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Original Paper

Electrochemotherapy with Cisplatin: Potentiation of Local Cisplatin Antitumour Effectiveness by Application of Electric Pulses in Cancer Patients

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This study was aimed at assessing the response to electrochemotherapy with cisplatin of cutaneous tumour nodules in patients with malignant melanoma, squamous cell carcinoma and basal cell carcinoma. In 4 patients, 30 tumour nodules of different sizes were treated; five without treatment, one with electric pulses, five with cisplatin injected intratumorally and 19 with electrochemotherapy, i.e. intratumoral administration of cisplatin followed by delivery of electric pulses to the tumour nodule. After 4 weeks, a complete response (CR) in all 19 electrochemotherapy treated nodules was obtained. All electrochemotherapy treated nodules remained in CR (range 7–11 months), regardless of histological type, except for the metastasis of a squamous cell carcinoma that progressed after 9 months. CR was also obtained in two of five tumour nodules treated with cisplatin intratumorally, but the other three nodules progressed within 3–7 months. Exposure of the tumour nodule to electric pulses without cisplatin treatment had no effect on tumour growth. Electrochemotherapy was well tolerated by all patients and a good cosmetic effect was obtained, with only minimal scarring and a slight depigmentation of the skin. Electrochemotherapy with cisplatin has proved to be effective in patients with cutaneous tumour nodules. Furthermore, electrochemotherapy is easy to perform and can be carried out on an out-patient basis. © **1998 Elsevier Science Ltd. All rights reserved.**

Key words: drug delivery system, cisplatin, electroporation, electrochemotherapy, melanoma, basal cell carcinoma, squamous cell carcinoma
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INTRODUCTION

A PROPORTION OF patients who have undergone extensive tumour treatment, including all conventional treatment modalities, face the problem of metastases. Progression of the disease in cutaneous tissue, without dissemination to other organs, raises the problem of which treatment to choose. In these cases, a simple and effective treatment approach, with no or minimal systemic side-effects and good antitumour effectiveness is desirable.

There are several reasons why many of the chemotherapeutic drugs presently used are not sufficiently effective. One reason is the hampered transport of the drugs through the plasma membrane. Therefore, new ways to facilitate chemotherapeutic drug delivery into cells are being sought in order to potentiate their effectiveness, while lowering systemic toxicity. Among the drug delivery systems presently under investigation [1] is the use of electric pulses [2].

In electrochemotherapy, electric pulses are used as a means of increasing chemotherapeutic drug delivery into cells [2]. Exposure of cells to electric pulses under specific conditions increases plasma membrane permeability, temporarily and reversibly, without affecting cell viability [2, 3]. This technique, termed electroporation, has been used successfully for the insertion of drugs, dyes, genes, oligonucleotides and monoclonal antibodies into cells [4]. Among several chemotherapeutic drugs tested for potentiation of their cytotoxicity by electroporation, bleomycin and cisplatin have been found to be very suitable, due to their limited transport through the plasma membrane [5–7]. *In vitro* data have

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demonstrated that electroporation potentiates the cytotoxicity of bleomycin several hundred times [8] and the cytotoxicity of cisplatin up to 70 times [7]. In preclinical studies on animal tumour models, a marked antitumour effect was observed on tumour nodules exposed to electric pulses after the application of bleomycin or cisplatin [7, 9–13]. A high degree of tumour complete responses (CR), as well as tumour cures, were observed at low drug concentrations without systemic toxicity.

Following these preclinical data, several clinical studies on electrochemotherapy with bleomycin were performed on cutaneous and subcutaneous tumour nodules of head and neck carcinoma, malignant melanoma, basal cell carcinoma, adenocarcinoma and Kaposi's sarcoma [2, 14–16]. Objective responses were obtained for the majority of the electrochemotherapy treated nodules, whereas nodules that were only exposed to electric pulses, or only treated with bleomycin did not respond.

Based on the promising results of the preclinical data on electrochemotherapy with intratumoral injection of cisplatin [17, 18], the aim of this study was to perform a first clinical study of electrochemotherapy with intratumoral cisplatin on cutaneous tumour nodules of patients with malignant melanoma, squamous cell carcinoma and basal cell carcinoma.

PATIENTS AND METHODS

Study design

To evaluate antitumour effectiveness of electrochemotherapy, i.e. intratumoral administration of cisplatin followed by delivery of electric pulses to tumour nodules, its antitumour effectiveness was compared with intratumoral administration of cisplatin only, delivery of electric pulses only and growth of untreated tumour nodules. The treatment was performed on tumour nodules of different histogical types and different sizes.

Electrochemotherapy treatment

Electrochemotherapy consisted of intratumoral administration of *cis*-diamminedichloroplatinum II (cisplatin) (Platinol, Bristol-Myers Squibb, Austria) followed by exposure of tumour nodules to electric pulses. Cisplatin was dissolved in 0.9% NaCl solution at a concentration 2 mg/ml. Cisplatin was administered intratumorally at doses ranging from 0.25 to 2 mg depending on the size of the tumour nodule. The interval between cisplatin administration and the application of electric pulses was 1–2 min. Square wave electric pulses of 100 μsec, 910 V amplitude (amplitude to electrode distance ratio 1300 V/cm), frequency 1 Hz were delivered through two parallel stainless steel electrodes (thickness 1 mm; width 7 mm; length 14 mm, with rounded tips and inner distance between them 7 mm) with an electropulsator Jouan GHT 1287 (Jouan, France). Electrical parameters were controlled using an oscilloscope HM 205-3 (Hameg Instruments, Germany). Each run of electric pulses was delivered in two trains of four pulses, with 1 sec interval, in two perpendicular directions. Good contact between the electrodes and the skin was assured by means of a conductive gel. Nodules larger than 7 mm in diameter were treated with several runs of electric pulses, administered in adjacent positions in such a way that the whole tumour area was covered. Electrochemotherapy was performed on an out-patient basis with a 3 week interval between sessions.

Patient selection

Between July 1995 and October 1996, 4 patients entered a phase I and phase II trial of electrochemotherapy with cisplatin (Table 1). National ethics committee approval and written informed consent from each patient were obtained before beginning treatment. To be eligible, the patients had to have recurrent or progressive disease, be affected by measurable cutaneous nodules, be pretreated or refused other standard treatment, i.e. radiotherapy, surgery or systemic chemotherapy. All patients were excluded from conventional chemotherapy protocols and from any other kind of treatment. Eligibility criteria also included WHO performance status 0–1, as well as normal blood tests and biochemistry. Patients with visceral, bone or brain metastases were not included.

Patient 1

A 59-year-old male patient presented a pigmented lesion on his left forearm. The lesion was radically excised in September 1993. Histopathological examination revealed nodular melanoma, Clark level V, of Breslow thickness 5.0 mm. Two months later a left axillary lymph node dissection was performed due to cytologically proven metastases.

The patient received adjuvant immunotherapy (interferon-alpha (IFN-α)) for 6 months. Multiple metastatic nodules in the thoraco-abdominal region of the skin were noted in November 1995. The patient was treated with four cycles of immunochemotherapy (vinblastine, lomustine (CCNU), cisplatin and IFN-α) and complete remission was achieved after the second cycle. In April 1996, multiple new metastatic cutaneous nodules were recorded on the upper thorax and neck and were cytologically confirmed. Because of the large number of metastatic nodules, surgical treatment was not feasible. The patient refused second-line chemotherapy, as well as radiation, and began treatment by electrochemotherapy in April 1996.

Patient 2

A 54-year-old female patient, with a history of UICC stage II breast carcinoma, treated with mastectomy and adjuvant chemotherapy 16 years ago, had a pigmented lesion on the left foot, suspected to be melanoma, completely excised in

Table 1. *Summary of patient characteristics*

Patient no.	Age/sex	Tumour type	Previous treatment	Wash out (months)*	Number of tumours
1	59/M	Melanoma	Surgery, immunochemotherapy	3	17
2	54/F	Melanoma	Surgery	1	2
3	52/M	Squamous cell carcinoma	Surgery, radiotherapy	4	2
4	58/M	Basal cell carcinoma	Surgery, radiotherapy, immunotherapy	2	9

*Time from last treatment.

July 1995. Histological examination revealed a superficial spreading type of malignant melanoma, Clark level III, of Breslow thickness 0.78 mm. Recurrent metastatic nodules were removed by surgery, first in January and then in June 1996. One month later, a new metastasis was detected near the operation scar. As the patient refused surgical intervention, she was treated by electrochemotherapy. In January 1997, another recurrence of malignant melanoma was noted, approximately 8 cm from the previously treated lesion, and was treated by electrochemotherapy.

Patient 3

A 52-year-old male patient was treated by supraglotic laryngectomy with unilateral radical neck dissection in September 1994 due to squamous cell carcinoma of the supraglotis, UICC pathological stage III. Four weeks later, radiotherapy was performed using a Cobalt-60 unit, through opposing lateral fields covering the area of the primary tumour and regional lymph nodes. The anterior field was added to cover the lower third of the neck and supraclavicular region. A tumour dose of 50 Gy was delivered in 2 Gy daily fractions, five times per week and an electron boost of 12.5 Gy was given in five fractions of 2.5 Gy to the tumour site. Regional recurrence was recorded in March 1995. The patient underwent additional radiotherapy on an ortovoltage machine. A total radiation dose of 36 Gy was delivered in 4 Gy daily fractions. Three months later, two recurrent nodules occurred within the radiation field. The patient was not receptive to additional surgery or radiotherapy and began treatment with electrochemotherapy.

Patient 4

In January 1993, a 58-year-old male patient with a diagnosis of nevoid basal cell carcinoma syndrome (Gorlin–Goltz syndrome) was presented at our institute for the first time. Detailed clinical and radiological examination revealed multiple basal cell carcinoma nodules over the whole body, a bone cyst on the mandibule, intracranial calcifications, coloboma and a cataract in the left eye. Since 1972 he has been subjected to more than 50 surgical treatments, which have included electrocautery, simple excision and Mohs micrographic surgery. At our institute, the patient was treated with IFN-α injected intralesionally and high doses of vitamin A. The treatment was stopped in May 1995 when complete remission of the treated nodules was observed. One year later, more than 100 basal cell carcinoma nodules were detected over the whole body and the treatment with IFN-α and vitamin A was restarted. All but nine nodules located on the neck regressed. Since the patient refused another surgical intervention and treatment with IFN-α or vitamin A, treatment with electrochemotherapy was started.

Treatment evaluation

After the treatment, patients were kept in a recovery room for 2 h, when they were examined and released. As outpatients, they were examined weekly in order to evaluate the treatment and possible side-effects. Tumour nodules were measured with a calliper and photographed. The volume of tumour nodules was calculated by the formula $V = a \times b \times c \times \pi/6$, where a, b and c represent the diameters of the tumour nodule. Response to treatment was scored after 4 weeks, according to WHO guidelines [19], as follows: complete response (CR), absence of any trace of tumour; partial response (PR), more than 50% reduction in tumour volume; no change (NC), reduction of tumour volume less than 50% and no enlargement more than 25%; and progressive disease (PD), tumour volume enlarged more than 25%. Duration of response was calculated as the interval between the date of the first treatment and the date of tumour progression. Observation time was calculated as the interval between the date of the first treatment and the date of the last examination of the patient.

RESULTS

Treatment response

In 4 patients, 19 tumour nodules of different sizes and different histological types were treated by electrochemotherapy. In order to evaluate objectively the antitumour effectiveness of electrochemotherapy, additional nodules were selected as controls. The control groups included untreated nodules (five nodules), a nodule exposed to electric pulses only and nodules treated with cisplatin only (five nodules) (Table 2).

Four weeks after treatment, PD was observed in two untreated nodules and one nodule exposed to electric pulses only, NC in three untreated nodules and one cisplatin treated nodule, PR in two cisplatin treated nodules, and CR in 19 electrochemotherapy treated nodules and two cisplatin treated nodules (Table 2). The two cisplatin treated nodules remained in CR for 8 months, while others treated only with cisplatin progressed within 3–7 months. All 19 electrochemotherapy treated nodules remained in CR (7–11 + months) regardless of the histological type, except for one metastasis of a squamous cell carcinoma that progressed after 9 months (patient 3, nodule 2).

Several electrochemotherapy sessions on the same tumour nodule, because of its large size, were performed on patient 3. The exophytic part of the exulcerated tumour was treated in eight sessions over a 3 week period. After the first session, the pain around the tumour location was relieved to the extent that the patient no longer required analgesics. Six months after the start of treatment, CR of the tumour nodule was obtained and cytologically confirmed. However, 3 months later regrowth of the metastasis was observed.

Treatment tolerance

Electrochemotherapy was well tolerated by all patients. The intratumoral route of cisplatin injection was tolerable, although associated with some pain, which dissipated after several minutes. The application of electric pulses induced instantaneous contractions of muscles located beneath the site of treatment, which disappeared immediately afterwards. During the application of electric pulses, some patients complained of a 'burning' pain and itching on the spot where the electrodes were in contact with the skin. 1 patient, with a nodule on the left side of the back, at the level of the heart, complained of discomfort in the chest during treatment. Electrocardiography showed no abnormalities and the patient had no breathing disturbances.

After electrochemotherapy the patients did not complain of fatigue or any kind of discomfort. The treatment with intratumoral cisplatin did not result in any local or systemic toxicity. No muscle ache associated with the application of the electric pulses was noted. After treatment, none of the patients suffered from a localised or systemic infection. Electrochemotherapy did not result in exulceration of the tumour

Table 2. Summary of tumour characteristics, treatment parameters and tumour response

Patient no.	Nodule	Treatment	Number of sessions	CDDP dose (mg/nodule)	Number of EP/nodule	Tumour volume (mm^3)	Response*	Duration of response (months)†	Observation time (months)‡
1	1	None	—	—	—	31	PD	—	11
	2	None	—	—	—	44	PD	—	10
	3	EP	1	—	8	50	PD	—	10
	4	CDDP	1	1.0	—	55	CR	11+	11
	5	CDDP	1	1.0	—	56	CR	10+	10
	6	CDDP	1	0.5	—	20	NC	7	9
	7	ECT	1	1.0	8	88	CR	11+	11
	8	ECT	1	2.0	16	168	CR	11+	11
	9	ECT	1	2.0	16	157	CR	11+	11
	10	ECT	1	0.5	8	31	CR	11+	11
	11	ECT	1	2.0	16	157	CR	10+	10
	12	ECT	1	2.0	16	188	CR	10+	10
	13	ECT	1	0.5	8	51	CR	10+	10
	14	ECT	1	1.0	8	106	CR	9+	9
	15	ECT	1	2.5	16	262	CR	9+	9
	16	ECT	1	2.5	56	262	CR	7+	7
	17	ECT	1	2.0	16	188	CR	7+	7
2	1	ECT	1	0.25	8	5	CR	11+	11
	2	ECT	1	0.25	8	7	CR	7+	7
3	1	ECT	3	3.5§	90¶	376	CR	11+	11
	2	ECT	8	8.5§	432¶	837	CR	9	11
4	1	None	—	—	—	82	NC	1	8
	2	None	—	—	—	51	NC	1	8
	3	None	—	—	—	106	NC	1	8
	4	CDDP	1	1.0	—	109	PR	3	8
	5	CDDP	1	0.5	—	51	PR	3	8
	6	ECT	1	2.0	8	190	CR	8+	8
	7	ECT	1	2.0	8	190	CR	8+	8
	8	ECT	1	0.5	8	1	CR	8+	8
	9	ECT	1	1.0	8	84	CR	8+	8

CDDP, cisplatin; EP, electric pulses; ECT, electrochemotherapy; PD, progressive disease; CR, complete response; NC, no change; PR, partial response.

*Determined 4 weeks after treatment. †Interval between the date of treatment and the date of tumour progression (+ indicates CR continuing). ‡Interval between the date of treatment and the date of last examination of the patient. §Total cisplatin dose injected over three or eight sessions. ¶Total number of electric pulses applied in three or eight sessions.

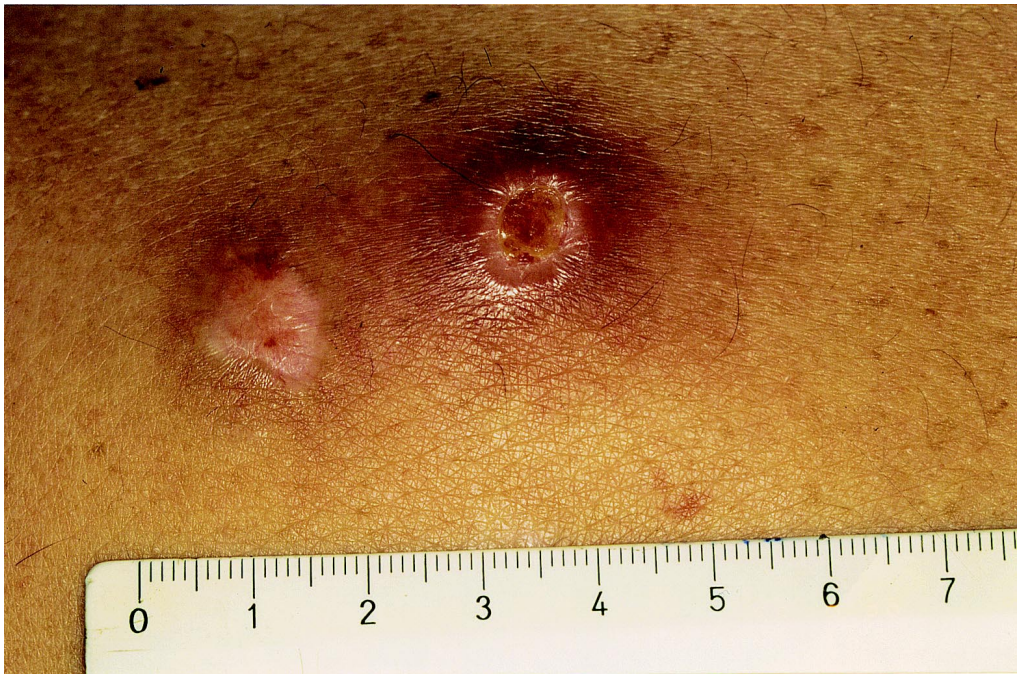

Figure 1. Response to treatment of two electrochemotherapy treated nodules in patient 1 with malignant melanoma. Depigmentation and minimal scarring of the skin is visible on the left tumour nodule 4 months after treatment. On the right tumour nodule 2 months after treatment, an erythema of the skin, after the superficial scab had fallen off, is visible.

nodules, but a slight erythema up to 1 cm occurred in the surrounding tissue, with the formation of a superficial scab which fell off within 5 weeks. A good cosmetic effect was obtained, with minimal scarring and a slight depigmentation of the skin (Figure 1).

DISCUSSION

This is the first clinical study demonstrating that electrochemotherapy with cisplatin is effective in eradicating cutaneous tumour nodules of malignant melanoma, squamous cell carcinoma and basal cell carcinoma. In the 4 cancer patients, CR of all 19 electrochemotherapy treated nodules was obtained which lasted for at least 7 months, and only one nodule progressed after 9 months. A good antitumour effect was also obtained after intratumoral cisplatin treatment, but three of five tumour nodules progressed within 3–7 months. Exposure of nodules to electric pulses without cisplatin treatment had no effect on tumour growth, as also demonstrated in previous preclinical studies of electrochemotherapy [7, 9–13, 17, 18].

In cancer treatment, one of the major problems is to deliver a sufficient concentration of the chemotherapeutic drug into the tumour cells for effective cytotoxicity, while minimising drug concentration in normal tissues. In view of this, there are several approaches to selective tumour drug delivery, including local application of the drugs [20], targeting by binding the drugs to tumour specific antibodies [21], magnetic drug targeting [22], incorporation of the drugs into liposomes or other vehicles [20, 23, 24], or selectively permeabilising the plasma membrane of tumour cells by employing either chemical [25] or physical (electroporation) methods [2]. In our study, we combined two of these approaches, intratumoral injection of cisplatin and electroporation of tumour cells by application of electric pulses to the tumour site.

In our preclinical studies, it was demonstrated that intratumoral cisplatin treatment is more effective than intravenous treatment at the same dose [17]. Furthermore, exposure of cutaneous tumours to electric pulses after intravenous or intratumoral, low non-toxic cisplatin doses potentiates the antitumour effectiveness of cisplatin several-fold [7, 17, 18]. Electrochemotherapy with intratumoral cisplatin injection proved to be more effective than with intravenous cisplatin injection, resulting in a higher percentage of tumour cures due to the higher cisplatin concentration in the tumours [7, 17, 18]. By both routes of cisplatin administration, electrochemotherapy proved to be dependent on the cisplatin dose, and on the amplitude of the electric pulses, as well as on the sequencing and interval of cisplatin administration, relative to the application of electric pulses [7, 18]. In accordance with these experimental data, we chose intratumoral cisplatin administration, an electric pulse amplitude to electrode distance ratio of 1300 V/cm and an interval between cisplatin and application of electric pulses of 1–2 min.

In the study, plate electrodes with a distance of 7 mm between them were used for percutaneous delivery of electric pulses. When electrochemotherapy is performed with such electrodes, the size of the tumour nodule represents a specific problem in cases where the whole tumour nodule cannot be encompassed between the electrodes, leaving deeper parts of the tumour ineffectively electroporated. This problem was solved by two approaches. First, the tumour nodule was treated with several runs of electric pulses positioned in adjacent areas in order to cover the whole tumour area [14, 15, 26]. The second approach was to treat thicker tumour nodules by repeating the electrochemotherapy over several sessions [14, 27]. As in the case of patient 3, electrochemotherapy was repeated eight times on the remaining tumour tissue before CR of the tumour nodule was obtained. On all other tumour nodules treated by electrochemotherapy, no repetitive treatment was needed, but several runs of electric pulses were needed, in order to obtain CR. The problem of treating thicker nodules and also deeper seeded tumours can also be approached by treating the nodules with fine needle electrodes [28, 29]. Such electrodes have already been tested in preclinical studies, as well as being used in the treatment of basal cell carcinoma [28–30]. In addition, such electrodes enable a better electric field distribution within the tumour and, consequently, a higher percentage of effectively electroporated tumour cells [28].

In clinical trials, electrochemotherapy has already been performed using bleomycin as the chemotherapeutic drug. In malignant melanoma, squamous cell carcinoma, basal cell carcinoma and Kaposi's sarcoma patients, 85% of objective responses has been observed [14–16]. In our study, electrochemotherapy with cisplatin was performed for the first time. The same tumour types have been treated and it was shown to be as effective as electrochemotherapy with bleomycin, indicating that electrochemotherapy, regardless of the chemotherapeutic drug used, is an effective treatment of cutaneous tumour nodules. In addition, new electrodes being developed will provide even better treatment opportunities, enabling the treatment of tumour nodules of varying size and thickness, and also the treatment of internal tumours. Therefore, intra-operative treatment of either liver or lung tumours will be possible. Because electric pulses can temporarily shut down tumour blood flow [31], this therapy will probably not cause excessive bleeding. Furthermore, perturbation of tumour blood flow may even contribute to the antitumour effectiveness of electrochemotherapy by prolonging exposure of the cells to chemotherapeutic drugs entrapped within the tumour after application of electric pulses.

Electrochemotherapy, in the studies published so far, was used as a single treatment modality [14–16]. However, because of the broad clinical applicability of cisplatin, electrochemotherapy with cisplatin can also be used as an adjunct to on-going cisplatin treatment in patients for whom the antitumour effect needs to be potentiated locally. Our preliminary results have demonstrated that exposure of tumours to electric pulses in patients undergoing cisplatin containing chemotherapy is very effective, but needs further evaluation.

Although one of the limitations of electrochemotherapy is that it is a local treatment, it may nevertheless find various applications. In clinical situations, where repeated surgical procedures and irradiation are difficult to perform due to progression of the disease in cutaneous tissue only, both intratumoral cisplatin treatment and potentiation of its effectiveness by electric pulses are feasible. Therefore, electrochemotherapy could find its place in the management of locally recurrent disease, e.g. breast cancer, in addition to malignant melanoma. With regard to systemic treatment, electrochemotherapy offers a significant advance in the case of malignant melanoma and basal cell carcinoma. In cases such as squamous cell carcinomas, the application of electrochemotherapy is less clear because there are less cases that need such local treatment. However, the treatment is easy to

perform, can be carried out on an out-patient basis and is inexpensive. The patients tolerate the treatment well and, as yet, no side-effects have been recorded.

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