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AGNIESZKA CHWIŁKOWSKA[1], JOLANTA SACZKO[1], JULITA KULBACKA[1],
MATEUSZ ŁUGOWSKI[1], MAŁGORZATA DRĄG-ZALESIŃSKA[2], TERESA WYSOCKA[2],
TERESA BANAŚ[1]

Influence of Photofrin® on Survival and Apoptosis of the Jurkat Leukemic T-Cell Line Undergoing Photodynamic Therapy

Wpływ Photofrinu® na przeżywalność i apoptozę w ludzkich komórkach chłoniaka T-komórkowego poddanych terapii fotodynamicznej

[1] Department of Medical Biochemistry, Silesian Piasts University of Medicine in Wrocław, Poland
[2] Department of Histology and Embryology, Silesian Piasts University of Medicine in Wrocław, Poland

Abstract

Background. Photodynamic therapy (PDT) offers the advantage of an effective and selective method of malignant tissue destruction. In PDT, a tumor-selective photosensitizer is administered, followed by activation by exposure to a light source of suitable wavelength.
Objectives. The aim of this study was to evaluate the effect of PDT on the survival and apoptosis of the Jurkat leukemic T-cell line.
Material and Methods. Various incubation times with the photosensitizer before PDT, Photofrin® (Ph) concentrations, and irradiation doses were applied. Cell survival was examined by MTT assay and the alkaline comet assay was used to evaluate the mode of cell death.
Results. The results of the MTT assay showed that incubation time does not significantly affect mitochondrial function. Jurkat cell survival was dependent on Ph concentration and light dose. Results of the comet assay indicated that short incubation with Ph (1 h) induced apoptosis in leukemia cells.
Conclusions. Jurkat cells are sensitive to Ph-mediated PDT and undergo death by apoptosis (**Adv Clin Exp Med 2006, 15, 6, 999–1002**).

Key words: photodynamic therapy, Photofrin®, leukaemia cells, apoptosis, survival.

Streszczenie

Wprowadzenie. Terapia fotodynamiczna (PDT) jest metodą miejscowego, wybiórczego leczenia nowotworów oraz stanów przedrakowych za pomocą środków fotouczulających i światła o określonej długości fali. Jest to metoda pozwalająca na selektywne niszczenie nowotworu, bez uszkadzania zdrowych tkanek.
Cel pracy. Oceniano wpływ terapii fotodynamicznej z zastosowaniem Photofrinu® (Ph) na przeżywalność i apoptozę komórek chłoniaka T-komórkowego Jurkat.
Materiał i metody. Zastosowano różne czasy inkubacji z Ph, różne stężenia fotouczulacza i dawka naświetlania. Przeżywalność komórek oceniano testem MTT, apoptozę w komórkach badano metodą kometkową.
Wyniki. Badanie aktywności oksydoredukcyjnej mitochondriów wykazało, że czas inkubacji nie ma znaczącego wpływu na przeżywalności komórek. Przeżywalność komórek chłoniaka zależała od stężenia Ph i dawki światła. Wyniki uzyskane z testu kometkowego wskazują, że już krótki czas inkubacji indukuje w komórkach apoptozę.
Wnioski. Komórki Jurkat są wrażliwe na PDT z użyciem Ph oraz są uśmiercane na drodze apoptozy (**Adv Clin Exp Med 2006, 15, 6, 999–1002**).

Słowa kluczowe: terapia fotodynamiczna, Photofrin®, chłoniak T-komórkowy, apoptoza, przeżywalność.

The primary objective of photodynamic therapy (PDT) is to kill unwanted tumor cells. PDT causes hardly perceptible damage to surrounding healthy tissues in comparison with current anticancer treatment methods. The light-activated photosensitizer leads to a massive generation of reactive oxygen species (ROS) which consequently induces oxidative stress in the malignant cells [1]. These events generate cell structure destruction. Primary antioxidant enzymes protect cells from oxidative stress. Oxidative stress-induced mitochondrial damage is the key event involved in the apoptosis, resulting in morphological and nuclear apoptotic changes [2]. Appropriate PDT conditions could change the redox balance and initiate cell death, particularly via apoptosis [3]. The main purpose of this study was to estimate the dependencies between the parameters of PDT which contribute to the efficacy of the therapy. The MTT assay was employed to examine the cytotoxic effect of PDT by evaluating mitochondrial function. The alkaline comet assay detected the DNA fragmentation associated with apoptosis.

Material and Methods

Cell Line

The Jurkat human leukemic T-cell line established from the peripheral blood of a 14-year-old boy by Schneider et al. [4] was used.

Cell Line Culture

Cells were cultured in RPMI complete growth medium with addition of 10% fetal bovine serum in 25-cm^2 flasks. The cells were maintained in a humidified atmosphere at 37°C with 5% CO_2. RPMI 1640 medium was adjusted to contain 1.5 g/l sodium bicarbonate, 4.5 g/l glucose, 10 mM HEPES, and 1.0 mM sodium pyruvate.

Photodynamic Treatment

The cells were treated with various concentrations (30, 15, and 6 μg/ml) of Photofrin® (QLT PhotoTerapeutics, Inc., Vancouver, Canada) in complete media in the dark. They were then irradiated with 1.5, 3.5, and 7 J/cm^2 using a lamp (OPTEL, Poland) with a red filter (λ_{max} = 632.8 nm). The fluence rate at the level of the cell monolayer was 10 mW/cm^2. All irradiations were performed at room temperature. After irradiation, the cells were incubated in a humidified atmosphere at 37°C with 5% CO_2.

MTT Assay

The cell response to Ph-PDT was measured by the MTT assay (TOX-1, Sigma). The cells were grown in 25-cm^2 flasks, then incubated from 1 to 4 h with Ph. After PDT the cells were diluted in PBS and plated in 96-well microtiter plates. The absorbance of each well was determined after two hours using a multiwell scanning spectrophotometer at 570 nm (Multiscan MS microplate reader).

Alkaline Comet Assay

For the detection of DNA fragmentation associated with apoptosis, the alkaline comet assay method was used [5]. Cells were incubated for 0, 1, 5, and 24 h with Ph (30 μg/ml), irradiated (3 J/cm^2), and then washed in PBS three times. Cells at a concentration of 1x10^5/ml were mixed with low-melting-point agarose (Sigma) at a ratio of 1:10 (v/v) and spread on a slide. The slides were submerged in pre-cooled lysis solution (2.5 M NaCl, 100 mM EDTA, pH 10, 10 mM Tris base and 1% Triton X-100) at 4°C for 60 min. After lysis and rinsing, the slides were equilibrated in TBE solution (40 mM Tris/boric acid, 2 mM EDTA, pH 8.3), electrophoresed at 1.0 V/cm^2 for 20 min, and then silver staining was performed [6]. For scoring the comet pattern, 100–200 nuclei on each slide were counted.

Results

The effects of different incubation times with Ph, doses of irradiation, and concentrations of photosensitizer on Jurkat cell survival were studied. The mode of cell death was also investigated in order to devise strategies to enhance the cell killing efficiency of PhotofrinPDT. The results of the MTT assay showed that the time of incubation did not significantly affect mitochondrial function (Fig. 1). After 4 hours incubation with Ph the percentage of Jurkat cell survival was dependent on Ph concentration and light dose. The highest survival was observed for 6 μg/ml of Ph; for all light doses the cell viability was higher than 60%. The lowest cell survival was noted for 30 μg/ml of Ph: about 30% of the control level at 1.5 J/cm^2, decreasing to 4.61% at 7 J/cm^2 (Fig. 2).

Discussion

According to these experiments, cell survival during PDT is dependent on the photosensitizer concentration and the amount of light. Similar studies were performed on the HL60 cell line with ALA-PDT (5-aminolevulinic acid-PDT). The influence of incubation time with ALA, irradiation wavelength and dose, and the concentration of photosensitizer was investigated. In contrast to our

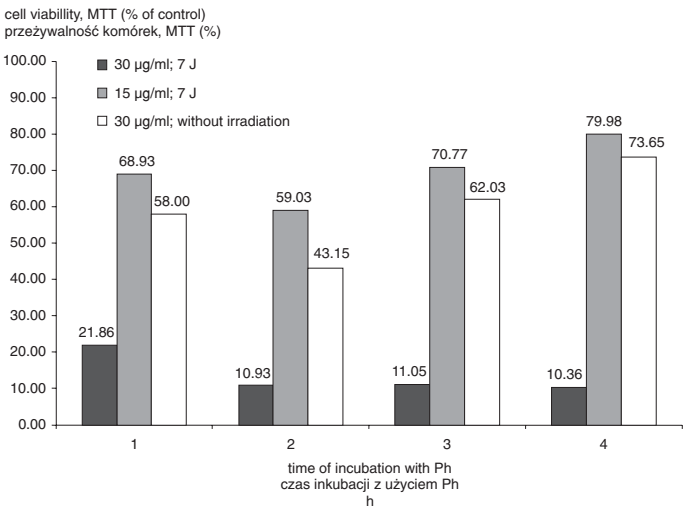

Fig. 1. Viability of Jurkat cells subjected to Ph-PDT. Cells were incubated from 1 to 4 h with Ph (15 or 30 µg/ml), irradiated (7 J/cm^2), and their viability was determined using the MTT assay as described. Viability is expressed as the percentage of the control cells (non-irradiated and without Ph)

Ryc. 1. Wpływ czasu inkubacji komórek z Ph na ich przeżywalność. Komórki inkubowano 1–4 godz. z Ph (15 lub 30 µg/ml), naświetlano (7 J/cm^2), następnie przeprowadzono test MTT według instrukcji. Wyniki podano jako procent komórek kontrolnych (nienaświetlanych bez Ph)

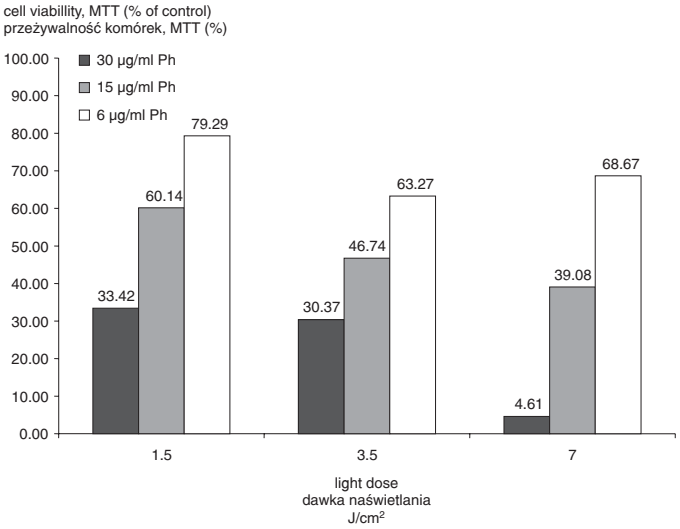

Fig. 2. Viability of Jurkat cells subjected to Ph-PDT. Cells were incubated 4 h with different Ph concentration (6, 15, or 30 µg/ml) and light dose (1.5, 3.5, and 7 J/cm^2), followed by determination of viability using the MTT assay as described. Viability is expressed as the percentage of the control cells (non-irradiated and without Ph)

Ryc. 2. Wpływ stężenia fotouczulacza i dawki światła na przeżywalność komórek. Komórki inkubowano 4 godz. z Ph o różnym stężeniu (6, 15 i 30 µg/ml) i naświetlano (1,5; 3,5 i 7 J/cm2), następnie przeprowadzono test MTT według instrukcji. Wyniki podano jako procent komórek kontrolnych (nienaświetlanych bez Ph)

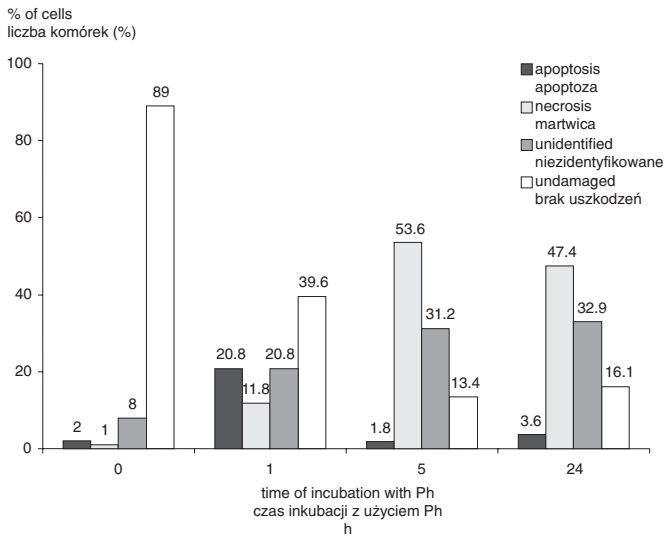

Fig. 3. The Effect of Ph-PDT on DNA degradation in Jurkat cells. Cells were incubated 0, 1, 5, and 24 h with Ph (30 µg/ml), irradiated (3 J/cm^2) and then DNA degradation was determined using the comet assay as described

Ryc. 3. Badanie degradacji DNA metodą kometkową w komórkach Jurkat poddanych Ph-PDT. Komórki inkubowano 0, 1, 5 i 24 godz. z Ph (30 µg/ml), naświetlano (3 J/cm2), następnie badano degradację DNA jak opisano w rozdziale Materiał i metody

studies, the influence of incubation time with photosensitizer was significant. In accord with the present data it was shown that the concentration of photosensitizer and irradiation fluence rate fulfilled the crucial roles in the effectiveness of photodynamic therapy [7]. Tremblay et al. [8] showed that Jurkat cell viability did not depend on TBO (toluidine blue) concentration (0.15 and 0.5 µg/ml) at a light dose of 11 J/cm^2.

The MTT test provides some information concerning the function of mitochondria. Therefore late, irreversible changes that point to the mode of

cell death were not assessed. Mitochondria-localized photosensitizers are able to induce apoptosis very rapidly [9].

It was noticed in this study that, according to the alkaline comet assay, the highest percentage of apoptotic cells (20.8%) was after 1 h of incubation with Ph (Fig. 3). The number of undamaged cells decreased to 40%. Longer incubation times (5 and 24 h) induced increases in necrotic cells (53.6% and 47.4%, respectively) and the number of apoptotic cells decreased to the control value (Fig. 3). Tremblay et al. [8] also observed increases in apoptotic cells with increasing incubation times with photosensitizer and its concentration. Woods et al. [10] applied the comet assay to indicate Ph–PDT-induced DNA damage and observed apoptosis at very low doses of photosensitizer (1 μg/ml). Gad and colleagues noted that the percentage of DNA fragmentation increased with increasing doses of ALA, red fluence, as well as longer incubation time with ALA [11]. They observed DNA fragmentation as early as three hours after ALA-PDT.

In conclusion, the present study showed that Ph-based photodynamic therapy induces phototoxicity and apoptosis in leukemic Jurkat cells. Determining the precise time to administer photosensitizer to tissue during therapy is difficult. Another problematic issue is determining the quantity of the photosensitizer in malignant tissue after intravenous delivery. These *in vitro* experiments suggest that clinical applications of PDT with Photofrin injection might be useful. The efficacy of Ph-PDT requires further studies in the treatment of various classes of tumor.

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Address for correspondence:

Agnieszka Chwiłkowska
Department of Medical Biochemistry,
Silesian Piasts University of Medicine
Chałubińskiego 10
50-368 Wrocław
Poland
Tel.: +48 71 784 13 75
fax: +48 71 784 00 85
E-mail: achwil@bioch.am.wroc.pl

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