Electrochemotherapy and Its Clinical Applications

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Abstract

Electrochemotherapy has grown out of simple cell experiments through preclinical studies to experimental clinical studies, to an accepted treatment modality for cutaneous metastases for any type of cancer. Electrochemotherapy has had a dynamic spread from few initial centers using custom-made electrodes to the current situation where standard operating procedures and clinically approved equipment are being used in many cancer centers. Treatment results have been remarkably consistent, possibly due to a robustness of the technology, and helped by the availability of clinically approved equipment and the early definition and adoption of standard operating procedures. Based on successful treatment of cutaneous metastases, a number of efforts have been initiated to expand treatment to tumors in internal organs. This requires development of specialized equipment, as well as clinical trials. The first of these trials are now published showing very promising results, and many further studies are ongoing. Finally a number of other avenues are being explored, including new drugs to be used, advanced use of imaging techniques, as well as combination with other treatments.

Keywords

Electrochemotherapy • Clinical studies • SOP • Clinical indications • Technical development

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Introduction

The first mention of the use of electric pulses to permeabilize cell membranes in biology dates back to the late 1970s (Kinosita and Tsong 1977), followed by a groundbreaking paper describing gene transfer by the use of electroporation in 1982 (Neumann et al. 1982). A second wave of the use of electric pulses for permeabilization came with the first papers describing increased uptake of chemotherapeutic agents (Preclinical Studies on Electrochemotherapy: Sersa, G., Bosnjak, M., Cemazar, M., Heller, R.) in cells exposed to permeabilizing electric pulses (Okino and Mohri 1987; Orlowski et al. 1988), showing that in particular the effect of the drug bleomycin could be enhanced several hundred fold. Bleomycin is a known chemotherapeutic agent, which has been shown to act as an enzyme creating 5–10 double-strand breaks in DNA per molecule internalized into the cell, yet this drug is hydrophilic and charged, with limited internalization into the cells without permeabilization of the membrane (Gothelf et al. 2003). The further expansion of this work into animal models confirmed the activity of bleomycin combined with electroporation. Simultaneously, the work trying to help patients with this new treatment entity ensued, and in 1993 (Belehradek et al. 1993), the first results on patients with cutaneous metastases of head and neck cancer were published, using a standard intravenous dose of bleomycin combined with electric pulses to the cutaneous metastases. Also around this time, electrochemotherapy using bleomycin by intratumoral delivery was being developed for the treatment of primary skin cancers (Glass et al. 1996), as well as cutaneous metastases of malignant melanoma (Heller et al. 1996; Gehl and Geertsen 2000) (Fig. 1).

Along with the use of bleomycin, also cisplatin was demonstrated to be effective in electrochemotherapy, based on extensive preclinical studies demonstrating its effectiveness, either by intravenous or intratumoral administration. The clinical data obtained demonstrated its effectiveness on melanoma metastases as well as on various other tumor types (Sersa et al. 2008).

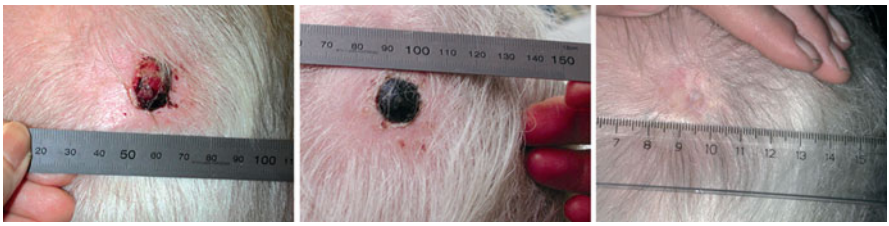

Fig. 1 Course of treatment for patient with malignant melanoma metastases, treated with intravenous bleomycin and type III electrodes, having an array of needle electrodes fixed on a holder in a hexagonal pattern, under general anesthesia. One of eight treated metastases is shown. Before treatment the metastasis was ulcerated and caused hemorrhage, pain, and discomfort. One month after treatment, the lesion was covered by a crust. Around the crust, needle marks in normal tissue are visible, because the tumor was covered including a margin of normal tissue. Whereas the tumor area became necrotic, the normal tissue was very little affected, indicating a therapeutic window. Six months after treatment, the treated nodule was in CR. The crust fell off after 10 weeks, revealing normal skin that had healed underneath the nodule (From Gehl, Ugeskrift for Laeger 2005, with permission)

At this time it became evident that (1) electrochemotherapy was highly efficient in cutaneous tumors, but also (2) that there was a need for commercialization to allow purchasable clinically approved equipment, and (3) that the different methods used in the initial studies needed to be tested in a larger setting in order to obtain standard operating procedures. In other words, electrochemotherapy needed to move from custom-made equipment in small investigator-initiated trials to a technology-driven clinical acceptance of the technology.

To this end, the ESOPE (European Standard Operating Procedures on Electrochemotherapy) was formed as a consortium supported by the European Commission. Here, four European cancer centers gathered and defined a way to examine standard operating procedures through experiences obtained by a common clinical trial (Marty et al. 2006), where different electrodes (hexagonal, linear array, plate electrodes), two drugs (bleomycin and cisplatin), and different methods of anesthesia (general anesthesia, local anesthesia, surface analgesia) were tested and results in terms of tumor response and patient-reported toxicity were measured. At the end of this study, the first set of standard operating procedures were defined (Mir et al. 2006). This first manual for electrochemotherapy is extensively quoted and used and made a very important contribution to the dissemination of electrochemotherapy.

In 2013, the National Institute of Health and Care Excellence (NICE) of the UK released a guidance (National Institute for Health and Care Excellence 2013) stating that "There is sufficient evidence of efficacy of electrochemotherapy for treating metastases in the skin from tumors of non-skin origin and melanoma to support its use as a palliative treatment. There are no major safety concerns." This 2013 paper marked the 20-year anniversary since the publication of the first clinical trial. In this period, electrochemotherapy has progressed from a purely investigational treatment performed with home-designed and produced equipment to a treatment that was recognized as important for patients with cutaneous metastases (Spratt et al. 2014).

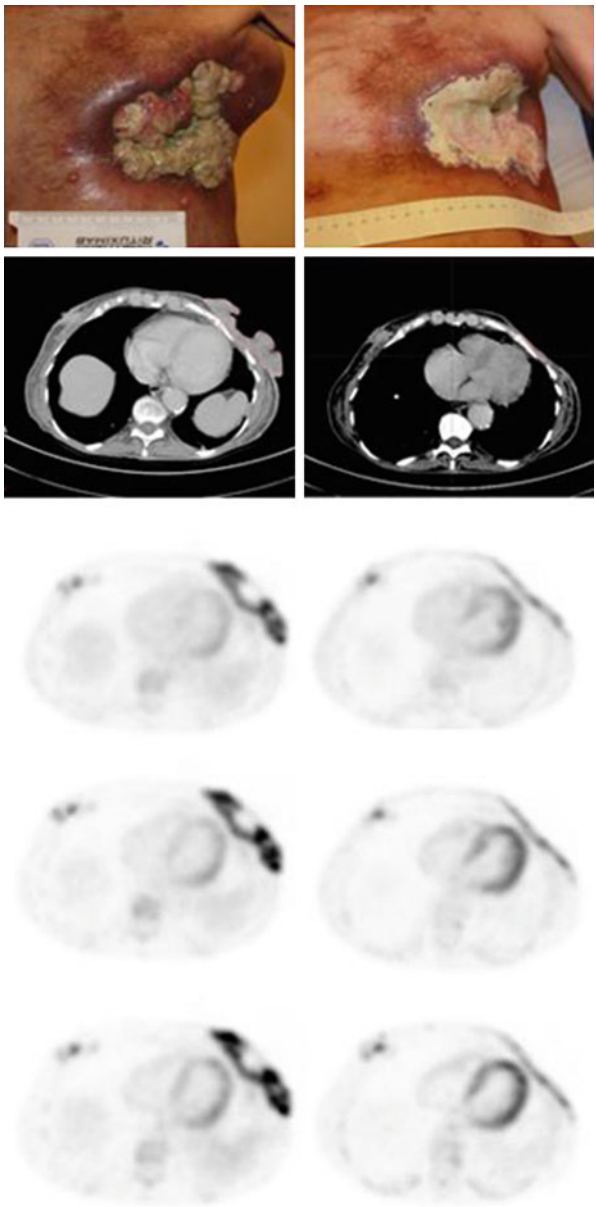

Fig. 2 Large recurrence with varying depth and inflammation on the left chest wall of a 68-year-old female. *Left column* shows from the top clinical presentation, CT-scan, PET scan at 60 min p.i., PET scan at 120 min p.i., and PET scan 180 min p.i. *Right column*. Same patients after one treatment. Change in SUVmax in target lesion at baseline compared to follow-up was 29.7% at 60 min p.i., 71.2% at 120 min p.i., and 83.1% at 180 min p.i. (From Matthiessen, Radiol Oncol, 2013, with permission)

Further developments have focused on transferring the success obtained in the treatment of cutaneous metastases to tumors in internal organs (Miklavcic et al. 2012). And this is a challenge in different ways; on the one hand, there is great benefit from the development of clinical-grade equipment, as well as extensive clinical experience; on the other hand, all tissues and all presentations of metastatic

lesions are different, and this poses new challenges. To give an example, the challenge in treating bone metastases is the rigidity of the tissue, whereas soft tissue metastases in the liver may be accessed, but is situated just below the heart.

Other new developments include new drugs, such as calcium electroporation (▶ Chap. 85, "New Drugs for Electrochemotherapy with Emphasis on Calcium Electroporation": Frandsen, S., Gehl, J.), as discussed elsewhere in this book. Important advances also include an understanding of the use of imaging modalities such as MRI (▶ Chap. 41, "Diffusion Weighted Magnetic Resonance Imaging for Detection of Tissue Electroporation In Vivo": Mahmood, F.), and PET/CT Fig. 2, and CT-guided placement of electrodes (▶ Chap. 54, "Treatment Planning for Electrochemotherapy and Irreversible Electroporation of Deep-Seated Tumors": Kos, B.). Furthermore, combination with other treatments, such as immunotherapy (▶ Chap. 103, "Adjuvant Immunotherapy as a Tool to Boost Effectiveness of Electrochemotherapy": Kamensek, U., Kos, S., Sersa, G.), is currently being investigated.

This chapter describes the many aspects of clinical use of electrochemotherapy, as well as future avenues.

Electrochemotherapy as a Cancer Treatment

What Types of Cancer Can Be Treated?

Due to the dramatic enhancement of cytotoxicity that can be achieved, electrochemotherapy has been shown to work across a wide range of tumor histologies including malignant melanoma, breast cancer, colorectal cancer, planocellular carcinoma, basocellular carcinoma, and transitocellular carcinoma (Campana et al. 2014a). As electrochemotherapy is so highly efficient, most treatments are being given as a once-only treatment. A concern in chemotherapy is the buildup of drug resistance over time, but with electrochemotherapy there really is not much of chance of building secondary resistance as a function of treatment exposure.

There is however a different challenge; in order to treat the tumor efficiently, the whole tumor must be covered by the electric field, and the drug must be available in sufficient quantities. In other words, although all *types* of solid cancers could be treated, there is an anatomical restriction as the tumor in its entirety needs to be covered by the electric field. In this way, electrochemotherapy carries similarities to surgery – although electrochemotherapy may be used also in cases of inoperable cancer or metastases.

The Effect on Normal Tissues Relative to Malignant Tumors

Interestingly, there is a striking difference between normal tissue and tumor sensitivity to electrochemotherapy. As evidenced in Fig. 1, there is typically a rapid and

extensive effect on tumors, while normal tissue treated with the same drugs and electrical parameters in the perimeter of the tumor shows very limited response.

The scientific background for this difference is currently being investigated, and there are likely several factors involved in this differential response. Important factors include differential drug uptake in malignant and normal tissues as in the case of bleomycin, slower resealing of membranes in malignant than in normal cells allowing higher uptake in malignant cells, differences in sensitivity to in vivo electroporation, and differences in response of normal endothelial vasculature and tumor endothelial vasculature (▶ Chap. 39, "Blood Flow Modifying and Vascular-Disrupting Effects of Electroporation and Electrochemotherapy": Cemazar, M., Markelc, B.; ▶ Chap. 78, "Preclinical Studies on Electrochemotherapy": Sersa, G., Bosnjak, M., Cemazar, M., Heller, R.).

Some of these effects would be specific to electrochemotherapy, but most would be relevant for different electroporation technologies. Thus, selectivity in treatment of malignant tumors is also described for irreversible electroporation.

Principles of Electrochemotherapy

Electrochemotherapy is based on the principle that chemotherapeutic drug cytotoxicity is increased by exposure of cells to externally applied electric field, which permeabilizes the cell membrane and allows diffusion of the drug into the cells. The whole procedure is based on a few basic principles:

- The first principle is that with electrochemotherapy, only drugs that have impaired transport through the cell membrane can be used, such as bleomycin, cisplatin, and as recently introduced calcium (▶ Chap. 85, "New Drugs for Electrochemotherapy with Emphasis on Calcium Electroporation": Frandsen, S., Gehl, J.). Several preclinical studies confirm this principle at least for the clinically approved chemotherapeutics. These preclinical studies also unequivocally show that lipophilic drugs, which have unhampered access to cytosol, are not suitable for electrochemotherapy, and lack of its potentiation by electroporation was confirmed. The potentiation of bleomycin and cisplatin was demonstrated by the increased drug uptake in cells and tumors, as well as by the increased DNA binding, and several fold potentiation of cytotoxicity was demonstrated.
- The second overall principle is that the drug needs to be present in the tumor when electric pulses are being applied to the tumors. In the clinics two routes of drug administration are being used, intravenous and intratumoral, and in a few studies also the combination of both. The combination of both routes of drug administration is not well established and needs to be used with great care not to overdose the tumors. The time for drug to reach the entire tumor depends on the route of its administration, after intravenous approximately 8 min, whereas after intratumoral only after a few minutes. Due to the differences in the vascularization of different tumors, the drug uptake and distribution vary. Predominantly tumors in heavily

pretreated areas, by surgery, or radiotherapy, may have lower drug uptake, which may affect tumor response.

- The third principle is the necessity of coverage of the tumor with the adequate electric field in order to permeabilize most of the tumor cells (Miklavcic et al. 2006). In principle with adequate electric field distribution and the optimal drug distribution, complete response of the treated tumors is obtained. In principle based on numerical modeling of the electric field and based on the clinical experience for the commercially available electrodes with fixed geometry, the electric field is sufficient to permeabilize the area of the electrode used. When the tumors are bigger than the geometry of the electrodes, a repetitive application of electric pulses, with repositioning of the electrodes to different places of the tumor, can also achieve adequate coverage of the electric field. As will be discussed later on, for the single electrode positioning, a treatment plan is needed with electric field calculation. The choice of the electrode is predominantly dependent on the anatomical location of the tumors to be treated. Different sets of electrodes are available for the treatment of superficial tumors. These tumors may differ both in size and depth, and other considerations may also affect electrode choice including the anatomical position of the tumor on the body, e.g., in sensitive areas, and in the face, smaller electrodes should be chosen (Campana et al. 2014a).

Importance of the ESOPE Study

In 2006, standard operating procedures for electrochemotherapy (SOP) were published (Mir et al. 2006). The SOP were derived from the experience of the groups that have had the experience with electrochemotherapy and were adopted to at that time the only clinically certified electric pulse generator with electrodes with fixed geometry.

The SOP standardized electrochemotherapy concerning the drug dosage, administration route, and selection of the electrodes according to the size and the number of the tumor nodules to be treated. The SOP also took into account whether local or general anesthesia is needed. It provided an algorithm what to do in specific situations and how to proceed throughout the treatment, including also the necessary equipment and consumables.

With the generator, the clinicians had access to an instrument that helped perform the treatment. In the ESOPE study, a center without previous experience in electrochemotherapy took part and in the study provided the same success rate as the other already experienced centers. Furthermore, the clinical results that have followed the ESOPE study have provided very similar tumor control rate as the ESOPE study and the previous ones. This supports the view that electrochemotherapy is a robust technology, with a high likelihood of success in different settings.

The clinical study ESOPE published alongside the SOP was possible by the availability of the electric pulses generator that was designed and then produced and

certified for clinical use. The availability of this generator, in fact, enabled the fast spread of electrochemotherapy throughout Europe.

The ESOPE study was conducted in order to develop the new SOP using electric pulse generator and electrodes developed within Cliniporator project (QLK-1999-00484). It had an ambitious goal to compare the effectiveness of electrochemotherapy based on the different drugs used (bleomycin, cisplatin), on different cutaneous metastases, with different drug administrations (intravenous, intratumoral), with the use of different sets of electrodes. In spite of the different variables in the study, the ESOPE study has demonstrated that regardless of the tumor type treated or the drug or its administration route, the local tumor control probability was 73% (complete response rate) (Marty et al. 2006). This was the first nonrandomized, prospective multicenter study on electrochemotherapy with the standardized treatment protocol. Its value is that it was so convincing that many cancer centers throughout Europe started to use electrochemotherapy. Furthermore, they have obtained similar results as the ESOPE study and the studies before that (Sersa 2006). All these studies provided solid evidence on effectiveness and safety of electrochemotherapy of cutaneous tumors of different histology.

Recent Developments in Electrochemotherapy

The technology of electrochemotherapy has spread out through the Europe into more than 140 centers. This was possible based on the SOP that showed safety of the procedure, with no reported serious adverse effects.

After the ESOPE study, several case controlled studies demonstrated effectiveness of electrochemotherapy on different tumor histologies. The studies confirmed effectiveness of electrochemotherapy in breast cancer (Matthiessen et al. 2012; Campana et al. 2012) and malignant melanoma other than skin cancers, including basal cell carcinoma, squamous cell carcinoma of the skin, Merkel cell carcinoma, Kaposi's sarcoma as well as soft tissue sarcoma, squamous cell carcinoma of the head and neck, cutaneous metastases of adenocarcinoma from, e.g., colorectal cancer, as well as bladder cancer and renal cancer. The data provided evidence on the effectiveness of electrochemotherapy on different superficial tumor types. However prospective, controlled studies were needed in order to explore more in depth the clinical value of electrochemotherapy.

Such studies were possible by pooled data from different cancer centers. In order to do that, an InSPECT database (► Chap. 95, "International Network for Sharing Practices on Electrochemotherapy (InspECT): An Integrative Patients Treatment Consortium": Qualigno, P., Gehl, J.) was created. The database provided the basis of pooling the data on different tumor types treated in different cancer centers. The number of centers is steadily increasing and the number of patients in the database as well. The InSPECT consortium was then able to provide clinical evidence on the

treatment of different patients in larger cohort. The first paper provided evidence on the effectiveness of the different tumor types after ESOPE study (Matthiessen et al. 2012). However later on there were papers published on specific tumor types, for example, on chest wall breast cancer (▶ Chap. 111, "Electrochemotherapy for Breast Cancer": Campana, L., Matthissen, L.) recurrences and lately on cutaneous head and neck tumors (▶ Chap. 102, "Electrochemotherapy of Head and Neck Cancer": Benazzo, M., Bertino, G., Groselj, A.). Further details are given in respective chapters of this book (▶ Chap. 96, "Electrochemotherapy of Cutaneous Metastases": Snoj, M., Farricha, V., Matthissen, L.; ▶ Chap. 97, "Electrochemotherapy of Basal Cell Carcinoma": Clover, J.; ▶ Chap. 98, "Electrochemotherapy of Liver Tumors: Colorectal Liver Metastasis": Edhemovic, I.; ▶ Chap. 99, "Electrochemotherapy of Primary Liver Tumors": Trotovsek, B., Đokić, M.; ▶ Chap. 100, "Electrochemotherapy of Locally Advanced Pancreatic Cancer": Granata, V., Leongito, M., Fusco, R., Piccirillo, M., Palaia, R., Lastoria, S., Petrillo, A., Izzo, F.; ▶ Chap. 102, "Electrochemotherapy of Head and Neck Cancer": Benazzo, M., Bertino, G., Groselj, A.).

The other repository of the clinical data was established in Italy. More than 30 centers share the data and have published some interesting clinical reports. The first one was predominantly on melanoma and pointed out on the effectiveness of electrochemotherapy in repetitive treatment (Campana et al. 2009); the second one has included a variety of tumor types and represents currently the largest number of patients evaluated in one series (Campana et al. 2012). There is also a comprehensive report on sarcoma tumors, which were bigger in size and were a challenge for treatment with electrochemotherapy (Campana et al. 2014b).

Most of the papers have also touched upon the side effects and the quality of life of electrochemotherapy-treated patients. In a series from InSPECT database, it was reported that tumor especially preexisting pain before treatment, but also previous irradiation, tumor size, and location, can predict the posttreatment pain related to regression of tumors (Quaglino et al. 2015). Most of the recent studies have, however, also evaluated the patients' improvement of quality of life. It is a general conclusion that electrochemotherapy is well accepted by the patients and that the quality of life of these patients is significantly improved. Taken together, since electrochemotherapy is currently applied predominantly in patients with palliative intent, the benefit of the quality of life increase in combination with the effective local tumor control provides significant benefit to these patients.

Based on this evidence, the National Institute for Health and Care Excellence, NICE, recommended electrochemotherapy in treatment of cutaneous tumors and also primary basal cell carcinoma in specific clinical situations. Since comparative studies are currently underway and not yet published, a meta-analysis that was published recently has compared the effectiveness of electrochemotherapy to other local therapies of skin tumors. Based on the analysis, it is evident that electrochemotherapy is comparable to other local ablative techniques and radiotherapy, although the data did not allow for direct comparison (Spratt et al. 2014).

Prognostic Factors and Clinical Features

Due to its principles of action, electrochemotherapy should be effective on all tumor types. The use of powerful drugs like bleomycin and cisplatin that are effective on most types of tumors and the physical drug delivery system that should not be dependent on the type of the tumor cells enable that.

Tumor Type

The first studies, due to the low number of patients, could not detect a difference in response between different tumor types. However, through the meta-analysis and systematic review of all the data, some differences in responsiveness of tumors have been discerned (Mali et al. 2013a). Basal cell carcinoma tumors have been identified as the most responsive ones, with response rate close to 100%. Less responsive are the sarcomas and carcinomas, with 70% response rate, and even lower are melanoma metastases. Therefore when treating different types of tumors, one has to bear in mind that tumor type is a predictive factor and that tumors with lower response rate need to be retreated and more closely monitored for recurrences. Several underlying mechanisms may play a role, but notably basal cell carcinomas also respond better to, e.g., radiotherapy, than do melanomas. Recent data indicate that mutational status or adaptation to previous treatments like radiotherapy can be the intrinsic tumor cells' factors that are involved. Other factors that might be involved are the structure of tumor stroma and tumor vascularization, which might affect the drug distribution in the tumors and hamper adequate drug quantity and distribution in the tumors. And the third one is the electric field distribution that can be dependent on the size and also the tissue structure of the tumor, as well as the surrounding tissue. All these factors need to be explored in the future to refine electrochemotherapy for specific tumors.

Tumor Size

With the size of the treated tumor, the parameters needed for the treatment change. First the perfusion of the tumors is impaired and therefore also the drug distribution to all the tumor cells. With intermittent and chronic hypoxia, this is well explained. This holds true for intravenous drug administration. When the drug is administered into the tumors directly, bigger tumors are difficult to infiltrate entirely as well. So both routes have a drawback in tumors larger than 3 cm in diameter. The electric field distribution adequately throughout the bigger tumors is difficult as well. Either the electrodes are placed several times in order to cover the whole tumor, including the safety margin, or long needle electrodes need to be placed, which require the planning of the electric field distribution. All these factors are involved in the treatment outcome of bigger tumors. In the ESOPE trial, tumors of up to 3 cm in diameter were treated, however, due to the impressive results physicians stated

to treat also bigger tumors. Of course the response as measured in cutaneous tumors reflects both the treatment efficacy and the time of healing of the treated area which may be prolonged in large lesions, especially in previously irradiated areas.

The first analysis on the responsiveness of the tumors depending on the tumor size indicated that tumors bigger than 3 cm in diameter have lower response rate (Matthiessen et al. 2011) which was later confirmed/elaborated (Mali et al. 2013b). All the following studies have confirmed that tumors that are bigger than 3 cm have lower response rate, the latest study was on head and neck tumors (▶ Chap. 102, "Electrochemotherapy of Head and Neck Cancer": Benazzo, M., Bertino, G., Groselj, A.) (Bertino et al. 2016).

The question is how to approach this problem? First of all, this may be a reporting problem in that complete responses take a long time to achieve if the lesions have to heal completely and the lesions are large. But also factors involving the treatment can be discussed, including repeated treatment. The other approach would be to combine local and systemic drug administration, which has already been explored, but more evidence is needed for the appropriate drug dosage. The third approach would be to combine electrochemotherapy with other local or systemic treatments. This approach is being already explored with the new immunomodulatory systemic treatments.

Previous Treatments

As discussed above, previous treatments can affect the tumor response to electrochemotherapy. The recent study by Bertino et al. has clearly indicated a higher responsiveness of naïve tumors than ones that were previously treated by radiotherapy, systemic treatment, or both (Bertino et al. 2016). The difference is substantial; the naive tumors had a complete response rate of 70%, whereas the previously treated tumors had a complete response rate of 55%. Again, several reasons can contribute to the observed difference.

The first one is the immunological status of the organism. It is now known that with the progression of tumors, immunoediting selects the resistant clones of tumors that might have additional mutations than the naïve tumor cells. In addition, the immune response is modulated, and since it is blocked at several immune checkpoints, the immune response of the organism that is needed for the eradication of the last tumor cells for complete response is lacking.

The second is that the previous treatments have induced drug resistance or radioresistance in the remaining tumor cells, which also play a role in treatment with electrochemotherapy. The molecular mechanism involved in such cells that affect response to electrochemotherapy is just in the early phases of exploration. The first reports indicate that BRAF-mutated cells are also sensitive to electrochemotherapy and that the recurrent tumors after therapy with BRAF inhibitors as well (Valpione et al. 2015). In vitro data also indicate that concomitant electrochemotherapy and BRAF inhibitors treatment on BRAF-mutated cells may even

have a synergistic effect. Studies exploring the responsiveness of, for example, radioresistant cells or HPV-infected cells are already underway.

The third aspect is the modulation of tumor perfusion by previous treatments. It is well known the tumor bed effect of radiotherapy, where radiation therapy affects also the normal tissue perfusion, therefore recurrent tumors in pre-irradiated areas are less perfused, and in such case it seems to be more appropriate to give the drug intratumorally than intravenously.

Current Developments for Treatment of Deep-Seated Tumors

Electrochemotherapy is now well established in treatment of cutaneous tumors, but needs to be introduced also into the treatment of tumors located deeper in the body in order to be universal and to compete with other ablative techniques, such as radiofrequency ablation and irreversible electroporation.

In order to translate electrochemotherapy from cutaneous to deep-seated tumors, technological development needs to provide equipment first. Usually, deep-seated tumors are bigger, and therefore the current electrodes do not suffice. In order to approach this, e.g., single needle electrodes are used that are individually placed into the tumor and around it and then connected to the new pulse generator that is able to produce larger electric fields, due to the bigger distance between the electrodes. For certain indications, it is important to have synchronization of the pulse with the heartbeat to be within the refractory phase of the heartbeat in order to avoid heart fibrillation. This is important predominantly in treatment of tumors that are close to the heart, such as liver tumors (Edhemovic et al. 2014; Mali et al. 2015).

Besides this technological approach, there is also development of endoluminal electrodes for the treatment of colorectal tumors and esophageal tumors. The clinical studies in this domain are not yet published, but the first patients have already been treated. Therefore, for specific clinical situations also specific electrodes are needed, such as umbrellalike, for the cranial tumors, which have already been developed. Obviously the translation of electrochemotherapy into deep-seated tumors will have to go hand in hand with technological developments (Miklavcic et al. 2012).

Nevertheless some clinical studies have already reported successful and safe treatment of deep-seated tumors, like liver tumors, pancreatic tumors, bone metastases, and a case of deep-seated head and neck tumor.

The first clinical study in treatment of colorectal liver metastases has already been published. In the first 16 patients, it proved to be effective and safe. The procedure is based on long needle electrode insertion into and around the tumor and delivery of pulse in between the pairs of electrodes consecutively in order to cover the whole tumor and the safety margin. For the appropriate electrode placement and the electric field strength, treatment planning (► Chap. 54, “Treatment Planning for Electrochemotherapy and Irreversible Electroporation of Deep-Seated Tumors”: Kos, B.) is used. The results of this study are described in detail in another chapter; in brief, the study indicates that electrochemotherapy as nonthermal ablative technique is safe and effective even when close or between the major hepatic vessels, where

radiofrequency ablation is contraindicated. The study is continuing into phase II, where basically the same observations as in the phase I study are confirmed, but also on tumors that are bigger than 3 cm in diameter, up to 6–7 cm. Another study is ongoing on primary hepatocellular carcinoma tumors (▶ Chap. 99, "Electrochemotherapy of Primary Liver Tumors": Trotovsek, B., Đokić, M.), with also promising results.

Pancreatic cancer is difficult to treat with currently available treatments and with low success rate. An effective ablative technique is needed, and electrochemotherapy may provide that. The first clinical study has been published, which indicated predominantly on safety of the use of electrochemotherapy, with partial responses in tumors. The details of the study are elaborated in separate chapter (▶ Chap. 100, "Electrochemotherapy of Locally Advanced Pancreatic Cancer": Granata, V., Leongito, M., Fusco, R., Piccirillo, M., Palaia, R., Lastoria, S., Petrillo, A., Izzo, F.).

The long needle electrode approach could be useful also in treatment of bigger head and neck tumors (▶ Chap. 102, "Electrochemotherapy of Head and Neck Cancer": Benazzo, M., Bertino, G., Groselj, A.). The first report on that is published (Groselj et al. 2015). However in this body region, due to many sensitive structures, exact placement of electrodes is a prerequisite; therefore the navigation of the electrodes according to the treatment plan is needed. The technology is there and can be applied in the future studies.

Preparation of New Standard Operating Procedures and Introduction of Electrochemotherapy into Standard Clinical Practice

The SOP that were published were prepared with the first experience that the first centers had with electrochemotherapy. They were prepared for the treatment of tumors that are less than 3 cm in diameter and for single treatment. Nowadays, with accumulated knowledge and experience from many cancer centers, the technology has developed, and updated SOP is needed.

The centers with the most experience with electrochemotherapy have gathered, and the experts have discussed the new findings, in order to organize them into new SOP. The intent was to prepare more general guidelines, for treatment of also bigger tumors, and repetitive treatment as well. Issues like pain control, anesthesia, and drug administration are pointed out as well. In this respect, the new SOP are being prepared and will hopefully enable further spread of electrochemotherapy also outside of Europe, where it is already well established.

The introduction of electrochemotherapy is already underway; since with NICE guidelines, it is being introduced also into many national guidelines and disease-specific international guidelines. Further progress in the use of electrochemotherapy is expected pending randomized studies on the specific indications. In order to guide the authors, thorough reporting of data recommendations for the data reporting have already been published (Campana et al. 2016).

Another aspect is that electrochemotherapy needs to be introduced along the other standard treatments. This can be expected in several ways. Ongoing is the use of

electrochemotherapy as adjuvant treatment to other treatments, like neoadjuvant as tumor debulking, or as adjuvant to emerging immunotherapies. In this respect, electrochemotherapy can be seen as in situ vaccination (▶ Chap. 103, "Adjuvant Immunotherapy as a Tool to Boost Effectiveness of Electrochemotherapy": Kamensek, U., Kos, S., Sersa, G.) where immune checkpoint inhibitors would enhance the immune response elicited by electrochemotherapy. We can also look at it the other way around; electrochemotherapy might enhance or help targeted drugs such as the described recurrences after the treatment with BRAF inhibitors.

Other avenues seem to be feasible as well. The use of electroporation technology is used also for gene therapy. In this way, both drug and plasmids encoding immunomodulatory molecules can be introduced into and around the tumors. The approach seem to work well; the concept is sound and is proved in preclinical study in spontaneous tumors in dogs (▶ Chap. 106, "Electrochemotherapy and Gene Electrotransfer in Veterinary Oncology": Tamzali, Y., Cemazar, M., Tozon, N.). However, also other immunostimulatory approaches in combination with electrochemotherapy can be used.

Conclusions

Electroporation-based technology provides an effective and safe approach for drug or gene delivery. Electrochemotherapy is by far the most advanced one. In the two decades of its development, it has reached the point to be accepted as standard treatment modality, an ablative approach for cutaneous tumors. Continued refinement of the standard operating procedures and the technological development will help its spread, and also translation into the treatment of deep-seated tumors. However, there are some detailed data still missing that will explain subtle differences in tumor responses and bring the approach on higher level of evidence-based approach.

The new direction is to explore electrochemotherapy with the standard treatments either targeted therapies, immune checkpoint inhibitors, or immunostimulants. Another combined modality approach that also awaits clinical testing is radiosensitization of tumors (▶ Chap. 89, "Combined Treatment of Electrochemotherapy with Irradiation": Kranjc, S., Kamensek, U., Cemazar, M., Sersa, G.), where solid preclinical data already exist.

The development is relatively slow, but it has to be so, due to the necessity of solid preclinical and clinical data, extensive data analysis, and for the most of all, for the safety of its use in patients.

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