ORIGINAL PAPERS

Adv Clin Exp Med 2008, **17**, 6, 599–605
ISSN 1230-025X

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The Effect of Photodynamic Treatment on the Levels of Aldehydic Lipid Peroxidation Products in Human Tumor Cells

Wpływ reakcji fotodynamicznej na zawartość aldehydowych produktów peroksydacji lipidów w ludzkich komórkach nowotworowych

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Abstract

Background. Reactive oxygen species generated during photodynamic treatment (PDT) initiate membrane lipid peroxidation, leading to the formation of saturated and unsaturated aldehydes which act as bioactive molecules under physiological and pathological conditions. In pigmented melanoma cells this process is influenced by melanin.
Objectives. The aim was to investigate the relationship of particular aldehyde levels in pigmented and unpigmented tumor cells and their survival after PDT.
Material and Methods. The survival of human melanoma (SK-MEL) and human colon adenocarcinoma (Caco-2) cells after incubation with delta-aminolevulinic acid (5-ALA) or illumination with visible light in the presence or absence of the photosensitizer was measured by a fluorimetric assay based on DAPI staining. Aldehydic lipid peroxidation products were determined as their dinitrophenylhydrazone derivatives in the cells by HPLC/MS/MS with atmospheric pressure ionization.
Results. After PDT nearly 60% of the Caco-2 cells were killed, while PDT insignificantly influenced the survival of SK-MEL cells. There was also no significant difference in cell survival and aldehydic lipid peroxidation product levels of cells exposed to 5-ALA or light in both cell lines. Aldehyde levels in Caco-2 cells significantly increased after PDT compared with the control, particularly of 4-hydroxyhexenal (3.9 times) and 4-hydroxynonenal (5.7 times). In contrast, PDT caused a statistically significant increase in SK-MEL cells only in 4-hydroxynonenal (2.0 times). The other aldehyde levels in these cells remained almost unchanged.
Conclusions. The observed increases in aldehyde levels, especially of 4-hydroxyalkenals, correlated with decreased cell survival, particularly of Caco-2 cells, which do not contain melanin. Excessive formation of 4-hydroxyalkenals and other highly reactive peroxidation products should be considered when developing photodynamic treatment protocols (**Adv Clin Exp Med 2008, 17, 6, 599–605**).

Key words: lipid peroxidation, photodynamic treatment, tumor.

Streszczenie

Wprowadzenie. Wytwarzane podczas reakcji fotodynamicznej reaktywne formy tlenu inicjują proces peroksydacji lipidów błonowych, który prowadzi do powstania biologicznie aktywnych, zarówno w warunkach fizjologicznych, jak i patologicznych, nasyconych i nienasyconych aldehydów. W upigmentowanych komórkach czerniaka na proces ten wpływa melanina.
Cel pracy. Zbadanie zależności między przeżywalnością upigmentowanych i nieupigmentowanych komórek nowotworowych poddanych reakcjom fotodynamicznym a ilością wytworzonych aldehydów.
Materiał i metody. Przeżywalność ludzkich komórek nowotworowych czerniaka (SK-MEL) i gruczolakoraka jelita grubego (Caco-2) poddanych inkubacji z 5-ALA, naświetlaniu w obecności i nieobecności fotouczulacza mierzono, stosując metodę fluorymetryczną po wybarwieniu komórek fluorochromem DAPI. Aldehydowe produkty peroksydacji lipidów po przekształceniu ich w dinitrofenylohydrazony oznaczano techniką HPLC/MS/MS z zastosowaniem jonizacji w ciśnieniu atmosferycznym.
Wyniki. W odróżnieniu od komórek Caco-2, gdzie prawie 60% komórek zostało zniszczonych wskutek reakcji fotodynamicznej, w komórkach SK-MEL nie obserwowano istotnych zmian w ich przeżywalności. W obydwu li-

niach komórkowych poddanych działaniu kwasu delta-aminolewulinowego lub naświetlaniu nie stwierdzono różnic w przeżywalności komórek i w zawartości aldehydowych produktów peroksydacji lipidów. W komórkach Caco-2 poddanych reakcji fotodynamicznej, w odniesieniu do grupy kontrolnej, znacząco zwiększyła się zawartość aldehydów, szczególnie 4-hydroxyheksenalu (3,9-krotnie) i 4-hydroksynonenalu (5,7-krotnie). W wyniku reakcji fotodynamicznej w komórkach SK-MEL znacząco zwiększyła się tylko zawartość 4-hydroksynonenalu (2-krotnie). Zawartość pozostałych oznaczanych aldehydów nie wykazała statystycznie istotnych zmian.
Wnioski. Zaobserwowano, że wzrostowi zawartości poszczególnych aldehydów, zwłaszcza 4-hydroksyalkenali, towarzyszył spadek przeżywalności komórek, w szczególności niezawierających melaniny komórek Caco-2. Powstawanie tych wysoko reaktywnych związków należałoby uwzględniać podczas ustalania warunków terapii fotodynamicznej (**Adv Clin Exp Med 2008, 17, 6, 599–605**).

Słowa kluczowe: peroksydacja lipidów, terapia fotodynamiczna, nowotwory.

Photodynamic lipid peroxidation has been associated with pathological conditions such as phototoxic skin injury and carcinogenesis as well as therapeutic treatments such as antitumor photodynamic therapy. During photodynamic therapy, the specific accumulation of a photosensitizer in the tumor tissue and its subsequent illumination at appropriate wavelengths induce the production of reactive oxygen species, predominantly singlet oxygen, responsible for the peroxidation of cell organelles and cell death. Cell death caused by photodynamic therapy can occur either by apoptosis and/or necrosis, depending on the cell type, the concentration and intracellular localization of the sensitizer, and the light dose [1–2]. Reactive oxygen species generated during photodynamic therapy can initiate the process of membrane lipid peroxidation, which leads to the formation of a complex of saturated and unsaturated aldehydes and other carbonyl compounds of different chain lengths, mainly secondary aldehydic lipid peroxidation products. These compounds contribute to peroxidative cell damage by inhibiting DNA, RNA, protein synthesis, and cell respiration and by depleting glutathione [3]. They are sufficiently long-lived to damage target molecules distant from the site of their formation and are capable of impairing protein function, causing cell lysis and affecting cellular division [4].

Aldehydic lipid peroxidation products, especially the 4-hydroxyalkenals (HAKs), due to their high reactivity, display marked biological effects in a concentration-dependent manner. The two most toxic 4-hydroxylkenals are 4-hydroxynonenal (HNE) and 4-hydroxyhexenal (HHE). Experimental and clinical evidence suggests that HAKs can act as bioactive molecules under physiological and/or pathological conditions. These compounds can affect and modulate, even at very low, nontoxic concentrations, several cell functions, including signal transduction, gene expression, cell proliferation and differentiation, cellular growth inhibition, and apoptosis [5–7].

Unfortunately, data pertaining to the amounts of different aldehydes in tumor cells after photodynamic therapy are very limited. In pigmented cells, melanin is believed to protect against oxidative damage by acting as an antioxidant neutralizing oxidizing radicals and other reactive oxygen species which may be generated via chemical and photochemical reactions [8]. Therefore it was decided, using cultured cells under laboratory conditions, to determine aldehydic lipid peroxidation product levels in pigmented human melanoma cells (SK-MEL) and human colon adenocarcinoma cells (Caco-2), which do not contain melanin, illuminated with visible light or not illuminated and in the presence or absence of delta-aminolevulinic acid (5-ALA), used as a photosensitizer. The purpose of this study was to investigate the relationship of particular aldehydes levels in pigmented and unpigmented tumor cells and their survival after photodynamic treatment (PDT).

Material and Methods

Cell Cultures and Photodynamic Treatment

The human colon adenocarcinoma cell line Caco-2 was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ). The pigmented human melanoma SK-MEL cells were obtained from Jagiellonian University of Cracow (Poland). Both cell lines were grown in monolayer cultures in Ham's F-10 (Sigma) and RPMI (Gibco) medium, respectively. Both media were supplemented with 10% heat-inactivated bovine serum (FBS, Gibco), 100 U/ml penicillin, and 100 μg/ml streptomycin. The cells were grown at 37°C in a humidified 5% CO_2 atmosphere. The cells were plated on 96-well plates (Nunc) at a density of 5000/well. After 24 hours the exponentially growing cells were incubated with freshly prepared 5-ALA solution (at a final concentration of 100 μg/ml; Wirkstoff) in serum-free medium at 37°C in the dark for 4 h. Then the cells were washed twice with phosphate-buffered saline (PBS) and fresh serum-free medium was added followed by exposure to a PDT TP-1 inco-

herent light source (400–750 nm, Cosmedico Medizintechnik GmbH, Schwenningen, Germany). The light intensity at the level of the cells was 77 mW/cm^2, giving a total light dose of 18.48 J/cm^2 during a 4-minute illumination period. After incubation with 5-ALA or after illumination with visible light in the presence or absence of this photosensitizer, the culture medium was removed and the cells were cultured using fresh medium for 24 hours as described above. Controls consisted of untreated native cells and medium alone.

Quantification of Cell Survival

Cell survival was evaluated at 24 h post-treatment on a 96-well culture plate using a fluorimetric assay based on DAPI-staining [9]. The cells were washed three times with PBS, fixed in 10% formalin-buffered saline for 10 minutes, and subsequently stained with 4',6-dianidino-2-phenylindole dihydrochloride (DAPI, 1 μg/ml, Sigma). After 30 minutes the fluorescence was measured using a Wallac 1420 VICTOR2™ (Perkin Elmer) fluorescence plate reader at 460 nm after excitation at 355 nm. In all experiments the cytostatic/cytotoxic effect was expressed as the relative survival of tumor cells (percent of control cultures incubated with medium only) and was calculated as follows: relative survival = (Ae – – Ab)/(Ac – Ab) × 100, where Ab is the background fluorescence, Ae the experimental fluorescence, and Ac the fluorescence of the untreated controls.

Preparation of the Dinitrophenylhydrazone Derivatives

DNP derivatives of the standard aldehydes were prepared according to the commonly used method described by Esterbauer et al. [10]. After 24 hours in culture, the cells were washed twice with PBS, harvested by scraping, centrifuged (10,000 *g*, 10 min), and immediately frozen in liquid nitrogen. The frozen cells (40–60 mg) were mixed with 1 ml methanol, vortexed (10 minutes) for extraction, and centrifuged (10,000 *g*, 10 minutes). The extraction was repeated two times. The supernatants were combined and added to an equal volume of the DNPH reagent. Derivatization was carried out for 1 hour in the dark at room temperature. The DNPH reagent was freshly prepared by dissolving 35 mg of 2,4-dinitrophenylhydrazine in 100 ml of 1 M HCl and extracted two times with 50 ml of hexane to remove impurities. The obtained derivatives, after subsequent centrifugation (10,000 × *g*, 15 minutes), were dissolved in 0.3 ml of acetonitrile and filtered (0.22 μm, Millipore). This procedure was adapted from a similar method proposed for malondialdehyde (MDA) and HNE derivatization by Deighton et al. [11].

Liquid Chromatography/Mass Spectrometry Determination

The 20-μl samples containing DNP derivatives were injected on a C-18 column (Saulentechnik Knauer; 250 × 2 mm, 5 μm) and separated using a gradient mixture of water, methanol, and acetonitrile acidified with formic acid on an HP1100 HPLC system with a diode array detector from which the effluent was directed to an API 2000 PE-Sciex mass spectrometer. The following methanol:acetonitryle:water:formic acid gradient program was used for elution: 0 min. 20:30:50:0.1, 10 min. 20:60:20:0.1, and 14 min. 20:80:0:0.1. The other chromatographic parameters were: flow rate 0.4 ml/min, temperature 35°C, run time 35 min., post time 12 min., and DAD detection range 200–600 nm.

The mass spectrometer was equipped with an atmospheric pressure ionization source and worked in the negative ionization mode. The ion source and detector parameters were identical in each run (CUR 50, IS –3800 V, TEM 350, GS1 60, GS2 85, CAD 2, CE 18 eV, DP –25, FP –200, EP 10). Detection was performed in multiple reaction monitoring (MRM) mode using characteristic mass pairs specific to each analyzed compound (m/z 267→182 for 2-hydroxybutanal, m/z 295→182 for 2-hydroxyhexanal, m/z 293→167 for 4-hydroxyhexenal, m/z 309→182 for 2-hydroxyheptanal, m/z 335→167 for 4-hydroxynonenal, m/z 265→163 for pentanal, and m/z 279→163 for hexanal).

Statistical Analysis

The data obtained from three independent series of experiments (each in triplicate) were expressed as mean values ± standard deviations. Statistical significance analysis was based on analysis of variance (ANOVA) followed by Tukey's HSD test. A *P*-value of less than 0.05 was considered significant. Statistical analysis was performed using Statistica 6 PL software for Windows (StatSoft, Poland).

Results

Effect of PDT on Cell Survival

Figure 1 shows the cell survival of Caco-2 and SK-MEL cells after 5-ALA incubation and after

illumination with visible light in the presence or absence of the photosensitizer. There was no significant difference in Caco-2 cell survival of cells exposed to 5-ALA and cells illuminated with visible light in the absence of the photosensitizer ($P > 0.05$). After PDT, nearly 60% of the Caco-2 cells were killed. In contrast, SK-MEL cells were less sensitive to this treatment. The effects of 5-ALA and of illumination with visible light in the presence or absence of the photosensitizer on the survival of SK-MEL cells were not significant ($P > 0.05$).

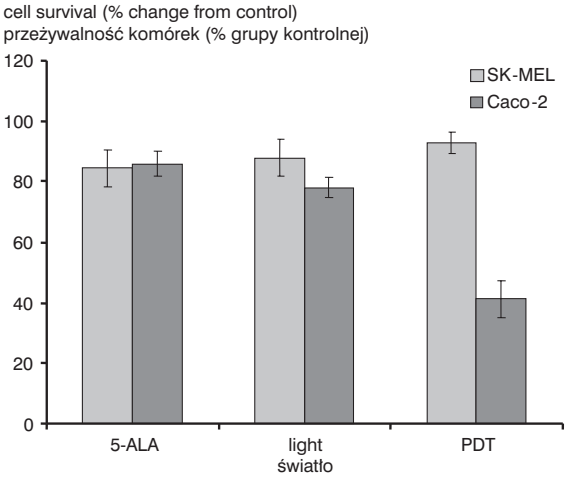

Fig. 1. Survival (% change from control) of Caco-2 and SK-MEL cells after 5-ALA incubation (5-ALA) or after illumination with visible light in the absence (light) or presence (PDT) of the photosensitizer. Data are expressed as the means ± *SD* from three independent series of experiments

Ryc. 1. Przeżywalność (% zmian względem grupy kontrolnej) komórek Caco-2 i SK-MEL poddanych inkubacji z 5-ALA (5-ALA), naświetlaniu w nieobecności (światło) i obecności fotouczulacza (PDT). Wyniki są wyrażone jako średnie arytmetyczne ± SD trzech niezależnych serii eksperymentów

Effect of PDT on Aldehydic Lipid Peroxidation Product Levels

The effects of 5-ALA or illumination with visible light in the presence or absence of the photosensitizer on the formation of aldehydic lipid peroxidation products in Caco-2, and SK-MEL cells are summarized in Tables 1 and 2. There were no significant differences in aldehydic lipid peroxidation product levels in cells exposed to 5-ALA or illuminated with visible light without the photosensitizer in both cell lines ($P > 0.05$). After PDT the amounts of the aldehydes in Caco-2 cells significantly increased compared with controls (2-hydroxybutanal 1.9 times, 2-hydroxyhexanal 1.9 times, 4-hydroxyhexenal 3.9 times, 2-hydroxyheptanal 2.1 times, 4-hydroxynonenal 5.7 times, and n-hexanal 1.7 times), whereas n-pentanal, and n-hexenal levels remained unchanged. In contrast, PDT caused a significant increase only in 4-hydroxynonenal (2.0 times) in SK-MEL cells.

Discussion

In 5-aminolevulinic acid-based photodynamic treatment, 5-ALA taken up by tumor cells is metabolized to protoporphyrin IX, which acts as a sensitizer of photooxidative damage leading to cell death. The exact mechanism of tumor destruction is not entirely clear, but evidence suggests that multiple mechanisms contribute to the clinical efficacy of photodynamic therapy. Its antitumor effect results from three interdependent processes: direct tumor cell killing, damage to the vasculature, and activation of a nonspecific immune response [12–14]. Direct cellular damage occurs via the generation of radical oxygen species and subsequent lipid peroxidation. In pigmented tumors, melanin, a free radical scavenger, additionally influences the behavior and effect of reactive oxygen species [8].

Several methods have been developed to measure lipid peroxidation products and lipid peroxidation damage in tissues, cells, and body fluids [15]. Malondialdehyde (MDA) reactivity with 2-thiobarbituric acid (TBA) is the basic analytical method for evaluating lipid peroxidation. The specificity of this method can be questioned because aldehydes other than MDA can also react with TBA. Moreover, TBA assay conditions, such as high temperature and low pH, may themselves cause oxidation of lipids [16]. Therefore, several analytical methods have been proposed using chromatographic techniques coupled with sensitive detectors for a direct determination of aldehydic products in biological systems [15–17]. In this study, the HPLC/MS/MS method was applied for the quantitative analysis of aldehydic lipid peroxidation products extracted from tumor cells after PDT. This is the first study in which analysis of DNP derivatives was used to assess lipid peroxidation after photodynamic treatment.

Data have been accumulated on the association of PDT-induced lipid peroxidation, measured as MDA formation, and survival of different cell lines [18–19]. Sakharov et al. [20] found that lipid peroxidation in cells did not happen instantaneously during PDT, but continued to increase during a 15–30 min period. PDT can generate lipid

Table 1. Aldehydic lipid peroxidation product levels after 5-ALA incubation (5-ALA) or illumination with visible light in the absence (light) or presence (PDT) of the photosensitizer in human colon adenocarcinoma cells (Caco-2). The amounts of DNP-aldehydes are expressed as mean values ± *SD* from three independent series of experiments

Tabela 1. Zawartość aldehydowych produktów peroksydacji lipidów w ludzkich komórkach gruczolakoraka jelita grubego (Caco-2) poddanych inkubacji z 5-ALA (5-ALA), naświetlaniu w nieobecności (światło) i obecności fotouczulacza (PDT). Ilości DNP-aldehydów są wyrażone jako średnie arytmetyczne ± SD trzech niezależnych serii eksperymentów

Amounts of DNP-aldehydes [nmol/g of cells] (Ilości DNP-aldehydów [nmol/g komórek])	Control (Grupa kontrolna)	5-ALA (5-ALA)	Light (Światło)	PDT (Obecność fotouczulacza)
2-hydroxybutanal (2-hydroksybutanal)	3.78 ± 0.20	4.10 ± 0.09	3.84 ± 0.16	7.10 ± 0.06 a
2-hydroxyhexanal (2-hydroksyheksanal)	0.52 ± 0.03	0.58 ± 0.07	0.64 ± 0.07	0.99 ± 0.12 a
4-hydroxyhexenal (4-hydroksyheksenal)	1.82 ± 0.17	2.01 ± 0.25	2.36 ± 0.06	7.17 ± 0.23 a
2-hydroxyheptanal (2-hydroksyheptanal)	2.59 ± 0.23	2.97 ± 0.29	3.12 ± 0.24	5.43 ± 0.45 a
4-hydroxynonenal (4-hydroksynonenal)	0.08 ± 0.01	0.13 ± 0.04	0.14 ± 0.06	0.43 ± 0.05 a
n-pentanal (n-pentanal)	3.15 ± 0.53	3.27 ± 0.25	3.26 ± 0.38	3.79 ± 0.33
n-hexenal (n-heksenal)	0.04 ± 0.02	0.05 ± 0.02	0.04 ± 0.01	0.12 ± 0.08
n-hexanal (n-heksanal)	0.66 ± 0.07	0.71 ± 0.07	0.76 ± 0.07	1.12 ± 0.19 a

a – significant difference in comparison with control, 5-ALA, and light.

a – istotna statystycznie różnica w porównaniu z grupą kontrolną, 5-ALA i światłem.

and cholesterol hydroperoxides capable of inducing apoptosis, which can be prevented by antioxidants added to the cells during or immediately after PDT [21]. Moreover, phospholipid hydroperoxide glutathione peroxidase provided significant protection against singlet oxygen-generated lipid peroxidation via removal of lipid hydroperoxides (LOOHs), major mediators in this cell injury process [22]. On the other hand, supplementation of tumor cells with unsaturated fatty acid, such as linoleic acid, γ-linolenic acid, and arachidonic acid, can act as an amplification factor for photofrin-mediated photodynamic therapy. These specific polyunsaturated fatty acids, via generation of the largest amounts of LOOHs or thiobarbituric acid-reactive substances (TBARS), may be potential tools for improving the efficacy of PDT [23]. Although the induction of lipid peroxidation is not a general mechanism responsible for the cytotoxicity of 5-ALA-PDT, it may be important in cell lines with an inherent sensitivity to lipid peroxidation products [18].

The oxidation of polyunsaturated fatty acids during oxidative stress gives rise to a series of toxic unsaturated aldehydes. Of these, 4-hydroxynonenal and 4-hydroxyhexenal have been shown to mediate oxidative stress-induced cell death in many cell lines [24–25]. The results of the present study showed that the amounts of aldehydes formed in Caco-2 and SK-MEL cells after 5-ALA incubation or illumination with visible light in the absence of the photosensitizer were similar and that there was no significant difference in survival of these cells. Exposure of Caco-2 cells to 5-ALA before illumination led to aldehydes accumulation, especially HAKs, compared with control cells. The levels of 4-hydroxyhexenal and 4-hydroxynonenal increased 3.9 times and 5.7 times, respectively, and cell death was consequently enhanced. In contrast, in the pigmented melanoma cells (SK-MEL) the initial levels of particular aldehydes remained unchanged after PDT, except for 4-hydroxynonenal, which increased 2 times. The effect of photodynamic treatment on the survival of these cells was insignificant.

The results of the presented study correspond to previous observations that melanin in cultured skin melanocytes was able to protect against PDT-induced TBARS formation [26], UV-B-induced DNA lesions [27], and membrane damage induced by UV-A [28]. The present findings support the hypothesis that aldehydic lipid peroxidation prod-

Table 2. Aldehydic lipid peroxidation product levels after 5-ALA incubation (5-ALA) or illumination with visible light in the absence (light) or presence (PDT) of the photosensitizer in human melanoma cells (SK-MEL). The amounts of DNP--aldehydes are expressed as mean values ± *SD* from three independent series of experiments

Tabela 2. Zawartość aldehydowych produktów peroksydacji lipidów w ludzkich komórkach nowotworowych czerniaka (SK-MEL) poddanych inkubacji z 5-ALA (5-ALA), naświetlaniu w nieobecności (światło) i obecności fotouczulacza (PDT). Ilości DNP-aldehydów są wyrażone jako średnie arytmetyczne ± SD trzech niezależnych serii eksperymentów

Amounts of DNP-aldehydes [nmol/g of cells] (Ilości DNP-aldehydów [nmol/g komórek])	Control (Grupa kontrolna)	5-ALA (5-ALA)	Light (Światło)	PDT (Obecność fotouczulacza)
2-hydroxybutanal (2-hydroksybutanal)	2.38 ± 0.51	2.37 ± 0.12	2.27 ± 0.32	2.40 ± 0.25
2-hydroxyhexanal (2-hydroksyheksanal)	1.57 ± 0.08	1.30 ± 0.24	1.12 ± 0.36	1.30 ± 0.39
4-hydroxyhexenal (4-hydroksyheksenal)	1.28 ± 0.15	1.84 ± 0.31	1.54 ± 0.36	1.99 ± 0.50
2-hydroxyheptanal (2-hydroksyheptanal)	0.37 ± 0.19	0.42 ± 0.04	0.52 ± 0.10	0.82 ± 0.17
4-hydroxynonenal (4-hydroksynonenal)	0.09 ± 0.01	0.12 ± 0.01	0.13 ± 0.02	0.17 ± 0.02 a
n-pentanal (n-pentanal)	1.11 ± 0.34	1.12 ± 0.16	1.30 ± 0.32	1.51 ± 0.17
n-hexenal (n-heksenal)	0.15 ± 0.05	0.19 ± 0.03	0.15 ± 0.03	0.19 ± 0.02
n-hexanal (n-heksanal)	0.52 ± 0.05	0.87 ± 0.22	0.97 ± 0.22 b	0.98 ± 0.18 a

a – significant difference in comparison with control, 5-ALA, and light.
b – significant difference in comparison with control.

a – istotna statystycznie różnica w porównaniu z grupą kontrolną, 5-ALA i światłem.
b – istotna statystycznie różnica w porównaniu z grupą kontrolną.

ucts, especially HAKs, can play an important role in PDT-induced cell death. Melanin present in pigmented tumors might protect cells against PDT-induced lipid peroxidation. A better understanding of the mechanisms occurring in different tumors after PDT may have clinical implications for the development of protocols used in photodynamic therapy.

References

[1] Girotti AW: Photodynamic lipid peroxidation in biological systems. Photochem Photobiol 1990, 51, 497–509.

[2] Girotti AW: Photosensitized oxidation of membrane lipids: reaction pathways, cytotoxic effects, and cytoprotective mechanisms. J Photochem Photobiol B Biol 2001, 63, 103–113.

[3] Comporti M: Lipid peroxidation. Biopathological significance. Mol Aspects Med 1993, 14, 199–207.

[4] Comporti M: Lipid peroxidation and biogenic aldehydes: from the identification of 4-hydroxynonenal to further achievements in biopathology. Free Radic Res 1998, 28, 623–635.

[5] Dianzani MU: 4-Hydroxynonenal and cell signaling. Free Radic Res 1998, 28, 553–560.

[6] Rinaldi M, Barrera G, Spinsanti P, Pizzimenti S, Ciafre SA, Parella P, Farace MG, Signori E, Dianzani MU, Fazio VM: Growth inhibition and differentiation induction in murine erythroleukemia cells by 4-hydroxynonenal. Free Radic Res 2001, 34, 629–637.

[7] Barrera G, Pizzimenti S, Laurora S, Briatore F, Toaldo C, Dianzani MU: 4-hydroxynonenal and cell cycle. Biofactors 2005, 24, 151–157.

[8] Scalia M, Geremia E, Corsaro C, Santoro C, Baratta D, Sichel G: Lipid peroxidation in pigmented and unpigmented liver tissues: protective role of melanin. Pigment Cell Res 1990, 3, 115–119.

[9] Blaheta RA, Franz M, Auth MK, Wenisch HJ, Markus BH: A rapid non-radioactive fluorescence assay for the measurement of both cell number and proliferation. J Immunol Methods 1991, 142, 199–206.

[10] Esterbauer H, Cheesman KH, Dianzani MU, Poli G, Slater TF: Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe^{2+} in rat liver microsomes. Biochem J 1982, 208, 129–140.

[11] Deighton N, Magill WJ, Bremner DH, Benson EE: Malonodialdehyde and 4-hydroxy-2-nonenal in plant tissue cultures: LC-MS determination of 2,4-dinitrophenylohydrazone derivatives. Free Radic Res 1997, 27, 255–265.
[12] Nowis D, Makowski M, Stoklosa T, Legat M, Issat T, Golab J: Direct tumor damage mechanisms of photodynamic therapy. Acta Biochim Pol 2005, 52, 339–352.
[13] Chen B, Pogue BW, Hoopes PJ, Hasan T: Vascular and Cellular Targeting for Photodynamic Therapy. Crit Rev Eukaryot Gene Expr 2006, 16, 279–306.
[14] van Duijnhoven FH, Aalbers RI, Rovers JP, Terpstra OT, Kuppen PJ: The immunological consequences of photodynamic treatment of cancer, a literature review. Immunobiology 2003, 207, 105–113.
[15] Esterbauer H: Estimation of peroxidative damage. A critical review. Pathol Biol (Paris) 1996, 44, 25–28.
[16] Liu J, Yeo HC, Doniger SJ, Ames BN: Assay of aldehydes from lipid peroxidation: gas chromatography-mass spectrometry compared to thiobarbituric acid. Anal Biochem 1997, 245, 161–166.
[17] Andreoli R, Manini P, Corradi M, Mutti A, Niessen WMA: Determination of patterns of biologically relevant aldehydes in exhaled breath condensate of healthy subjects by liquid chromatography/atmospheric chemical ionization tandem mass spectrometry. Rapid Commun Mass Spectrom 2003, 17, 637–645.
[18] Gederaas OA, Lagerberg JW, Brekke O, Berg K, Dubbelman TM: 5-aminolevulinic acid induced lipid peroxidation after light exposure on human colon carcinoma cells and effects of alpha-tocopherol treatment. Cancer Lett 2000, 159, 23–32.
[19] Hjelde A, Gederaas OA, Krokan HE, Brubakk AO: Lack of effect of hyperoxia on photodynamic therapy and lipid peroxidation in three different cancer cell lines. Med Sci Monit 2005, 11, BR351–356.
[20] Sakharov DV, Elstak ED, Chernyak B, Wirtz KW: Prolonged lipid oxidation after photodynamic treatment. Study with oxidation-sensitive probe C11-BODIPY581/591. FEBS Lett 2005, 579, 1255–1260.
[21] Kessel D, Luo Y: Delayed oxidative photodamage induced by photodynamic therapy. Photochem Photobiol 1996, 64, 601–604.
[22] Wang HP, Qian SY, Schafer FQ, Domann FE, Oberley LW, Buettner GR: Phospholipid hydroperoxide glutathione peroxidase protects against singlet oxygen-induced cell damage of photodynamic therapy. Free Radic Biol Med 2001, 30, 825–835.
[23] Voigt A, Agatha G, Zintl F: Polyunsaturated but not conjugated linoleic acid supplementation of leukemic U937 cells can act as an amplification factor for photofrin-mediated photodynamic therapy. Photochem Photobiol 2006, 82, 763–769.
[24] Lee JY, Je JH, Kim DH, Chung SW, Zou Y, Kim ND, Ae-Yoo M, Suck-Baik H, Yu BP, Chung HY: Induction of endothelial apoptosis by 4-hydroxyhexenal. Eur J Biochem 2004, 271, 1339–1347.
[25] West JD, Ji C, Duncan ST, Amarnath V, Schneider C, Rizzo CJ, Brash AR, Marnett LJ: Induction of apoptosis in colorectal carcinoma cells treated with 4-hydroxy-2-nonenal and structurally related aldehydic products of lipid peroxidation. Chem Res Toxicol 2004, 17, 453–462.
[26] Hadjur C, Richard MJ, Parat MO, Jardon P, Favier A: Photodynamic effects of hypericin on lipid peroxidation and antioxidant status in melanoma cells. Photochem Photobiol 1996, 64, 375–381.
[27] Kowalczuk C, Priestner M, Baller C, Pearson A, Cridland N, Saunders R, Wakamatsu K, Ito S: Effect of increased intracellular melanin concentration on survival of human melanoma cells exposed to different wavelengths of UV radiation. Int J Radiat Biol 2001, 77, 883–889.
[28] Kvam E, Dahle J: Pigmented melanocytes are protected against ultraviolet-A-induced membrane damage. J Invest Dermatol 2003, 121, 564–569.

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Conflict of interest: None declared

Received: 7.10.2008
Revised: 10.11.2008
Accepted: 21.11.2008