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The influence of photodynamic therapy on the peroxidation-antioxidation balance of the organism and the phagocytic activity

Photodynamic therapy (PDT) is a slightly invasive, alternative method of treating cancers and precancerous conditions. It is used in many medical specialities.Although the beginnings of photodynamic therapy go back to the ancient times, this therapeutic method became more popular in the 1960s. In 1966 Lipson et al. applied haematoporphyrin derivatives as photosensitizing substances in breast tumour treatment (17). In 1975 attempts were made to cure urinary bladder cancer using the photodynamic method (12). In 1976 Roswell Park Cancer Institute in Buffalo started wide-range studies on the uselulness of photodynamic therapy not only in oncology (5, 7).

The mechanism of photodynamic therapy consists in the activation by visible light of photosensitive substance deposited in the treated tissue. During this reaction reactive oxygen forms are produced and they have a toxic effect on the cells of the treated cancer and the vessels of the tumour. Although photooxidation of the affected tissue is highly selective, the free radical mechanism can spread outside the area of the treated tissue in the form of inducing the cytokine synthesis, the mechanisms of phagocytosis or apoptosis.

A significant element of the success of the photodynamic method is the choice of an appropriate photosensibilizer which should be characterized by a short period of tissular photosensitivity and selective accumulation in the treated tissue. Photosensitizing substances used in photodynamic therapy are porphyrin derivatives and are characterized by absorption of light of various wavelengths. To photosensitizing substances of the 1st generation belong haematoporphyrin derivatives Photophrin I (HPD) and Photophrin II (P-II-polymer sodium). HPD and P II are usually activated with the light of 630 nm wavelength, coming from argon, copper or gold lasers (14). The drawbacks of 1st generation photosensibilizers are their nonselective accumulation in the tissues (unaffected tissues, especially reticuloendothelial system, have also the ability to take up these substances) and persisting photosensitivity 6–10 weeks following their application (4, 5).

The 2nd generation photosensibilizers are among others: 5-aminolevulinic acid (ALA), protoporphirin IX precursor (PpIX) in the heme transformation cycle, its good point being a selective accumulation in the treated tissue and short period of photosensitivity (about 24 h after the application). The application of exogenous ALA on the affected tissue increases the intracellular production of endogenous PpIX, with its particular accumulation in dysplastic and neoplastic tissues. Optimal induction (activation) wavelength is also 630 nm here (9, 10).

Other photosensitizing substances are: benzoporphyrin derivatives (BPD), for which 690 nm wavelength is applied and photosensitivity period lasts 3–5 days (10), m-tetrahydroxyphenylchlorin (mTHPC) activated by 652 nm wavelength (5, 6) and MACE (chlorine aspartate esther), activated by 660 nm (20).

The aim of this paper was to evaluate the intensity of lipid peroxidation process and antioxidant status in the plasma following the photodynamic therapy in patients with Bowen's disease or superficial basal cell carcinomas. The activity of neutrophils in the patients' blood after the therapy was also assessed.

MATERIAL AND METHODS

The studies were carried out in 10 patients aged 60–70 with Bowen's disease or basal cell carcinoma diagnosed histopathologically. The area of the skin exposed to radiation did not exceed 10% of total skin area. The patients were subjected to the photodynamic therapy using 20% 5-aminolevulin acid (5-ALA). Twenty per cent cream with ALA was applied onto the affected skin 6 h before the treatment in a light-resistant dressing and then the skin was irradiated with a 30+/– 5 W Omnilux lamp (Photo Therapeutics Ltd) emitting the light of 633+/– 3nm wavelength, for 16 min 40 sec. The distance from the light source to the surface of the exposed skin was 25 mm.

The intensity of lipid peroxidation was determined on the basis of the content of lipid hydroperoxides (HPETE). The antioxidant efficiency was evaluated as total antioxidant status (TAS).

The above mentioned value parameters were determined in the plasma before the treatment and 30 min after starting the treatment. HPETE contents were assessed according to the Buege and August method (3) with the Ward modification (21), as described in our previous papers, and expressed in optical density (OD) units on 1 g of plasma proteins.

TAS was determined spectrophotometrically with Randox reagents (No.Nx 2332, Randox Lab. Ltd, UK). In this method 2,2-azine-di-3-ethylbenzothiazoline sulphate (ABTS) is incubated with peroxidase and hydrogen peroxide. As a result, ABTS is formed in the cation form (AB .+) giving blue-green colour of the sample and the colour intensity is measured at 600 nm. Proportionally to their amount, the plasma antioxidants inhibit the formation of the ABTS.+ concentration-dependent colour. The TAS value is determined from the standard curve for trolox and expressed in U Trolox/ml of plasma. Total antioxidant status (TAS) in the plasma was determined with TAS kits (Cat. No. Nx 2332, Randox Lab. Ltd, UK). In this assay ABTS (2,2-azino-di-[3-ethylbenzthiazoline sulphate]) is incubated with a peroxidase (metmioglobin) and hydrogen superoxide to produce the blue-green colour radical cation ABTS*+. Antioxidants in the test plasma sample cause the suppression of this colour production to the proportional degree to their concentration and the change of absorbance is measured at 600 nm. TAS was described in mmol (of Trolox units)/l.

The phagocytic activity of neutrophils in peripheral blood was determined using on the basis of the test of the absorption and the reduction of NBT (18). The NBT reduction test determines the number of neutrophil containing dark-blue deposits of formazan in 100 neutrophils. The results are expressed in per cent. The data are expressed as mean (SD). Statistical analysis for significant differences was performed according to Wilcoxon rank test, and the correlation of variables using Spearman correlation coefficient test. P=0.05 was assumed as the significance level.

RESULTS

In the studies the increase in the concentration of lipid hydroperoxides (HPETE) in the patients' plasma was found 30 min after the photodynamic therapy was finished, compared to their concentration before the treatment (Fig. 1). This increase was not statistically significant. The concentration of total antioxidant status TAS was not significantly changed after the patients' exposure to radiation (Fig. 2). The activity of peripheral blood neutrophils, measured with the NBT test of absorption and reduction increased significantly following the PDT (Fig. 3).

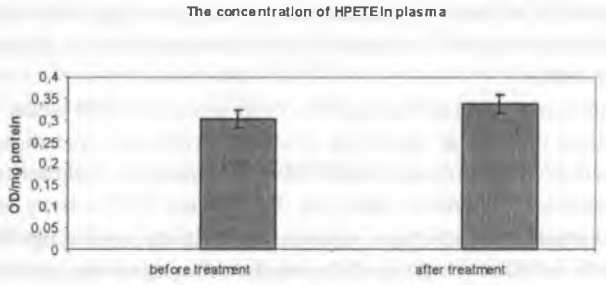

Fig. 1. HPETE concentration before and 30 min after photodynamic treatment.
The data are expressed as mean +/– SD, statistical difference between groups is not significant

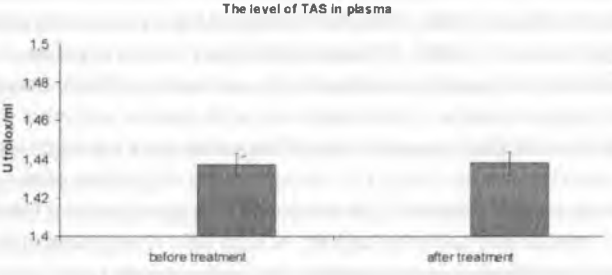

Fig. 2. The level of TAS expressed in units of trolox per ml of plasma, before and 30 min after photodynamic treatment. The data are expressed as mean +/– SD

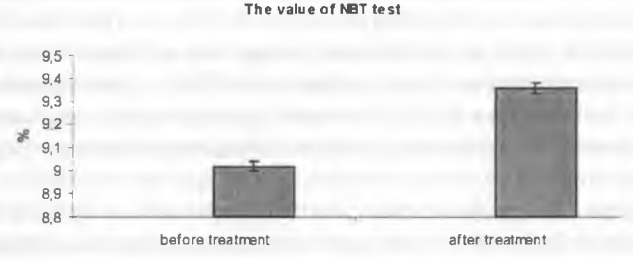

Fig. 3. The value of NBT test. The data are expressed as mean +/– SD,
statistical difference is very significant (Wilcoxon rank test p – 0.0020,
Spearman corelation coeficient (r) = 0.85, p=0.0014)

DISCUSSION

The studies showed that as a result of the photodynamic therapy (PDT) the phagocytic activity of peripheral blood neutrophils was increased, accompanied by a rise in lipid hydroperoxides (HPETE) concentration in the plasma. Total anioxidant status (TAS) of the plasma was not significantly increased.

The obtained results are, in our opinion, the effect of the chain of free radical reactions released during the PDT. In the photodynamic reaction a point of departure is the activated triplet state of photosensibilizer particles, which occurs following the light absorption, whereas the effect of the reaction is the appearance of peroxide radicals, mainly singlet oxygen. This first chain releases biological effects – cellular, vascular and immunological, both immediate and distant, which inluence the therapeutic success (8).

The direct destruction of cells undergoing PDT occurs as a result of the action of singlet oxygen or other free radicals on the cellular structures. A few hours after the irradiation the inhibition of normal cell functions and formation of membrane vesicles were observed, followed by the cell division arrest and decomposition. The critical places for the cytotoxic PDT activity are mitochondrial, lysosomal and Golgi apparatus membranes, endoplasmic reticulum, nuclear membrane and cellular membrane itself. Both unsaturated organic acids and plasmatic membrane proteins undergo quick oxidation under the influence of the therapy and the result of their depolarization is the appearance of hyperpermeability of cell membranes. This leads to the increased inflow of Ca and Na ions to the inside of the cell from the intercellular space and the outflow of Mg and K ions from the cell.

The ATP synthesis in the cell is impaired. The inactivation of membrane mitochondrial enzymes (cytochromaloxidase C, succinoxidase, FoF1 ATP synthase) takes place in the mitochondria, while the lysosome damage causes the output of lysosomal enzymes to the cytoplasm.

DNA and chromosome damage takes place in the cell nucleus. From among nucleinic acids components singlet oxygen reacts in a preferential way with guanine and other purine derivatives, unlike hydroxide radical attacking nonselectively all the purine and pyrimidine residues (2).

PDT therapy quickly leads to changes in blood vessels, regardless of the photosensibilizer applied. After several dozen seconds of light activity platelet aggregates are formed in the vessels and then vascular stenosis and occlusion appear. At high light energy doses (about 250 J/cm^2 of tissue) and high photosensibilizer concentration the acute occlusion takes place in the tissue. At lower doses (about 135 J/cm^2) the occlusion appears a few hours after the exposure. The vascular occlusion is the cause of tissue necrosis.

Cellular and vascular mechanisms depend first of all on free radical reactions. Those reactions, first localized in the treated tissue and depending on the local distribution of photosensibilizer, can spread outside the treated area, which can be connected among others with the activation of phagocytosis after PDT and another mechanism influencing the results of PDT – the immunological mechanism. In the neoplastic cells there is a high lipid content: lipid alkyloesthers and neutral lipids in the surface cell membrane and the degradation products of neoplastic tissue contain high concentrations of alkylolipid derivatives which strongly stimulate macrophages and neutrophils to chemotaxis and pathogen destruction. Our studies revealed that the concentration of lipid peroxidation markers (HPETE) was increased after PDT. At the same time the intensification of phagocytic activity of neutrophils was found, so it is likely that the observed increase in lipid peroxidation is both the effect of release of arachidonic acid metabolites from the cells after PDT and the oxygen explosion of phagocytes.

The immunological mechanism of PDT results from the fact that oxygenic free radicals are particles acting on the principle of secondary cellular transmitters, thus influencing the expression of acute phase protein genes, cytokine genes and their receptors, growth factors, adhesive particles and antioxidative enzymes (1, 15). They also activate the cascade of arachidonic acid metabolism and are one of the death signals, leading to cellular apoptosis (10).

Karrer et al. found that in the supernatant of keratinocyte culture undergoing PDT with ALA, the amount of IL-1 increased six times and the amount of alpha-TNF – three times. Skin fibroblasts undergoing the activity of the supernatant from this culture produced a large amount of metaloproteinases having a destructive effect on collagen (11). Cytokines released outside the tumour area have a direct effect on the neutrophil activation (high values of NBT test). These reactions, however, do not lead to a significant decrease in TAS values. The concentration of secondary forms of oxygenic radicals – lipid hydroperoxides (HPETE) shows a growth tendency, although the growth is not statistically significant.

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SUMMARY

Photodynamic therapy (PDT) is a recognized method of treatment for Bowen's disease, actinic keratosis and superficial basal cell carcinomas. Other oncological indications for PDT are among others: Barrett's oesophagus, urinary bladder carcinoma, cervical and vaginal endothelial neoplasia (CIN) and (VIN) and metastatic foci of breast cancer to the skin. The mechanism by which photodynamic therapy leads to the neoplastic damage is complex and consists of cellular, vascular and immunological components. The first stage of photodynamic reaction is the activation through visible light of photosensitive substance previously placed in the treated tissue; as a result of that reactive oxygen forms are produced and start the selective process of tissue damage. The aim of the study was to determine the influence of photodynamic therapy on the activation of phagocytosis, lipid peroxidation in the plasma and the total antioxidative status of the plasma. The studies showed that as a result of the applied therapy in the patients with Bowen's disease or BCC phagocytic activity of peripheral blood neutrophils was increased, accompanied by the increase in the concentration of lipid hydroperoxides (HPETE) in the plasma. Total antioxidant status of the plasma (TAS) in the treated patients did not significantly change following the therapy.

Wpływ terapii fotodynamicznej na równowagę peroksydacyjno-antyoksydacyjną ustroju oraz na aktywność fagocytów

Terapia fotodynamiczna (PDT) jest uznaną metodą leczenia choroby Bowena, nowotworów, rogowacenia słonecznego i powierzchownych raków podstawnokomórkowych. Inne onkologiczne wskazania do PDT to leczenie nowotworów przełyku (Barrett's oesophagus), raka pęcherza moczowego, szyjkowej oraz pochwowej neoplazji śródbłonkowej (CIN i VIN) oraz ognisk przerzutów raka sutka do skóry. Mechanizm, w którego wyniku terapia fotodynamiczna prowadzi do uszkodzenie nowotworu, polega na aktywacji reakcji komórkowych, naczyniowych i immunologicznych. Pierwszym etapem reakcji fotodynamicznej jest aktywacja przez światło widzialne substancji fotowrażliwej umieszczonej uprzednio w leczonej tkance. W wyniku tego procesu dochodzi do reakcji chemicznych, podczas których produkowane są reaktywne formy tlenu, które zapoczątkowują selektywne niszczenie komórek neoplastycznych. Celem naszej pracy była ocena wpływu terapii fotodynamicznej na procesy peroksydacji lipidów w osoczu krwi, całkowity potencjał antyoksyda-

cyjny osocza oraz na aktywność fagocytów. Badania wykazały, że po zastosowaniu terapii fotodynamicznej u pacjentów z chorobą Bowena lub BCC aktywność fagocytów krwi obwodowej wzrosła, a stężenie HPETE w osoczu zwiększyło się. Całkowity potencjał antyoksydacyjny (TAS) nie uległ istotnej zmianie po terapii PDT.