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review

Electrochemotherapy of tumours

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Electrochemotherapy consists of chemotherapy followed by local application of electric pulses to the tumour to increase drug delivery into cells. Drug uptake can be increased by electroporation for only those drugs whose transport through the plasma membrane is impeded. Among many drugs that have been tested so far, only bleomycin and cisplatin found their way from preclinical testing to clinical trials. In vitro studies demonstrated several fold increase of their cytotoxicity after electroporation of cells. In vivo, electroporation of tumours after local or systemic administration of either of the drugs, i.e. electrochemotherapy, proved to be an effective antitumour treatment. In preclinical studies on several tumour models, electrochemotherapy either with bleomycin or cisplatin was elaborated and parameters for effective local tumour control were determined. In veterinary medicine, electrochemotherapy also proved to be effective in the treatment of primary tumours in cats, dogs and horses. In human clinical studies, electrochemotherapy was performed on the patients with progressive disease and accessible tumour nodules of different malignancies. All clinical studies demonstrated that electrochemotherapy is an effective treatment for local tumour control in cancer patients.

Key words: neoplasms – drug therapy; electroporation; electrochemotherapy, drug delivery systems; bleomycin; cisplatin,

Introduction

Treatments for cancer may be divided into different categories based on their goals and mode of action. Very often the different types of treatment are used in combination, either simultaneously or sequentially. In general, cancer treatment includes three main treatment modalities, surgery and radiation, which are local treatment modalities and chemotherapy which is a systemic treatment modality.

Chemotherapy, a systemic treatment modality for cancer is effective if the drugs that have intracellular targets readily pass the plasma membrane. However, among highly cytotoxic chemotherapeutic drugs there are some whose transport through the plasma membrane is hampered. These drugs are good candidates for electroche-

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motherapy. Electrochemotherapy is a local treatment combining chemotherapy and application of electric pulses to the tumour, thus increasing plasma membrane permeability. In electrochemotherapy, the optimal antitumour effectiveness is achieved when electric pulses are given at the time of the highest extracellular concentration of hydrophilic chemotherapeutic drug in the tumour, thereby increasing their transport through the plasma membrane towards their intracellular targets.[1-4]

Preclinical data

In vitro studies

Electroporation proved to be effective in facilitating the transport of different molecules across the plasma membrane. Different biochemical and pharmacological studies on chemotherapeutic drug transport facilitated by means of electroporation, report that the increased intracellular drug accumulation improves the cytotoxicity of the drug. Since electroporation can facilitate the drug transport through the cell membrane only for poorly or non-permeant molecules, suitable candidates for electrochemotherapy are limited to those drugs that are hydrophilic and lack transport system in the membrane. Several chemotherapeutic drugs were tested *in vitro* on cells for potential application in combination with electroporation; some of them are daunorubicin, doxorubicin, etoposide, paclitaxel, actinomycin D, adriamycin, mitomycin C, 5-fluorouracil, vinblastine, vincristine, gemcitabine, cyclophosphamide, carboplatin, cisplatin and bleomycin. Electroporation of cells increases the cytotoxicity of some of these drugs ranging from 1.1 to up to several thousand fold. However, only two of these drugs have been identified as potential candidates for electrochemotherapy of cancer patients.[1,2,4] The first is bleomycin; it is hydrophilic and has very restricted transport capacity through the cell membrane, thus its cytotoxicity can be potentiated up to several 1000 fold by electroporation of cells. Few hundred internalized molecules of bleomycin are sufficient to kill the cell. [1,2,5] The second is cisplatin whose transport through the cell membrane is also hampered. Only 50% of cisplatin is transported through the plasma membrane by passive diffusion, the rest is transported by carrier molecules. The overall flux across the plasma membrane is thus limited. Electroporation of the plasma membrane enables greater flux and accumulation of the drug in the cells which results in increase of cisplatin cytotoxicity by up to 80-fold.[3-6] These promising preclinical data obtained *in vitro* on a number of different cell lines have paved the way for testing these two drugs in electrochemotherapy *in vivo* on different tumour models.

In vivo studies

Bleomycin and cisplatin were tested in electrochemotherapy protocol on a number of animal models *in vivo* (Figure 1). Extensive studies on different animal models with different tumours, either transplantable or spontaneous were performed. Antitumour effectiveness of electrochemotherapy was demonstrated on tumours in mice, rats, hamsters, cats and rabbits. Tumours treated by electrochemotherapy were either subcutaneous, grew in the muscle, brain or in the liver, and were of different types, *e.g.* sarcomas, carcinomas, glioma or melanoma.[1,2,4]

In these studies, different factors controlling antitumour effectiveness were determined:

- The drugs can be given by different *routes of administration*, they can be injected either intravenously or intratumourally. The prerequisite is that, at the time of the application of electric pulses to the tumour, a sufficient

amount of drug is present in the tumour. Therefore, after intravenous drug administration into small laboratory animals (4 mg/kg of cisplatin or 0.5 mg/kg bleomycin), only a few minutes' interval is needed to reach the maximal drug concentration in the tumours. After intratumoural administration, this interval is even shorter and the application of electric pulses has to follow the administration of the drug as soon as possible (within a minute). [1,2,4]

- Good antitumour effectiveness may be achieved by good tissue electroporation. The plasma membrane electroporation is obtained if the cell is exposed to a sufficiently high electric field. This depends on the *electric field distribution in the tissue* which is controlled by the geometry of electrodes and tissue. The electric field distribution in the tissue and cell electroporation can be improved by rotating electric field. Surface tumours can be effectively treated by plate electrodes, whereas appropriate electric field distribution in deeper parts of the tumour is assured by using needle electrodes.[7-11]
- The antitumour effectiveness is dependent on the *amplitude, number and duration of the electric pulses applied*. Several studies in which parallel plate electrodes were used for surface tumours showed that an amplitude over distance ratio above 1000 V/cm is needed for tumour electroporation, and that above 1500 V/cm, irreversible changes in the normal tissues adjacent to the tumour occur; so, the window for effective and safe electrochemotherapy is between 1000 -1500 V/cm. in most studies the amplitude over distance ratio of 1300 V/cm induced good antitumour effectiveness without sub-optimal electroporation of the tissue or damage to the tissue due to irreversible cell permeabilization.[7] For other types of electrodes, the electric field distribution and thus also the necessary amplitude of electric pulses need to be determined by numerical calculations.[10] *Repetition frequencies of the pulses* for electrochemotherapy are either 1 Hz or 5 kHz. The minimal number of the pulses used is 4; most studies use 8 electric pulses of 100 μs.[11,12]

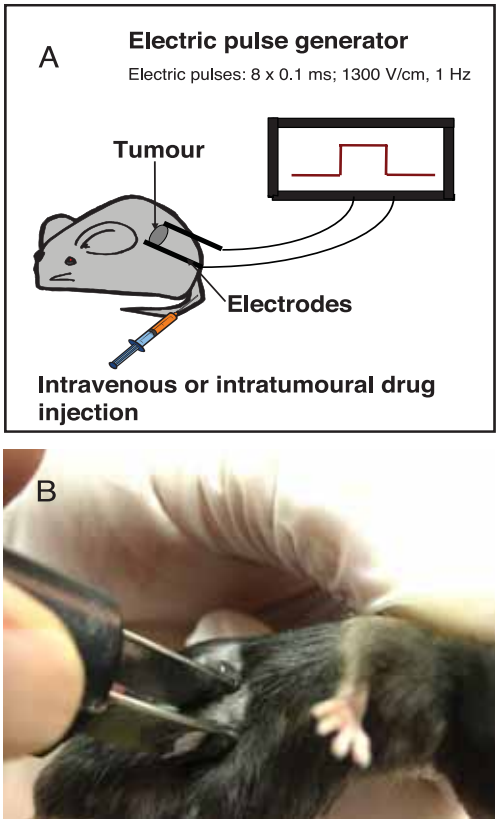

Figure 1. Protocol of electrochemotherapy of experimental tumours presented schematically (A). The drug is injected either intravenously or intratumorally, at the doses that do not exert antitumour effect. After the interval that allows sufficient drug accumulation in the tumours, electric pulses are applied to the tumour either by plate or needle electrodes (1300 V/cm, 100 μs, 1 Hz or 5 kHz, 8 pulses). The plate electrodes are placed in that way that the whole tumour is encompassed between the electrodes, providing good electric field distribution in the tumours for an optimal electroporation of the cells in the tumours (B).

All the experiments conducted *in vivo* on animals provided sufficient data to demonstrate that electrochemotherapy with either bleomycin or cisplatin is effective in the treatment of solid tumours, using drug concentrations which without application of electric pulses have no or minimal antitumour effect. Already a one-time treatment by electrochemotherapy induces partial or complete regression of tumours, whereas the treatment with bleomycin or cisplatin alone or application of electric pulses alone has no or minimal antitumour effect (Figure 2).

Mechanisms of action

The principal mechanism of electrochemotherapy is *electroporation* of the cells in the tumours, which increases the drug effectiveness by enabling the drugs to reach its intracellular targets. This was demonstrated in the studies that measured the intratumoural drug accumulation and the amount of the drug bound to DNA. Basically, the amounts of bleomycin and cisplatin in the electroporated tumours were up to 2-4 fold higher than in those without application of electric pulses.[13,14,15]

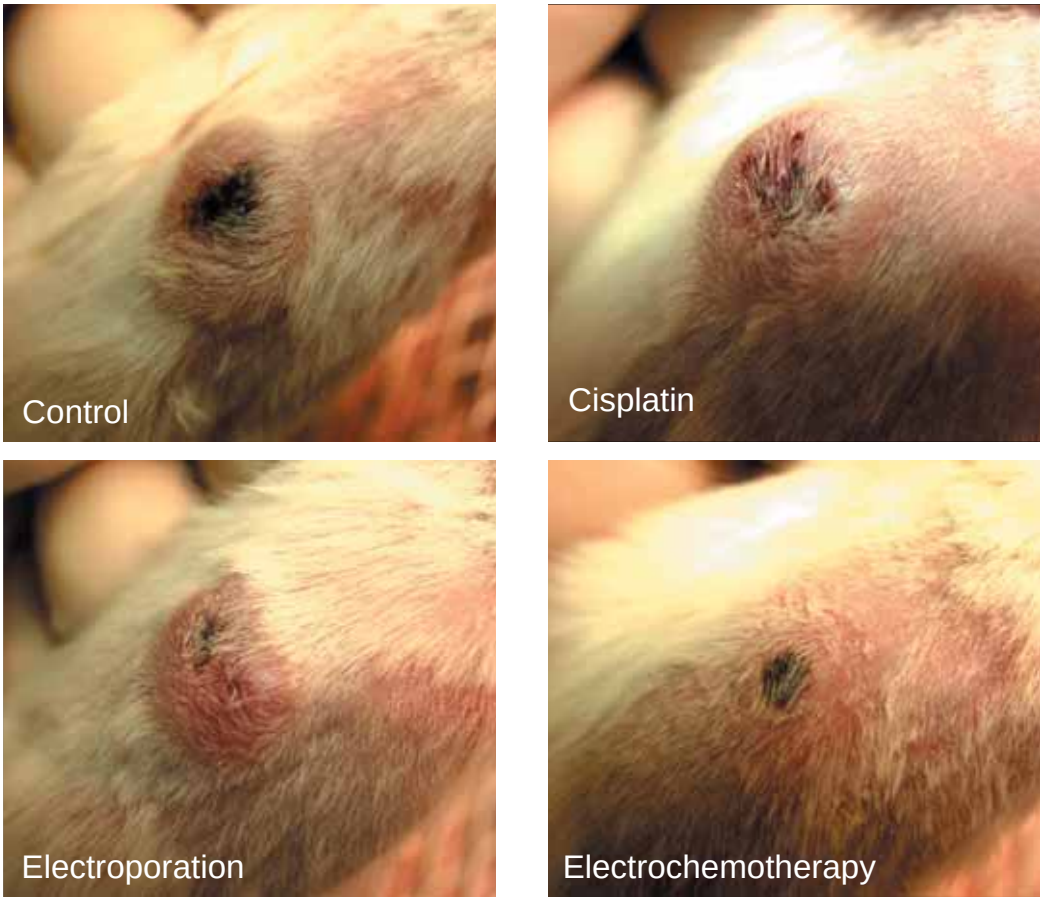

Figure 2. Example of good antitumour effectiveness of electrochemotherapy with cisplatin on SA-1 tumours. Cisplatin was given intravenously (4 mg/kg), 3 min thereafter 8 electric pulses were applied to the tumour with plate electrodes. Electric pulses were applied in two directions; 4 pulses in one and the other 4 in the perpendicular direction. Eight days after the treatment, good antitumour effectiveness of electrochemotherapy with cisplatin is evident, compared to the treatments with cisplatin or electric pulses alone.

Besides membrane electroporation, which facilitates drug transport and its accumulation in the cell, other mechanisms that are involved in antitumour effectiveness of electrochemotherapy were described. The application of electric pulses to the tissues induces a transient, and reversible *reduction of blood flow*.[16] The restoration of the blood flow in normal tissue is much faster than of that in tumours.[17] The decrease in tumour blood flow induces *drug entrapment* in the tissue, providing more time for the drug to act. Besides, this phenomenon prevents bleeding from the tissue, which is important in clinical situations of haemorrhagic tumours.[17]

The cytotoxic effect of electrochemotherapy is not limited only to tumour cells in the tumours. Electrochemotherapy acts also on stromal cells, including endothelial cells in the lining of tumour blood vessels.[5] This represents yet another mechanism involved in the antitumour effectiveness of electrochemotherapy, i.e. *vascular disrupting effect.*[18]

The difference in antitumour effectiveness of electrochemotherapy was observed between immunocompetent and immunodeficient experimental animals, indicating to the involvement of *immune response* in antitumour effectiveness.[19,20] Due to the massive tumour antigen shedding in the organisms after electrochemotherapy, systemic immunity can be induced, and upregulated by additional treatment with biological response modifiers like IL-2, GM-CSF and TNF-α.[21-23]

To sum up, the electrochemotherapy protocol was optimized in preclinical studies *in vitro* and *in vivo*, and basic mechanisms were elucidated. In addition to the electroporation of cells, the tumour drug entrapment, vascular disrupting effect and involvement of immune response were also demonstrated. Based on all these data, electrochemotherapy with bleomycin and cisplatin was promptly evaluated in clinical trials.

Other biomedical applications of electroporation and electrochemotherapy in cancer treatment

Knowledge about the mechanisms involved in the antitumour effectiveness of electrochemotherapy opened new possibilities for the application of electric pulses or electrochemotherapy in the treatment of cancer.

The chemotherapeutic drugs that increase effectiveness of radiation therapy are radiosensitizing drugs. Among them are also bleomycin and cisplatin. Since drug delivery induced by electroporation is site-specific, it could be used for tumour-specific delivery of radiosensitizing drugs. By the increased radiosensitizing drug delivery into the tumours and not in the surrounding normal tissue the therapeutic index of tumour irradiation is increased. In our recent studies, we combined electrochemotherapy either with bleomycin or cisplatin with radiotherapy and demonstrated a good potentiation of tumour radiation response: 1.9 fold for electrochemotherapy with bleomycin and 1.6 fold for electrochemotherapy with cisplatin.[24-26]

The application of electric pulses was shown to modulate the tumour blood flow. Both, reduced blood flow and lowered partial oxygen pressure (pO_2) in the tumours are consequences of the applied electric pulses.[18] The reduced pO_2 can activate bioreductive drugs to exhibit cytotoxic effect on hypoxic cells.[27] In well oxygenated cells, the drug remains inactive. On the other hand, tumour hypoxia induced by application of electric pulses can improve therapeutic conditions for the use of hyperthermia since tumour cells are more sensitive to heat in sub-optimal physiological conditions.[28]

Electrochemotherapy with cisplatin or bleomycin was successfully used also in the veterinary medicine. It was used to treat different tumours, such as mammary adenocarcinoma, fibrosarcoma, cutaneous

mast cell tumour, hemangioma, hemangiosarcoma, perianal tumours, neurofibroma and sarcoids in dogs, cats, hamsters, rabbits and horses.[29-33] Recent reports demonstrated a successful treatment of different neoplasms in companion animals and sarcoids in horses.[30-33] Hopefully, electrochemotherapy will be broadly used in veterinary medicine for the treatment of different malignancies, both primary and metastatic disease.

Electrochemotherapy is an effective cytoreductive treatment; however, its curative effect is dependent on the permeabilisation of possibly all cells in the tumours. Since permeabilisation of every single cell in the tumour is virtually impossible, electrochemotherapy could be combined with other cytoreductive treatments. Another approach is a combination of electrochemotherapy with electrogene therapy. The first promising reports and data are already available, supporting the effectiveness of this concept. [23,34]

In conclusion, the electroporation in electrochemotherapy has already been very well exploited; however, there are new biomedical applications of electroporation in cancer treatment that still need testing and development.

Clinical studies on electrochemotherapy

The first clinical study on electrochemotherapy was published in 1991, reporting good treatment effectiveness of electrochemotherapy on cutaneous tumour nodules of head and neck tumours.[35] The results of this study by the group from the Institute Gustave Roussy, have stimulated other groups to initiate their own clinical studies. The first clinical centres which performed electrochemotherapy were Villejuif and Toulouse in France, the group in Tampa in USA, and our group at the Institute of Oncology Ljubljana in Slovenia. Recently, also new centres reported clinical experience on electrochemotherapy, *e.g.* Copenhagen in Denmark, Mexico City in Mexico, Chicago in USA, Vienna in Austria, Matsumoto and Jamagata in Japan, Sydney in Australia and Cork in Ireland.[35-63]

In all clinical studies, 247 patients were included; 202 patients with 655 tumour nodules were treated by electrochemotherapy with bleomycin and 45 patients with 354 tumour nodules were treated by electrochemotherapy with cisplatin. The majority were malignant melanoma patients, and also the patients with metastases in head and neck region, mammary carcinoma, skin cancer, ovarian cancer, Kaposi sarcoma and chondrosarcoma were treated by electrochemotherapy. The results of the studies can be summarized as supporting the assumption that electrochemotherapy has good antitumour effectiveness either using bleomycin or cisplatin, resulting in ~80% objective responses of the treated tumour nodules.[3,60]

Based on these results, the European project that was aimed at developing and producing electric pulses generator was launched. In the CLINIPORATOR project, this electric pulses generator was developed and is now commercially available for those who would like to perform electrochemotherapy. This generator under the same name as the project - CLINIPORATOR™ (IGEA S.r.l., Carpi, Italy) is certified as a medical devices and is therefore appropriate for clinical use. Along with the development of the electric pulse generator, also plate and needle electrodes were developed (Figure 3).

The next step was to gather clinical experience of four cancer centres in Villejuif, Copenhagen, Cork and Ljubljana and prepare Standard Operating Procedures (SOP) of electrochemotherapy. This was a prerequisite step to bring electrochemotherapy into standard clinical practice. SOP is now completed and the drug licensing for electrochemotherapy in process, so electroche-

Figure 3. CLINIPORATOR™, an electric pulse generator for clinical use in electrochemotherapy. For application of electric pulses plate and needle electrodes were developed.

motherapy can be used as standard procedure for local tumour treatment.

Treatment procedures for electrochemotherapy

Treatment advantages and clinical uses

Electrochemotherapy is used for the treatment of cutaneous and subcutaneous tumour nodules of different malignancies. The treatment advantages and clinical uses for electrochemotherapy can be summarized:

- ❖ easy and effective treatment of single or multiple tumour nodules of any histology in the cutaneous and subcutaneous tissue,[3,60]
- ❖ treatment that improves quality of life of patients with progressive disease,[3,60]
- ❖ treatment of choice for tumours refractory to conventional treatments,[3,60]
- ❖ neoadjuvant treatment in form of cytoreductive therapy before conventional treatment,[62]
- ❖ organ sparing and function saving treatment,[62,63]
- ❖ treatment of hemorrhagic or painful nodules, since it reduces bleeding and in some cases pain level.[50,62]

Treatment procedure

The treatment procedure is as follows: based on SOP, tumour nodules can be treated by electrochemotherapy with injection of bleomycin intravenously or intratumourally and by electrochemotherapy with cisplatin given intratumourally. The choice of the chemotherapeutic drug in not based on tumour histology, but depends on the number and size of the nodules. After drug injection the tumour nodules are exposed to electric pulses. The interval between the intravenous drug injection and application of electric pulses is 8-28 min, and after the intratumoural injection, as soon as possible. Different sets of electrodes are available for application; plate electrodes for smaller tumour nodules and needle electrodes for the treatment of larger (3 cm) and thicker tumour nodules. The treatment can be performed in one-session or can be repeated in case of new emerging nodules or on those nodules that relapsed in some regions not well treated in the first treatment.

Electrochemotherapy does not induce side effects due to chemotherapeutic drugs since the drug dosage is very low. However, the application of electric pulses to the tumours induces contraction of the underlying muscles. For electroporation, square wave electric pulses of the amplitude over distance ration of 1000-1300 V/cm, duration of 100 μs, frequency 1 Hz or 5 kHz are used. These muscle contractions are painful, but the pain dissipates immediately after electric pulses application. Nevertheless, in SOP, the procedures for alleviating the pain by local anaesthesia

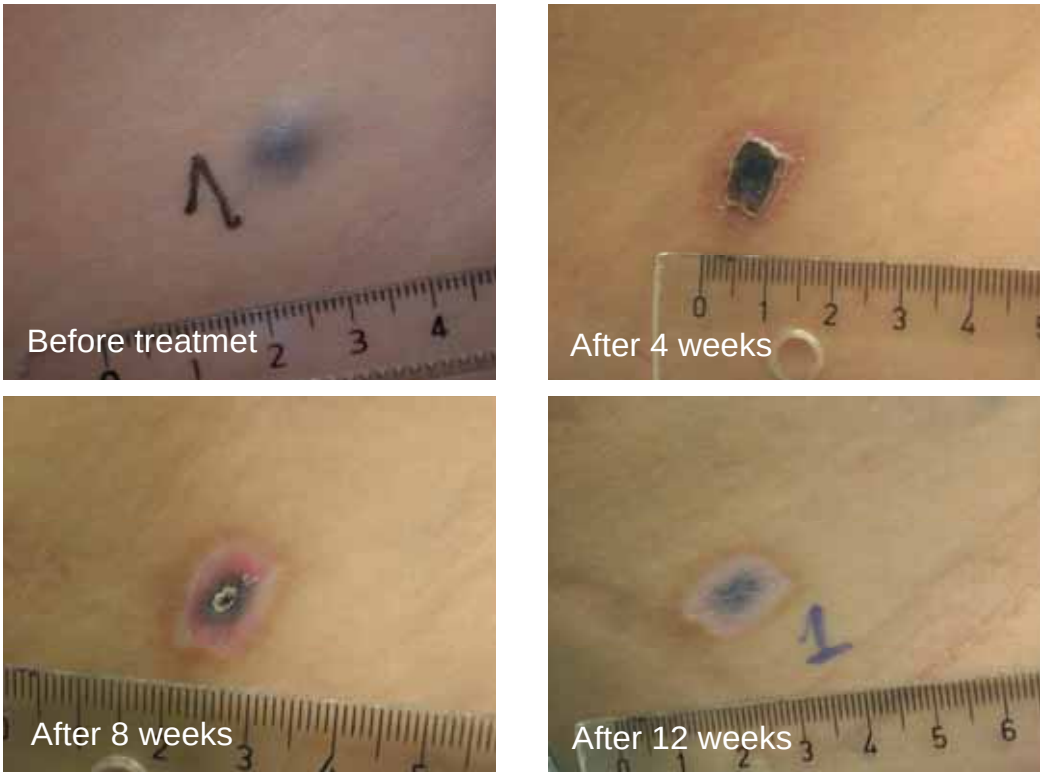

Figure 4. Antitumour effectiveness of electrochemotherapy with intratumoural injection of cisplatin in a subcutaneous metastasis of the patient with malignant melanoma. Twelve weeks after the treatment the tumour nodule was in complete response (CR), with pigmentation and good cosmetic effect.

or by general anaesthesia in case of treating multiple nodules are also described.

Treatment effectiveness

The treatment after single electrochemotherapy session results in most cases in complete tumour eradication. When necessary, treatment can be repeated at 4-8 weeks intervals with equal antitumour effectiveness. The treatment has a good cosmetic effect without scaring the treated tissue (Figure 4,5).

Conclusion

Electrochemotherapy is now on the verge being standard treatment in palliative treatment of cutaneous and subcutaneous tumour nodules of different malignancies. However, further progress of electrochemotherapy will continue by developing new electrodes that will enable the treatment of larger tumours and tumours in internal organs. Consequently, the indications for electrochemotherapy may be extended.

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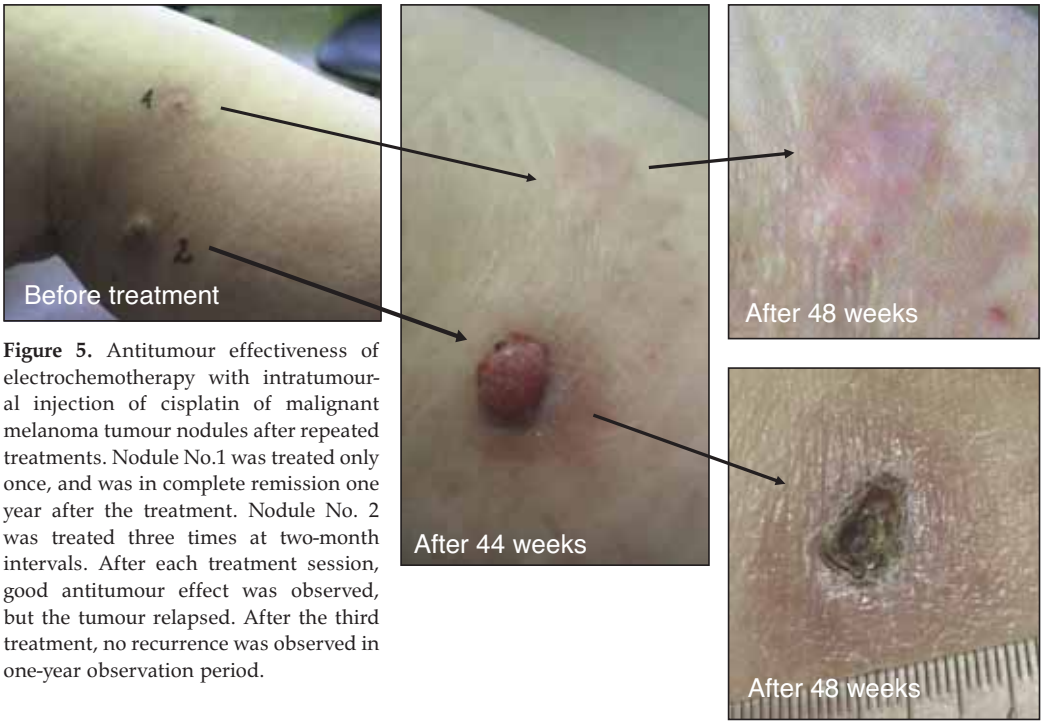

Figure 5. Antitumour effectiveness of electrochemotherapy with intratumoural injection of cisplatin of malignant melanoma tumour nodules after repeated treatments. Nodule No.1 was treated only once, and was in complete remission one year after the treatment. Nodule No. 2 was treated three times at two-month intervals. After each treatment session, good antitumour effect was observed, but the tumour relapsed. After the third treatment, no recurrence was observed in one-year observation period.

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Kardiotoksičnost kemoterapije. Nove rešitve starega problema

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Izhodišča. Kardiotoksičnost, ki jo povzroča kemoterapija ima raznolike zgodnje in kasne oblike. Zmanjšuje možnost učinkovitega zdravljenja z namenom ozdravitve pa tudi paliativnega zdravljenja. Onkološka zdravila, ki jih najpogosteje povezujemo s kadiotoksičnostjo so antraciklini, trastuzumab, 5-flurouracil in taksani. Nekatere oblike kardiotoksičnosti, ki jih lahko povzroča večina protitumorskih zdravil, pa avtorji redko opisujejo in navajajo. Velika verjetnost je, da bo širša uporaba novih bioloških zdravil privedla do odkritja drugih manj poznanih stranskih pojavov.
Zaključki. Ker srce razvrščamo med organe z omejeno regeneracijsko sposobnostjo, je pomembno, da poznamo incidenco, klinično sliko in patogene mehanizme, ki so povezani s stranskimi učinki zdravil na srce. To nam lahko pomaga pri ugotavljanju, prevenciji in zdravljenju kardiotoksičnosti, ki jo povzroča kemoterapija. Ob novih načinih zdravljenja so nujno potrebne še nadaljnje raziskave.

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Elektrokemoterapija tumorjev

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Elektrokemoterapija je način zdravljenja raka, ki združuje uporabo standardnih kemoterapevtikov in aplikacijo električnih pulzov na območje tumorja. Z aplikacijo električnih pulzov na tumor povzročimo destabilizacijo celične membrane, s čimer omogočimo, da citostatiki, ki imajo slabo prehajanje skozi membrano, lažje vstopajo v celico. Tako se večkrat poveča citotoksičnost citostatikov, kot sta cisplatin ali bleomicin, s tem pa se poveča tudi njihova protitumorska učinkovitost, posebno na mestu aplikacije električnih pulzov. Zaradi selektivno povečanega vnosa samo na območju tumorja je terapevtski indeks elektrokemoterapije zelo dober, dobra je namreč lokalna protitumorska učinkovitost brez lokalnih ali sistemskih stranskih pojavov, zaradi kemoterapevtikov ali aplikacije električnih pulzov. Po številnih predkliničnih raziskavah je bila elektrokemoterapija preizkušena tudi v mnogih kliničnih raziskavah. V veterinarski onkologiji je bila uspešnost elektrokemoterapije dokazana pri zdravljenju različnih primarnih tumorjev mačk, psov in konjev.V humani onkologiji se je elektrokemoterapija izkazala pri zdravljenju kožnih in podkožnih tumorjev pri bolnikih z napredovalo boleznijo različnih vrst rakov. Rezultati vseh teh študij dokazujejo uspešnost elektrokemoterapije v onkologiji za pri lokalnem nadzoru rasti kožnih in podkožnih lezij različnih vrst raka.

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