Journal of Photochemistry & Photobiology, B: Biology 183 (2018) 266–274

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Verteporfin mediated sequence dependent combination therapy against ovarian cancer cell line

S. Sibel Erdem[a,b,*], Vildan Akgul Obeidin[c], Turkan Yigitbasi[c], S. Serranur Tumer[a], Pakize Yigit[d]

[a] İstanbul Medipol University, International School of Medicine, Medical Biochemistry, Istanbul, Turkey
[b] Regenerative and Restorative Medicine Research Center (REMER), Istanbul, Turkey
[c] İstanbul Medipol University, School of Medicine, Medical Biochemistry, Istanbul, Turkey
[d] İstanbul Medipol University, School of Medicine, Department of Biostatistics, Istanbul, Turkey

ARTICLE INFO

Keywords:
Photodynamic therapy
Combination therapy
Cationic peptide
Verteporfin
Ovarian Cancer

ABSTRACT

Ovarian Cancer is one of the deadliest gynecological cancer showing high resistance to chemotherapy. Non-overlapping and synergistic combination therapies are the best option to overcome this multi-pathological silent disease. Cationic peptides (CPs) with high targeting feature and ability to pass through cell membrane induce apoptosis via disruption of cancer cell membrane. Photodynamic Therapy (PDT) is a noninvasive clinically approved treatment modality combining light activated photosensitizer, light and oxygen. In this study we present, combination therapy composed of 9-mer +4 charge bearing CP and Benzoporphyrin derivative monoacid, (BPD-MA, Verteporfin) mediated PDT. In order to evaluate the effect of sequence on the outcome of the therapy, CP and BPD-MA mediated PDT was applied in two different sequence: 'CP first' 'BPD-MA first'. Treatment efficacy of combination therapy in SKOV-3 ovarian cancer cell line has been evaluated based on cell inhibition, cell death pathway, Combination index (CI), and Dose Reduction Index (DRI) values.

When SKOV-3 ovarian cancer cell line treated with BPD-MA mediated PDT (5 J/cm^2) and CP individually, IC30 values for each drug were determined as 1.1 μM and 240 μM respectively and apoptosis was the major death cell pathway for both of the drugs. In the case of combination therapy, SKOV-3 cell line treated with drugs in constant ratio yet on different sequence. Drugs were used in constant ratio so that one of them would not de-emphasize the effect of other in any concentration point. Our theoretical and experimental results were in agreement and showed that the treatment outcome significantly depends on the order of the treatment. For instance, while BPD-MA mediated PDT was applied prior to CP, cell inhibition at IC_{30} value of BPD-MA was roughly 28% with CI = 3.3 suggesting antagonistic interaction between each therapy. When the sequence of treatment was changed to CP first, cell inhibition at IC_{30} concentration of CP was determined as 98% with CI = 0.3 creating substantial synergism between the drugs. Moreover, synergistic interactions were observed at all concentration points at CP first scenario. DRI value for CP first treatment option was much higher compared to BPD-MA first treatment making the former treatment sequence more attractive option for clinically relevant combination therapies.

Based on our results, we strongly believe that 9-mer CP and BPD-MA-PDT based combination therapy, offering synergistic therapeutic outcome, may increase chances of treatment of ovarian cancer in comparison to 9-mer CP and/or BPD-MA alone case.

1. Introduction

Ovarian cancer is known as the most lethal gynecological cancer [11] and is the fifth most common cancer in woman. Due to the its silent progress, the disease is not diagnosed until it advances to stage III or IV, at which patient's 5 year survival rate drops to < 20% [3]. Due to its non-symptomatic advancement, high metastases rate, and its resistance to chemotherapy, treatment of ovarian cancer is a challenge for clinicians. Therefore, it is critical to develop new approaches and treatment options for ovarian cancer.

Photodynamic therapy (PDT) is FDA approved local and non-invasive treatment modality and is used in clinics for treatment of actinic keratosis, age related macular degeneration and various types of cancers [20]. PDT combines visible light, non-toxic dye, called photosensitizer, and oxygen to kill malignant cells. As a result of irradiation of photosensitizer with specific wavelength of light, cytotoxic species

* Corresponding author at: İstanbul Medipol University, International School of Medicine, Medical Biochemistry, Istanbul, Turkey.
E-mail address: serdem@medipol.edu.tr (S.S. Erdem).

https://doi.org/10.1016/j.jphotobiol.2018.04.039
Received 3 March 2018; Received in revised form 24 April 2018; Accepted 26 April 2018
Available online 01 May 2018
1011-1344/

such as reactive oxygen species (ROS) and/or singlet oxygen are generated via two different mechanisms [5]. Photosensitizer's preferential localization in the neoplastic tissue via enhanced permeability retention (EPR) effect and/or targeting agents as well as very short life time of newly generated singlet oxygen (10–320 ns) [8] along with its limited diffusion ability to only 10–55 nm in cell [8] make PDT local treatment only in the illuminated tissues. While PDT's immediate response is apoptosis or necrosis, long term effects of PDT is also very critical for cancer treatment. For instance, PDT damages the microvasculature and halts tumor's nutrient and oxygen supply. As a result of acute inflammation generated, immune response is triggered to recruit immune cells to the tumor site [5]. Combination of all these promotes cell death in the neoplastic tissue. In addition to be a safe and local treatment, PDT's one other advantage is that it can be easily combined with conventional therapies such as chemotherapy, radiotherapy and/or surgery to improve treatment outcome.

Combination therapy is a multi-component treatment option that is frequently used in clinics. Rationale behind the combination therapy is to engage multiple drugs or treatment modalities with different mode of actions in order to increase therapeutic effectiveness via achievement of synergism between the drugs. In addition, combination therapy aims to deliver not only the advantage of increased efficacy with dose reduction but also reduced toxicity to eliminate intolerable side effects. These therapeutic benefits have been widely utilized to develop new PDT based combination therapy regimens for cancer including but not limited to PDT/chemotherapy [18], PDT/radiation therapy [14], PDT/gene therapy [2], PDT/surgery [22] and PDT/immunotherapy [21]. In these PDT bearing combination therapies, PDT affects the treatment via two different pathways: It either suppresses survival signaling in resisting tumor cells or it deteriorates tumor cells which makes them more susceptible towards follow up PDT sessions [17].

One of the most important components of PDT is photosensitizer. To date, different generations of photosensitizers have been approved for clinical use and several novel agents' tests are being conducted in vitro as well as in clinics [4,15]. Among these photosensitizers, Benzoporphyrin derivative monoacid A (BPD-MA, Verteporfin) is a second generation photosensitizer approved for the PDT-based treatment of age related macular degeneration in US, Japan, most of the European Countries and Canada. Due to its favorable photophysical and photochemical properties BPD-MA has also been extensively studied as PDT agent for treatment of numerous cancer types [1,15,21].

Another way to induce apoptosis in cancer cells is to destroy flawless architecture of the cell membrane by employing cell membrane disrupting agents one of which is cationic peptides (CPs). CPs and their analogs are widely used to treat bacterial infections due to their ability to disrupt negatively charged cell membrane [13,24]. The same principle can be applied to cancer cells by targeting phosphatidylserine in the cell membrane. Unlike healthy mammalian cells in which mostly zwitterion phospholipids occupy the outer leaf of the membrane, pathological cells have elevated levels of negatively charged phosphatidylserine making the cell attractive to positively charged molecules [23]. As a result of electrostatic interactions, the cell membrane's permeability increases and/or apoptosis is induced [12,16].

The present study investigated the combination therapy composed of apoptotic, cationic 9 amino acid bearing peptide (CP) and BPD-MA against ovarian cancer cell line using Chou and Talalay analysis [6]. For the first time, we showed enhanced anti-tumor activity of CP when combined with BPD-MA mediated PDT. The combination effect of BPD-MA and CP, working in two different mechanism of action, showed that the sequence dependent combination therapy was much more effective compared to treatment results of PDT or CP alone.

2. Materials and Methods

2.1. Materials

Unless otherwise stated, all reagents and disposables were purchased from commercial sources. 9-mer CP composed of D amino acids (RLLLRIGRR-NH2) was purchased from GL Biochem. Human ovarian cells (SKOV-3) were purchased from ATCC (American Type Culture Collection). McCoy's medium supplemented with L-glutamine, penicillin, and streptomycin were purchased from Capricorn. BPD-MA was purchased from Sigma Aldrich. FBS was purchased from Biowest. MTT was purchased from Fischer. ApopTag® Peroxidase In Situ Apoptosis Detection Kit was purchased from Millipore. LysoHunt Green®, MitoHunt Green® and ER-Hunt Green organel markers were purchased from Setareh Biotech. As a light source, 150 W QTH lamp equipped with 610 nm long pass filter and liquid light guide was used. Electronic absorption spectra were measured on microplate reader (Moleclar Devices, Spectramax i3). Microscope images were taken using a laser scanning confocal microscope (Zeiss LSM 510 Meta).

2.2. Cell Cultures

SKOV-3 cells were maintained in McCoy's medium supplemented with L-glutamine (2 mmol/L), penicillin (100 U/mL), streptomycin (100 g/mL), and 10% FBS. The cells are incubated in 5% CO_2 incubator at 37 °C. The cells were subcultured twice weekly to maintain subconfluent stocks. In vitro cell studies were performed by seeding 10,000 SKOV-3 cells per well in a 96-well plate. All experiments were performed in triplicate.

2.3. Cytotoxicity of Apoptotic CP and BPD-MA

SKOV-3 cells (1×10^4 cells per well) were plated in a 96 well plate and allowed to adhere for 24 h. CP stock solution was prepared at 2.6 mM in water and diluted with McCoy's medium to yield 7 different concentrations ranging from 10 μM to 640 μM. The cells were incubated with the peptide for 24 h. The loading medium was removed and the wells were washed with medium. 100 μL of MTT solution (5 mg/mL in PBS) were then introduced to each well and allowed to incubate for another 3 h to yield formazan crystals. Following the removal of MTT solution, dimethyl sulfoxide (DMSO) (100 μL) was added to the crystals and the plate was left in shaker for 30 min. Absorbance intensity at 570 nm measured by a plate reader. The relative cell viability (%) was expressed as a percentage relative to the untreated control cells (100% viable) and the cells treated with detergent Triton X (0% viable). For BPD-MA, the cells were prepared as described. BPD-MA stock solution was prepared at 10 mM in negligible amount DMSO containing medium and further diluted with medium to yield 4 different concentrations ranging from 1 μM to 10 μM. The cells were incubated with BPD-MA for 90 min. and submitted to MTT assay as described above. Data analysis were done using Graphpad Prism software.

2.4. Phototoxicity

The cells were prepared as described in cytotoxicity assay. The cells were treated with 4 different concentrations of BPD-MA solution, ranging from 0.25 μM to 5 μM. After 90 min. Incubation, BPD-MA solution was aspirated and fresh medium was added to each well. The cells were exposed to 150 W QTH lamp filtered through a 610 nm long pass filter to provide 5 J/cm^2 light dose. Following light exposure (PDT), the cells were incubated for 24 h after which toxicity measured as described above. It is important to note that the conditions described both for cytotoxicity and phototoxicity above were chosen after having done