Cancer* 69 (1994) 875–878.
- [49] C.-K. Chou, J.A. McDougall, C. Ahn, N. Vora, Electrochemical treatment of mouse and rat fibrosarcomas with direct current, *Bioelectromagnetics* 18 (1997) 14–24.
- [50] J.O'M. Bockris, S.U.M. Khan, *Surface Electrochemistry: A Molecular Level Approach*, Plenum, New York, 1993.
- [51] A.J. Bard, *Encyclopedia of Electrochemistry of the Elements Vol. 4* Marcel Dekker, New York, 1975.
- [52] S. Srinivasan, G.L. Cahen Jr., G.E. Stoner, Electrochemistry the past thirty and the next thirty years, in: H. Bloom, F. Gutmann (Eds.), *Electrochemistry in the Biomedical Sciences Vol. 57* Plenum, New York, 1977, pp. 57–84.
- [53] K.H. Li, Y.L. Xin, Y.N. Gu, B.I. Xu, D.J. Fan, B.F. Ni, Effects of direct current on dog liver: possible mechanisms for tumor electrochemical treatment, *Bioelectromagnetics* 18 (1997) 2–7.
- [54] H. von Euler, E. Nilsson, A.-S. Lagerstedt, J.M. Olsson, Development of a dose-planning method for electrochemical treatment of tumors. A study on mammary tissue in healthy female CD rats, *Electro-Magnetobiol.* 18 (1999) 93–104.
- [55] L. Samuelsson, T. Olin, N.O. Berg, Electrolytic destruction of lung tissue in the rabbit, *Acta Radiol.* 21 (1980) 447–454.
- [56] R. Lemberg, M. Legge, *Hematin Compounds and Bile Pigments*, Interscience, New York, 1949.
- [57] J. Berendson, D. Simonsson, Electrochemical aspects of treatment of tissue with direct current, *Eur. J. Surg. Suppl.* 574 (1994) 111–115.
- [58] J. Berendson, J.M. Olsson, Bioelectrochemical aspects of the treatment of tissue with direct current, *Electro-Magnetobiol.* 17 (1998) 1–16.
- [59] P. Cvirn, J. Reščič, D. Miklavčič, Tumour pH changes due to electrotherapy — experimental results and mathematical model, *ELVEA* 61 (1994) 37–42.
- [60] E. Nilsson, J. Berendson, E. Fontes, Electrochemical treatment of tumours: a simplified mathematical model, *Electroanal. Chem.* 460 (1999) 88–99.
- [61] 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.
- [62] L. Samuelsson, L. Jönsson, Electrolytic destruction of tissue in normal lung of the pig, *Acta Radiol.* 22 (1981) 9–14.
- [63] L. Samuelsson, L. Jönsson, Electrolytic destruction of lung tissue. Electrochemical aspects, *Acta Radiol.* 21 (1980) 711–714.
- [64] D.T. Griffin, N.J.F. Dodd, S. Zhao, B.R. Pullan, J.V. Moore, Low-level direct electrical current therapy for hepatic metastases: Part I. Preclinical studies on normal liver, *Br. J. Cancer* 72 (1995) 31–34.
- [65] W.M. Thompson, D.S. McAlister, M. Miller, S.V. Pizzo, D.C. Jackson, I.S. Johnsrude, Transcatheter electrocoagulation: experimental evaluation of the anode, *Invest. Radiol.* 14 (1979) 41–47.
- [66] U. Batista, D. Miklavčič, G. Serša, The effect of low-level direct current on V-79 cell line in vitro, *Period. Biol.* 93 (1991) 225–226.
- [67] U. Batista, D. Miklavčič, G. Serša, Low level direct current — cell culture fibroblast model, *Bioelectrochem. Bioenerg.* 35 (1994) 99–101.
- [68] Y. Yen, J.-R. Li, B.-S. Zhou, F. Rojas, J. Yu, C.K. Chou, Electrochemical treatment of human KB cells in vitro, *Bioelectromagnetics* 20 (1999) 34–41.
- [69] M. Lyte, J.E. Gannon, G.D. O'Clock Jr., Effect of in vitro electrical stimulation on enhancement and suppression of malignant lymphoma cell proliferation, *J. Natl. Cancer Inst.* 83 (1991) 116–119.
- [70] R.O. Becker, C. Esper, Electrostimulation and undetected malignant tumors, *Clin. Orthop.* 161 (1981) 336–339.
- [71] A.K. Vijn, Electrochemical treatment of tumours (ECT): electroosmotic dewatering (EOD) as the primary mechanism, *Drying Technol.* 17 (1999) 585–596.
- [72] G.S.M. 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. Cancer* 85 (1998) 1212–1216.
- [73] E. Nilsson, J. Berendson, E. Fontes, Impact of chlorine in the electrochemical treatment of tumours, *J. Appl. Electrochem.* (1999) Submitted for publication.
- [74] P.A. Riley, Free radicals in biology: oxidative stress and the effects of ionizing radiation, *Int. J. Radiat. Biol.* 65 (1994) 27–33.
- [75] F. Zywietz, W. Reeker, E. Kochs, Changes in tumour oxygenation during a combined treatment with fractionated irradiation and hyperthermia: an experimental study, *Int. J. Radiat. Oncol., Biol., Phys.* 37 (1997) 155–162.