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Original article

Combination of therapy with 5-fluorouracil and cisplatin with electroporation in human ovarian carcinoma model in vitro

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ABSTRACT

High electric field, applied to plasma membrane, affects organization of the lipid molecules, generating transient hydrophilic electropores. The application of the cell membrane electroporation in combination with cytotoxic drugs could increase the drug transport into cells. This approach is known as electrochemotherapy (ECT). Our work shows new data concerning the influence of electrochemical reaction with cisplatin or with 5-fluorouracil (5-FU) on cancer ovarian cells resistant to standard therapy with cisplatin, in comparison to ECT effect on human primary fibroblasts. We investigated the effect of electroporation and electrochemotherapy with 5-FU and cisplatin on human ovarian clear-cell carcinoma cell line (OvBH-1) and epithelial ovarian carcinoma cell line (SKOV-3) – both resistant to cisplatin typically used in ovarian cancers. As control cells, human gingival fibroblasts (HGF's) from primary culture were used. Electropermeabilization efficiency was determined by FACS analysis with iodide propidium. Efficiency of electrochemotherapy was evaluated with viability assay. The cytotoxic effect was dependent on the electroporation parameters and on drug concentration. Electroporation alone only insignificantly decreased cells proliferation in OvBH-1 line; SKOV-3 line was more sensitive to the electrical field. Electrochemotherapy with cisplatin and 5-FU showed promising effects on both ovarian cell lines with recovery of normal cells revealed after 72 hours.

1. Introduction

Ovarian cancer is the most lethal type among the gynecological cancers. Despite recent developments in aggressive surgery and chemotherapy (CT), the prognosis remains poor because ovarian cancers are often not diagnosed until advanced stages. Moreover, the cancer cells often have primary or secondary drug resistance [1]. The successful treatment of cancer is dependent on effectiveness of cytotoxic anticancer therapies, either alone or in combination with other treatments. Therefore, new diagnostic and therapeutic techniques, with significantly improved efficacy, are required. Electrochemotherapy (ECT) is a relatively new method of anticancer therapy [2]. This method offers an alternative treatment to conventional therapies, and it is becoming increasingly accepted as a therapeutic modality in oncology [3–7]. This technique is a combination of conventional chemotherapy with cell membrane electroporation (EP), increasing drug transport into cells.

In electrochemotherapy, cells exposed to EP may display 300–700-fold increase of intracellular drug concentration, as in case of bleomycin – the drug applied the most frequently in the electrochemotherapy, which is normally non-permeant through plasma membrane [8,9]. In ECT, a weakly permeant drug is generally absorbed by cancer cells and shows the effect only in the cancer cells, significantly reducing side effects of the standard systemic chemotherapy. An important contribution to the therapy efficiency comes from the vascular lock in ECT, which keeps elevated drug concentrations in the vicinity of the cancer for several hours [10]. The appropriate therapy conditions could limit necessity of surgical intervention, and give better prognoses in treatment of the tumors. In contrast to chemotherapy and

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radiation therapy, ECT has fewer adverse side effects and higher selectivity. The selective application of ECT coincides with the primary site of cells damage. Nowadays, researchers combine electroporation with bleomycin or cisplatin. In the latter case, the drug transport can be significantly enhanced, although plasma membrane is permeable for cisplatin, and cisplatin is also successfully used in standard therapies without electroporation. Other cytostatic drugs require advanced biological investigations before their use in ECT.

Nowadays, 5-FU is extensively used in the treatment of a wide range of solid tumors – its efficacy makes it one of the most widely used agents against solid tumors: colon, rectum, head and neck cancers, breast, stomach and pancreas carcinomas and infrequently ovarian cancer [11]. It has also been tested in ovarian cancer. Generally, despite widespread use of 5-FU, the single-agent response rates are only 10–30% [11,12]. Therefore, recent research focus on the biomodulation of 5-FU to improve its cytotoxicity and therapeutic effectiveness [11]. Furthermore, at the concentrations used in anticancer treatment, 5-FU leads to several toxicities: myelotoxicity, hand-foot syndrome (palmar-plantar erythrodysesthesia), stomatitis, and neuro- and cardiotoxicities, which are associated with continuous infusions [11,13]. Cisplatin is one of the most effective chemotherapeutic drug applied to treat several types of cancers, including some carcinomas (e.g. small cell lung cancer, and ovarian cancer), lymphomas, and germ cell tumors. The high number of side effects and acquired cancer cells resistance forces searching for new alternative solutions [14]. Hence, a potential dose reduction of 5-FU and cisplatin in the systemic treatment is of great interest. Additionally, electroporation probably can affect multidrug resistance mechanisms, enabling better drug absorption by cancer cells.

Nanopores formed in cell membrane during EP may cause faster and easier transport of drug molecules into cells, which results in faster response to the treatment [7,9]. An attempt of combining the 5-FU therapy with electroporation, on human gastric cancer cell line GCIY, showed that it could increase the therapy efficiency of ca. 25% [14].

The aim of this study was to evaluate the cytotoxic effect of 5-FU and cisplatin on two human ovarian cancer cell lines: OvBH-1 and SKOV-3. As a control, normal cells HGF's were used. Both cell lines are resistant to cisplatin treatment which is used in standard chemotherapy of ovarian cancers [15]. We test whether 5-FU could be an alternative to cisplatin for these cell lines, if electroporation is capable of reducing the effective drug dose, and how electroporation affects the transport of 5-FU and cisplatin into ovarian cancer cells, i.e. whether ECT with 5-FU or cisplatin can potentially be considered for treatment of those cancers.

2. Materials and methods

2.1. Cell lines

Human malignant cell line OvBH-1 was established at the Department of Clinical Immunology, Wroclaw Medical University, Poland, from ascetic fluid cells of a 54-year-old woman with ovarian clear cell adenocarcinoma. The tumor was poorly differentiated and the disease stage was FIGO IV. The patient had not received chemotherapy. The morphological and phenotypic characterization and clonal homogeneity of this cell line have been described before. The morphological and immunophenotypic characteristics as well as temperature-sensitive behavior of OvBH-1 cells have also been described previously [16]. The second cell line, SKOV-3, was a kind gift from Prof. J. Golab from Department of Immunology, Center of Biostructure Research and Medical University of Warsaw. HGF's (human gingival fibroblasts) cells were isolated according to the procedure described and patented by Saczko et al. (Patent N°.: P 3812045) [17]. HGF's and OvBH-1 were maintained in the growth medium: Dulbecco's modified Eagle' medium (DMEM, Sigma); SKOV-3 grew on McCoys' (Sigma) supplemented with 10% fetal calf serum (Cambrex) in 25 cm^2 TC flasks (Falcon) at 37 °C, in a 5% CO_2 humidified atmosphere.

2.2. Electropermeabilization (EP)

We performed EP with ECM 830 Square Wave Electroporation System (manufactured by BTX Harvard Apparatus). As electrodes, two aluminum parallel plates were used, 4 mm apart (BTX Harvard Apparatus). The electric field was applied in the series of amplitudes: 1000 ÷ 3000 V/cm, 5 impulses in a sequence, 50 μs each, at the frequency of 1 Hz. Suspension of cells was electroporated in 800 μL of electroporation buffer [18]. The cuvettes were first sterilized with 70% alcohol, and after that sterilized for 20 minutes with UV radiation.

2.3. Electropermeabilization efficiency – iodide propidium uptake (FACS)

EP of cells was quantified by penetration of impermeant dye – propidium iodide (PI, P4170, Sigma), using FACS. Immediately before electroporation, PI was added to the cuvette with the electroporation buffer at the concentration of 10 μmol/L. After electroporation, cells were incubated for 15 min at 37 °C in a humidified atmosphere containing 5% CO_2. Then cells were washed twice in PBS and resuspended in 1 mL PBS. Samples were analyzed immediately after permeabilization on a FACS Calibur flow cytometer (Becton Dickinson). At least 10,000 viable cells were measured from each sample at a rate up to 1000 cells/s. The samples were excited using the 488 nm line of argon laser and red detection of fluorescence was performed at 650 nm. Light-scatter and fluorescence measurements were used for indication of object size and shape, allowing discrimination between cells, microspheres, and debris. Data were analyzed using CellQuest software (Becton Dickinson) and presented as fluorescent emission intensities of positive cells.

2.4. Chemotherapy

First, cells were seeded into 96-well microculture plates and allowed to attach for 24 hours. Next, DMEM was removed and 5-FU (Sigma, Poland) or cisplatin (Sigma, Poland) added in adequate concentration (5-FU: 1–100 μM; cisplatin: 0.1–600 μM) for 24 hours or 72 hours continuous incubation with drug.

2.5. Electrochemotherapy (ECT)

We investigated the effect of electroporation on all cell lines, alone and with 5-FU or cisplatin. The electric field was applied in the series of amplitudes: 1000 ÷ 3000 V/cm, 5 impulses in a sequence, 50 μs each, at the frequency of 1 Hz. The electroporation conditions in ECT were selected according to the sensitivity of cells to the electric field. 5-FU was added to cell suspension before electroporation, at concentrations of 1, 5, 10, 20, 50 and 100 μM. Similarly, cisplatin was added to cell suspension before electroporation, at concentrations of 5, 25, 75 and 150 μM. After pulsation, cells were left for 10 minutes, centrifuged, than resuspended in culture medium with no drug, seeded into 96-well microculture plate and incubated for 24 hours or 72 hours in 37 °C.

2.6. MTT assay – determination of cells proliferation

The viability of OvBH-1 and SKOV-3 was examined using the viability test - MTT assay (Sigma, In Vitro Toxicology Assay), which indicates mitochondrial metabolic functioning (NADH activity).