Ulinastatin reduces elevation of cytokines and soluble adhesion molecules during cardiac surgery

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***Purpose**: To investigate whether ulinastatin pretreatment (6000 $U \cdot kg^{-1}$ before CPB and before declamping of aorta) influenced the production of cytokines and adhesion molecules in the peripheral circulation.*

***Methods**: This prospective randomized study was performed in 22 patients undergoing cardiac surgery. They were divided into two groups. Patients in Group I were untreated and in Group II treated with ulinastatin. The soluble intercellular adhesion molecule-1 (S-ICAM-1), soluble endothelial leukocyte adhesion molecule-1 (S-ELAM-1), interleukin8 and 6 (IL-8, 6) were measured using ELISA kits.*

***Results**: Serum S-ICAM-1 concentration in Group I increased from the preoperative value of 297 ± 27 $ng \cdot kg^{-1}$ to 418 ± 106 $ng \cdot kg^{-1}$ at 60 min after declamping of the aorta ($P < 0.01$) but did not change in Group II. Serum S-ELAM-1 concentration did not change in either group. Serum concentration of IL-8 and IL-6 in Group I (37 ± 44 $pg \cdot kg^{-1}$, and 59 ± 59 $pg \cdot kg^{-1}$, preoperatively) increased to 169 ± 86 $pg \cdot kg^{-1}$ and 436 ± 143 $pg \cdot kg^{-1}$ at 60 min after declamping of the aorta ($P < 0.001$, $P < 0.001$). The increases were greater than those from 25 ± 6 $pg \cdot kg^{-1}$ and 30 ± 26 $pg \cdot kg^{-1}$ to 56 ± 36 $pg \cdot kg^{-1}$ and 132 ± 78 $pg \cdot kg^{-1}$ in Group II ($P < 0.001$, $P < 0.001$). The levels of S-ICAM-1 correlated with those of IL-8 ($r = 0.5$, $P < 0.001$).*

***Conclusion**: These results suggest that ulinastatin may suppress the increase in IL-8 production and the expression of ICAM-1 during cardiac surgery.*

***Objectif**: Rechercher si le l'administration préalable d'ulinastatin (6000 $U \cdot kg^{-1}$ avant la CEC et au déclampage de l'aorte) influençait la production de cytokines et de molécules adhésives dans la circulation périphérique.*

***Méthodes**: Cette étude prospective et aléatoire a été réalisée chez 22 patients soumis à une chirurgie cardiaque. Ils ont été répartis entre deux groupes. Les patients du groupe I n'ont pas reçu de l'ulinastatin alors que le groupe en a reçu. La molécule-1 adhésive intercellulaire soluble (S-ICAM-1), la molécule-1 endothéliale leucocytaire soluble (S-ALAM-1), les interleukines 8 et 6 (IL-8, 6) ont été mesurées à l'aide d'une trousse ELISA.*

***Résultats**: La concentration sérique de S-ICAM-1 du groupe I a augmenté 60 min après le déclampage de l'aorte de la valeur préopératoire de 297 ± 27 $ng \cdot kg^{-1}$ à 418 ± 106 $ng \cdot kg^{-1}$ ($P < 0,01$) mais est demeurée inchangée dans le groupe II. La concentration sérique de IL-8 et IL-6 n'a pas changé dans les deux groupes. Les concentrations sériques de IL-8 et IL-6 dans le groupe I (valeurs préopératoires 37 ± 44 $pg \cdot kg^{-1}$ et 59 ± 59 $pg \cdot kg^{-1}$) ont a augmenté à 169 ± 86 $pg \cdot kg^{-1}$ et 436 ± 43 $pg \cdot kg^{-1}$ 60 min après le déclampage de l'aorte ($P < 0,001$, $P < 0,001$). Les niveaux de S-ICAM-1 étaient en corrélation avec ceux de IL-8 ($r = 0,5$, $P < 0,001$).*

***Conclusion**: Ces résultats suggèrent que l'ulinastatin peut supprimer l'augmentation de la production de IL-8 et se ICAM-1.*

Key words

ANAESTHESIA: cardiac, cardiopulmonary bypass;
DRUG: ulinastatin, protease inhibitor;
HEART: reperfusion injury;
IMMUNOLOGY: cytokines, adhesion molecules (S-ICAM-1, S-ELAM-1).

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Our previous studies showed[1,2] that the production of IL-8 and IL-6 increased after declamping the aorta in patients who had undergone cardiac surgery with CPB, and that the increase in these factors correlated with CK-MB concentration, suggesting that IL-8 and IL-6 contributed to reperfusion injury following ischaemia. These inflammatory cytokines are known to potentiate the expression of various adhesion molecules on neutrophils and vascular endothelial cells[3,4] and, as a result,

to activate neutrophils and produce or release oxygen free radicals and elastase, which may cause tissue injury. In the present study, we determined soluble adhesion molecules, and evaluated the effect of ulinastatin on the production of S-ICAM-1, S-ELAM-1, IL-8 and IL-6.

Methods

With institutional approval and informed consent, this prospective randomized study was performed in 22 patients undergoing cardiac surgery: Group I, 10 patients (CABG five, valve disease five cases) underwent standard CPB; Group II, 12 patients (CABG five, valve disease seven cases) received 6000 $U \cdot kg^{-1}$ ulinastatin (Miracrid®, Mochida Ltd., Japan) *iv* before CPB and before declamping of the aorta.

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

The perfusion apparatus included a hollow fibre membrane oxygenator (Terumo, Capiox) and nonpulsatile roller-pump (Pemco Inc.) A mixture of mannitol 20%, sodium bicarbonate 7%, electrolyte solution, and CPD-added preserved blood was used for priming, and then perfused at a flow of 2.4 $L \cdot m^{-2} \cdot min^{-1}$. Haematocrit concentrations were maintained at 20% or more throughout CPB. Body temperature was cooled down to below 30°C by core cooling through CPB. Crystalloid and blood cardioplegia were 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 pulmonary artery catheter was inserted via the internal jugular vein and a catheter was placed in the radial artery to measure arterial pressure directly. Six arterial blood samples were drawn after induction of anaesthesia at the following times: before surgery, immediately before starting CPB, 60 min after aortic occlusion, and 60, 120 and 180 min after declamping of the aorta. In each sample, S-ICAM-1, S-ELAM-1, IL-8, and IL-6 concentrations were measured by ELISA kits (S-ICAM-1, S-ELAM-1: British Biotechnology Products Ltd., Abingdon, U.K., IL-8: R&D systems Minneapolis, MN, USA, IL-6: Toray Fujibionics Inc., Tokyo, Japan). For statistical analyses, repeated measures ANOVA was used for multiple within group comparisons and Student's t test for between group comparison. Significant difference was defined as $P < 0.05$. Data are presented as mean ± standard deviation (mean ± SD).

TABLE I Clinical characteristics

Group	*Control*	*Ulinastatin*
Patients	$n = 10$	$n = 12$
Age (yr)	54 ± 17	63 ± 11
BW (kg)	62 ± 11	55 ± 8
EF (%)	50 ± 23	60 ± 12
CPB (min)	159 ± 47.4	159 ± 27
Ao clamp (min)	101 ± 32.6	97 ± 26

Mean ± SD; BW: body weight; EF: ejection fraction; CPB: cardiopulmonary bypass; Ao. clamp: aortic clamp time.

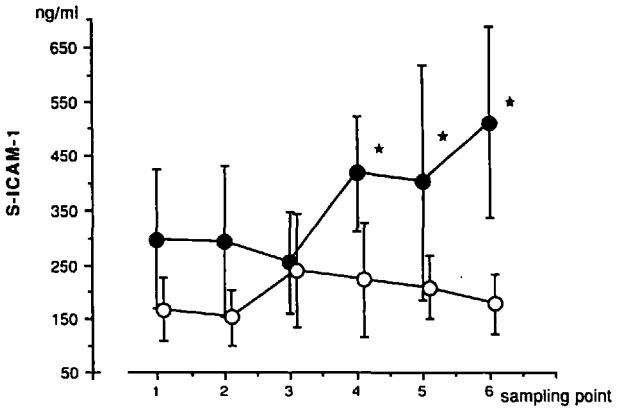

FIGURE 1 Changes of serum soluble ICAM-1 concentration mean SD. ● Group I ($n = 10$), ○ Group II ($n = 12$). ★$P < 0.01$ vs (1) (3). Sampling times: 1. before surgery; 2. before CPB; 3. 60 min after aortic occlusion; 4. 60 min after reperfusion; 5. 120 min after reperfusion; 6. 180 min after reperfusion.

Results

Patients from the two groups did not differ in terms of age, body weight, ejection fraction, duration of CPB, or aortic clamping time (Table I). The serum concentrations of S-ICAM-1 did not change in Group II but increased at 60, 120, 180 min after reperfusion (declamping of aorta) compared with the value before surgery and 60 min after aortic occlusion in Group I ($P < 0.01$, Figure 1). Serum S-ELAM-1 concentration did not change in either group (Table II). Serum IL-8 concentration increased at 60, 120, and 180 min after reperfusion compared with the values before surgery and 60 min after aortic occlusion in both groups ($P < 0.001$). The IL-8 concentration in Group II was less than that in

TABLE II Concentrations of serum IL-8, IL-6 and S-ELAM-1.

		Sampling points					
	Group	*1*	*2*	*3*	*4*	*5*	*6*
IL-8 ($pg \cdot ml^{-1}$)	Group I	37.7 ± 44.2	37.2 ± 27.0	60.3 ± 36.9	169.5 ± 86.5*	169.0 ± 77.0*	113.6 ± 78.6*
	Group II	25.0 ± 6.6	25.1 ± 18.5	26.1 ± 24.7	56.3 ± 36.4*†	49.8 ± 30.8*†	44.9 ± 31.2*†
IL-6 ($pg \cdot ml^{-1}$)	Group I	59.8 ± 59	64.0 ± 52.1	252.1± 159.6	436.5 ±143.5*	390.3 ± 114.2*	332.4 ± 109.6*
	Group II	30.7 ± 26.8	25.0 ± 25.1	22.3 ± 19.4	132.8 ± 78.0*†	167.9 ± 146.0*†	153.0 ± 66.7*†
S-ELAM-1 ($ng \cdot ml^{-1}$)	Group I	38.0 ± 12.7	40.3 ± 13.8	41.9 ± 14.7	50.6 ± 17.8	41.5 ± 5	51.1 ± 21.7
	Group II	37.3 ± 21.3	39.5 ± 23.1	42.3 ± 22.9	40.9 ± 21.4	35.4 ± 15.3	40.3 ± 14

Sampling Points (1) before surgery (2) before CPB (3) 60 min after Aortic occlusion (4) 60 min after reperfusion (5) 120 min after reperfusion (6) 180 min after reperfusion. Mean ± SD. *$P < 0.001$ vs (1), (3). †$P < 0.01$ vs Group I.

Group I at each time after reperfusion ($P < 0.01$, Table II). Serum concentrations of IL-6 increased at 60 min after reperfusion compared with the values before surgery and 60 min after aortic occlusion and remained increased until 180 min after reperfusion in both groups ($P < 0.001$, Table II). The IL-6 concentrations in Group II were less than those in Group I at each time after reperfusion ($P < 0.02$, Table II). The serum S-ICAM-1 concentration correlated with the IL-8 concentration ($r = 0.5$, $P < 0.01$, $n = 60$, Figure 2), but not with the IL-6 concentration in Group I. The cardiac index (CI) was higher in Group II (3.4 ± 0.36 $L \cdot min^{-1} \cdot m^{-2}$) than in Group I (2.9 ± 0.69 $L \cdot min^{-1} \cdot m^{-2}$) ($P < 0.05$) and pulmonary capillary wedge pressure (PCWP) was lower in Group II (10.4 ± 4.8 mmHg) than in Group I 13.7 ± 3.0 mmHg ($P < 0.05$) on the first day after cardiac surgery. Doses of catecholamine used after cardiac surgery were not different between groups.

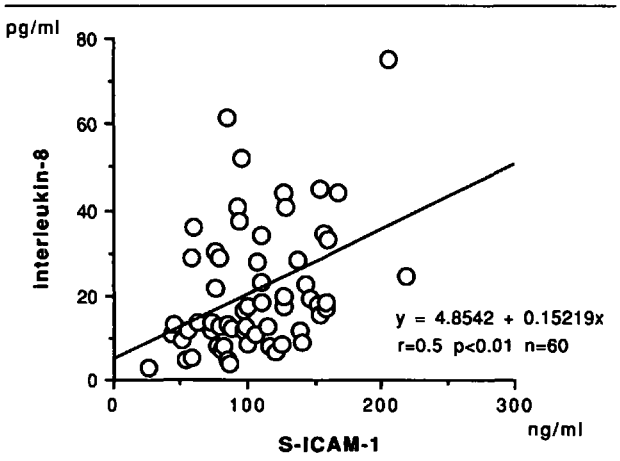

FIGURE 2 Correlation between serum IL-8 and soluble ICAM-1 concentration in Group I. $n = 60$ $r = 0.5$ $P < 0.001$.

Discussion

Neutrophil and endothelial cell adhesion is mainly mediated by two pathways[5]: one is the LECAM (leukocyte-endothelial cell adhesion molecules) family molecules and sugar residue, and the other is CD-18 and ICAM-1. Adhesion takes place in several steps: (1) tethering, (2) triggering, (3) adhesion, and (4) migration.[6] The LECAM family molecules, such as LECAM-1 and ELAM-1, play an important role in tethering, while integrin family molecules, such as LFA-1 and Mac-1 on leukocytes contribute to the adhesion of leukocytes and vascular endothelial cells.[7] The LECAM family molecules attract unstimulatd leukocytes in the circulation to the activated vascular endothelium. They induce rolling of leukocytes on the endothelium,[8] and then activate them with leukocyte chemotactic factors, such as IL-8 and platlet activating factor (PAF) which are produced and released from the endothelium. Activated leukocytes express Mac-1 on their surfaces, and then adhere dependently to LECAM-1 or CD-18/ICAM-1 which proceeds to migration. The expression and function of these adhesion molecules may be regulated by cytokines. Cytokines such as TNF-α, IL-1, IL-4, IL-6 and IL-8 have been reported to potentiate the expression of VCAM-1, ICAM-1 and ELAM-1 on endothelial cells.[9] The present study showed that IL-8, IL-6 and S-ICAM-1 increased after ischaemic reperfusion and that IL-8 correlated positively with S-ICAM-1. These results support the hypothesis that the production of IL-8 is increased after ischaemia and that S-ICAM-1 is then up-regulated. It has been reported that IL-8 is induced in the myocardium after ischaemia and reperfusion *in vivo*[10] and up-regulates adhesion molecules.[9] Smith *et al.*,[11] reported that the ELAM-1 mediated pathway was quickly suppressed after adhesion of neutrophils to endothelial cells, and disappeared within 30 sec, to be replaced by the CD-18 and ICAM-1 mediated pathway. In our study, S-ELAM-1 did not change. This may be explained as follows: S-ELAM-1 may have been elevated but disappeared quickly and, in addition, ELAM-1

was not detected directly on the endothelium, but soluble ELAM-1 was measured indirectly, and therefore its changes could not be followed. Ulinastatin, which is isolated from human urine, inhibits trypsin, and pancreatic elastase activity,[12] polymorphonuclear leukocyte elastase activity[13] and the endotoxin-stimulated production of tumour necrosis factor alpha and interleukin 1.[14] Moreover, it has an anti-shock effect similar to steroid hormones.[15] Endo *et al.*,[16] reported that ulinastatin inhibited production of polymorphonuclear leukocyte elastase and IL-8. In a previous study, we investigated the inhibitory effects of ulinastin on the increased production of IL-8 and IL-6 during cardiac surgery with cardiopulmonary bypass.[17] Also, IL-8 and IL-6 have been reported to potentiate the expression of adhesion molecules. In the present study, we observed the effects of ulinastatin on the soluble adhesion molecules following suppression of IL-8 and IL-6 production. As a result, in patients receiving pretreatment with ulinastatin, the increases in the production of IL-8 and IL-6 were suppressed, and S-ICAM-1 did not change after reperfusion. There was a positive correlation observed between IL-8 and S-ICAM-1. These results suggest that ulinastatin prevented up-regulation of S-ICAM-1 by inhibiting the increased production of IL-8.

Hennein *et al.*,[18] reported that inflammatory cytokines, such as IL-8 and IL-6, are elevated during coronary revascularization and may contribute to postoperative myocardial ischaemia and segmental wall motion abnormalities. We have reported a negative correlation between serum IL-8 and postoperative cardiac index and suggested that elevation of IL-8 during cardiac surgery caused postoperative cardiac dysfunction.[2] Also, the expression of ICAM-1 on cardiac myocytes was induced by IL-6 and adhesion of leukocytes to myocytes was potentiated by IL-6. In this respect, it is suggested that IL-6 is involved in reperfusion injury. The clinical importance of these findings is that suppression of increased production of inflammatory cytokines and adhesion molecules by pretreatment with ulinastatin may produce myocardial protection. We observed, in ulinastatin-treated patients, that postsurgical cardiac index was higher and PCWP was lower than in untreated patients. Nevertheless, the total doses of catecholamines used after cardiac surgery were not different between groups. As C.I. is negatively correlated with maximum concentration of IL-8 during surgery,[2] ulinastatin pretreatment may improve post-surgical cardiac function by suppressing increased the production of IL-8 and S-ICAM-1.

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