ORIGINAL PAPER

Effect of photodynamic therapy with hypericin on the secretion of selected cytokines of colorectal cancer cells tested *in vitro*

Anna Międzybrodzka [1], Aleksandra Kawczyk-Krupka [1], David Aebisher [2],
Grzegorz Cieślar [1], Zenon Paweł Czuba [3]

[1] Department of Internal Medicine, Angiology and Physical Medicine, Center for Laser Diagnostics and Therapy, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Katowice, Poland
[2] Department of Photomedicine and Physical chemistry, Faculty of Medicine, University of Rzeszow, Rzeszow, Poland
[3] Department of Microbiology and Immunology, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia in Katowice, Zabrze, Poland

ABSTRACT

Introduction and aim. Photodynamic therapy is a complex process involving the introduction of photosensitizers into the patient's body and irradiation of them in order to destroy the lesion, and activate the immune system. An important role in photodynamic therapy is played by photobiochemical and physical mechanisms that affect the tumor vessels and lead to the death of the damaged cell. The aim of the study is to determine the effect of photodynamic therapy with the use of Hypericin (Hyp) on the secretion of selected cytokines by colorectal cancer cells.
Material and methods. Two colorectal cancer cell lines SW480 and SW620 were used in the study. Cells treated Hypericin were exposed to visible light. Then cell viability was determined by the MTT assay with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium. Assays were performed for control samples without hypericin and light exposure, with Hyp without light exposure, without Hyp and irradiated with light, and test samples with Hyp and light exposure.
Results. In the experiment we reveal, that Hyp- photodynamic activity does not influence the secretion of cytokines.
Conclusion. The obtaining results confirming the destructive effect of Hyp- PDT on the colon cancer cells, show a possibility of extending the indication for photodynamic therapy using Hyp, qualification of precancerous changes.
Keywords. colorectal cancer, hypericin, photodynamic therapy
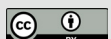
Introduction

The photosensitizer (PS) is one of the most important factors responsible for the successful performance of photodynamic therapy (PDT). An ideal PS should have characteristics such as: be a pure chemical substance, commercially available, low toxicity in the dark, but strong photocytotoxicity, have good selectivity for cancer cells, react with the wavelength range – 600–800 nm, which allows for deeper light penetration, should be cleared quickly from the body and be administered in a number of ways: directly to the skin, orally, intravenously or inhaled. *Hypericum perforatum* is a herbaceous perennial, commonly known as St. John's wort. The main photoactive compounds found in *H. perforatum* are hypericin and its analog pseudohypericin (hypericin).[1] Hypericin with the general formula $C_{30}H_{16}O_8$ and molecular weight 504.44 are polycyclic aromatic phenan-throperylenediones. It selective-

ly accumulates in tumor tissue by diffusion, pinocytosis or endocytosis.[2] Hypericin also exhibits antiproliferative and cytotoxic properties in many cancer cell lines.[3] Although hypericin is classified as a photoactive compound, its use and clinical application is limited due to a decrease in its photosensitizing activity in the presence of serum.[4-9] However, no evidence of phototoxic potential has been found in humans after oral administration of *Hypericum* extract.[10] Hypericin mainly accumulates in the membranes of the endoplasmic reticulum, lysosomes, Golgi apparatus and mitochondria due to its hydrophobic nature. PDT is becoming the routine treatment for some types of non-melanoma skin cancer. The most important components of the method are: photosensitizer, light and molecular oxygen.[11-15] PDT mechanism of tumor cell death is a two-step process in which interactions with cells take place upon activation of PS with light. The physicochemical properties of the photosensitizer have a great influence on the processes of their destruction. Hydrophilic PS interacts with albumin and globulins, while hydrophobic PS tend to bind to low-density lipoprotein receptors. Anionic PSs tend to accumulate in the organelles of cell lysosomes, while cationic molecules are absorbed by mitochondria. Upon activation of PS with a defined wavelength of light, three main mechanisms of cell death are induced: apoptosis, necrosis and autophagy. Two of them, the molecular mechanisms of apoptosis and necrosis, have been thoroughly investigated. Initially, they were considered to be mutually exclusive cellular states.[16] It is now known that there is a balanced interaction between these two modes of cell death. Initiating and effector factors, signaling pathways and subcellular sites were identified as key mediators in both processes. They work by building common modules or as a switch that allows cells to decide which route to take depending on the situation. Autophagy, which is mainly a cytoprotective process, has been associated with both types of cell death, serving as a guide to cell survival or death. The process of cell death after PDT is influenced by: the type of PS and its location in cells, the concentration of PS uptake and its physicochemical features, the concentration of cellular oxygen, as well as the wavelength and intensity of the light used.[17] It is worth adding that there are cases when neoplastic cells become more sensitive to PDT, and sometimes PDT is able to disrupt cellular defense processes, increasing the effectiveness of the therapy. Photodynamic therapy, especially when used in lower doses, has been shown to be effective energy, stimulates the immune response and – by activating the system immune – recognition of tumor antigens (tumor-associated antigens – TAA), even leading to complete remission of adjacent and distant neoplastic lesions from the site of PDT action. These observations prompted the authors to combine photodynamic therapy with immunother-

apy, which was anti-cancer effect, and at the same time prevents the recurrence of the disease.[18,19] Interleukin 2 (IL-2) is the most important cytokine stimulating the growth of T lymphocytes, especially those with cytotoxic properties. It means that IL-2 indirectly stimulates the process of programmed cell death (apoptosis) infected with viruses and cancer cells. Stimulation of T lymphocytes increases the production of apoptosis-stimulating molecules on its surface. Interleukin 2 has been considered in research as an anti-cancer drug. Interleukin 4 (IL-4) is of great importance in the process of developing an allergic reaction. It has a broad effect and stimulates many different cells of the immune system. It is produced by basophils, mast cells and Th2 lymphocytes. Due to the stimulation of monocytes and macrophages and the induction of the secretion of pro-inflammatory cytokines, IL-4 is directly and indirectly involved in the formation of an inflammatory focus. Its presence stimulates the activity of macrophages and monocytes. IL-4 is involved in the formation of an inflammatory focus. Positive effect on the production of cytokines stimulating hemopoiesis. An increase in the concentration of interleukin 4 therefore stimulates hematopoietic processes. Interleukin 6 is a pleiotropic cytokine, affecting the innate immune system and acquired, but above all by activating the inflammatory response. Among many functions, the most important role of IL-6 is participation in immunological processes. It is the body's main pyrogen. Mobilization of the immune system to the anticancer defense effect of IL-6 is manifested in the activation of maturation megakaryocytes, bone marrow progenitor cells and growth inhibition cancer cells by increasing the lytic activity of NK lymphocytes and by an increase in the expression of MHC class II proteins. Interleukin-6 stimulates lymphocyte differentiation B targets immunoglobulin-releasing cells of various classes and stimulates lymphocytes antigen-recognizing T, acting synergistically with TNF to induce antitumor response.[18,19]

Aim

The aim of the study was to determine the effect of HYP-PDT on colorectal cancer cells in terms of the secretion of selected cytokines involved in the processes occurring in the tumor environment.

Material and methods
Cell culture

Human colorectal cancer cells SW480 and SW620 were used in the study. They come from the ATCC bank (American Type Cell Culture-ATCC LGC Limited. Queens Road, Teddington, Middlesex, TW11 0LY, UK). Two cell lines of adenocarcinoma colorectal cancer with varying degrees of malignancy were used in the experiments: • SW 480 (ATCC, cat. no. CCL-228)

from a 50-year-old Caucasian male with primary colorectal adenocarcinoma, expresses carcinoembryonic antigen (CEA), transformation factor TGF-β and oncogenes: *myc, myb, ras, fos, sis, p53, abl, ros, src*, and • SW620 (ATCC, Cat. No. CCL-227) from a 51-year-old Caucasian male with lymph node metastases from colorectal cancer expressing the carcinoembryonic antigen (CEA) of the following oncogenes: *myc, myb, ras, fos, sis, p53, abl, ros, src*. In the experiment, cells of the SW480 and SW620 lines were used (American-type culture of the ATCC collection. The growth medium of Leibovitz's L-15 culture fluid with the addition of 10% inactivated fetal bovine serum FBS, 100 U/ml of penicillin and 100 μg/ml of streptomycin was used to culture cells of the SW 480 and SW 620 lines. The culture was carried out in plastic bottles with an area of 25 cm^2 and 75 cm^2 in a culture medium and then incubated at 37 degrees C in 100% air humidity without carbon dioxide. The cells adhered in the form of a monolayer, the growth medium was exchanged 2-3 times a week. Cells adhered to the bottom of culture bottles were detached from the substrate with 0.25% trypsin solution with 0.53 mM EDTA and incubated for 5-10 minutes at 37°C. After the trypsinization process, fresh growth medium was added to the cell suspension and centrifuged for 5 to 7 minutes (125xg). The cells prepared in this way were suspended in the culture medium, bringing the suspension to the appropriate density, experimentally determined for each line SW 480 500,000/ml, SW 620 250,000/ml, using a Burker hemocytometer, then cells of a certain density were adhered to 96-well plates and incubated at 37°C, in conditions of 100% humidity for 24 hours. Then, the culture medium was removed, rinsed once with PBS, and transferred to a room with limited access to light. Hypericin was added to the culture medium at concentrations of: 1 μM; 0.5 μM; 0.25 μM, 0.1 μM, photosensitizer was aspirated from the monolayer after one hour of incubation. The monolayer was washed 2 times with PBS.

Incubation of cells with Hyp
After 24-hour incubation of the test cells, the growth medium was removed, the cells were washed with calcium and magnesium free PBS solution. They were then treated with hypericin (Hypericin Calbiochem) at 0.1 μM, 0.25 μM, 0.5 μM and 1 μM for 1 hour. After incubation with Hyp, the medium was removed, the cells were washed with calcium and magnesium free PBS twice, and then complete growth medium was added.

Cellular uptake of hypericin
Cellular uptake of hypericin was assessed using an Olympus IX51 inverted microscope with a reflected fluorescence system (Olympus Corp) and a Color View III digital camera with Cell F imaging software (Soft Imaging System GmbH). In the experiment, the intra-cellular fluorescence intensity of Hyp was determined as a function of time with a flow cytometer (Becton Dickinson, LSR II) using the PerCP channel.

Exposure of cells to light
In the next stage of the experiment, cells were exposed to VIS visible light (400-750 nm) from an incoherent light source PDT TP-1 (Fig. 1) (Cosmedico Medizintechnik GmbH, Chwenningen, Germany) equipped with infra-red filters and an orange filter, light emission in the 600-720 nm wavelength range was obtained. After adding the culture medium, exposure to light with an intensity of 5 J/cm^2 was started; 10J/cm^2; 20J/cm^2; 40J/cm^2. The photodynamic therapy lamp PDT TP1 (Cosmedico Medizintechnik GmbH, Schwenningen, Germany) was used for irradiation. To avoid hyperthermia with long exposure times, irradiation was conducted through a 270 mL water filter. The following doses were irradiated: 5J/cm^2, 10J/cm^2, 20J/cm^2, 40J/cm^2. Cultivation was then continued at 37°C, 100% humidity without CO_2 for 24 hours.

Cells viability
• Vital staining with 0.2% trypan blue in 0.85% NaCl solution. Trypan blue diluted 1:1 was added to the cell suspension on the glass slide after 3 minutes for the percentage of viable cells that stained white and dead cells that stained blue. Cells stained with trypan blue were counted in the automated counter BioTek Instruments.

 • Mitochondrial dehydrogenase measurement – MTT test. After that, the cell viability was assessed with the MTT and LDH test. Cell viability was assessed by the 3-(4,5-DIMETHYLTHIAZOL-2-YL)-2,5-DIPHENYLTETRAZOLIUM (MTT) bromide conversion method (Sigma-Aldrich, St. Louis, MO, USA). The principle of the method is to assess the ability of living cells to convert the yellow, water-soluble MTT tetrazolium salt into the colored blue formazan under the influence of the osido-reductases present in the mitochondria (Tetrazole Succinate Reductase) and the cytoplasm. The color intensity of the water-insoluble formazan is proportional to the number of viable cells. After culturing, the supernatant from the superadhered cells was removed and then supplemented with culture medium and MTT was added – the final concentration was 1.2 mM, cultured for 4 hours. The supernatant was removed and the formed formazan was extracted with DMSO. MTT was determined in 96-well polypropylene plates (Corning, NY, USA). The formazan absorbance was measured with a Biotek Eon™ microplate spectrophotometer at a wavelength of 550 nm. Measurements were replicated 6 times in 2 independent experiments for each hypericin concentration and light intensity.

Concentration IL-2, IL-4 and IL-6 assessment
The following concentrations in the tested cell culture were analyzed: interleukin

2 (IL-2), interleukin 4 (IL-4), interleukin 6 (IL-6). The concentration of the tested parameters was determined using two types of kits Bio-Plex Pro™ Assay (according to the above-mentioned list of determined parameters) and apparatus Bio-Plex Suspension Array System (BIO-RAD Laboratories Inc, Hercules, CA, USA). Experiment performed in accordance with the kit manufacturer's procedural recommendations. The Bio-Plex system allows the simultaneous determination of up to 100 cytokines in one well 96-well plate for 3 hours in 50 µl of cell culture supernatant. The method is similar to the ELISA enzyme immunoassay technique. Opposites and directed against a specific cytokine are covalently bound on fluorescent dye-encoded polystyrene beads with a diameter of 5.6 µm. Conjugated specific antibodies the bead reacts when incubated with a sample containing a specific cytokine at an unknown concentration. Then, specific detection antibodies are biotinylated, which during the next one incubation binds to other epitopes of the cytokines assayed than coated antibodies on the balls. After carrying out a series of successive washings, in order to remove unbound known antibodies, a complex of streptavidin with phycoerythrins is added to the reaction system, which binds to biotinylated detection antibodies. After another incubation and series washed, the beads with complexed cytokines are resuspended in the appropriate buffer and then introduced into the Bio-Plex instrument for fluorescence reading corresponding to the concentration of individual cytokines assayed. Concentration of each determined cytokine concentration is automatically calculated by the Bio-Plex Manager software based on the domain of the relevant standard curve.

Statistical methods

Measurement results were presented as mean and standard deviation (SD). Statistical significance was determined using Student's t-test (Microsoft Office, Warsaw, Poland). The obtained results were presented in a descriptive form and as tables and graphs. The significance level was set at < 0.05. Graphs contain a description of the coordinate axes and the study groups, a description of the data, numerical values and a graphical representation of standard deviations and statistical significance for the groups compared. The tables show mean values, standard deviation and level of statistical significance measured by Student's t-test. Distribution of variables was checked using the Shapiro-Wolf test.

Results

The levels of the analyzed progression factors

IL-2 concentration was determined using photodynamic therapy in light doses of 1.5 and 10 J/cm^2 and hypericin in concentrations of 0.25 and 0.5 µM. The use of Hyp-PDT did not affect the concentration of IL-2 secreted by SW480 and SW620 cells (Fig. 1 and 2).
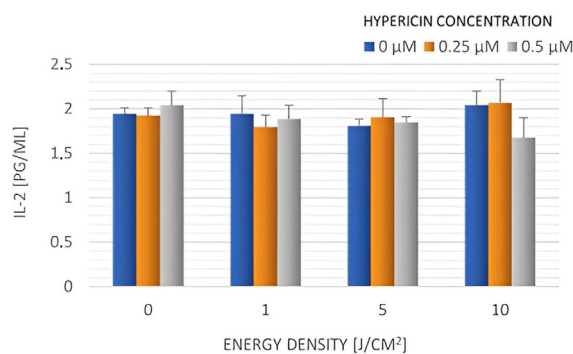

Fig. 1. Diagram presenting the concentration of IL-2 in the tested supernatants from SW480 cancer cells under certain conditions: without Hyp and light (control), after treatment with Hyp PDT at various concentrations and after irradiation at various doses

Fig. 2. Diagram presenting the concentration of IL-2 in the tested supernatants from SW620 cancer cells under certain conditions: without Hyp and light (control), after treatment with Hyp PDT at various concentrations and after irradiation at various doses

Fig. 3. Diagram presenting the concentration of IL-4 in the tested supernatants from SW480 cancer cells under certain conditions: without Hyp and light (control), after treatment with Hyp PDT at various concentrations and after irradiation at various doses
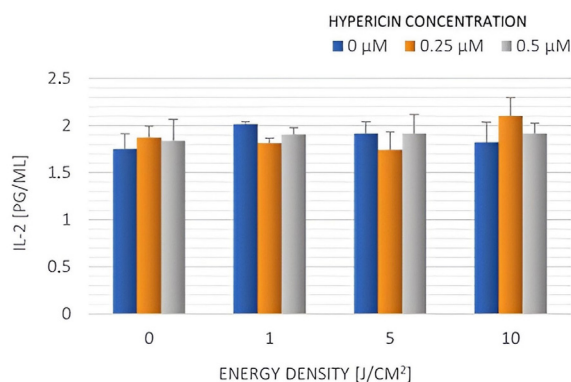
IL-4 concentration was determined using photodynamic therapy in light doses of 1.5 and 10 J/cm^2 and hypericin in concentrations of 0.25 and 0.5 µM. The use

of Hyp-PDT did not affect the concentration of IL-4 secreted by SW480 and SW620 cells (Fig. 3 and 4).
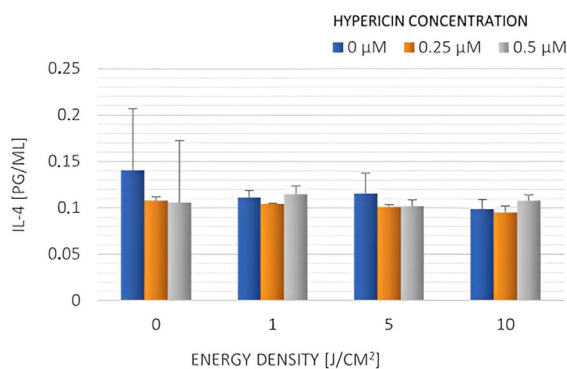
Fig. 4. Diagram presenting the concentration of IL-4 in the tested supernatants from SW480 cancer cells under certain conditions: without Hyp and light (control), after treatment with Hyp PDT at various concentrations and after irradiation at various doses
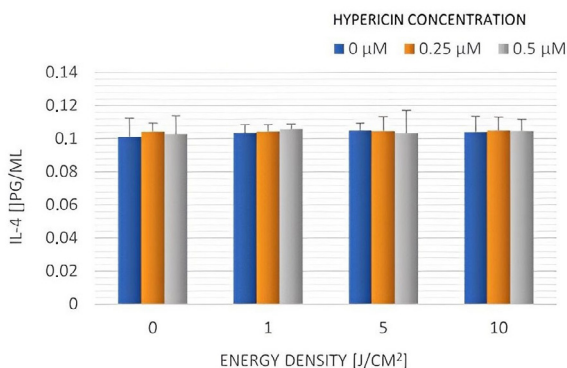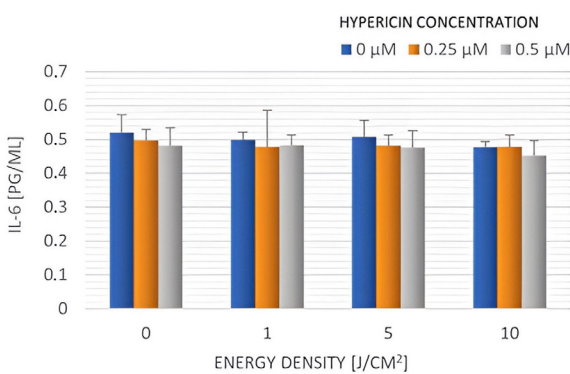

Fig. 5. Diagram presenting the concentration of IL-6 in the tested supernatants from SW480 cancer cells under certain conditions: without Hyp and light (control), after treatment with Hyp PDT at various concentrations and after irradiation at various doses.

Fig. 6. Diagram presenting the concentration of IL-6 in the tested supernatants from SW620 cancer cells under certain conditions: without Hyp and light (control), after treatment with Hyp PDT at various concentrations and after irradiation at various doses

IL-6 concentration was determined using photodynamic therapy in light doses of 1.5 and 10 J/cm² and hypericin in concentrations of 0.25 and 0.5 μM. The use of Hyp-PDT did not affect the concentration of IL-6 secreted by SW480 and SW620 cells (Fig. 5 and 6).
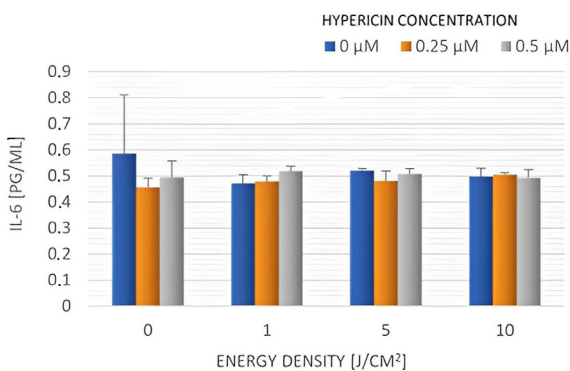
Discussion

The action of photodynamic therapy (PDT) is related not only to the direct effect cytotoxic (through necrosis and apoptosis), indirect, using vascular damage, but also immunomodulatory affecting the activity secretion of both immune cells and cancer cells.[17-20] The synergism the cooperation of these mechanisms results in remission in clinical trials not only the primary lesion, but also distant metastases.[21-25] PDT is effective, selective and minimally invasive, that's the above attributes hide possibility its underperformance due to limited penetration light to the tumor tissue. Therefore, the fundamental question remains as to how PDTs may act on the activation of secreting tumor cells that have not undergone cytotoxic action and, thanks to their activity, may cause recurrence of local and distant metastases through auto- and paracrine secretion of active cytokines, growth factors and pro-angiogenic substances.[26-31] The modern cancer treatment strategy is based on knowledge of cell biology cancer, including its secretory activity, especially the factors determining progression diseases. The author determined a necrosis of induced colorectal cancer cells with cytotoxic doses of Hyp PDT and the effect of therapy used in sublethal doses influences the secretion of cells related to the secretion of responsible factors for growth, invasion, angiogenesis, and metastasis. Determination of biomarkers for colon cancer and the influence of photodynamic therapy with Hyp on their secretion may explain the mechanism of tumor progression and determine if Hyp-PDT has a regressive or progressive effect on the remaining colon cancer cells, which have not been destroyed. Interleukins play an important role in cancer progression. Activate them detaching cells from the cancerous tumor, stimulate the production of VEGF by cells cancer, which induces their proliferation and angiogenesis. Research described in the in vitro and in vivo prove that photodynamic therapy affects cell adherence, especially stimulates the process of neutrophils adhesion to cells, also with the participation 2-integrins. Interleukin-6 - through its pleiotropic action - participates in the regulation of many processes, often causing opposite effects. On the one hand, IL-6 takes active participation in the mobilization of the immune system for anti-cancer defense through activation maturation of megakaryocytes, bone marrow progenitor cells, increased activity natural killer cells and by increasing the expression of MHC class II proteins.[31-32]

Taking into account this aspect of IL-6 activity, no differences in IL-6 concentration caused by the Hyp-

PDT may imply unfavorable, in terms of anticancer defense. On the other hand, the participation of IL-6 has been proven in the malignant transformation of tissues characterized by chronic inflammation, and this process very often it is the cause or a phenomenon that accompanies gastrointestinal cancers.[33] The autocrine production of IL-6 by tumor cells reveals its pejorative the nature of the action, manifested in the acceleration of tumor growth, inhibition of the process apoptosis of cancer cells, induction of angiogenesis and participation in the formation metastases. The author showed in the conducted experiment both lines of cancer cells large intestine: SW480 and SW620 produce IL-6. The results of this study confirm earlier reports of IL-6 secretion by colorectal cancer cells and cells many other cancer lines, e.g. breast cancer.[34,35] In the conducted experiment, the author did not show the effect of HYP PDT on the secretion IL-2, IL-4 and IL-6 via both SW480 and SW620 lines. Contrary to our results Jenny Lou et al. found a 46.64-fold and 61.33-fold increase in IL-6 after repeated photodynamic therapy (R-PDT) using porphyrin lipoprotein (PLP) as a photosensitizer.[36] This effect of the R-PDT and combination R-PDT + αPD-1 relative to PBS respectively, suggesting broad innate immune activation. Kawczyk-Krupka et al. demonstrated, apart from the production of IL-6 by both colon cancer lines, cells of the SW620 line react to the use of light energy alone – increased secretion IL-6, while the application of ALA PDT suppresses the release of this cytokine by cells colorectal cancer line SW620, but does not affect its secretion by cells line SW480. ALA PDT-induced IL-6 reduction may work regressively on colorectal cancer cells, which implies additional benefits of using photodynamic therapy in gastrointestinal cancers.[37] Author concluded that the cytotoxic effect of PDT is supported by an additional mechanism that stops proliferation and migration processes and colorectal cancer angiogenesis, which are coordinated by IL-6. In another study Kawczyk-Krupka et al. reveal that PDT performed in hypoxia-like condition in vitro not only effectively destroy malignant tissue, but also used in sublethal dose can develops its anticancer activity through the reduction of IL-6 and IL-10 secretion.[38] In our study we achieved also in certain parameters a cytotoxic effect, however, when using sublethal doses, we did not notice the effect of Hyp-PDT on the secretion of interleukins. Kaleta-Richter et al. described that based on the identification of immunological cancer biomarkers, the therapy of combining various forms of treatment, including immunotherapy and PDT, may be a justified strategy for colorectal cancer treatment that focuses on individualized comprehensive therapy.[39] Researcher pronounced that after Hyp PDT there was a statistically significant amplification of IL-8 secretion during Hyp-PDT in the SW620

cell line and a statistically significant decrease in IL-8 during Hyp-PDT in the SW480 cell line, with no statistically significant differences in IL-10 concentration following Hyp-PDT in the SW480 or in the SW620 cell.[40] Thus, as in our experiments, the effect of Hyp-PDT on cytokine secretion may have different effects, and it may not have any effect on cytokine secretion.

We confirmed that Hyp-PDT can eliminate a primary tumor not only via cytotoxic effects, but due to immunological mechanism. This influence depended not only of the physical condition of photodynamic therapy, but also on photosensitizer type.

Conclusion

Neoplastic diseases are a constant challenge of modern medicine. Due to the constantly increasing incidence and mortality, alternative methods of treatment are sought and improved. PDT as a method without serious side effects is gaining more and more popularity mainly in pediatric studies in children with various types of cancer. PDT with Hyp- successfully inhibits tumor growth via apoptosis and necrosis in various models and clinical trials. What's more it is much cheaper in comparison with the currently used photosensitizers. The photodynamic effect of Hyp is directed at various subcellular organelles, primarily the mitochondria and the endoplasmic reticulum complex. Depending on the conditions of drug administration and light PDT effect leads to cell death which occurs by induction of necrosis, apoptosis, or autophagy-related cell death. Exposure of colorectal cancer cells to Hyp-PDT in sublethal doses did not affect the progression of tumor cells dependent on the release of IL-2,4,6, which is related to the lack of differences in IL concentration for both the SW480 and SW620 cell lines after PDT, compared with controls. In the conducted experiment, the author did not show the effect of Hyp- PDT on the secretion IL-2, IL-4 and IL-6 via both SW480 and SW620 lines, nevertheless, further experiments should be performed, not only in vitro but also in vivo, to fully evaluate the above immunological effect of photodynamic therapy.

Declarations

Conflicts of interest
The authors declare no conflict of interest.

Data availability
Data available on request from the authors.

Ethics approval
Not applicable.

References

1. Jendželovská Z, Jendželovský R, Kuchárová B, Fedoro-čko P. Hypericin in the light and in the dar: two sides of the same coin. *Front Plant Sci.* 2016;7:560. doi: 10.3389/fpls.2016.00560

2. Dong X, Zeng Y, Zhang Z, et al. Hypericin-mediated photodynamic therapy for the treatment of cancer: a review. *J Pharm Pharmacol.* 2021;73:425-436. doi: 10.1093/jpp/rgaa018

3. Huntosova W, Stroffekova K. Hypericin in the Dark: Foe or Ally in Photodynamic therapy? *Cancers (Basel).* 2016;8:93. doi: 10.3390/cancers8100093

4. Vandenbogaerde AL, Kamuhabwa A, Delaey E, Himpens BE, Merlevede WJ, De Witte PA. Photocytotoxic effect of pseudohypericin versus hypericin. *J Photochem Photobiol B Biol.* 1998;45:87-94. doi: 10.1016/s1011-1344(98)00163-8

5. Schempp CM, Winghofer B, Müllerm K, et al. Effect of oral administration of Hypericum perforatum extract (St. John's wort) on skin erythema and pigmentation induced by UVB, UVA, visible light and solar simulated radiation. *Phytother Res.* 2003;17:141-146. doi: 10.1002/ptr.1091

6. Agostinis P, Vantieghem A, Merlevede W, De Witte PA. Hypericin in cancer treatment: More light on the way. *Int J Biochem Cell Biol.* 2002;34:221-241. doi: 10.1016/s1357-2725(01)00126-1

7. Nestor MS, Gold MH, Kauvar AN, et al. The use of photodynamic therapy in dermatology: results of a consensus conference. *J Drugs Dermatol.* 2006;5:140-154.

8. Braathen LR, Szeimies RM, Basset-Seguin N, et al. International Society for Photodynamic Therapy in Dermatology. Guidelines on the use of photodynamic therapy for nonmelanoma skin cancer: an international consensus. International Society for Photodynamic Therapy in Dermatology, 2005. *J Am Acad Dermatol.* 2007;56:125-143. doi: 10.1016/j.jaad.2006.06.006

9. Henderson BW, Dougherty TJ. How does photodynamic therapy work? *Photochem Photobiol.* 1992;55:145-157. doi: 10.1111/j.1751-1097.1992.tb04222.x

10. Dolmans DE, Fukumura D, Jain RK. Photodynamic therapy for cancer. *Nat Rev Cancer.* 2003;3:380-387. doi: 10.1038/nrc1071

11. Wołuń-Cholewa M, Szymanowski K, Markwitz-Nowak E, Warchoł W. Photodiagnosis and photodynamic therapy of endometriotic epithelial cells using 5-aminolevulinic acid and steroids. *Photodiagnosis Photodyn Ther.* 2011;8:58-63. doi : 10.1016/j.pdpdt.2010.12.003

12. Ostańska E, Aebisher D, Bartusik-Aebisher D. The potential of photodynamic therapy in current breast cancer treatment methodologies. *Biomed Pharmacother.* 2021;137:1-10. doi: 10.1016/j.biopha.2021.111302

13. Kawczyk-Krupka A, Bugaj AM, Potempa A, Wasilewska K, Latos W, Sieroń A. Vascular targeted photodynamic therapy in the treatment of neovascular age-related macular degeneration: clinical perspectives. *Photodiagnosis Photodyn Ther.* 2015;12:161-175. doi: 10.1016/j.pdpdt.2015.03.007

14. Mhawech P, Renaud A, Sene C, Lüdicke F, Herrmann F, Szalay-Quinodoz I, van den Bergh H, Major AL. High efficacy of photodynamic therapy on rat endometrium after systemic administration of benzoporphyrin derivative monoacid ring A. *Hum Reprod.* 2003;18:1707-1711. doi: 10.1093/humrep/deg341

15. Olek M, Kasperski J, Skaba D, Wiench R, Cieślar G, Kawczyk-Krupka A. Photodynamic therapy for the treatment of oral squamous carcinoma- clinical implications resulting from in vitro research. *Photodiagnosis Photodyn Ther.* 2019;27:255-267. doi: 10.1016/j.pdpdt.2019.06.012

16. Szygula M, Pietrusa A, Adamek M, Wojciechowski B, Kawczyk-Krupka A, Cebula W, Duda W, Sieron A. Combined treatment of urinary bladder cancer with the use of photodynamic therapy (PDT) and subsequent BCG-therapy: a pilot study. *Photodiagnosis Photodyn Ther.* 2004;1:241-246. doi: 10.1016/S1572-1000(04)00067-5

17. Morton CA, Brown SB, Collins S, et al. Guidelines for topical photodynamic therapy: report of a workshop of the British Photodermatology Group. *Br J Dermatol.* 2002;146:552-567. doi: 10.1046/j.1365-2133.2002.04719.x

18. Kiesslich T, Krammer B, Plaetzer K. Cellular mechanism and prospective applications of hypericin in photodynamic therapy. *Curr Med Chem.* 2006;13:2189-2204. doi: 10.2174/092986706777935267

19. Miccoli L, Beurdeley-Thomas A, De Pinieux G, et al. Light-induced photo-activation of hypericin affects the energy metabolism of human glioma cells by inhibiting hexokinase bound to mitochondria. *Cancer Res.* 1998;58:5777-5786.

20. French CS, Smith JHC, Virgin HI, Airth RL. Fluorescence-spectrum curves of chlorophylls, pheophytins, phyco-erythrins, phycocyanins and hypericin. *Plant Physiol.* 1956;31:369-374. doi: 10.1104/pp.31.5.369

21. Seyed MA, Malini O, Ganyik Y, Khee CS. Induction of apoptosis by hypericin through activation of caspase-3 in human carcinoma cells. *Int J Mol Med.* 2001;8:521-530. doi: 10.3892/ijmm.8.5.521

22. Couldwell WY, Gopalakrishna R, Hinton DR, He S, Weiss MH, Law RE, Apuzzo ML. Hypericin: A potential antiglioma therapy. *Neurosurgery.* 1994;35:705-710. doi: 10.1227/00006123-199410000-00017

23. Jarvis WD, Turner AJ, Povirk LF, Traylor RS, Grant S. Induction of apoptotic DNA fragmentation and cell death in HL-60 human promyelotic leukaemia cells by phar-

macologic inhibitors of protein kinase C. *Cancer Res.* 1994;54:1707-1714.

24. Koren H, Schenk GM, Jindra RH, Alth G, Ebermann R, Kubin A, Koderhold G, Kreitner M. Hypericin in phototherapy. *J Photochem Photobiol B.* 1996;36:113-119. doi: 10.1016/s1011-1344(96)07357-5

25. Zhang W, Lawa RE, Hintona DR, Su Y, Couldwell WT. Growth inhibition and apoptosis in human neuroblastoma SK-N-SH cells induced by hypericin, a potent inhibitor of protein kinase C *Cancer Lett.* 1995;96:31-35. doi: 10.1016/0304-3835(95)03914-i

26. Fox FE, Niu Z, Tobia A, Rook AH. Photoactivated hypericin is an anti-proliferative agent that induces a high rate of apoptotic death of normal, transformed, and malignant T lymphocytes: Implications for the treatment of cutaneous lymphoproliferative and inflammatory disorders. *J Invest Dermatol.* 1998;111:327-332. doi: 10.1046/j.1523--1747.1998.00278.x

27. Huygens A, Kamuhabwa AR, De Witte PA. Stability of different formulations and ion pairs of hypericin. *Eur J Pharm Biopharm.* 2005;59:461-468. doi: 10.1016/j.ejpb.2004.09.014

28. Liu F, Pan C, Drumm P, Ang CY. Liquid chromatography–mass spectrometry studies of St. John's wort methanol extraction: Active constituents and their transformation. *J Pharm Biomed Anal.* 2005;37:303-312. doi: 10.1016/j.jpba.2004.10.034

29. Alecu M, Ursaciuc C, Hălălău F, et al. Photodynamic treatment of basal cell carcinoma and squamous cell carcinoma with hypericin. *Anticancer Res.* 1998;18:4651-4654.

30. Chen WR, Carubelli R, Liu H, Nordquist RE. Laser immunotherapy: a novel treatment modality for metastatic tumors. *Mol Biotechnol.* 2003;25:37-44. doi : 10.1385/MB:25:1:37

31. Gallagher G, Stimson WH, Findlay J, AI-Azzawi F. Interleukin-6 enhances the induction of human lymphokine--activated killer cells. *Cancer Immmunol Immunother.* 1990;31:49-52. doi: 10.1007/BF01742495

32. Takai Y, Wong GG, Clark SC, Burakoff SJ, Herrmann SH. B cell stimulatory factor-2 is involved in the differentiation of cytotoxic T lymphocytes. *J Immunol.* 1988;140:508-512.

33. Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002;420:860-867. doi: 10.1038/nature01322

34. Esfandi F. Mohammadzadeh Ghobadloo S, Basati G. Interleukin-6 level in patients with colorectal cancer. *Cancer Lett.* 2006;244:76-78. doi: 10.1016/j.canlet.2005.12.003

35. Zhang GJ, Adachi I. Serum interleukin-6 levels correlate to tumor pro-gression and prognosis in metastatic breast carcinoma. *Anticancer Res.* 1999;19:1427-1432.

36. Lou J, Aragaki M, Bernards N, et al. Repeated photodynamic therapy mediates the abscopal effect through multiple innate and adaptive immune responses with and without immune checkpoint therapy. *Biomaterials.* 2023;292:121918. doi: 10.1016/j.biomaterials.2022.121918

37. Kawczyk-Krupka A, Czuba Z, Latos W, et al. Influence of ALA-mediated photodynamic therapy on secretion of interleukins 6, 8 and 10 by colon cancer cells in vitro. *Photodiagnosis Photodyn Ther.* 2018;22:137-139. doi: 10.1016/j.pdpdt.2018.02.021

38. Kawczyk-Krupka A, Latos W, Oleś P, et al. The influence of 5-aminolevulinic photodynamic therapy on colon cancer cell interleukin secretion in hypoxia-like condition in vitro. *Photodiagnosis Photodyn Ther.* 2018;23:240-243. doi: 10.1016/j.pdpdt.2018.07.007

39. Kaleta-Richter M, Kawczyk-Krupka A, Aebisher D, Bartusik-Aebisher D, Czuba Z, Cieślar G. The capability and potential of new forms of personalized colon cancer treatment: Immunotherapy and Photodynamic Therapy. *Photodiagnosis Photodyn Ther.* 2019;25:253-258. doi: 10.1016/j.pdpdt.2019.01.004

40. Kaleta-Richter M, Aebisher D, Jaworska D, Czuba Z, Cieślar G, Kawczyk-Krupka A. The Influence of Hypericin-Mediated Photodynamic Therapy on Interleukin-8 and -10 Secretion in Colon Cancer Cells. *Integr Cancer Ther.* 2020;19:1534735420918931. doi: 10.1177/1534735420918931