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Short communication

Electrochemotherapy of Mycosis fungoides by interferon-α

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Abstract

Eight patients with 29 lesions of histologically verified 1st stage of Mycosis fungoides were successfully treated by electrochemotherapy with interferon-α. For this purpose 8 biphasic pulses were used, each of 50+50 μs duration with 900 μs interpulse intervals, resulting in a burst of 7.1 ms total duration. Compared to the traditional monoimmunotherapy with interferon-α applied three times weekly for a total of 4 weeks, the electrochemotherapy was very efficient. Complete response (CR) was observed in 25 (86%) of the 29 treated lesions by single-act electrochemotherapy with interferon-α. At the end of the 12-month period, all 29 lesions showed 100% complete response (CR). New lesions for a period of 12 months were not observed.

The expected mechanism involved in multiple cytotoxic action of interferon-α could be the local increased concentration in the tumour and prolongation of the time of its action after the application of pulses.
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1. Introduction

Electrochemotherapy could be considered as an established method for the treatment of malignant cutaneous and subcutaneous tumours. Since its introduction by Mir et al. [1], several clinical trials (for example [2–5]) confirmed its high efficiency. Recently we also applied electrochemotherapy and bleomycin to basal and spin cell carcinoma and malignant melanoma metastases with complete response [6]. These results stimulated us to treat other skin tumours by electrochemotherapy.

Mycosis fungoides is related to the skin T-lymphoma, which in itself is a low-grade malignant lymphoma with 3 clinical phases of the disease. The first stage is expressed by premycosal eruptions and tumour-like formation and is initiated by a generalized and constant skin itching. Erythemas and urticaria-like rashes appear and are later transformed in erythema-squamos infiltrations. Sometimes the lesions are like swellings.

Interferon-α was the first cytokine to be used in clinical trials that proved to be useful in the treatment of several types of cancer [7–9]. However, the half-life of interferon recombinant protein is so short that high doses need to be administered repeatedly to obtain an effective concentration. Such administrations lead to the repetitive fluctuation between extremely high peak levels to basal level of interferon-α in the serum and could cause untoward systemic effect [10].

In view of these considerations, we were tempted to try and use predominantly a single-act electrochemotherapy combining interferon-α with a short duration burst of biphasic pulses. Some of our previous experience in electrical stimulation of innervated muscles [11] suggested that biphasic stimuli were of better tolerance. A new type of biphasic electrical pulse sequence was introduced for electrochemotherapy, resulting in improved efficiency and toleration by patients [4,12]. In addition, bidirectional electrical field might induce permeabilisation of a greater number of tumour cells. Moreover, pore formation is considered a process that develops with microseconds [13,14]. The enhanced effect of biphasic pulses applied at higher frequencies was subsequently proven by independent theoretical and experimental studies of other authors [15–17].

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2. Method and patients

Electrical stimulation was carried out with 8 biphasic pulses that were used each of 50+50 μs duration with 900 μs interpulse intervals (the period of repetition of pulses was 1 ms), resulting in a burst of 7.1 ms total duration [12]. The electrical pulses were applied 10 min after the injection of interferon-α, according to the accepted electrochemotherapy protocol by the National Oncological Centre in Bulgaria. The electrodes were a pair of parallel stainless steel wires of 0.8 mm in diameter and 15 mm of length. The interelectrode distance was adjustable (calliper type) in the range of 5–30 mm.

The threshold sizes of treated Mycosis fungoides tumours in 1st clinical stage were 30 mm in diameter. Lesions bigger than 30 mm were treated with radiation and/or chemotherapy.

The patients were selected according to the following conditions:

- diagnosis confirmed by histology;
- absence of critical heart rhythm disturbances;
- informed consent to acceptance of the type of treatment, including comparison with other treatment modalities of the disease;
- post-treatment follow-up of at least 12 months.

Eight patients with 29 lesions, being in the 1st clinical stage of Mycosis fungoides were treated.

We applied the already established procedure for electrochemotherapy [6], approved by the Ethical Committee of the National Centre of Oncology. Shortly, we injected a local anesthetic (lidocaine 1%) and interferon-α into the tumour. The dosage of the used interferon α-2a with trade name Roferon A (Roche) was 3–6 MU, its volume approximately corresponding to the lesion volume: 3 MU for tumour volume up to 100 mm^3, 4–5 MU for volume in the range of 100 to 150 mm^3 and 6 MU for volumes of 150 to 500 mm^3. One MU contains 3.7 μg interferon α-2a (Roche). The volume of tumour lesions was calculated according to the formula $V=a\times b\times c$, where a is the length, b is the width and c is the thickness of the tumour, used in our former investigation [6]. The size of the lesions was measured by a calliper instrument (Arimedex). Good contact between the skin and electrodes was assured by a conductive gel. The treatment response was evaluated at least 4 weeks (one month) after the treatment according to WHO guidelines as follows: 1) Complete response (CR): the absence of any trace of tumour; 2) Partial response (PR): decrease in the tumour volume by 50% or greater; 3) No change (NC): decrease of < 50% or an increase of < 25% in the tumour volume; 4) Progressive disease (PD): tumour volume enlarged more than 25%.

The field strength was considered taking into account some parameters: the longest length, place, and conciseness of the lesions. The applied field intensity was lower when the lesions were near to the heart. Low field strengths were used in cases when the lesions were on the face (on the cartilaginous tissue) or near to the mucous tissue to avoid necrosis. The field intensity was the same when a need to treat the same lesion again was indispensable.

Control experiments were done on 3 patients with 5 tumours. During the first experiments, only 8 electrical pulses were applied (Table 1, A). After one month the same patients were treated with interferon-α injected in the lesions but without electrical pulses (Table 1, B). The results were accounted after 24 h, 72 h, 2 weeks and one month. After that the same patients were treated with interferon-α and electrical pulses (Table 1, C).

The next 5 patients (N 4–8) were treated only by interferon-α and electrical pulses (Table 1, C).

The patients were subjected to monthly examinations for the first 6 months and to three-month check-ups for the remaining period of up to one-year total post-treatment follow-up.

The treated lesions in one experiment were no more than three in number. Other lesions were treated after a period of three days.

3. Results and discussion

After one month, when only electrical pulses were applied no change in the volume, pigmentation, or epidermal atrophy was observed (Table 1, A). Therefore, we decided to continue the chemotherapy with interferon-α because with this kind of tumour the lesions could increase in size and number. The tumour size was reduced by one third in a period of one month when treated with interferon-α injected in the lesions, but no electrical pulses were used (Table 1, B). It gives 100% complete response (CR) when applying combination of interferon-α plus electrical pulses to the same patients from group B (Table 1, C, patients 1–3). In cases A* and C* the patient was treated with two electrical pulse applications using inter-rod distances up to 15 mm and field intensity of 1500 V cm^{-1}.

The 18 mm (diameter) lesion on the chest of patient N4 was treated at the end of the 3rd month and at the end of the 5th month. There were no signs of recurrence up to the end of the follow-up period.

The 20 mm lesion on the back of patient N6 was treated at the end of each control month from the beginning of the first three months. Full resorption was observed during the remaining 8 months of follow-up. After each electrochemotherapy, the volumes of the tumours were reduced approximately to 30%.

Patient N8 lesions on the left thigh and right gluteus were treated once more at the 1st month of the control examination. They were fully healed during the remaining 10 months. In addition, this patient had a marked side effect after the injection of interferon-α. His temperature rose to 39°, with headache and apparent weakness for about 12 h. He also had pronounced erythema around the right gluteus lesion, which was near to the mucous membrane.

Here we have to mark down that, when NC or PR were scored (Table 1#) lesions were still present either in the treated lesion center or at its periphery.

At the end of the 12-month period, all 29 lesions showed 100% complete response. New lesions for a period of 12 months were not observed.

Application of electrical pulses only or treatment only with drug had minimal or no effect upon tumour growth.

Table 1
Parameters of the treatments by electrochemotherapy with interferon-α

Patient N, age, gender	Lesion location	Lesion sizes (*a*,*b*,*c*) [mm]	Interferon-α [MU]	Field strength [V cm^{-1}]	Response after one month	Duration of CR response (months)
A						
1. Age 72, F	Back*	30/10/1.5	–	1500	NC	
2. Age 47, M	Back	8/6/2	–	1000	NC	
	Right arm	15/12/2	–	1200	NC	
	Left arm	11/8/1.6	–	1200	NC	
3. Age 27, M	Nose	4/4/3	–	800	NC	
B						
1. Age 72, F	Back	30/10/1.5	6	–	NC	
2. Age 47, M	Back	8/6/2	3	–	NC	
	Right arm	15/12/2	6	–	NC	
	Left arm	11/8/1.6	5	–	NC	
3. Age 27, M	Nose	4/4/3	3	–	NC	
C						
1. Age 72, F	Back*	22/8/1.5	6	1500	CR	11
2. Age 47, M	Back	6/5/1.5	3	1000	CR	11
	Right arm	12/10/1	6	1200	CR	11
	Left arm	9/7/1	5	1200	CR	11
3. Age 27, M	Nose	4/3/2	3	800	CR	11
4. Age 58, F	Low back	7/7/2	3	1500	CR	11
	Left arm	15/8/1.2	4	1000	CR	11
	Back	8/8/1.6	3	1500	CR	11
	Chest-left side-low	10/8/1.1	3	1300	CR	11
	Chest-centrum[#]	18/8/1.1	5	890×3	PR	6
5. Age 42, M	Right arm	9/8/1.4	3	1100	CR	11
	Back	6/5/3.5	3	1500	CR	11
	Back	12/10/1.2	4	1000	CR	11
6. Age 30, M	Back-left side[#]	20/13/1.8	6	850×3	NC	8
	Back	9/6/2	3	1300	CR	11
	Back	5/5/4	3	1500	CR	11
	Left forearm	13/10/1.3	4	1000	CR	11
7. Age 65, M	Neck	10/10/1.5	4	1100	CR	11
	Right shoulder	4/4/5	3	1500	CR	11
	Right wrist	6/6/3	3	1500	CR	11
	Back-left side	17/15/2	6	890	CR	11
	Back	10/8/1.8	5	1100	CR	11
8. Age 25, M	Back	5/5/4	3	1500	CR	11
	Back	9/7/1.8	4	1200	CR	11
	Back	13/10/2	6	1000	CR	11
	Left shoulder	8/8/3.2	6	1500	CR	11
	Right upper arm	12/8/3	6	1200	CR	11
	Right thigh[#]	15/10/1.2	6	1000×2	NC	10
	Right gluteus[#]	25/10/1.2	6	700×2	NC	10

*Lesion with two electrical pulse applications in one experiment; [#]lesions treated two (×2) or three (×3) times (see Results and discussion); *a*: length, *b*: width and *c*: thickness of the lesion; Response, determined according to WHO classification: Complete (CR) and Partial (PR) responses, and No change (NC); Duration of CR in months after the first documented CR.

When looking at Table 1, it can be seen that the lesions which required more than one treatment procedure were of relatively large dimensions, placed near the heart, the mucous tissue or the cartilage. In these cases a considerably lower field intensity was applied. The reason for this was to avoid possible skin necrosis or erythema under the electrodes when higher voltage was applied in body regions with tender skin or to avoid some fibrillation of the heart.

In our practice the standard procedure until 1998 for treatment of Mycosis fungoides at its 1st stage was with interferon-α, applied 3 times weekly for a total of 4 weeks (unpublished data). Compared to the traditional monoimmunotherapy, the electrochemotherapy was very efficient (Table 1).

The mechanism of action of electrochemotherapy is the electropermeabilisation of cells and thus it increases the concentration of cytotoxic drugs as bleomycin and cisplatin in the tumour cells [2–5]. The target of the cytotoxic effect of the above drugs is attacking of DNA in the nucleus. Interferon-α is another candidate that possesses antitumourous properties [7–9]. According to the latest reviews, the variety of biological functions of interferon-α is expressed by the interaction with interferon-α receptors, which are composed of two identified subunits, resulting in the activation of a number of signalling pathways [18–21]. The difference between bleomycin and/or cisplatin and interferon-α is that the latest drug expresses its

cytotoxic effect on the surface of the cells by interactions with the receptors. Furthermore, the higher concentration of interferon-α in the serum could give some side effects and develop serum antibodies [10]. An appropriate technology for improving the practical application of interferon-α could involve the increase of its local delivery in the tumours, also to overcome systematic side effects.

Other mechanisms that are involved in the antitumour effectiveness of electrochemotherapy with bleomycin or cisplatin have been reported [22,23]. The application of electric pulses with high field intensities induces transient but reversible reduction of blood flow. Restoration of blood flow in normal tissues is much faster than in tumours. The decreased blood flow in the tumour might induce the entrapment in the tumour of drug injected intratumourally, providing more time for the drug to exert its cytotoxic activity. For this reason, we supposed that the expected mechanism of the observed positive effect of interferon-α with the use of high voltage electrical pulses is due to the increase of the local concentration of interferon-α and the prolongation of the time for the action of this cytokine inside the tumour.

4. Conclusions

Taking into account the 100% complete response for the 29 lesions treated by electrochemotherapy with interferon-α, the method can be recommended for treatment of Mycosis fungoides in the 1st stage. Compared to other treatments for this condition, this non-traumatic and predominantly single-act procedure has definite advantages. The expected mechanism involved in multiple cytotoxic action of interferon-α could be the increased concentration for a longer time in the tumour after the application of pulses.

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