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Medicine in focus

Potential role of electrochemotherapy for the treatment of soft tissue sarcoma: First insights from preclinical studies in animals

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Abstract

Local management of soft tissue sarcoma in humans generally involves multi-modality approaches whose cornerstones are surgery combined with radiation therapy. The usual radiation protocols are based on preoperative, intraoperative, or postoperative external beam treatment or adjuvant brachytherapy. The aim of these strategies is to maximize tumor control while minimizing side effects, especially in the case of limb sarcomas. Unfortunately, the rate of local wound complication associated with aggressive surgical management and radiation therapy are still elevated. Electrochemotherapy is an anticancer technique that gained popularity over the past 15 years. It involves the administration of anticancer agents to the application of permeabilizing pulses so to increase the uptake of antitumor molecules. Goal of this review is to underline the advances in this field obtained from animal studies in order to point out the possible therapeutic applications of this technique for the treatment of soft tissue sarcomas in humans.

Keywords: Adjuvant; Bleomycin; Cat; Dog; Electrochemotherapy; Sarcoma

1. Introduction

Human and veterinary oncologists acutely perceive the difficulty of achieving local tumor control in cancer patients affected by various neoplasms. In fact, lack of awareness, inadequate screenings and the sudden onset of rapidly growing neoplasms often prevent the clinician from curing tumors with surgery alone. Due to these and other reasons, cancer is considered (with the exception of some benign tumors) a disease that requires multi-modality treatment. In humans, such approaches have been developed through multi-institutional phase II and III trials and usually consist of the association of surgery and radiation therapy (depending on the clinical situation, usually brachytherapy with radiation beam) (Banfi & Lombardi, 1994; Bujko, Suit, Springfield, & Convery, 1993; Hellman, 1997; O'Sullivan et al., 2002; Sadoski, Suit, Rosenberg, Mankin, & Efird, 1993; Strohbehn, 1994). Chemotherapy is usually combined with the aforementioned treatments in an adjuvant fashion for those cancers with high tendency to metastasize (i.e. high-grade sarcoma or breast cancer) (Edmonson et al., 2002). In selected cases, chemotherapy can be added to the protocol in a neoadjuvant approach to maximize the possibility of eradication (Edmonson et al., 2002). Other modalities explored to find new, synergistic combinations led to the investigation of local and whole body

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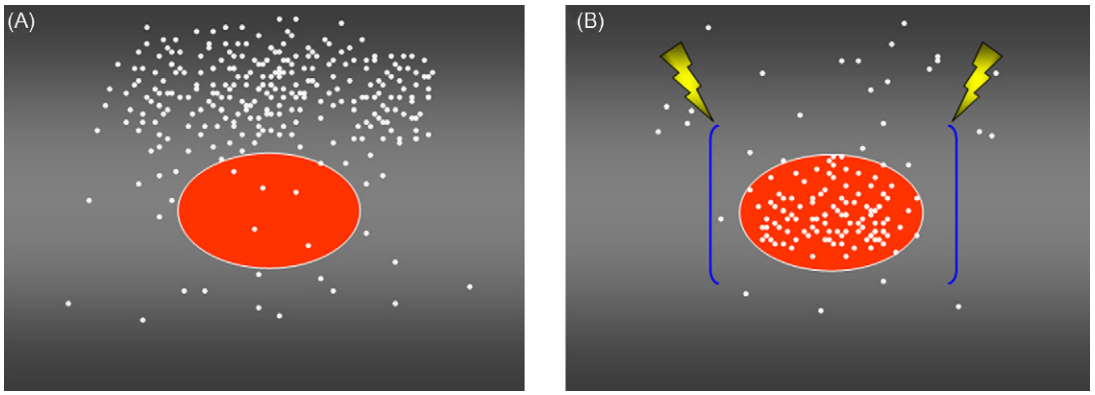

Fig. 1. Schematic representation of the enhanced uptake of lipophobic drug (white dots) by a tumor cell (red circle) following the creation of an electric field (blue square parenthesis) and the administration of permeabilizing pulses (yellow sparks). (A) Drug uptake by the tumor cell before the administration of permeabilizing pulses. (B) Drug uptake by the tumor cell after administration of permeabilizing pulses.

hyperthermia; however, the high costs of this treatment as well as the lengthiness of the procedure and the need for highly skilled operators confined this therapy to a small number of research institutions (Strohbehn, 1994).

Another critical point when evaluating local control modalities in cancer patients is the biological cost paid to accomplish such a goal. Many patients have to undergo disfiguring or mutilating surgeries and often the side effects of radiation therapy can leave sequelae that may lead to a poor quality of life. The most commonly reported side effects of radiation therapy are: (1) gradual side effects, usually dose-dependent (local fibrosis, necrosis, nerve damage, etc.) and (2) the so-called "statistically demonstrable side effects", also known as "radiation induced tumors" (Banfi & Lombardi, 1994; Hellman, 1997).

This is particularly true for the high-grade soft tissue sarcomas (STS) (Grade III with high mitotic rate) of humans where the aim of these strategies is to maximize tumor control while minimizing side effects, especially in the case of limb location (O'Sullivan et al., 2002). For these reasons low dose external beam fractionation is usually preferred. However, in case of large neoplasms that involve deep underlying structures, preoperative radiation therapy might be chosen in the attempt to shrink the tumor volume and to reduce the satellite infiltrations (Sadoski et al., 1993). Unfortunately the rate of local wound complication associated with aggressive surgical management and radiation therapy are still elevated (Bujko et al., 1993). Recent publications advocated a trend toward increased disease free interval and survival in patients receiving chemotherapy within a multi-modality treatment as a result of improved systemic and intra-arterial chemotherapy (Edmonson et al., 2002).

1.1. Electrochemotherapy

A new treatment modality being further explored that can achieve high rates of remission without the associated problems of high financial and biological cost of previous procedures is electrochemotherapy (ECT). It involves the administration of anticancer agents to the application of permeabilizing pulses so to increase the uptake of antitumor molecules (Fig. 1).

In vitro studies showed that the application of high voltage, exponentially decaying electric pulses to cells in suspension could induce pores in the cell membrane, thus resulting in cross-membrane flow of material (electroporation, electroinjection) or even in cell fusion if the cells were adjacent (Lo et al., 1984; Sugar & Neumann, 1984; Zimmermann & Scheurich, 1981). These methods were initially used to transfect bacterial cells with plasmids and subsequently exploited to produce monoclonal antibodies through fusion of eukaryotic cells. Later, researchers realized that electroporation could enhance the transport of drugs and genes through the cytoplasmic membrane by exposing animal cells in culture and plant protoplasts to non-cytotoxic electric pulses (Lo et al., 1984; Neuman, Schaefer-Ridder, Wang, & Hofschneider, 1982; Sugar & Neumann, 1984; Zimmermann & Scheurich, 1981). Subsequently, electroporation has been proven to be very effective at enhancing the *in vitro* cytotoxicity of anticancer molecules (Pron, Belehradec, & Mir, 1993; Tounekti, Pron, Belehradek, & Mir, 1993).

The first and most actively studied chemotherapy agent in ECT has been bleomycin. This drug can penetrate the cell membrane only through protein receptors due to its lipophobic nature, thus resulting in slow and quantitatively limited uptake under normal conditions

(Pron et al., 1993). The complex formed by bleomycin and its carrier is transported in the cytosol by means of endocytotic vesicles, but the mechanism of its release is still unknown. Bleomycin induced DNA fragmentation is a very rapid phenomenon: it happens within 30 s of drug entry within the cell (Pron et al., 1993; Tounekti et al., 1993). The high toxicity of bleomycin when it reaches the intracellular environment is impaired by its inability to freely diffuse through the cytoplasmic membrane (Pron et al., 1993; Tounekti et al., 1993). *In vitro* studies evidenced that less than 0.1% of bleomycin added to culture medium becomes associated to the cell (Tounekti et al., 1993). It is on this background that the cytotoxicity of bleomycin can be enhanced by 300–700-fold by electroporation (Tounekti et al., 1993). Another drug that is carried into cells by this mechanism is cisplatin (CDDP), however its captation is less influenced by the concurrent application of electric pulses, as a result, compared to the current literature investigating bleomycin, the *in vivo* use of CDDP is still limited (Hyacinthe et al., 1999; Kranjic, Cemazar, Grosel, Sentjurc, & Sersa, 2005; Mir et al., 1997; Sersa et al., 2002; Spugnini, Citro, & Porrello, 2005; Spugnini & Porrello, 2003; Spugnini, Vincenzi, Baldi, Citro, & Baldi, 2006; Spugnini, Vincenzi, Citro, Santini et al., in press; Spugnini et al., 2007; Tozon, Sersa, & Cemazar, 2001; Zaharoff, Barr, Li, & Yuan, 2002).

Several electroporation protocols have been adopted, mostly involving sequences of repeated decaying or square single pulses until the desired number of permeabilizing electric stimulations was reached (Lo et al., 1984; Mir et al., 1997; Neuman et al., 1982; Pron et al., 1993; Sugar & Neumann, 1984; Tounekti et al., 1993; Zimmermann & Scheurich, 1981). More recently, a novel protocol involving the adoption of bursts of rectangular but biphasic pulses with selectable period of repetition has been successfully used (Spugnini, Baldi et al., 2007; Spugnini, Citro et al., 2007; Spugnini & Porrello, 2003; Spugnini, Vincenzi, Citro, Santini et al., in press; Spugnini, Vincenzi, Citro, Tonini et al., 2007; Spugnini et al., 2006, 2005, 2007). This schedule offers advantages in decreasing the morbidity of the treated animals and human beings as well as improving the clinical outcome (Daskalov, Mudrov, & Peycheva, 1999; Spugnini, Baldi et al., 2007; Spugnini, Citro et al., 2007; Spugnini & Porrello, 2003; Spugnini, Vincenzi, Citro, Santini et al., in press; Spugnini, Vincenzi, Citro, Tonini et al., 2007; Spugnini et al., 2005, 2007, 2006).

The exact mechanism of this therapy at the membrane level is still not well understood, however a recent study underlined the occurrence of membrane changes following the exposure to electric pulses (Spugnini, Arancia et al., 2007). In this paper, a number of ultrastructural alterations in the cellular membranes following the exposure of orthotopic melanomas and red blood cells to trains of biphasic pulses are described. Specifically, the freeze-fracturing analysis of both cell types evidenced defects in the dynamic assembly of lipids and proteins, which generated "areas with rough structure" and intensive clustering of intramembrane proteins. Such modifications are suggestive of lipid and protein alterations, of protein cohesion reduction, and of changes in lipid orientation inside cell membranes.

1.2. Animal studies

Differently from other cancer investigations, electrochemotherapy has conducted in tandem studies in rodents and companion animals.

The first *in vivo* study involved the use of ECT as a rescue protocol in cats with recurring soft tissue sarcoma after radiation therapy and surgical ablation (Mir et al., 1997). In that trial, cats were randomized to receive bleomycin ± the implant of cells secreting interleukin 2 followed by the delivery of square pulses. A small cohort of untreated cats was selected as well. The authors described only one partial response; however, they claimed a prolonged survival in 12 cats receiving ECT versus 11 untreated controls. This low response rate could be partially ascribed to the previous treatment since resistance to radiation therapy through increased DNA repair is one of the described mechanisms of resistance to bleomycin as well, at least in cell lines (Spugnini & Porrello, 2003).

After this preliminary investigation in cats, several studies in laboratory animals evidenced a potential therapeutic application of ECT for sarcomas. The first article involved mice carrying limb sarcoma xenografts, treated with rectangular pulses and intralesional bleomycin. This study showed a high response rate in mice with both small and large sarcomas, and evidenced the need of multiple sessions for the control of larger neoplasms (Hyacinthe et al., 1999). Further murine studies evidenced a vasoconstrictive and a potential radiosensitizing effect of ECT (Kranjic et al., 2005; Sersa et al., 2002).

Few years after the first preliminary investigation in cats, two phase I/II studies were conducted in companion animals; in the first a cohort of dogs and cats were treated with intralesional cisplatin coupled with square electric pulses (Tozon et al., 2001). At the same time a preliminary study, including several cases of softi tissue sarcomas, was conducted using trains of

biphasic pulses associated with intralesional bleomycin (Spugnini & Porrello, 2003). The overall response rate of this preliminary investigation was 80% with a 40% of long lasting remissions, in particular, canine hemangiopericytomas showed a remarkable sensitivity to this approach. This work pointed out two important issues to be addressed: the need of specifically tailored electrodes for the therapy of soft tissue neoplasms and the obstacle to a smooth permeabilization represented by the high content of connective tissue within solid tumors and soft tissue sarcomas in particular (Zaharoff et al., 2002).

After the development of novel electrodes (Spugnini et al., 2005), several phase II studies were conducted in our Institution to evaluate the potential of ECT as adjuvant treatment after surgical cytoreduction of STS.

In a large cohort of cats with STS, intraoperative and postoperative ECT has been evaluated, mimicking the radiation therapy protocols adopted in humans (Spugnini et al., 2007). Cats were assigned to the following groups: surgery alone, surgery plus intraoperative ECT and surgery plus postoperative ECT. The study evidenced a significant advantage of adjuvant ECT in terms of local control and overall survival compared to surgery single modality of treatment. Time to recurrence was 12 and 19 months for the intraoperative and postoperative cohorts, while the cats treated with surgery alone experienced tumor recurrence within 2 months.

A similar study in 22 dogs with STS yielded a median time to recurrence of 730 days with a 95% response rate (Spugnini et al., 2007).

At the same time, a similar investigation was performed in 28 dogs with mast cell sarcomas that resulted in a mean time to recurrence of 52.7 ± 6.5 months; at the time of writing the median time to recurrence was not reached since 24 of the patients were still disease free (Spugnini et al., 2006).

2. Conclusion

ECT has proven to be a safe and efficacious therapy for the local control of STS in companion animals, especially when administered in an adjuvant fashion through the generation of trains of biphasic pulses (Spugnini & Porrello, 2003; Spugnini, Vincenzi, Citro, Santini et al., in press; Spugnini et al., 2006, 2005, 2007). ECT is currently being assayed for different spontaneous tumors in companion animals showing promising results and identifying patterns of response and prognostic factors (Spugnini, Baldi et al., 2007; Spugnini, Citro et al., 2007; Spugnini, Vincenzi, Citro, Tonini et al., 2007). Further studies are currently ongoing to evaluate new drugs and delivery systems to improve the responses obtained so far, also in view of its future translation to human patients.

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