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EXPERIMENTAL ELECTROCHEMOTHERAPY USING NOVEL DESIGN SINGLE NEEDLE DEVICE

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Abstract

This is a feasibility study for the application of a novel concept of single-needle device for localized chemotherapy.

Systemic chemotherapy has numerous and severe side effects. To conduct localized (electro)chemotherapy, we designed a novel device that does not currently exist on the market. Electrochemotherapy is based on the cell membranes temporary or permanent permeabilization using an electric current of defined characteristics. Electroporation can be reversible, when after a period of opened pores and membrane permeability increasing, membranes and cells return to their original state without damage. Electroporation can be an irreversible process when the pores on the membrane remain permanently open, electrolyte imbalance occurs resulting in cell death. Electrochemotherapy involves a combination of cytostatics and reversible electroporation, when pores on the cell membrane are temporarily opened and, during that short period, a large amount of cytostatic is entered into the cell, which is a macromolecule that would not normally penetrate the cell. After closing the pores, the cytostatic remains trapped in the cell in large quantities, multiplying its effect. In this paper, we present a feasibility study of electroporation application in irreversible mode without the use of cytostatics. Fresh porcine liver tissue was used to show that the constructed equipment was effective, thus opening the way for further investigations using reversible electroporation with the application of cytostatics, which would represent localized electrochemotherapy. We penetrated the virtual tumor area (liver metastases) with a specially designed needle with electrodes that generate an electric field and apply electroporation in the target tissue. We have shown that the constructed novel design single needle equipment for electroporation is effective on the experimental model of isolated porcine liver. Further steps in our study will be the testing of electrochemotherapy in an experimental animal model *in vivo*.

Keywords: Electroporation; electrochemotherapy; tumor treatment; liver

1. Introduction

Treatment of liver metastases, primarily colorectal but also tumors of other primary localizations, due to its high incidence and mortality, is still a challenge for clinicians. These are often patients in poor general condition, due to advanced underlying malignancy, comorbidities. Extensive surgical procedures to remove large volumes of liver tissue, and aggressive chemotherapy with its well-known side effects are certainly not something that this group of patients easily tolerate (Yarmush et al. 2014). The mentioned concept implies that the minimally invasive method achieves the greatest effect. The most important is to help a patient who is already in a difficult general condition due to the underlying disease with a method that will solve the problem with the least trauma to the body. A patient who is often exhausted, hemodynamically unstable, intoxicated and malnourished often cannot tolerate any extensive procedures treating tumor near large blood vessels when surgical resection is complicated or impossible. We tried to develop a methodology and appropriate hardware and software equipment that would help us treat metastases with a minimally invasive technique which is based on the phenomenon of electroporation and electrochemotherapy. Electroporation has been used in medicine and biology primarily for gene transfection (Chopinet et al. 2013). It is based on the fact that the pores on the cell membrane under the characteristic electric current open allowing larger molecules to pass through the membrane. The substance of interest remains "trapped" in the cell after pores restitution and cell normally continues with its cycle and function. Electroporation can be reversible when, after a short period of increased membrane permeability, membranes and cells return to their original state without damage. Electroporation can be an irreversible process when the pores on the membrane remain permanently open, and electrolyte imbalance occurs resulting to cell death. To be reversible or irreversible electroporation depends on the properties of the applied electric current /frequency, strength, voltage, pulse duration. If cytostatic molecules or any substance capable of killing a cell is inserted into a cell by electroporation, that process is called electrochemotherapy (Cvetković et al. 2017). It is known that the molecules of many cytostatic are macromolecules, so that they otherwise penetrate poorly into the cell despite their large dose in the circulation, with unfortunately numerous and severe side effects on the human body. By electroporation, i.e., electrochemotherapy, we would be able to selectively induce absorption of high doses of cytostatic in the region of interest (area of metastasis) by brief and reversible increasing the permeability of the cell membrane. The data show that the concentration of Paclitaxel in the tissue treated with electroporation can increase the concentration of the drug up to 1000 times. The proposed technique would achieve such a high concentration of cytostatic only in the area of metastasis, while the drug in the free circulation is practically not present or at least in minimal amounts, thus preventing side effects of systemic chemotherapeutic agents, which is often a reason to terminate therapy. It is known that systemically applied chemotherapy has a non-selective effect to a greater or lesser extent on all tissues of the organism, applying localized chemotherapy as a highly desirable form of therapy.

2. Material and Methods

We offer two original designs of single-needle devices for localized electrochemotherapy. The concept of this treatment methodology is to penetrate the tumor area (for example, deeply seated liver metastasis percutaneously) with a specially designed needle with electrodes that generate an electric field, and then perform electroporation according to certain protocols, exclusively in the volume of the tissue exposed to the electric field and administer a small amount of cytostatic which should be almost completely absorbed by the targeted tissue.

The body of the needle in the first concept will be made with a 3D printer, 3D Printer Form2 - manufacturer: FormsLab, printing accuracy 0.16mm (https://formlabs.com/3d-printers/form-

2/), and the material that will be used to make the needle itself does not conduct electricity / material - rubber: rigid / (https://formlabs.com/materials/engineering/#rigid-resin). 3d model made in the SolidWorks 2020 CAD software package. Longitudinally, grooves pass through the body of the needle, through which movable electrodes (cathode and anode) pass, and which are used for electroporation. In the middle of the needle body lies a channel for injecting a chemotherapeutic agent (Fig. 1.).

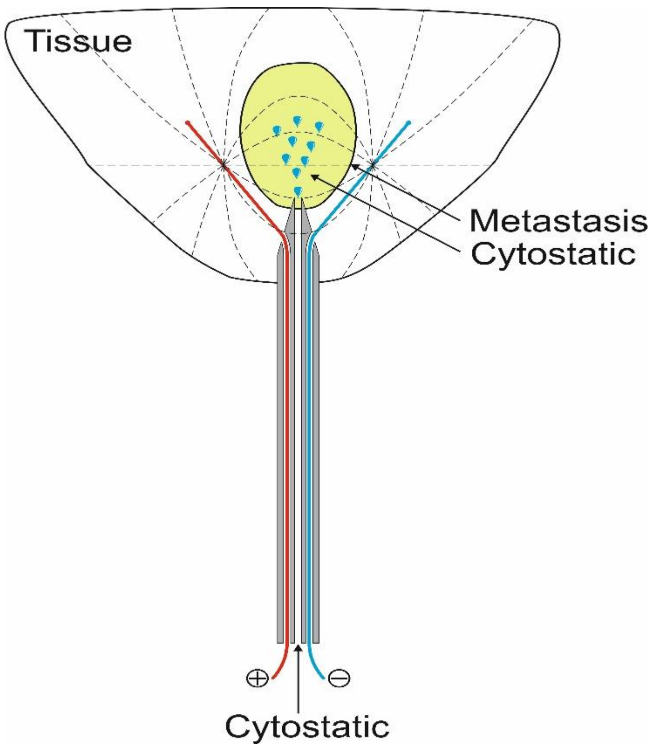

Fig. 1. Single needle with two linear electrodes.

Schematic representation of the needle, which would be introduced into the area of metastasis under the guidance of ultrasound, electrodes applied on both sides of the metastasis, while a small amount of chemotherapeutic agent applied to the target area. The pores of cells in the field of electroporation open for a short period, allowing cytostatic molecules that are otherwise macromolecules to enter the cell in much greater extent than with systemic drug administration when it is distributed by circulation to tumor, but also to all other tissues.

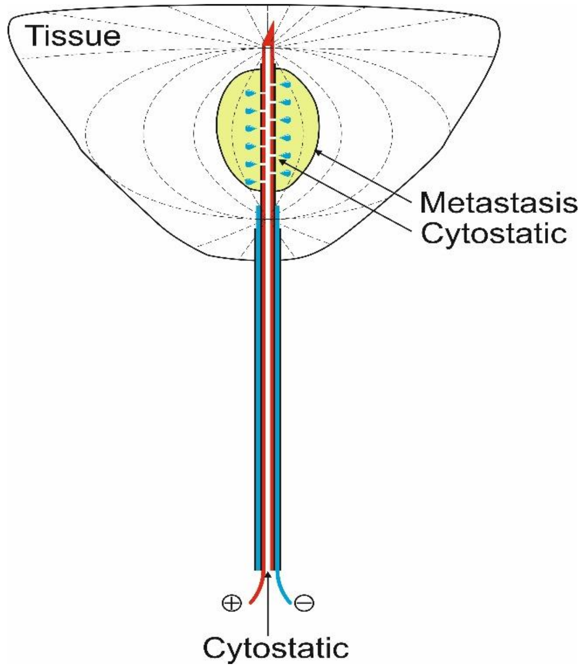

Fig. 2. Telescopic needle electrode for localized electrochemotherapy.

Concept II is a telescopic needle, two needles, coaxially placed, 25G inside 16G (cathode and anode), which are coated with insulating material except the tips of both needles, which are electrodes (Fig. 2.). The part of the inner thin needle, which is located between the electrodes, is perforated in several places, and is used to inject a chemotherapeutic agent. By inserting and extracting the inner needle, the distance between the electrodes is changed and in this way the device adapts to the size of the area in which the electroporation is to be performed, depending on the size of the metastasis. In this paper, we just wanted to perform electroporation without electrochemotherapy.

3. Ex vivo electroporation on experimental animals. Porcine isolated liver specimen electroporation.

Liver preparations were procured from a local slaughterhouse, where the animals are reared and stationed in standard conditions provided for animals used in the food industry. Since these are nonperfused preparations, the liver was carefully explanted immediately after sacrificing the animal (which would otherwise be done without participating in the experiment), immersed in 0.9% NaCl solution and delivered to the laboratory of BioIRC (*Research and Development Center for Bioengineering*) to perform the experiment. Irreversible electroporation has been performed with second proposed device (Concept II, telescopic needle), and then preparations for pathohistological analysis have been made. The preparations would clearly mark the zones in which tissue necrosis occurred due to needle trauma, as well as the zones where complete, irreversible electroporation with tissue necrosis was achieved and the zone in which partial electroporation was achieved. The size of the electroporation field will be analyzed depending on the applied current parameters (strength, voltage, length, pulse, pulse duration), the size of the electrodes, their positions, etc. The obtained results will be used for calibration of the computer simulation model and will represent one type of training for working with the mentioned equipment in the next step of the experiment, in vivo electroporation of liver tumors in rats.

4. Results

Formaldehyde-fixed, porcine liver tissue was trimmed and processed by dehydration with increasing concentrations of alcohol (70%-80%-96%), and further embedded into paraffin blocks suitable for cutting on a microtome. A 5 μm tissue sections from each samples have been deparaffinized with bioclir and decreasing concentrations of alcohol (96%-80%-70%) and finally stained with Hematoxylin and Eosin (Sigma Aldrich) for light examination by microscope.

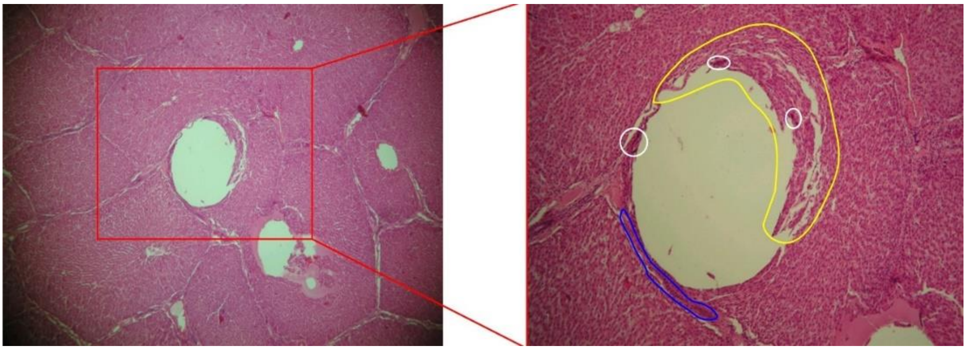

Fig. 3. The pictures show a histological preparation, a porcine liver specimen that was subjected to electroporation with the previously described device. The site of penetration of the device into the liver tissue is visible, and radially from the site of the needle penetration, cells with altered cytoplasm and condensation of the nucleus can be seen as an effect of electroporation.

5. Discussion and Conclusions

Since cancer is one of the most frequent pathologies of today, the interest in this area is understandable. Other local ablative techniques such as cryoablation, radiofrequency ablation (RFA), microwave ablation, as well as HIFU (high-focus ultrasound therapy) are thermal techniques, i.e., through locally generated heat, they damage target tissue, but can therefore easily damage surrounding sensitive structures (Wagstaff et al. 2015). On the other hand, electroporation is a non-thermal ablative method that protects the surrounding healthy tissue and vital structures such as blood vessels, nerves, surrounding organs. This is the most important advantage of electroporation (Wagstaff et al. 2016). The literature mainly describes experiments using high voltages, of the order of 200–1000 V cm^{-1}, where cell adhesion and survival after electroporation treatment were analyzed with crystal violet and MTT essay (Pehlivanova et al., 2012). Electroporation is also in clinical application for superficial tumors, primarily skin tumors, as well as hollow organ tumors that can be relatively easily accessed and in which superficial electroporation makes sense. All electroporation techniques described in the literature use electrochemotherapy modality for superficial tumors using multiple needle electrodes that are brought into contact with the skin tumor with the application of a chemotherapeutic agent. For the treatment of lesions that are not superficial but are in internal organs, for example, pancreatic examinations, are in the phase of experiments on animals, but even then, only irreversible electroporation is used as an ablative method. According to the available data, there is only one study of a similar concept but with a significantly different device for conducting electrochemotherapy and one with the use of multiple needles (Pedersoli et al. 2019; Tarantino et al. 2018).

When performing the experiment, we encountered a couple of problems. First, the expected level of ablation in the tissue was not completely achieved. One of the possible causes is that it was an unperfused tissue sample, and electroporation requires some time for the flux changes through the membrane to give results. Our next research will go in that direction, so we will work on an *in vivo* animal model. We also encountered a problem of a technical nature due to poor isolation and close contact between the two telescopic electrodes, which can be solved by a better choice of isolation material.

If the described methodology would prove effectiveness in practice, the benefits would primarily reflect on patients with malignant diseases. In addition to this primary and undoubtedly the most important goal, the manufacturers of equipment and consumables used in this procedure would also benefit, so that the mutual benefit in the cooperation of medicine and industry is obvious. The health budget would also be profitable as the funds saved by using smaller amounts of chemotherapeutic agents, which are very expensive, could be allocated where needed. We should not forget shorter hospital stays, shorter stays in health institutions with fewer complications, faster return of the patient to daily activities, etc. The treatment of inoperable tumors that are difficult to access even according to current attitudes (due to the local and general condition of the patient) is certainly a great challenge for clinicians. When we consider that the presented methodology achieves greater efficiency with fewer side effects, expectations from such treatment modalities are high. If efficacy in an *in vivo* animal model will be demonstrated, without significant side effects, the next logical phase would be testing in the human population. Here, the research would understandably be raised to a higher level, with the inclusion of sophisticated imaging diagnostics, as well as molecular profiling of tumors, monitoring the response to therapy, which would enable this methodology to be patient-specific, i.e. to suit each patient individually, considering the characteristics of the patient and his disease, and not just rough and approximate oncological protocols.

The proposed concept achieves the maximum effect in the treatment with a minimally invasive procedure, while minimizing the side effects of the applied therapy. We offer two innovative needle concepts with electrodes for electrochemotherapy, which do not currently exist commercially on the market. The topic is very current, and the proposed concept of treatment is very logical and feasible, so we believe that in the relatively near future it will experience commercialization due to its obvious advantages over many standard types of cancer treatment.

Conflict of interest The authors have declared that no competing interests exist.

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