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Electrochemotherapy of solid tumors – preclinical and clinical experience

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Abstract— Electrochemotherapy consists of administration of the chemotherapeutic drug followed by application of electric pulses to the tumor, in order to facilitate the drug uptake into the cells. Only two chemotherapeutics are currently used in electrochemotherapy, bleomycin and cisplatin, which both have hampered transport through the plasma membrane without electroporation of tumors. Based on extensive preclinical studies, elaborating on parameters for effective tumor treatment and elucidating the mechanisms of this therapy, electrochemotherapy is now in clinical use. It is in standard treatment of melanoma cutaneous metastases in Europe. However it is effective also for cutaneous metastases of other tumor types. Currently the technology is being developed also for treatment of bigger, deep seated tumors. With long needle electrodes and new electric pulse generators, clinical trials are on-going for treatment of liver metastases, bone metastases and soft tissue sarcomas.

I. Introduction

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

II. Pre-clinical data on electrochemotherapy

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. However, only two of these drugs have been identified as potential candidates for electrochemotherapy of cancer patients [2, 3]. The first is bleomycin; it is hydrophilic and has very restricted transport capacity through the cell membrane, but its cytotoxicity can be potentiated up to several 1000 fold by electroporation of cells. Several hundred internalized molecules of bleomycin are needed to kill the cell [2]. 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 the cisplatin cytotoxicity increase by up to 80-fold [4]. These promising preclinical data obtained *in vitro* on a number of different cell lines set the stage for testing these two drugs in electrochemotherapy *in vivo* on different tumor models.

Extensive *in vivo* studies of electrochemotherapy were performed on different animal tumor types, either transplantable or spontaneous; on fibrosarcomas, melanoma, and carcinomas in mice, rats and rabbits [5, 6]. Furthermore, good clinical results were obtained in veterinary medicine on cats, dogs and horses [7]. In these studies drug administration route (intravenous or intratumoral), drug dosage, timing of its administration and electrical parameters were elaborated. It was determined that the drugs may be injected either intravenously or intratumorally and electric pulses applied to the tumors within a few minutes thereafter (8 electric pulses; amplitude over distance 1300 V/cm, duration 100 μs; frequency 1 Hz). In these studies it was demonstrated that with drug doses that have minimal or no antitumor effectiveness, high (up to 75%) complete responses of the electrochemotherapy-treated tumors were obtained on different tumor types. In addition, the drug doses used were so low that they had no systemic toxicity. Furthermore, the application of electric pulses to the tumors had no antitumor effectiveness and no systemic side effects.

Besides the principal mechanism of increased drug accumulation in tumor cells, other mechanisms are also involved in the antitumor effectiveness of electrochemotherapy that may substantially contribute to overall treatment response. By application of electric pulses to the tumors up to four-fold increase in bleomycin or cisplatin accumulation in tumors was observed [5, 8]. However, electroporation of tumors does not affect only tumor cells, it can electroporate also stromal cells in the tumors, like endothelial cells. In relation to this,

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electrochemotherapy induces abrogation of tumor blood flow due to cytotoxic effect on endothelial cells. Besides this, application of electric pulses induces vasoconstriction, contributing to drug entrapment in the tumors. Both of these vascular effects result in vascular disrupting effect of electrochemotherapy [9].

Immune response is also involved in overall antitumor effectiveness of electrochemotherapy. Based on the differential effectiveness in immunocompetent and immunodefficient mice, it was presumed that elicited immune response by shedding of antigens from the destructed tumor cells, may contribute to complete eradication of the last remaining tumor cells and lead to complete responses of the tumors [10].

III. Clinical studies on electrochemotherapy

The first clinical study was published in 1991 on head and neck tumor nodules, [11] which was thereafter followed by several other studies that have demonstrated good local tumor control of electrochemotherapy in melanoma skin metastases and also on skin metastases of breast cancer, basal cell carcinoma, hypernephroma, ovarian cancer, Kaposi's sarcoma, urogenital carcinoma and Merkel cell cancer [12-15]. The hallmark of introduction of electrochemotherapy into clinical practice was clinical European Standard Operating Procedures on electrochemotherapy (ESOPE) study published in 2006 [16]. In this study procedures for electrochemotherapy were standardized according to the bleomycin or cisplatin selection and dosing, route of their administration (intratumoral or intravenous), the anesthesia used (local or systemic) and electric pulses protocol according to the electrodes used (plate, needle row or hexagonal) and frequency of applied pulses (1Hz or 5kHz). Based on these standardized procedures Standard Operating Procedures (SOP) for electrochemotherapy were prepared, for electric pulses generator Cliniporator™ (IGEA, Italy), that was developed for clinical use [17]. The four cancer centers involved in the study have, based on these SOP, conducted a clinical trial. Results of this trial confirmed the previously published clinical results. Namely the study has demonstrated an objective response (OR) obtained in 145 of the treated nodules (84.8%), with 11.1% being partial responses and 73.7% complete responses (CR) after a single treatment. Only in a very small number of cases a negative response was observed, with either no response in 10.5% or progressive disease in 4.7% of cases. Predominant tumor type treated was melanoma. The study showed no statistical difference in local tumor control at 150 days after the treatment (median follow-up was 133 days and range 60-380 days) between bleomycin given intravenously (88% OR) or intratumorally (73% OR), or cisplatin given intratumorally (75% OR).

The ESOPE study set the stage for introduction of electrochemotherapy in Europe. After the encouraging results of the ESOPE study, several cancer centers have started to use electrochemotherapy and reported the results of their studies. Collectively, the results were again similar as reported in the ESOPE study. However some advances in the treatment were reported. Predominantly it was reported that tumors bigger than 3 cm in diameter can be successfully treated by electrochemotherapy in successive chemotherapy sessions [18, 19], and that electrochemotherapy provides a benefit to patients especially in quality of life [18], because electrochemotherapy is nowadays used predominantly in palliative intent.

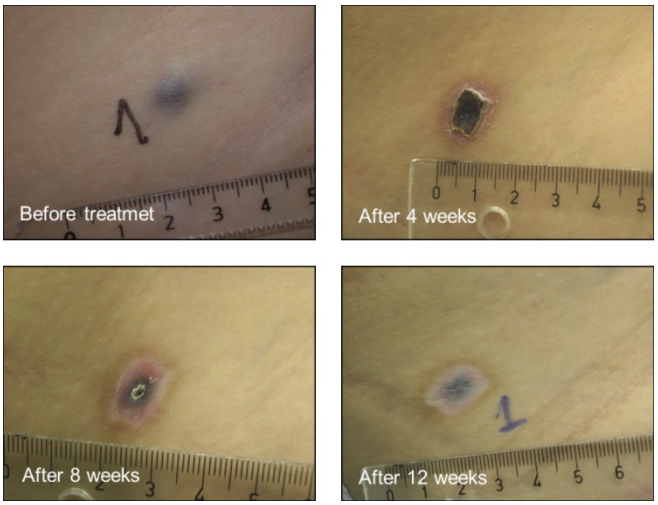

Fig. 1. Antitumor effectiveness of electrochemotherapy with intratumoral injection of cisplatin on a subcutaneous metastasis of the patient with malignant melanoma. Twelve weeks after the treatment the tumor nodule was in complete response (CR), with pigmentation and good cosmetic effect. With permission from Radiol Oncol [21].

Based on all these reports, electrochemotherapy has been recognized as treatment option for disseminated cutaneous disease in melanoma, and accepted in many national and also international guidelines for treatment of melanoma [20]. Treatment advantages and clinical use for electrochemotherapy can be summarized as follows:

- Effective in treatment of tumors of different histology in the cutaneous or subcutaneous tissue.
- Palliative treatment with improvement of patient's quality of life.
- Treatment of choice for tumors refractory to conventional treatments.
- Cytoreductive treatment before surgical resection in an organ sparing effect.
- Treatment of bleeding metastases.

In summary, electrochemotherapy has been recognized as a valid treatment approach; over 60 cancer centers have

started to use it and have reported positive results. So far the effectiveness of the therapy is on case based evidence and further controlled and randomized studies are needed for the translation of this technology into broader and standard clinical practice.

IV. New Clinical Applications of Electrochemotherapy

Based on clinical experience that electrochemotherapy can be effectively used in treatment of cancer with different histology, when appropriately executed, the treatment could be used also for treatment of deep seated tumors. Prerequisite for that is further development of the technology in order to reach and effectively treat the tumors located either in the muscle, liver, bone, esophagus, rectum, brain or other internal organs.

The first steps in technological development have already been made. For example, there is already the first report in treatment of melanoma metastasis in the muscle, 2 cm under the skin. With long needle electrodes and new electric pulses generator Cliniporator Vitae™ it was possible to treat this deep seated metastasis 2 x 1.4 cm in diameter [22]. Further development of such electrodes enabled treatment of liver metastases. At the Institute of Oncology Ljubljana, Slovenia a clinical trial was launched, where liver metastases of colorectal tumors are treated and effectiveness evaluated at the two stage operation (NCT01264952). So far 6 patients were enrolled. No immediate or late side-effects of electrochemotherapy were observed [23]. The delivery of electric pulses during open surgery was synchronized with ECG in order to avoid possible arrhythmias. Specific treatment plan is prepared for the treatment, in order to predict the exact location of the electrodes for sufficient coverage of the tumors with the electric field in the tumor and in the safety margins of the tumor [24].

Similar technology is being used in treatment of bone metastases or soft tissue sarcoma. The tumors are similarly as in treatment of liver metastases punctured by long needle electrodes, so that electrodes are placed around and in the tumors. The clinical trials are still ongoing but according to the preliminary report at the First Users meeting of Electrochemotherapy in Bologna Italy (2010) the technology is feasible, safe and also effective.

Another approach that is in development is the use of endoluminal electrodes for the treatment of tumors in esophagus or in rectum. The first reports demonstrate that the technology is available, and was tested also in dogs. The translation of this technology into the human clinics is underway; the clinical trial for the treatment of unresectable colon tumors is ongoing [25].

The last but not least also electrodes for treatment of brain tumors are developed [26]. They will enable treatment of brain tumors, minimally invasively. The clinical trial is being prepared and will soon start enrolling patients.

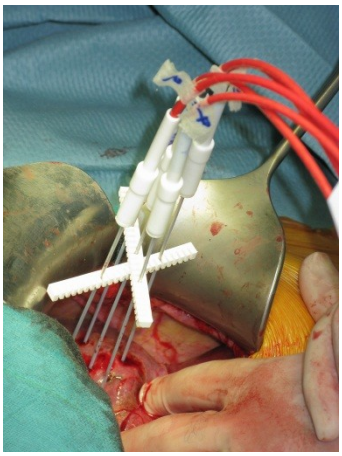

Fig. 2. Electrochemotherapy of liver metastasis. Electrodes were inserted into the tumor and around the tumor in healthy liver tissue and connected to electric pulse generator. Electric pulses were delivered between the pairs of electrodes according to the treatment plan.

V. Conclusion

Electrochemotherapy is one of the biomedical applications of electroporation. Its development has reached clinical application and is an example of successful translational medicine. However its development is not finished yet; new technical developments will certainly enable further clinical uses and eventually clinical benefit for the patients. Another application of electroporation is still awaiting such translation, electrogene therapy. In relation to this, first clinical results are encouraging, but standard clinical use is still far away.

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