RESEARCH ARTICLE

Effect of pentoxifylline on expression of proinflammatory cytokines

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Proinflammatory cytokines play a dominant role as local or systemic regulators in the acute inflammatory response. Tissue injury causes the release of proinflammatory cytokines, which are involved in many aspects of inflammation. Pentoxifylline, a non-specific phosphodiesterase inhibitor, has anti-inflammatory properties and can suppress the production of some factors of inflammatory response and presumably prevent certain diseases. The aim of this study was to investigate the potential impact of the phosphodiesterase inhibitor, pentoxifylline, on expression of proinflammatory cytokines and whether this effect is mediated by activation of transcription factors NFκB and AP-1. Experiments were performed in human and murine monocyte and endothelial cell lines stimulated with lipopolysaccharide, in the presence or absence of pentoxifylline. Synthesis of cytokines was measured by using enzyme-linked immunosorbent assay. Treatment with pentoxifylline did not cause any cytotoxicity. Pentoxifylline inhibited lipopolysaccharide-induced DNA binding of NFκB and AP-1 transcription factors. It also decreased production of TNFα and VEGF and showed some tendency to inhibit basal production of IL-8. In contrast, it upregulated release of IL-1β and IL-6. The mechanisms in charge of action of pentoxifylline on cytokine network are still not entirely understood. Nevertheless, it seems that those regulatory pathways are cell-specific, type of stimulus- or time of stimulation-dependent, and variable for different inflammatory mediators.

Keywords: Pentoxifylline; inflammation; endothelial cells; monocytes/macrophages; cytokines.

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Introduction

Inflammation is the reaction of immune system toward the infection and injury, involved in the pathogeneses of various maladies as arthritis, cancer, stroke, neurodegenerative, and cardiovascular diseases. The acute-phase reaction is mediated by proinflammatory cytokines and involves increased blood flow and vascular permeability as well as the accumulation of fluid or leukocyte infiltration [1]. In healthy conditions, immune response is controlled to restrict the potentially injurious effects of sustained or surplus inflammatory reactions. However, in pathologic conditions, these immunomodulatory mechanisms may either exert insufficient control or compensate and block the immune response, rendering the host at risk from systemic infection [2].

Various cytokines, such as tumor necrosis factor-α (TNFα), interleukins (IL-6, IL-1, IL-8, IL-11), granulocyte colony stimulating factor (G-CSF), and granulocyte-macrophage colony stimulating factor (GM-CSF) play a role in mediating acute inflammatory reactions. Among these, TNFα, IL-

1α and IL-1β are potent primary cytokines that mediate acute inflammation induced in animals by bacterial lipopolysaccharide (LPS).

Pentoxifylline (PTX) is a tri-substituted purine (3,7 -dimethyl-1-(5-oxohexyl)-3,7-dihydro-1H-purine-2,6-dione) along with caffeine, theophylline, and theobromine. It is a methylxanthine derivative and competitive nonselective phosphodiesterase (PDE) inhibitor, with hemorheological and immunomodulating properties. PTX induces relaxation of smooth muscles and stimulates diuresis, affecting the functioning of the cardiovascular and central nervous systems. Importantly, it exhibits anti-inflammatory properties in many experimental and clinical settings [3, 4]. PTX modulates the cytokine network and inhibits expression of some proinflammatory cytokines. Especially, PTX was found to potently inhibit AP-1 and NFκB transcription factors, the major regulators of proinflammatory cytokines such as TNF, IL-6, and IL-8 [3]. A very important effect of PTX is inhibition of expression of TNF, demonstrated at mRNA and protein levels in several cell lines. Inhibition of TNFα results in attenuation of inflammation and reduction of oxidative stress [3, 5]. PTX was also reported to inhibit interleukins IL-1β, IL-2, IL-6, IL-8, IL-12 or interferon-γ (INFγ), and GM-CSF [6]. On the other hand, PTX induces the expression of anti-inflammatory IL-10 [7]. Moreover, PTX inhibits adhesion of immune cells to endothelium, which affects the functions of other cell types, such as vascular smooth muscle cells (VSMC), pericytes, platelets, and resident macrophages through the production of several mediators [8, 9]. Dysfunction of the endothelium associated with increased leukocytes adhesion and enhanced inflammatory reaction is an important factor in the pathogenesis of vascular diseases.

Because PTX influences the processes in which endothelium plays a crucial role, it seems reasonable to compare the effect of PTX on endothelial cells and inflammatory cells. Thus, the aim of this study was to investigate the potential impact of PTX on the expression of proinflammatory cytokines and activation of NFκB and AP-1 transcription factors.

Materials and methods

Cell culture

The human monocyte U937 cell line was kindly provided by Dr. Guenter Weigel, Medical University of Vienna, Austria. The cells were cultured in RPMI-1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL), streptomycin (10 μg/mL), and L-glutamine (2 mM). Both murine macrophage J774 cell line (ATCC, Manassas, VA, USA) and murine brain microvascular endothelial cell-1 (MBEC-1) cell line (kindly provided by Dr. Joanna Bereta, Jagiellonian University, Krakow, Poland) were cultured in Dulbecco's modified eagle's medium (DMEM)-HG (PAA Laboratories, GmbH, Pasching, Austria) supplemented with 10% FBS, L-glutamate (2 mM), penicillin (100 U/mL), and streptomycin (100 μg/mL). Human microvascular endothelial cells (HMEC-1) were obtained from Center for Disease Control in Atlanta, Georgia, USA. They were cultured in MCDB-131 medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% FBS, L-glutamate (2 mM), epidermal growth factor (EGF) (10 ng/mL), hydrocortisone (1 μg/mL), penicillin (100 U/mL), and streptomycin (10 μg/mL) (Sigma, Poznan, Poland). Human umbilical vein endothelial cells (HUVEC) were freshly isolated from umbilical veins of newborn babies by collagenase digestion. HUVEC were grown in M199 medium (PAA Laboratories, GmbH, Pasching, Austria) supplemented with 20% FBS, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (20 mM), L-glutamine (2 mM), heparin/endothelial cell growth supplement (30 ng/mL), penicillin (100 U/mL), and streptomycin (100 μg/mL) (Sigma, Poznan, Poland). Cells of the second to fourth passages were used in experiments. For differentiation, U937 cells were grown overnight on a 6-well plate at a density of 8×10^5 cells per well. Cells were then incubated with Phorbol Myristate Acetate (PMA) for 24 hours and

washed with phosphate buffer saline (PBS) to remove non-adherent cells. The number of adherent cells were counted. All experiments were performed in resting and LPS-stimulated cells cultured at a confluence of 80-90% in standard conditions (humidified atmosphere, 21% O_2, 5% CO_2). Some experiments were performed under hypoxic conditions to generate environment more relevant to *in vivo* conditions. For such purpose, cells were incubated for the last 24 h under hypoxic conditions (2% O_2, 5% CO_2)

Cell viability assays

Viability of the cells was assessed for different concentrations of PTX, ranging from 0.1 µM to 1,000 µM. Cells were seeded in 96-well plates and cultured up to confluence of 80-90% when PTX was added for 24 h. After this time, viability was measured by using lactate dehydrogenase (LDH) release or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay.

MTT reduction assay

This colorimetric test is based on the reduction of a yellow tetrazolium salt to navy blue formazan, catalyzed by mitochondrial dehydrogenases. Amounts of the reduced product reflect activity of mitochondria and are considered as a measure of cell viability. Cells were cultured in 96-well plates in 90 µL of medium per well. To each well, 10 µL of MTT (Sigma, Poznan, Poland) solution (5 mg/mL) was added and the cells were incubated for further 1-3 hours until formazan crystals were formed. Subsequently, medium was aspirated and crystals were dissolved in 100 µL/well of acidic isopropanol (0.2 M HCl in isopropanol). The absorbance of samples was measured at 562 nm using VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA). Results were expressed as the percentages of reduced MTT, assuming the absorbance of control cells as 100%.

LDH release assay

Cell viability was also determined by using CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega, Madison, WI, USA). This test measures the activity of the cytosolic enzyme lactate dehydrogenase (LDH), which is released from dead cells. Cells were cultured in 96-well plates in 100 µL of medium per well. To measure the LDH activity, 30 µL of medium from each well were transferred to 96-well assay plate and mixed with 30 µL of Substrate Mix. After color developing in the dark at room temperature (~20 minutes), 30 µL of Stop Solution was added to the samples. The absorbance of samples was read at 492 nm using VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Preparation of nuclear extract

The nuclear extract kit (Active Motif, Rixensart, Belgium) was used for this purpose. The procedure was performed according to the vendor's protocol. Protein concentration was determined using the Bradford assay.

Evaluation of NFκB and AP-1 activity

NFκB activity was measured in nuclear protein extracts (2-20 µg) by using TransAMTM NFκB p65 protein assay (Active Motif, Rixensart, Belgium), an enzyme-linked immunosorbent assay (ELISA)-based method designed to specifically detect and quantify NFκB p65 subunit activation. The assay was performed according to vendor's protocol and analyzed by using VersaMax microplate reader (Molecular Devices, Sunnyvale, CA, USA). Similar assay was performed for AP-1.

Measurement of inflammatory mediators

To measure the release of murine and human vascular endothelial growth factor (VEGF) and cytokines (IL-1β, IL-6, and IL-8), cells were seeded into 24-well plates and grown up to 80-90% confluence. Then, fresh medium was introduced and treated with PTX at concentrations of 0.1 µM to 1,000 µM and/or LPS (100 ng/mL). Culture media were collected after 24 h, and concentrations of cytokine proteins in the culture media were quantified by using ELISA (R&D Systems (Minneapolis, MN, USA) following the manufacturer's instructions.

Statistical analysis

Statistical analysis of the data was performed with Microsoft Excel 2013 software. All experiments were performed in duplicates or triplicates and, if not reported otherwise, they were repeated at least three times. Results were expressed as mean ± SD unless otherwise stated. Data obtained in the experiments were analyzed with Student's t-test. Statistical significance was accepted at $P<0.05$.

Results

PTX does not affect cell viability

To check the potential cytotoxicity of PTX, HUVEC, HMEC-1, and MBEC-1 cells were treated with the wide range of PTX doses, namely 0.1 µM – 1,000 µM, which are commonly used for *in vitro* experiments. Viability of cells after a 24-hour stimulation was measured by using MTT reduction assay. PTX did not cause any cytotoxicity in the endothelial cell lines studied (Figure 1A). Very similar results were obtained by using LDH release assay (data not shown). Also, no significant changes in cell viability were observed in both U937 human monocytes and J774 murine macrophages treated with different doses of PTX, even at the very high doses applied (Figure 1B).

PTX inhibits NFκB and AP-1 transcription factors function

To check the effect of PTX on the activation of NFκB and AP-1, the U937 cell-line were pre-incubated with PTX (100 µM or 1,000 µM), and then, stimulated with 100 ng/mL of LPS. The cells treated with PTX alone showed a slight upregulation of DNA binding activity of NFκB, although a statistically significant difference was only found at the concentration of 1,000 µM PTX when comparing to the control, untreated cells (Figure 2A). Cells stimulated with LPS, without pre-treatment with PTX, showed two-fold elevation in DNA binding activity of NFκB in comparison to control cells. Importantly, addition of 100 µM and 1,000 µM PTX resulted in a dose-dependent downregulation of DNA NFκB binding with a statistically significant difference reached at the concentration of 1,000 µM PTX in comparison to the cells treated with LPS alone (Figure 2A). Furthermore, PTX dose-dependently downregulated DNA binding of AP-1 transcription factor. Again, the difference was statistically significant when the cells exposed to 1,000 µM PTX were compared to the untreated counterparts (Figure 2B). Noticeably, AP-1 activity was 1.5-fold stronger in the cells treated alone with 100 ng/mL LPS than that in control cells. This upregulation was significantly reduced by pre-treatment with 100 µM or 1,000 µM PTX (Figure 2B).

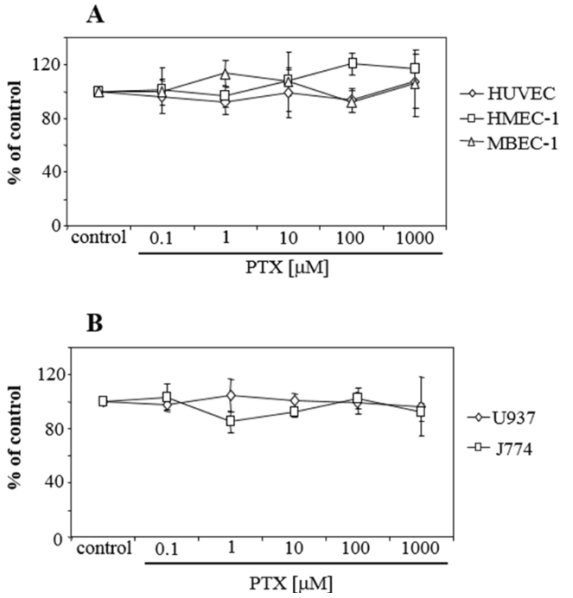

Figure 1. Effect of PTX on viability. **A:** HMEC-1, HUVEC, and MBEC. **B:** U937 monocytes and J774 macrophages. Cells were cultured for 24 hours in the presence of PTX (0.1 µM – 1,000 µM). Cytotoxicity was measured by using MTT reduction assay. Each point represents mean ± SD of three experiments done in triplicates.

PTX inhibits expression of TNFα

Inhibitory effect on NFκB and AP-1 activities suggested that PTX may also decrease the production of proinflammatory cytokines. First, the effect of PTX on TNFα was analyzed. As shown in Figure 3, production of TNFα in the undifferentiated U937 monocyte cells was very low, even after stimulation with LPS. However, production was highly increased after cell differentiation by phorbol myristate acetate

(PMA) 24 hours prior to stimulation with PTX and LPS. Similarly, unstimulated J774 macrophage-like cells produced only small amounts of TNFα, but they responded effectively to the stimulation with LPS (Figure 3A). Expression of TNFα was not induced by hypoxia (Figure 3A). As expected, PTX significantly and concentration-dependently inhibited production of TNF in U937 (Figure 3B) and J774 (Figure 3C). Moreover, a similar, dose-dependent effect was demonstrated for the primary endothelial cells, HUVECs (Figure 3D). These results support and supplement our observation on the effect of PTX on TNFα production described earlier [3].

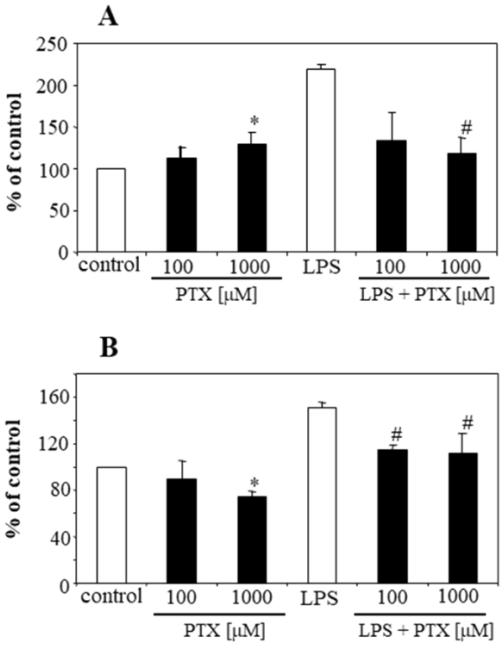

Figure 2. Effect of PTX on activity of transcription factors. NFκB (**A**) and AP-1 (**B**) in cells incubated with 100 μM and 1,000 μM PTX and/or 100 ng/mL LPS. Binding activities of the transcription factors in extracts of nuclear proteins were measured by using TransAM ELISA. Data are presented as percentage of the control value (control – untreated cells). *: P<0.05 in comparison with control. #: P<0.01 in comparison with control.

PTX inhibits expression of VEGF under normoxic and hypoxic conditions

Further experiments were designed to check whether PTX can modulate production of vascular endothelial growth factor (VEGF), the proangiogenic mediator involved in regulation of inflammatory response. To this purpose, cells were exposed to LPS or hypoxia with or without pre-treatment with different doses of PTX. First, the generation of VEGF in a steady state was measured. Resting undifferentiated U937, differentiated U937, and J774 cells cultured in 24-well plates released approximately 600 pg/mL, 500 pg/mL, and 400 pg/mL of VEGF protein, respectively (Figure 4A). This production was not changed by LPS (100 ng/mL) but was significantly increased up to ~800 pg/mL in response to hypoxia (2% O_2). Production of VEGF by HUVECs, even cultured under hypoxic condition, was below the detection threshold (data not shown). Both in U937 monocytes and J774 macrophage-like cell lines cultured under normoxic conditions, PTX dose-dependently decreased the production of VEGF even by 50% at the highest dose used (Figure 4B and 4C). In cells cultured under hypoxia, the effect of PTX was slightly less pronounced (Figure 4B and 4C). Noteworthy, although stimulation of cells with LPS did not change production of VEGF, in the LPS-activated monocytes and macrophages PTX was much less effective (data not shown).

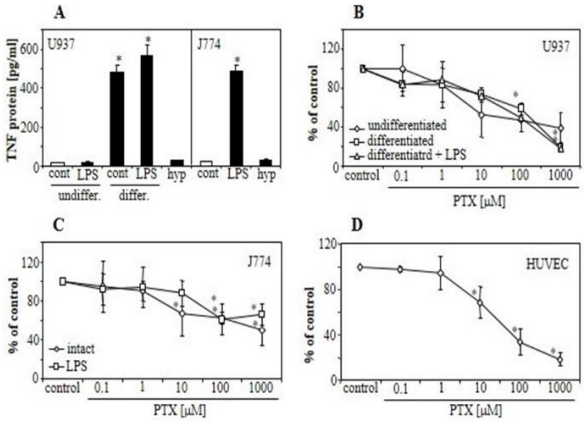

Figure 3. Effect PTX on TNFα synthesis. **A:** Concentration of TNFα protein in cultured media harvested from U937 monocytes and J774 macrophages. Some cells were treated with LPS (100 ng/mL) or kept in hypoxia (2% O_2). **B:** Effect of PTX on release of TNFα protein in LPS-stimulated undifferentiated and differentiated U937 monocytes. **C:** Effect of PTX on release of TNFα protein in LPS-stimulated J774 macrophages. **D:** Effect of PTX on release of TNFα protein in LPS-stimulated HUVEC. TNFα was measured by using ELISA. Cells were exposed to PTX for 24 hours. Data represents mean ± SD of 3-5 experiments. *: P<0.05 in comparison with control.

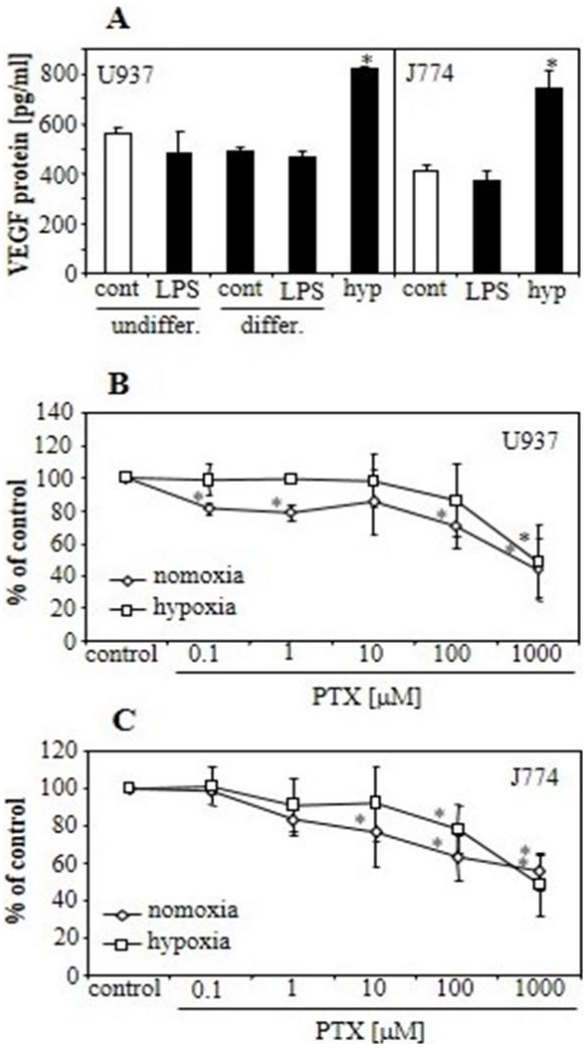

Figure 4. Role of PTX on growth factor modulation. **A:** Concentration of VEGF protein in cultured media harvested from U937 monocytes and J774 macrophages. Some cells were treated with LPS (100 ng/mL) or kept in hypoxia (2% O_2). **B:** Effect of PTX on release of VEGF protein in LPS-stimulated U937 monocytes. **C:** Effect of PTX on release of VEGF protein in LPS-stimulated J774 macrophages. VEGF was measured by using ELISA. Cells were exposed to PTX for 24 hours. Values represents mean ± SD of 3-5 experiments. *: P<0.05 versus control cells.

PTX increases IL-1β release

The next cytokine studied was IL-1β. Production of IL-1β in undifferentiated U937 monocytes was measurable but very low and was not changed by LPS. While resting differentiated U937 monocytes and J774 macrophages released only small amounts of IL-1β, its synthesis was strongly induced in response to LPS. On the other hand, both cell lines produced relatively small amount of IL-1β in response to hypoxia (Figure 5A). Both in U937 and J774 cells, PTX tended to increase IL-1β release in a dose-dependent manner, although this effect did not reach statistical significance (Figure 5B and 5C). In the endothelial cells treated with LPS, PTX at low concentrations (up to 10 μM) showed a similar tendency to induce IL-1β, although this upregulation was diminished by the highest concentrations of PTX (100 μM and 1 mM) (Figure 5D).

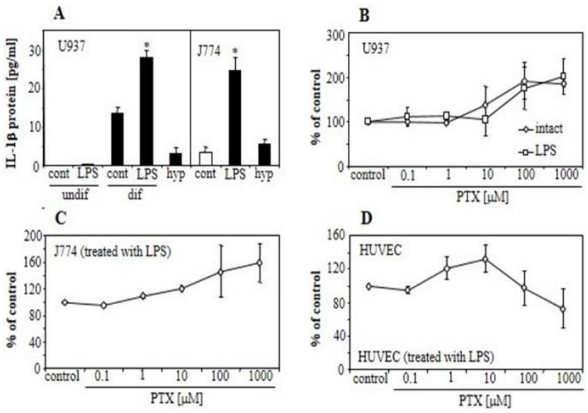

Figure 5. Effect PTX on IL-1β modulation. **A:** Concentration of IL-1β protein in cultured media harvested from U937 monocytes and J774 macrophages. Some cells were treated with LPS (100 ng/mL) or kept in hypoxia (2% O_2). **B:** Effect of PTX on release of IL-1β protein in LPS-stimulated U937 monocytes. **C:** Effect of PTX on release of IL-1β protein in LPS-stimulated J774 macrophages. **D:** Effect of PTX on release of IL-1β protein in LPS-stimulated HUVECs. IL-1β was measured by using ELISA. Cells were exposed to PTX for 24 hours. Data represents mean ± SD. *: P<0.05 in comparison with control.

PTX increases synthesis of IL-6 in LPS-stimulated cells

Additionally, the production of proinflammatory IL-6 cytokine was evaluated in several cell lines. Like in case of IL-1β, resting monocytes and macrophages produced relatively small amounts of IL-6. However, its synthesis was strongly induced in differentiated U937 and J774 cell lines in response to LPS. Like IL-1β, U937 monocytes and J774 macrophages produced small amounts of IL-6 in response to hypoxia (Figure 6A). The role of PTX in IL-6 modulation was assessed in a steady-state and after stimulation of cells with LPS. In resting undifferentiated U937 cells, PTX revealed some tendency to enhance IL-6

production in a dose-dependent manner, however, this production was reduced by the highest dose of PTX (Figure 6B). Similar tendency was observed in differentiated U937 cells treated with PTX, regardless of stimulation with LPS (Figure 6B). Importantly, very similar effect was observed in resting and LPS stimulated J774 macrophages (Figure 6C). On the other hand, all endothelial cell lines produced measurable amounts of IL-6. This synthesis was not significantly influenced by PTX at the doses of 0.1 µM to 100 µM. Surprisingly, the highest dose of PTX (1,000 µM) strongly increased IL-6 generation in human and murine microvascular cell lines (Figure 6D). A similar, but not statistically significant, trend was visible also in HUVEC, the venous endothelial cells.

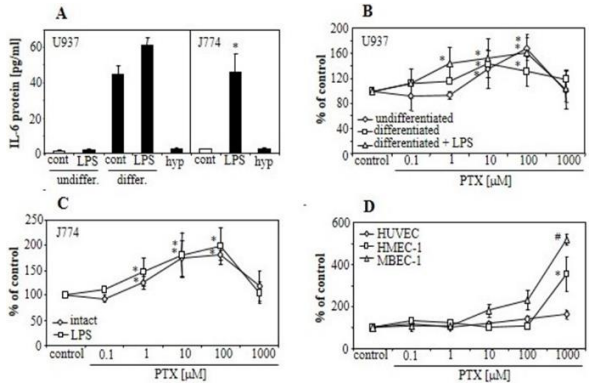

Figure 6. Effect PTX on IL-6 synthesis. **A:** Concentration of IL-6 protein in cultured media harvested from U937 monocytes and J774 macrophages. Some cells were treated with LPS (100 ng/mL) or kept in hypoxia (2% O_2). **B:** Effect of PTX on release of IL-6 protein in LPS-stimulated undifferentiated and differentiated U937 monocytes. **C:** Effect of PTX on release of IL-6 protein in LPS-stimulated J774 macrophages. **D:** Effect of PTX on release of IL-6 protein in LPS-stimulated HUVEC, HMEC-1, and MBEC-1. IL-6 was measured by using ELISA. Cells were exposed to PTX for 24 hours. Data represents mean ± SD. *: P<0.05 in comparison with control. #: P<0.01 in comparison with control.

PTX does not affect constitutive production of IL-8 in human and murine endothelial cells

The level of chemokine IL-8 was scanned in different cell lines. As shown in Figure 7A, resting undifferentiated monocytes U937 and J774 macrophage-like cells produced relatively small amounts of IL-8, whereas differentiated U937 showed about two-fold augmentation in IL-8 in comparison to undifferentiated U937. LPS did not affect synthesis of IL-8 in U937, but strongly induced it in J774 cells. In both cell lines the production of IL-8 remained at the low level under hypoxic conditions (2% O_2). In the control undifferentiated U937 cells, PTX showed some tendency to reduce IL-8 production in a dose-dependent manner (Figure 7B). In contrast, PTX did not influence IL-8 generation in the differentiated U937 cells (Figure 7B). On the other hand, production of IL-8 was diminished by the highest concentrations of PTX (100 µM and 1,000 µM) in J774 cells (Figure 7C). Noteworthy, in cells treated with LPS, PTX did not affect IL-8 expression either in U937 cells (Figure 7B) or in J774 macrophages (Figure 7C). Again, all endothelial cell lines produced considerable amounts of IL-8. PTX did not show any meaningful effects on IL-8 production, in any cell line, even at the highest dose used (Figure 7D). Only in HMEC-1, some tendency of increase in IL-8 release was observed, however, it did not reach statistical significance (Figure 7D).

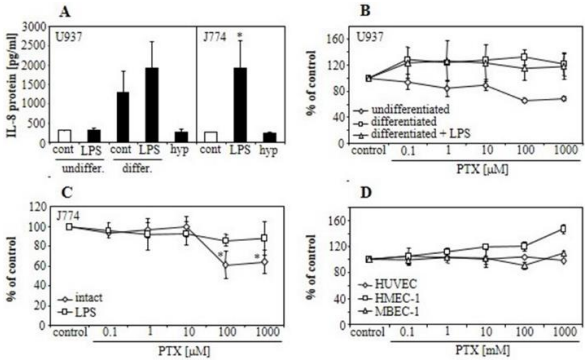

Figure 7. Effect PTX on IL-8 production. **A:** Concentration of IL-8 protein in cultured media harvested from U937 monocytes and J774 macrophages. Some cells were treated with LPS (100 ng/mL) or kept in hypoxia (2% O_2). **B:** Effect of PTX on release of IL-8 protein in LPS-stimulated undifferentiated and differentiated U937 monocytes. **C:** Effect of PTX on release of IL-8 protein in LPS-stimulated J774 macrophages. **D:** Effect of PTX on release of IL-8 protein in LPS-stimulated HUVEC, HMEC-1, and MBEC-1. IL-8 was measured by using ELISA. Cells were exposed to PTX for 24 hours. Data represents mean ± SD of 3-5 experiments. *: P<0.05 in comparison with control.

Discussion

The obtained results revealed that the applied doses of PTX (0.1 μM - 1 mM) were well tolerated by all tested cell lines. This may suggest that even higher doses of PTX do not exert significant side effects. Similar conclusion was claimed after a trial in volunteers [12]. The results of the current study are in concordance with the observation of cytokine-specific and cell-type specific action of PTX. However, earlier experiments gave contradictory results regarding anti-inflammatory effects of PTX [10–12].

It was shown that anti-inflammatory efficacy of PTX is independent of heme oxygenase-1 (HO-1) pathway [3]. Instead, PTX may inhibit NFκB and AP-1 transcription factor activities, the major regulators of proinflammatory cytokines [12, 14-16]. Earlier observations, in line with the current results, demonstrated that the transcription factors were activated by LPS and attenuated by PTX. Such a regulation of NFκB and AP-1 activity was reflected by the production of TNFα, encoded by their target genes. Importantly, inhibition of TNFα synthesis was observed not only in monocytic or macrophage-like cell lines but also in primary human endothelial cells.

Earlier report postulated [17] that PTX can inhibit the expression of VEGF, the most potent proangiogenic mediator known so far, induced under hypoxic conditions and involved in the regulation of inflammatory response [18]. The current results fully support these observations, showing that PTX inhibits expression of VEGF under normoxic and hypoxic conditions, and indicating that PTX can act as anti-angiogenic compound.

Furthermore, it has been shown that PTX can modulate IL-1β synthesis in response to LPS, the most effective proinflammatory stimuli [19], although the exact influence can be different in various tissues [11, 20, 21]. The results of the current study revealed that in all LPS-stimulated cell lines, PTX increased IL-1β release in a dose-dependent manner. However, this induction was diminished by the highest concentrations of PTX (100 μM and 1 mM) in endothelial cells.

Recent studies have provided significant insight into the inflammatory actions of IL-6, a potent pro-inflammatory, pyrogenic mediator, which is secreted by several tissues [22]. Despite contradictory results [3, 12, 15, 23], some of anti-inflammatory activities of PTX are ascribed to IL-6 inhibition. However, it should be noticed that most papers concerning inhibition of IL-6 by PTX describe the expression of IL-6 stimulated by some inflammatory mediators. Similarly, our results also demonstrated the increased synthesis of IL-6 in LPS-stimulated cells treated with PTX. Moreover, we showed, for the first time, that high concentration of PTX increased constitutive synthesis of IL-6 in endothelial cells. These data are very similar to those reported earlier for another cell type, namely peripheral blood mononuclear cells (PBMC) [24]. Probably, the induction of IL-6 by PTX may result directly from the inhibition of enzymatic activity of the intracellular cyclic nucleotide phosphor-diesterases, leading to the increase in cAMP levels. Such a mechanism is possible as IL-6 expression is regulated by cAMP responsive elements present in the promoter, and elevation of cAMP augments IL-6 production [12].

Many reports showed that PTX can decrease the release of IL-8 in response to inflammatory stimuli [25, 26]. However, it seems that this relationship is not a general feature and published data are very often inconsistent [24, 27]. Current results confirm that PTX displays a tendency to inhibit expression of IL-8 in resting monocytes and macrophages. Despite that, in cells stimulated with LPS, such an inhibition was not effective. Moreover, the current data showed, for the first time, that PTX does not affect constitutive production of IL-8 in human and murine endothelial cells. One possible explanations of the observed insensitivity of IL-8 and IL-6 syntheses to inhibitory effects of PTX treatment can be lack of production of TNFα in the murine brain microvascular endothelial cell-1 (MBEC-1) and human microvascular endothelial cells (HMEC-1) cell lines. TNFα is a primary cytokine induced in the inflamed tissues, which in turn stimulates production of other mediators. In

most studied cell lines, PTX is an inhibitor of TNFα synthesis, thus the decrease in other pro-inflammatory cytokines' generation could result from blocking of the TNFα pathway [12, 28].

In summary, the effects of PTX on cytokine expressions are cell-specific and type of stimulus- or time of stimulation-dependent. The reduced production of main proinflammatory mediator, namely TNFα, is observed not only in monocytes or macrophages but also in endothelial cells. Anti-inflammatory activity of PTX is associated with inhibition of NFκB and AP-1 function.

Acknowledgement

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