Differential Expression of VEGF and IL-1alpha After Photodynamic Treatment in Combination with Doxorubicin or Taxotere

DAIVA DABKEVICIENE[1], AUSRA SASNAUSKIENE[1], EVA LEMAN[1,2], RAIMONDA KVIETKAUSKAITE[1] and VIDA KIRVELIENE[1]

[1]*Department of Biochemistry and Molecular Biology, Vilnius University, Vilnius, Lithuania;*
[2]*Department of Evolutionary and Environmental Biology, University of Haifa, Haifa, Israel*

Abstract. *Aim: To show the impact of chemotherapeutic drugs doxorubicin and taxotere on the molecular pattern of cell response to photodynamic treatment (PDT). Materials and Methods: Human squamous cell carcinoma cells A-431 were studied. Apoptosis was investigated by recording caspase-3 activity. Expression of IL-1alpha and VEGF on mRNA and protein levels was measured by qPCR and ELISA. Results: PDT in combination with either doxorubicin or taxotere was found to be more cytotoxic in comparison to either single-treatment. The expression of IL-1alpha and VEGF was up-regulated in PDT-treated cells, either alone or in combination with doxorubicin or taxotere. Addition of doxorubicin to the cytokine induction after PDT was not detected, however, taxotere promoted significant over-expression of IL-1alpha and VEGF on the protein level. Conclusion: Contribution of chemotherapeutic drugs to IL-1 alpha and VEGF release from cells which received dual treatment involving PDT could be significantly different, despite the same level of cytotoxicity.*

Combination regimens for cancer treatment have shown increased response rates in comparison to monotherapy. These strategies aim to exploit effects arising from the non-overlapping targets and mechanisms of action of two or more treatment modalities. Due to a specific mode of action, photodynamic therapy (PDT) offers the attractive addition to the conventional strategies of cancer treatment. PDT is based on the use of non-toxic photosensitizing compounds that localize quite selectively in neoplastic/hyperplastic tissues and become cytotoxic when exposed to light inducing production of reactive oxygen species (ROS) in the illuminated area. The first attempts to incorporate PDT into multi-treatment regimens were recorded decades ago. Currently, antitumor efficacy of combined regimens of PDT is an active subject of both pre-clinical and clinical research (1).

It has been shown that molecular responses triggered by PDT in surviving tumor or stromal cells could be reduced by combination of PDT with some chemotherapeutic agents (2). In order to reveal the effects of such combination, we studied the response of A-431 cells to PDT *in vitro* combined with the administration of the conventional anti-tumor drugs doxorubicin (Dox) and taxotere (Tax).

The photosensitizer used in this study, m-tetra(3-hydroxyphenyl)-chlorin (mTHPC, generic name Temoporfin, trade name Foscan®) (3) is one of the most potent photosensitizers currently available for clinical use. mTHPC localizes to cellular membranes and induces damage to endoplasmic reticulum, Golgi apparatus (4) and mitochondria (5). Doxorubicin ([Dox], adriamycin) is one of the most extensively studied chemotherapeutic drugs used as single-agents in therapy for various cancers (6). It accumulates mainly in the cell nucleus and its main molecular target is DNA topoisomerase II (7). The potential of Dox to enhance the effects of PDT has already been investigated with some photosensitizers. In almost all cases, the combined treatment was more effective than either single treatment (8-11).

Another chemotherapeutic agent used in the present study is taxotere ([Tax], docetaxel), a semi-synthetic microtubule inhibitor of the taxane family. Disturbance of cytoskeleton structure and cellular transport, inhibition of cell division and induction of apoptosis are considered to be the modes of action of microtubules-bound Tax (12). Recently, it was shown that photodynamic treatment of cells preloaded with docetaxel and a photosensitizer zinc-phthalocyanine produced an increased experimental therapeutic effect in comparison with docetaxel as a single treatment (13).

Correspondence to: Vida Kirveliene, Vilnius University, Department of Biochemistry and Molecular Biology, Ciurlionio 21/27, Vilnius, LT-03101, Lithuania. Tel: +370 67343676, Fax: +370 52398231, e-mail: vida.kirveliene@gf.vu.lt

Key Words: Photodynamic therapy, doxorubicin, taxotere, IL-1alpha, VEGF, mTHPC.

ROS produced by PDT stimulates multiple signal transduction pathways, which may promote either cell death or cell survival or an immune response *in vivo* (2). Our recent study revealed a significant up-regulation of two "crosstalking" cytokines, vascular endothelial growth factor A (VEGF), and interleukin-1alpha (IL-1alpha) in human epidermoid carcinoma A-431 cells following mTHPC-mediated PDT *in vitro* (14). VEGF is a strong promoter of angiogenesis *in vivo* providing tumor with a potential to grow and spread. The combination of PDT and anti-angiogenic treatment has already given promising experimental results (15-17). IL-1alpha expression after PDT is less studied (18). The data on the biological role of IL-1alpha in tumor progression are controversial (for a comprehensive review see Ref. (19)). In general, it is a pleiotropic cytokine that regulates immune and inflammatory responses (20), and its role in angiogenesis has been noted (21).

The main question of the current study was whether the administration of Dox or Tax affected the PDT-induced production of VEGF and IL-1alpha, which might hinder tumor eradication.

Materials and Methods

Materials. A-431 human epidermoid carcinoma cells (DSMZ, Braunschweig, Germany) were routinely cultured in Dulbecco's modified Eagle's medium (DMEM) (Biochrom AG, Berlin, Germany) supplemented with 5% FBS (Biochrom AG, Berlin, Germany) and antibiotics (Chinoin, Budapest, Hungary). Meso-tetrakis(3-hydroxyphenyl)-chlorin (mTHPC) was a generous gift of R. Bonnett, Queen Mary, University of London, UK). mTHPC was dissolved in ethanol as 1 mg ml^{-1} stock solution and stored at –20˚C in the dark. Pharmaceutical solutions of Doxorubicin hydrochloride (Adriamycin, Ebewe Arzneimittel, Unteracht, Austria, 2 mg ml^{-1}) and taxotere® (Rhone-Poulenc, Courbevoie, France, 40 mg ml^{-1}) were stored at 4˚C. All experiments were performed using dilutions of the stock solutions with the culture medium.

Cell treatment. The treatment was carried out according to the experimental schedule shown in Figure 1.

For photosensitized treatment, the cell cultivation medium was changed for FBS-free DMEM containing 100 ng ml^{-1} of mTHPC and the cells were incubated in the dark. After 18 h, the cells were washed with Dulbecco's phosphate-buffered saline (DPBS), and DMEM/FBS was added. The cells were exposed to light for 60 or 90 s from a LED array UNIMELA-1 (λ=660±5 nm, VU Laser Research Centre, Vilnius, Lithuania). The fluence rate at the level of the cells was 1.6 mW cm^{-2}, as measured using an irradiation power meter Nova (Ophir, Jerusalem, Israel). For combined treatment, Dox was added to the cell incubation medium immediately after light exposure to a final concentration of 50-500 ng ml^{-1}, while Tax was added 24 h prior to light exposure to final concentration of 1-2 ng ml^{-1}.

Cytotoxicity assay. Toxicity was estimated by staining with crystal violet. Briefly, the cells were fixed with 96% ethanol for 10 min, 0.05% crystal violet solution in 20% ethanol was added for 30 min, the cells were rinsed, the remaining cell-attached dye was dissolved in 0.1 % acetic acid solution in 50 % ethanol and absorbance recorded at 585 nm (22).

DEVDase activity assay. The Caspase-3 Cellular Activity Assay Kit PLUS (AK-703) (Biomol, Hamburg, Germany) was used. Cells were harvested and treated following the manufacturer's instruction. Fluorescence of 7-amino-4-trifluoromethyl coumarin (AFC), corresponding to the DEVD ase activity, was recorded on fluoroscan Ascent FL (Labsystems, Helsinki, Finland) at excitation/emission wavelengths of 390/510 nm, respectively.

Quantitative RT-PCR (qPCR). Total RNA was isolated, cDNA was synthesized and qPCR reactions were performed as described in our previous study (14). Levels of *VEGFA* and *IL1A* were normalized to *ACTB* and *GAPDH*. Results were analyzed by the Comparative C_T (Threshold Cycle) method.

Enzyme-linked immunosorbent assay (ELISA) was carried out as described in our previous study (14). Results were normalized for 10^6 cells. Based on standard curves, detection limits of ELISA for recombinant human IL-1alpha and VEGF proteins were determined to be 8 and 62 pg ml^{-1}, respectively. The intra-assay coefficients of variation were less than 15%.

Statistical analysis. The SigmaPlot 12.3 software was used for statistical analysis (Systat Software, Alfasoft AB, Goteborg, Sweden). The data are presented as mean±standard deviation (SD) (unless otherwise noted) from at least two independent assays, each one at least in duplicate. Significance was accepted with a p-value <0.05. Data of two groups were compared using the t-test. Multiple comparisons were performed by ANOVA and *post-hoc* tests.

Results

Cytotoxic effects. The cytotoxic effects of PDT and/or Dox or Tax on A-431 cells were characterized in a dose-response manner. Cytotoxicity was measured by the crystal violet staining technique, which was shown to be the most sensitive assay for evaluation of the combined action (23). A number of pilot experiments were performed to establish the optimal concentrations and administration sequence of mTHPC and Dox or Tax. The selected regimens reduced the number of viable cells to 75-10% at 24 h after light exposure. If cytotoxicity differed from the preset value by more than 10%, the results of the experiment were not taken into account. It should be noted, that we aimed to study the pattern of response to the combination and not to achieve a full loss of cell viability. No significant toxicity of mTHPC at the selected concentration without light exposure or that of light without mTHPC was observed. No photocytotoxicity of Dox or Tax due to light exposure was registered.

The effect of the combined treatment was studied by full factorial experiments (Figure 2a and b). Both duration of light exposure of the cells pre-loaded with the fixed concentration of mTHPC and concentration of the Dox or Tax were significant determinants.

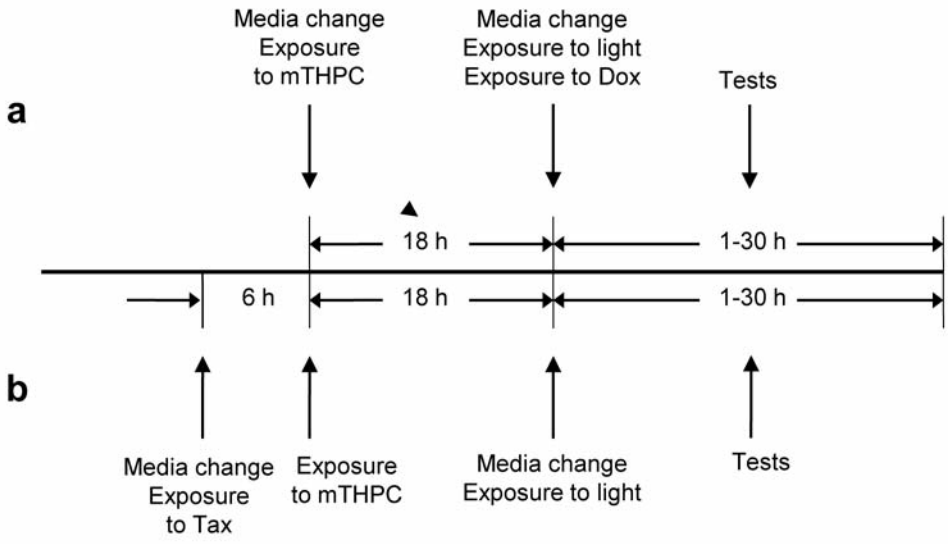

Figure 1. *Schedule for cell treatment. a, Treatment with Dox and PDT, alone and in combination; b, treatment with Tax and PDT, alone and in combination. For PDT, 100 ng ml^{-1} mTHPC was added to cells and incubated in the dark for 18 h. Then the medium was replaced with the fresh one, the cells were exposed to light at 660±5 nm, 1.6 mW cm^{-2}, and incubated in the dark. The chemotherapeutic drugs were administrated as shown in the scheme. After the treatment, the cells were incubated for 1-30 h as follows: 1) 24 h for cell viability assay; 2) 1-30 h for DEVDase activity assay; 3) 1-24 h for mRNA assay, 4) 6 or 24 h for assessment of protein amount.*

In addition, the cytotoxic effect was examined by registering the activation of caspase-3, the executive peptidase of apoptosis (Figure 2c and d). Cell exposure to either Dox or Tax as mono-treatment induced a delayed apoptotic response, since the increase in DEVDase activity was detected only at 24 h post-exposure. The apoptotic response to PDT was more pronounced and prompt, while the highest DEVDase activity was detected at 9 h post-exposure. After either combined treatment, the DEVDase activity was detected at an earlier point and the maximum activity exceeded that induced by PDT-alone.

Expression of IL-1alpha. In order to evaluate *IL1A* and *VEGFA*, we determined the levels of the corresponding mRNA using the qPCR technique. If the ratio of the mRNA levels between treated and non-treated cells exceeded two-fold, the difference was considered to be significant. The effect of light-alone on *IL1A* and *VEGFA* mRNA levels in cells without mTHPC, as well as that of mTHPC without light exposure was not significant.

We found that variations of *IL1A* mRNA level in cells treated with single Dox or Tax was not significant (Figure 3a). Meanwhile, in case of PDT (60% toxicity) and combined treatments PDT+Dox (65% toxicity) and Tax+PDT (90% toxicity), at 4 to 24 h after light exposure, the levels of *IL1A* mRNA were significantly increased. The highest *IL1A* mRNA values were registered at 8 h after light exposure.

To validate the PDT-stimulated production of IL-1alpha in cells, the protein amount was determined using the ELISA method. Since the pronounced accumulation of *IL1A* mRNA was registered at 4 h post-exposure, the protein amount was assayed at 6 h post-exposure, giving two more hours for protein translation to occur. In addition, the protein assessment at 24 h post-exposure was carried-out in order to evaluate the time-course response to treatment. In general, data of *IL1A* expression on the protein level corresponded to the results of mRNA analysis, since all treatment modalities, which involved PDT, resulted in a significant increase of IL-1alpha both in cell lysates (Figure 3b) and cell growth medium (Figure 3c). However, in cells treated with Tax, a difference between mRNA levels and protein amounts was observed; treatment with Tax as a single modality induced a significant accumulation of IL-1alpha protein inside the cells.

Expression of VEGF. Analyzing the time course of *VEGFA* changes at mRNA level, we found that variations in cells after treatment with either Dox (25% toxicity) or Tax (60% toxicity) were not significant. The significant *VEGFA* mRNA increase was detected only when cells were treated with PDT as a single (60% toxicity) or combined-treatment, *i.e.* PDT+Dox (65% toxicity) and Tax+PDT (90% toxicity). In all cases, the highest increase of the mRNA levels was observed at 8 h after light exposure (Figure 4a).

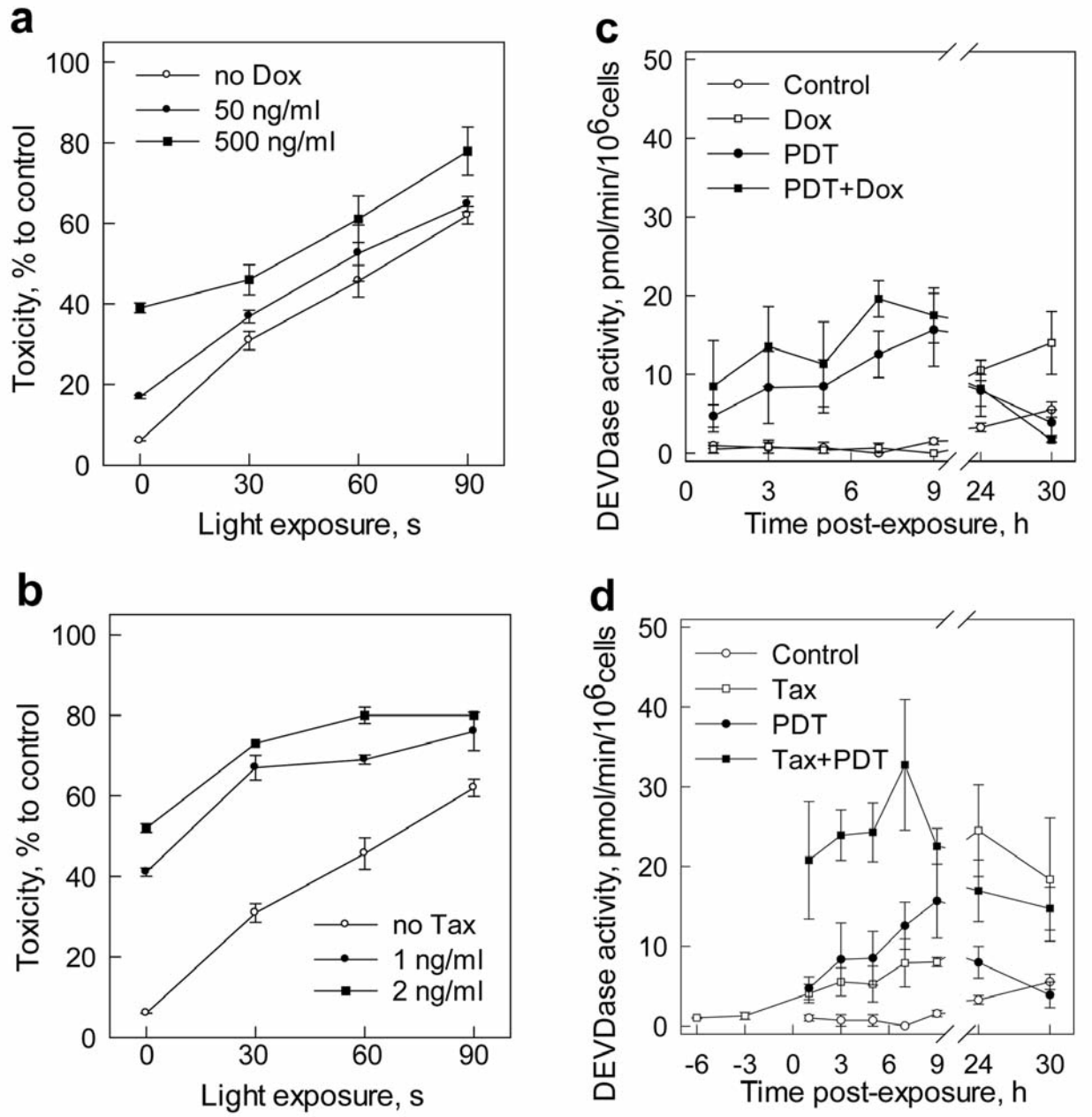

Figure 2. *Cell viability and DEVDase activity in A-431 cells treated with PDT and/or drugs. The cells were incubated with 100 ng ml^{-1} mTHPC (open symbols) or/and Dox or Tax (closed symbols), and exposed to light at 660±5 nm, and 1.6 mW cm^{-2}, when appropriate, according to the scheme in Figure 1. Cell viability was evaluated by staining with crystal violet following incubation for 24 h in the dark. Cell viability at light exposure for 0 s corresponds to that of "no PDT". a, Cell viability after PDT and Dox; b, Cell viability after PDT and Tax; c, DEVDase activity after PDT and Dox (50 ng ml^{-1} Dox, light exposure at 660±5 nm for 60 s, fluence 96 mJ cm^{-2}), d, DEVDase activity after PDT and Tax (2 ng ml^{-1} Tax, light exposure at 660±5 nm for 60 s, fluence 96 mJ cm^{-2}); the point '0 h post-exposure' corresponds to 24 h incubation with Tax. Control, non-treated cells. Error bars, ±SE, n=3.*

The amount of VEGF protein in cell lysates was below the detection limit. Meanwhile, in the cell culture medium the VEGF protein amount was significantly increased at 24 h after PDT-alone and in combination with cytotoxic drugs (Figure 4b). Dox as a single-treatment or in combination with PDT did not affect VEGF amount in cell cultivation medium. However, 2 ng×ml^{-1} Tax-alone or in combination with PDT significantly increased VEGF protein amount at 24 h after light exposure.

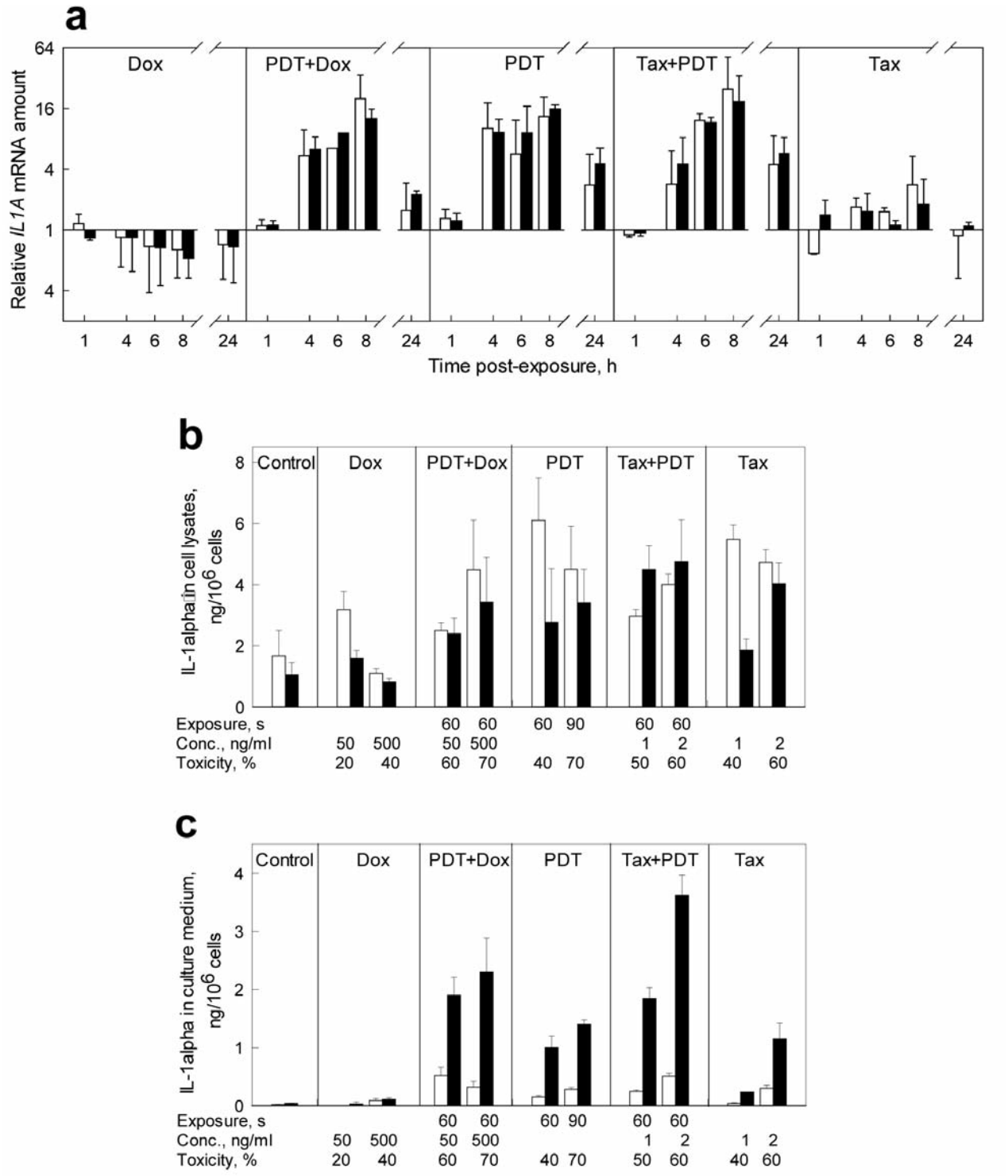

Figure 3. *Time- and dose-dependent expression of IL-1alpha in A-431 cells following PDT or/and administration of drugs. The cells were treated according a scheme shown in Figure 1. a, Time-course of IL1A mRNA accumulation in cells following PDT after light exposure for 60 s or/and incubation with 50 ng ml^{-1} Dox or 2 ng ml^{-1} Tax, related to control cells. Data normalized to ACTB (open bars) and GAPDH (closed bars); b, Dose dependence of IL-1alpha protein accumulation in cells following PDT or/and incubation with Dox or Tax; The cells were harvested at 6 h (open bars) and 24 h (closed bars) after treatment; c, Dose dependence of IL-1alpha protein accumulation in cell culture medium following PDT or/and incubation with Dox or Tax; The cells were harvested at 6 h (open bars) and 24 h (black bars) after treatment. Control, non- treated cells; Error bars, ±SD. Data of PDT as a single treatment are reproduced from our previous study (14) for comparison.*

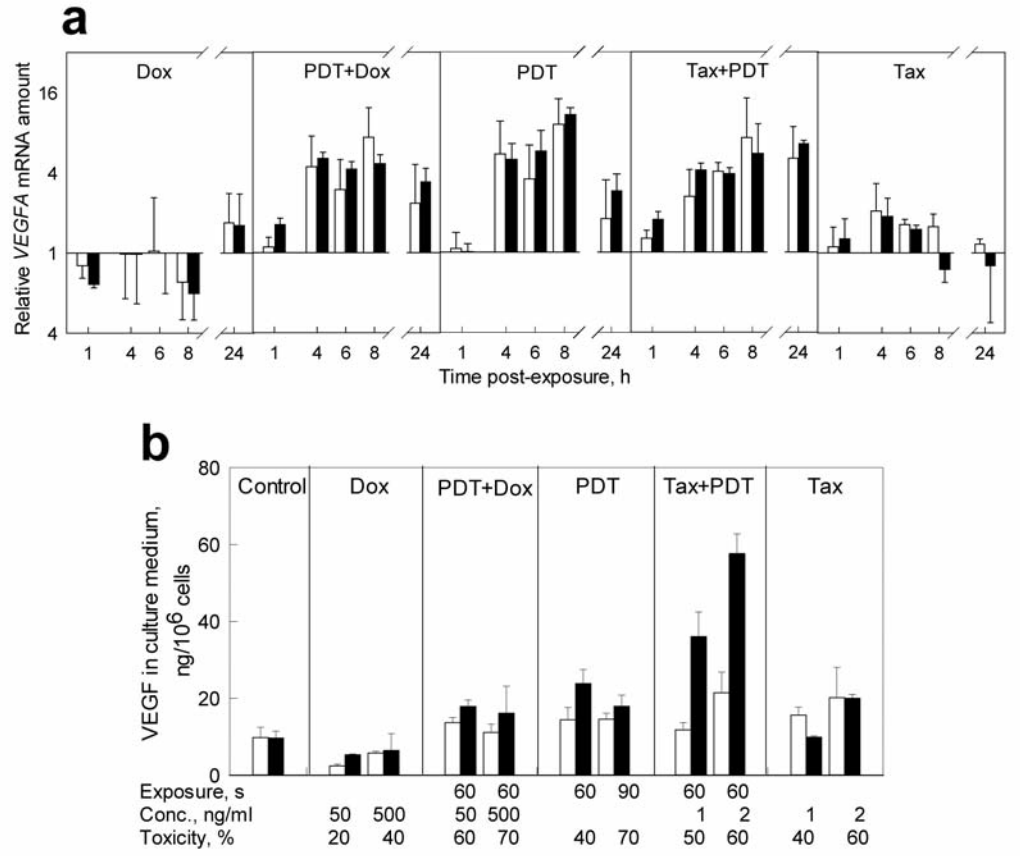

Figure 4. *Time-and dose-dependent expression of VEGF in A-431 cells following PDT or/and administration of drugs. The cells were treated according a scheme shown in Figure 1. a, Time-course of VEGFA mRNA accumulation in cells following PDT after light exposure for 60 s or/and incubation with 50 ng ml^{-1} Dox or 2 ng ml^{-1} Tax, related to control cells; b, Dose-dependence of VEGF protein accumulation in cell culture medium following PDT or/and incubation with Dox or Tax; The cells were harvested at 6 h (open bars) and 24 h (closed bars) after treatment. Control, non treated cells; Error bars, ±SD. Data of PDT as a single treatment are reproduced from our previous study (14) for comparison.*

Discussion

Each treatment adds its anti-tumor action as well as side-effects to the overall result. In the present study, we aimed to estimate some pros and cons of PDT combination with two efficacious chemotherapeutic drugs *in vitro*, since alongside the induction of direct tumor eradication, PDT stimulates the expression and release of cytokines that could affect the tumor response to the treatment (24). A human epidermoid carcinoma cell line has been chosen as a model system because it responds to stress by increased expression of numerous cytokines. Our goal was to reveal a contribution of the widely used chemotherapeutic agents Dox and Tax to the PDT-induced overexpression of two "crosstalking" cytokines, IL-1alpha and VEGF.

The enhanced cytotoxicity of the combined treatments *versus* single ones with involvement of increased apoptosis (we did not study other modes of cell death) was an expected outcome of our experiments in accordance with the findings of many other authors (25). The main value of our study lies in data on accumulation of *IL1A* and *VEGFA* mRNAs and their proteins (IL-1alpha and VEGF, respectively) in PDT-treated cells, and, especially, the contribution of Dox and Tax. The effects of these two chemotherapeutic agents on PDT-treated cells were different. In general, PDT was the main factor inducing the overexpression of both IL-1alpha and VEGF. Dox treatment as a single modality or combined with PDT had no significant effect on the expression of the studied cytokines. Tax as a single modality, slightly but significantly increased the amount of both cytokines in cells treated with a high dose of the drug, whereas the combined treatment with Tax and PDT resulted in more than twice as high amount of IL-1alpha and VEGF in cell culture medium as after PDT-alone. In summary, (i) cell treatment involving PDT induced increased amounts of

IL-1alpha and VEGF, (ii) Dox and Tax contribution to cytokine expression in PDT-treated cells was different.

What could be the outcome of the stimulated cytokine production? There are plenty of published studies regarding the pro-tumor activity of VEGF due to its strong angiogenic action. The effects of IL-1alpha are less studied and less predictable. The precursor form of IL-1alpha is constitutively expressed and retained inside resting cells. Its activity can be greatly enhanced by proteolysis to two mature components (26). The N-terminal pro-piece has the nuclear localization sequence and can probably influence the transcription of other genes. The so-called C-terminal component can be actively exported out of the cell or be passively released upon cell death (27). It can attach to the host cell membrane in tumor and activate anti-tumor responses by increasing immunogenicity. On the other hand, it can enter the tumor microenvironment and induce invasiveness of tumor cells (28). Thus, it is hard to predict the final effect of this cytokine on tumor eradication.

The parallel increase of IL-1alpha and VEGF in the treated cells was not accidental. Recently, we have shown that recombinant extracellular IL-1alpha contributes to overexpression of VEGF in cells after mTHPC-PDT (14). Besides, a close connection between VEGF and pro-inflammatory cytokines has been demonstrated by using an antibody against VEGFR-2, which suppress IL-1alpha activity (29).

The distinct cellular response to Dox and Tax may be induced by different mechanisms of action. In addition to different cellular targets, these drugs possibly trigger diverse signalling pathways. It has been shown that Dox elicits immunogenic cell death (ICD) (30). Immunogenic effects of Tax sensitize the tumor to cytotoxic T lymphocyte killing by a pathway, which is distinct from ICD (31).

In conclusion, contribution of the chemotherapeutic drug Tax to IL-1alpha and VEGF release from cells, which received the dual treatment involving PDT, is significantly higher than that of Dox, despite the same level of produced cytotoxicity. Thus, the final outcome of the combined treatment *in vivo* could depend not only on the cytotoxic potential of a drug, but the miscellaneous responses of the treated cell as well. The combination of PDT with a particular chemotherapeutic drug should be applied after considering the possible hazard of consequences, *i.e.* after proper targeted-studies.

Acknowledgements

DD and AS contributed equally to this work. We are indebted to Prof. R. Bonnett (Queen Mary, University of London, UK) for the generous gift of mTHPC and V. Piskarskiene for the excellent care of the cells. This work was funded by the European Social Fund under National Integrated Programme Biotechnology & Biopharmacy, grant VP1-3.1-SMM- 08-K01-005. EL gratitudes ERASMUS MUNDUS, EDEN project for scholarship.

References

1 Huang YY, Vecchio D, Avci P, Yin R, Garcia-Diaz M and Hamblin MR: Melanoma resistance to photodynamic therapy: new insights. Biol Chem *394*: 239-250, 2013.

2 Oleinick NL and Evans HH: The photobiology of photodynamic therapy: cellular targets and mechanisms. Radiat Res *150*: S146-156, 1998.

3 Bonnett R, White RD, Winfield UJ and Berenbaum MC: Hydroporphyrins of the meso-tetra(hydroxyphenyl)porphyrin series as tumour photosensitizers. Biochem J *261*: 277-280, 1989.

4 Teiten MH, Bezdetnaya L, Morliere P, Santus R and Guillemin F: Endoplasmic reticulum and Golgi apparatus are the preferential sites of Foscan localisation in cultured tumour cells. Br J Cancer *88*: 146-152, 2003.

5 Sasnauskiene A, Kadziauskas J, Vezelyte N, Jonusiene V and Kirveliene V: Apoptosis, autophagy and cell cycle arrest following photodamage to mitochondrial interior. Apoptosis *14*: 276-286, 2009.

6 Minotti G, Menna P, Salvatorelli E, Cairo G and Gianni L: Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev *56*: 185-229, 2004.

7 Pommier Y, Leo E, Zhang H and Marchand C: DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. Chem Biol *17*: 421-433, 2010.

8 Brophy PF and Keller SM: Adriamycin enhanced *in vitro* and *in vivo* photodynamic therapy of mesothelioma. J Surg Res *52*: 631-634, 1992.

9 Canti G, Nicolin A, Cubeddu R, Taroni P, Bandieramonte G and Valentini G: Antitumor efficacy of the combination of photodynamic therapy and chemotherapy in murine tumors. Cancer Lett *125*: 39-44, 1998.

10 Casas A, Fukuda H, Riley P and del C Batlle AM: Enhancement of aminolevulinic acid based photodynamic therapy by adriamycin. Cancer Lett *121*: 105-113, 1997.

11 Kirveliene V, Grazeliene G, Dabkeviciene D, Micke I, Kirvelis D, Juodka B and Didziapetriene J: Schedule-dependent interaction between Doxorubicin and mTHPC-mediated photodynamic therapy in murine hepatoma *in vitro* and *in vivo*. Cancer Chemother Pharmacol *57*: 65-72, 2006.

12 Jordan MA: Mechanism of action of antitumor drugs that interact with microtubules and tubulin. Curr Med Chem Anticancer Agents *2*: 1-17, 2002.

13 Conte C, Ungaro F, Maglio G, Tirino P, Siracusano G, Sciortino MT, Leone N, Palma G, Barbieri A, Arra C, Mazzaglia A and Quaglia F: Biodegradable core-shell nanoassemblies for the delivery of docetaxel and Zn(II)-phthalocyanine inspired by combination therapy for cancer. J Control Release *167*: 40-52, 2013.

14 Dabkeviciene D, Sasnauskiene A, Leman E, Kvietkauskaite R, Daugelaviciene N, Stankevicius V, Jurgelevicius V, Juodka B and Kirveliene V: mTHPC-mediated photodynamic treatment up-regulates the cytokines VEGF and IL-1alpha. Photochem Photobiol *88*: 432-439, 2012.

15 Zhou Q, Olivo M, Lye KY, Moore S, Sharma A and Chowbay B: Enhancing the therapeutic responsiveness of photodynamic therapy with the antiangiogenic agents SU5416 and SU6668 in murine nasopharyngeal carcinoma models. Cancer Chemother Pharmacol *56*: 569-577, 2005.

16 Tirand L, Frochot C, Vanderesse R, Thomas N, Trinquet E, Pinel S, Viriot ML, Guillemin F and Barberi-Heyob M: A peptide competing with VEGF165 binding on neuropilin-1 mediates targeting of a chlorin-type photosensitizer and potentiates its photodynamic activity in human endothelial cells. J Control Release *111*: 153-164, 2006.
17 Ferrario A and Gomer CJ: Avastin enhances photodynamic therapy treatment of Kaposi's sarcoma in a mouse tumor model. J Environ Pathol Toxicol Oncol *25*: 251-259, 2006.
18 Koon HK, Lo KW, Leung KN, Lung ML, Chang CC, Wong RN, Leung WN and Mak NK: Photodynamic therapy-mediated modulation of inflammatory cytokine production by Epstein-Barr virus-infected nasopharyngeal carcinoma cells. Cell Mol Immunol *7*: 323-326, 2010.
19 Rider P, Carmi Y, Voronov E and Apte RN: Interleukin-1alpha. Semin Immunol *25*: 430-438, 2013.
20 Dinarello CA: IL-1: discoveries, controversies and future directions. Eur J Immunol *40*: 599-606, 2010.
21 Voronov E, Carmi Y and Apte RN: The role IL-1 in tumor-mediated angiogenesis. Front Physiol *5*: 114, 2014.
22 Ito M: Microassay for studying anticellular effects of human interferons. J Interferon Res *4*: 603-608, 1984.
23 Mickuviene I, Kirveliene V and Juodka B: Experimental survey of non-clonogenic viability assays for adherent cells *in vitro*. Toxicol In Vitro *18*: 639-648, 2004.
24 Agostinis P, Berg K, Cengel KA, Foster TH, Girotti AW, Gollnick SO, Hahn SM, Hamblin MR, Juzeniene A, Kessel D, Korbelik M, Moan J, Mroz P, Nowis D, Piette J, Wilson BC and Golab J: Photodynamic therapy of cancer: an update. CA Cancer J Clin *61*: 250-281, 2011.
25 Zuluaga MF and Lange N: Combination of photodynamic therapy with anti-cancer agents. Curr Med Chem *15*: 1655-1673, 2008.
26 Afonina IS, Tynan GA, Logue SE, Cullen SP, Bots M, Luthi AU, Reeves EP, McElvaney NG, Medema JP, Lavelle EC and Martin SJ: Granzyme B-dependent proteolysis acts as a switch to enhance the proinflammatory activity of IL-1alpha. Mol Cell *44*: 265-278, 2011.
27 Jiang S, Dupont N, Castillo EF and Deretic V: Secretory *versus* degradative autophagy: unconventional secretion of inflammatory mediators. J Innate Immun *5*: 471-479, 2013.
28 Voronov E, Shouval DS, Krelin Y, Cagnano E, Benharroch D, Iwakura Y, Dinarello CA and Apte RN: IL-1 is required for tumor invasiveness and angiogenesis. Proc Natl Acad Sci USA *100*: 2645-2650, 2003.
29 Salven P, Hattori K, Heissig B and Rafii S: Interleukin-1alpha promotes angiogenesis *in vivo* via VEGFR-2 pathway by inducing inflammatory cell VEGF synthesis and secretion. Faseb J *16*: 1471-1473, 2002.
30 Casares N, Pequignot MO, Tesniere A, Ghiringhelli F, Roux S, Chaput N, Schmitt E, Hamai A, Hervas-Stubbs S, Obeid M, Coutant F, Metivier D, Pichard E, Aucouturier P, Pierron G, Garrido C, Zitvogel L and Kroemer G: Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. J Exp Med *202*: 1691-1701, 2005.
31 Hodge JW, Garnett CT, Farsaci B, Palena C, Tsang KY, Ferrone S and Gameiro SR: Chemotherapy-induced immunogenic modulation of tumor cells enhances killing by cytotoxic T lymphocytes and is distinct from immunogenic cell death. Int J Cancer *133*: 624-636, 2013.

Received June 20, 2014
Revised July 25, 2014
Accepted July 28, 2014