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Proceeding Paper

Synergistic Effect of the Combination of the recombinant toxin DARPin-LoPE and PDT against HER2-positive breast cancer *in vitro*

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Abstract: A promising strategy to enhance the therapeutic effectiveness for the treatment of oncological diseases is development of combined therapeutic schemes. In our work, we showed the therapeutic potential of the combined action of the anticancer targeted toxin and PDT against HER2-positive breast cancer *in vitro*. Photodynamic treatment led to photoinduced cell death with IC50 0.64 μM and after incubation with the toxin for 48 h IC50 was 2.8 pM. When using two therapeutic agents at IC50 doses, it led to significant increase in the effectiveness; the viability of the combination-treated cell culture did not exceed 10%. The calculated combination index was 0.07 indicating a significant synergistic effect of the agents.

Keywords: targeted therapy; recombinant anticancer toxin; DARPin-LoPE; photodynamic therapy; Photodithazine; breast cancer; HER2 receptor

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1. Introduction

The human epidermal growth factor receptor 2 (HER-2) is overexpressed in 30% of breast cancer and associated with malignant cell transformation. Currently, targeted agents are used in clinical practice for the treatment of this cancer subtype [1]. Targeted agents block certain molecular pathways of the tumor cell that lead to the arrest of tumor proliferation and spread [2]. However, monotherapeutic approach has limited effectiveness due to the development of acquired drug resistance by tumor cells. A promising strategy to enhance the therapeutic effectiveness for the treatment of oncological diseases is development of combined schemes. This approach involves using several therapeutic agents with different mechanisms of action. It can lead to an additive or synergistic effect at a reduced therapeutic dose of each individual agent [3]. The high potential was recently shown for combination of targeted therapy and photodynamic treatment. Photodynamic therapy (PDT) is an attractive therapeutic approach for treatment of oncological diseases. PDT bases on interaction of a photosensitizer, light and molecular oxygen (O_2). A photosensitizer produces reactive oxygen species (ROS), which lead to an oxidative stresses in cells and their death [4].

In this work, we studied the therapeutic potential of the combined action of the anticancer targeted toxin and PDT against HER2-positive breast cancer *in vitro*.

2. Materials and Methods

2.1 Culturing of cell line

The experiments were performed on cell line of human breast adenocarcinoma SK-BR-3, which is characterized by HER2 overexpression. Cells were cultured in DMEM

medium with 10% (v/v) fetal calf serum and 2 mM L-glutamine in 5% CO_2 at 37°C. At each passaging stage, the cells were treated with Trypsin-EDTA (1:1) solution.

2.2 Study of Combination Cytotoxicity

For the cytotoxicity study, SK-BR-3 cells were seeded in a 96-well plate, 2000 cells per well, and grown overnight. The medium was then exchanged with the fresh one containing different concentrations of DARPin-LoPE [5], and the cells were incubated for 48 h. Then the medium was exchanged with the medium containing different concentrations of Photodithazine (PD, OOO VETA-GRAND, Russia). The cells were incubated with PD for 4 h and after changing the medium to the fresh one irradiated at a dose of 20 J/cm^2 (32 mW/cm^2 for 10 min 25 s) in the spectral range 655–675 nm using a light-emitting diode (LED) under thermostatically controlled conditions (37°C). The MTT assay was used for estimation the viability of cell cultures 24 hours after treatment. The combined action of targeted and photodynamic therapy was compared with monotherapeutic options.

3. Results and Discussion

The DARPin-LoPE is a recombinant protein agent, which consists of a targeting protein (DARPin9.29) and a cytotoxic moiety (LoPE). The DARPin9.29 provides high-affinity specific binding to the extracellular domain of HER2. The LoPE is low-immunogenic fragment of the Pseudomonas exotoxin A, which irreversibly blocks protein synthesis in the cell at the translation level [6]. We have shown that after incubation with DARPin-LoPE for 48 h the half-maximal inhibitory concentration IC_{50} was 2.8 pM. Photodynamic treatment with PD and irradiation at a dose of 20 J/cm^2 led to photoinduced cell death with IC_{50} 0.64 μM. Sequential application of two therapeutic agents at IC_{50} doses (Fig. 1a) led to significant increase in the effectiveness; the viability of the combination-treated cell culture did not exceed 10% (Fig. 1b).

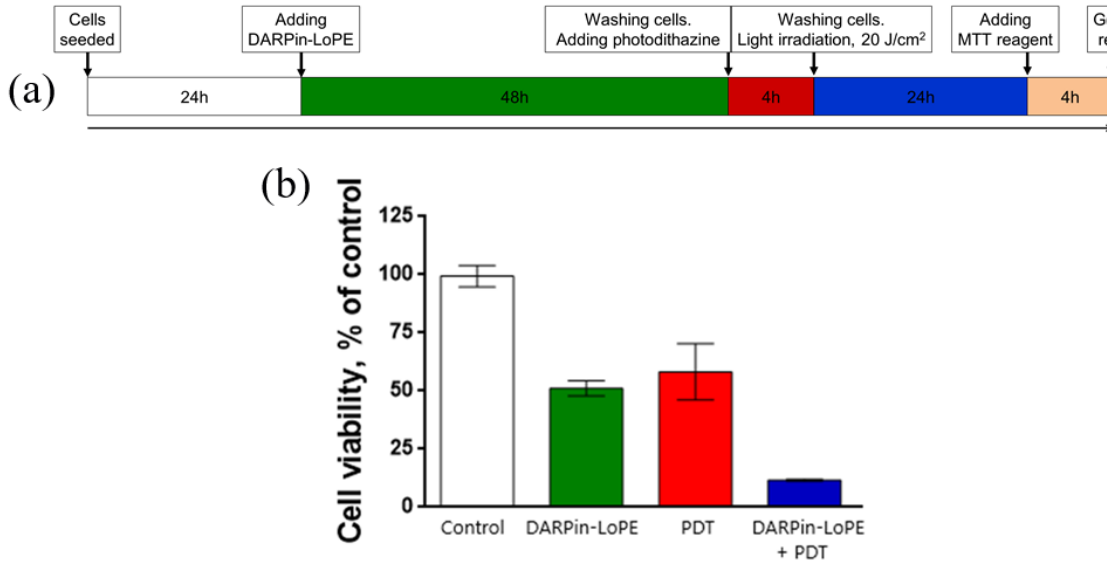

Figure 1. (a) Scheme of the experiment to evaluate the combined action of DARPin-LoPE and PDT (20 J/cm2) *in vitro*; (b) dependence of the relative viability of SK-BR-3 cells on the concentration of DARPin-LoPE and PDT under illumination at a dose of 20 J/cm^2 individually and after their combination at doses corresponding to IC_{50}.

To analyze the mode of the drug interaction, we compared the dose-response curves for DARPin-LoPE and Photodithazine at monotherapeutic or combined treatment. The ratio between agents' concentrations was maintained equal (1:250), which corresponds to the IC_{50} ratio (Fig. 2).

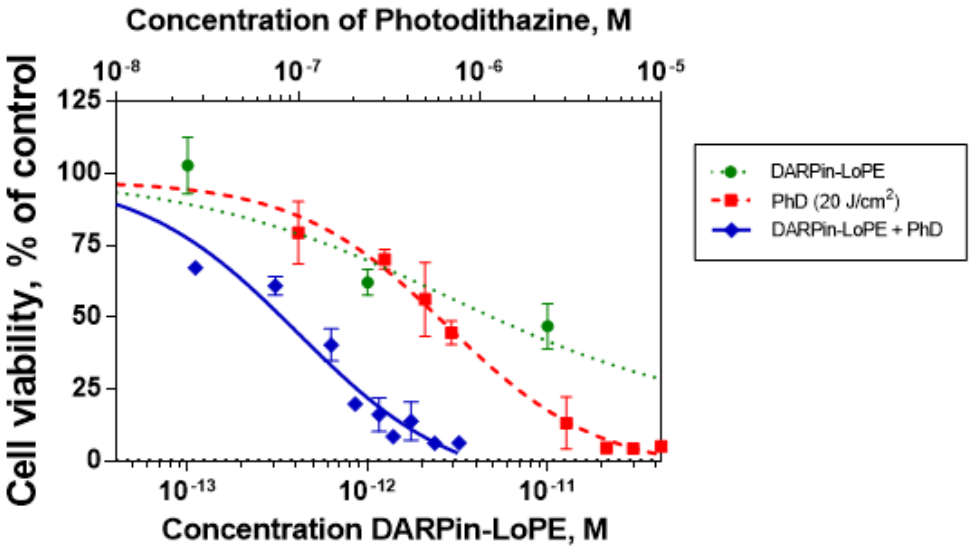

Figure 2. Relative viability of SK-BR-3 cell after the treatment with DARPin-LoPE and PDT (20 J/cm^2) and their combined effects.

The calculated combination index was 0.07 indicating a significant synergistic effect of the agents [7]. The half-maximal decrease in the viability of the SK-BR-3 cell culture was observed at concentrations of the toxin and photosensitizer that are virtually non-toxic to cells when the agents are used separately. We hypothesize that the protein synthesis arrest by DARPin-LoPE toxin is the main cause for sensitization of the cells to the subsequent PDT treatment.

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