Letter to the Editor

Effect of Various Pentoxiphylline Concentrations on Macrophage Inflammatory Protein 1 Alpha Production

Macrophage inflammatory protein 1 alpha (MIP-1α) is a newly described 6- to 8-kDa lipopolysaccharide (LPS)-inducible monocyte and neutrophil chemotactic protein that may be important in acute and chronic inflammation (3). It is interesting that MIP-1α has been found to induce the production of other inflammatory proteins such as tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), which are thought to play a major role in the development of septic shock (2). It was shown by Waage et al. (7) that oxpentoxiphylline inhibits the endotoxin-induced production of TNF in a dose-dependent manner, suggesting a beneficial treatment for patients with septic shock (8). The aim of our in vitro study was to investigate the effect of different concentrations of pentoxiphylline on MIP-1α production in a whole-blood model.

Blood from healthy volunteers was collected in endotoxin-free tubes (Kabi Tube; Chromogenix) (4) containing 120 IU of heparin (n = 8 volunteers). Afterwards, 10^7 CFU of Escherichia coli ATCC 35218 per ml, 5 µg of gentamicin per ml, and pentoxiphylline at final concentrations of 100, 50, and 10 µg/ml were added in the stated order (we used this system to mimic bacterial treatment of a patient with bacteremia). The tubes were then incubated at 37°C for 6 h. After the incubation period, concentrations of MIP-1α, TNF-α, soluble TNF receptor (60 kDa), LPS, and viable CFU per milliliter were determined. MIP-1α, TNF-α, and sTNF-R were measured by an enzyme immunosorbent assay technique using commercially available kits (Quantikine R&D systems for the first two cytokines and Bender Med-Systems for the last). Endotoxin was measured by using the chromogenic Limulus amoebocyte lysate assay (Chromogenix) as recommended by the manufacturer. Values are expressed as nanograms per milliliter. Viable counts of E. coli (CFU/ml) were determined by diluting and plating on Mueller-Hinton agar.

Data (n = 8 in each group) were analyzed by using the two-tailed Wilcoxon rank test. A P of <0.05 was considered statistically significant.

We found concentration-dependent inhibition of MIP-1α production (Fig. 1). Significantly lower concentrations of MIP-1α were observed using pentoxiphylline concentrations of 100 and 50 µg/ml in comparison with the control group using gentamicin alone (Values are expressed as means ± standard deviations [SD]; bacteria [B] = 79.5 ± 47.8 ng/ml; B + gentamicin [G] = 89.9 ± 53.2 ng/ml; B + G + 100 µg of pentoxiphylline [100P] = 37.9 ± 23.6 ng/ml; B + G + 50P = 44.5 ± 31.5 ng/ml; B + G + 10P = 59.24 ± 33.5 ng/ml; whole blood [WB] = 14 ± 9 pg/ml). Although with pentoxiphylline at 10 µg/ml lower values of MIP-1α were determined, the results were not significant. The TNF-α production determined showed a concentration-dependent suppression, as described previously by other study groups (Values are expressed as mean ± SD; B = 18.24 ± 5.74 ng/ml; B + G = 14.93 ± 3.97 ng/ml; B + G + 100P = 2.73 ± 1.52 ng/ml; B + G + 50P = 3.92 ± 2.08 ng/ml; B + G + 10P = 10.27 ± 4.1 ng/ml; WB = 24 ± 12 pg/ml). Significantly lower levels were observed using 100 and 50 µg of pentoxiphylline per ml in comparison with the control group using gentamicin alone. Concerning endotoxin release, we found a significant difference between bacteria alone and the other treatment groups ([mean ± SD]: B = 475 ± 113 ng/ml; B + G = 129 ± 81 ng/ml; B + G + 100P = 132 ± 72 ng/ml; B + G + 50G = 117 ± 50 ng/ml; B + G + 10G = 141 ± 81 ng/ml; WB = 8 ± 4 pg/ml [endotoxin-negative cells]).

No significant difference in soluble TNF receptor levels of the groups was observed ([mean ± SD]: B = 3.75 ± 1.87 ng/ml; B + G = 3.68 ± 1.99; B + G + 100P = 4.32 ± 2.25 ng/ml; B + G + 50G = 4.46 ± 2.46 ng/ml; B + G + 10P = 4.19 ± 2.42 ng/ml; WB = 3.61 ± 1.87 ng/ml).

Since sTNF-R (60-kDa) levels in all groups were similar, their influence on the TNF-α enzyme-linked immunosorbent assay could be excluded.

Viable counts of bacteria could not be detected in any group treated with gentamicin after the incubation period; in the bacteria group, an increase of CFU to 10^8 was observed. These findings support the thesis that pentoxiphylline may be of benefit in the early phase of sepsis.

MIP-1α, a chemotactic cytokine which belongs to the immunoregulatory proteins the C-C chemokines, is a potent chemotactant of lymphocytes and monocytes, as well as an activator and attractant of eosinophils and basophils (5). Increasing information suggests that MIP-1α mediates acute neutrophilic inflammation (1), although the mechanism is unknown. The production of polymorphonuclear leukocyte-derived MIP-1α, in association with the expression of appropriate adhesion molecules at the site of inflammation, may be one of the central events that contributes to the temporal shift from predominantly polymorphonuclear leukocytes to monocytes during the evolution of inflammation. Excessive production of cytokines such as MIP-1α or TNF-α triggered by massive LPS release may be the cause of sepsis syndrome (6). Thus, it would be of benefit in the early phase of septic shock to suppress an
excessive production of cytokines. It has been shown previously that pentoxiphylline is able to inhibit the TNF-α production (7). We demonstrate that pentoxiphylline also inhibits the production of MIP-1α. Whether this suppression is a direct mechanism or is caused by other cytokines remains unclear.

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REFERENCES