Reports of Investigation

Plasma lipid concentrations correlate inversely with CPB-induced interleukin-6 release

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Purpose: Cardiopulmonary bypass (CPB) is characterized by translocation of intestinal endotoxin and subsequent endogenous production of the pro-inflammatory cytokine interleukin-6 (IL-6). Plasma lipid fractions, especially high density lipoproteins, bind and neutralize endotoxin and, therefore, inhibit endotoxin-induced macrophage cytokine production, including IL-6. Increased IL-6 plasma levels have been implicated in adverse consequences associated with CPB. Previous studies demonstrated large interpatient variability in IL-6 plasma levels after CPB. The purpose of this study was to evaluate the relationship between plasma lipid concentrations and the concentrations of IL-6 following CPB in humans.

Methods: In a prospective study, a group of 15 patients selected to exclude variables known to influence post-CPB plasma levels of IL-6 (preoperative left ventricular ejection fraction >45%, similar durations of aortic cross clamping and total CPB time, similar temperature control during CPB, and avoidance of platelet transfusion and shed mediastinal blood re-infusion), IL-6 was measured at baseline, one and 24 hr post-CPB.

Results: Interleukin-6 plasma concentrations (mean ± SD) increased at one (142 ± 89 $pg \cdot ml^{-1}$, $P < 0.05$) and 24 (129 ± 82 $pg \cdot ml^{-1}$, $P < 0.05$) hr post-CPB compared with baseline (1.5 ± 1 $pg \cdot ml^{-1}$) concentrations. An inverse correlation was found between IL-6 plasma concentrations at one hour post-CPB and plasma cholesterol concentrations ($r = -0.592$, $P = 0.02$), high density lipoprotein ($r = -0.595$, $P = 0.02$), and low density lipoprotein ($r = -0.656$, $P = 0.01$).

Conclusions: These results suggest that plasma lipids attenuate the production of IL-6 during CPB and may partly explain the variability of interpatient levels of IL-6 reported post-CPB by others.

Objectif : La circulation extracorporelle (CEC) est caractérisée par la translocation de l'endotoxine intestinale et la production endogène subséquente de la cytokine interleukine-6 (IL-6) pro-inflammatoire. Des fractions de lipides plasmatiques, surtout les lipoprotéines de haute densité, se fixent à l'endotoxine et la neutralisent et, par conséquent, inhibent la production de cytokine macrophage induite par l'endotoxine, incluant IL-6. L'accroissement des niveaux plasmatiques de IL-6 a été présumé comme responsable des conséquences défavorables associées à la CEC. Des études antérieures ont démontré une grande variabilité dans les niveaux plasmatiques de IL-6 à la suite d'une CEC. Le but de cette étude était d'évaluer la relation entre les concentrations de lipides plasmatiques et les concentrations de IL-6 après une CEC chez les humains.

Méthodes : Dans une étude prospective, un groupe de 15 patients a été sélectionné en excluant les variables connues pour influencer les niveaux plasmatiques de IL-6 post CEC (fraction d'éjection ventriculaire gauche préopératoire > 45%, durées similaires de clampage aortique et de temps total de CEC, contrôle similaire de la température pendant la CEC et suppression de la transfusion de plaquettes ainsi que de la reperfusion du sang médiastinal dérivé), IL-6 a été mesurée au début, puis une heure et 24 heures post CEC.

Résultats : Les concentrations plasmatiques d'inlerleukine-6 (moyenne ± écart type) s'accroissent à une heure (142 ± 89 $pg \cdot ml^{-1}$, $P < 0,05$) et à 24 heures (129 ± 82 $pg \cdot ml^{-1}$, $P < 0,05$) post CEC par rapport aux concentrations de base (1,5 ± 1 $pg \cdot ml^{-1}$). On a constaté une corrélation inverse entre les concentrations plasmatiques de IL-6 à une heure post CEC et les concentrations de cholestérol plasmatique ($r = -0,592$, $P = 0,02$), de lipoprotéines de haute densité ($r = -0,595$, $P = 0,02$) et de lipoprotéines de basse densité ($r = -0,656$, $P = 0,01$).

Conclusion : Ces résultats suggèrent que les lipides réduisent la production de IL-6 pendant la CEC et peuvent expliquer partiellement la variabilité interindividuelle des niveaux de IL-6 post CEC rapportés par d'autres chercheurs.

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THE institution of cardiopulmonary bypass (CPB) generates several pro-inflammatory cytokines, including tumour necrosis factor- (TNF), and the interleukins 1 and 6 (IL-1, IL-6).[1] The importance of translocation of intestinal endotoxin in the initiation of pro-inflammatory cytokine generation is demonstrated by the reduction of plasma endotoxin and IL-6 concentrations following pre-CPB gut sterilization with antibiotics.[2] While the generation of these pro-inflammatory cytokines during CPB is well established, there is a large reported interpatient plasma concentration variability.[3] Several clinical variables have been demonstrated to have an impact on IL-6 plasma concentrations during and after CPB including preoperative left ventricular (LV) systolic function, determined by ejection fraction (EF),[4] duration of CPB and aortic cross-clamp times, temperature control during CPB, duration of mechanical ventilation post-CPB, the use of epinephrine or norepinephrine post CPB,[3-5] and possibly blood component (platelet) infusion.[6]

Another potential source of variability in the IL-6 response to CPB may be blood lipid concentration. Previous studies demonstrated that high density lipoproteins (HDL) neutralize endotoxin by surface binding, thereby reducing macrophage and/or monocyte TNF release,[7] which may decrease macrophage IL-6 production and release.[1] Low density lipoproteins (LDL) also reduce LPS-induced macrophage TNF and IL-1 expression,[8] while clinical studies demonstrated that, following surgery, humans who exhibit low plasma cholesterol and lipoprotein concentrations have an elevated infection rate.[9] These reports suggest endogenous lipids may have a role in sepsis prevention. This study was designed to evaluate if a correlation exists between preoperative lipid profiles and the endogenous production of IL-6 during CPB in a group of adults selected to exclude the variables previously described which are known to influence CPB-induced IL-6 production. We chose to measure the plasma levels of IL-6 because previous reports demonstrated that IL-6 is the single best predictor among the pro-inflammatory cytokines of post-CPB LV systolic dysfunction, myocardial ischaemic episodes,[5] and cardiovascular haemodynamic abnormalities,[3] while correlating best with mortality rates in other systemic inflammatory states, like sepsis.[1]

Methods

After obtaining Institutional Review Board approval and patient informed written consent, 15 adult male patients scheduled for first time myocardial revascularization surgery were included in a prospective study. In order to eliminate several variables previously reported to influence the CPB-induced release of IL-6, the following criteria were employed: males between 50-65 yr of age with no current history of anti-inflammatory (NSAIDs or glucocorticoids) or lipid lowering drug therapy. In addition, patients were eliminated from the study if: any intra- or postoperative platelet infusion was administered, the duration of aortic cross-clamp was < 35 or > 45 min, total CPB times were < 80 or > 100 min, left ventricular ejection fraction was < 45%, epinephrine or norepinephrine were used at any time, and mechanical ventilation or total intensive care stay exceeded 24 and 48 hr, respectively. No anti-inflammatory therapy (aprotinin or glucocorticoids) were used in conjunction with CPB, and no shed mediastinal blood was reinfused in any patient.

On the morning of surgery, each patient was given 0.1 $mg \cdot kg^{-1}$ morphine sulphate and 0.2-0.4 mg scopolamine *im* prior to admission to the operating room. On arrival, a radial artery catheter, a right internal jugular vein pulmonary artery catheter, and large-bore intravenous lines were placed. Standard anaesthesia consisting of 50-100 $\mu g \cdot kg^{-1}$ fentanyl as a short *iv* infusion, 0.1-0.3 $mg \cdot kg^{-1}$ midazolam, a volatile anaesthetic (usually isoflurane) as needed, and 0.1-0.15 $mg \cdot kg^{-1}$ pancuronium were used. Cardiopulmonary bypass was completed with a centrifugal pump (Biomedicus, Inc., Eden Prairie, MN), and a hollow-fibre membrane oxygenator (Baxter Health Care, Irvine, CA) with arterial line filtration and mild hypothermia (32 C core temperature) in each study patient. Perfusion, flow rate and mean arterial pressure during CPB were maintained between 2.2 and 2.4 $L \cdot min^{-1} \cdot m^{-2}$, and 60 to 80 mmHg respectively. Myocardial protection was achieved through both antegrade and retrograde administration of cold hyperkalaemic blood (8:1 blood to crystalloid mixture) cardioplegia. A terminal dose of normothermic continuous cardioplegia was administered approximately 15 min before reperfusion. Anticoagulation was obtained by the administration of 300 $IU \cdot kg^{-1}$ bovine lung heparin, and kaolin-based activated clotting times (ACT) were maintained at 480 sec or greater by the addition of heparin when necessary. At the termination of CPB, protamine was administered in a ratio of 1.3 mg for every 100 U total heparin administered, and confirmed by the return of the ACT to baseline values. Ten millilitres heparinized whole blood were drawn at three time periods: 1) baseline (after placement of the arterial and intravenous catheters but before anaesthetic drug administration); 2) one hour after termination of CPB; 3) 24 hr after termination of CPB.

Arterial blood for IL-6 levels was collected in sterile 10 ml syringes, spun at 2000 × g for 15 min, the

plasma separated, frozen at -80C, and batched. Interleukin-6 was quantified by using a "sandwich" enzyme-linked immunosorbent assay (ELISA) using specific monoclonal antibodies (Quantikine HS, R&D Systems, Minneapolis, MN) as described previously [3-5] and according to the manufacturer's protocol. Sensitivity (minimal detectable dose) for IL-6 is < 0.70 pg·ml^{-1}, with a normal human plasma concentration of < 3.13 pg·ml^{-1}. All samples were assayed in duplicate, and concentrations were calculated from a standard curve prepared concurrently with each assay. The intra-assay coefficient of variation was 2.1% at 186 pg·ml^{-1} and the inter-assay coefficient of variation was 3.8% at 191 pg·ml^{-1}.

Baseline serum lipids were measured colorimetrically the day before cardiac surgery by a Kodak Ektachem 700 analyser (Rochester, NY). Ranges considered normal are low density lipoproteins < 130 mg·dL^{-1}, high density lipoproteins > 35 mg·dL^{-1} and cholesterol < 200 mg·dL^{-1}.

Statistical analysis was performed by determining the Spearman correlation coefficients between each lipid component and IL-6 at each time interval. ANOVA was used to determine changes in plasma IL-6 concentrations over time. Results were expressed as the mean ± SD. A P 0.05 was considered significant.

Results

Interleukin-6 plasma concentrations (mean ± SD) increased at one and 24 hr post-CPB compared with baseline concentrations (*P < 0.05) (Figure 1).

When serum cholesterol concentrations (mg·dL^{-1}) were compared with IL-6 plasma concentrations by regression analysis, an inverse relationship (P = 0.02, r = -0.592) was found one hour post-CPB (Figure 2, Table).

When serum high density lipoprotein concentrations (mg·dL^{-1}) were compared with IL-6 plasma concentrations by regression analysis, an inverse relationship (P = 0.02, r = -0.595) was found at one hour post- CPB (Figure 3, Table).

When serum low density lipoprotein concentrations (mg·dL^{-1}) were compared with IL-6 plasma concentrations by regression analysis, an inverse relationship (P = 0.01, r = -0.656) was found at one hour post-CPB (Figure 4, Table).

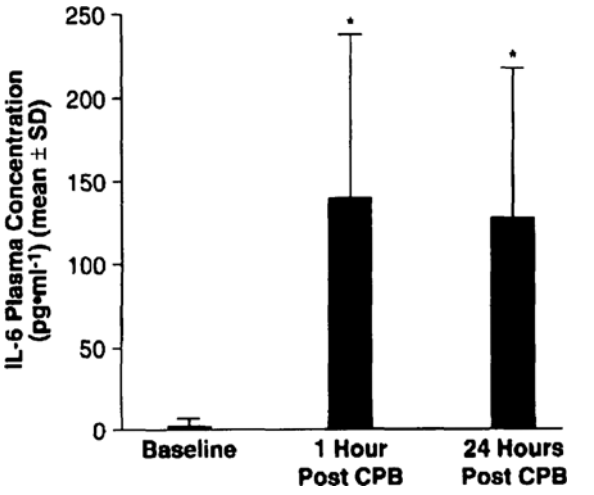

FIGURE 1 Plasma interleukin-6 (IL-6) concentrations in pg·ml^{-1} (mean ± SD) at baseline, one and twenty-four hours post cardiopulmonary bypass. *P < 0.05, compared with baseline.

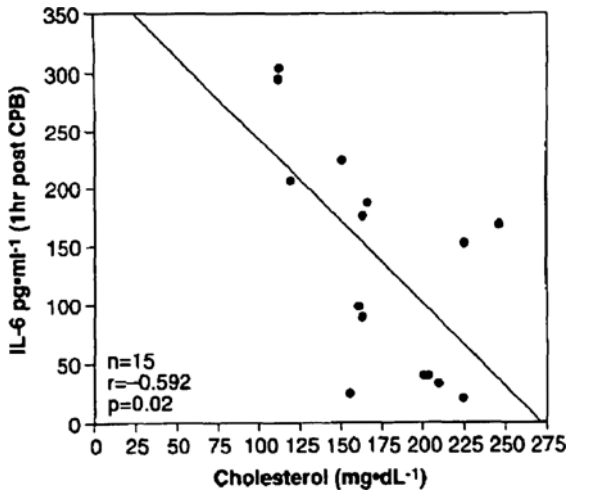

FIGURE 2 Regression analysis of interleukin-6 (IL-6) plasma levels (pg·ml^{-1}) and serum cholesterol (mg·dL^{-1}) concentration (r = -0.592, P = 0.02).

Discussion

The data reported in this study are consistent with the concept that plasma lipids bind and neutralize absorbed endotoxin during CPB, thus reducing CPB-induced pro-inflammatory cytokine production and release, as determined by plasma levels of IL-6. Wurfel *et al.*[11] demonstrated that endotoxin (or lipopolysaccharide, LPS) absorbed systemically adheres to LPS-binding protein (LBP), which, in turn, may transfer endotoxin or LPS to either inflammatory cells (neutrophils, macrophages, monocytes, etc) or to lipoproteins. The lipoprotein fraction primarily demonstrated to bind the transferred endotoxin is HDL,[11] thus HDLs reduce endotoxin-induced release of TNF and IL-6.[7,8,12] A similar mechanism of action has been described for LDL.[7,12,13] Low serum cholesterol, HDL, and LDL concentrations correlate with post-operative infection and sepsis rates, while the addition

of high density lipoprotein to patient blood *ex vivo* suppresses endotoxin-induced TNF production.[9]

The systemic endotoxaemia resulting from increased bowel mucosal permeability during CPB initiates a cascade of events resulting in the generation and systemic release of pro-inflammatory cytokines, including TNF, IL-1 and IL-6.[1] The importance of the systemic endotoxaemia is emphasized by demonstration that low plasma antiendotoxin core antibody level is an independent predictor of poor outcome following CPB (defined as postoperative length of stay over 10 days or in-hospital death).[14] Similarly, removal of the pro-inflammatory cytokines, including IL-6, by haemofiltration during CPB results in reduced time to extubation, improved lung function, reduced blood loss, reduced febrile response,[15] and increased mean arterial blood pressure[16]

TABLE Plasma concentration of cholesterol, high density lipoproteins, and low density lipoproteins compared to plasma IL-6 concentrations at baseline, one and 24 hr post-CPB.

Plasma lipid	*Baseline*	*1 Hr Post-CPB*	*24 Hr Post-CPB*
	IL-6	*IL-6*	*IL-6*
Cholesterol	r=-0.128	r =-0.592	r = 0.032
	P = 0.65	*P* = .02*	*P* = 0.91
High Density Lipoproteins	r = 0.293	r = -0.595	r = -0.226
	P = 0.29	*P* = 0.02*	*P* = 0.42
Low Density Lipoproteins	r = -0.139	r = -0.656	r = 0.002
	P = 0.62	*P* = 0.01*	*P* = 0.99

*$P < 0.05$

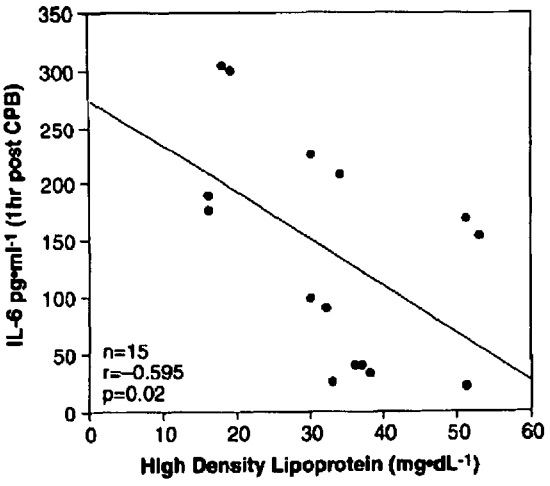

FIGURE 3 Regression analysis of interleukin-6 (IL-6) plasma levels (pg·ml^{-1}) and serum high density lipoprotein (mg·dL^{-1}) concentration (r = -0.595, P = 0.02).

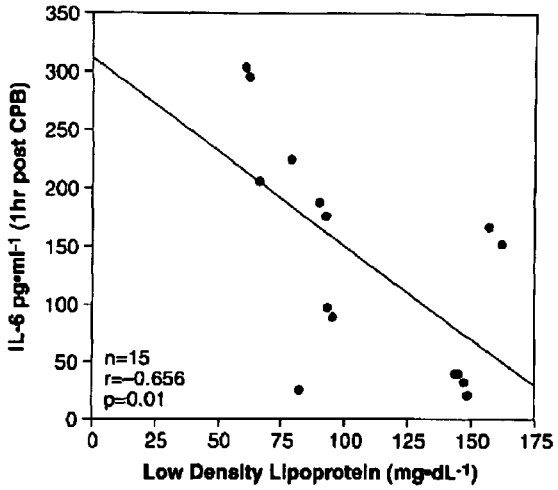

FIGURE 4 Regression analysis of interleukin-6 (IL-6) plasma levels (pg·ml^{-1}) and serum low density lipoprotein (mg·dL^{-1}) concentration (r = -0.656, P = 0.01).

post-CPB. Interleukin-6 plasma levels have been positively correlated with reduction in systemic vascular resistance,[3] need for vasopressor support,[17] worsening post-CPB left ventricular wall motion abnormality scores and frequency of myocardial ischaemic episodes[5] post-CPB. Reduction of IL-6 plasma concentrations by nafamostat (a serine protease inhibitor) reduces the incidence of post-CPB myocardial ischaemic episodes and infarction.[18] In addition, IL-6 plasma levels correlate with mortality rates in other systemic inflammatory states, like sepsis.[10] Thus, the generation of pro-inflammatory cytokines, in particular IL-6, during CPB correlates with adverse outcomes while reduction of these cytokines will improve outcomes following CPB.[1,12,18]

Large variations in the plasma levels of IL-6 during and following CPB have been reported. For this study, patients were selected to exclude variables known to influence post-CPB IL-6 levels in order to evaluate the effect of plasma lipid concentrations as a predictor of post-CPB IL-6 production and systemic release. Since red blood cell concentrates do not contain IL-6,[20] this variable was not used as an elimination criteria. Peak IL-6 plasma levels are reported to occur between one to four hours following CPB termination[3–5] and remain elevated for at least 24 hr post-CPB.[3–5] Therefore, the time intervals chosen for this study were 1 and 24 hr post-CPB.

In summary, this study demonstrated that the plasma levels of cholesterol, LDL, and HDL lipoproteins are inversely correlated with the levels of IL-6 produced and released during CPB. Since all study patients were carefully matched in variables previously demonstrated to

effect IL-6 plasma levels, these data demonstrate that plasma concentrations of these lipids may be a predictor of IL-6 release during CPB. Variations in plasma concentrations of these lipids may explain some of the inter-patient variations in plasma IL-6 concentrations post-CPB reported by different investigators. Since IL-6 correlates with adverse outcomes post-CPB, higher plasma concentrations of these lipids may be advantageous in patients undergoing cardiac surgery requiring CPB. Since lower IL-6 plasma levels post-CPB result in reduced myocardial ischaemic and infarction rates,[18] patients with low preoperative plasma lipid concentrations may be at increased risk for adverse events post-CPB. Therefore, low preoperative plasma lipid concentrations may be a clinical marker for a group of patients that antiinflammatory therapy in conjunction with CPB is indicated.[18,21]

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