

Electrochemotherapy: advantages and drawbacks in treatment of cancer patients

Review Article

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Summary

Electrochemotherapy combines administration of nonpermeant or poorly permeant chemotherapeutic drugs with application of electric pulses to the tumors in order to facilitate the drug delivery into the cells. Thus, enhanced drug delivery can substantially potentiate chemotherapeutic drug effectiveness, locally at the site of the cell electroporation by electric pulses, without affecting drug effectiveness in the tissues that were not exposed to electric pulses. Vast amount of information gathered on effectiveness and mechanisms of action of electrochemotherapy facilitated clinical trials using bleomycin and cisplatin in electrochemotherapy protocols. All studies provided evidence that electrochemotherapy is effective treatment for local tumor growth in patients with different cancer types. In this review we gathered the data of the clinical trials that have been published so far, and presented our latest clinical experience on electrochemotherapy with cisplatin at the Institute of Oncology in Ljubljana, pointing out the advantages and drawbacks of this treatment.

I. Introduction

What is electrochemotherapy?

Electrochemotherapy consists of chemotherapy followed by local application of electric pulses to the tumor to increase drug delivery into the cells. In late eighties were published the first reports using different sets of electric pulses, both exponential and square wave, with high amplitude, demonstrating that antitumor effectiveness of chemotherapeutic drug bleomycin can be potentiated, resulting in tumor cures (Belehradek et al, 1991; Mir et al, 1991a; Okino and Mohri, 1987). The idea was to apply electric pulses through a set of metal plate electrodes to the limited area of the tissue, *i.e.* tumor, in order to permeabilize the membrane of tumor cells and increase uptake and effectiveness of the drug injected before the application of electric pulses. The drug that was used in these first studies, bleomycin, is a hydrophilic drug, that has very limited transport through the cell membrane, but is very cytotoxic once bound to DNA (Mir et al, 1996). Consequently, for good antitumor effectiveness, drug doses could be drastically reduced, because electroporation increased drug effectiveness several fold, locally at the site of electric pulses application. As a result of reduced drug dosage minimal or no side effects were observed in animals and patients

treated by electrochemotherapy (Heller et al, 1999; Mir, 2000).

II. Preclinical studies on electrochemotherapy

Some review papers have already dealt with this subject, but briefly we will summarize again (Mir, 2000; Sersa, 2000a). *In vitro* studies tested several chemotherapeutic drugs for potential application in combination with electroporation of the cells (Cemazar et al, 1998a; Gehl et al, 1998; Jaroszeski et al, 2000a; Orłowski et al, 1988; Sersa et al, 1995). Since electroporation can facilitate drug transport through cell membrane for only those molecules that are poorly or non-permeant, the selection is limited to those drugs that are hydrophilic, and lack transport systems in the membrane. The result of these studies was that only two drugs have been identified as potential chemotherapeutic drugs for electrochemotherapy. The first being bleomycin, that is hydrophilic, has very restricted transport through the cell membrane, but its cytotoxicity could be potentiated several 1000 times with electroporation of cells (Cemazar et al, 1998b; Gehl et al, 1998; Jaroszeski et al, 2000a; Kambe et al, 1996; Kuriyama et al, 2000; Orłowski et al,

1988). The second being cisplatin that has also hampered transport through the cell membrane (Gately and Howell, 1993). The exact mechanisms of the transport for cisplatin are not fully understood. However, electroporation of cells demonstrated increased cisplatin cytotoxicity up to 80 fold (Cemazar et al, 1998a, 2001; Gehl et al, 1998; Jaroszeski et al, 2000a; Kambe et al, 1996; Kuriyama et al, 2000; Melvik et al, 1986; Sersa et al, 1995). Besides these two drugs other platinum containing compounds, actinomycin D, adriamycin, mitomycin C, 5-FU and cyclophosphamide showed promising results in *in vitro* studies and in some *in vivo* studies, but didn't reach clinical testing (Kambe et al, 1996; Kuriyama et al, 2000; Orłowski et al, 1988; Yabushita et al, 1997).

Both drugs, bleomycin and cisplatin, have been tested on animal models *in vivo*. Their effectiveness was demonstrated on several tumor models, in mice, rats, rabbits, cats, dogs, horses and guinea pigs. In these studies solid subcutaneous tumors, in muscle, liver and brain, being either sarcomas, carcinomas, melanoma or neuroblastoma were used to demonstrate antitumor effectiveness of electrochemotherapy (Rols et al, 2002; Mir et al, 1995; Sersa, 2000a). It was established that electric pulses have to be applied at the time of maximal drug concentration in the tumor, in order to achieve the best antitumor effect. Depending on the route of the drug administration, the best timing for intravenous injection of the drug is 3 minutes before application of electric pulses, and for intratumoral administration electric pulses should be applied immediately after drug injection (Cemazar et al, 1998c; Domenge et al, 1996; Heller et al, 1997; Sersa et al, 1995). Electroporation of the tissue before drug administration has minimal or no antitumor effectiveness. In addition, drug dosage dependency of antitumor effectiveness and dependency on amplitude, number of pulses and electric field distribution in the tissues were elaborated in the preclinical studies (Cemazar et al, 1998c; Heller et al, 1997; Jaroszeski et al, 2001; Miklavcic et al, 1998; Mir et al, 1991a; Sersa et al, 1995). Furthermore, elaborated were also other electrical parameters such as the threshold for reversible and irreversible permeabilization of the tissue and frequency of the pulses (Gehl et al, 1999; Macek Lebar et al, 2002; Miklavcic et al, 2000; Pucihar et al, 2002). Based on all these data, we can summarize that for good antitumor effectiveness using plate electrodes with the distance from 4 to 8 mm between them, optimal set of pulses is 8 pulses with amplitude 1100 to 1300 V/cm, pulse duration 100 μ s, and frequency 1Hz. For better effect 8 electric pulses should be delivered in two perpendicular directions in two sets of 4 pulses (Cemazar et al, 1995; Miklavcic et al, 1998; Sersa et al, 1996a). Application of electric pulses only or treatment with the drug only had minimal or no effect on tumor growth. Needle electrodes with different configuration of the needles were also developed for electrochemotherapy (Gehl et al, 1999; Gilbert et al, 1997; Mir et al, 1997). Due to the different setup of the electrodes it is difficult to prescribe optimal electric pulses parameters for good antitumor effect of electrochemotherapy. However, basically, these types of electrodes require lower electric field intensity than plate electrodes. This is because with

needle electrodes there is no need to overcome the resistance of stratum corneum, since these electrodes are inserted directly in the tumor tissue.

Basic mechanism of action of electrochemotherapy is electroporation of cells in tumors, which increases drug effectiveness by enabling the drugs to reach intracellular targets (Belehradek et al, 1994; Cemazar et al, 1998c, 1999). Besides this principal one, other mechanisms that are involved in antitumor effectiveness of electrochemotherapy were described. Application of electric pulses to the tissues induces transient but reversible reduction of blood flow. Restoration of blood flow in normal tissue is much faster than in tumors (Gehl et al, 2002; Sersa et al, 1999a). The decrease in tumor blood flow induces drug entrapment in the tissue, providing more time for drug action (Sersa et al, 1999a,b). Besides, this phenomenon prevents bleeding from the tissue (Gehl and Geertsen, 2000). The effect of electrochemotherapy is not only on tumor cells in the tumors, but also on stromal cells, including endothelial cells in the lining of tumor blood vessels. Therefore, another mechanism involved in antitumor effectiveness of electrochemotherapy is its vascular targeted effect (Cemazar et al, 2001; Sersa et al, 1999b, 2002). Due to the massive tumor antigen shedding in the organisms, electrochemotherapy can induce also some systemic immunity, that can be up-regulated by additional treatment with biological response modifiers like IL-2 and TNF- α (Heller et al, 2000; Mir et al, 1992, 1995; Sersa et al, 1996b, 1997).

Summarizing, electrochemotherapy protocols were optimized in preclinical studies *in vitro* and *in vivo*, and basic mechanisms elucidated, such as electroporation of cells, tumor drug entrapment, antivascular effect and involvement of immune response. Based on all these data, electrochemotherapy with bleomycin and cisplatin was promptly evaluated in clinical trials.

III. Overview of clinical studies

The first report of L.M. Mir on treatment of head and neck patients with electrochemotherapy using bleomycin, published in 1991, stimulated other groups that have already tested electrochemotherapy in preclinical studies, to launch their own clinical studies (Belehradek M et al, 1993; Domenge et al, 1996; Glass et al, 1996ab, 1997; Heller et al, 1996; Mir et al, 1991; Reintgen et al, 1996; Rudolf et al, 1995). At that time, groups from Villejuif and Toulouse in France, and groups in Tampa, USA and Ljubljana, Slovenia were involved in electrochemotherapy studies. Based on the first experience, a joint clinical paper was published, summarizing clinical results (Mir et al, 1998). The results of the joint study indicated that electrochemotherapy with bleomycin in patients, given either intravenously or intratumorally, is feasible, effective and without side effects. The treatment was performed on skin tumor nodules originating from different malignant tumors; however predominant tumor type was malignant melanoma and squamous cell carcinoma. Observed were 85% objective responses, with high percentage (56%) of

long lasting complete responses. Thereafter, other groups reported on effectiveness of electrochemotherapy with bleomycin, with similar results as published in the joint study (Burian et al, 2003; Gehl et al, 2000; Heller et al, 1998; Kubota et al, 1998; Panje et al, 1998; Rodriguez et al, 2001; Rols et al, 2000; Sersa et al, 2000b) (**Table 1**).

Our group published the first clinical data on electrochemotherapy with intratumorally injected cisplatin in 1998 and thereafter with intravenous injection (Rebersek et al, 2000; Sersa et al, 1998; 1999c, 2000c, d).

The study with intravenously given cisplatin was designed to verify whether electroporation of tumors in patients with progressive disease of malignant melanoma can increase antitumor effectiveness of a standard cisplatin based chemotherapy protocol (Sersa et al, 2000d). Good antitumor effectiveness was observed, but not very high percentage of objective responses, predominantly because big tumor nodules were included, where electrochemotherapy was less effective (**Table 2**). However, the study demonstrated that electrochemotherapy could be used as an adjunct to systemic ongoing cisplatin treatment, predominantly in patients in whom antitumor effectiveness needs to be potentiated locally.

Compared to electrochemotherapy with intratumorally injected cisplatin, electrochemotherapy with intravenously injected cisplatin was less effective (**Table 2**). Electrochemotherapy with intratumorally injected cisplatin was equally or more effective compared to electrochemotherapy with bleomycin given intratumorally (Glass et al, 1996b, 1997; Heller et al,

1998; Mir et al. 1998; Sersa et al, 1998, 1999c, 2000c). Furthermore, the data obtained on electrochemotherapy with cisplatin demonstrated that when cisplatin was given intravenously it was less effective compared to electrochemotherapy with bleomycin given either intravenously or intratumorally (Glass et al, 1996a, b, 1997; Heller et al, 1998; Mir et al, 1998; Sersa et al, 2000d).

Studies on electrochemotherapy with intratumorally injected cisplatin were carried out on patients with squamous cell carcinoma of the neck, basal cell carcinoma and adenocarcinoma of the breast and tubae, however the predominant group was 10 patients with malignant melanoma (Sersa et al, 1998, 2000c). The results on malignant melanoma patients proved that electrochemotherapy with cisplatin is effective in controlling local tumor growth, and that it has a much higher probability for local tumor control than intratumoral cisplatin injection (78% and 19%, respectively) (Sersa et al, 2000c). **Table 2** summarizes results of these studies. Long lasting complete responses of the treated nodules up to two years were induced, without scarring of the tissue and good cosmetic effect. Nodules that were bigger than the distance between the electrodes were treated by consecutive application of electric pulses to the tumor nodules, until the whole tumor area was covered in one or in consecutive sessions. If the tumors regrew it was possible to retreat the nodules in the next session with equal effectiveness.

Table 1. Results of clinical studies on electrochemotherapy with bleomycin, given intravenously or intratumorally.

Tumor	No. Pts.	No. Tumors	OR (%)	CR (%)
Intravenous				
BLM dose: 10-15 mg/m² or 18-27 U/m²				
Head and neck squamous cell carcinoma	17	77	62	43
Malignant melanoma	14	94	89	34
Basal cell carcinoma	2	6	100	17
Adenocarcinoma (breast, salivary gland, hypernephroma)	4	31	100	97
<i>Total</i>	<i>37</i>	<i>208</i>	<i>62-100</i>	<i>17-97</i>
Intratumoral				
BLM dose: 0.2-0.55 mg/cm³ or 0,25-1.0 U/cm³				
Head and neck tumors	14	14	86	50
Squamous, adeno and adenid cystic carcinoma				
Malignant melanoma	11	106	95	60
Squamous cell carcinoma	1	1	100	0
Kaposi sarcoma	1	4	100	100
Breast cancer	2	14	100	58
Bladder; trans. cell ca.	1	17	100	82
Total	25	116	80-100	0-100

Table 2. Results of clinical studies on electrochemotherapy with cisplatin, given intravenously or intratumorally.

Tumor	No. Pts.	No. Tumors	OR (%)	CR (%)
Intravenous				
Cisplatin based chemotherapy protocol				
Malignant melanoma	9	27	48	11
Intratumoral				
Cisplatin 1mg/cm³				
Head and neck squamous ca.	1	2	100	100
Malignant melanoma	10	82	78	68
Basal cell carcinoma	1	4	100	100
Adenocarcinoma (breast, ovary)	2	6	100	78
Total	14	94	78-100	68-100

Many of the patients were treated as out-patients, since they tolerated the treatment well. The patients described the sensation of applied electric pulses as painful. But, the pain dissipated immediately after application of electric pulses and it could be alleviated by xylocaine. Nevertheless none of the patients demanded to stop the treatment, or refused the treatment in the next session.

IV. Our experience with electrochemotherapy with cisplatin from 2000 to 2002

After some of the initial studies that have compared the effectiveness of electrochemotherapy with cisplatin to antitumor effectiveness of cisplatin only given intratumorally, we initiated another clinical study that had three goals:

ñTo use electrochemotherapy with cisplatin given intratumorally to treat patients with progressive disease in order to alleviate side effects to the patients.

ñTo re-evaluate the effectiveness of electrochemotherapy on similar group of patients as in the previous study (Sersa et al, 2000c).

ñTo gain experience in order to be able to further optimize treatment procedure based on assessment of advantages and drawbacks of electrochemotherapy. The study was performed on 14 patients with progressive disease of malignant melanoma. The enrolled patients had local recurrent disease and all standard treatments had been exhausted. Before enrolment, the patients were informed about the principles and procedure of the treatment, and signed an informed consent.

The treatment was performed on outpatient basis, without any pre or post medication, or need for hospitalization after the treatment. Before, and in regular intervals after the treatment, tumor nodules were measured and photographed. The treatment was performed by intratumoral injection of cisplatin using hypodermic needle. The dose of cisplatin was app. 1mg/cm³ of the

tumor. In the case of bigger nodules cisplatin was injected in several different locations in the tumor area in order to obtain better distribution of the drug. Cumulative dose was adjusted to the size of the tumor nodule. Intratumoral injection was in most cases successful, without leakage from the tumor. Electric pulses were applied first with custom-made plate electrodes, later with IGEA s.r.l. (Carpi, Modena) made plate electrodes. The distance between the electrodes was 4 or 7 mm. Electric pulses generator Jouan GHT 1287 (Jouan Saint Herblaine, France) was used, which delivered 8 electric pulses, amplitude/distance ratio 1300 V/cm, 100 µs long, with frequency 1 Hz. In order to assure good contact between the electrodes and the skin, ultrasonographic paste was used (**Figure 1**).

Tumor nodules that were treated were of varying size, from 4 mm up to 3 cm in diameter. Electric pulses were delivered in two sets of four pulses in perpendicular direction with 1-second pause in-between. Nodules that were bigger than the distance between the electrodes were treated by consecutive application of electric pulses to the tumor nodules until the whole tumor area was covered. Immediate effects of the treatment were marks of the electrodes on the skin that disappeared after few minutes, and unpleasant sensation, predominantly caused by muscle contractions. The pain was bearable, therefore patients did not require special pain control, and the pain dissipated immediately after application of electric pulses.

The patients were regularly checked for the response to the treatment in 2-4 weeks intervals. Some tumors needed retreatment. If the tumors were big, retreatment was needed every 2-4 weeks in order to eradicate the whole tumor mass. In the case of tumor regrowth after complete response, retreatment was performed at the time of tumor progression. The observation time of the patients varied, depending on the time of inclusion into the study, from few weeks to up to one and a half years. In **Table 3** are listed the patients that were treated in this study. The number of lesions that were treated in the patient, number of the consecutive treatment sessions, response to the

treatment, and observation time are indicated. In most cases the response to the treatment after 4 weeks was partial or complete regression of the treated nodules (objective response: 82%). New electrochemotherapy sessions were needed in order to treat tumor nodules that regrew in the time between the sessions or to treat new tumor nodules that emerged between the two visits. Therefore, in some patients up to 13 consecutive sessions were needed in order to control tumor growth locoregionally. In some cases electrochemotherapy was

effective in controlling growth of specific nodules, however patient's disease progressed to other sites.

Results of the treatment in these 14 patients are in accordance with our previously published results. In our previous study on 10 malignant melanoma patients, 82 tumor nodules were treated with electrochemotherapy with intratumorally injected cisplatin; 78% of the treated nodules were in objective response, from these 68% were in complete response.

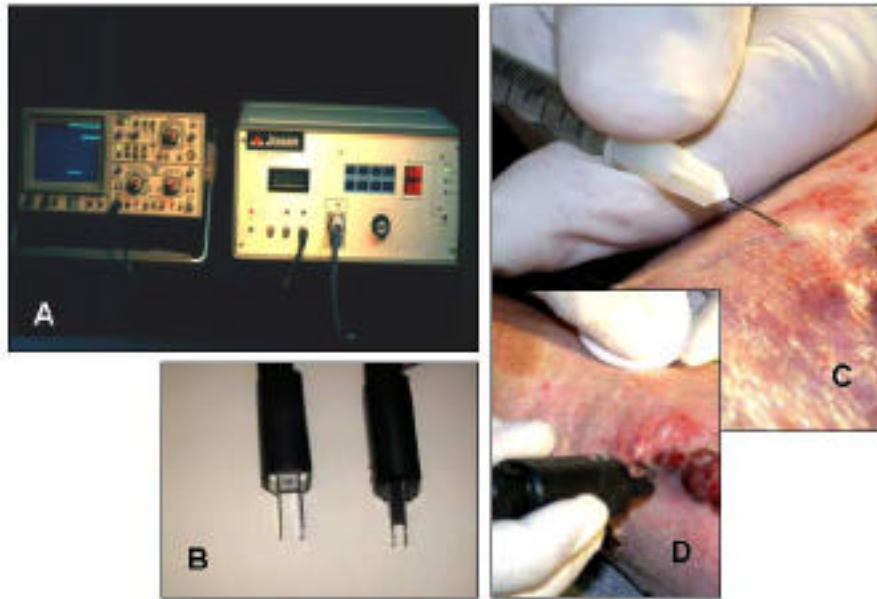


Figure 1. Electrochemotherapy treatment procedure on the patient. A.– Electric pulses generator with oscilloscope for the control of the applied electric pulses. B. – two sets of electrodes with 4 mm and 7 mm distance between the plate electrodes. C. – Intratumoral injection of cisplatin in tumor nodule. Noticeable is whitening of the tumor area in cases with successful drug administration. D. – Application of electric pulses to the tumors by placing the electrodes to the tumor, possibly embracing the tumor mass between the electrodes for better electric field distribution.

Table 3. Summary of electrochemotherapy with cisplatin in malignant melanoma patients; in the years 2000-2002.

Treatment Patients	No. of treated lesions	No. of treatment sessions	Response to treatment				Observation time (weeks)
			PD	NC	PR	CR	
No. 1	6	1			1	5	4 - 8
No. 2	8	4				8	64 - 66
No. 3	3	2	3				7
No. 4	2	2	1		1		6
No. 5	5	2				5	16 - 18
No. 6	7	3	3			4	2 - 6
No. 7	18	5	8	1	4	5	2 - 15
No. 8	26	11	1	1		24	6 - 36
No. 9	34	7				34	42
No. 10	21	9				21	3 - 33
No. 11	19	5		1		18	7 - 57
No. 12	16	3				16	4 - 54
No. 13	1	1			1		2
No. 14	45	9		21	16	8	2 - 21
Total	211	64	16	24	23	148	2 - 66
			7.6%	11.4%	10.9%	70.1%	median - 13

Only 7% of nodules were in progressive disease and 15% in no change (Sersa et al, 2000c). Therefore, we can conclude that the described protocol for electrochemotherapy is effective and reproducible, confirmed on two groups of patients in two separate clinical studies. However, we have gained additional experience that we can summarize in two categories: advantages and drawbacks that will be discussed in the next two subheadings.

A. Advantages of electrochemotherapy

Concerning the treatment procedure, electrochemotherapy is easy and quick to perform, and is inexpensive. The requirements are a suitable room for patient preparation and treatment, and an electric pulse generator with different sets of electrodes that are used for different sizes of tumor nodules. After the treatment, patients do not require special attention or post-treatment medication. They can wait for a while in the hospital in order to be in the position to obtain medical attention, if needed, but so far no side effects were observed or medical attention of the patients required. Concerning the personnel, a M.D. in charge, a nurse and an assistant trained in handling the electric pulses generator are required to perform the treatment.

Electrochemotherapy with cisplatin was successful in controlling the growth of the treated nodules. Tumors regressed in most cases within 4-6 weeks, when superficial scab fell off. Good cosmetic effect was observed, with light depigmentation of the skin (Figure 2). During regression of smaller tumor nodules there was no exulceration, therefore no special wound dressing was required, and also no extra visits to the supervising oncologist. Most of the tumor nodules that were up to 1 cm in diameter regressed completely after single treatment, and remained in complete response for a long period of time, the longest that could be followed was 66 weeks, almost 1.5 year. In one treatment session it was feasible to treat up to 15 tumor nodules. It was possible to retreat tumor nodules that did not show typical signs of regression or progressed within 2-4 weeks after therapy. On bigger tumor nodules, it was possible to control tumor growth or reduce the size of the nodules by consecutive treatments in 2- 4 weeks interval (Figure 3).

The treatment can be performed on any part of the body. In our study most of the tumor lesions were located on the limbs. However we have treated also tumor nodules that were located on the thorax, stomach, back and head and neck region. The only experience that was demanding abrogation of the treatment was in a patient that was treated in the early beginning of our studies.



Figure 2. Example of good local tumor control in a patient with two tumor nodules on the leg. The first tumor nodule (No. 1) was treated once and tumor regressed, after one year there is no recurrence and good cosmetic effect. The second tumor nodule (No. 2) was treated three times in a two-month interval and each time good response was obtained although the tumor in the intervals grew substantially.

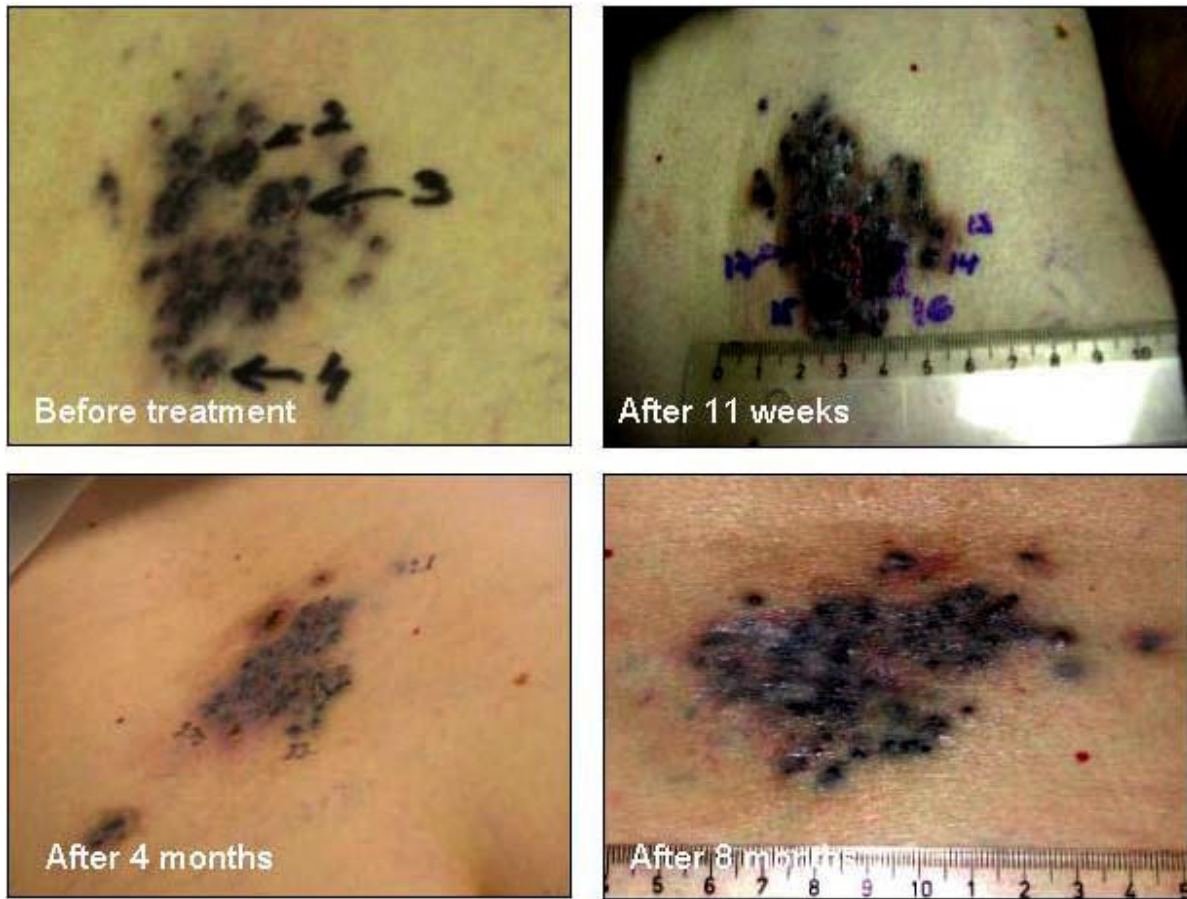


Figure 3. Retreatment of the nodules can provide good local tumor control. A plaque on the back of the malignant melanoma patient was treated by electrochemotherapy with cisplatin in 9 consecutive sessions in 2 to 4 weeks intervals. After 8 months, good local tumor growth was obtained.

A patient had a tumor nodule on the back in the region of the diaphragm. During application of the electric pulses, spasm of the diaphragm occurred and breathing was interrupted. After the abrogation of the treatment the patient recovered within a few minutes.

B. Disadvantages of electrochemotherapy

Besides the advantages, there are also some disadvantages of electrochemotherapy. Pain is a limiting factor in most of the patients. Pain can be avoided by lifting the treated tumor nodule while applying electric pulses. In addition, it was observed that patients that were obese had less sensation, because adipose tissue prevented electric field distribution deeper into the underlying tissue, therefore less muscle contractions were observed. There was also a difference in sensations between the electrodes that had smaller gap (4 mm) than those that had bigger gap (7 mm), because electrodes with smaller gap required lower electric field intensity for electroporation of the tissue.

Electrochemotherapy is local treatment that can be effective in treatment of limited number of tumor lesions that are not bigger than 3 cm in diameter. Therefore, it can be effective in those patients that have few or up to 15 skin metastases in transit. In the case of more nodules electrochemotherapy cannot be performed on all nodules

in one session. Electrochemotherapy is however effective on those nodules that were treated, but has no effect on the general progression of the disease. Furthermore, because of occasional quick progression of the disease, new nodules emerge, that were not detectable in previous sessions. Electrochemotherapy can be performed on these new nodules, and taken collectively it can be effective in local control of the disease, but cannot affect general progression of the disease.

Currently, the electrodes that are used are effective in treatment of superficial nodules, whereas they are not quite appropriate for deeper seeded or big nodules. Bigger nodules need application of several sets of electric pulses, and also several treatment sessions, in order to cover the whole tumor area and to be able to remove deeper layers of the tumor. The problems have to be solved, if electrochemotherapy is to be applied to the treatment of nodules that are more than 3 cm in diameter and thicker than 0.5 cm. This issue has been already addressed in the studies performed in Tampa and Villejuif, where they used needle electrodes (Gilbert et al, 1997; Mir et al, 1997).

V. Conclusion

Electrochemotherapy cannot be the only biomedical application of tissue electroporation. It has to be envisioned as the first step toward a broader use of

electroporation in clinical use, predominantly in electrogene therapy and transdermal drug delivery (Jaroszeski et al, 2000b).

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