

THE EFFECTS OF PHOTODYNAMIC THERAPY IN BIOLOGICAL SYSTEMS

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Keywords:

photodynamic therapy, apoptosis, reactive oxygen species, immune response

Abstract: Photodynamic therapy (PDT) is a recent acquisition in the treatment of cutaneous premalignant and malignant lesions, based upon the administration or induction of the synthesis of a photosensitizer in the tumoral tissue, followed by the illumination of the target area using a radiation with an adequate wavelength. This paper aims to review the main molecular, cellular and systemic mechanistic aspects involved in the *in vivo* effects of PDT, with emphasis on apoptosis and autophagy induction in tumoral cells, local vascular destruction and immune response alteration.

Cuvinte cheie: terapie fotodinamică, apoptoză, specii reactive de oxigen, răspuns imun

Rezumat: Terapia fotodinamică (PDT) este o achiziție recentă în tratamentul leziunilor cutanate premaligne și maligne, ce presupune administrarea sau inducerea sintezei unui compus fotosensibilizator la nivelul țesutului tumoral, urmată de iluminarea zonei-țintă utilizând o radiație de lungime de undă adecvată. În urma activării fotosensibilizatorului se declanșează reacțiile fotodinamice care conduc la distrugerea tumorii. Lucrarea de față își propune să treacă în revistă principalele mecanismele intime moleculare, celulare și sistemice ale efectelor terapiei fotodinamice *in vivo*, cu accent pe inducerea apoptozei și a autofagiei în celulele tumorale, alterarea vascularizației locale și modificarea răspunsului imun.

Photodynamic therapy (PDT) is a modern antineoplastic treatment modality used in numerous medical specialties (dermatology, gastroenterology, urology or ophthalmology). The method consists in administering or inducing the synthesis of a photosensitizer in the tumoral tissue, followed by the illumination of the target area, using a radiation with an adequate wavelength. The activation of the photosensitizer on this pathway triggers complex photochemical processes, resulting in the production of reactive oxygen species (ROS) that produce extensive tissue destruction and local vascularisation alterations, apoptosis, necrosis and autophagy induction and triggering of the immune response, all of the above contributing to the destruction of tumour cells.

For the achievement of the destructive effects of PDT, the concurrent presence of sufficient quantities of photosensitizer and molecular oxygen in the target area is necessary, as well as the use of a light source of an adequate emission spectrum that would correspond to the absorption spectrum of the photosensitizer and, at the same time, it would allow a good tissue penetration of the light to its level, with minimal absorption by other cutaneous chromophores (melanin, oxi/hemoglobin and water).

Effects of PDT on tumour vascularisation

The anti-tumoral *in vivo* effects of PDT have a greater amplitude than anticipated considering the hypoxia present in most of the tumours, which suggests the fact that tumour cells are not only destroyed through direct photodynamic effects but also through other mechanisms, most likely through the destruction of blood vessels that supply nutrients to the tumour.(1) The destruction of tumour vascularisation causes the shortage of the nutrition and energetic support needed by the

tumour cells, leading to the partial or total destruction of the tumour. The reduction of the blood supply secondary to the vascular destruction is also accompanied by an equivalent decrease of the oxygen quantity delivered to the tumour; the lack of oxygen will lead to the decrease or abolishment of the efficiency of subsequent PDT light pulses as PDT effects are dependent on the production of oxygen reactive species (ROS) in the target area, especially of singlet oxygen, through photochemical processes. Moreover, the photochemical reactions triggered by a light pulse consume the tumour tissular oxygen, emphasizing tumour hypoxia and limiting the photodynamic effect of subsequent light pulses. The variations of the illumination parameters produce controllable modulations of the vascular effects, phenomenon that could be therapeutically exploited through the variation of the used illumination fluence.(2)

Becker et al. demonstrated that the initial blood flow decreases after applying PDT, possibly as a consequence of the nitric oxide (NO) reduction following tissular oxygen consumption during the photochemical reactions. Subsequently, there appears a sudden increase in the blood flow in the lesion and a slow relapse to the usual flow.(3) PDT also induces vascular thrombosis. Given that hypothesis, Yang and colab. showed in 2010 in a study performed on BALB/c mice subcutaneously injected with EMT6 mammary carcinoma cells that previously anticoagulation with heparin determined a better anti-tumour response to PDT using porphyrin derivatives.(4)

The hypoxia caused by PDT induces angiogenesis by increasing the levels of HIF-1 α (Hypoxia inducible factor), the α subunit of a dimeric transcription factor (α - β) that determines the synthesis of VEGF (vascular endothelial growth factor), the

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Article received on 25.06.2012 and accepted for publication on 23.08.2012
ACTA MEDICA TRANSILVANICA December 2012;2(4):313-315

latter stimulating the angiogenesis after binding to the VEGF receptor situated on the endothelial cells. In conditions of adequate oxygenation HIF-1 α is decomposed by the tissular oxygen, but in case of hypoxia, HIF-1 α persists and binds to the β subunit, stimulating the synthesis of VEGF and as a consequence, the angiogenesis.(5)

The administration of anti-angiogenetic factors determines an improvement of the tumoral response to PDT. In 2006, Ferrario and Gomer proved on a murine model of Kaposi sarcoma that PDT determines the increase of VEGF produced by the tumour cells and, to a smaller extent, of the VEGF produced by the other tumour cells of the organism, along with an increase in the level of HIF-1 α and TNF- α , and that the use of bevacizumab (Avastin), a VEGF inhibitor, improves the efficacy of PDT.(6)

The effects of PDT on the immune system

PDT attracts macrophages and neutrophils in the target area, leading to an antitumor immune response towards the residual neoplastic tissue. The neutrophils attracted in the target area after PDT are activated and reach the lymph nodes, where they initiate the dendritic cells which activate the CD8+ lymphocytes.(7) Other authors consider that the dendritic cells will mature secondary to the cytokines released from the PDT focus and will reach the regional lymphatic nodes, where they will present the captured antigens to the CD8+ T lymphocytes, which consequently become effector T lymphocytes that will be attracted in the PDT focus by chemokines and will participate in the destruction of the residual tumour tissue.(8)

PDT leads to the enhancement of the tumour immunogenicity through various mechanisms. One of those consists in an increase of the expression of the major histocompatibility complex MHC type I molecules in the tumour cells, which increase their susceptibility to the subsequent action of the immune NK (natural killer) cells.(9) PDT also determines the activation of the complement on the alternate pathway. Moreover, through the destruction of the tumour cells during PDT, new antigens are revealed to the immune system and the tumour cells, which are very weak immunogens, acquire a greater capacity of eliciting an immune response from the organism. These effects of PDT on the immunity are subject to active research in order to obtain antitumoral vaccines.(10)

On the other hand, PDT can also determine the abolishment of certain types of immune reactions. Simkin et al. showed that benzoporphyrine derivatives PDT determines the inhibition of cutaneous sensibilisation to 2,4-dinitrofluorobenzene (DNFB) in laboratory mice. The same authors have also demonstrated that this effect of inhibition on the immune contact system is due to the IL-10 production stimulation by PDT.(11) Subsequently, Gollnick and colab. showed that IL-10 is not significantly involved in the contact hypersensitivity inhibition(12) In both studies though the administration of IL-12 leads to the reemergence of contact hypersensitivity, suppressing the immune inhibition caused by PDT.(11,12)

In animal studies, it was observed that PDT determines the reversible decrease of the Langerhans cells number in the irradiation spot, with nadir 5 days post-PDT; this effect appears as a consequence of the migration of Langerhans cells from the skin in the lymph nodes - explaining, at least partially, the contact reaction suppression. The PDT action determines an inhibition of the immune contact response not only locally, but also systemically, the amplitude of the reaction depending merely on the used dose. The effect is to a greater extent similar to the one determined by UVB during phototherapy.(13) The immunosuppressive effect of PDT was also verified on human skin; this effect could be speculated in

cutaneous inflammatory conditions, while in antitumoral treatment it could contribute to the decrease of the PDT efficacy.(14)

Apoptosis induction by PDT

Apoptosis is an active process, taking place with energy consumption, highly phylogenetically conserved, through which the cell responds to the presence (or the absence) of exterior stimuli or to internal structural alterations by destroying itself, following the steps of a well established process, similar in all the tissues of the organism. Tumoral cells develop accommodation mechanisms that suppress apoptosis; apoptosis induction in neoplastic cells is a desirable effect of PDT that significantly contributes to tumour destruction. PDT can produce cellular death on different pathways, following one or another depending on various factors. Therefore, based on the intensity of the cellular injury, PDT may induce necrosis - in case of a great intensity oxidative stress, or apoptosis, programmed cellular death - in case of moderate intensity oxidative stress.(15)

The intracellular location of the photosensitizer also determines the response modality to PDT; the photosensitizers located in the mitochondrias trigger apoptosis by binding to the mitochondrial membrane and releasing the cytochrome c in the cytosol, leading to the cleavage of procaspase 9 and finally to the activation of caspase 3, the main effector of apoptosis involved in the degradation of the laminines from the nuclear membrane and, by deteriorating poly-(ADP-ribosyl)-polymerase, in activating the endonucleases that will cleave the DNA.(16)

Apart from this mechanism, apoptosis can be also induced through the alteration of the anti-apoptotic proteins Bcl-2, the increase of the membrane expression of CD95/FAS, the activation of the FAS/FAS ligand pathway and of the FLICE molecules pathway with the cleavage of procaspase 8 and the activation of the ceramide signaling pathway.(17,18) Once activated, caspase 8 can cleave the Bid protein which will migrate from the cytosol to the mitochondria where it will determine the additional release of cytochrome c. In the case of the photosensitizers located in the endoplasmatic reticulum, the injury of its membrane determines the increase of the intracellular calcium, mechanism that also triggers apoptosis. Apoptosis can also be initiated on the mitogen activated protein kinase (MAPK) pathway. On the other hand, PDT can also determine the activation of the nuclear transcription factor NF κ B, which has an anti-apoptotic role. Acting especially in the terminal phase of apoptosis, usually by injuring the mitochondria, PDT is also efficient in destroying the neoplastic cells otherwise resistant to radiotherapy and most chemotherapies.(19)

PDT induced autophagy

The induction of autophagy appears as a protection mechanism of the cell against PDT effects. Autophagy is a highly conserved process in eukaryotes, in which certain regions of the cytoplasm - including abnormal or excessive organelles- are surrounded by double membranes, resulting in autophagosomes that will fuse with lysosomes, contributing to the recycling of various components by the cell. Autophagy is altered when using photosensitizers located preferentially in the lysosomes; suppressing the expression or the inactivation of the pro-autophagy proteins may result, in the future, in significant enhancement of PDT efficiency.(17)

Generating reactive species of oxygen

The reactive oxygen species (ROS) produced during PDT attack the lipids leading to their oxidation, a cyclic process triggered by the hydroxyl radical (HO), by the hydrogen peroxide or by the singlet oxygen (1O_2), during which peroxy or

hydroperoxyl radicals will result from the non-saturated fatty acids, the latter reacting with other fatty acid molecules forming cycle finalization products like malondialdehyde or acrolein, which can react to nucleic acids producing their structural alterations, or to proteins, producing carbonilated proteins.(20,21) The glucides attacked by ROS lead to the production of keto-aldehydes, keto-amines and deoxyozones, which can also contribute to the formation of carbonilated proteins. Moreover, ROS produce structural alterations of the proteins, acting preferentially through oxidation in the lateral amino acid residues, with the formation of cross-linkage between the proteins, or through the fragmentation of the proteic chain, either on the α -amination pathway either on the diamine formation pathway. The aminoacids most susceptible to oxidation are cistein, fenilalanine, tyrosine, histidine, lysine and proline. The proteins can be carbonylated by glycation, glycooxidation or by reacting to the lipid peroxidation products.(21)

ROS also attack the nucleic acids, producing significant structural alterations resulting in mutations or ruptures of the chain. Nucleic acids are attacked by the hydroxyl radicals HO, the most common reaction being the oxidation of guanine to 8-oxo-guanine, which in the next replication cycle attach to adenine instead of cytosine leading to the appearance of mutations.(22) The mutations that cannot be repaired by the cellular enzymatic apparatus determine the start of apoptosis.

Conclusion remarks and future outlook

The *in vivo* effects of PDT are very complex and are exerted on different levels, the molecular effects determined by the structural and functional alteration produced by generating ROS intrincating with the cellular effects, represented by the induction of apoptosis and autophagy and with the tissular and systemic effects, consisting in alteration of the vascularisation and the immune response. Future research on these effects may become the foundation for optimising current PDT protocols and may lead to the production of anti-tumour vaccines.

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