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Suppressive effect of pentoxifylline on natural killer cell activity; experimental and clinical studies

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Abstract The methylxanthine derivative pentoxifylline, widely used in the treatment of vascular diseases, also has numerous immunological effects. In in vitro experiments, the human natural killer cell cytotoxicity was investigated in the presence of pentoxifylline. A clinical trial involved an investigation of the natural killer cell activity in patients to whom pentoxifylline had been administered for different periods. The natural cytotoxicity in macroangiopathic patients treated with pentoxifylline was compared with that in healthy controls and that in patients with vascular diseases who did not receive pentoxifylline therapy. A total of 62 macroangiopathic patients and 20 healthy controls were investigated. The natural killer cell activity in patients receiving pentoxifylline therapy for more than a year proved to be significantly lower ($P<0.005$). The presence of vascular disease did not influence the natural killer activity. In the in vitro cytotoxicity reaction, pentoxifylline at a concentration of 100 µg/ml was found to suppress the natural killer cell cytotoxicity at any stage of the reaction. The influence of pentoxifylline on the natural killer cell activity was not due to inhibition of the expression of intercellular adhesion molecule-1. However, this drug significantly decreases ($P<0.05$) the apoptosis of target cells. It is presumed that the suppressor effect of pentoxifylline on natural killer cell activity should be taken into consideration in the treatment of clinical diseases where this drug is administered chronically.

Key words Natural killer · Pentoxifylline · Macroangiopathic patients · Apoptosis

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Introduction

The methylxanthine derivative pentoxifylline (PTX) has been widely used for years in the treatment of various diseases (Ely 1994; Samlaska and Winfield 1994). Its hemorheologic properties include an increased red blood cell flexibility, a decreased blood viscosity and inhibited platelet aggregation (Dettelbach and Aviado 1985; Hammerschmidt et al. 1988).

Among its immunological effects, the inhibition of tumor necrosis factor (TNF) production is of great importance (Doherty et al. 1991). PTX might therefore be beneficial in the treatment of septic patients, in tumor-induced cachexia, in organ transplantation, in graft-versus-host disease, and in patients with AIDS and asymptomatic HIV-seropositivity (Clerici et al. 1997). We recently reported the results of complementary PTX therapy in the treatment of patients with sepsis syndrome (Mándi et al. 1995; Nagy et al. 1997).

The effector cells of the immune response associated with infection and cancer are the macrophages and the natural killer (NK) cells (Brittenden et al. 1996). The most important characteristic feature of NK cells is their ability to lyse a variety of virus-infected and tumor cells without prior sensitization (Lanier et al. 1986; Trinchieri 1989; Robertson and Ritz 1990; Yokoyama 1995). It may therefore be concluded that a long-lasting decrease of NK activity may increase the incidence of neoplastic disorders. Consequently, there are clinically relevant reasons for study of the mechanisms regulating the NK cell activity, and of the influence of drugs on such an important cellular defense as the NK cell activity.

The aims of this investigation were to establish the influence of PTX on the NK cell activity, to elucidate the pathomechanism of the in vitro immunomodulatory effect and to study this effect in vivo during the regular administration of PTX to macroangiopathic patients for different periods.

Table 1 Data of groups of patients

Group	Male/female	Average age (in years)	Duration of PTX therapy (in years)	Diagnosis rate (in years)
n=15	8:7	59.1 (43–77)	0	Generalized arteriosclerosis obliterans (*n*=12) Arteriosclerosis in lower extremities (*n*=2) Supraaortic arteriosclerosis (*n*=1)
n=19	15:4	59.2 (36–78)	0–1 (mean, 0.7)	Generalized arteriosclerosis obliterans (*n*=17) Atherosclerosis of the aorta (*n*=1) Visceral arteriosclerosis (*n*=1)
n=28	23:5	57.4 (44–74)	1–10 (mean, 3.6)	Generalized arteriosclerosis obliterans (*n*=19) Arteriosclerosis in lower extremities (*n*=6) Thromboangiitis obliterans (*n*=1) Aneurysm of the abdominal aorta (*n*=1) Supraaortic arteriosclerosis (*n*=1)

Materials and methods

Effector cell separation. Human peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood samples from normal blood donors and macroangiopathic patients by gradient centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden), following a standard procedure (Böyum 1968).

Target cells and culture medium. The most frequently applied NK-sensitive cell line, K 562, a human tumor cell line derived from a patient with chronic myelogenous leukemia, was used as target cell (Robertson and Ritz 1990). The cells were suspended in an RPMI 1640 (Gibco Brl, Grand Island, N.Y., USA) medium supplemented with 10% fetal calf serum (Gibco).

Cytotoxicity assays. The natural killer cell activity against K 562 target (T) cells was measured in a 4-h standard chromium-51 release assay. Cytotoxicity assays were performed in 96-well U-bottom microplates (Nunc, Denmark) in a total volume of 0.2 ml. K 562 target cells were labeled with 100 µCi $Na_2{}^{51}CrO_4$ (Amersham) for 45 min at 37°C and washed three times with phosphate-buffered saline (pH 7.4). A total of 1×10^4 target cells were used in each well. Effector (E) cells were titrated to obtain E:T ratios of 50:1, 25:1 and 12.5:1. After a 4-h incubation period at 37°C in 5% CO_2, the plates were centrifuged at 300 *g* for 5 min. Supernatant (0.1 ml) was collected and the released radioactivity was counted in a gamma counter (Autogamma, Cobra II; Packard). Spontaneous release was measured after the incubation of target cells with the appropriate assay medium. The percentage cytotoxicity was calculated according to the formula:

$$\%\ \text{cytotoxicity} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{total incorporated radioactivity}} \times 100$$

Pentoxifylline (*Trental; Hoechst, Germany*). In the in vitro experiments, PTX was used at a concentration of 100 µg/ml, at which it has no toxic effect on the effector or target cells. PTX was added to the wells before the target cells were added to the effector cells.

Immunofluorescence analysis of intercellular adhesion molecule-1 (*ICAM-1*) *expression.* The expression of ICAM-1 on K 562 target cells was analyzed by flow cytometry in a FACStar Plus (Becton Dickinson) machine at an excitation wavelength of 488 nm. The K 562 cells (2×10^6 cells in 50 µl) were incubated for 45 min on ice with 50 µl fluorescein-isothiocyanate (FITC)-conjugated anti-ICAM-1 antibody (Serotec).

Determination of CD56+ cell number by immunofluorescence analysis. The expression of CD56+ on peripheral mononuclear blood cells was investigated in a FACStar (Becton-Dickinson) by immunofluorescence assay. Mononuclear cells (2×10^6 cells in 50 µl) were incubated for 45 min on ice with 20 µl phycoerythrin (PE)-conjugated anti-CD56 antibody (Becton-Dickinson). The background fluorescence was determined by using mouse isotypes as negative controls. Cells were washed three times with ice-cold PBS containing 1% FCS, and fluorescence analysis was performed with a FACStar Plus at 488 nm excitation.

Determination of target cell apoptosis. The target cell apoptosis was determined by flow cytometry, the apoptotic cells being marked with FITC-labeled Annexin V (R&D Systems) and the necrotic cells with propidium-iodide (PI), according to the instructions of the manufacturer (R&D Systems).

TNF assay. TNF was titrated in a bioassay on cell line WEHI-164 (Espevik and Niessen-Meyer 1986). The activities were calibrated against rh TNF (Genzyme, Cambridge, England).

Statistical method. Data were analyzed by using the paired Student's *t*-test. The level of significance (two-tailed) was set at 5%.

Patients. In a clinical study, the natural cytotoxicity in macroangiopathic patients treated with pentoxifylline for different periods was compared with that in healthy controls and that in patients with vascular diseases not receiving PTX therapy. The trial involved 62 macroangiopathic patients (Table 1) and 20 healthy volunteers (10 men, 10 women; mean age 40.2 years, range 22–58 years) who had never

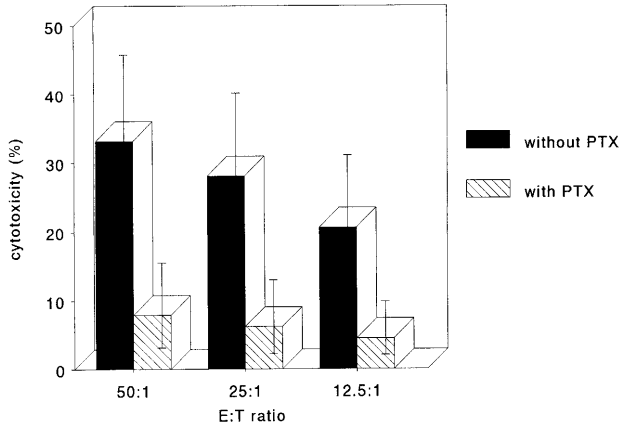

Fig. 1 In vitro effect of PTX on NK cell activity. The effector cells were obtained from healthy controls (n=20). K 562 cells were used as target cells. E:T ratios were 50:1, 25:1 and 12.5:1. Cytotoxicity was mesured by the 4-h ^{51}Cr release assay. PTX was added to the NK cell cytotoxicity assay at a concentration of 100 µg/ml at the onset of the reaction. The data are means ±SD

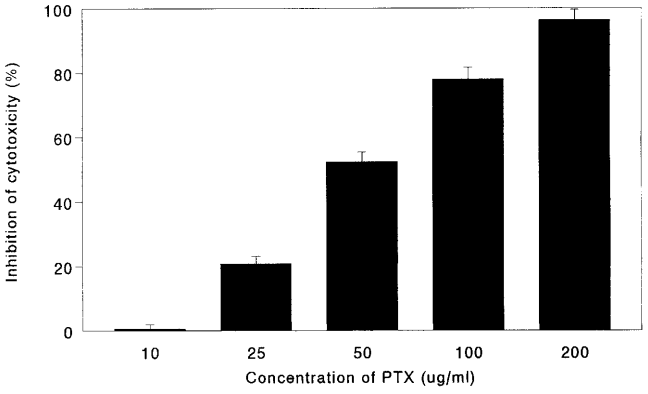

Fig. 2 Concentration dependence of inhibitory effect of PTX. Before mixing of the effector and target cells, PTX was added to the reaction in different concentrations (10, 25, 50, 100 and 200 µg/ml). The inhibition of cytotoxicity was calculated via the following formula:

$$\frac{\text{control cytotoxicity} - \text{cytotoxicity in the presence of PTX}}{\text{control cytotoxicity}} \times 100\ (\%)$$

E:T ratio=50:1. Target cell: K 562. Data are means ±SEM of six similar experiments

received PTX. The macroangiopathic patients were divided into three groups:
Group 1: patients not receiving PTX therapy, n=15.
Group 2: patients receiving PTX therapy for less than 1 year (mean, 0.7 years), n=19.
Group 3: patients receiving PTX therapy for more than 1 year (range 1–10 years; mean, 3.6 years), n=28.

PTX was administered in an oral dose of 800–1200 mg/day, divided into three parts. Factors affecting NK cell activity, such as immunosuppression, diabetes mellitus, major depression, autoimmune diseases and virus infections, were excluded. These patients were examined in the preoperative diagnostic period.

Results

In vitro effect of PTX on NK cell activity

Figure 1 demonstrates the results relating to the in vitro NK cell cytotoxicity in healthy volunteers. The presence of PTX at a concentration of 100 µg/ml significantly (P<0.0001) suppressed the NK activity against K 562 target cells at all E:T ratios.

The effect of PTX on NK activity was demonstrated to be concentration-dependent (Fig. 2). A PTX concentration of 10 µg/ml was still ineffective, while a concentration of 200 µg/ml decreased the cytotoxicity by 96±3.2%. The concentration of 100 µg/ml was chosen for further experiments because our previous studies suggested that this was optimal from the point of view of the inhibition of TNF production (Mándi et al. 1995). This concentration decreased the cytotoxicity by 78±3.5% ($\bar{x}$±SEM of six experiments).

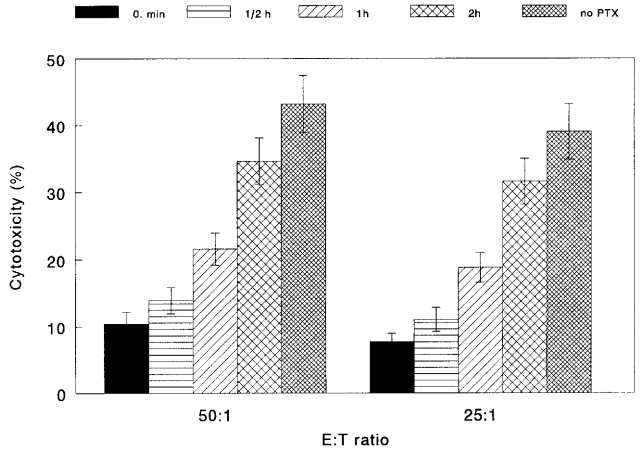

Fig. 3 Time-dependence of the effect of PTX during the NK cell reaction. PTX was added to the reaction at 0 min or 0.5, 1 or 2 h after mixing the effector and target cells. Supernatants were collected after 4-h incubation. The E:T ratios were 25:1 and 50:1. The data are means ±SD of three similar experiments

As concerns the time-dependence of the effect of PTX, it was found that PTX suppressed the cytotoxicity at all stages of the 4-h reaction (Fig. 3).

Effect of PTX on ICAM-1 expression

Intercellular adhesion molecule-1 plays an important role in binding the effector cells to the target cells. Chong et al. (1994) reported that specific cell adhesion molecule interactions can provide costimulation to enhance the natural cytotoxicity in NK cells. We therefore investigated the ef-

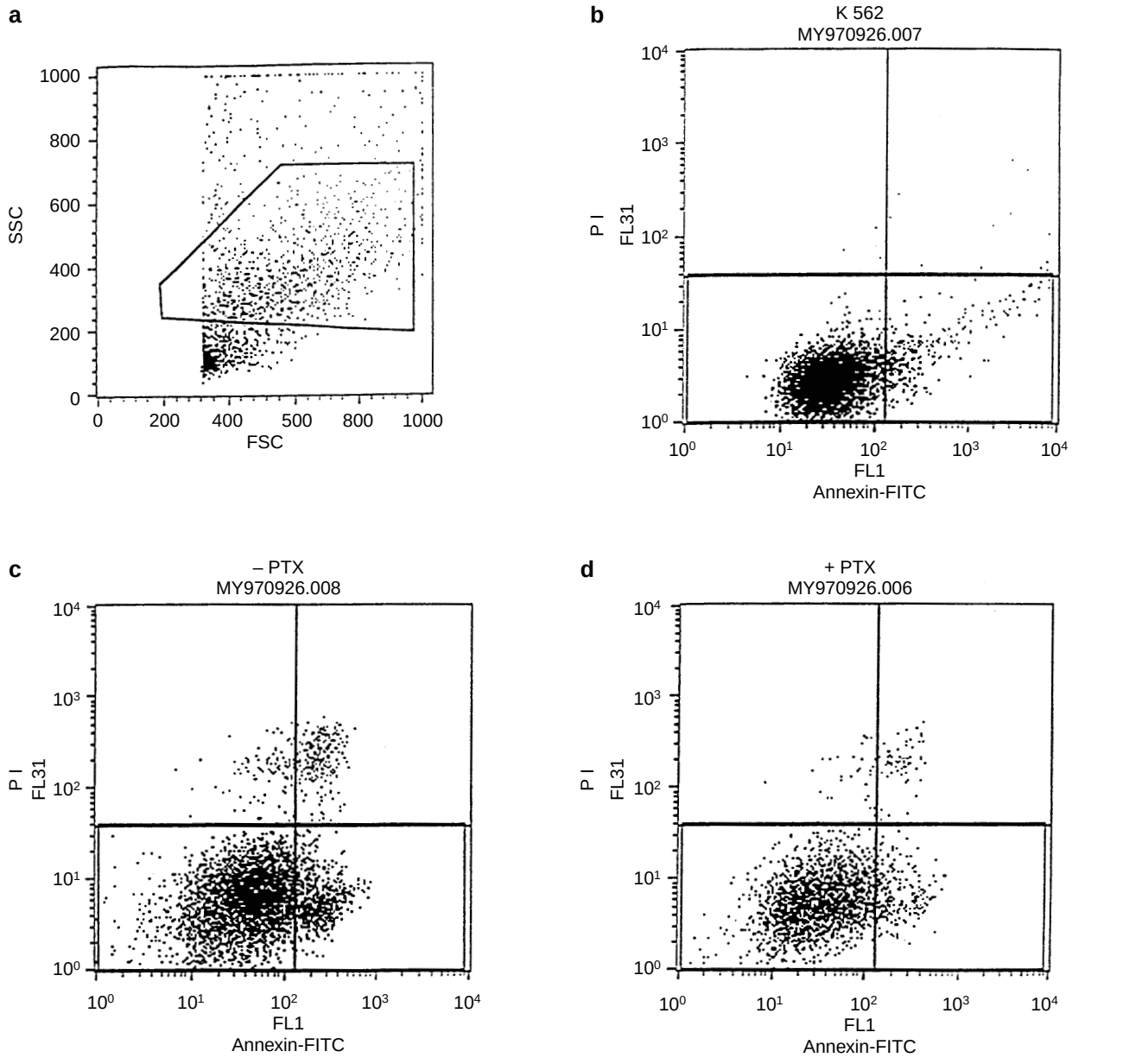

Fig. 4a–d Apoptosis of K 562 target cells, as analyzed by flow cytometry. The effector cells were incubated with the target cells for 4 h with or without PTX. The E:T ratio was 10:1. **a** Target cells were gated according to their size (*FSC*) and shape (*SSC*). **b** The spontaneous apoptosis of K 562 cells labeled with Annexin V and propidium-iodide. **c** Fluorescence intensity of target cells due to the NK cell reaction without PTX. **d** The same as c, but with PTX at a concentration of 100 µg/ml

fect of PTX on the ICAM-1 expression of K 562 target cells. Flow cytometric analysis revealed that K 562 cells exerted a considerable expression of ICAM-1 on their surface. PTX did not influence the fluorescence intensity of K 562 cells after a 4- to 18-h pretreatment at the concentration used in the NK cell reaction. In five separate experiments with or without PTX the mean fluorescence intensity was 44.8±10.3 as well. Accordingly, it seems that PTX does not inhibit the binding of the effector cells to the target cells.

Effect of PTX on target cell apoptosis

Apoptosis was detected through the changes in cell membrane structure via Annexin V labeling (Koopman et al. 1994; Vermes et al. 1995). Annexin V binds specifically to phosphatidylserine (Andree et al. 1990) while propidium-iodide is a DNA-binding fluorochrome. Among the double-stained cells, the cells undergoing apoptosis were represented in the lower right quadrant in flow cytometric measurements, exhibiting considerable green fluorescence (FL1). Necrotic cells stained by PI were represented in the upper right quadrant, exhibiting red fluorescence (FL2).

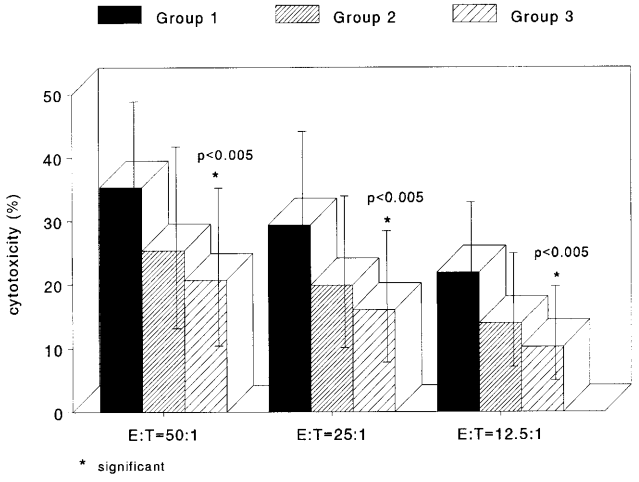

Fig. 5 NK cell activity in macroangiopathic patients. *Group 1*: patients receiving no PTX therapy (n=15); *Group 2*: patients receiving PTX therapy for less than 1 year (n=19); *Group 3*: patients receiving PTX therapy for more than 1 year (n=28). The E:T ratios were 50:1, 25:1 and 12.5:1. The data are means ±SD. *Significant relative to the healthy controls (P<0.005)

Only a small proportion of the K 562 cells (5%) displayed spontaneous apoptosis (Fig. 4b). When these cells were incubated with lymphocytes in a ratio of 10:1, 15.8% of the target cells were apoptotic (Fig. 4c). It should be noted that K 562 cells were gated from lymphocytes according to their forward scattering (FSC) and side scattering (SSC) because of the differences in size and shape. Only gated K 562 cells were investigated further. In the presence of PTX, the apoptotic proportion decreased to 8.5% (Fig. 4d). Therefore, we presume that PTX inhibited the apoptosis of the target cells in the NK cell reaction. In contrast, in separate experiments PTX did not inhibit the camptothecin-induced apoptosis of K 562 cells (data not shown).

In vivo effect of PTX on NK cell activity

Figure 5 demonstrates the cytotoxicity reactions of certain groups of macroangiopathic patients. The NK cell activity in patients to whom PTX had been administered for more than 1 year was significantly (P<0.005) lower than that in healthy blood donors at all E:T ratios (20.7±12.4% at an E:T ratio of 50:1). In patients who received PTX therapy for less than 1 year, the difference in cytotoxicity was not significant (P>0.05; 25.3±14.3%). The cytotoxicity in the patients not receiving PTX therapy (35.2±11.5%) did not differ from that in the healthy controls (33.1±11.2%); it may therefore be stated that the presence of vascular disease does not influence the NK cell activity. Cytotoxicity values below 20% were regarded as reflecting a decreased cytotoxicity. In the macroangiopathic group not receiving PTX therapy, none of the patients displayed a decreased cytotoxicity. In the groups of patients receiving PTX therapy for less than 1 year or for 1–10 years, 42.1% (9 of 19) and 53.6% (15 of 28), respectively, of the patients exhibited a decreased NK cell activity.

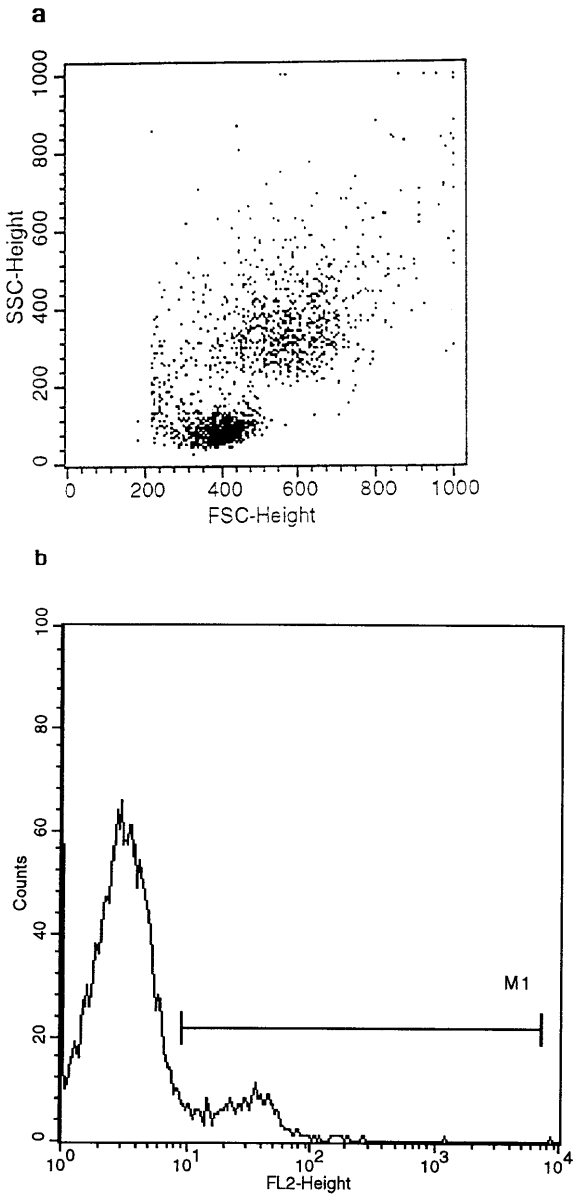

Fig. 6a,b Determination of CD56+ cells in mononuclear cells. **a** Dot-plot analysis of mononuclear cell population according to FSC and SSC by FACStar Plus (Becton-Dickinson). **b** CD56 expression on mononuclear cells. The figure presents the results obtained by analysis of the mononuclear cells of a healthy blood donor, a typical representative of ten similar experiments; mean fluorescence intensity 56.7

Determination of the number of NK cells in the peripheral blood

The number of CD56+ NK cells was determined by flow cytometric analysis. Figure 6a depicts the results of a typical dot-plot analysis of mononuclear cells originating from

a healthy blood donor, while Fig. 6b presents the histogram obtained after the staining of these cells with PE-conjugated CD56-antibodies. A total of 13.5% of the mononuclear cells were positively stained with the antibody. A comparison of the results with those of an analysis of cells originating from macroangiopathic patients treated with PTX for more than 1 year revealed no significant difference as concerns the percentage of CD56+ cells: 13.2±1.5% in healthy volunteers vs. 12.8±1.6% in the PTX-treated group (n=10 in each group). It can therefore be presumed that the decrease in NK cell activity observed after long-term PTX therapy is not due to a decrease in the NK cell number in vivo, but rather to a direct effect of PTX on NK cells.

Discussion

Pentoxifylline is applied in the treatment of many different systemic disorders (Ely 1994; Samlaska and Winfield 1994). Its beneficial effect has been demonstrated mainly in chronic obliterative arterial diseases (Poggesi et al. 1985), cerebrovascular insufficiency, hypercoagulation, diabetic retinopathy, autoimmune diseases, Graves ophthalmopathy (Balázs et al. 1997) and andrological conditions (Jayaprakash et al. 1997). The numerous immunological effects of PTX (Balázs and Kiss 1994) include the inhibition of TNF production (Doherty et al. 1991) and the activation of granulocytes (Hammerschmidt et al. 1988; van Leenen et al. 1993). The in vitro effect of PTX on NK cell activity was first reported by Reed and DeGowin (1992), who found that the suppression of NK cell activity by PTX occurred at drug concentrations higher than those achieved in patients, and appeared to be mediated by an increase in prostaglandin E_2 production from PBMCs.

As far as we are aware, the present report is the first publication concerning the modulation of cytotoxicity by PTX during chronic administration to macroangiopathic patients. The NK cell activity was significantly lower in macroangiopathic patients receiving PTX therapy for more than 1 year. Peak plasma drug levels in patients receiving oral doses of 400 mg PTX are reported to range from 0.9 ng/ml up to more than 1000 ng/ml (Reed and DeGowin 1992); at that concentration, no demonstrable effects of PTX on NK cell activity were manifested. We hypothetize that the plasma concentration of PTX during chronic administration might not be the same as that on the surface of the leukocytes and in the tissues. Just because of the differences in PTX concentrations present in vitro and in vivo, we presume that a longer administration of PTX would be necessary to inhibit the NK activity in vivo. This might be the explanation of why the inhibition of NK cell activity in vitro can be seen immediately, whereas the “same effect” in vivo was evident in patients receiving PTX therapy only after more than 1 year.

The present in vitro experiments demonstrated the inhibitory effect of PTX on the NK cell reaction. Previous studies revealed suppressive effect against other target cells, too, such as VERO cells (African green monkey kidney cell line; Nagy and Mándi 1998). The PTX concentration of 100 µg/ml was chosen because this was the most effective in previous studies relating to the inhibition of TNF production (Mándi et al. 1995). PTX significantly decreased the apoptosis of target cells. Contradictory data have been published concerning the effect of PTX on apoptosis (Belloc et al. 1995). In our experiments, PTX had no effect on the apoptosis of K 562 cells induced by camptothecin (unpublished data), but it exerted an inhibitory effect on target cell apoptosis during the NK cell reaction. In this reaction, the target cell death due to the NK cell activity was inhibited by PTX; this could be detected either on the basis of ^{51}Cr release or by examining the apoptosis of target cells. It is noteworthy that in parallel experiments there was a moderate production of TNF by lymphocytes incubated together with K 562 cells (60–100 u/ml), but this production decreased in the presence of PTX (0–10 u/ml).

Flow cytometric analysis revealed that K 562 cells exerted a considerable expression of ICAM-1 on their surface. PTX did not influence the fluorescence intensity of K 562 cells after a 4- to 18-h pretreatment at the concentration used in the NK cell reaction. Neuner et al. (1997) reported that pentoxifylline in vivo and in vitro down-regulates the expression of ICAM-1 in monocytes, but in our system the ICAM-1 expression was investigated on K 562 cells, which is not a monocytic cell line. The effect of PTX on the ICAM-1 expression might be dependent on the type of cell. In addition, Neuner et al. applied a higher concentration of PTX (200 µg/ml).

In the NK cell reaction, the effect of several signal mechanisms and mediators lead to the death of target cells (Arase et al. 1995; Berke 1995; Oshimi et al. 1996; Moretta 1997). The apoptosis of target cells and the TNF production by effector cells are only parts of this process, and it seems that PTX can inhibit both. The possibility of further effects of this drug that could result in the suppression of NK cell activity remains an issue to be solved in the future.

Our investigations indicate that PTX suppresses the in vitro NK cell activity to a considerable extent, but also that the NK cell activity of patients chronically treated with PTX is significantly lower than that of untreated patients. It can therefore be concluded that the suppression of NK cell activity by PTX should be taken into consideration in clinical diseases where this drug is administered chronically.

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References

Andree HA, Reutelingsperger CP, Hauptmann R, Hemker HC, Hermens WT, Williems GM (1990) Binding of vascular anticoagulant alpha (VAC alpha) to planar phospholipid bilayers. J Biol Chem 265:4923–4928

Arase H, Arase N, Saito T (1995) Fas-mediated cytotoxicity by freshly isolated natural killer cells. J Exp Med 181:1235–1238

Balázs C, Kiss E (1994) Immunological aspects of the effect of pentoxifylline (Trental). Acta Microbiol Immunol Hung 41: 121–126

Balázs C, Kiss E, Vámos Á, Molnár I, Nadir RF (1997) Immunomodulatory effect of pentoxyfilline on thyroid associated ophthalmopathy. (A pentoxifyllin immunmoduláns hatása Graves-ophthalmopathiában). Orv Hetil 138:2869–2874

Belloc F, Jaloustre C, Dumain P, Lacombre F, Lenoble M, Boisseau MR (1995) Effect of pentoxifylline on apoptosis of cultured cells. J Cardiovasc Pharmacol 25:S71–S74

Berke G (1995) Unlocking the secrets of CTL and NK cells. Immunol Today 16:343–346

Böyum A (1968) Separation of leukocytes from blood and bone marrow. Scand J Clin Lab Invest 21 [Suppl 97]:77–89

Brittenden J, Heys SD, Ross J, Eremin O (1996) Natural killer cells and cancer. Cancer 77:1226–1243

Chong AS-F, Boussy LA, Jiang XL, Lamas M, Graf LH Jr (1994) CD54/ICAM-1 is a costimulator of NK cell-mediated cytotoxicity. Cell Immunol 157:92-105

Clerici M, Piconi S, Balotta C, Trabattoni D, Capetti A, Fusi ML, Ruzzante S, Longhi R, Colombo MC, Moroni M, Milazzo F (1997) Pentoxifylline improves cell-mediated immunity and reduces Human Immunodeficiency Virus (HIV) plasma viremia in asymptomatic HIV-seropositive persons. J Infect Dis 175: 1210–1215

Dettelbach HR, Aviado DM (1985) Clinical pharmacology of pentoxifylline with special reference to its hemorheologic effect for the treatment of intermittent claudication. J Clin Pharmacol 25:8–26

Doherty GM, Jensen JC, Alexander HR, Buersh CM, Norton JA (1991) Pentoxifylline suppression of tumor necrosis factor gene transcription. Surgery 110:192–198

Ely H (1994) Is pentoxifylline the drug of the decade? J Am Acad Dermatol 30:639–642

Espevik T, Niessen-Meyer JA (1986) A highly sensitive cell line WEHI-164 for measuring cytotoxic factor/tumor necrosis factor. J Immunol Methods 95:99–105

Hammerschmidt DE, Kotasek D, McCarthy T, Huh P-W, Freyburger G, Vercelotti GM (1988) Pentoxifylline inhibits granulocyte and platelet function, including granulocyte priming by platelet activating factor. J Lab Clin Med 122:254–261

Jayaprakash D, Kumar KS, Shivaji S, Seshagiri PB (1997) Pentoxifylline induces hyperactivation and acrosome reaction in spermatozoa of golden hamsters: changes in motility kinematics. Hum Reprod 12:2192–2199

Koopman G, Reutelingsperger CP, Kuijten GA, Keehnen RM, Pals ST, Oers MH van (1994) Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. Blood 84:532–540

Lanier LL, Philips JH, Hackett J, Tutt M, Kumar V (1986) Natural killer cells: definition of a cell type rather than a function. J Immunol 137:2735–2739

Leenen D van, Poll T van der, Levi M, Cate H ten, Deventer SJH van, Hack CE, Aarden LA, Cate JW ten (1993) Pentoxifylline attenuates neutrophil activation in experimental endotoxaemia in chimpanzees. J Immunol 151:2318–2325

Mándi Y, Farkas G, Ocsovszky I, Nagy Z (1995) Inhibition of tumor necrosis factor production and ICAM-1 expression by pentoxifylline: beneficial effects in sepsis syndrome. Res Exp Med 195:297–307

Moretta A (1997) Molecular mechanisms in cell-mediated cytotoxicity. Cell 90:13–18

Nagy Z, Mándi Y (1998) Natural killer cell-mediated cytotoxicity against VERO target cells; the suppressive effect of pentoxifylline. Immunol Lett (in press)

Nagy Z, Mándi Y, Gyulai Z, Farkas G, Márton J, Balogh Á (1997) Beneficial effects of pentoxifylline in sepsis syndrome. In: Faist E (ed) The immune consequences of trauma, shock and sepsis. Monduzzi Editore, Bologna, pp 903–908

Neuner P, Klosner G, Pourmojib M, Knobler R, Schwarz T (1997) Pentoxifylline in vivo and in vitro down-regulates the expression of the intercellular adhesion molecule-1 in monocytes. Immunology 90:435–439

Oshimi Y, Oda S, Honda Y, Nagata S, Miyazaki S (1996) Involvement of Fas ligand and Fas-mediated pathway in the cytotoxicity of human natural killer cells. J Immunol 157:2909–2915

Poggesi L, Scarti L, Boddi M, Masotti G, Serneri GGN (1985) Pentoxifylline treatment in patients with occlusive peripheral arterial disease: circulatory changes and effects on prostaglandin synthesis. Angiology 36:628–637

Reed WR, DeGowin RL (1992) Suppressive effects of pentoxifylline on natural killer cell activity. J Lab Clin Med 119:763–771

Robertson MJ, Ritz J (1990) Biology and clinical relevance of human natural killer cells. Blood 76:2421–2438

Samlaska MCP, Winfield EA (1994) Pentoxifylline. J Am Acad Dermatol 30:603–621

Trinchieri G (1989) Biology of natural killer cells. Adv Immunol 47:187–376

Vermes I, Haanen C, Steffensnakken H, Reutelingsperger C (1995) A novel assay for apoptosis – flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labeled Annexin V. J Immunol Methods 184:39–51

Yokoyama WM (1995) Natural killer cell receptors. Curr Opin Immunol 7:110–120