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Electrochemotherapy with cisplatin: the systemic antitumour effectiveness of cisplatin can be potentiated locally by the application of electric pulses in the treatment of malignant melanoma skin metastases

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The application of electric pulses to skin tumour nodules enhances the antitumour effectiveness of cisplatin. This treatment approach, known as electrochemotherapy, was employed in the treatment of skin metastases and lymph node metastases in malignant melanoma patients. Electric pulses were applied to tumour nodules in order to potentiate locally the antitumour effectiveness of systemic cisplatin-based chemoimmunotherapy. The study included nine malignant melanoma patients with skin metastases and metastases in lymph nodes not amenable to surgery, undergoing cisplatin-based chemoimmunotherapy. The antitumour effectiveness of the chemoimmunotherapy was compared with the antitumour effectiveness of electrochemotherapy, i.e. application of electric pulses to tumour nodules together with cisplatin-based chemoimmunotherapy. Application of electric pulses to the 27 skin tumour nodules potentiated locally the antitumour effectiveness of cisplatin. Four weeks after the treatment, 48% of the tumour nodules had an objective response (OR), compared with 22% of the 18 tumour nodules treated with cisplatin-based chemoimmunotherapy alone. Furthermore, the median time to progression was longer in the electrochemotherapy-treated nodules (21 weeks) than in the chemoimmunotherapy-treated nodules (4 weeks). This study shows that application of electric pulses to malignant melanoma tumour nodules can potentiate the antitumour effectiveness of cisplatin in patients undergoing systemic cisplatin-based chemoimmunotherapy. Therefore, electrochemotherapy may be used as an adjunct to systemic ongoing cisplatin treatment, predominantly in patients in whom antitumour effectiveness needs to be potentiated locally. © 2000 Lippincott Williams & Wilkins

Key words: cisplatin, drug delivery system, electrochemotherapy, electroporation, malignant melanoma

Introduction

The response of melanoma patients to chemotherapy is usually low, with a complete response rate less than 5%.[1,2] There are several reasons why many of the chemotherapeutic drugs presently used in clinical chemotherapy are not sufficiently effective. One is that the plasma membrane can be a significant barrier that hampers the transport of drugs into cells, hindering the cytotoxicity of drugs that have intracellular targets. 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[3] is the use of electropermeabilization.[4]

In order to facilitate chemotherapeutic drug delivery into cells, electroporation performed by the application of short intense electric pulses has been demonstrated to be very effective. Exposure of cells to electric pulses under specific conditions increases plasma membrane permeability, temporarily and reversibly, without affecting cell viability.[5,6] Enhanced delivery of chemotherapeutic drugs into the cells is termed electrochemotherapy.[4]

Electrochemotherapy has been shown to be successful for drugs such as bleomycin and cisplatin, which normally exhibit impeded transport through the plasma membrane.[7,8] Due to the increased accumulation of bleomycin and cisplatin in tumours exposed to electric pulses, the potentiation of bleomycin and cisplatin antitumour effectiveness has been demonstrated in several animal tumour mod-

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els.[4,8–11] These two drugs have also proved their clinical application in electrochemotherapy protocols, both drugs being very effective in the treatment of different cutaneous tumour nodules in cancer patients.[12–20]

Our recent study has provided evidence that electrochemotherapy with cisplatin, when injected intratumorally, is very effective in the treatment of skin tumour nodules in malignant melanoma.[14,16] The objective response rate, its median duration and the local control rate of electrochemotherapy-treated nodules were significantly higher compared with nodules treated with cisplatin alone.[16]

However, many malignant melanoma patients treated with chemoimmunotherapy also present with cutaneous metastases or metastatic involvement in lymph nodes. In such patients, local potentiation of the cytotoxicity of cisplatin would be beneficial because of the common low effectiveness of chemoimmunotherapy. This study was undertaken to evaluate whether application of electric pulses to malignant melanoma tumour nodules can locally potentiate the antitumour effectiveness of cisplatin in patients undergoing systemic cisplatin-based chemoimmunotherapy.

Patients and methods

Patient eligibility

Nine patients (five men and four female) with histologically confirmed cutaneous malignant melanoma were included in this phase I–II clinical trial. All of the patients had measurable skin metastases and lymph node metastases not amenable to surgery. Three of them had metastases in lung and liver, three in lung and one in bone. National ethics committee approval and written informed consent from each patient were obtained before the beginning of the study.

Study design and treatment plan

The aim of the study was to evaluate the antitumour effectiveness of cisplatin-based chemoimmunotherapy on measurable skin tumour nodules and compare this with the antitumour effectiveness on skin tumour nodules treated in combination with the application of electric pulses (electrochemotherapy). In each patient tumour nodules were enrolled in both treatment groups. Selection of the tumour nodules for enrolment into the treatment groups was random, but balanced in number. In one patient the treatment was repeated three times due to the development of new metastases. Eighteen tumour nodules were included in the cisplatin-based chemoimmunotherapy-treated group, and 27 tumour nodules were included in the electrochemotherapy-treated group. In each group only three tumour nodules were lymph node metastases.

Chemoimmunotherapy

Patients were treated with vinblastine 4 mg/m^2 intravenously (i.v.) and lomustine (CCNU) 80 mg/m^2 orally on day 1, cisplatin (CDDP) 20 mg/m^2 on days 2–5 and interferon-α2b 3 MIU/m^2 on days 4–7.

Electrochemotherapy

Electrochemotherapy was performed on day 4 of the chemoimmunotherapy protocol during the daily chemotherapy infusion, at least 3 h after the beginning of the infusion in order to provide optimal cisplatin concentration in the tumour nodules. Interferon was given to the patients at least 6 h after the electrochemotherapy session. Eight square-wave electric pulses of 100 µs, 910 V amplitude (amplitude to electrode distance ratio 1300 V/cm), frequency 1 Hz were applied to the tumour nodules. Electric pulses were delivered through two parallel stainless steel electrodes (thickness 1 mm, width 7 mm, length 14 mm, with rounded tips with an inner distance between them of 7 mm) using the electropulsator Jouan GHT 1287 (Jouan, France). Electrical parameters were controlled using a HM 205-3 oscilloscope (Hameg Instruments, Germany). Each run of electric pulses was delivered in two trains of four pulses, with a 1 s interval, in two perpendicular directions, as reported previosly.[14] 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 to assure adequate coverage of the whole tumour area.[14] Patients received lidocaine spray over the treated surface before application of the electric pulses.

Treatment evaluation

Patients were examined at 4 week intervals on an outpatient basis, by the same team of experts that

performed the therapy, in order to evaluate the treatment response and possible side effects. Tumour nodules were measured with a calliper. The volume of tumour nodules was estimated using 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 World Health Organization guidelines,[21] 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% or enlargement no more than 25%; and progressive disease (PD), tumour volume enlargement more than 25%. The objective response (OR) was calculated as the sum of PR and CR. The time to progression was calculated as the interval between the date of treatment and the date of tumour progression or patient death. The duration of CR was calculated as the interval between the date when the CR was first recorded and the date of tumour progression.[21]

Statistical analysis

Statistical calculations were performed using a PC computer and a Sigma Stat statistical software package (SPSS Inc.). The differences in median tumour volume before and 4 weeks after treatment and the median time to progression were tested using the Mann-Whitney rank sum test.

Results

Treatment response

Electrochemotherapy was evaluated as an adjunct to ongoing cisplatin-based chemoimmunotherapy of tumour nodules in nine malignant melanoma patients. The local antitumour effectiveness of electrochemotherapy was compared with the antitumour effectiveness of cisplatin-based chemoimmunotherapy alone.

Cisplatin-based chemoimmunotherapy proved to be moderately effective. The median tumour volume of the 18 tumour nodules did not significantly reduce ($P = 0.376$); however ORs were obtained in 22% of the tumour nodules 4 weeks after treatment and the median time to progression was 4 weeks (Table 1).

Nodules treated with electrochemotherapy, i.e. the application of electric pulses to the tumour nodules during cisplatin-based chemoimmunotherapy, responded better than nodules that did not receive electric pulses. Their median tumour volume was significantly smaller 4 weeks after electrochemotherapy ($P = 0.04$). The majority of the nodules were NCs or ORs; the treatment was ineffective in only three nodules. The time to progression of the electrochemotherapy-treated nodules was prolonged from 4 weeks in the cisplatin-based chemoimmunotherapy-treated group to 21 weeks in the electrochemotherapy group ($P = 0.046$).

Treatment tolerance

Chemoimmunotherapy was well tolerated by the patients. Application of the electric pulses induced instantaneous contractions of the muscle located beneath the site of the treatment, which dissipated immediately afterwards. There was no ulceration of the tumour nodules, only slight erythema surrounding the nodule and sometimes formation of a superficial scab. A good cosmetic effect was obtained, with minimal scarring and slight depigmentation of the skin.

Discussion

Results of this study show that electrochemotherapy with cisplatin is effective when cisplatin is given systemically. It was found that the application of

Table 1. Response of tumours treated by electrochemotherapy compared with intravenously injected cisplatin alone in nine malignant melanoma patients

Treatment	No. of nodules	Mean tumour volume (mm^3) ($\pm$ SE)	Mean tumour volume after 4 weeks (mm^3) ($\pm$ SE)	Response to treatment after 4 weeks					Median time and range to progression (weeks)
				PD	NC	PR	CR	OR	
Cisplatin	18	502 $\pm$ 201	437 $\pm$ 335	7	7	2	2	4 (22%)	4.0 (0.0–52.0)
Electrochemotherapy	27	1010 $\pm$ 474	474 $\pm$ 307	3	11	10	3	13 (48%)	21.0 (8.0–52.0)

electric pulses to malignant melanoma tumour nodules potentiates the antitumour effectiveness of cisplatin in patients undergoing systemic cisplatin-based chemoimmunotherapy. A greater reduction in tumour volume, a higher percentage of ORs and a longer time to progression were found in the electrochemotherapy- than in the cisplatin-treated group.

Electrochemotherapy with cisplatin has already found to be effective in the treatment of skin tumour nodules of various types of tumours, including malignant melanoma.[14–16] In our recent study on 10 malignant melanoma patients it was demonstrated that electrochemotherapy using intratumoral cisplatin injection resulted in 78% ORs, and that intratumoral cisplatin injection as a single treatment was also effective, resulting in 38% ORs.[16] This clinical trial was based on preclinical studies that included both intratumoral and intravenous electrochemotherapy.[8,22] Comparison of these results demonstrated that both routes of cisplatin administration were effective; however, the intratumoral route proved to be more effective than the intravenous route.[11] The results of the present study are also in line with our preclinical results. Electrochemotherapy resulted in 48% ORs and cisplatin treatment in 22% ORs, being less effective than after itntratumoral cisplatin injection. It is difficult to explain the three tumour nodules that did not respond to electrochemotherapy. The most probable reason could be that the tumour nodules were inadequately perfused, resulting in an insufficient cisplatin concentration being achieved in the tumour nodules. The tumour nodules that did not respond after electrochemotherapy treatment were found in a patient with superficial, exophytic tumour nodules. It is difficult to speculate, but macroscopically these nodules were found to be poorly vascularized and perfused. However, they were not so big that this caused an inadequate intratumoral cisplatin concentration. Nevertheless, these results demonstrate that electrochemotherapy can be used as an adjunct to cisplatin-based chemotherapy.

Bleomycin, another drug that is used in electrochemotherapy, has also been employed in the treatment of skin metastases in malignant melanoma as well as in the treatment of many other tumour types.[12,13,17,19,20] In the first multicentre report on electrochemotherapy from three centres involving 13 patients, the response to electrochemotherapy with bleomycin given intravenously was found to be very effective, with the OR rate ranging from 50–96%.[13] In that study bleomycin was used for the electrochemotherapy as the treatment of choice for a particular tumour type, and not as part of a chemotherapy scheme. Also, bleomycin was ineffective without the application of electric pulses. In contrast, the aim of our study was to determine whether the application of electric pulses can be used in order to increase drug delivery into tumour nodules during on-going cisplatin-based chemotherapy routinely used in the treatment of metastatic disease in malignant melanoma patients. The application of electric pulses to tumours has already been demonstrated to have no effect on tumour growth, but is only a means to increase drug delivery into cells.[12–16]

All these clinical studies are based on preclinical data demonstrating that electroporation is an effective drug delivery system for local potentiation of bleomycin and cisplatin cytotoxicity. In many studies it has been demonstrated that electroporation increases drug accumulation in cells and tissues,[7,10,22] that application of electric pulses decreases tumour blood flow and results in prolonged drug entrapment,[23,24] and that, especially with bleomycin, electrochemotherapy is also a vascular-targeted therapy.[25] All the three mechanisms listed are involved in the antitumour effectiveness of electrochemotherapy and, in addition, stimulation of the immune response or the immune responsiveness of the organism can also contribute to the effectiveness.[26,27]

In conclusion, electrochemotherapy with cisplatin was found to be effective when cisplatin was given systemically, in addition to the studies demonstrating its effectiveness when cisplatin is given intratumorally. Therefore, electrochemotherapy with cisplatin can be used as a single modality or, as demonstrated in this study, as an adjunct to systemic ongoing cisplatin treatment in patients where antitumour effectiveness needs to be potentiated locally. In general, the current limitation of electrochemotherapy, either with bleomycin or cisplatin, is that it can be performed only on superficially accessible tumour nodules. In the future, optimization of electric field distribution in tumours[6] and the development of new types and configuration of electrodes[28,29] will enable the treatment of tumours located deeper in the body, either during open surgery or by catheterization.

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