Review

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Electrochemotherapy in Veterinary Oncology

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Electropermeabilization is a method that uses electric field pulses to induce an electrically mediated reorganization of the plasma membrane of cells. Electrochemotherapy combines local or systemic administration of chemotherapeutic drugs such as bleomycin or cisplatin that have poor membrane permeability with electropermeabilization by direct application of electric pulses to the tumors. Preclinical studies have demonstrated excellent antitumor effectiveness of electrochemotherapy on different animal models and various tumor types, minimal toxicity, and safety of the procedure. Based on results of preclinical studies, clinical studies were conducted in human patients, which demonstrated pronounced antitumor effectiveness of electrochemotherapy with 80–85% objective responses of the treated cutaneous and SC tumors. Clinical studies in veterinary oncology have demonstrated that electrochemotherapy is very effective in the treatment of cutaneous and SC tumors of different histologic types in cats, dogs, and horses. The results of these studies have also demonstrated approximately 80% long-lasting objective responses of tumors treated by electrochemotherapy. Primary tumors of different histologic types were treated. Electrochemotherapy in veterinary oncology has future promise to be highly effective, and could be used to treat primary or recurrent solitary or multiple cutaneous and SC tumors of different histology or as an adjuvant treatment to surgery.

Key words: Companion animals; Electropermeabilization; Electroporation; Electropulsation; Horses.

Electropulsation is the direct delivery of electric pulses to cells. Under controlled conditions, it brings targeted permeabilization to the cell membrane (ie, electropermeabilization, electroporation).[1,2] This is true for cells not only in culture but also in vivo by direct electric field pulse delivery to the organ or across the skin of the animal. Electropermeabilization allows exogenous chemotherapeutic drugs to enter cells. It has, therefore, received considerable attention in the last 15 years as an emerging way to deliver chemotherapeutic agents into different types of tumors in vivo.[3–7] This treatment was named electrochemotherapy.[2] Clinical studies performed in veterinary medicine started soon after the beginning of the 1st clinical trials in human oncology. To date, approximately 15 papers have been published that describe electrochemotherapy in the treatment of dogs, cats, and horses.

Preclinical Studies on Electrochemotherapy

In Vitro Studies

Electropulsation of cells in culture, aimed at increasing the cytotoxicity of bleomycin, was first described by Orlowski et al.[8] Thereafter, the cytotoxicity of several other chemotherapeutic agents was tested in vitro on cells in combination with electropermeabilization. Cisplatin was shown to be very suitable for electrochemotherapy. Electropulsation of cells increased the cytotoxicity of bleomycin up to several 1,000-fold and the cytotoxicity of cisplatin up to 70-fold. The prerequisite for a drug to be effective in combination with electropulsation is that the drug cannot cross the cell membrane because of its chemical properties or lack of a transport mechanism for crossing the cell membrane.[2,6,9]

Increased cytotoxicity of cisplatin caused by electropulsation of cells was also obtained in cell lines resistant to cisplatin.[10] Furthermore, it was demonstrated that endothelial cells are sensitive to bleomycin and to cisplatin delivered by electropulsation.[11] These data are important for an explanation of the vascular-disrupting effect of electrochemotherapy.[11–13]

In Vivo Studies

Bleomycin and cisplatin were tested by an electrochemotherapy protocol in a number of animal models in vivo (Fig 1). The antitumor effectiveness of electrochemotherapy was tested on tumors in mice, rats, hamsters, and rabbits. Tumors treated by electrochemotherapy were SC, and grown in muscle, brain, or liver, and were of different types (eg, sarcomas, carcinomas, glioma, and melanoma).[3–7,14] Studies demonstrated that drug doses that have minimal or no antitumor effectiveness produced nearly 80% complete responses when delivered by electrochemotherapy. The drug doses used were so low as to have no systemic toxicity. The route of administration was either IV (for bleomycin) or intratumoral (bleomycin and cisplatin). Although none of the studies compared the different routes of administration directly, the antitumor effectiveness of electrochemotherapy with intratumoral cisplatin or bleomycin or with IV bleomycin was comparable. Electrochemotherapy with IV injection of cisplatin was less effective.[14] The time interval between drug injection and application of electric pulses was important because at the time of the application of electric pulses to the tumor, a sufficient amount of drug must be present in the tumor. After IV administration of the drug into small laboratory animals (4 mg/kg of cisplatin or 0.5 mg/kg bleomycin), an interval of only a few minutes is needed to reach maximal drug concentra-

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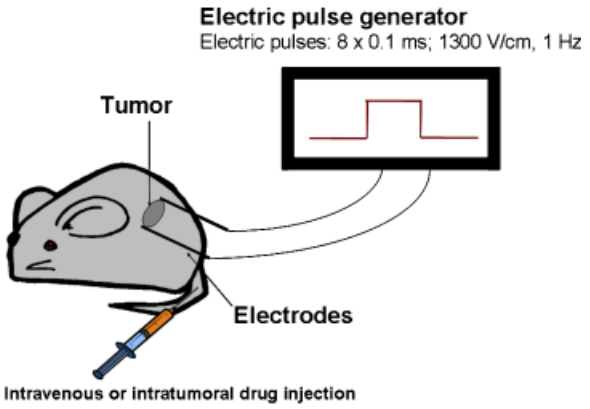

Fig 1. Protocol of electrochemotherapy of tumors presented schematically. The drug is injected either IV or intratumorally at doses that do not exert an antitumor effect. After an interval that allows sufficient drug accumulation in the tumors, electric pulses are applied to the tumor by plate, contact, or needle electrodes.

tion in the tumors. After intratumoral administration (2 mg/cm^3 of cisplatin and 3 mg/cm^3 of bleomycin), this interval is even shorter and the application of electric pulses must follow administration of the drug within a minute.[14]

The application of electric pulses with parameters adequate to produce sufficient electrical field distribution in the tumor to obtain electropermeabilization had no antitumor effectiveness and no systemic adverse effects.[15] Local adverse effects were contractions of the muscles underlying the treated area, but these were present only during the application of electric pulses and were tolerable; hence, in most instances, anesthesia of the laboratory animals was not necessary.[16]

Mechanisms of Action

The principal mechanism of electrochemotherapy is electropermeabilization of the cells in the tumors, which enables the drug to reach intracellular targets. In preclinical studies on murine tumors, increased uptake of bleomycin and cisplatin in the electropulsated tumors was demonstrated as compared with tumors not treated by electropulsation.[17,18] Furthermore, a 2-fold increase in cisplatin DNA adducts was determined in electropulsated tumors.[18]

In preclinical studies, application of electric pulses to the tissues induced a transient but reversible reduction of blood flow, which induced drug entrapment in the tissue for several hours, providing more time for the drug to act. This phenomenon also prevented bleeding from the tissue, which was important in the case of hemorrhagic tumors.[12,13]

The cytotoxic effect of electrochemotherapy not only was limited to cells in the tumors themselves but also acted on stromal cells, including endothelial cells of blood vessels, resulting in their death, disruption of tumor blood flow, and consequently death of tumor cells surrounding the vessels. This vascular-disrupting action of electrochemotherapy contributed to antitumor effectiveness.[13]

Furthermore, involvement of the immune system in the antitumor effectiveness of electrochemotherapy was also demonstrated.[19] In addition, because of the massive tumor antigen shedding in the animals after electrochemotherapy, systemic immunity can be induced and upregulated by additional treatment with biological response modifiers such as interleukins (ILs) 2 and 12, granulocyte–macrophage colony-stimulating factor, and tumor necrosis factor α.[20–22]

Theoretical Background of Electroporation

The theoretical knowledge of electropulsation is crucial to obtain the most suitable protocols for drug delivery. The molecular mechanisms remain rather obscure,[23] but 2 key phenomena are induced in the cell membrane: the induced transmembrane voltage, which is crucial for electropermeabilization, and the transport of the drug molecules through the permeabilized cell membrane after electrical pulse delivery.

Induced Transmembrane Potential

When a cell is exposed to an external electrical field, as used in electrochemotherapy, an induced transmembrane voltage is generated across the cell membrane because of the differences among the electrical properties of the cell membrane, cytoplasm, and external medium. The induced transmembrane voltage is not uniform on the cell surface and is maximal at the surfaces of the cell facing the electrodes. This was demonstrated experimentally by videomicroscopy using potential difference-sensitive fluorescent probes.[24–28] The induced transmembrane potential is also dependent on cell size and shape. The membrane cannot withstand the increase in potential and appears to become permeabilized when a critical value is reached. The surface area of caps where the threshold transmembrane voltage is reached is under the control of the shape of the cell and on the leakiness of its membrane.[28]

The local electrical field is the critical parameter for permeabilization because it defines the area of the membrane that is permeabilized and through which transport occurs. This local field is different from the macroscopic definition of the field (the ratio between the delivered voltage and the width between the electrodes). The reorganization of the membrane associated with this strong increase in transport persists for a minute after the pulse delivery.

Transport through the Permeabilized Membrane

Molecular transfer of small molecules (<4 kDa) across the permeabilized area is mostly driven by their concentration difference across the membrane by simple diffusion. The structural alterations in membrane organization that occur during electropulsation are able to reseal by a metabolic process.[29] The diffusion of ions through the permeabilized region is a relatively slow process that occurs mainly after the pulse application. The theoretical predictions were assayed on cell suspensions by measuring the leakage of metabolites (eg, adenosine triphosphate) or observed at the single-cell level by digitized fluorescence microscopy.[30,31]

Again, the conclusion is that it is the local electrical field that is the driving force in inducing permeabilization and the resulting transport. Another limiting factor in

tissue is that diffusion in the bulk is limited by the narrow space present between cells.[32]

Conclusion

In summary, the basic principle of electropermeabilization is that the electric field pulse brings about an electrically mediated membrane reorganization (so-called "electropores" or "transient permeant defects"). This is a localized event on the cell surface. It is induced only when the local field strength exceeds a critical threshold. Polar molecules can then cross the membrane, giving a loading effect of drug in the cell cytoplasm. This loading is under the control of the field strength and of the cumulated pulse duration. The membrane alteration remains present after the pulse, but the defects will spontaneously reseal, leaving cell viability unaffected if proper electrical parameters are chosen.

Table 1. Summary on clinical trials on electrochemotherapy as a single treatment or as an adjuvant to surgery.

Type of ECT	Species	No. of Patients	Tumor Histology	Clinical Stage	Response/Duration of Response	References
ECT bleomycin IV in combination with adjuvant immunotherapy	Cats	12	Soft tissue sarcoma	Grades I–II	Partial response and stable disease up to7 months	Mir et al[33]
ECT cisplatin IT	Dogs	6	MAC, mast cell tumor, hemangioma, hemangiosarcoma, perianal adenocarcinoma, neurofibroma	MAC: stage IV; others: stages I–III	84% complete response <14 months; 16% partial response	Tozon et al[34]
ECT cisplatin IT	Cats	1	MAC	Stage II	Progressive disease	Tozon et al[34]
ECT cisplatin IT	Cats	1	Lingual squamous cell carcinoma	T3aN0M0–stage III	Stable disease >4 months	Pavlica et al[35]
ECT cisplatin IT	Horses	3	Sarcoid	T1–T2	100% complete response >18 months	Tamzali et al[36], Rols et al[37]
ECT cisplatin IT	Horses	25 + 58	Sarcoid	T1–T4 N0M0	100% complete response >24 months	Tamzali et al[38,39]
ECT cisplatin and bleomycin IT	Dogs	12	Perianal adenoma, perianal adenocarcinoma	T1–T2 N0M0	65% complete response >10 months; 27% partial response up to 32 months	Tozon et al[40]
ECT bleomycin and cisplatin IT	Cats	1	Ganglioneuroblastoma	NA	Complete response >15 months	Spugnini et al[41]
ECT bleomycin IT	Dogs	8	Lymphosarcoma, hemangiopericytoma, neurofibrosarcoma, liposarcoma, acanthomatous epulis, melanoma	NA	38% complete response >5 months; 50% partial response up to 19 months	Spugnini and Porello[42]
ECT bleomycin IT	Cats	9	Squamous cell carcinoma, tricoepithelioma, melanoma, fibrosarcoma, adenocarcioma, anaplastic sarcoma	NA	33% complete response >3 months; 67% partial response >1.5 months	Spugnini and Porello[42]
ECT bleomycin IT	Dogs	10	Mucosal melanoma	T2–T3 N0–N1 M0	70% complete response >6 months; 10% partial response up to 4 months	Spugnni et al[43]
ECT bleomycin IT	Cats	9	Squamous cell carcinoma	T2–T4 N0M0	78% complete response >3 months; 22% partial response up to 1.5 months	Spugnini et al[41]
ECT bleomycin IT as an adjuvant therapy to surgery	Dogs	28	Mast cell tumor	Grades I–III	82% complete response >22 months	Spugnini et al[43]
ECT bleomycin IT as an adjuvant therapy to surgery	Cats	1	Hemangiopericytoma	NA	Complete response >12 months	Baldi and Spugnini[47]
ECT bleomycin IT as an adjuvant therapy to surgery	Dogs	1	Soft tissue sarcoma	Grade III	Complete response >24 months	Spugnini et al[46]
ECT bleomycin IT as an adjuvant therapy to surgery	Cats	58	Soft tissue sarcoma	T2–T4 N0M0	Median time to recurrence: 12–19 months	Spugnini et al[41]

ECT, electrochemotherapy; IT, intratumoral; MAC, mammary adenocarcinoma; NA, not available.

Clinical Studies on Electrochemotherapy

Clinical Results

A summary of clinical trials performed until now is presented in Table 1. In the 1st veterinary clinical trial, conducted in 1997, 12 cats with spontaneous large soft tissue sarcomas that had relapsed after treatment with conventional therapies were treated with electrochemotherapy combined with immunotherapy consisting of an intratumoral injection of CHO (IL-2) living cells that secreted IL-2, which makes this study substantially different from other studies. Electrochemotherapy involved bleomycin-injected IV followed by application of electrical pulses.[33]

In most of the studies on electrochemotherapy in small animals, cisplatin was used as a chemotherapeutic agent. In these studies, electrochemotherapy was used as a single treatment and not as an adjuvant treatment. Only recently were studies with intratumorally injected bleomycin performed either alone or as an adjuvant treatment to surgery. From 2001, groups from Ljubljana and Toulouse reported the successful use of electrochemotherapy with cisplatin as a direct single treatment of tumors in dogs, cats, and horses.[34–40]

Electrochemotherapy with bleomycin injected intratumorally in pets with spontaneous tumors of different histological types was reported from 2003.[40–44] In the case of adjuvant treatment, electrochemotherapy proved to be very effective as an adjunct to surgery for treatment of mast cell tumors and soft tissue sarcoma in dogs and hemangiopericytoma and soft tissue sarcoma in cats.[45–49] The latter study was a randomized study comprising 72 cats that were assigned to surgical treatment alone (14 cats), intraoperative electrochemotherapy (19 cats), or postoperative electrochemotherapy (39 cats). The median time to recurrence was 4 months for cats treated with surgery alone, 19 months for the postoperative electrochemotherapy, and 12 months for the intraoperative group.[48] Electrochemotherapy with cisplatin injected intratumorally was tested in several clinical trials on larger numbers of equine sarcoids. The results of the studies confirmed that electrochemotherapy with cisplatin is a highly effective treatment with long-lived antitumor effects and good treatment tolerance.[36–39]

Summary of Treatment Protocols

Drug administration was very uniform in all of the above-mentioned studies except for the 1st study on cats. An intratumoral injection of very low doses of chemotherapeutic agents was always used to obtain a high enough concentration of the drug in the tumor cells after electropulsation and at the same time to avoid the occurrence of systemic adverse effects.

Electropulsation protocols were quite similar among the groups. Uni- or bipolar square wave electric pulses were used with amplitudes of approximately 1,000 V/cm and a pulse duration of up to 100 μs with a repetition frequency of 1 Hz. Two different types of electropulsators were used in the studies: a Jouan electropulsator,[a] which produces unipolar pulses, and the Chemopulse,[b] which produces bipolar pulses. Both electropulsators produce square wave electrical pulses. Electropermeabilization of tumors requires an effective field value inside the tissue where the tumor is growing. Square wave pulses where a constant voltage is delivered are always applied to avoid problems associated with changes in tissue impedance during the pulse, as in the case of the use of capacitor discharge systems. The protocols vary mostly in the choice of electrodes. Plate and needle electrodes were used for electrochemotherapy of dogs and cats, whereas wire contact electrodes were used for the treatment of horses.[34,36] Needle electrodes produced a high field depth in the tissue but with a heterogeneous distribution. The needles are invasive and their use is difficult when the skin is tough (eg, as in the case of horses). Plate electrodes are suitable for surface tumors of different sizes because the electrodes can be moved around the tumor to cover the whole tumor area. The electrical field distribution is rather uniform.[15] Especially for treatment of horses, contact wire electrodes are easy to bring in contact with the shaved skin (the electrical contact being obtained with a conductive gel) (Fig 2).[38] They can be moved easily on the tumor surface in different orientations to take advantage of the increased drug delivery obtained with crossed orientation of the field. Their drawback is that only a limited amount of the tissue is affected by the field discharge and successive treatments are required for eradication. The biphasic pulses in currently adopted protocols are administered in bursts

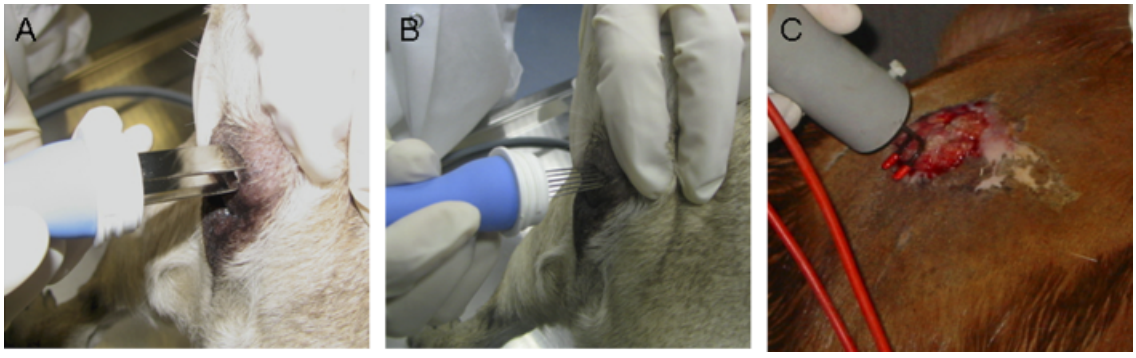

Fig 2. Application of electrical pulses with different types of electrodes: (A) plate, (B) needle, and (C) contact electrodes.

and are not delivered in crossed orientation.[42] A proper design of the electrodes and an accurate evaluation of the field distribution in the tissue are still required. Electrode configuration affects electrical field distribution in tissue. Because of its anatomy and electrical properties, tissue reacts to the applied external electric field, making it difficult to choose the optimal electrode configuration and pulse parameters for the particular target tissue. Empirical methods of design are developed frequently.[49–51] A safe approach is to compute in advance the electric field distribution in tissue by means of numerical modeling techniques.[52] This is demanding because of the heterogeneous properties and morphology of tissue.

Clinical Results in Human Patients

Considerable experience with electrochemotherapy has already been gathered in humans.[53] A recent prospective nonrandomized multi-institutional study, European Standard Operating Procedures of the Electrochemotherapy-ESOPE, demonstrated a good treatment response regardless of the tumor type treated, drug used, route of administration, and type of electrodes used.[54] An objective response rate of 85% of the tumors (74% complete responses and 11% partial responses) was achieved. The results of this study are comparable to other studies in centers such as the Melanoma unit in Sydney.[55] Overall, approximately 1,200 tumor nodules in 300 patients were treated in the studies published so far, with an objective response rate of 84%.[56]

In humans, electrochemotherapy is aimed at treatment of cutaneous or SC tumor nodules of progressive disease, with palliative intent also in previously irradiated or surgically treated areas.[54] Nodules can be located in any part of the body, including perianal and perineal locations.[57,58] The advantages of electrochemotherapy are that it is easy to perform on an outpatient basis and it has high treatment effectiveness. Tumors regress completely after 1 session, but if the tumors are large or the 1st session has not eradicated the tumor completely, electrochemotherapy can be repeated with improved treatment effectiveness.

Conclusion

Electrochemotherapy proved to be highly effective against different primary tumors or metastases in dogs and cats and sarcoids in horses. Electrochemotherapy can be used with curative intent for solitary or multiple cutaneous or SC tumor nodules or as an adjuvant treatment to surgery. Because of results that are comparable to other standard treatment approaches and the low cost and relative ease of the procedure, it may be a very suitable treatment option for veterinary oncology. However, controlled randomized studies should be performed to fully confirm these promising results of electrochemotherapy tested in a wide variety of different histological tumor types.

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Footnotes

[a] Jouan electropulsator, Jouan, St Herblain, France
[b] Chemopulse, Centre of Biomedical Engineering of Sofia, Sofia, Bulgaria

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