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The inhibition of pro-inflammatory cytokines with pentoxifylline in the cardiopulmunary bypass lung

H. ÜSTÜNSOY, M. C. SIVRIKOZ, K. BAKIR[†], I. ŞENKAYA[‡], B. ERBAĞCI[§], R. UÇAK[†] AND F. NURÖZLER[¶]

*Department of Cardiovascular and Thorax Surgery, [†]Department of Pathology, Gaziantep University School of Medicine, Gaziantep; [‡]Department of Cardiovascular and Thorax Surgery, Uludağ University School of Medicine, Bursa; [§]Department of Biochemistry, Gaziantep University School of Medicine, Gaziantep, Turkey; [¶]Department of Cardiothoracic Surgery, Columbia University Presbyterian Hospital, New York, NY, USA

Abstract In addition to preventing tissue energy loss during cardiopulmonary bypass, pentoxifylline (Ptx) prevents the production of pro-inflammatory cytokines as well. The aim of this study was to investigate whether Ptx decreases the inflammatory effects of cardiopulmonary bypass on the lungs during open-heart surgery. The patients in the study group (n=15) who were going through an open-heart surgery had 500 mg l^{-1} of Ptx added to their prime solution, whereas the patients in the control group (n=10) only received prime solution. Pre-pump and post-pump blood samples were obtained from both groups and assayed for interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor alpha (TNFα). Lung tissue samples that were obtained after the pump were examined with light microscopy and stained for tissue TNFα. Non-parametric Wilcoxon test was utilized for statistical evaluation. In the post-pump period, the difference in the IL-6, IL-8 and TNFα levels of the two groups was found to be statistically significant ($P<0{\cdot}005$). The tissue samples from the control group had significant staining with TNFα. We think that Ptx has important protective effects on the lungs during cardiopulmonary bypass.

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Keywords pentoxifylline; lung; inflammatory response; cardiopulmonary bypass.

INTRODUCTION

During cardiopulmonary bypass the lungs are exposed to several types of insults such as ischaemia–reperfusion injury, inflammatory responses and re-expansion injury (1). These factors interact in an attempt to increase each other's damaging capacity, and tissue injury occurs as a result of a complex metabolic chain of events. Pentoxifylline (Ptx) avoids dephosphorylazation of adenosine monophospate (AMP) to adenosine and inosine monoposphate (IMP) to inosine via inhibition of 5′-nucleotidase (5′-NT) and exterminate these precursors from cell during cardiopulmonary bypass.*Ptx also inhibits the production of pro-inflammatory cytokines and have anti-inflammatory properties. Ptx decreases tissue injury by exerting its effect at different steps of this metabolic chain (2). Our aim was to investigate these effects of Ptx on lungs during cardiopulmonary bypass.

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Correspondence should be addressed to: Haşim Üstünsoy MD, Gaziantep Universitesi Tip Fakültesi, Göğüs Kalp Damar Cerrahi Anabilim Dali, 27070 Kolejtepe – Gaziantep/Turkey. Fax: +90 (0) 342, 3365505; E-mail: hustunsoy@yahoo.com

MATERIALS AND METHOD

The study was conducted on the patients who had gone through open-heart surgery in our department between December 1999–May 2000 with the authority of the Board of Ethics of the Gaziantep University Medical Faculty Hospital issued as of 9 June 1999 and after their written consents were obtained. According to demographic data, the patients were blindly divided into two subgroups. Both groups had approximately the same average age, diagnosis and female–male ratio. The surgeon, the biochemist and the pathologist were blind to the grouping

The average age of the patients in the study group (n=15) was 36·5 yrs, the female to male ratio was 2/1 and their diagnoses were valvular heart disease (n=13), atrial septal defect (ASD) ($n=1$) and ventricular septal defect ($n=1$). Control group patients ($n=10$) had an average age of 34·4 yrs, female to male ratio of 3/2 and their diagnoses were valvular heart disease ($n=7$), ASD ($n=1$) and VSD ($n=2$).

All the patients received intravenous anesthesia with fentanyl (10 mg kg^{-1}), Midazolam (0·05 mg kg^{-1}) and Vecuronium (0·1 mg kg^{-1} induction, 0·05 mg kg^{-1} maintenance).

The average duration of pumping was 125 min for the study group and 110 min for the control group. The average duration of cross-clamp was calculated as 85 min for the study group and 80 min for the control group. Standard prime solutions that consisted of ringer lactate, $NaHCO_3$ (1 mg kg^{-1}), corticosteroid (1 mg kg^{-1}), mannitol (20 mg kg^{-1}) and antibiotics were used in the control group. Five hundred mg l^{-1} Ptx was added to the prime solution for the study group (2,3).

According to the preliminary study results, pre-pump and post-pump left atrium blood samples were collected from both groups and interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor alpha (TNFα) levels were determined with chemiluminescence enzyme immunometric assays on an Immulite immunoassay analyser (Immulite IL-6, IL-8, TNFα assays, DPC, Los Angeles, CA, USA). In these assays the chemiluminescent substrate, a phosphate ester of adamantyl dioxetane, undergoes hydrolysis in the presence of alkaline phosphatase resulting in the sustained emission of light.

The analytical sensitivity of the IL-6, IL-8 and TNFα assays were 1 pg ml^{-1}, 2 pg ml^{-1} and 1.7 pg ml^{-1} and the lower reportable ranges were 5 pg ml^{-1}, 5 pg ml^{-1} and 4 pg ml^{-1} respectively. Control sera ILCO 10009 and ILC0 20009 were included in each analytic run. Intra-assay and interassay precision performances of the assays that were determined on 10 replicates in a single run and 20 different runs, respectively, yielded coefficients of variation within 3·4–8·8% at 13·3 and 31·4 pg ml^{-1} concentrations for IL-6, at 117·0 and 584·0 pg ml^{-1} concentrations for IL-8 and at 65.0 and 281·0 pg ml^{-1} concentrations for TNFα. The lung tissue samples that were obtained after the pump were stained for tissue TNFα with immunohystochemical streptavidin–biotin method. Five-micron tissue sections were placed on the adhesive slides (poly-L-lysine, Sigma no P 8920) and microwaved with antigen retrieval solution for 10 min. The monoclonal antibodies to TNFα (Neomarkers MS 1073, USA) was used for immunohistochemical staining of the sections. Streptavidin–biotin, horseradish peroxidase method (DAKO, LSAB 2, K 0675) and DAB chromogene was used for immunohistochemical staining. The sections were washed with phosphate-buffered saline (PBS) to prevent drying. Finally Mayer's haematoxylin was used as counterstain.

To control the lung tissues stained, we used the tonsil tissue macrophages that were recommended on the data sheet of Neomarkers MS 1073, and also transsuced the antigen retrieval solutions at the same time as the lung preparations.

We observed light and dark brown areas in the stoplasmic tonsil tissue macrophages and the lung tissue at the light microscopic examination. The brown area contained tissue TNFα and the nuclei were stained blue and violet.

For the statistical comparison of the data that were obtained from both groups, the non-parametric Wilcoxon test was utilized.

RESULTS

In the left atrium blood samples that were obtained before the pump, mean IL-6, IL-8 and TNFα values did not show any significant difference between the two groups ($P>0·05$) (Table 1, Fig. 1). In the left atrium blood samples that were obtained after the pump, mean IL-6, IL-8 and TNFα values showed significant differences between the two groups ($P<0·005$) (Table 1, Fig. 1).

In the control group, the tissue samples had significant staining for TNFα, whereas there was only mild accumulation of tissue TNFα in the study group (Figs 2 and 3).

DISCUSSION

During a cardiopulmonary bypass operation, the metabolites that are produced as result of inflammation and ischaemia–reperfusion injury damage the lung tissue. With the activation of 5′nucleotidase enzyme in the tissue's ischaemia phase, ATP is converted to AMP and IMP, adenosine, inosine and hypoxanthine.

TABLE 1. Measurements of cytokines in the both groups*

	Before CPB**	After CPB**
IL–6 (pg ml^{-1})		
Control group	8·34 ± 2·08	718 ± 116·78
Study group	10·8 ± 3·01	205 ± 48·81
IL–8 (pg ml^{-1})		
Control group	60·0 ± 17·02	831 ± 148·68
Study group	48·6 ± 14·92	43·7 ± 13·11
TNFα (pg ml^{-1})		
Control group	8·8 ± 2·02	104·6 ± 29·82
Study group	10·5 ± 2·92	10·8 ± 2·51

*Values are mean ± SEM.
** CPB = Cardiopulmonary bypass.

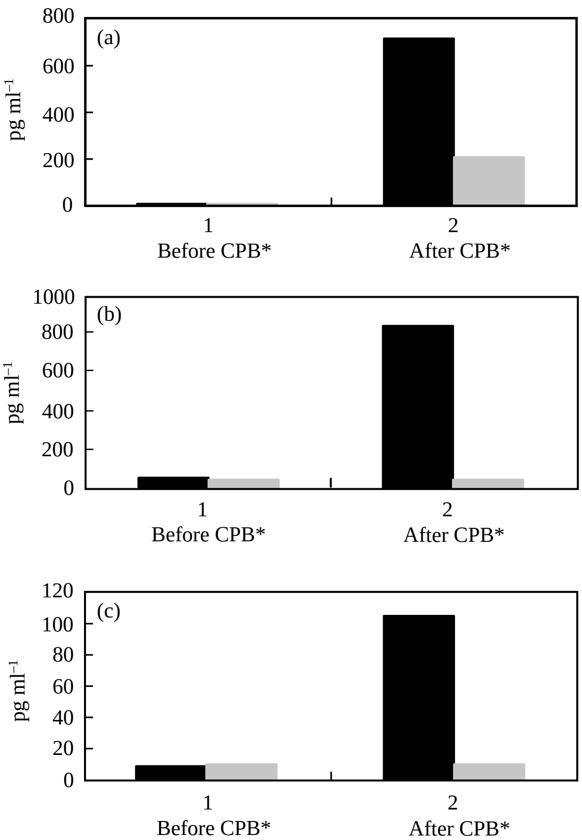

FIG. 1. IL-6 blood levels of left atrium; (b): IL-8 blood levels of Left atrium; (c): Blood TNFα levels of Left atrium. CPB=cardiopulmonary bypass; control, ■; study, □.

Meanwhile, Ca^{2+} accumulates within the cell due the altered ion balance. During the reperfusion period, the reaction of hypoxanthine with the accumulated Ca^{2+} results in the formation of reactive oxygen metabolites within the cell (4). Reperfusion of the ischaemic tissue increases the concentrations of chemotactic factors such as lipid mediators, polypeptide mediators and immune complexes (5,6). Chemotactic factors attract monocytes, polymorphonuclear leukocytes (PNL) and macrophages to the environment.

The lung is one of the largest reservoirs for monocytes, macrophages and PNLs. During cardiopulmonary bypass, in addition to ischaemia–reperfusion injury, an inflammatory reaction occurs as a result of PNL accumulation in the lungs. The PNLs that migrate to the tissue as a result of the inflammatory process initiates the tissue damage by triggering several reactions (7).

PNLs that accumulate in the tissue secrete myeloperoxidase, which produces hydroxychloride (HOCl). HOCl has a direct cytotoxic effect and, by inactivating α 1-protease inhibitor, it participates in the production of collagenases and elastases from the PNLs (8–10).

The PNLs that are in the environment, favour the secretion of pro-inflamatory cytokines such as IL-6, IL-8 and TNFα (11).

Ptx has been reported to inhibit the production of pro-inflammatory cytokines and to have anti-inflammatory properties. It protects the mitochondria structures of the cell at the same time (12).

Animal and human myocardial studies have demonstrated that newborn myocardium had higher resistance to ischaemia. This finding is supported by the low levels of 5′nucleotidase activity in the myocardium of the newborns (13).

Ptx exerts its effect by inhibiting the activity of 5′nucleotidase enzyme. It prevents the evolution of future metabolic chains that have potential hazards while preserving the energy stores which will be required at the time of reperfusion. This shows us that Ptx could be used during cardiopulmonary bypass to protect the myocardium (2,11,13–15).

The reaction that occurs during cardiopulmonary bypass is not limited to the myocardium, but can affect other organs of the body as well. As a result of this, peripheral organ systems including the lungs can be harmed to varying degrees (1,13,16).

In order to evaluate the protective effect of Ptx the present study determined the IL-6, IL-8 and TNFα levels in blood samples obtained from the left atrium and TNFα stain was performed at the tissue level.

In an *in vivo* study performed by Yoshiki *et al*. (14) in parallel to the inflammation that occurred during the ischaemia, the levels of IL-6, IL-8 and blood TNFα increased as a result of the activation of PNLs and monocytes and macrophages were reported. In the same study, with the administration of Ptx, IL-6, IL-8 and blood TNFα levels decreased when compared to those of the control group. The results of Cain *et al*. also supported these findings (15).

In the present study, if the levels of IL-6, IL-8 and TNFα are analyzed, there is a statistically significant difference between the two groups. In the light of this observation, we think that the tissue inflammatory response and ischaemia–reperfusion damage has been reduced to a great extent in the control group patients.

In the study by Türköz *et al*., it was reported that Ptx, by inhibiting the PNL activation in the lungs, prevented the accumulation of the cells, thereby reducing the damage to the lungs (17). Kleinschmidt *et al*. stated that Ptx could decrease endothelial damage and permeability (1).

TNFα, which is a pro-inflammatory cytokine, can accumulate in the tissue as well as being released to the circulation. The tissue staining for TNFα was minimal in the study group, whereas the control group showed a great degree of staining.

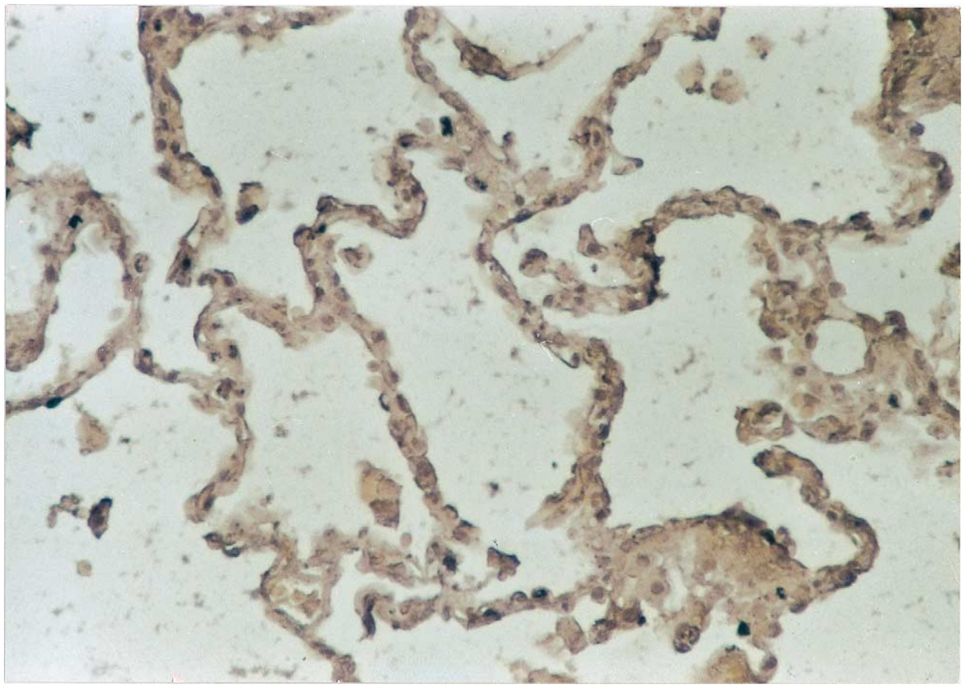

Fig. 2. Increased levels of TNFα, meant that expanded brown areas were visualized in the alveolar wall of control group samples (Immunohistochemistry, ×200).

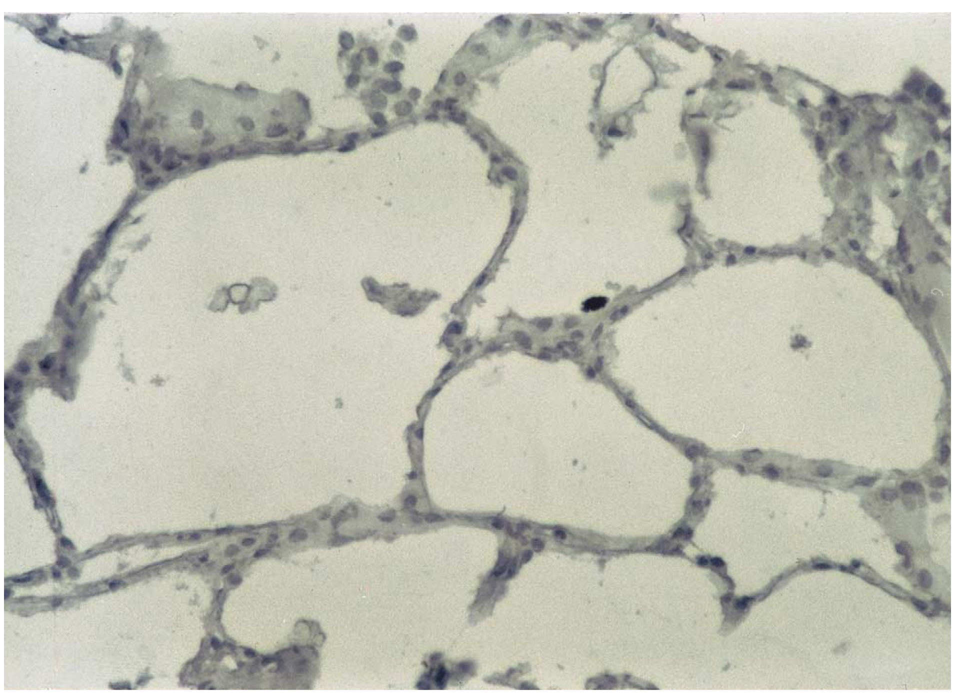

Fig. 3. In the alveolar wall of study group samples, small brown areas were visualized (Immunohistochemistry, ×200).

According to the evidence collected from the biochemical and microscopic data, the tissue inflammatory response and ischaemia-reperfusion injury was significantly diminished in the study group.

In conclusion, in the light of the results we have obtained, we think that Ptx can play an important role in the prevention of lung damage that can occur during cardiopulmonary bypass.

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