Novel electroporation-based treatments for breast cancer

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Abstract

Breast cancer (BC) is the most common cancer in women, and its incidence is increasing every year. Current treatment is based on surgical resection, chemotherapy (CT), radiotherapy, and hormone therapy (HT). Unfortunately, these methods are ineffective and are associated with a wide range of side effects (e.g., nausea, hair loss and fertility disorders). Electrochemotherapy (ECT), which exposes tumor cells to electric pulses (known as electroporation (EP)) in combination with cytostatic drugs, enables the reduction of cytotoxic drug doses while increasing their efficacy. Electroporation-based treatment methods are applied in breast carcinoma and are the subject of intensive research globally. Irreversible EP has shown promising therapeutic potential in the absence of cytotoxic drugs, as has EP associated with molecules such as calcium ions that are already present in the human body. The application of EP-based methods seems to be a safer and more effective treatment for BC in vitro and in vivo. Indeed, they have found applications in the treatment of BC and its metastases. Moreover, their palliative effects have also been established, and pain reduction has been noted in patients.

Key words: breast cancer, calcium ion, electroporation, electrochemotherapy

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Introduction

Breast cancers (BCs) are the most common carcinomas in developing countries, and they consist of a wide range of heterogeneous diseases, both intertumorally and intratumorally. Existing morphological and molecular differences between the different types of BC affect their susceptibility to therapy.[1,2] Consequently, contemporary medicine is still facing the challenge of mounting an effective fight against BC, and this neoplasm remains one of the most common types of cancer and the leading cause of cancer-associated mortality in women.[3]

Conventional first-line treatment for BC includes surgery, radiation and chemotherapy (CT). While the first two treatments target the tumor region, CT involves the systemic administration of cytotoxic agents to either inhibit the growth of cancer cells or trigger their apoptosis.[4] However, default CT is noneffective due to the occurrence of multidrug resistance (MDR) against commonly used cytostatic agents, and the severe toxicities induced by them. Therefore, researchers started to look for new treatment strategies. One such strategy, endocrine therapy, exploits the fact that a wide group of BCs are characterized by the expression of estrogen and/or progesterone receptors. This adjuvant treatment reduces BC-associated mortality. Moreover, hormone therapy (HT) is the basic treatment in the advanced phases of BC. However, a subgroup of hormone receptor-positive (HR^+) BCs do not benefit from endocrine therapy, and all HR^+ metastatic BCs develop resistance to HT.[5,6] Due to the lack of efficiency in current methods, there is an urgent need to look for alternative, affordable and simple techniques to treat neoplasms refractory to conventional treatment standards.

Electroporation (EP) is a biophysical method based on the introduction of high-voltage electric pulses to cells in vitro or tissues in vivo, which results in permeabilization of the plasma membrane (PM). The occurrence of hydrophilic pores in the PM results in enhanced influx of a variety of molecules into the cell's cytosol. Pulse duration and electric field strength determine whether structural rearrangements in the cell membrane are reversible, enabling the re-establishment of the cell homeostasis, or irreversible, leading to cell death due to the loss of essential organelles through pores in the PM. Thus, 2 main types of EP may be distinguished: reversible EP (RE) and irreversible EP (IRE).[7–9]

The use of EP-based treatment methods can have a significant impact on increasing the amount of drug delivered into cells, which prevents the emergence of active drug efflux-based resistance mechanisms in cancer cells.[10] To this end, electrical pulse-mediated CT, known as electrochemotherapy (ECT), is a novel way to improve cancer treatment efficiency. Optimal EP parameters for use in clinical practice were defined as part of the European Standard Operating Procedures on Electrochemotherapy (ESOPE) multicenter trial,[11] and include the delivery of 8 rectangular pulses, each lasting 100 μs, with an electric field intensity ranging from 1.0 kV/cm to 1.5 kV/cm. Depending on whether a drug is administered locally or intravenously, pulses have to be delivered immediately following or a few minutes after the drug is delivered, respectively.[12,13] Hitherto, only 3 compounds have been used in clinical practice for ECT protocols: cisplatin, bleomycin, and recently, calcium chloride ($CaCl_2$). Moreover, it should be noted that ECT may be combined with immunotherapy[14,15] or radiotherapy.[16]

Studies on EP and ECT in breast cancer

Due to the phenomenon of MDR and other difficulties associated with BC (e.g., histological variety, hormonal dependence and resistance of cancer cells), EP has been highlighted as one of the methods that can increase the effectiveness of conventional treatment. Furthermore, EP decreases the number of side effects of conventional cancer treatments. Electroporation of BC cells may provide an efficient and feasible drug delivery system, enabling the reduction of dosage and drug exposure time. Electroporation and ECT have been investigated in BC in vitro, as well as in clinical studies.[17–20] Pehlivanova et al. examined the influence of electrical treatment on the cell adhesion of BC cells and fibroblasts. The application of suitable electric pulses triggered rearrangements in cytoskeleton organization and cell adhesiveness. Such variations could lead to the restriction of tumor metastasis rate, which contributes to the increased antitumor effect of EP-based therapy.[21] In other research, high-frequency irreversible EP (H-FIRE), an effective tumor ablation strategy, used bipolar bursts of ultrashort (0.25–5 μs) pulses characterized by different polarity.[22] It has been discovered that H-FIRE induced immune-mediated cell death and promoted systemic anti-tumor immunity. Cell death and tumor ablation following H-FIRE treatment activated the local innate immune system, causing the tumor microenvironment to change from an anti-inflammatory to a pro-inflammatory state.[23,24]

The analysis of the impact of EP without the use of anticancer drugs was conducted on human triple-negative BC (MDA-MB-231) and human colon cancer (SW-480 and HCT-116) cells, and these results were compared with studies investigated human fibroblast cell line (MRC-5), primary human aortic smooth muscle cells (hAoSMCs) and human umbilical vein endothelial cells (HUVECs).[25] The inhibition of cell proliferation was observed after EP was applied, and the intensity of this effect was dependent on the parameters of the protocol used. The use of a lower voltage (up to 0.5 kV/cm) induced a fast but temporary disturbance in viability of MDA-MB-231 cells, and apoptosis was the predominant type of cell death. However, the cells started to proliferate again after several hours. Only IRE with high voltages resulted in permanent BC cell

degradation. Different results were obtained for colon cancer cells, in which exposure to pulse intensities of up to 0.5 kV/cm caused permanent damage. Healthy cells (MRC-5s, hAoSMCs and HUVECs), similar to the MDA-MB-231 cell line, recovered after 72 h. This research indicates that EP might be a promising treatment method; however, more precise analyses are needed to develop an optimal EP protocol.[25]

Electrochemotherapy investigations on BC cell lines have mainly been conducted with the use of bleomycin or cisplatin. Increasing intracellular concentrations of these 2 drugs lead to cell death by apoptosis,[26] necrosis[27] or by other pathways, depending on the drug used.[26,28] A local inflammatory reaction has been observed within the area of the electric field application after ECT,[29,30] and the cytotoxicity of the anti-cancer agents used increased by 80–100-fold.[31,32]

Electroporation-based methods are a promising alternative for human breast adenocarcinoma therapy, especially in those resistant to drugs. Electroporation reduces the effective dose of the drug and drug exposure time; thus, it reduces the number of side effects. Rembiałkowska et al. conducted an in vitro investigation into the use of doxorubicin (DOX) as an anti-cancer drug alongside EP in the human estrogen receptor-positive (ER^+) BC cell line (MCF-7/WT), which is sensitive to DOX. They also used a DOX resistance cell line (MCF-7/DOX), and an increased effectiveness of the drug was observed in these cells after ECT. Indeed, the resistant cell line was shown to be more sensitive to electric pulses. Furthermore, electron microscopic examination of both cell lines revealed some interesting results. Combining electric pulses with DOX led to the appearance of heterogeneous materials with irregular shapes characteristic of secondary lysosomes and vacuoles.[33]

Combining EP with calcium ions (Ca^{2+}) instead of cytotoxic agents has been investigated as a potential treatment modality, and is known as calcium EP (CaEP). An in vitro study demonstrated that an EP-driven influx of supraphysiological doses of Ca^{2+} into cells caused necrotic cell death associated with a severe energy reduction.[34,35] In another study, an enhanced antiproliferative effect on MCF-7 and MCF-7/DX cells electroporated using nanosecond pulsed electric field (nsPEF) protocols in combination with Ca^{2+} was noted.[36] In general, the use of CaEP revealed similar effects.[35,37]

Electrochemotherapy has a promising potential and can be used for inoperable, chemoresistant and radioresistant tumors that do not respond to the current standard of treatment.[38] Preliminary clinical studies on BC metastasis to the skin and subcutaneous tissue demonstrated the high effectiveness of ECT as a palliative treatment, with a significant improvement in the patient's quality of life.[19] However, such a small number of applicable drugs is a limiting factor of ECT, as its efficacy may be abolished by side effects such as pulmonary toxicity after the application of bleomycin,[39] or extensive tumor necrosis following EP with Ca^{2+}.[40]

Conclusions

The application of ECT, CaEP and IRE shows promising results as a safer and more effective treatment option for BC both in vitro and in vivo, with specific success seen for ECT in the treatment of BC and its metastases. Moreover, the palliative effects of ECT and pain reduction have been noted in patients.

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