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IL-6 and TNFα Release in Association with Neutrophil Activation after Cardiopulmonary Bypass Surgery

Summary: The serum IL-6 and TNFα response to cardiopulmonary bypass surgery was studied in 12 patients. Human neutrophil elastase was also measured in order to detect the presence of neutrophil activation. Peripheral venous blood samples were obtained before, during, and 1, 2, 3 and 6 days after surgery. Intra-operative samples were also obtained from the coronary sinus and pulmonary artery. Cardiopulmonary bypass stimulated the immediate release of IL-6 into the coronary, pulmonary and systemic circulations. TNFα was transiently detected in the pulmonary circulation in seven patients. Surgery also induced early and sustained activation of neutrophils, which peaked 24 h following maximum IL-6 release. Both IL-6 and TNFα not only enhance neutrophil activation, but also stimulate an adhesive neutrophil-cardiac myocyte interaction which is associated with the release of toxic oxygen radicals. Their detection, in association with concomitant neutrophil activation, suggests a possible pathway for enhanced neutrophil mediated myocardial damage following cardiopulmonary bypass surgery.

Editorial Remarks: This is not an infectious disease study, but comes nevertheless from an author who is intensively concerned with infections and their consequences. We thought that an understanding of IL-6, TNFα and neutrophil activation immediately after cardiopulmonary surgery, after which it is well known that infections can play a fatal role, may be of importance to an infectious disease expert in his assessment of data in this connection.

W. M.

Introduction

The extracorporeal circulation of blood during cardiopulmonary bypass induces a systemic inflammatory reaction which may lead to the "postperfusion syndrome" of pulmonary, renal and cardiac dysfunction [1–3]. Although previously thought to be mainly due to activation of the complement system following blood membrane contact [4], recent evidence suggests that these changes are mediated, in part, by activated leukocytes [5–7]. Leukocytes appear to contribute to myocardial injury under conditions where ischaemic tissue is reperfused with oxygenated blood [8, 9, 9a]. Further studies suggest that removal of these cells from the circulation [10, 11], or pharmacological inhibition of their function [12, 13] not only significantly reduces the extent of myocardial damage after ischaemia and reperfusion, but also improves renal and pulmonary function [3].

Although the precise mechanism underlying leukocyte-mediated tissue damage remains unclear, experimental evidence suggests that activated neutrophils may either physically obstruct capillaries limiting reperfusion [6, 7] or alternatively, mediate tissue injury through the release of secretory products which are cytotoxic to myocytes [8, 9, 9a]. Alterations in leukocyte adherence contribute to both mechanisms, as adhesion to capillaries not only promotes retention within vessels, but also promotes emigration into extravascular sites [14–16], phenomena which are known to occur after reperfusion of the ischaemic myocardium [17–19]. Neutrophil adherence also appears to trigger the release of a number of inflammatory mediators which include toxic oxygen radicals [20, 21]. Recent *in vitro* experimental evidence suggests that the cytokines tumour necrosis factor α (TNFα), interleukin 1 (IL-1), and interleukin 6 (IL-6) stimulate an adhesive interaction between neutrophils and cardiac myocytes, which is followed by the release of the toxic metabolite, H_2O_2 [22, 23]. Although much attention has been focused on the mechanisms of neutrophil activation following ischaemia and reperfusion, no attempt has been made to evaluate the role of these cytokines as possible co-factors in mediating myocardial tissue injury in patients following cardiopulmonary bypass.

In this study, we addressed the hypothesis that cytokine-stimulated adhesion of activated neutrophils to cardiac myocytes may play an important role in mediating reperfusion injury following cardiopulmonary bypass. Neutrophil activation, as measured by human neutrophil elastase, TNFα, and IL-6 were assayed in the systemic, pulmonary, and coronary circulations of 12 patients undergoing cardio-

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Table 1: Demographic data on 12 consecutive patients undergoing coronary artery bypass grafting.

Variable	n	Mean (range)
Age (years)		62.5 (53–70)
Ejection fraction		65.7% (48–82)
Male	11	
Two vessel disease	4	
Three vessel disease	8	
History of myocardial infarction	6	
Hypercholesterolaemia/hyperlipidaemia	8	
Diabetes	4	
History of smoking	7	
New York Heart Association, Grade 3	6	
Grade 4	6	
Left main vessel disease	2	
Unstable angina	9	

pulmonary bypass. The data suggest that cardiopulmonary bypass stimulates the immediate release of IL-6 in all patients. TNFα was also transiently detected in pulmonary circulation in some patients. Their detection, in association with concomitant neutrophil activation, suggests a possible pathway for both neutrophil activation, and neutrophil mediated myocardial damage following cardiopulmonary bypass surgery.

Patients and Methods

Patients: Twelve consecutive patients undergoing coronary artery bypass grafting in the Department of Cardiothoracic Surgery, University Hospital, Giessen, were prospectively studied. Informed consent was obtained from each patient according to the protocol of the Human Study Committee. Patient details are outlined in Table 1.

Anaesthesia and cardiopulmonary bypass: Premedication and anaesthesia procedures were standardized and comparable for all patients, and consisted of weight dependent dosages of fentanyl, midazolam, and pancuronium. Controlled mechanical ventilation was performed with a PEEP of 5 cm H_2O and an FIO_2 of 0.5 during anaesthesia. Cardiopulmonary bypass was instituted with membrane oxygenators and nonpulsatile roller pumps, using a circuit primed with Ringer's lactate. Moderate hypothermia (rectal temperature 32.0 ± 0.8°C; oesophageal temperature 33.1 ± 0.5°C) was used, and a flow of 2.4 l/min/m² was maintained during bypass. Myocardial preservation was accomplished with multiple doses of Bretschneider's cardioplegia solution, and the myocardial surface was cooled with ice-cold saline solution. The mean duration of anaesthesia was 284 min (range 220–350), the mean duration of cardiopulmonary bypass was 91 min (47–117), and the mean duration of aortic cross clamping i.e. ischaemia, was 47 min (27–68). All patients were operated on by the same surgical team.

Blood sampling protocol: Peripheral venous blood was sampled pre-operatively and again on days 1, 2, 3 and 6 following surgery. Peripheral, coronary sinus and pulmonary artery blood samples were also taken after induction of anaesthesia but before institution of cardiopulmonary bypass, and 5 min after removal of the aortic cross clamp. Parameters measured included haemoglobin, neutrophil and platelet counts, and serum glutamate oxaloacetate transferase (SGOT). Serum samples were stored at –70°C until assayed for elastase, IL-6 and TNFα.

Assays: Neutrophil activation was determined by measuring the release of human neutrophil elastase, a neutrophil derived azurophilic granule marker [24, 25]. Enzyme activity was quantitated in complex with α_1-proteinase inhibitor using a commercially available enzyme-linked immunosorbent assay (ELISA) (Merck, Darmstadt, Germany).

Serum IL-6 was measured using a commercially available ELISA assay which has a lower limit of sensitivity of 10 pg/ml (Quantikine Human IL-6 Immunoassay, R&D Systems, USA). TNF was determined using a similar ELISA assay, which was specific for human TNFα (free circulating TNF), and had a lower limit of sensitivity of 5 pg/ml (Quantikine Human TNFα, R&D Systems, USA).

Statistics: Statistical analysis was performed using the Stata® statistics programme (Computer Resource Center, Santa Monica, CA). Data were correlated using the Spearman rank correlation coefficient, and the Student's paired *t* test was used for comparison of changes within the group. Significance was taken at the 5% level.

Results

Haemoglobin fell from a mean pre-operative value of 14.5 g/dl (range: 12.4–16.4) to 10.7 g/dl (9.1–13) prior to discharge from the hospital. There was a similar fall in the post-operative platelet counts, although counts had recovered by the time of discharge (mean [range] x 10^3/ml: pre-op 225 [167–292]: day 6 post-op 324 [204–436]). Surgery was followed by transient leukocytosis, which peaked on the first post-operative day, and was maintained for 48 h (Figure 1).

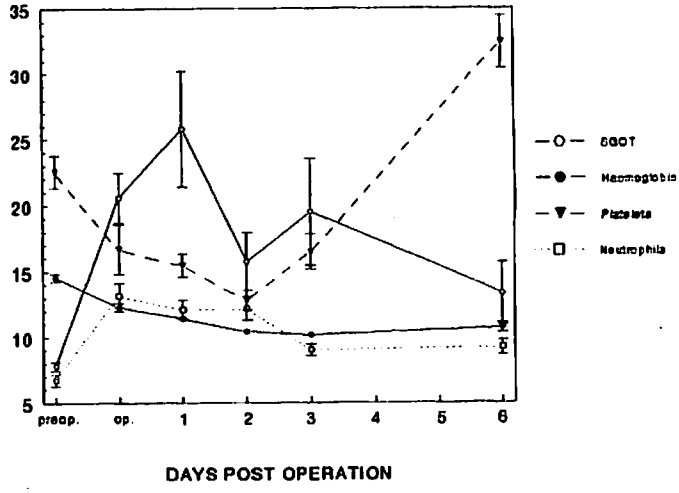

Figure 1: Peri-operative peripheral venous blood indices in 12 patients undergoing cardiopulmonary bypass surgery. Values expressed as a mean (SEM) (SGOT U/ml; haemoglobin g/dl: platelets x 10^{10}/l: neutrophils x 10^9/l).

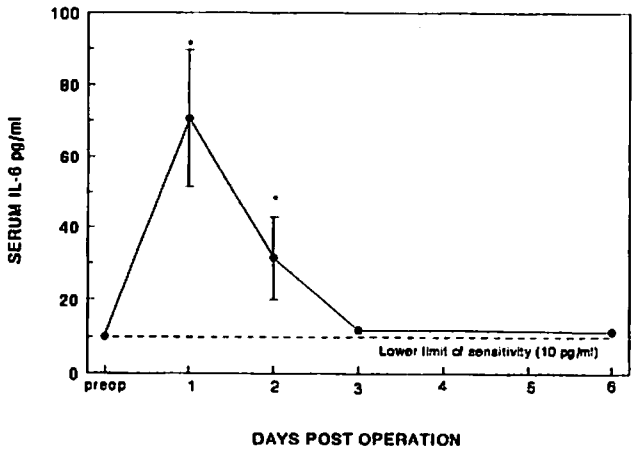

Figure 2: Mean (SEM) peri-operative serum IL-6 in peripheral venous blood in 12 patients undergoing cardiopulmonary bypass surgery (* $p < 0.05$).

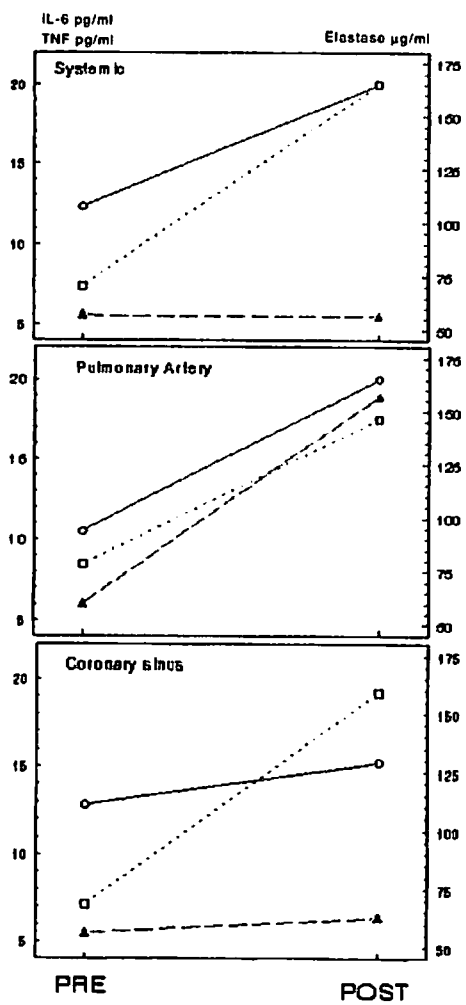

Figure 3: Mean intra-operative elastase, IL-6, and TNFα after induction of anaesthesia but before cardiopulmonary bypass (PRE), and 5 min following removal of the aortic cross clamp (POST), in the systemic, pulmonary, and coronary circulations.

SGOT was significantly elevated immediately following surgery, and peaked on the first post-operative day (U/ml mean [range]: pre-op 7.8 [6–10]: day 1 post-op 25.8 [12–63]. SGOT activity had returned to pre-operative values in almost all patients at the time of discharge on day 6 (Figure 1). The duration of ischaemia correlated with subsequent SGOT activity on the first ($r = 0.67$) and second ($r = 0.76$) postoperative days.

IL-6 was not detected in the peripheral venous blood samples of any patient prior to surgery. Post-operatively, there was a rapid rise in serum IL-6, which was detected in 10 of 12 patients on day 1 following surgery (mean [SEM] pg/ml: 70.7 [19]), but declined over the subsequent 48 h (Figure 2). Cardiopulmonary bypass stimulated the early release of IL-6 into the pulmonary (mean [sem] pg/ml; pre-cross clamp 10.5 [0.5]: post-cross clamp 20 [4.4], $p < 0.05$), coronary (pre 12.8 [2]; post 15.2 [2], $p < 0.05$), and systemic circulations (pre 12.3 [2]; post 20 [6]), in all patients (Figure 3).

TNFα was detected pre-operatively, at levels just above the lower limit of sensitivity of the assay in four of the 12 patients (mean [sem]; 6.3 [1.2]). This cytokine was not detected in significantly increased quantities in either the coronary sinus or systemic circulation following reperfusion, or on any subsequent post-operative day (Figure 3) . TNFα was detected in the pulmonary artery in seven patients following removal of the cross clamp. A maximum value of 160 pg/ml was recorded in one (mean [sem] pg/ml; pre-cross clamp < 5: post-cross clamp 18.9 [12.8], $p < 0.05$).

Serum elastase was also significantly elevated in the systemic, coronary and pulmonary circulations following reperfusion (Figure 3). In contrast to the early rise in IL-6, elastase peaked in the systemic circulation on the second postoperative day (mean [SEM] µg/ml: pre-op 75.5 [15]: day 2 post-op 139.5 [26], $p < 0.05$) (Figure 4).

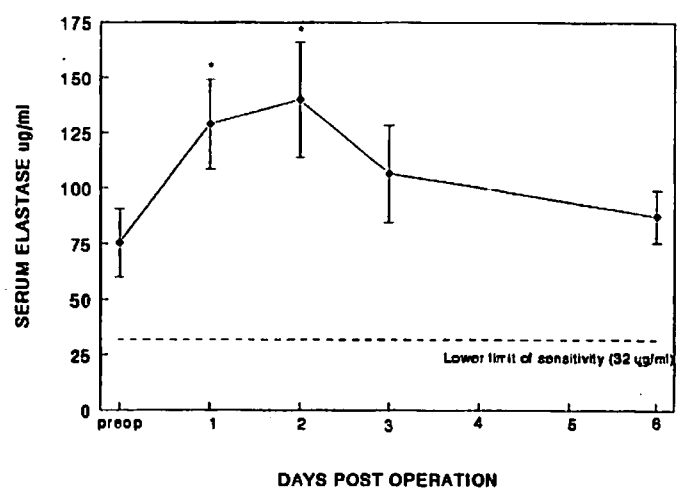

Figure 4: Mean (SEM) peri-operative serum elastase concentrations (* $p < 0.05$).

Discussion

Neutrophil activation following cardiopulmonary bypass is a well documented phenomenon, and appears to occur at two distinct time points: a) following blood membrane contact, which is associated with an increase in elastase and C3a, and b) following reperfusion of the ischaemic myocardium, which is also followed by the release of elastase, C3a, leukotriene B4, and tissue plasminogen activator [26]. Experimental evidence suggests that pre-existent activation in the terminal capillary bed of the lung is a necessary requirement for full activation to occur [27], and is supported by the work of *Quiroga* et al. who reported increased pulmonary uptake of neutrophils as blood flow decreases immediately after going on bypass, and again after restoration of flow. Both events were associated with complement activation and release of C3a [28]. In the present study, serum elastase was also used as a marker for neutrophil activation. Cardiopulmonary bypass was followed by early and persistent neutrophil activation. Serum activity of the enzyme was significantly increased in the systemic, pulmonary, and coronary circulations following removal of the aortic cross clamp, and remained elevated in the systemic circulation for up to 72 h after surgery (Figures 3 and 4).

Although much attention has focused on the nature and timing of neutrophil activation following cardiopulmonary bypass, recent studies suggest that both TNFα and IL-6 are also capable of inducing and enhancing neutrophil activation [29, 30]. TNFα is a mononuclear phagocyte and neutrophil derived cytokine which is released in response to a wide variety of stimuli including bacterial and viral infections, major traumatic and thermal injury, and some tumours. Similary, IL-6 is released by these same cells in response to a variety of conditions which are associated with an inflammatory response, including infection, major surgery, thermal injury, and some tumours including cardiac myxoma [31, 32]. Both cytokines are felt to play a pivotal role in modulating the host's response to infection and trauma, and have wide ranging effects on haematologic, metabolic, and immune functions [33]. *Shenkin* et al. noted an inconsistent TNF response to elective surgery, but found IL-6 to be a valuable early marker of tissue damage after abdominal surgery [32].

In the present study, serum IL-6 peaked on the first postoperative day, and remained elevated in the systemic circulation for up to 72 h after surgery, which is in keeping with the findings of *Butler* et al. who also noted an early rise in IL-6 in the systemic circulation after cardiopulmonary bypass [34].

Although TNFα was detected at very low levels in the pulmonary artery in some patients after removal of the aortic cross clamp, TNF has a very short half-life in the circulation (approximately 20 min). Frequent serial measurements would be necessary in order to evaluate the precise role, if any, of TNF after cardiopulmonary bypass. The present study, however, suggests that under certain circumstances the cardiopulmonary bypass may stimulate the release of TNF into the pulmonary circulation. The concomitant detection of elastase release and IL-6 and, to a lesser extent, TNF in the pulmonary circulation following reperfusion suggests that they may play an important role in the activation of the neutrophil population within the lung during cardiopulmonary bypass. These data also suggest that IL-6 may continue to modulate neutrophil function in the immediate post-operative period as this cytokine peaked in the systemic circulation 24 h prior to maximum elastase release.

Although neutrophil activation and secretion of toxic metabolites has been well documented following cardiopulmonary bypass, it is unlikely that the systemic release of these leukocyte derived factors directly mediates impaired organ function. These mediators, which include H_2O_2 and other oxygen radicals, have a very short half life, and are rapidly diluted following entry into the systemic circulation. Activated neutrophils are more likely to cause tissue inflammation and damage by direct cell to cell interaction, where toxic metabolities are released in close proximity to susceptible cells. In contrast to neutrophil-endothelial adhesion which only requires neutrophil activation [35, 36], neutrophil-myocyte adhesion requires activation of both cells [22]. This form of adhesive interaction, which is stimulated by the cytokines TNFα and IL-6, is followed by the release of H_2O_2 [22, 23]. Such an interaction would yield a relatively high local concentration of this cytotoxic oxygen radical, thus further enhancing the detrimental effect of neutrophil activation following cardiopulmonary bypass. Recent evidence points to intercellular adhesion molecule-1 (ICAM-1) as the most likely adhesive ligand on myocytes stimulated by these cytokines [22, 23]. *Youker* et al. reported that lymph from the reperfused ischaemic canine myocardium stimulated myocytes for ICAM-1 dependent adhesion to neutrophils. Activity was highest within the first hour after reperfusion and persisted for up to 72 h. This action was inhibited by the addition of anti-IL6 antibody [23]. These findings suggest that extracellular fluid from the reperfused canine myocardium contains factors (e. g. IL-6), which are capable of not only stimulating emigration of neutrophils [37], but also inducing myocyte activation. Although neutrophil activation is a characteristic feature of clinical cardiopulmonary bypass, it is unclear if surgery is also associated with the concomitant release of either TNFα or IL-6. The present findings suggest that cardiopulmonary bypass is followed not only by early and persistent activation of neutrophils, but also stimulates the early and widespread release of both IL-6 and, to a lesser extent, TNFα.

Pharmacological inhibition of neutrophil activation using high dose corticosteroids, or leukocyte depletion by filtration, has been shown to improve haemodynamic stability and ameliorate free radical induced lung injury after cardiopulmonary bypass [3, 26, 38]. Although such therapies hold promise for the future prevention of the post perfusion syndrome, it remains to be determined whether leukocyte depletion by mechanical filtration will lead to serious post-

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operative infections, and whether the simultaneous depletion of platelets by these filters will predispose to a bleeding diathesis. Similarly, the administration of high dose corticosteroids is likely to predispose to infectious complications due to immunosuppression. The present data suggest a potential therapeutic role for the use of anti-IL-6 antibodies in the prevention of the post perfusion syndrome following cardiopulmonary bypass. Such therapy has previously been used to good effect and without apparent toxicity in both experimental and clinical trials of gram-negative sepsis [39, 40].

In summary, we report that clinical cardiopulmonary bypass induces early and persistent activation of the neutrophil population. The concomitant detection of IL-6 and TNFα suggests that these cytokines, which induce neutrophil activation [29, 30] and stimulate cardiac-myocyte adhesion to activated neutrophils [22, 23] may play an important role in mediating myocardial damage after cardiopulmonary bypass. Although therapies designed to downregulate either the number or function of activated neutrophils are of proven benefit in preventing such injury [13, 14], the present data suggest a potential therapeutic role for the use of anti-IL-6 (or anti-TNF) therapy in the prevention of myocardial ischaemia/reperfusion injury following cardiopulmonary bypass.

Zusammenfassung: Ausschüttung von IL-6 und TNFα bei Neutrophilen-Aktivierung nach kardiopulmonaler Bypass-Operation. Serum Interleukin-6 und TNFα-Spiegel wurden während und nach koronarer Bypass-Operation bei 12 Patienten untersucht. Neutrophilen-Elastase wurde als Marker für die Neutrophilen-Aktivierung bestimmt. Peripher-venöses Blut wurde vor, während und 1, 2, 3 und 6 Tage nach der Operation entnommen. Intraoperativ wurden Proben von dem Koronarsinus und der Pulmonalarterie gewonnen. Kardiopulmonale Bypass-Operation führte zu einer Ausschüttung von IL-6 in die koronare, pulmonale und systemische Zirkulation. TNFα war vorübergehend in der pulmonalen Arterie erhöht. Durch die Operation wurde auch frühzeitig eine Aktivierung von Neutrophilen bewirkt, die 24 Stunden nach der Operation ihr Maximum nach der maximalen IL-6-Ausschüttung erreichte. Der Nachweis von IL-6 und TNFα zusammen mit der Neutrophilen-Elastase weist auf die Möglichkeit einer Myokardschädigung nach kardiopulmonaler Bypass-Operation durch Aktivierung von Cytokinen hin, die zu Komplikationen wie Sepsis und Multiorganversagen führen könnte.

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