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The influence of 5-aminolevulinic photodynamic therapy on colon cancer cell interleukin secretion in hypoxia-like condition in vitro

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ABSTRACT

Photodynamic therapy (PDT) becomes a method of personalized cancer treatment, based on the individual determination of cancer biomarkers. The aim of the study was to evaluate the influence of PDT with δ-aminolevulinic acid (ALA-PDT) used in sub-lethal dose on the interleukins secretion (IL-6, IL-8 and IL-10) by the residual colon cancer cells (CCC) under hypoxia-like conditions (addition of cobalt chloride- $CoCl_2$). CCC: SW480 and SW620 cells were incubated with ALA, $CoCl_2$ and irradiated with red light. The cells viability was detected using MTT assay, LDH and apoptosis tests. Determination of interleukins was carried out using the Bio- Plex Assay Pro™ kit on the Bio- Plex Suspension Array System. After ALA-PDT we found no change in the IL-6 level secreted by SW480 cells, but decrease of IL-6, IL-10 secretion by SW620 cells, an increase in the IL-8 secreted by both cells lines. The levels of IL-6, IL-8 and IL-10 secreted by more aggressive SW620 cells were higher than released by SW480 cells. We concluded, that PDT not only effectively destroy malignant tissue, but also used in sub-lethal dose can develops its anticancer activity through the reduction of IL-6 and IL-10 secretion. On the other hand, we reveal an unfavorable PDT effect, connected with increase of IL-8 secretion by both treated colon cancer cell lines, which implicates the use of adjuvant immunotherapy against IL-8, as a part of individualized colon cancer therapy.

1. Introduction

A problem in cancer treatment is the recurrence of cancer associated with the presence of residual cancer cells, which may lead to the progression of tumor growth and metastasis. Photodynamic therapy (PDT) efficaciously destroys cancer tissue, and can also induce a complex immune response, that potentiates antitumor immunity, which could be a chance to exterminate residuals cancer cells [1]. Three cytokines; IL-6, IL-8 and IL-10, play a crucial role in cancer progression and human anticancer defense, and the question is, how idoes secretion of these cytokines by residual colon cancer cells during photodynamic therapy impact therapy. Interleukin-6 (IL-6) is a pleiotropic keystone cytokine with unquestionable participation in the carcinogenesis process by the inhibiting tumor cell apoptosis, inducing cell proliferation and angiogenesis within the tumor, and by the acceleration the invasion of tumor cells into the lymph nodes and to the liver [2–6]. In colorectal cancer the carcinogenesis is accompanied by increased synthesis and release of not only IL-6 but also interleukin-8 (IL-8), that is responsible for inflammation, migration, tumor growth, angiogenesis and metastases, especially in solid tumors, including colorectal cancer [7–11]. Interleukin-10 (IL-10) is initially proved to have immunosupressive activity, which involves limiting the synthesis of important cytokines by

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lymphocytes, monocytes and macrophages, and by impairing the expression of MHC class II molecules on human monocytes [12]. It was also showed that, depending on the genetic variant, the specific alleles of the IL-10 gene may have a different effect on the risk of colorectal cancer, i.e. the C allele in rs1800872 may have protective effects and reduce the risk of cancer development [13].

2. Aim

The main objective of our study was to evaluate the effect of sub-lethal dose PDT using the photosensitizer-precursor δ-aminolevulinic acid (ALA-PDT) on the IL-6, IL-8 and IL-10 secretion of residual colon cancer cells under hypoxia-like conditions, achieved by the presence of cobalt chloride ($CoCl_2$).

3. Methods

3.1. Photodynamic treatment

The investigations were carried out using the human colon cancer cell lines SW480 and SW620 as model cells from ATCC (American Type Cell Culture - ATCC LGC Limited, UK) and cultured in accordance with standard protocols as previously described [14–17].

After 24 h of adhesion, the SW480 and SW620 cells were separately incubated with 1000 μM of ALA, a precursor of the photosensitizer protoporphyrin IX (PpIX), for 4 h. 100 μM of $CoCl_2$ were added to cell culture plates at this point. The cells were irradiated with visible light from an incoherent light source PDT TP-1 (Cosmedico Medizintechnik GmbH, Schwenningen, Germany) equipped with infrared and orange filters permeable to wavelengths of 600–720 nm, according to the manufacturer's statement. At an average power density of 1.5 $mW.cm^{-2}$, total fluence of 10 $J.cm^{-2}$ were used in the present study. Light dose controller automatically calculated exposure time. This protocol was precisely previously described [14–17].

3.2. Cell viability assessment and quantification of apoptosis and necrosis

The MTT assay was performed to assess viability of the cells in both lines, using the tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma Aldrich, St. Louis, MO, USA). Furthermore, cell viability was also assessed by measuring lactate dehydrogenase (LDH) activity, using a Lactate Dehydrogenase Activity Assay Kit (Roche Diagnostic GmbH, Mannheim, Germany). In addition to viability, determination of the cell death mode, i.e. apoptosis and necrosis, was implied. Quantification of cell death types was performed 24 h after irradiation and/or ALA treatment using Apoptotic, Necrotic & Healthy Cells Quantification Kit from Biotium, Inc. (Hayward, USA). These protocols were previously described [14–17].

3.3. Concentration of IL-6, IL-8 and IL-10

The concentrations of IL-6, IL-8 and IL-10 released from cancer cells were determined 24 h after ALA-treatment and/or irradiation using the Bio-Plex sets Pro™ Cytokine Assay (BIO-RAD Laboratories, Inc.) and the Bio-Plex Suspension Array System (BIO-RAD Laboratories, Inc.) according to the manufacturer's instructions. This protocol was previously described [18].

3.4. Statistical analysis

All measured values are presented as means + SD (standard deviation). The two-tailed Student's *t*-test was used for comparison between groups in all experiments. Values of $p < 0.05$ were considered as statistically significant. All experiments were repeated 4 times, n = 4.

4. Results

4.1. MTT cell viability results

The viability after ALA-PDT in sub-lethal dose (1000 μM 10 $J.cm^{-2}$) in hypoxic-like condition was compared to the native-control group (80 ± 1.2% SW620 and 94 ± 2.1% SW480) ($p < 0.001$). The cell viability of SW480 and SW620 cell lines decreased exponentially with an increase of ALA concentration and PDT fluences. These results were previously described [14–17].

4.2. LDH cytotoxicity results

The same doses of ALA-PDT as in MTT assay were evaluated in the LDH assay relative to two colorectal cancer cell lines in hypoxia-like (with CoCl2) environment. The cytotoxicity after exposure SW480 cells to 1000 μM ALA and fluence 10 $J.cm^{-2}$ in an hypoxia-like environment was 8 ± 0.4% and for SW620 cell line 4 ± 0.3% ($p < 0.001$). These results were previously described [14–17].

Taking into account the above results, ALA of 1000 μM concentration and fluence 10 $J.cm^{-2}$ were chosen for use in the next steps of experiment, since those ALA-PDT parameters were a sub-lethal doses.

4.3. Apoptosis detection via fluorescent microscope results

To confirm the sub-lethal effect of previous viability and cytotoxicity test results (1000 μM of ALA, fluence of 10 $J.cm^{-2}$, with $CoCl_2$), the cells apoptosis was performed. These sub-lethal doses of ALA-PDT didn't cause differences in the number of apoptotic cells in comparison with the control group. In hypoxia like conditions apoptosis percentage of SW620 cells was 6.4 ± 0.4% when in SW480 line it was 3.0 ± 1.3%, what was previously presented [14].

4.4. IL-6, IL-8, IL-10 concentration after ALA PDT in hypoxia- like conditions

1 After ALA-PDT we found no change in the IL-6 level secreted by SW480 cells (14.17 ± 0.58 pg. mL^{-1} vs control cells 15.67 ± 4.65 $pg.mL^{-1}$) however 35% decrease of IL-6 secretion by ALA-PDT treated SW620 cells under hypoxia-like conditions, compared to the respective untreated control (20.17 ± 3.79 $pg.mL^{-1}$ vs control cells 3083 ± 375 $pg.mL^{-1}$) (Fig. 1). In addition, the general level of IL-6 secretion by SW620 cells is significantly higher (30.83 ± 3.75 pg. mL^{-1}) than that by SW480 cells (15.67 ± 4.65 $pg.mL^{-1}$) ($p < 0.05$). The number of experiments was n = 4.

2 After ALA-PDT we found an increase in the IL-8 level secreted by SW480 (311.33 ± 34.07 pg. mL^{-1} vs control cells 191.00 ± 46.81 pg. mL^{-1}) and SW620 cells compared to the untreated control (2285.50 ± 208.50 pg. mL^{-1} vs control cells 537.67 ± 168.64 pg. mL^{-1}) (Fig. 2). The general level of IL-8 secretion by SW620 cells is significantly higher than that by SW480 cells (537.7 ± 168.64 pg. mL^{-1} vs 191.0 ± 46.81 pg. mL^{-1}) ($p < 0.05$). The number of experiments was n = 4.

3 After ALA-PDT we found the decrease in the IL-10 level secreted by SW480 (829.17 ± 49.49 pg. mL^{-1} vs control cells 1095.83 ± 141.62 pg. mL^{-1}) and SW620 cells compared to the untreated control (980.33 ± 103.50 pg. mL^{-1} vs control cells 1360.67 ± 19,790 pg. mL^{-1}) (Fig. 3). The general level of IL-8 secretion by SW620 cells is significantly higher than that by SW480 cells (1360.7 ± 197.90 pg. mL^{-1} vs 1095.8 ± 141.62 pg. mL^{-1}) ($p < 0.05$). The number of experiments was n = 4.

5. Discussion and conclusion

The recurrence of cancer is associated with the presence of residual cancer cells and with the progression of tumor growth and metastasis