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Pentoxifylline reduces plasma tumour necrosis factor-alpha concentration in premature infants with sepsis

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Abstract Increased plasma tumour necrosis factor α (TNF) concentration correlates with mortality in sepsis. We suggested that pentoxifylline (PTXF), which is known to inhibit TNF production, may improve survival and attenuate clinical symptoms of sepsis in neonates. Plasma TNF levels were evaluated in 29 newborn infants with sepsis. Patients were randomly assigned into two groups, receiving PTXF in a dose of 5 mg/kg per hour for 6 h or placebo (saline), on 3 successive days. Both groups were subjected to the same conventional therapy. TNF was evaluated before and after PTXF or placebo administration on the 1st and 3rd days of therapy. There was a statistically significant decrease in plasma TNF level in the PTXF group when the values before the first and after the last PTXF infusion were compared [mean: 671.5 pg/ml; SD: 438; med: 729.6 vs mean: 41.0 pg/ml; SD: 64.1; med: 11.5; $P < 0.000004$]. In the placebo group, decrease was not significant [mean: 633.0 pg/ml SD: 488.6; med: 618.9 vs 246.9 pg/ml; SD: 243.9; med: 191.0]. A significantly higher plasma TNF level, evaluated after the last PTXF infusion, was found in the placebo group [246,9 pg/ml vs 41.0 pg/ml; $P < 0.001$]. Only one of four infants with signs of shock in the placebo group survived, whereas all of five newborns with symptoms of shock in the PTXF group survived [$P < 0.04$]. An increased incidence of metabolic acidosis [$P < 0.05$], necrotizing enterocolitis [$P < 0.04$] and renal insufficiency [$P < 0.05$] was observed in infants in the placebo group.

Conclusion PTXF inhibits production of TNF and may have therapeutic value in the treatment of premature infants with sepsis complicated by shock.

Key words Pentoxifylline · Shock · TNF · Human · Neonate

Abbreviations FiO_2 fraction of inspired oxygen · *NEC* necrotizing enterocolitis · *PTXF* pentoxifylline · *TNF* tumour necrosis factor-alpha

Introduction

Pentoxifylline (PTXF), a methylxanthine derivative, has been widely used in the treatment of intermittent claudication for the last two decades. Recently, human and animal studies have shown that PTXF therapy results in a variety of physiological changes at the cellular level, which may be important for treatment of sepsis. PTXF inhibits production of monocyte and macrophage-derived tumour necrosis factor-alpha (TNF), increases leucocyte deformability and chemotaxis, decreases endothelial leucocyte adhesion, neutrophil degranulation and release of superoxides [11, 16]. Moreover, PTXF reverses the endotoxin-in-

duced alterations of red cell deformability and blood viscosity [9]. Administration of PTXF to animals with sepsis improves the survival rate and attenuates circulatory shock [8, 18]. Zabel et al. [19] demonstrated attenuation of symptoms of experimental endotoxaemia in man by PTXF infusion. Our preliminary clinical observations [7], suggested that PTXF treatment significantly decreased mortality rate in premature infants with sepsis.

It is known that plasma TNF level is elevated in infants with sepsis and that the magnitude of elevation correlates with the mortality rate [4, 14]. Therefore, inhibition of TNF production by PTXF administration may influence survival rate and attenuate symptoms of disease.

The prospective, randomized study was designed: (1) to determine plasma TNF concentration in premature infants with sepsis and to compare it with the value obtained in a control population of healthy, gestational age and birth weight-matched infants: (2) to compare the plasma TNF level between the infants with sepsis who were treated with PTXF or placebo; (3) to compare mortality rate between both groups of neonates; and (4) to evaluate the magnitude of elevation of TNF in relation to clinical symptoms and outcome.

Patients and methods

All prematurely delivered (gestational age < 36 weeks) infants with suspected sepsis, admitted to the neonatal intensive care unit of The Neonatal Department of The Medical College, Jagiellonian University between 1 March and 30 July 1994, were candidates for the study. To minimize the influence of maternal or placenta infection on the infant's plasma cytokine concentration, only neonates with sepsis diagnosed after the 1st week of life were enrolled in the study. Inclusion criteria included: physical signs of infection and respiratory or cardiovascular dysfunction. Physical symptoms of infection were defined by the presence of at least two of the following: feeding intolerance, abdominal distension, lethargy, irritability, temperature instability, hyperbilirubinaemia and hepatosplenomegaly. All neonates with abdominal distention had an X-ray examination at the outset. There was no evidence of necrotizing enterocolitis (NEC) at that stage of infection (according to Bell et al. [1]). Respiratory dysfunction was diagnosed by presence of tachypnoea (> 70 breaths/min) or episodes of apnoea (> 20 s). Circulatory dysfunction was determined by the presence of tachycardia (heart rate > 190 beats/min), bradycardia (heart rate < 90 beats/min), disordered peripheral circulation, mean arterial blood pressure < 30 mm Hg and an increase in fraction of inspired oxygen (FiO_2).

Shock was defined by the presence of a mean arterial blood pressure < 20 mm Hg with sudden deterioration in clinical status (apnoea, FiO_2 > 0.8, pallor, anuria, metabolic acidosis) requiring higher doses of dopamine (> 5 μg/kg/min) and/or volume infusion. Exclusion criteria included: major congenital malformation, intraventricular haemorrhage grade III, IV and congenital infection.

All infants with the presumptive (before the results of blood culture were obtained) diagnosis of sepsis were randomly assigned to receive PTXF (PTXF group) or saline (placebo group). The PTXF (Trental, Hoechst, Germany) was given intravenously in a dose of 5 mg/kg per hour for 6 h. The first infusion of PTXF or saline started about 30 min before antibiotics were administered. Identical infusions were repeated on the 2nd and 3rd days of treatment. The infused volumes of PTXF or saline were comparable in both groups. The medical carers and laboratory workers were blinded as to which infant received PTXF or placebo.

The routine medical management of sepsis in both groups was comparable. Augmentin and amikacin were used as first line antibiotics. After the results of blood cultures were obtained, antibiotics were adjusted according to the sensitivity of the isolated bacteria. Protocols for dopamine and dobutamine administration were identical for both groups. On the 1st day of therapy, all neonates received a single dose of immunoglobulin (Bioglobulin, Biomed, Poland) intravenously. Dosage was dependent on the weight of the infant. Premature infants weighing less than 1000 g obtained 0.9 g/kg of body weight and neonates with weight above 1000 g received 0.5 g/kg. Neither steroids nor granulocyte transfusions were given. The criteria for artificial ventilation and for weaning from the ventilator were comparable.

Follow up

Infants treated with PTXF were monitored for adverse reactions, (i.e. hypotension, irritability, deterioration of vital signs). Systemic arterial pressure was recorded continuously (Hewlett Packard 1290c monitor) during the 3- to 5-day period beginning at the first onset of sepsis in both groups. Acid-base balance was determined by arterial blood sampling at similar intervals of time (4–8 h) in both groups. To determine the white blood cell, platelet count and plasma protein level, blood samples were taken daily from peripheral veins. Samples for blood culture were obtained simultaneously to the first infusion of PTXF or saline. When *Staphylococcus epidermidis* was cultured, only a significant growth of bacteria was considered for diagnosis of sepsis.

Daily urinary volume was measured during the first 3–5 days of sepsis. Renal insufficiency was defined when the urine volumes measures were below 20 ml/kg per day.

TNF determination

For TNF determination, four plasma samples were collected from newborns: on the 1st day of treatment immediately before and after the PTXF or saline infusion and similarly on the 3rd day of treatment.

Blood samples were also obtained from 10 healthy newborn infants, weight and gestational age-matched, on the 3rd–17th day of life and used for comparative TNF determination. The volume of plasma required for each TNF analysis was 30 μl.

All samples were stored at –70° C and thawed once at the time of analysis. TNF was determined by immuno-enzymetric test (TNF-alpha EASIA, Medgenix, Fleurus, Belgium) as described previously [21]. The minimum detectable concentration was estimated to be 3 pg/ml and was defined as the TNF concentration corresponding to the mean OD of 20 replicates of the zero standard + 2 standard deviations. Interassay variations were 8%–9.8%.

The randomization was calculated for a sample size of 40 infants with sepsis (two groups of 20 subjects). Infants were assigned to the PTXF or placebo group by means of a permuted block randomization scheme.

The study was approved by the Ethical Committee of the Jagiellonian University Medical College. Informed written consent was obtained from parents of all infants.

End point of the study; clinical condition of infants with sepsis at discharge.

Statistical analysis

Categorical data were analysed by the Fisher Exact Test. Continuous variables were analysed with the Student *t*-test when normally distributed and with the Wilcoxon rank sum test if not.

Table 1 Comparison of infants at randomization (*uti* urinary tract infection, *NS* statistically not significant)

Characteristic	PTXF group $n = 16$	Placebo group $n = 16$	P
Birth weight (g)	mean 1752.09; SD 483.4 med 1690.0	1861.29; SD 511.7 1740.00	NS
Apgar score	mean: 7.48; range 5–9	mean 8.0; range 5–9	NS
Gestational age in weeks	mean 31.54 med 31.00 SD 2.87	32.35 33.00 2.95	NS
Symptoms of shock	5	4	NS
Hypotension < 30 mm Hg observed > 5 min	7	6	NS
Neutropenia < 2000/mm^3	4	4	NS
Hypoproteinaemia < 5 g/l	2	1	NS
Metabolic acidosis arterial blood sample (defined as pH < 7.23 and plasma bicarbonate value < 17 mEq/l)	6	7	NS
Thrombocytopenia < 100 × 10^3/mm^3	3	4	NS
Abdominal distension	6	5	NS
Accompanying focal infections	pneumonia 5 omphalitis 1	pneumonia 3 uti 2	NS

Results

In 4 of 20 infants in the PTXF group and in 7 of 20 newborns in the placebo group, recruited for the study, sepsis was not confirmed by blood cultures and they were excluded from further analysis.

A total number of 29 patients was analysed: the PTXF group and the placebo group consisted of 16 and 13 subjects, respectively. The onset of sepsis was between the 8th and 12th day of life. Plasma TNF concentration determined before the first infusion of PTXF or saline was within the range of 20.3–1457.0 pg/ml, whereas in the comparative group of 10 healthy, birth weight and gestational age-matched infants, it was not detectable.

Table 1 shows that there were no significant differences at randomization between the PTXF and placebo group with regard to the birth weight, gestational age, Apgar score, and occurrence of shock, hypotension, neutropenia, hypoproteinaemia, metabolic acidosis, thrombocytopenia, abdominal distension and frequency of focal infections. The mean values of plasma TNF concentrations determined before the first infusion were also similar in both groups (mean: 671.52 pg/ml; median: 729.65; SD: 438.0 in the PTXF group vs mean: 633.09 pg/ml; median: 618.9; SD: 488.6 in the placebo group). The comparison of plasma TNF level (mean ± SD), before and after PTXF or saline administration on the 1st and 3rd days of therapy is shown in Fig. 1. In the PTXF group, plasma TNF level evaluated immediately after PTXF infusion, was significantly lower when compared to the value obtained before the drug administration either on the 1st or 3rd day of treatment. In contrast, in the placebo group, plasma TNF concentration determined before and after saline infusions showed no significant difference. The most pronounced difference was found between the values of plasma TNF, measured before the first and after the last infusion of PTXF (671.5 pg/ml vs 41.0 pg/ml; $P < 0.000004$; Wilcoxon test). In contrast, these values were not significantly different in the placebo group (633.0 pg/ml vs 246.9 pg/ml; $P < 0.3$; Wilcoxon test). The difference in plasma TNF level between the PTXF and the placebo group, evaluated after the last infusion, was also significant (41.0 pg/ml in the PTXF group vs 246.9 pg/ml in the placebo group; $P < 0.001$; Wilcoxon test).

The effect of PTXF on plasma TNF level in infants with Gram-positive sepsis was similar to those with a Gram-negative one. However, the numbers were too small to give statistically valid results.

The values of plasma TNF levels determined before the first infusion were significantly higher ($P < 0.000003$; Wilcoxon test) in cases of sepsis caused by Gram-negative bacteria when compared to infections caused by Gram-positive micro-organisms (Gram-negative: mean = 881.4 pg/ml, SD: 346.5 pg/ml; med: 817.1; vs Gram-positive:mean = 149.4 pg/ml; SD:111.4 pg/ml; med: 135.3).

The alterations in individual data of TNF plasma level are shown in Fig. 2.

Only Gram-negative bacteria caused sepsis complicated by septic shock. The causative organisms isolated from blood culture are listed in Table 2.

In the PTXF group, none of 16 treated infants died, whereas in the placebo group, 3 of 13 patients died (23%) of sepsis and diagnosis was confirmed by autopsy. The difference in mortality was not significant. However, three of four infants with septic shock in the placebo group

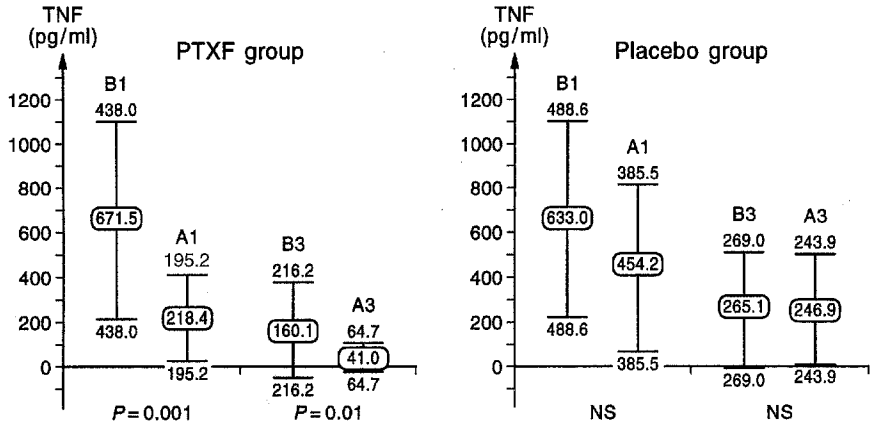

Fig. 1 Plasma TNF levels (mean ± SD) before *(B)* or after *(A)* PTXF or saline infusion on the 1st (*B1* or *A1*) and 3rd (*B3* or *A3*) days of therapy

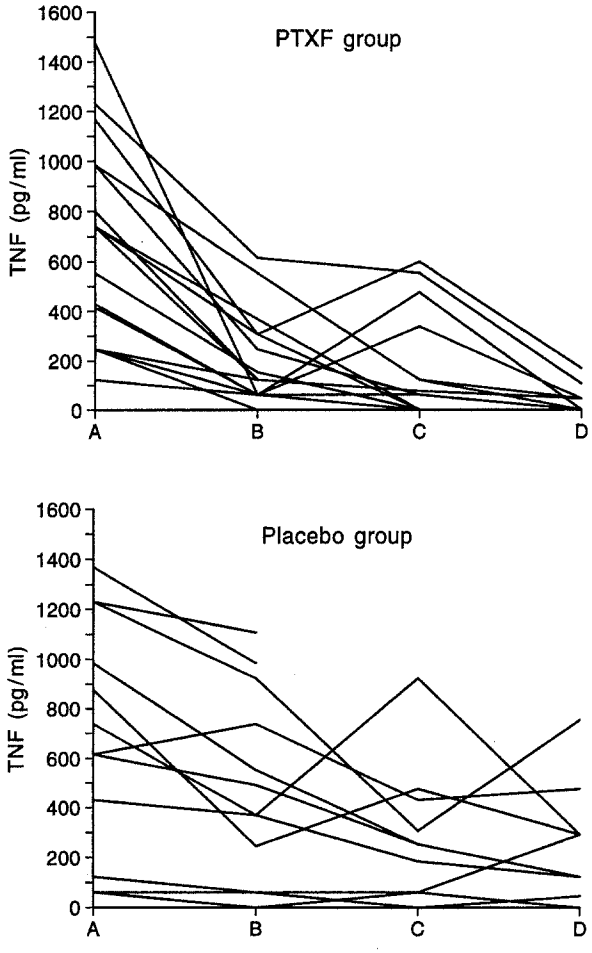

Fig. 2 The alterations in individual date of plasma TNF levels (*A* before the first infusion, *B* after the first infusion, *C* before the third infusion, *D* after the third infusion)

died. In contrast, all of five infants with shock in the PTXF group survived. The difference in mortality rate for sepsis complicated by shock was statistically significant ($P < 0.04$; Fisher Exact Test).

Table 2 The causative organisms isolated from blood culture

Organism	PTXF group $n = 16$	Placebo group $n = 13$
Escherichia coli	3	3
Enterobacter cloacae	2	3
Pseudomonas aeruginosa	1	1
Klebsiella pneumoniae	3	3
Serrattia marcescens	1	0
Staphylococcus epidermidis	4	3
Staphylococcus aureus	2	0

In 9 of 18 infants with plasma TNF levels above 500 pg/ml, determined at the beginning of infection, signs of shock were found whereas none of 11 infants with plasma TNF concentration below 500 pg/ml developed shock. The occurrence of the clinical symptoms of shock was significantly higher in infants with plasma TNF levels above 500 pg/ml ($P < 0.004$; Fisher Exact Test).

In the PTXF group, 1 of 16 treated infants developed NEC in the course of sepsis. However, in the placebo group there were five neonates who developed NEC. Three of them had abdominal signs at the beginning of infection. The occurrence of the clinical and radiological symptoms of NEC was significantly higher in the placebo group ($P < 0.04$; Fisher Exact Test).

Also, significant differences were found between the neonates in the PTXF and the placebo group, with respect to the incidence of metabolic acidosis ($P < 0.05$; Fisher Exact Test), and anuria or oliguria ($P < 0.05$; Fisher Exact Test) observed during the 3–5 days of treatment.

There was no evidence of PTXF toxicity, including decreased blood pressure or irritability in all infants treated with PTXF.

Discussion

Previous studies on adults have implicated TNF as an important mediator in the pathogenesis of sepsis [13]. De-

bets et al. [3] demonstrated the increased plasma TNF levels in adults with bacterial sepsis, which were associated with an increased incidence of shock and death. There are only a few studies on TNF levels in neonates [4, 5]. Our findings showed that plasma TNF levels were highly elevated in newborn infants with sepsis. It was especially suggestive when infection was caused by Gram-negative bacteria. This is in keeping with data of Girardin et al. [4]. We also found the continuous decline of plasma TNF concentration on 3 successive days in the course of sepsis in both groups. However, the decrease in plasma TNF concentration found in the PTXF group was markedly statistically significant ($P < 0.000004$), whereas in the placebo group the difference was not significant. As shown in Table 1, the characteristics of the two groups at randomization were comparable. Thus, differences in severity of illnesses at randomization could be ruled out. It allowed us to conclude that statistically significant reduction of plasma TNF levels, found in the PTXF group, was likely caused by PTXF.

However, plasma TNF concentration still remained elevated. Since the half-life of TNF in plasma is approximately 6–9 min [2], we conclude that production of TNF was continued during therapy but was markedly suppressed. One possible explanation for continued generation of TNF may be antibiotic therapy leading to destruction of bacteria and following endotoxin release.

In our study a highly elevated plasma TNF level (> 500 pg/ml) was significantly associated with the occurrence of shock ($P < 0.004$). This is in keeping with data obtained in adults [3] as well as in newborn infants with bacterial sepsis [4].

The difference in the overall mortality rate between both groups was not statistically significant. However, the mortality in the course of sepsis complicated by shock was significantly higher in the placebo group ($P < 0.04$). It may suggest the beneficial effect of PTXF on the course of severe systemic infections in premature infants and is in keeping with our previous results [7]. The clinical effectiveness of PTXF in inhibiting TNF response during OKT3 antibody therapy was also demonstrated in renal transplant recipients [20].

This study may also suggest that a highly elevated plasma TNF level is associated with metabolic acidosis and may increase the risk of the occurrence of NEC. None of six infants, treated with PTXF, who had abdominal distention at the beginning of sepsis, developed NEC. Whereas in the placebo group, in three of five neonates with abdominal signs at the outset of infection, NEC was recognized in the course of sepsis. On the grounds of these results, one may suspect that PTXF protects septic neonates against the NEC development. Similar results were obtained in animal models of sepsis [15]. The attenuation of metabolic acidosis and a decrease in the occurrence of NEC, found in infants treated with PTXF, was likely associated with an influence of PTXF either on plasma TNF level or increase in deformability as well as decrease in endothelial adhesion of leucocytes [11, 16]. According to Hewett et al. [6], neutrophils and neutrophil-derived oxidants have been implicated in the development of acute tissue injury in sepsis. Also, Steeb et al. [12] found that PTXF prevented small intestine vasoconstriction and preserved microvascular blood flow during sepsis. Moreover, PTXF has been shown to improve cardiac output as well as hepatic blood flow in an animal model of shock [17, 18].

Apart from the dosage, the period of time between the endotoxin release into the blood and the infusion of PTXF, is crucial for the outcome. PTXF was unable to protect mice when administered beginning 4 h after the endotoxin injection [10]. In our study, PTXF was administered as soon as sepsis was presumptively diagnosed, what could be also the reason for the beneficial effect of this drug.

In summary, we conclude that plasma TNF levels are significantly increased in infants with bacterial sepsis and seem to be an important factor in the pathophysiology of disease. The reduction in TNF level as well as rheological properties of the drug may have a therapeutic value in the treatment of sepsis in premature infants. However, blind-controlled, multicentre studies to assess the dosage, efficacy and safety of PTXF are required.

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ANNOUNCEMENT

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