Elevation of cytokines during open heart surgery with cardiopulmonary bypass: participation of interleukin 8 and 6 in reperfusion injury

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Myocardial ischaemia is one of the major causes of low output syndrome during open heart surgery. Injury associated with ischaemia and reperfusion has been considered to result, in part, from the action of neutrophils, the interaction of neutrophils with vascular endothelial cells, and the effects of cytokines which are mediators that induce and modify reactions between these substances. We investigated cell injury in relation to the concentrations of interleukins 6 and 8 (IL-6 and IL-8), which have recently received attention as neutrophil activators. Neutrophil counts, granulocyte elastase (GEL), IL-6, IL-8, tumour necrosis factor-α (TNF-α), CK, and CK-MB concentrations were determined serially in 11 patients undergoing open heart surgery with cardiopulmonary bypass (CPB). Neutrophil counts (mean ± SD 2717 ± 2421 μl^{-1} preoperatively) peaked 60 min after declamping the aorta at 7432 ± 4357 μl^{-1} ($P < 0.01$) and remained elevated 7136 ± 5194 μl^{-1} at 180 min ($P < 0.01$). Plasma GEL level (168 ± 71 $\mu g \cdot L^{-1}$ preoperatively) peaked at 1134 ± 453 $\mu g \cdot L^{-1}$ 120 min after declamping of the aorta ($P < 0.01$) and remained elevated, 1062 ± 467 $\mu g \cdot L^{-1}$, after 180 min ($P < 0.01$). Serum IL-6 level (118 ± 59 $pg \cdot ml^{-1}$ preoperatively) peaked at 436 ± 143 $pg \cdot ml^{-1}$ 60 min after declamping of the aorta ($P < 0.01$) and remained elevated, 332 ± 109 $pg \cdot ml^{-1}$, after 180 min. Serum IL-8 level (37 ± 44 $pg \cdot ml^{-1}$ preoperatively) peaked at 169 ± 86 $pg \cdot ml^{-1}$ at 60 min after declamping of the aorta ($P < 0.001$) and remained elevated at 113 ± 78 $pg \cdot ml^{-1}$ 180 min after declamping of the aorta. Serum TNF-α was decreased at 60 min after aortic occlusion but otherwise did not change. Plasma GEL concentrations correlated with serum IL-8 levels ($R = 0.7$, $P = 0.001$) and the IL-6 and IL-8 concentrations correlated with the duration of aortic clamping ($R = 0.64$, $P = 0.01$, $R = 0.7$, $P = 0.01$). We conclude that the increases of IL-6 and IL-8 occur as a result of ischaemia, and suggest that these cytokines participate in reperfusion injury by activating neutrophils.

L'ischémie myocardique est une des principales causes du syndrome de bas débit pendant la chirurgie à coeur ouvert. On pense que la lésion associée à l'ischémie et la reperfusion résulte en partie de l'action des neutrophiles, l'interaction des neutrophiles avec les cellules vasculaires endothéliales et l'activité de médiateurs, les cytokines qui induisent et modifient les réactions entre ces substances. Nous avons examiné la relation de la lésion cellulaire avec la concentration des interleukines 6 et 8 (IL-6 et IL-8), qui ont récemment attiré l'attention comme activateurs de neutrophiles. Chez 11 patients soumis à une chirurgie cardiaque ouverte avec circulation extracorporelle (CEC), on mesure en série le décompte des neutrophiles, l'élastase granulocytaire (GEL), l'IL-6 et l'IL-8, le facteur-α de nécrose tumorale (TNF-α) et la concentration des CK et CK-MB. Le décompte des neutrophiles (moyenne ± SD: 2717 ± 2421 μl^{-1} en préopératoire) atteint un maximum de 7432 ± 435 μl^{-1} 60 min après le déclampage de l'aorte ($P < 0,01$) et demeure élevé, 7136 ± 5194 μl^{-1}, à 180 min ($P < 0,01$). Le niveau de la GEL plasmatique (168 ± 71 $\mu g \cdot L^{-1}$ en préopératoire) atteint un maximum de 1134 ± 453 $\mu g \cdot L^{-1}$ après 120 min du déclampage de l'aorte ($P < 0,01$) et demeure élevé, 1062 ± 467 $\mu g \cdot L^{-1}$ après 180 min de déclampage ($P < 0,01$). L'IL-6 sérique (118 ± 59 $pg \cdot ml^{-1}$) atteint un maximum de 436 ± 143 $pg \cdot ml^{-1}$ 60 minutes après le déclampage de l'aorte ($P < 0,01$) et demeure élevé, 332 ± 109 $pg \cdot ml^{-1}$ après 180 min. Le niveau sérique d'IL-8 (37 ± 44 $pg \cdot ml^{-1}$ en préopératoire)

Key words
HEART: ischaemia;
HORMONES: cytokines, interleukins.

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atteint un maximum de 169 ± 86 pg · ml^{-1} 60 min après le déclampage de l'aorte ($P < 0,01$) et demeure élevé, 113 ± 78 pg · ml^{-1} après 180 min. Le TNF-α décroît 60 min après le clampage aortique mais ne change plus par la suite. La concentration plasmatique de GEL est en corrélation avec le niveau sérique de l'IL-8 ($R = 0,7$, $P = 0,001$). Les concentrations d'IL-6 et d'IL-8 sont en corrélation avec la durée du clampage ($R = 0,64$, $P = 0,01$, $R = 0,07$, $P = 0,01$). Nous concluons que les augmentations d'IL-6 et d'IL-8 résultent de l'ischémie et nous suggérons qu'en activant les neutrophiles, ces cytokines participent à la genèse de la lésion de reperfusion.

Since it was reported that infarct size could be reduced by suppressing leukocyte infiltration into the ischaemic myocardium with anti-leukocyte antibodies[1] and non-steroidal anti-inflammatory agents,[2] attention has been focused on the role of leukocytes in progressive ischaemic myocardial damage and reperfusion injury. The mechanism of local leukocyte infiltration is complicated. The substances C5a, leukotriene B4 and various cytokines are known as endogenous leukocyte chemotactic factors which finally guide leukocytes to the target site of action. Also, tumour necrosis factor-α (TNF-α), IL-6, and IL-8 have been reported to be involved in tissue injury but detailed studies of their action in relation to reperfusion injuries have not been performed. Accordingly, in an attempt to clarify their participation in reperfusion injury, we examined the changes in circulating concentrations of these cytokines in relation to leukocyte count, creatine phosphokinase (CK), CK-MB and granulocyte elastase (GEL) in patients during CPB.

Methods

With institutional approval and informed consent, we studied 11 patients who underwent open heart surgery (Table I).

Preanaesthetic medications included diazepam (0.2 mg · kg^{-1}), hydroxyzine (1 mg · kg^{-1}), dumeor (1 mg · kg^{-1}) and atropine (0.01 mg · kg^{-1}). Anaesthesia was induced with fentanyl (30 μg · kg^{-1}), and tracheal intubation was facilitated with vecuronium (0.15 mg · kg^{-1}). Anaesthesia was maintained using oxygen, and high-dose fentanyl (total 100 μg · kg^{-1}). Ventilation was controlled to maintain $PaCO_2$ at approximately 40 mmHg.

The perfusion apparatus included a Hollow fibre membrane oxygenator (Terumo, Capiox) and nonpalsatile roller pump (Pemco Inc). A mixture of 20% mannitol, 7% sodium bicarbonate, electrolyte solution, and CPD-added preserved blood was primed, and then perfused at a flow rate of 2.4 L · m^{-2} · min^{-1}. Haematocrit levels were maintained at 25% or more throughout CPB. Crystaloid cardioplegia was used for cardiac preservation.

The ECG, EEG, and oesophageal and rectal temperatures were monitored continuously. Arterial blood oxygen saturation was also monitored continuously with a pulse-oximeter (Datex, Satlite), and end-tidal carbon dioxide concentrations with capnography (Datex, Capnomac). A catheter was placed in the radial artery to measure arterial pressure directly, and from which blood samples were drawn. Six blood samples were drawn after inducing anaesthesia, at the following times: before operation, immediately before starting CPB, 60 min after aortic occlusion, and 60, 120 and 180 min after declamping the aorta. In each sample, CK, CK-MB, peripheral WBC, neutrocyte, GEL, IL-6, IL-8, and TNF-α concentrations were measured. The IL-8, IL-6 and TNF-α were measured by ELISA kits (IL-8: R & D systems, Minneapolis, MN, USA[3] IL-6: Toray Fujibionics Inc, Tokyo, Japan,[4] TNF-α: Medgenix Diagnostics, Belgium) and GEL by enzyme linked immunosorbent assay as GEL-α_1-PI complex. Data were analyzed by analysis of variance and Neuman-Keuls multiple comparison tests. Significant difference was defined as $P < 0.05$. All data are expressed as the mean ± SD.

Results

The patient's age, body weight, ejection fraction, cardiopulmonary bypass time, aortic clamp time and cardioplegic solution are shown in Table I. The mean serum CK and CK-MB concentrations increased at 60 min after declamping of the aorta compared with those before operation and 60 min after aortic occlusion and increased continuously until 180 min after declamping of the aorta ($P < 0.01$, Table II).

The polymorphonuclear leukocyte (PMN) count increased from 60 min after declamping of the aorta compared with that before operation and 60 min after aortic occlusion and remained elevated at 180 min after declamping of the aorta ($P < 0.01$, Table II).

The plasma granulocyte elastase concentration was increased 60 min after declamping of the aorta compared with that before operation and 60 min after aortic occlusion and high levels were maintained 180 min after declamping of the aorta ($P < 0.01$, Figure 1).

Serum IL-8 and IL-6 concentrations increased 60 min after declamping of the aorta and remained high at 180 min after declamping of the aorta ($P < 0.001$, Figures 2 and 3). Furthermore, the concentrations of IL-8 at 60, 120, 180 min after declamping of the aorta were higher than at 60 min after aortic occlusion ($P < 0.001$, Figure 2) and the serum IL-6 concentration at 60 min after declamping of the aorta was higher than that 60 min after aortic occlusion ($P < 0.001$, Figure 3).

TNF-α was decreased at 60 min after aortic occlusion but otherwise did not change (Table II).

TABLE I Characteristics of patients and results (mean ± SD)

Patient	Age (y)	Weight (kg)	Diagnosis	EF (%)	CPB (min)	Ao.-clamp (min)	L.-temp (°C)	C.-plegia (ml)
1	71	37	AS	64	180	120	28.9	2050
2	66	53	AR	72	184	85	29.4	2100
3	55	68	AP	56	115	63	30	1700
4	63	51	AR	35	260	140	27.9	2800
5	66	54	AR	50	190	109	27.8	1800
6	24	45	VSD, PH	68	135	79	29.9	1640
7	66	41	AP	58	180	58	28	1200
8	47	48	AR, MS	52	180	80	28	1640
9	47	72	AR	68	170	120	28.2	3100
10	70	40	AP	68	240	43	25.7	2300
11	61	65.5	MS	52	160	60	29.3	1600
Mean	58	52		58	181	87	28.4	1993
SD	13	11		10	41	31	1.2	560

AS: aortic stenosis; AR: aortic regurgitation; AP: angina pectoris; VSD: ventricular septal defect; PH: pulmonary hypertension; MS: mitral stenosis; EF: ejection fraction; CPB: cardiopulmonary bypass; AO-clamp: aortic clamp time; L-temp.: lowest temperature; C-plegia.: cardioplegia.

TABLE II Concentrations of serum creatine phosphokinase (CK), CK-MB, polymorphonuclear leukocyte (PMN) and serum tumor necrosis factor-α (TNF-α) in each period.

	1	2	3	4	5	6
CK ($IU \cdot L^{-1}$)	161 ± 116	229 ± 124	246 ± 73	428 ± 130*†	519 ± 240*†	592 ± 179*†
CK-MB ($IU \cdot L^{-1}$)	0.5 ± 0.7	0.7 ± 0.9	0.7 ± 1.1	4.1 ± 3.2*†	6.9 ± 3.2*†	10.9 ± 6.8*†
PMN (μl^{-1})	2717 ± 2421	3257 ± 2181	3016 ± 1786	7432 ± 4357*†	7192 ± 4829*†	7136 ± 5194*†
TNF-α ($pg \cdot ml^{-1}$)	11.4 ± 15	10.9 ± 12	5.7 ± 6*	6.9 ± 8	9.8 ± 9	6.9 ± 7

*$P < 0.01$ versus before operation (1).
†$P < 0.01$ versus 60 min after aortic occlusion (3).
Sampling point (1) before operation; (2) pre-CPB; (3) 60 min after aortic clamp; (4) 60 min after declamping of the aorta; (5) 120 min after declamping of the aorta; (6) 180 min after declamping of the aorta.

Regression analysis demonstrated a relationship between GEL and IL-8 (R = 0.7, $P = 0.001$, Figure 4), and between GEL and CK-MB (R = 0.47, $P = 0.02$, Figure 5).

Correlations were obtained between IL-8 and aortic clamping time (R = 0.7, $P = 0.01$, Figure 6), and between IL-6 and aortic clamping time (R = 0.64, $P = 0.01$, Figure 7). No correlation was found between IL-8 and IL-6.

Correlations were observed between IL-6 and CK-MB (R = 0.62, $P = 0.002$, Figure 8) and between IL-8 and CK-MB (R = 0.64, $P = 0.001$, Figure 9).

No correlation was found between IL-6 and GEL nor between TNF-α and GEL.

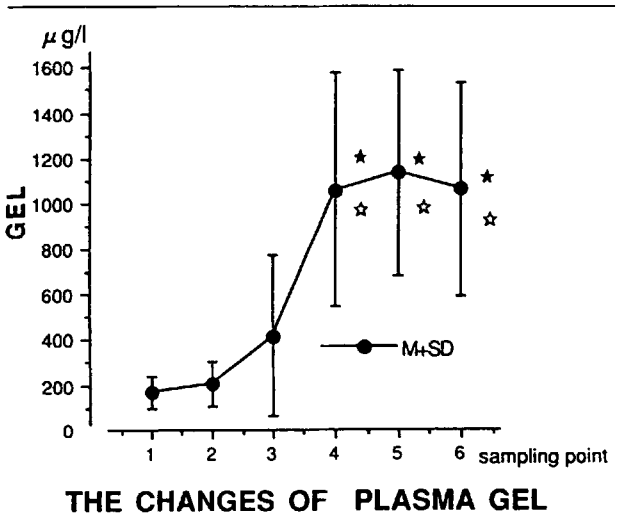

FIGURE 1 Absolute levels of plasma granulocyte elastase level in each period. ★$P < 0.01$ versus before operation (1). ☆$P < 0.01$ versus 60 min after aortic occlusion (3). Sampling point (1) before operation; (2) pre-CPB; (3) 60 min after aortic occlusion; (4) 60 min after declamping of the aorta; (5) 120 min after declamping of the aorta; (6) 180 min after declamping of the aorta.

Discussion

Many studies have shown that cardiac infarct size was decreased in reperfusion models when neutrophil infiltration was suppressed.[1,2,5] As a result, attention has focused on the possible role of neutrophils in reperfusion injury. Our study demonstrated an increase in CK, CK-MB, neutrophils and GEL from 60 min after declamping

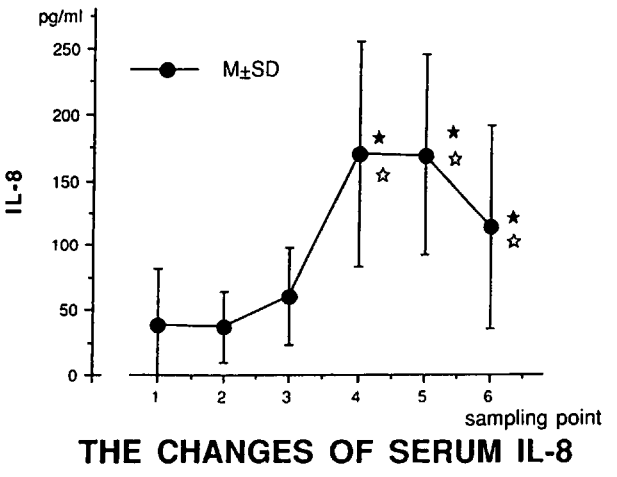

FIGURE 2 Absolute levels of serum interleukin-8 concentration in each period. ★$P < 0.001$ versus before operation (1). ☆$P < 0.001$ versus 60 min after aortic occlusion (3).

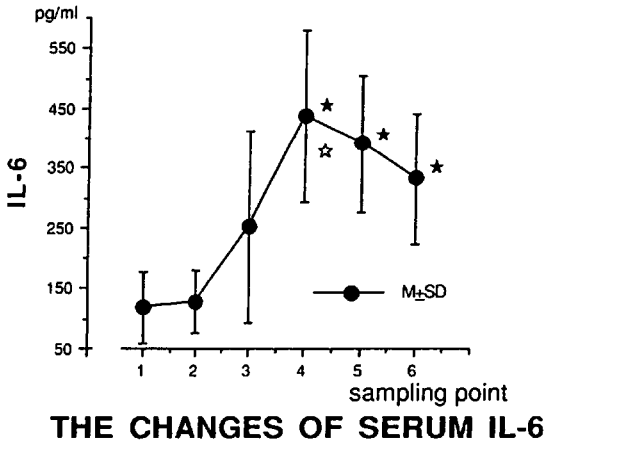

FIGURE 3 Absolute levels of serum interleukin-6 concentration in each period. ★$P < 0.001$ versus before operation. ☆$P < 0.001$ versus 60 min after aortic occlusion (3).

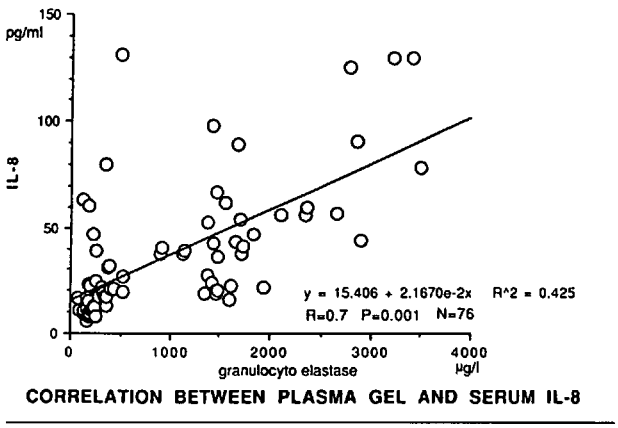

FIGURE 4 Relationship between absolute values in granulocyte elastase and interleukin-8 ($R = 0.7$, $P = 0.001$).

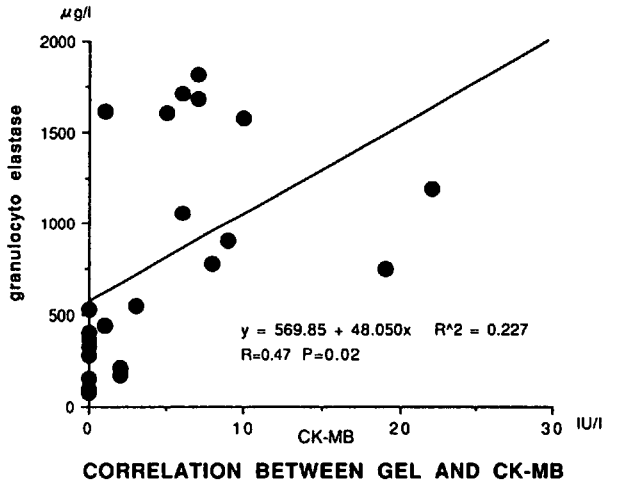

FIGURE 5 Relationship between absolute values in granulocyte elastase and CK-MB ($R = 0.47$, $P = 0.02$).

of the aorta which suggests that neutrophils participate in reperfusion injury.

Before leukocytes infiltrate into tissues, appropriate cell adhesion molecules[6] should be expressed first on the surface of the leukocytes, and endotherial cells and then the leukocytes should adhere to the vascular endothelial cells, penetrate them, and then destroy their basal membrane, followed by migration to the target site of action. Interleukin 8 is a factor which may finally guide leukocytes to the target site of action. It has been shown, *in vitro*, to be neutrophil chemotactic and, in addition, it is known to have other biological actions including neutrophil activation. Activated neutrophils induce the release of lysosomes[7,8] and the production of leukotriene B4.[9] They also augment the expression of CR-1 and Mac-1 which are involved in cell adhesion, and promote the adhesion of neutrophils to unstimulated vascular endothelial cells.[10,11]

The present study showed an increase in IL-8 as well as in neutrophils and GEL concentrations. In addition, IL-8 was positively correlated with GEL. This observation suggests that GEL was released from neutrophils activated by IL-8, and the cells were injured. The results are consistent with those presented by Endo *et al.*[12] who reported that IL-8 was correlated with GEL in septic ARDS, indicating that IL-8 was closely involved in the production and release of GEL. IL-8 is produced from monocytes, macrophages, fibroblasts and vascular endothelial cells following stimulation with TNF and IL-1.[13-15] There is also a recent report that IL-8 is produced

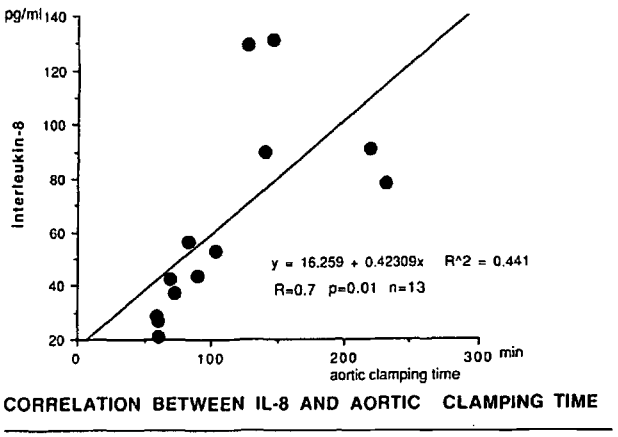

FIGURE 6 Relationship between absolute values in interleukin-8 and aortic occlusion time (R = 0.7, $P = 0.01$).

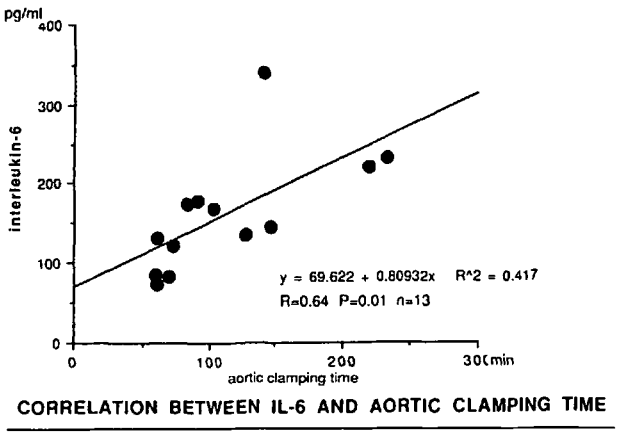

FIGURE 7 Relationship between absolute values in interleukin-6 and aortic occlusion time (R = 0.64, $P = 0.01$).

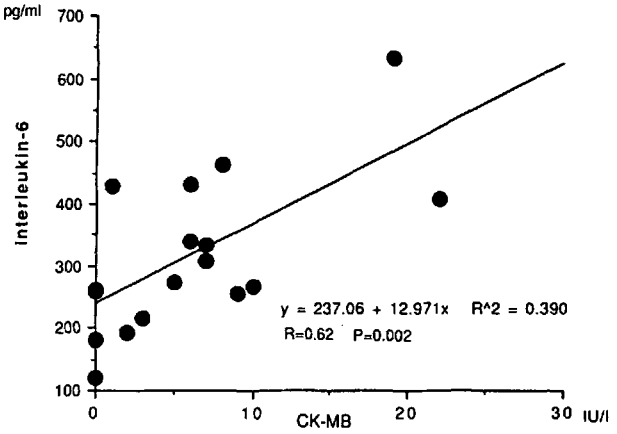

FIGURE 8 Relationship between absolute values in interleukin 6 and CK-MB (R = 0.62, $P = 0.002$).

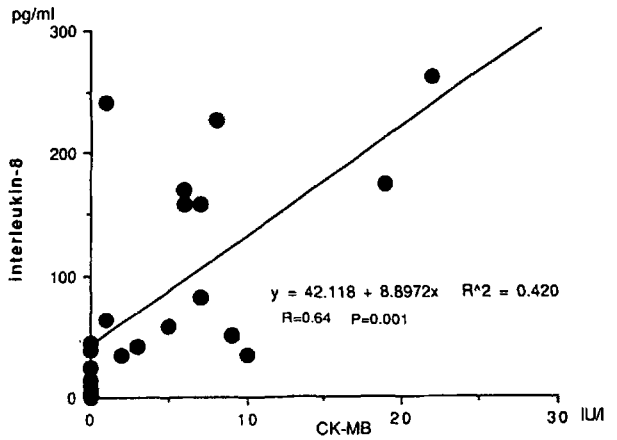

FIGURE 9 Relationship between absolute values in interleukin 8 and CK-MB (R = 0.64, $P = 0.001$).

in tissue hypoxia associated with ischaemia.[16] In our study, TNF-α did not increase and positive correlation between IL-8 and aortic clamping time was observed, suggesting that IL-8 production increased in ischaemia but was not stimulated with TNF-α. Reperfusion injury may develop by the pathway starting from ischaemia → increased production of IL-8 → activation of neutrophils → release of GEL → cell injury.

Interleuken-6 is produced from T cells, B cells, macrophages and other immunocomponent cells.[17,18] It plays an important role not only in the immune reaction, but also in acute phase reaction and the defence mechanism.[19] TNF-α has also been reported to be involved in tissue injury in a similar fashion to IL-1 and IL-8. Our study showed that IL-6, like IL-8, increased from 60 min after declamping of the aorta. These results suggest that IL-6 may also be involved in the production and release of GEL.

Attempts[9,20-22] have been made to explain ischaemic myocardial injury associated with neutrophils by (1) oxygen free radicals, (2) metabolites of arachidonic acid produced by the lipoxygenase pathway, and (3) neutrophil lysozomes including GEL. These substances appear only after activation of neutrophils, therefore there is a strong possibility that reperfusion injury might be controlled if IL-6, IL-8 and other substances which activate neutrophils are inhibited. The results of our study seem to support this possibility. Glucocorticoid,[23] and IL-4[24] suppress the production of IL-8. If these substances are used in open heart surgery with cardiopulmonary bypass, myocardial injury might be relieved.

In conclusion, the possibility of the participation of cytokines and neutrophils in reperfusion injury was in-

vestigated in 11 patients with CPB. Sixty minutes after declamping of the aorta, CK, CK-MB, neutrophils and GEL began to increase suggesting that neutrophils might participate in reperfusion injury. At the same time IL-6 and IL-8 concentrations began to increase. IL-8 was positively correlated with GEL, indicating that it was involved in cell injury following neutrophil activation.

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