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Cytokine Responses and Anti-inflammatory Strategies in Coronary Artery Bypass Grafting

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ACADEMIC DISSERTATION

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ABBREVIATIONS

ADO	Adenosine
CABG	Coronary artery bypass grafting
CI	Cardiac index
CK-MB	Creatine kinase cardiac isoenzyme
cNOS	Constitutive nitric oxide synthase
CPB	Cardiopulmonary bypass
CRP	C-reactive protein
CS	Coronary sinus
CTX	Cardiac transplantation
E_2	17β-estradiol
HR	Heart rate
HRT	Hormone replacement therapy
ICAM-1	Intracellular adhesion molecule-1
IL	Interleukins
IL-1RA	IL-1 receptor antagonist
iNOS	Inducible nitric oxide synthase
LA	Left atrial
LPS	Lipopolysaccharide
MAC	Membrane attacking complex
MAP	Mean arterial pressure
MIDCAB	Minimally invasive direct CABG
MODS	Multiple organ dysfunction syndrome
MPAP	Mean pulmonary artery pressure
MPO	Myeloperoxidase
MUF	Modified ultrafiltration
NO	Nitric oxide
NYHA	New York Heart Association class
PAF	Platelet-activating factor
PCWP	Pulmonary capillary wedge pressure
PDEI	Phosphodiesterase inhibitor
PGE_2	Prostaglandin E_2
PVRI	Pulmonary vascular resistance index
sTNFR	Soluble TNF receptor
SIRS	Systemic inflammatory response syndrome
SVRI	Systemic vascular resistance index
TNF	Tumor necrosis factor

INTRODUCTION

Sirs, I have found you an argument, but I am not obliged to find you an understanding.

Dr Samuel Johnson (1709-1784)

Doctor Johnson addressed his observation to his colleagues, not to the “systemic inflammatory response syndrome” (SIRS), but the point he made is nonetheless relevant to in the present context. Although it has been clearly shown that cardiopulmonary bypass (CPB) is indispensable for most heart surgery, it is well established that conventional coronary artery bypass grafting (CABG) with CPB induces a systemic inflammatory response. Inflammation becomes injurious to multiple organ systems, and lead to pulmonary and renal dysfunction, neurological changes and fever of noninfective origin. This “systemic inflammatory response syndrome” following upon cardiac surgery has also been called the "post-perfusion syndrome" (Kirklin JK et al. 1983). Recent studies have revealed that severe generalized autodestructive inflammation may result in multiorgan failure after cardiac surgery. Awareness of this aspect of cardiac-surgical pathophysiology is increasing, but the mechanism involved is still less understood.

The inflammatory response of the body is designed as a protective mechanism to quarantine and destroy what the body recognizes as foreign. Nonimmunologic activation of this response is also induced by many factors in cardiac surgery. It is accomplished through a cascade of events involving many inflammatory mediators and the production of many deleterious substances. A thorough understanding of the pathogenesis of bypass-induced organ dysfunction is clearly a prerequisite in developing effective therapeutic interventions to attenuate this problem.

Humoral mediators such as cytokines are working in a complex network. A role for cytokines in both normal and abnormal physiologic responses has been clearly documented. Search of the scientific literature found that between 1991 and 2000, nearly 300,000 documents appeared in which the word "cytokine" was used. Cytokines have been implicated in such diverse and conflicting functions as precursor cell development and inhibition, clonal activation and tolerance induction, cellular apoptosis and enhanced survival, cellular differentiation, tumor rejection and metastasis, and proinflammatory

and anti-inflammatory states. They are also among the mediators which participate in the mediation of immuno-endocrine interactions. Recently, attention has focused on the role of cytokines as mediators of metabolic, immunological and endocrine responses to cardiac surgery.

Recent researches have evaluated different strategies affecting different stages of the inflammatory mediator network with various degrees of success. Aprotinin has long been used in cardiac surgery, and is found to have an anti-inflammatory effect, but the dosage used varies in different institutions. It has been shown that estrogen inhibits TNF production (Selzman CH et al. 1998) and reduces cardiac leukocyte accumulation in the myocardium (Squadrito F et al. 1997). Adenosine, a cardioprotective drug, was recently found to regulate cytokine release and neutrophil activation (Meldrum DR et al. 1997, Mathew JP et al. 1995). These may represent a novel anti-inflammatory property whereby inflammation could be modulated and ischemia-reperfusion injury limited. The present series of studies was designed to evaluate the cytokine responses in CABG, and to investigate the anti-inflammatory effects of aprotinin, estradiol and adenosine in patients undergoing CABG.

REVIEW OF THE LITERATURE

1. Mediators of inflammatory responses after CPB

It has been well documented that many factors present during CPB, either material-dependent (exposure of blood to nonphysiologic surfaces and conditions) or material-independent (surgical trauma, ischemia-reperfusion to the organs, changes in body temperature, and release of endotoxin), induce a complex inflammatory response. This response is designed as a protective mechanism to quarantine and destroy what the body recognizes as foreign. Elimination of such material is accomplished through a cascade of events involving both humoral and cellular inflammatory mediators (including complement activation, release of cytokines, leukocyte and endothelial cell activation, Table 1), and the production of many substances such as oxygen-free radicals, arachidonic acid metabolites, platelet-activating factor (PAF), nitric oxide (NO), and endothelins (Figure 1).

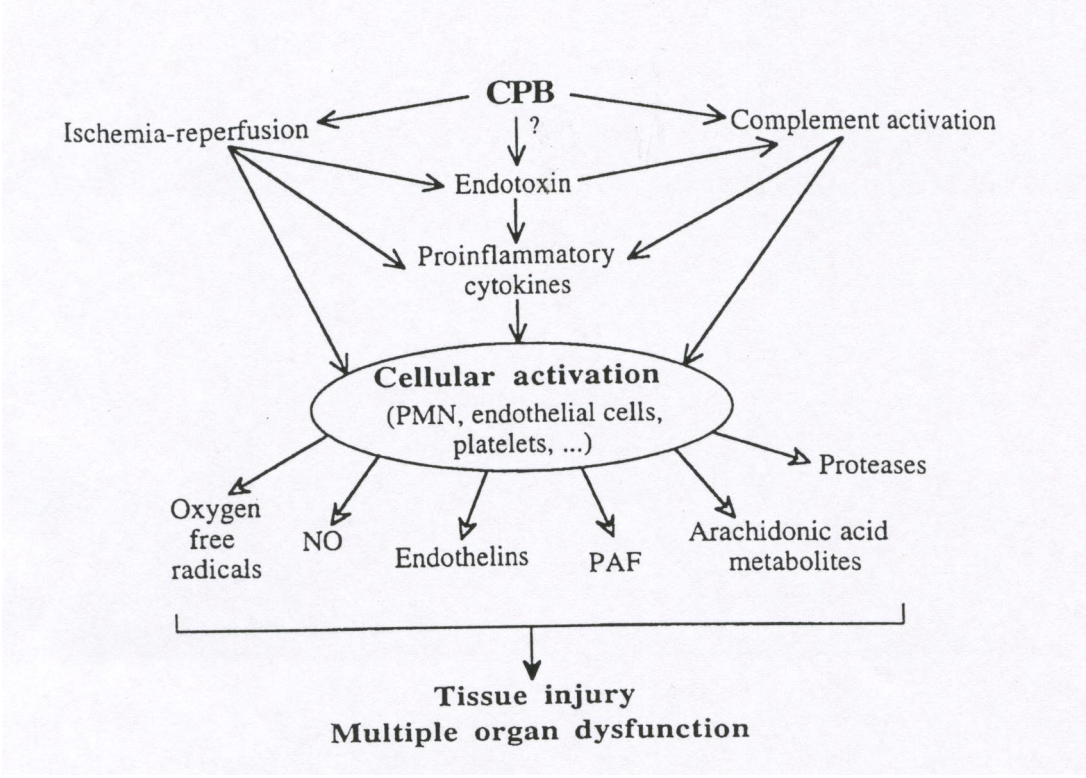

Figure 1 Inflammatory response to CPB (Wan S, 1997C)

Table 1 Inflammatory mediators (Miller BE, 1997)

Humoral
Contact activation products
Factor XIIa
Thrombin
Kallikrein
Fibrinogen degradation products
Complement
Cytokines
Tumor necrosis factor
Interleukins
Leukotrienes
Cellular
Neutrophils
Endothelial cells

1.1 Complement

The CPB circuit has a surface interface with the blood stream which is far from similar to normal endothelium, and this circumstance leads to adverse reactions. Activation of the complement system is an early-acting mechanism which triggers and amplifies the acute inflammatory response. In inactive form, the complement system consists of 9 components numbered C1-C9, while in its activated state it presents over 20 cleavage products. There are three recognized pathways of complement activation, termed the classical, alternative and common (or membrane attack), which represent a true cascade in that continuous low levels of turn-over are accelerated by activation. The 'classic pathway' of complement activation is triggered by binding of antibody-antigen complexes to C1, eventually resulting in cleavage of C3 and C5 with production of C3b and C5b and of the anaphylatoxins C3a and C5a. Anaphylatoxins may promote phenomena characteristic of acute inflammatory injury such as vasodilatation with increased vascular permeability, leukocyte activation, chemotaxis and adhesion, and phagocytosis of microorganisms by neutrophils and monocytes (Asimakopoulos G, 1999). A wide variety of stimuli (foreign surfaces, endotoxin etc.) induce alternative pathway activation, which is dependent upon a change in the molecular configuration of C3 (Frank MM, 1987). This results in the formation of the C3 convertases, which cleave C3 into C3a and C3b. After the

cleavage of C3, the two pathways join, proceeding to cleavage of C5 into C5a and C5b. Once C5b has been produced, it interacts with further complement components producing the complex C5b-9, also called the "membrane attacking complex" (MAC) (Muller-Eberhard HJ, 1984). This final common complement pathway is non-enzymatic. MAC causes lysis of cells and activates the endothelium and leukocytes (Moore FD Jr, 1994).

Complement activation during CPB, as reflected in elevated levels of plasma C3 activation products, has been repeatedly demonstrated. C3a levels are significantly increased within 10 minutes of the initiation of bypass and that levels continue to rise steadily thereafter until bypass was terminated (Chenoweth DE et al. 1981). Early studies showed that the incidence and degree of deranged function of the heart, lung and kidney could be related to the raised plasma C3a concentration. It has been suggested that complement activation was the pivotal key in the genesis of the damaging effects of cardiac surgery (Chenoweth DE et al. 1981, Kirklin JK et al. 1983). Soluble complement receptor type 1 suppressed post-ischemic myocardial inflammation and necrosis in a rat ischemia-reperfusion model (Weisman HF et al. 1990). It is quite conceivable that complement activation may have an important role in pathogenesis of CPB-induced organ dysfunction.

1.2 Cytokines

Cytokines are a group of hormone-like polypeptide or glycopeptide mediators which play a variety of regulatory roles both in host defense and normal and abnormal homeostatic mechanisms. They are small molecular proteins or glycoproteins with weights from 8 to 30,000 d. The major site of synthesis appears to be cells of the macrophage and monocyte series (Bendtzen K 1989), but virtually every nucleated cell can produce them in response to tissue injury. They are of importance in the metabolic response to injury or infection, potentiating the release of other cytokines and amplifying the injury cascade in relatively low endogenous concentrations (Billiau A and Vandekerckhove F, 1991). Cytokines control the intensity and length of the immune response by affecting the activation, proliferation and differentiation of various cells or by regulating antibody production or the secretion of other cytokines. TNF and several interleukins play significant roles in the regulation of inflammatory response. Their main effects are presented in Table 2.

Most cytokines act locally at very low concentrations in the vicinity of the production site. They are undetectable or only found at low concentrations in peripheral blood under

Table 2 Effects of pro- and anti-inflammatory cytokines

Cytokine	Effects
TNF-α	induction of fever, hypotension and leukopenia induces production of IL-1, IL-6, leukotrienes and PAF induction of hepatic acute-phase protein synthesis stimulates neutrophil degranulation and adherence to endothelialium induces endothelial dysfunction
IL-1	production of PGE_2 in the hypothalamus with resultant fever stimulation of IL-6 and IL-8 production activation of neutrophils and endothelial cells stimulation of antibody production from lymphocytes inhibition of the myocardial contractility
IL-6	induction of acute-phase proteins
IL-8	upregulation of neutrophil adhesion molecules stimulates neutrophil degranulation
IL-10	triggers the release of IL-1RA and sTNFR inhibits TNF, IL-1 and IL-6 release

physiological conditions. Some of the cytokines also act systemically as pleiotropic hormones that modulate functions of cells at distant locations. They may be potentially harmful mediators under pathological conditions such as major trauma, sepsis and shock, where significant plasma levels may be reached.

Functionally these cytokines can be divided into proinflammatory (TNF, IL-1, IL-8), and anti-inflammatory (IL-1RA, IL-10) molecules, while IL-6 has both of the properties. IL-8 is also considered as a chemokine as it evinces a potent chemoattractant activity for neutrophils. Positive correlations have been obtained between IL-10 and IL-8, and between IL-10 and IL-6, findings which demonstrate that pro- and anti-inflammatory cytokines increase to maintain their balance during cardiac surgery (Kawamura T et al. 1997). It is noteworthy that cytokines are likely to act both individually and within a complex network of interrelated and interacting signals (Leeuwenberg JF et al. 1994). Inflammatory reaction is an essential component of the body defense mechanisms, however, the network of the cytokines regulating inflammation must work at optimal

levels to quarantine and destroy what the body recognizes as foreign and to avoid excessive damage to the host. A prolonged or too strong an inflammation is deleterious.

1.3 Leukocyte activation

A critical step in the inflammation process is the activation of leukocytes and their mobilization toward the injured area. Leukocyte activation during CPB can be mediated by a number of factors, including C3a, C5a, and PAF. It has been shown that myeloperoxidase (MPO), lactoferrin and elastase are significantly elevated in patients undergoing CPB (Wachtfogel YT et al. 1987, Riegel W et al. 1998). In systemic inflammation, the generalized activation of neutrophils may often be one of the main causative factors leading to SIRS and MODS; activated neutrophils may damage endothelial cells with several toxic substances from intracellular granules of the neutrophils. Neutrophils may facilitate their migration into tissues by the extracellular damaging effect of oxygen-derived free radicals. Proteolytic enzymes in neutrophil granules may also act as mediators of endothelial and subendothelial damage (Weiss SJ, 1989).

Neutrophil adherence to endothelial cells is an important early step in tissue injury. Neutrophils interact with these cells via the expression of specific adhesion molecules on their surface (the selectin and intergrin families and the immunoglobulin superfamily) (Springer TA, 1990). Increased levels of CD11b/CD18 (also known as Mac-1, CR3, or Mo-1) after CPB have been demonstrated by experimental (Dreyer WJ et al. 1995) and clinical studies (Gu YJ, 1992). Other adhesion molecules such as E-selectin, P-selectin and intracellular adhesion molecule-1 (ICAM-1) may also be involved in these complex reactions during CPB. By their coordinate action, these molecules orchestrate the three-step sequence of events which comprise the interactions between neutrophils and the vascular wall. These three steps are known as rolling, adhesion and emigration. The damaging potential of the activated neutrophil depends on its ability to adhere to the endothelium.

Following the expression of adhesion molecules, activated neutrophils may be largely responsible for pulmonary damage (Gillinov AM et al. 1994) and myocardial ischemia-reperfusion injury (Youker KA et al. 1994). Activated neutrophils may depress myocardial function and contribute to impaired functional recovery after

global hypothermic ischemia (Myers ML et al. 1992). It has been shown that enhanced polymorphonuclear leukocyte adhesion in reperfusion leads to an increase in coronary permeability (Kupatt C et al.1996).

Though circulating levels of adhesion molecules may not be markedly increased during CPB (Boldt J et al. 1995), CD11b/CD18 expression on leukocytes increases immediately after the onset of CPB and has a second peak of expression after reperfusion to the myocardium (Gu YJ et al. 1992). Prevention of neutrophil adhesion may provide practical benefits. Leukocyte depletion filters significantly reduce ischemic damage during acute surgical revascularization in pigs (Lazar HL et al. 1995). Inhibition of neutrophil adhesion has been observed to reduce myocardial infarct size by 51% after transient left anterior descending artery occlusion in a porcine model (Curtis WE et al. 1993). Blocking neutrophil adhesion molecules during reperfusion reduces myocardial inflammation and edema, and improves ventricular function after heart preservation and transplantation (Byrne JG et al. 1992). Leukocyte depletion by means of a blood cell separator during CPB results in lower neutrophil elastase and thromboxane B_2 plasma levels and improved lung function after CPB in humans (Morioka K et al. 1996).

1.4 Endothelial cell activation

During CPB, the endothelium is potentially activated by endotoxin, humoral inflammatory mediators and ischemia-reperfusion. Plasma molecules derived from the activated endothelium, for examples von Willebrand factor and thromboxane, have been demonstrated. In one study using isolated cat hearts, the Langendorff model showed that endothelial dysfunction occurs initially upon reperfusion of the previously ischemic heart and that this condition is aggravated by superoxide radicals produced by activated neutrophils (Tsao PS et al. 1992).

Under resting conditions, the endothelial cell lining of blood vessels is a relatively inert surface which regulates the passage of intravascular substrates to the extravascular space and assures the unhindered flow of cellular and serum elements through capillary beds. In response to inflammatory signals such as cytokines, LPS, complement activation products (C5a), hypoxia or oxygen-derived free radicals, endothelial cells are converted to an activated state, resulting in profound changes in gene expression and cellular function. Activated endothelial cells release cytokines

and express proteins on their surface, these promoting inflammatory reactions and thrombosis (Virkhaus R et al. 1995).

Endothelial activation can be classified into two types. In one, in response to the abrupt restoration of blood flow to ischemic tissues, stimuli such as reactive oxygen species and activated complement fragments induce the transient expression of preformed proteins stored within the endothelium within seconds to minutes. This then promotes leukocyte-endothelial cell interactions and coagulation. Alternatively, in response to TNF, IL-1 and IL-6, transcriptional activation of several genes is initiated in endothelial cells and translation of specific transcripts into protein products on the endothelial surface is completed over the course of several hours. These proteins include leukocyte adhesion molecules which mediate the recruitment of neutrophils to sites of inflammation early in the course of an activation reaction, and tissue factor, which initiates the intravascular formation of thrombin (Pober JS and Cotran RS, 1990).

The endothelial cell alterations produced as a result of the inflammatory response lead to increased vascular permeability. A reversible and short-lived contraction of endothelial cells occurs rapidly after the inflammatory process is initiated as a result of the actions of thrombin, histamine and the leukotrienes. Subsequently, the actual cytoskeleton of endothelial cells is altered by the actions of TNF and IL-1. This produces an increased gap at the junction of endothelial cells and leads to a more prolonged increase in vascular permeability. Finally, proteolytic enzymes injure endothelial cells and toxic oxygen radicals liberated by activated neutrophils (Miller BE et al. 1997).

2 Cytokine release in coronary artery disease and coronary surgery

2.1 Cytokines and coronary artery disease

It has been suggested that chronic subclinical infection induces a low-grade systemic inflammation, with cytokines being the mediators. Such infections with Chlamydia pneumoniae, Helicobacter pylori, chronic bronchitis and chronic dental infection have been associated with raised values of the acute-phase reactant, C reactive protein (CRP), albeit within the normal range, and have been implicated as risk factors in coronary artery disease. There is indeed a relationship between chronic low-grade systemic inflammation, as indicated by serum levels of CRP, and mortality from coronary artery disease (Ridker P et al.1997, Mendall M et al. 1996). Non-infective conventional environmental risk factors, include age, low adult social class, smoking and obesity are also associated with low-grade acute phase responses (Mendall M et al. 1996).

The hepatic synthesis of CRP is largely under the regulation of the pro-inflammatory cytokines TNF-α and IL-6, and these also affect lipid metabolism in vivo. Mendall and colleagues suggest that serum concentrations of TNF-α and IL-6 are associated with cardiovascular risk factors and prevalent coronary artery disease. TNF-α concentrations have been positively related to Helicobacter pylori infection, and IL-6 concentrations with age, smoking and symptoms of chronic bronchitis. TNF-α is associated with increased triglycerides and reduced high-density lipoprotein cholesterol, while IL-6 is associated with raised fibrinogen, sialic acid and triglycerides. ECG abnormalities are independently associated with increases in IL-6 and TNF-α. These findings confirm the conception that many of the phenomena with which CRP is associated are also associated with serum levels of cytokines (Mendall M et al. 1997).

Cytokines may, however, also correlate with other factors. Levels of inflammation may respond to metabolic change and be influenced by various dietary factors. The body mass index is correlated with serum concentrations of TNF-α (Mendall M et al. 1997). Synthesis of TNF mRNA by adipocytes is higher in obese individuals. Body weight reduction in obese subjects, resulting in improved insulin sensitivity, has been found to be also associated with a decrease in TNF-α mRNA expression in fat tissue (Hotamisligil GS et al. 1995).

TNF-α and IL-6 generated in the lungs or gut in response to environmental stress could have direct effects which promote atherosclerosis and thrombosis at distant sites (Vallance P et al. 1997). Alternatively, inflammation may be located principally at the sites of the atherosclerotic lesion being directly influenced by environmental factors which can reach that location, for example smoking, alcohol, diet and Chlamydia pneumoniae, with the systemic inflammatory response being an epiphenomenon of this process. The data discussed above would suggest new markers of, or mechanisms for, the pathogenesis of atherosclerosis. A prolonged inflammation is deleterious. Acetylsalicylic acid, an agent which can influence inflammatory processes, has recently been suggested to have important therapeutic effects in atherosclerosis (Ridker PM et al.1997).

Inflammatory reaction may be also involved in the mechanisms for acute coronary syndrome or chronic heart failure. In patients with heart failure, elevated levels of TNF-α and IL-6 may be presented even in the absence of cachexia. A resent study demonstrates that in NYHA class IV heart failure there is a significant relation between IL-6 and TNF-α, and between levels of both IL-6 and TNF-α and plasma levels of norepinephrine, supporting the concept of a cytokine cascade, which in turn may be related to neurohumoral activation (MacGowan GA et al. 1997). The presence of inflammatory infiltrates in unstable coronary plaques suggests that inflammatory processes may contribute to the pathogenesis of these syndromes. Plasma IL-6 concentrations are higher in patients with unstable angina and acute myocardial infarction than in patients with stable angina, and are higher in acute myocardial infarction than in unstable angina (Manten A et al. 1998). Peripheral and coronary circulating IL-6 levels are higher in patients with acute coronary syndrome or chronic heart failure relative to control patients. The transcardiac IL-6 gradient is larger in patients with acute coronary syndrome compared with the controls (Deliargyris EN et al. 2000).

2.2 Cytokines and cardiac surgery

Considerable interest has recently been focused on the involvement of the cytokine network during and after CPB. Numerous reports document an increase in plasma levels of TNF (Journois D et al. 1994, Menasche P et al. 1994, Teoh KH et al. 1995), IL-1 (Haeffner-Cavaillon N et al. 1989), IL-6 (Kawamura T et al. 1993, Teoh KH et al.

1995), IL-8 (Teoh KH et al. 1995, Finn A et al. 1993) and IL-10 (Sablotzki A et al. 1997, Seghaye M et al. 1996) during CPB. The hypermetabolic state after CPB may be attributable to the release of these cytokines (Menasche P et al. 1994, Teoh KH et al. 1995). The time course of cytokine release after CPB is presented in table 3.

Table 3 Time course of cytokine release after cardiac surgery.

Cytokine	Initiation time	Peaking time	Duration
TNF	after initiation of CPB	2 and 18 hours after	24 hours
IL-1	after weaning off CPB	24 hours	-
IL-6	2 hours after initiation of CPB	4 hours	3 to 5 days
IL-8	during rewarming	1 to 3 hours	24 hours
IL-10	after weaning off CPB	1 hours after CPB	several hours

It has been reported that levels of cytokine release are associated with the duration of CBP time. TNF-α levels rise faster than those of any other cytokines, indicating its role as an initiator in this branch of the inflammatory response. Though this cytokine release may be a natural protector against bacterial or other pathogen exposure, a "double-edged sword" effect has been suggested by numerous studies showing a relationship between cytokine release and untoward post-CPB events (Table 4). An extensive cytokine release may thus be detrimental.

2.3 Triggers and sources of cytokines during cardiac surgery

The release of cytokine can be stimulated by a number of factors including endotoxin release, ischemia-reperfusion, complement activation and the effect of other cytokines (Wan S et al. 1997B). Endotoxin appears in the circulation during CPB as a result of contamination by extracorporeal circuits, pulmonary arterial catheters, intravenous fluids or banked blood products or by adsorption from the intestinal tract after periods of splanchnic hypoperfusion during the course of the observation (Jansen NJ et al. 1992). A major stimulus to TNF-α production seems to be the presence of endotoxin in the circulation. Endotoxin release in the circulation may be able to active both the classical and alternative pathway (Jansen NJ et al. 1992). This

Table 4 Some studies on cytokine release after cardiac surgery

References	Patients	Results and associations
Jansen NJ et al. 1991	Adults routine op	TNF associated with hemodynamic instability
Kawamura T et al. 1993	Adults routine op	IL-6/IL-8 correlated with CK-MB
Hennein HA et al. 1994	Adults routine op	IL-6 and IL-8 correlated with AC time and cardiac function
Menasche P et al. 1994 *	Adults routine op	Higher TNF, IL-6 and IL-1β associated with vasodilation and the use of pressure agent
te Velthuis H et al. 1995	Adults routine op	Higher TNF associated with suppressed myocardial performance in elderly patients
Deng MC et al. 1996	Adults routine op	IL-6 correlated with dose of norepinephrine and epinephrine support and complications
Wan S et al. 1999	Adults routine op	IL-8 correlated with troponin-I levels
Ashraf S et al. 1999	Pediatric	IL-6 and IL-8 correlated with S100B levels. Astroglial injury may be cytokine-mediated

* Normothermic CPB, op = operation, AC time = Aortic cross-clamping time, CPB = cardiopulmoanry bypass

endotoxin causes massive production of TNF-α from monocytes and macrophages which have already been primed by exposure to C5a (Schindler R et al.1990). Although endotoxin is a potent trigger of the inflammatory cascade of mediators, the role of endotoxin in the release of cytokines during CPB is not straightforward.

It has also been suggested that ischemia-reperfusion and complement activation (Haeffner-Cavaillon N et al. 1989) contribute to the release of cytokines. The important role of ischmia-reperfusion in the release of cytokines is supported by the observations relating the magnitude of proinflammatory cytokine response to the duration of ischemia, and the evidence of that the myocardium release cytokines after cardiac surgery (Wan S et al. 1996B, Liebold A et al. 1999). However, the relationship between myocardial injury and cytokine levels is relatively weak. Cytokine release during reperfusion of the ischemic myocardium may be dependent on complement fragment

C5a production (Ivey CL, 1995), however, the precise role of complement activation as a trigger of cytokine release under CPB condition is still unclear.

Animal models have shown that myocardium synthesizes and releases TNF-α and IL-10 in response to ischemia and reperfusion (Gurevitch J et al. 1996, Frangogiannis et al. 2000). Human studies showed that myocardium is a major source of TNF-α, IL-6 and IL-8, while IL-10 is mainly released from the liver after cardiac surgery (Table 5).

Table 5 The sources of cytokines after cardiac surgery

References	Operations	Cytokines	Source of cytokine
Wan S et al. 1996B	CABG	TNF-α, IL-6 and IL-8	Myocardium
Liebold A et al. 1999	CABG	IL-6 and E-selectin	Myocardium
Oz MC et al. 1995	CTX	IL-8	Myocardium
Karube N et al. 1996	CABG	IL-6 and IL-8	Not myocardium
Wan S et al. 1997	CABG, VR	IL-10	Liver

CABG = coroanry artery bypass grafting, CTX = Cardiac transplantation, VR = valve replacememt

3 Cytokines and myocardial injury

Myocardial stunning after heart surgery is frequently observed and is associated with increased morbidity and mortality, especially in patients of advanced age, and with decreased myocardial reserve. Although we now possess more data on myocardial cellular function, the problem of inadequate myocardial protection persists. The association between inflammation and myocardial ischemic injury has been recognized for over 50 years, and remains a matter of continued investigation.

The proinflammatory cytokines can significantly alter myocardial contractility. Importantly, these cytokines do not need to circulate, as the myocardium is capable of synthesizing biologically active TNF-α (Kapadia S et al. 1995, Sablotzki A et al. 1997). Local release of TNF in the myocardium may be involved in the postischemic myocardial stunning seen after CPB. Some of the effects in question may be mediated by an increased production of nitric oxide within cardiac myocytes (Finkel MS et al. 1992). Proinflammatory cytokines may profoundly alter the peripheral circulation, reducing vascular tone and thus resulting in postoperative low vascular resistance (Menasche P et al. 1994). Cytokines also exert a direct damaging effect on other organs and contribute to the development of multiorgan failure.

3.1 Mechanisms of cytokine-induced adverse events

First, proinflammatory cytokines induce increased neutrophil and endothelial surface adhesive molecule expression, thereby promoting enhanced neutrophil-endothelial adherence, which leads to organ injury (as reviewed in the previous chapter). Furthermore, proinflammatory cytokines also increase cellular expression of inducible nitric oxide synthase, thus increasing cellular production of nitric oxide, a known inflammatory mediator.

Nitric oxide production inhibits neutrophil-endothelial adhesion by downregulation of CD11/CD18 receptor expression and can inhibit platelet aggregation. An enzyme responsible for the synthesis of endogenous NO is constitutive nitric oxide synthase (cNOS), which synthesizes NO in very small (picomolar) concentrations. Though it has a very short half-life, NO is continuously synthesized and is rapidly oxidized to nitrite. It is important in the maintenance of capillary blood flow and the regulation of cell function. Another isoform of NOS is inducible (iNOS) by endotoxin and

cytokines, including TNF and IL-1, and results in larger (nanomolar) concentrations of NO (Hill GE, 1998B). These large amounts of endogenously produced NO secondary to cytokine activation of iNOS not only induce vasodilatation but may also cause tissue injury through formation of toxic peroxynitrites, activation of cyclooxygenase, DNA deamination, and other as yet unidentified mechanisms. An optimal level of endogenous NO may be necessary for normal cell integrity.

A study in isolated guinea pig cardiac myocytes has shown that NO attenuates contractility (Brady AJ et al. 1993). TNF and IL-6, through activation of iNOS and endogenous NO generation, cause myocardial depression (Finkel MS et al. 1992). Administration of the NO synthase inhibitor NG-nitro-L-arginine methyl ester to the extracorporeal circuit has afforded almost complete protection against myocardial reperfusion injury (Matheis G et al.1992). It has been postulated that the ischemic heart-generated proinflammatory cytokines result in the induction of iNOS, large amounts of NO production will reduce myocardial contractility and cause cell death or apoptosis. A vicious circle of events ensues with reduced cardiac output and coronary blood flow, leading to further ischemia and more cytokine release, which will result in intractable cardiac failure (Hill GE, 1998B).

Low NO concentrations may result from endothelial ischemia and impair NOS activity (Fukuda H et al. 1995). It has been shown that reperfusion after aortic cross-clamping during CPB may lead to G-protein dysfunction in the coronary endothelium, which may impair NO released by cNOS and thus lead to coronary vasospasm (Evora PR et al. 1995). NO donors can have protective effects against ischemia-reperfusion injury and improve cardiac function (Vinten-Johansen J et al. 1995).

3.2 TNF-α

TNF-α has been implicated in many problems arising after cardiac operations with CPB (Jansen NJ et al. 1992, Casey LC, 1993). Recent basic experimental and clinical evidence suggests that TNF-α is an important mediator of myocardial injury during acute myocardial infarction, chronic heart failure, cardiac allograft rejection and cardiopulmonary bypass operations. The heart produced TNF-α in response to ischemia-reperfusion. Isolated rat heart experiment have shown that ischemia-reperfusion induces a marked increase in myocardial TNF-α, which is associated with decreased myocardial contractility and coronary flow, and with increased end-

diastolic pressure and postischemic creatine kinase loss. Ischemia-induced TNF-α production may contribute to postischemic myocardial stunning, necrosis or both (Meldrum DR et al. 1998). Immunohistochemical staining has localized TNF-α to the cardiac myocytes and endothelial cells. Anti-TNF-α neutralizes local TNF-α from cardiac myocytes after ischemia and improves myocardial recovery during reperfusion. These circumstances would indicate that postischemic paracrine TNF-α release plays an active role in myocardial dysfunction after CPB (Gurevitch J et al. 1997).

TNF-α causes significant concentration-dependent depression of the maximum extent and peak velocity of myocyte shortening in vitro. Using immunoabsorption, removal of TNF-α from the serum reverses myocardial depression (Kumar A et al. 1996). It has also been proved that TNF-α depresses human myocardial function in a dose-dependent fashion, and affects systolic relatively more than diastolic performance (Cain BS et al. 1999). It has been suggested that therapeutic strategies to reduce the production of TNF may limit myocardial dysfunction. Kapadia S and colleagues (1995) found that endotoxin-treated cardiac myocytes synthesize TNF-α, and cell motion in isolated cardiac myocytes is depressed in those treated with superfusates from endotoxin-treated hearts. This negative inotropic effect could be completely abrogated by pretreatment with an anti-TNF-α antibody, which indicates that the myocardium synthesizes biologically active TNF-α and has negative inotropic effects on cardiac myocytes.

3.3 IL-6

IL-6 is a sensitive marker of the acute inflammatory response. It derives mainly from the myocardium in cardiac surgery (Wan S et al. 1996B, Liebold A et al. 1999). Plasma levels of IL-6 have been parallel to the severity of tissue damage induced by surgery and to the inflammatory response to CPB (Sakai T et al. 1993). Echo-cardiographic wall motion abnormalities and postoperative myocardial ischemic episodes have also been associated with increased levels of IL-6 (Hennein HA, 1994). The acute-phase response comprises substantial and diverse systemic and metabolic changes occurring in response to events such as trauma and infection. IL-6 has been found to be derived from hypoxic human myocytes and play a role in neutrophil-mediated myocardial ischemia-reperfusion injury (Sawa Y et al. 1998).

Peak IL-6 concentrations appear to be associated with cross-clamping time of the aorta. Plasma levels of IL-6 may be correlated with the severity of tissue damage induced by surgery and inflammatory response to CPB and also with echocardiographic wall motion abnormalities and postoperative myocardial ischemic episodes in adults (Sakai T et al. 1993, Hennein HA et al. 1994). Hence, IL-6 seems to be responsible for much of the morbidity associated with the inflammatory response to CPB. A progressive increase in peripheral circulating levels of IL-6 has been observed in direct relation to the patient's NYHA classification in a study of left ventricular dysfunction (Torre-Amione G et al. 1996). A positive correlation has been seen between creatine kinase-MB isoenzyme levels and IL-6 levels 90 minutes and 2 hours after declamping, but this correlation was relatively weak ($r=0.54$ and 0.56, respectively, both $p<0.02$) (Wan S, et al 1997B).

3.4 IL-8

IL-8 possesses a potent chemoattractant activity for neutrophils. Ischemia induces an acute inflammatory response in myocardial tissue, with an early phase of neutrophil accumulation which is accelerated by reperfusion. Study in a rabbit model has shown IL-8 to be an important neutrophil chemoattractant generated in the ischemic myocardium. It is evident that IL-8 is involved in myocardial ischemia reperfusion injury (Ivey CL et al. 1995). After 1 h of coronary occlusion, IL-8 mRNA is markedly and consistently induced in reperfused segments of canine myocardium, and it has been suggested that IL-8 participates in neutrophil-mediated myocardial injury (Kukielka GL et al. 1995). Use of antibodies to inhibit IL-8 has significantly reduced the degree of necrosis in a rabbit model of myocardial ischemia-reperfusion injury (Boyle EM Jr et al. 1998).

It has been suggested that the degree of myocardial injury may be related to IL-8 production in CABG (Wan S et al. 1999). In patients with acute myocardial infarction, a significant cardiac release of IL-8 has been noted (Neumann FJ et al. 1995). However, Takahashi and colleagues (1995) found that endogenous endothelial IL-8, secreted from activated endothelial cells into the apical side of endothelial cell monolayers, has an inhibitory effect on the transendothelial migration of neutrophils. This suggested that IL-8 may prevent excessive neutrophil infiltration to myocardial tissue from circulating blood in reperfusion injury.

3.5 IL-10

Recent studies have shown that IL-10 might be cardioprotective. A comparative study between wild-type and IL-10-deficient mice showed that endogenous IL-10 inhibits the production of TNF-α and NO, and serves to protect the ischemic and reperfused myocardium via suppression of neutrophil recruitment. (Yang Z et al. 2000). The genetic deletion of IL-10 enhances neutrophil infiltration into the reperfused tissues at 6 hours after reperfusion and increases infarct size and myocardial necrosis. An enhancement of the inflammatory response was seen in the absence of IL-10. Reperfusion for 24 hours was associated with a 75% mortality rate in IL-10-deficient mice, whereas no deaths occurred in the wild-type animals.

In a murine model of myocardial ischemia-reperfusion, administration of IL-10 prior to reperfusion reduced myocardial creatine kinase and myeloperoxidase activity in the ischemic-reperfused region. IL-10 treatment significantly attenuated neutrophil adherence to the rat superior mesenteric artery endothelium stimulated with IL-1β. It was suggested that IL-10 mediates its effects, in part, by inhibiting leukocyte-endothelial interactions (Hayward R et al. 1997).

Taken together, an inflammatory reaction is associated with myocardial injury and other complications after cardiac surgery. The humoral component of the inflammatory response to CPB is a complicated web of interacting cascades. Endotoxin release, completment activation and ischemia-reperfusion may play significant roles in initiating these cascades with subsequent generation of cytokines. TNF-α plays major regulatory roles in the cytokine network, with IL-6 and IL-8 having more specific effects. Therapies aimed to modulate the inflammatory reaction after cardiac surgery may limit myocardial injury and reduce the incidence of postoperative complication.

4 Anti-inflammatory strategies

Increasing understanding of the mechanisms described has facilitated the development of strategies aimed to attenuate the damaging effects of the systemic inflammatory response. Postoperative mortality after cardiac operations has been related to the damaging effects of cardiopulmonary bypass. These effects are mainly mediated by the activation of leukocytes and other mediators of inflammation. Alterations in perfusion technique or pharmacological interventions altering these activations might improve the clinical outcome (Table 6). Different strategies affect different stages of the inflammatory mediator network and in cardiac surgery with CPB; various approaches have been evaluated for anti-inflammatory purposes.

Table 6 Anti-inflammatory effect of different drugs

Drugs	Effects	Clinical relevance
Corticosteroids	↓TNF, IL-1, IL-6, IL-8 ↑IL-10	Reduces myocadrial and pulmonary damage
Aprotinin (high dose)	↓TNF, IL-1, IL-6, IL-8, iNOS ↑IL-10	Cardioprotective ↓ bleeding
Estrogen	↓TNF, IL-6	↑ cardiac performance
Adenosine	↓TNF, free radical, neutrophil activation	Cardioprotective
Sodium Nitroprusside	↓IL-6, IL-8, TNF,CD11b, C3, C5a	
PDEI	↓TNF, IL-1, IL-6	↑ Myocardial function
Amiodarone	↓TNF, IL-6	
Amlodopine	↓ IL-6	
Ketamine	↓TNF, IL-6, IL-8, CD18	
Vit C, Vit E and Allopurinal	Antioxidant	↓ myocardial injury ↓ perioperative morbidity

4. 1 Pharmacology

4.1.1 Corticosteroids

Thought corticosteroids have been administered in cardiac operations for over 30 years, their exact mechanism of action has not been well defined. Methylprednisolone (MP) and dexamethasone are the most commonly used corticosteroids. Early studies suggested a single bolus of a large dose of corticosteroids as a therapy for low-output syndrome after CPB, due to the effects of vasodilatation with increased venous capacitance (Dietzman RH et al. 1969). It has also been suggested that massive doses of steroids before, during and after CPB may exert ill-defined cellular protective effects, including stabilization of the lysosome membrane (Replogle RL et al.1966). Steroids can inhibit phospholipase activation and enhance membrane fluidity, which may result in improved myocardial preservation (Engelman RM et al. 1989). These effects may reduce myocardial and pulmonary damage following CPB.

More recent findings have proved the anti-inflammatory effects of steroids. Glucocorticoids will not prevent endotoxemia during CPB, but blunt subsequent complement activation and release of proinflammatory cytokines. Steroid administration before CPB has indeed been found to reduce complement activation (Andersen LW et al. 1989, Engelman RM et al. 1995), and steroids have been shown to reduce the production of TNF-α , IL-1, IL-6, IL-8 and to attenuate neutrophil activation. Jansen and colleagues (1991) reported that steroid administration before CPB can effectively reduce TNF-α production after reperfusion. Methylprednisolone pretreatment inhibits the increase in IL-6 and IL-8 to be anticipated after declamping of the aorta, and the postoperative cardiac index is higher than that of non-MP-treated patients (Kawamura T et al. 1995). Steroids can significantly increase IL-10 production during CPB (Wan S, 1997). This enhancement may bring some beneficial effects not only by inhibiting the release of other proinflammatory cytokines (Gerard C et al. 1993, Fiorentino DF et al. 1991), but also by diminishing the immunocyte hyperstimulation seen under CPB conditions (Stefano GB et al.1995). Steroids can inhibit the expression of adhesion molecules and prevent endothelial cells from becoming more adhesive for neutrophils (Cronstein BN et al. 1992). As a result of these actions, it has been shown that the use of steroids can improve hemodynamic stability, attenuate vascular leakage and subsequent fluid requirements, and preserve pulmonary vascular and alveolar architecture (Miller

BE and Levy JH, 1997). Glucocorticoids are also known to inhibit endotoxin-induced expression of iNOS in the vascular endothelium (Radomski MW et al. 1990).

Recently, several randomized studies have confirmed the hypothesis that steroid administration prior to cardiopulmonary bypass would reduce the inflammatory mediator release and improve the postoperative clinical course (Yilmaz M et al. 1999, Bronicki RA et al. 2000). The most extensively researched and widely accepted mode of intervention is the use of corticosteroids before the onset of CPB. The optimal timing of steroid administration, before rather than after CPB, is important in achieving the benefit (Wan S et al. 1996). Steroid pretreatment has become a fundamental strategy in the "fast-track recovery" protocol and has been shown to improve postoperative recovery in patients undergoing CPB and to reduce the length of ICU and hospital stay (Engelman RM, 1994).

4.1.2 Aprotinin

Aprotinin is a polypeptide protease inhibitor derived from the bovine lung. It antagonizes many of the body's proteolytic enzymes, including kallikrein and plasmin. It has been found to reduce blood loss after CPB when used in high, low, or pump prime-dosing protocols (Bidstrup BP et al. 1995). More recent data have indicated that the role for this serine protease inhibitor may go beyond reducing blood loss during and after CPB.

Serine protease inhibitors have the potential to moderate the inflammatory response to CPB by regulating cytokine release and leukocyte activation (Royston D, 1996). In vivo studies have shown that aprotinin has a dose-responsive inhibitory effect on complement activation (Dietrich W et al. 1990), and subsequent studies have discovered its ability to attenuate the production of TNF-α and IL-6, the upregulation of neutrophil integrin adhesion receptors, the kallikrein and complement-induced activation of neutrophils, and the release of elastase from activated neutrophils which occurs with CPB. Aprotinin appears to be as effective as corticosteroids in blunting these inflammatory responses to CPB (Hill GE et al. 1995). Serine protease inhibitors will reduce TNF-α release from endotoxin-stimulated macrophages (Kim KU et al. 1993). Aprotinin damps IL-8 production and neutrophil accumulation in lungs after CPB, and can reduce cytokine-induced nitric oxide production by inhibition of iNOS expression (Hill GE et al. 1996 and 1997). This might be the mechanism underlying the

anti-inflammatory effects reported with aprotinin (Hill GE et al. 1997). Furthermore, it has been demonstrated that full-dose aprotinin therapy enhances an endogenous anti-inflammatory response characterized by IL-10 release after CPB. These observations demonstrate a unique anti-inflammatory activity of aprotinin which may be of clinical significance.

The anti-inflammatory effect of aprotinin might be dose-dependent (Table 7). Hill GE and colleagues (1995) found that low-dose aprotinin has an anti-inflammatory effect similar to that of methylprednisolone in blunting CPB-induced systemic TNF-α and neutrophil integrin CD11b upregulation, while Diego (1997) found that only high-dose aprotinin has such an anti-inflammatory effect.

Table 7 Anti-inflammatory effect of aprotinin in CABG

References	Dosage	Case number	Results
Hill GE et al. 1995	Half	8 vs 8	↓ TNF and CD11b
Diego RP et al. 1997	Half	10 vs 10	similar IL-6
Diego RP et al. 1997	Full	10 vs 10	↓IL-6
Ashraf S et al. 1997	Pump	19 vs 19	similar IL-6, IL-8
Hill GE, et al. 1998	Full	10 vs 10	↑IL-10
Harig F et al. 1999	Full	10 vs 10	↓IL-6, ↓IL-8
Alonso A et al. 1999	Pump	12 vs 10	↓ CD11b
Defraigne JO et al. 2000*	Full	100 vs 100	similar TNF, IL-6, IL-8

* Normothermic CPB was used in some patients (number unknown)
Pump = pump prime only

4.1.3 Estrogen

Gender-specific differences in heart disease have long been known, but only since the advent of molecular biology has it become possible to investigate the molecular mechanisms involved. The inhibitory influence of estrogen on the development of atherosclerosis has been suggested by an abundance of human epidemiological and animal experimental data. Premenopausal women run a lower risk of cardiovascular disease than men. This cardiovascular protection is lost after the menopause. An early menopause also substantially increases the risk of cardiovascular disease. The precise mechanisms whereby estrogens favorably influence the arterial disease risk are not

fully understood. Much attention has been paid to changes in lipid metabolism, but adjustment for lipid levels explains only part of the protective effect (25% to 50%) (Barrett-Connor E and Bush TL, 1991).

In very recent years, it has been recognized that estrogens and androgens act on a much wider spectrum of tissues. Functional estrogen receptors have also been shown in vascular smooth muscle cells and in the endothelium (Pelzer T et al. 1997). Grohe and colleagues (1997) found that cardiac myocytes and fibroblasts contain functional estrogen receptors and that estrogen regulates expression of specific cardiac genes. This suggests a direct effect of estrogen on the heart. Animal studies have suggested that infiltration of neutrophils in the endotoxin-induced glomerular inflammatory response is under the control of estradiol (Faas MM et al. 1999). Recent research has demonstrated that estradiol reduces LPS-induced IL-6 and TNF-α production. This inhibition of cytokine production could have a profound effect on the immune response during the inflammatory reaction (Deshpande R et al. 1997). Clinically, Rosano and his workmates (1993 and 1997) found acute administration of 17β-estradiol to have a beneficial effect on myocardial ischemia in women with coronary artery disease. This beneficial effect of 17β-estradiol has been proved by several researches not only in women but also in men (Komesaroff PA et al. 1998) (Table 8).

Table 8 Acute effects of sublingual 17β-estradiol

References	Individuals	Dosage	Effects
Rosano GM (1993)	PM women with CAD	Sublingual (1 mg)	Useful adjunct to the treatment of angina
Riedel M (1995)	PM women	Sublingual (1 mg)	Vasodilation and increase of blood flow.
Volterrani (1995)	PM women	Sublingual (1 mg)	Increases peripheral blood flow
Rosano GM (1997)	PM women with CAD	Sublingual (1 mg)	Reduces the degree of pacing-induced myocardial ischemia
Pines A (1998)	PM women	Sublingual (4 mg)	The left heart cavities become smaller
Komesaroff (1998)	Healthy young men	Sublingual (2 mg)	Act on the male cardiovascular system in a clinically beneficial manner
Fisman EZ (1999)	PM women	Sublingual (4 mg)	Induces acute modifications in left ventricular diastolic function

PM = postmenopausal, CAD = Coronary artery disease

Cardiovascular surgeons have used conjugated estrogens to reduce the blood loss associated with open heart procedures. This is not, however, its only benefit. Pacific and colleagues (1991) reported that after surgical menopause patients had higher levels of IL-1 than those who received hormone replacement therapy (HRT). Evidence in humans generally supports an inhibitory effect of estrogens on TNF production (Selzman CH et al. 1998). Recent research on estrogen has shown that 17β-estradiol limits the deleterious ICAM-1-mediated binding of leukocytes to injured myocardium and protects against myocardial ischemia-reperfusion injury by inhibiting TNF-α production (Squadrito F et al. 1997). Administration of 17β-estradiol significantly improves cardiac performance, cardiac output and hepatocellular function and attenuates the increase in plasma IL-6 levels after traumatic hemorrhage in male rats (Mizushima Y et al. 2000).

4.1.4 Adenosine

Adenosine is known to regulate the activities of immune and inflammatory cells, and to be released by the transiently ischemic myocardium during preconditioning (Headrick JP, 1996). A considerable body of experimental evidence shows that adenosine is a cardioprotective agent independently of its well-known vascular smooth muscle relaxing effects (Belardinelli L et al. 1995). The phenomenon in question appears to be mediated by activation of the A_1-receptor coupled to guanine nucleotide inhibitory binding proteins (Belardinelli L, 1995, Mentzer RM Jr,1993).

Adenosine exhibits a broad spectrum of effects against neutrophil-mediated events and can therefore intervene in the ischemia-reperfusion response, a capacity which may offer therapeutic benefits (Jordan JE et al. 1999). It has been found in an animal model that intracoronary administration of adenosine after reperfusion significantly reduces neutrophil and red blood cell stagnation in capillaries, and is associated with reduced infarct size and improves regional ventricular function in the ischemic zone (Olafsson B et al. 1987). Adenosine reduces oxygen-derived free radical production by neutrophils, an effect which could minimize the free-radical-induced damage believed to occur during reperfusion (Cronstein BN et al. 1983).

Recently, Wagner and colleagues (1998) found that adenosine reduces LPS–induced secretion of TNF-α in neonatal rat myocytes and the failing human heart. An animal test has shown that adenosine lowers ischemia-induced cardiac TNF-α levels

and bioactivity after ischemia and reperfusion of the isolated rat heart (Meldrum DR et al. 1997). Therapy with acadesine, a drug which modulates adenosine levels in ischemic tissue, has shown inhibition of the upregulation of neutrophil CD11b adhesive receptors after CPB (Mathew JP et al. 1995).

There have been several reports of the clinical use of adenosine as a cardioprotective agent (Table 9). These data suggest a novel anti-inflammatory property of adenosine by which it could modulate inflammation and limit ischemia-reperfusion injury.

Table 9 Adenosine protocol used in CABG

Reference	Total dosage	Timing	CPB	Result
Lee et al. 1995	2450 µg/kg	Before CPB	No	↑ Ventricular performance ↓ CK release
Mentzer et al. 1997	1400 µg/kg	After AC	Yes	↓ Dopamine requirement
Mentzer et al. 1997	0.1 to 2 mM/L	Cardioplegia only	Yes	↓ Dopamine requirement
Mentzer et al. 1999	5000 µg/kg	after AC (2000 µg/kg) after DC (3000 µg/kg)	Yes	↓Postoperative complications
Belhomme et al. 2000	700 µg/kg	Before AC	Yes	No effect

CPB = Cardiopulmonary bypass, AC = Aortic cross-clamping, DC = Aortic declamping

4.1.5 Anticytokine monoclonal antibodies

The newest agents to be investigated for their abilities to attenuate the harmful effect of the inflammatory response to CPB are monoclonal antibodies directed at specific polypeptide mediators. In this novel and promising anti-inflammatory strategy, though still at the experimental stage, monoclonal antibodies have been developed for TNF-α, IL-1 receptors, IL-8 and ICAM-1 receptors. Their use has been found to attenuate the effects of endotoxin and the vascular permeability and tissue damage which ensue upon ischemia-reperfusion during CPB, as evidenced by prevention of the morphological changes in the lungs and heart seen after CPB as well as preserved

function of these organs (Miller BE et al. 1997). Soluble receptors (IL-1 or TNF), and receptor antagonist (IL-Ra) have been used in clinical trials. Specific blockade of TNF using neutralizing antibodies in animal models of SIRS reduces mortality and severity of disease. Similar results have been observed by blocking IL-1 using soluble IL-1 receptors or IL-1 receptor antagonists.

In animal studies, convincing data are now available showing that immunotherapy improves the prognosis of sepsis. A recent study in isolated rat hearts has shown that monoclonal hamster antimurine TNF-α antibodies limit myocardial TNF-α expression and improve postischemic left ventricular peak systolic pressures, the first derivative of the rise in left ventricular pressure, pressure-time integral, coronary flow and O_2 consumption, and lower creatine kinase levels, while myocardial structure is preserved. Anti-TNF-α neutralizes local TNF-α release from cardiac myocytes after ischemia and improves myocardial recovery during reperfusion (Gurevitch J et al. 1997). Blockade of neutrophil adhesion with NPC 15669 has reduced lung dysfunction after cardiopulmonary bypass in an ovine model (Friedman M et al. 1996).

Preliminary clinical studies suggest that blockade may be useful in treating human SIRS. Judging from animal studies and preliminary clinical trials, strategies to block IL-1 or TNF may benefit patients with the syndrome; however, further thorough clinical trials are warranted (Dinarello CA et al. 1993). In a rabbit model of lung reperfusion injury, the administration of a neutralizing monoclonal antibody against IL-8 has prevented neutrophil infiltration and tissue injury, proving a causal role of locally produced IL-8 in this model (Sekido N et al. 1993). Neutralization of IL-8 by a specific monoclonal antibody has significantly reduced the degree of necrosis in a rabbit model of myocardial ischemia-reperfusion injury (Boyle EM Jr et al. 1998). Much work remains to be done in this area, but it is hoped that specific blockade of these cytokines and receptors by monoclonal antibodies will become a life-saving mode of clinical intervention.

4.1.6 Sodium nitroprusside

Nitric oxide has been shown to reduce ischemia-reperfusion injury. NO appears to mitigate postischemic polymorphonuclear leukocyte adhesion and vascular injury (Kupatt C et al. 1996). A protective effect of endogenous nitric oxide has been demonstrated using canine models (Sato H et al. 1997). Animal studies have shown

that sodium nitroprusside has a cardioprotective effect by radical scavenging (Massoudy P et al. 1995). It has been hypothesized that the application of a NO donor reduces the inflammatory reaction. Intracoronary infusion with SPM-5185, a cysteine-containing nitric oxide donor compound, was found to reduce myocardial necrosis and neutrophil accumulation in an acute model of canine myocardial ischemia and reperfusion (Lefer DJ et al. 1993).

It has been suggested that sodium nitroprusside (a nitric oxide donor) has an inhibiting effect on complement activation and that this effect is mediated by nitric oxide release from sodium nitroprusside. In patients undergoing routine coronary artery bypass grafting, administration of sodium nitroprusside during early reperfusion reduces systemic IL-6 and IL-8 (Massoudy P et al. 1999). Treatment with sodium nitroprusside 0.5 μg/kg/min for the first 60 minutes of reperfusion in patients undergoing coronary artery bypass grafting reduces the transcardiac production of IL-6, IL-8, TNF-α, CD41, CD62 and CD11b (Massoudy P et al. 2000). Patients treated with sodium nitroprusside evinced significantly less C3 conversion during CPB and significantly less C5a liberation immediately after CPB than patients not treated with sodium nitroprusside. The leukocyte count during the rewarming period of cardiopulmonary bypass was significantly reduced in patients treated with sodium nitroprusside. In vitro experiences clearly demonstrate inhibition of complement hemolytic activity by sodium nitroprusside. The decrease in complement hemolytic activity measured was dose-dependent and was enhanced by sodium nitroprusside preincubation of the sera tested. This effect was related to the duration of preincubation. Zymosan-induced C3 conversion was inhibited by sodium nitroprusside. Nitroglycerin and isosorbide dinitrate (other nitric oxide donors) also have in vitro effects on complement hemolytic activity similar to those of sodium nitroprusside (Seghaye MC et al. 1996B).

However, the optimal timing and dosage of intervention with a donor of nitric oxide is unclear from the literature. Engelman and colleagues (1996) reported that L-arginine is most beneficial when given before cardioplegic arrest, effective during cardioplegic arrest, and detrimental during reperfusion in isolated rat hearts, while Massoudy and associates (1999, 2000) reported that the administration of sodium nitroprusside during early reperfusion reduces systemic inflammatory responses in

patients undergoing CABG. Further clinical trial is essential to define the best protocol regarding timing and dosage of the intervention.

4.1.7 Phosphodiesterase inhibitors

Phosphodiesterase inhibitor (PDEI) has been accepted as an inodilator with positive inotropic and vasodilating actions, but it also has anti-inflammatory action at the therapeutic concentrations used for heart failure. The PDE III inhibitors amrinone and milrinone and the non-specific phosphodiesterase inhibitor pentoxifylline, inhibit unstimulated and LPS-induced TNF-α secretion from the rat left ventricle (Bergman MR et al. 1996). PDE inhibitors have the potential to inhibit inflammatory cell activation (Banner KH et al. 1996).

Amrinone markedly reduces systemic cytokine release (TNF and IL-1) and LPS-mediated effects on myocardial function in rabbits (Takeuchi K et al. 1999). It inhibits the production of pro- and anti-inflammatory factors and NO production in endotoxin-stimulated cells in in vitro studies (Nemeth ZH et al. 1997A). Intraperitoneal treatment of animals with amrinone 30 minutes prior to LPS administration has been seen to reduce both plasma IL-6 and IL-10 concentrations in the first phase of the response, and to result in a marked inhibition of LPS-evoked plasma concentrations of TNF-α and nitrite/nitrate (breakdown products of nitric oxide) throughout the response (Nemeth ZH et al. 1997B). Pretreatment with amrinone at therapeutic concentrations significantly reduce the IL-1-induced elevation of E-selectin ICAM-1 on the endothelial surface (Fortenberry JD et al. 1997).

Milrinone suppresses cytokine production by elevating cyclic adenosine monophosphate levels in patients undergoing cardiopulmonary bypass (Hayashida N et al. 1999). Perioperative administration of low-dose milrinone may have anti-inflammatory properties. IL-1β (Hayashida N et al. 1999), IL-6 and amyloid A (Mollhoff T et al. 1999) values were attenuated by milrinone treatment in routine coronary artery bypass grafting.

Pentoxifylline has been reported to be an effective drug in inhibiting TNF-α responses during septic shock (Staudinger T et al. 1996). Pentoxifylline may attenuate the endothelial injury and permeability seen in CPB (Tsang GM et al. 1996). Olprinone, a new PDEI-III agent, can enhance the plasma levels of IL-10 and lead to a faster IL-6 clearance in cardiac surgery (Okuda K et al. 1997).

4.1.8 Other modulators

In vitro experience has shown that amiodarone significantly inhibits TNF-α and IL-6 production of peripheral blood mononuclear cells (Matsumori A et al. 1997).

The beneficial effect of amlodipine in heart failure is mediated by a reduction in IL-6 levels. IL-6 levels were significantly lower at 26 weeks in patients treated with amlodipine versus placebo (Mohler ER 3rd et al. 1997).

Ketamine directly suppresses LPS-induced TNF-α, IL-6 and IL-8 production in human whole blood (Kawasaki T et al.1999). Ketamine has inhibited stimulated up-regulation of CD18 in a concentration-dependent manner. It also causes a significant decrease in endotoxin-stimulated IL-6 production in human whole blood (Weigand MA et al. 2000).

As the release of oxygen-free radicals plays a certain role in the inflammatory responses, there may be a place for antioxidant intervention. Preoperative administration of a combination of vitamin C, vitamin E and allopurinol may reduce perioperative morbidity and myocardial injury (Sisto T et al. 1995). Reperfusion with a blood cardioplegic solution instead of crystalloid cardioplegia may reduce ischemia-reperfusion injury as it contains the endogenous oxygen-free radical scavengers that are present in erythrocytes (Julia PL et al. 1991). A recent randomized study has shown that use of blood cardioplegia attenuates systemic IL-6 compared to crystalloid cardioplegia, and the post-CPB cardiac index is superior in patients with blood cardioplegia (Liebold A et al. 1999B).

All these data suggest new research directions in efforts to evaluate the effect or mechanism of many pharmacological therapies.

4.2 Technique modifications

4.2.1 Heparin-coated CPB conduit

A crucial factor triggering the inflammatory response to CPB is material-related, being attributable to the exposure of blood to nonphysiologic surfaces and conditions. It has been suggested for a long time that the heparin-bonding surface can greatly reduce thrombus formation (Gott VL et al. 1963). Heparin-coated CPB circuits have been shown to "improve biocompatibility". They inhibit complement activation via the alternative pathway and terminal pathway (te Velthuis H et al. 1996). The use of heparin-coated circuits reduces leukocyte activation (Borowiec J, 1992), inhibits platelet adhesion and improves platelet function (Hatori N et al. 1994). Some studies have also shown reduced TNF-α (Gu YJ et al. 1993), IL-6 and IL-8 release (Steinberg BM et al. 1995) with the use of heparin-coated circuits. It remains unclear, however, whether these effects can lead to significant improvement in clinical outcome.

Heparin coating of the extracorporeal circuit significantly reduces rheologic damage of blood cell in low-risk patients undergoing routine bypass surgery (Belboul A et al. 2000). With the use of heparin-coated circuits, IL-6 and IL-10 release from activated peripheral blood mononuclear cells are less than with uncoated CPB (Giomarelli P et al. 2000). However, heparin-coated circuits offer minimal clinical and biological benefits for routine CABG surgery (Belboul A et al. 2000, Giomarelli P et al. 2000, Collart F et al. 2000). A recent randomized clinical trial with 200 patients showed that circulating TNF-α, IL-6, IL-8, myeloperoxidase and elastase levels were similar between the groups. No significant differences between groups were observed. CPB is associated with cytokine release and neutrophil activation, which are not attenuated by the use of heparin-coated circuits. Heparin-coated circuits show no additive effects (Defraigne JO et al. 2000). Recent data show that there are no major statistically significant clinical benefits of heparin-coated circuits in low-risk patients. They may prove beneficial for complex procedures or at-risk patients.

Reduced heparin dose may lead to less postoperative blood loss and transfusion requirements (Ovrum E et al. 1995A, Aldea GS et al. 1996), and may inhibit complement and granulocyte activation (Fosse E et al. 1994, Ovrum E et al. 1995B). These effects are believed to be markers of "improved biocompatibility". However, some studies have indicated that it may be important to maintain a full heparin dose during CPB (Gorman RC et al. 1996, Korn RL et al. 1996). Certain other differences

may be related to the different coating techniques. The design of the CPB circuit itself may have a major impact on activation of the complement cascade. Both Duraflo II (Baxter Healthcare Corp; Irvine, Calif) and Carmeda BioActive Surface (CBAS; Medtronic Cardiopulmonary Division; Anatheim, Calif) heparin-coated circuits have been applied to clinical use. Although both circuits reduce complement and neutrophil activation, the CBAS system was observed to be more effective than the Duraflo II system (Moen O et al. 1995, Baufreton C et al. 1998).

Further randomized double blind multicenter trial are essential to evaluate the effect of heparin-coated CPB conduit and define the best protocol regarding heparin dosage and related management during CPB.

4.2.2 Ultrafiltration

Ultrafiltration can remove water and certain low-molecular-weight substances from plasma. Although it is not a direct means of attenuating the inflammatory response to CPB, ultrafiltration has interesting applications when used before, during and after CPB in the attempt to reduce the accumulation of extravascular water commonly encountered (Magilligan DJ Jr, 1985). Ultrafiltratin may also remove some of the inflammatory mediators released during CPB. Conventional and modified ultrafiltration (MUF) techniques have been shown to lower plasma levels of complement components (C3a and C5a) and cytokines (TNF-α, IL-6 and IL-8). As a result, clinical improvement is seen in postoperative cardiac function and pulmonary function (Elliott MJ, 1993, Journois D et al. 1994). A prospective randomized study of 97 adult patients undergoing elective CABG has shown that MUF leads to a significant reduction in IL-6, IL-8, TNF-α and adhesion molecule (sE-selectin, sICAM-1) levels after hypothermic CPB. MUF is an efficient way to remove cytokines and adhesion molecules. However, the study in question could not demonstrate any significant impact of MUF on outcome of adults after elective CABG (Grunenfelder J et al. 2000).

The hemodilution effects of CPB are most pronounced in children, who may thus benefit most from the positive effects of ultrafiltration. A study of ultrafiltration effect during pediatric cardiac operations showed that ultrafiltratin was more efficient in removing TNF-α than the other mediators (IL-6 and IL-8) (Wang MJ et al. 1996). Other studies which have examined the effect of lowering concentrations of cytokines have

used hemofiltration at the end of surgery. These studies, in a pediatric setting, showed that hemofiltration during rewarming is associated with a reduction in plasma concentrations of C3a, C5a, IL-6 and IL-8. This in turn was associated with a higher arterial pressure, a reduction in bleeding and also pulmonary shunting (Journois D et al. 1994). However, Saatvedt and colleagues (1996) could not demonstrate any reduction in either complement activation or levels of TNF and IL-6 in children undergoing CPB with ultrafiltration. Tassani and associates (1999) concluded from their study that ultrafiltration diminished the inflammatory response over a very limited time period immediately after CPB and, probably as a consequence, slightly improved clinical parameters. The choice of filter material and pore size, the hemofiltration rate and the timing of the procedure may all influence its efficacy and effect on outcome (Journois D et al. 1996, Wang MJ et al. 1996).

4.2.3 Leukocyte depletion

Another circuit modification which has been used in an attempt to attenuate the CPB-induced inflammatory response is the placement of a leukocyte-depleting filter in the circuit's arterial line. The rationale for this modification is that elimination of leukocytes could interrupt the inflammatory sequence and attenuate subsequent tissue damage and organ dysfunction.

Leukocyte depletion has been shown in animals to be beneficial. In an isolated blood-perfused heart model from newborn rabbits, hearts reperfused with leukocyte-depleted blood showed higher percentages of recovery than the group of hearts reperfused with whole blood. The hearts reperfused with leukocyte-depleted blood showed significantly lower levels of malondialdehyde, chemiluminescence in the coronary sinus effluent, and counts of intracapillary neutrophils in the myocardium than did the whole-blood group (Sawa Y et al. 1994). It has been demonstrated that leukocyte depletion during initial reperfusion results in sustained improvement in postischemic left ventricular function despite the rapid return of granulocytes to the circulation (Wilson IC et al. 1993). Leukocyte depletion filters significantly reduce ischemic damage during acute surgical revascularization and appear to be most effective when placed in the CPB circuit before cardioplegic arrest in pigs (Lazar HL et al. 1995).

Removal of leucocytes by filtration during the reperfusion period may potentially reduce postoperative morbidity after CPB. However, clinical use of the leukocyte filter has caused no significant reduction in leukocyte count and no improvement of postoperative lung function in terms of oxygenation index, pulmonary vascular resistance and intubation time (Mihaljevic T et al. 1995). The leucocyte-depletion filter brought about no significant reduction in circulating C3-complement activation products and levels of myeloperoxidase and IL-6 and IL-8 (Baksaas ST et al. 1999). The clinical benefit of leucocyte filters in routine or high-risk patients remains to be demonstrated and may be dependent on both the efficacy and the biocompatibility of the filters.

4.2.3 Temperature of CPB

Lichtenstein and associates (1991) reported that warm aerobic arrest of the heart is safe and effective even in high-risk patients with aortic cross-clamping times longer than 3 hours, and that these patients are easily weaned from CPB without inotropic support. However, one clinical study has indicated that normothermic CPB is associated with a higher incidence of low systemic vascular resistance. In vitro and vivo experience shows that TNF and IL-6 levels are consistently higher in patients undergoing normothermic bypass, and the incidence of vasodilatation necessitating vasopressor support is two-fold higher in the normothermic group. Patients supported by pressor agents had significantly higher cytokine levels after bypass than those who did not require presser therapy. This would imply that vasodilatation occurring with the warm-heart operation is, at least partly, mediated by a temperature-dependency of cytokines (Menasche P et al. 1994A). Normothermic CPB was accompanied by higher circulating IL-1 receptor antagonist, ICAM-1 and elastase levels (Menasche P et al. 1994B).

However, data in the literature remain confusing with regard to the effects of perfusion temperature on the activity of the inflammatory response. It is reported that IL-8 and elastase levels in normothermic CPB patients are lower than hypothermic patients at 12 hours after CPB (Ohata T et al. 1995). This group also found that CPB with a tepid temperature (34 degree) showed an earlier decrease in IL-8 and neutrophil elastase levels as compared with hypothermic CPB (28 degree) (Ohata T et al. 1997). A recent prospective randomized study, with all patients receiving cold antegrade

crystalloid cardioplegia, found a similar pattern of elastase and IL-8 release after normothermic, hypothermic and moderately hypothermic CPB (Birdi I et al. 1999), while Chello and coworkers (1997), in a randomized study with all patients receiving warm-blood cardioplegia found significantly higher C3a, C5a, C5-9 and neutrophil activation in those undergoing normothermic CPB. Further randomized study with a larger study population is warranted to evaluate the effect of CPB temperature on inflammatory mediator release.

4.2.5 Off-pump technique

Cardiopulmonary bypass provides a high-quality and safety standard for coronary bypass surgery. However, CPB has a negative impact on different organ systems with a variety of biological pathways. Excessive activation of the inflammatory process might be avoided by minimally invasive surgery. Recently, new CABG techniques without CPB (off-pump CABG) have been introduced to reduce the invasiveness of the procedure (Subramanian VA, 1995), and thus to reduce the risk of postoperative morbidity. Minimally invasive direct CABG (MIDCAB) has been shown to be beneficial (Gu YJ et al. 1998). Off-pump CABG via median sternotomy may also result in fewer episodes of postoperative arrhythmia and pulmonary and neurological complications (Buffolo EE, 1997). Off-pump coronary bypass significantly reduces the incidence of transfusion requirement and has a consistent trend in reducing morbidity and mortality both overall and in high-risk subsets compared to the CPB counterparts (Yokoyama T et al. 2000). The incidence of postoperative overall infections is significantly lower in off-pump patients (Ascione R et al. 2000). Off-pump revascularization reduces myocardial cell damage and lipid peroxidation, and is associated with a reduced activation of the potent vasoconstrictor peptide endothelin. These approaches may contribute to improved myocardial function and faster postoperative recovery from surgical revascularization procedures, particularly in critically ill patients (Wildhirt SM et al. 2000).

A reduction in cytokine response has been found in MIDCAB via minithoracotomy (Gu YJ et al. 1998, Strüber M et al. 1999). Wan and colleagues (1999) found a delayed IL-6 release and statistically significantly lower IL-8 and IL-10 levels in off-pump patients. However, Diegeler and group (1998) found similar leukocyte activation in patients undergoing CABG through median sternotomy with or without

CPB, and concluded that the reaction of the leukocyte subsets to coronary bypass surgery is related more to the surgical trauma in general than to CPB in particular. In their study, there was no significant difference in cytokines (TNF-α receptors, IL-6, IL-8 and IL-10) measured in relation to the type of operative approach (Diegeler A et al. 2000). Fransen and colleagues (1998) compared the inflammatory response in patients undergoing CABG via median sternotomy with or without CPB, and found a similar pattern of IL-6 release and CRP levels in both groups on the first postoperative day. Recently, a prospective randomized study has shown that elastase, IL-8 levels and leukocyte, neutrophil and monocyte counts were significantly higher in the on-pump when compared with the off-pump group (Ascione R et al. 2000).

These data suggest that avoidance of CPB during CABG reduces cytokine response, and may thus be advantageous in the treatment of patients with a high degree of comorbidity.

AIMS OF THE STUDY

Based on the findings in literature, proinflammatory cytokine levels all appear to be elevated during cardiac surgery; but their relationship to myocardial injury and postoperative complications, however, is less well defined. A better understanding of this process and novel therapies aimed at limiting the inflammatory responses are clearly necessary. Therefore the purpose in this series was:

1. to study cytokine responses in low-risk CABG patients and their relationship to myocardial injury and postoperative complications.
2. to compare cytokine responses in CABG with and without CPB.
3. to investigate the anti-inflammatory effect of pump prime aprotinin in patients undergoing CABG.
4. to define the anti-inflammatory effect of estradiol in patients undergoing CABG
5. to evaluate the cardioprotective and anti-inflammatory effects of adenosine in low-risk CABG.

PATIENTS AND METHODS

1. Patient selection

The investigation was approved by the local ethics committee of Tampere University Hospital and informed written consent was obtained from all patients entering the study. The study was carried out from June 1999 to December 2000 in the Division of Cardiothoracic Surgery, the Department of Anesthesiology and Intensive Care Unit, the Department of Clinical Microbiology and the Laboratory Center, Tampere University Hospital, Tampere, Finland. Male patients with multiple-vessel coronary artery disease and stable angina admitted for the first time for elective coronary artery bypass surgery were invited to take part. Patients were randomized into different groups for studies of aprotinin, estrogen and adenosine.

Exclusion criteria were unstable angina pectoris, acute myocardial infarction present within previous 3 weeks, poor left ventricular function (ejection fraction < 35%), use of corticosteroid or estrogen within previous 4 weeks, presence of cancer, renal insufficiency or immunodeficiency syndromes, concomitant cardiac procedures (such as aortic valve replacement), previous heart operation, and history of hypersensitivity reactions to adenosine, estradiol or aprotinin. Patients with an aortic cross-clamping time exceeding 120 minutes, and/or any postoperative complication requiring re-exploration were also excluded.

Ninety-seven male patients were invited to take part, and 4 were subsequently excluded as aortic cross-clamping time exceeding 120 minutes. Another 3 patients were rejected owing to the necessity for postoperative re-exploration (Table 10). Finally, completed clinical and laboratory data were thus obtained from 90 patients. The clinical data on the 90 patients completing the study are presented in Table 11.

Table 10 Numbers of patients enrolled in studies I to V

Study number	Cases enrolled	Abandoned cases		Final cases
		AC time > 120 min	Re-exploration	
I	22	1	1	20
II	24	1	1	22
III	21	0	0	21
IV	21	0	1	20
V	32	2	0	30

Table 11 Patients clinical data.

	Study I	Study II		Study III		Study IV		Study V	
		CPB	off-pump	Control	Aprotinin	Control	Estrogen	Control	Adenosine
Number of patients	20	13	9	9	12	10	10	15	15
Age (years)	62.2 ± 7.6	63.5 ± 6.8	65.3 ± 5.4	62.4 ± 7.7	64.8 ± 7.3	62.9 ± 7.7	64.0 ± 7.6	64.7 ± 8.6	65.9 ± 7.9
Body surface area (m^2)	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.1 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.2 ± 0.2	2.0 ± 0.1	2.0 ± 0.2
Ejection fraction (%)	61.4 ± 11.7	58.6 ± 12.8	54.0 ± 19.1	63.1 ± 12.2	67.6 ± 10.6	64.9 ± 9.9	57.9 ± 15.9	63.1 ± 9.9	60.1 ± 12.6
NYHA class (II/III)	7/13	6/7	4/5	2/7	4/8	3/7	5/5	6/9	3 /12
Ischemia time (min)	79.5 ± 19.1	75.2 ± 14.6	--	72.6 ± 29.1	80.3 ± 27.9	74.6 ± 20.8	90.7 ± 20.7	89.6 ± 19.1	90.4 ± 19.7
CPB time (min)	106.3 ±27.0	98.8 ± 20.4	--	97.8 ± 30.7	104.3 ± 26.2	101.0 ± 27.0	112.6 ± 24.2	107.6 ± 21.9	109.9 ± 24.5
Number of grafts	3.5 ± 1.1	3.2 ± 0.8	1.4 ± 0.5	3.2 ± 0.8	3.5 ± 1.1	3.3 ± 1.1	3.9 ± 0.9	3.7 ± 1.0	3.7 ± 0.9

Data are mean ± standard deviation or absolute numbers, EF = ejection fraction, NYHA = New York Heart Association class,
CPB = cardiopulmonary bypass

2. CPB and CABG

In the evening before the operation, the patients received a single dose of lorazepam (2 mg) orally. Anti-hypertensive, anti-anginal and other cardiac medication was continued up to the day of surgery. Pre-medication consisted of morphine (8-12 mg) and scopolamine (0.2-0.4 mg), given i.m. prior to the induction of anesthesia.

Anesthesia was induced with lorazepam (1-2 mg), thiopentone (2 mg/kg) and fentanyl (7 μg/kg). Pancuronium (0.1 mg/kg) was given to facilitate intubation, with further increments (0.03 mg/kg) as required to maintain muscle relaxation. After endotracheal intubation, the lungs were mechanically ventilated with oxygen in air (FiO_2 = 0.40) using positive pressure ventilation. During surgery, additional bolus doses of fentanyl (total dose 20 μg/kg) were given to maintain adequate analgesia. Isoflurane was administered to deepen the anesthesia as required during sternotomy. During the CPB phase no isoflurane was administered.

A standard CABG operation was undertaken with one internal thoracic artery (ITA) and with one to four peripheral vein grafts taken in each case from the lower extremities. The patients were perfused at a temperature of 32°C with nonpulsative flow from a membrane oxygenator (Dideco, Mirandola, Italy). The circuit was primed with 2,000 mL of Ringer acetate. Cold-blood antegrade-retrograde cardioplegia (6-8°C) was delivered through a BCD-Plus device® (Dideco, Mirandola, Italy), which mixed blood with asanguineous solution in a ratio of 4:1. The potassium concentration of the induction cardioplegia was 21 mmol/L. After each distal anastomisis, additional cardioplegic solution was delivered for one minute through the vein graft and a coronary sinus catheter. Proximal anastomoses were completed before aortic declamping. Warm-blood retrograde cardioplegia was given immediately before the end of cross-clamping.

After weaning from the CPB, pharmacologic therapy with inotropes (Adrenaline, Dopaxamin) was used to maintain a cardiac index greater than 2.0 L/min/m^2. Vasopressor (Norepinephrine) would be used if systolic arterial pressure below 80 to 90 mmHg (depending on the preoperative values) and SVRI lower than 1000 dynes/sec/cm^{-5}/m^2 even though adequate cardiac filling pressure and CI (higher than 2.0 L/min/m^2) were guaranteed with volume infusions and inotropes. Corticosteroids were not administered perioperatively.

3. Sample collection and analysis

Blood samples for cytokine measurements were collected from the radial artery before induction of anesthesia (baseline), and 5 minutes and 1, 4 and 20 hours after myocardium reperfusion. All samples were anticoagulated with ethylenediaminetetraacetic acid, immediately cooled in 4°C, and centrifuged within 30 minutes (4000g for 10 minutes); plasma was transferred to polypropylene test tubes and stored at -70°C until assay. Laboratory personnel measured the plasma cytokine levels blindly. TNF-α, IL-6, IL-8 and IL-10 levels in plasma were determined by means of a commercially available enzyme-linked immunosorbent assay (CLB, Netherlands). The cytokines were measured with commercially available "sandwich-type" enzyme immunoassay kits according to the manufacturer's instructions (CLB, Netherlands). Plasma samples were put into polystyrene microtiter wells bounded with monoclonal anti-human cytokine antibody. Subsequently, a biotinylated second monoclonal antibody to human cytokine was added after the plasma samples, followed by horseradish peroxidase conjugated streptavidin. A substrate solution was added and absorbance was measured in a microtiter plate reader. The cytokine concentation were determined by interpolation with the standard curve. The detection levels were 3.0, 0.4, 3.0, 3.0 pg/ml for TNF-α, IL-6, IL-8 and IL-10, respectively.

Plasma myeloperoxidase (MPO) was analysed by radioimmunoassay techniques (Pharmacia, Sweden) as a parameter for perioperative leukocyte activation. Leukocyte counts were taken (ADVIA 120, Bayer, Tarrytown, New York, USA) the day before, and 6 and 20 h after the operation. Creatine kinase cardiac isoenzyme (CK-MB) release was analysed 3 times in each patient: 6 h after reperfusion, and on the 1st and 2nd postoperative days. The investigators in the laboratory were blind to the study.

4. Hemodynamic measurements and data collection

Hemodynamic monitoring comprised measurements of heart rate (HR), mean arterial pressure (MAP), mean pulmonary artery pressure (MPAP), pulmonary capillary wedge pressure (PCWP) and cardiac output. Derived cardiovascular variables such as cardiac index (CI), systemic vascular resistance index (SVRI) and

pulmonary vascular resistance index (PVRI) were calculated using standard formulas. All measurements were based on the thermodilution technique. Hemodynamic measurements and calculations were collected at 3 time-points: 1) baseline value, before anesthesia induction; 2) 15 minutes and 3) 6 hours after the end of CPB

5. Off-pump CABG

In the non-CPB group, after median sternotomy, coronary grafting was performed on a beating, normothermic heart. To dampen the movement of the beating heart and consequently to isolate the region for anastomosis, a custom-made U-shaped stabilizer was used. By means of vessel loops segmentary occlusion of the coronary artery to a length of 2 to 3 cm was effected to control bleeding during anastomosis. Proximal anastomosis of the aorta was performed using tangential clamping. Postoperative treatment in the intensive care unit was standardized and similar for both groups. Anesthesia for the off-pump operation was similar to that in conventional CABG except that Rocurouium was used as a muscle relaxant, with less fentanyl. Esmolol was given where necessary to reduce the heart rate.

1. Aprotinin protocol

In the aprotinin group, 2×10^6 KIU (280 mg) of aprotinin (Trasylol; Bayer AG, Leverkusen, Germany) was added to the priming solution in the extracorporeal circuit.

7. 17β-estradiol protocol

Patients randomized into the E_2 group were given 17β-estradiol (Progynova®, Schering AG, Berlin, Germany) 2 mg orally twice, at six o'clock in the evening before the operation and 1 hour before transfer to the operating room. Other routine pre-operative medication for anesthesia was the same in both groups.

8. Adenosine protocol

Routine preoperative medication for anesthesia was the same in both groups. The adenosine (ADO) group (n = 15) received an infusion of Adenoscan ® (Sanofi Winthrop, France) prior to initiation of CPB (after complete cannulation of appropriate vessels and before administration of cardioplegic solution) through a Swan-Ganz catheter to the superior vena cava using a computer-controlled pump infusion system. The initial infusion rate was a 50 μg/kg increment to the dose of 100μg/kg per minute at the second minute, whereafter the infusion lasted for 6 minutes or until the patient developed a systolic arterial pressure (SAP) < 70 mmHg. Three minutes after completion of adenosine infusion, the CPB machine was started. In patients with immediate hypotension (SAP < 70 mmHg) resulting from adenosine infusion, the infusion was stopped and CPB initiated at once.

9. Statistical analysis

Statistical analysis was carried out using SPSS software. Mann-Whitney U test was used to distinguished demographic differences between the groups. Continuous variables were analysed by ANOVA for repeated measures, which included the baseline value, if available, as a covariate. Logarithmic transformation was used when the variables were not normally distributed. The post-hoc testing, which evaluated the difference between groups in different time-points, was carried out calculating 95% confidence intervals, where the square root of the ANOVA mean square error was used as standard deviation. The calculation was made using CIA (confidence interval analysis) software. Correlations between variables were assessed using correlation coefficients. Statistical significance was attributed to *p* values lower than 0.05.

RESULTS

1. Cytokine release after low-risk CABG

In all studies, only traces TNF-α (lower than the lowest standard, 3.0 pg/mL) were detected in most of the patients perioperatively (data not shown) (I, II, III, IV, V).

Plasma levels of IL-6 were higher at all time-points as compared to preoperative levels, the highest increase being recorded at 4 hours after reperfusion. IL-8 levels were higher than baseline at 5 minutes and 1 and 4 hours after reperfusion. However, IL-8 levels at 5 minutes and 1 and 4 hours after reperfusion remained at the same increased level when compared to the baseline values. IL-10 levels rose at 5 minutes and at 1 hour after reperfusion, the highest level being reached at 1 hour after reperfusion (I) (Fig. 2).

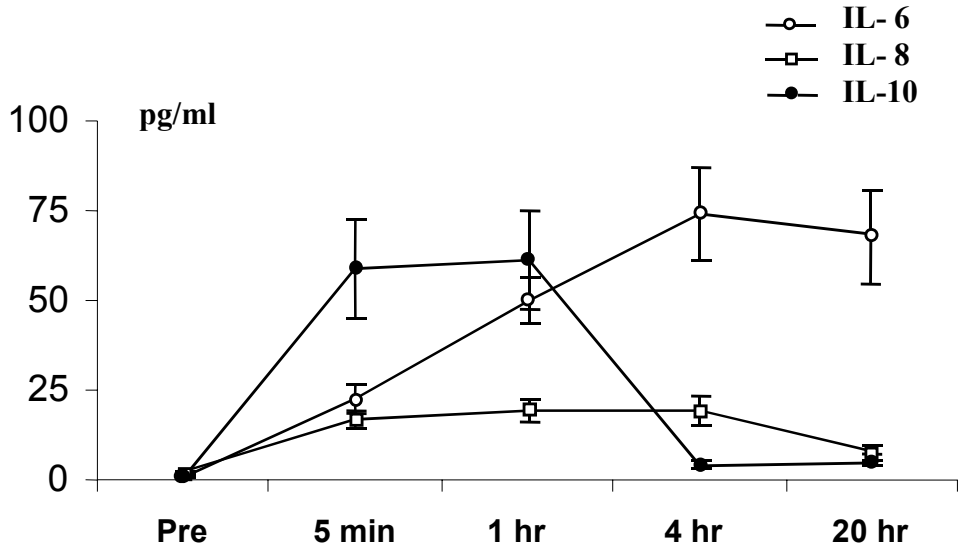

Figure 2 Perioperative plasma levels of IL-6, IL-8 and IL-10. Before anesthesia induction (Pre), 5 minutes and 1, 4 and 20 hours after reperfusion of the myocardium. Data are shown in mean ± standard error of the means.

It was found that plasma cytokine levels at 1 hour after reperfusion correlated with the maximum postoperative CK-MB value (IL-6, $r = 0.587$, $p < 0.01$; IL-8, $r = 0.460$, $p < 0.05$; and IL-10, $r = 0.570$, $p < 0.05$) (I). After CPB, cardiac indexes were improved or unchanged in 8 patients (Group 1) and decreased in 12 (Group 2) compared to the index before anesthesia induction. Mean changes in cardiac indices

were 0.68 ± 0.44 and -0.58 ± 0.54 L/min/m^2 in groups 1 and 2 respectively. Though none of the differences reached statistical significance, the plasma levels of IL-6 and IL-8 were lower at all study time-points in group 1 than in group 2. The absolute CI value did not correlate with circulating levels of measured cytokines perioperatively (Table 12) (I).

Table 12 Perioperative cytokine release in patients with different changes in cardiac index after cardiopulmonary bypass

Time-points	Group 1 (n = 8)	Group 2 (n = 12)
Before induction		
IL-6 (pg/ml)	0.73 ± 0.26	0.80 ± 0.33
IL-8 (pg/ml)	1.81 ± 0.97	1.86 ± 1.02
IL10 (pg/ml)	0.62 ± 0.59	0.75 ± 0.66
5 minutes after reperfusion		
IL-6 (pg/ml)	18.79 ± 15.82	23.79 ± 19.81
IL-8 (pg/ml)	14.62 ± 10.11	18.56 ± 9.70
IL10 (pg/ml)	52.09 ± 70.91	62.74 ± 54.56
1 hour after reperfusion		
IL-6 (pg/ml)	39.81 ± 17.62	56.59 ± 32.79
IL-8 (pg/ml)	16.02 ± 10.33	20.79 ± 13.33
IL10 (pg/ml)	74.18 ± 88.06	53.75 ± 32.40
4 hours after reperfusion		
IL-6 (pg/ml)	57.09 ± 25.42	88.63 ± 43.19
IL-8 (pg/ml)	13.82 ± 9.69	22.76 ± 15.91
IL10 (pg/ml)	2.19 ± 2.63	5.12 ± 5.24
20 hours after reperfusion		
IL-6 (pg/ml)	66.03 ± 55.97	68.75 ± 51.03
IL-8 (pg/ml)	7.17 ± 2.83	9.13 ± 5.78
IL10 (pg/ml)	5.67 ± 5.24	3.77 ± 2.60

Data are shown as mean ± standard deviation
Group 1, patients with improved or unchanged cardiac index after CPB
Group 2, patients with a decrease in cardiac index after CPB

2. Cytokine responses in CABG with or without CPB (II)

Nine patients underwent off-pump revascularization and 13 CABG with CPB. The duration of aortic cross-clamping and perfusion in the CPB group was 79 (mean, interquartile, 63, 88) and 95 (mean, interquartile, 82, 116) minutes, respectively (Table 1). There were no deaths or major complications in either group. Operation times were 230 (mean, interquartile, 205, 240) and 180 (mean, interquartile, 150, 193) minutes for the CPB group and off-pump group, respectively.

Though ANOVA for repeat-measurement showed the release of IL-6 to be similar in both groups, a delayed elevation was observed in the off-pump group (5 minutes, 16.7 (10.6, 40.0) versus 8.3 (5.4, 10.8) pg/mL, $p<0.05$) (Fig. 3A). Plasma IL-8 and IL-10 were significantly higher in the CPB than in the off-pump group (IL-8, $p = 0.006$; IL-10, $p = 0.001$, ANOVA for repeat measurement, Fig. 3B and 3C).

CK-MB levels in the off-pump group were lower than those in the CPB group 6 and 24 hours after declamping ($p = 0.001$, ANOVA for repeated measurement, Fig. 3D). CK-MB 6 hours after CPB correlated with cytokine values 5 minutes (IL-6, $r = 0.516$, $p = 0.01$; IL-8, $r = 0.495$, $p = 0.02$; IL-10, $r = 0.689$, $p < 0.001$) after reperfusion to the myocardium.

3. Effect of low-dose aprotinin on inflammatory response (III)

Though the result was not statistically significant, 24-hour total blood loss (833.0 ± 257.3 versus 666.4 ± 258.7 ml, $p = 0.13$) and maximum CK-MB levels (38.0 ± 19.0 versus 51.8 ± 10.1 U/L, $p = 0.09$) were reduced in the aprotinin group compared with the controls. The CRP value in the 1st day after surgery was similar in both groups. No postoperative myocardial infarction was observed in any of the patients. Preoperative leukocyte counts were not different between the groups. After the operation, significant increases in leukocyte counts were observed in both groups, reaching peak levels at 6 hours after reperfusion. The peak level was lower in the aprotinin group (9.3 ± 0.58 versus 11.2 ± 0.68 $\times 10^9$/L, $p = 0.01$), levels returning toward baseline after 20 hours (Fig. 4A).

Significant increases of IL-8 were observed at 5 minutes, 1 hour and 4 hours after reperfusion in both groups ($p< 0.05$), but levels returned toward baseline 8 hours after reperfusion. Mean IL-8 was two-fold higher in the controls than in the aprotinin group at 5 minutes (28.1 ± 9.2 vesus 12.0 ± 1.5 pg/ml, $p = 0.28$) after reperfusion. In the later measurements of IL-8 levels, no significant group differences emerged (Fig. 4B). Plasma IL-10 increased significantly at 5 minutes (49.6 ± 24.9 vesus 8.13 ± 2.8 pg/ml, $p = 0.01$) and at 1 hour (42.0 ± 10.1 vesus 23.7 ± 8.8 pg/ml, $p = 0.16$) after reperfusion in the controls, but decreased rapidly thereafter (Fig 4C). No significant differences were noted between the groups in serial IL-6 measurements perioperatively.

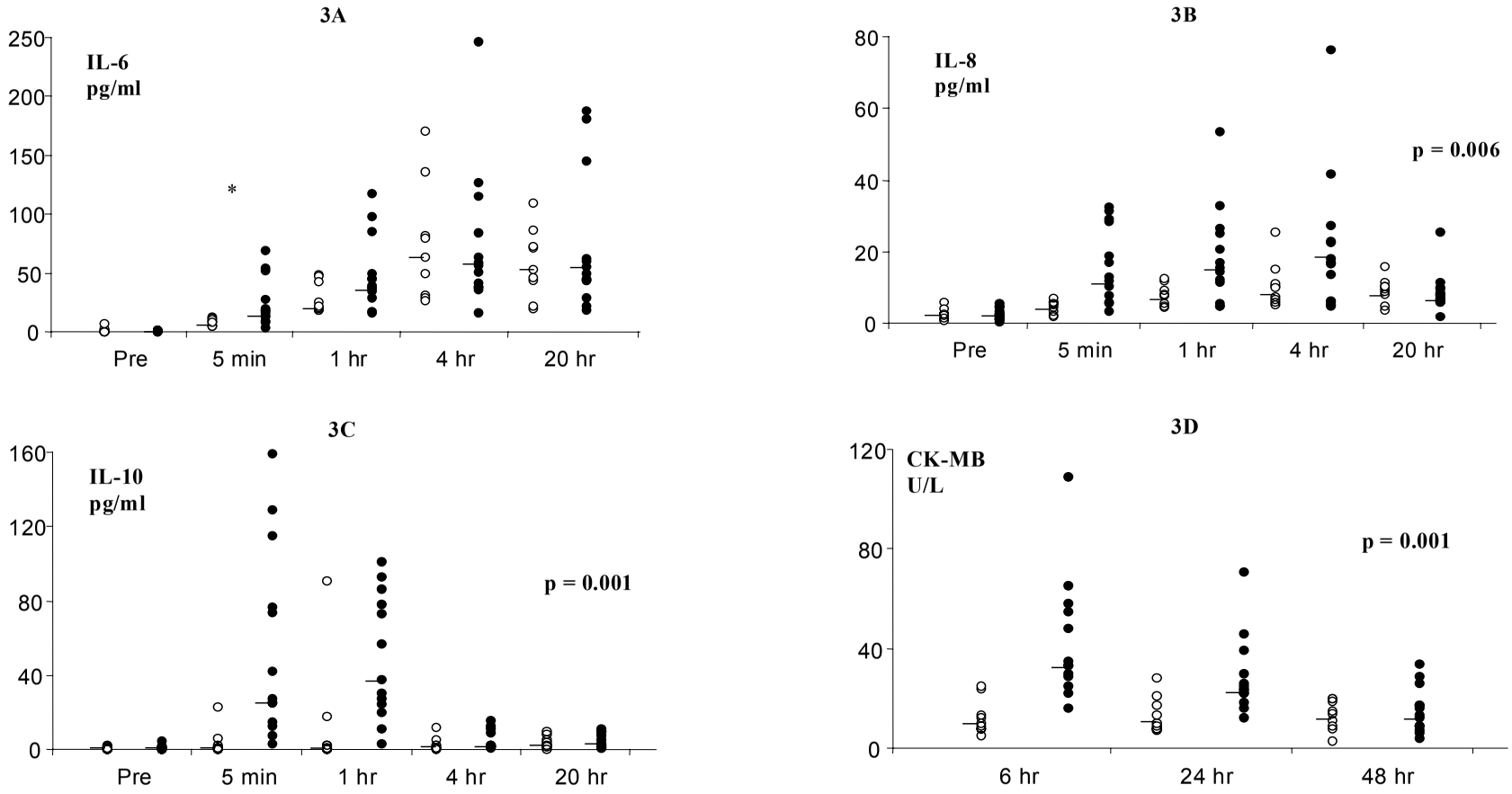

Figure 3 Systemic CK-MB and cytokine levels in patients undergoing CABG with CPB (black circles, n = 13) or without CPB (white circles, n = 9). The lines indicate the medians. * $p<0.05$, difference between groups at the same time-point. Sampling time-points: before anesthesia induction (Pre) and time after reperfusion to the myocardium

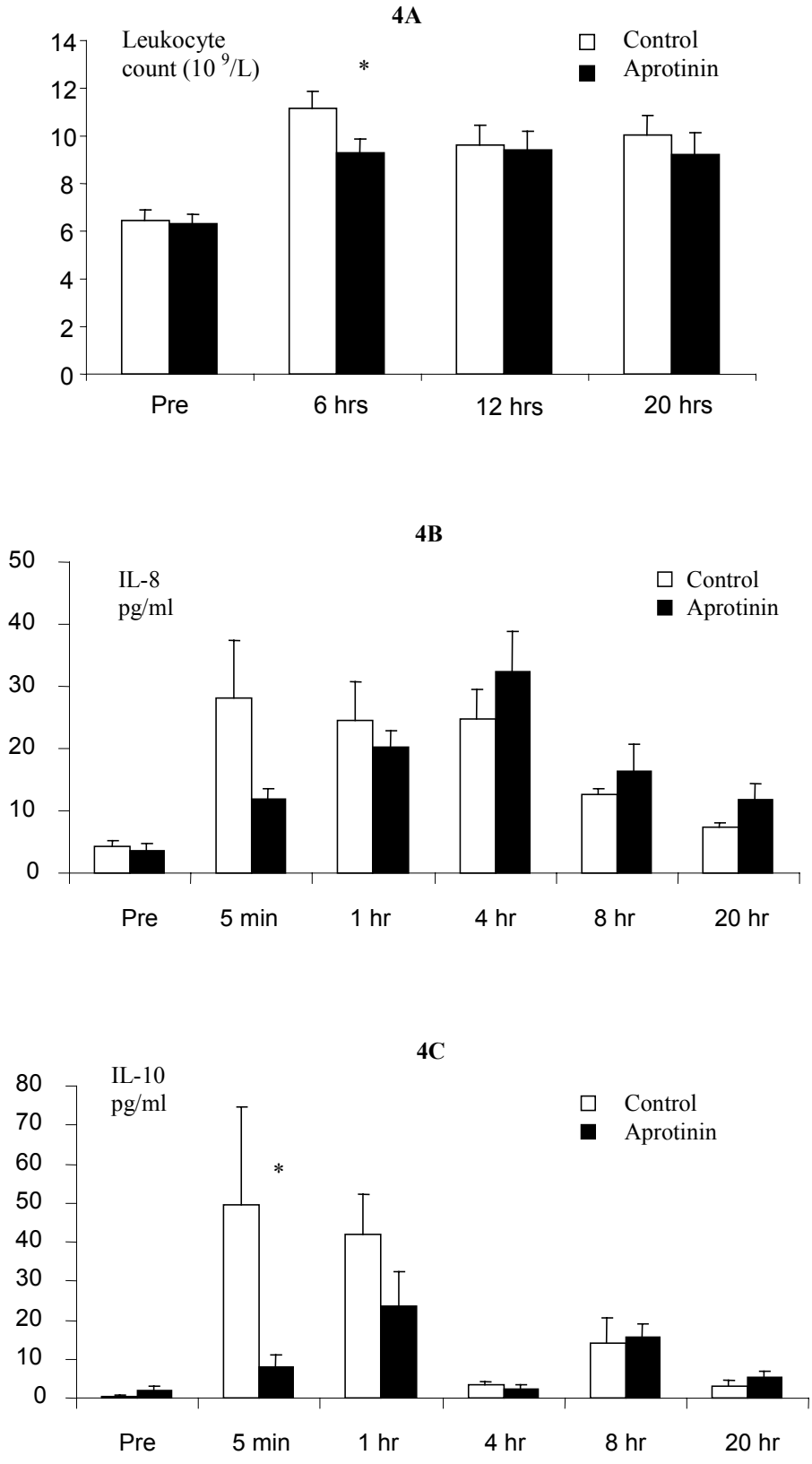

Figure 4 Changes in leukocyte count, IL-8 and IL-10 over time (Mean ± SEM). * $p < 0.05$, compared to the control group.

4. Anti-inflammatory effect of 17β-estradiol in CABG (IV)

Leukocyte counts rose after the operation in both groups. However, the counts were lower in the E_2 group than in controls at 6 (11.4 ± 2.0 versus 15.5 ± 4.7 $\times 10^9$/L) and 20 hours (11.6 ± 1.9 versus 13.6 ± 2.5 $\times 10^9$/L) after reperfusion (ANOVA for repeated measures, p = 0.03, Fig. 5). The release of MPO reached a peak level 5 minutes after reperfusion, with another peak level appearing at 4 hours after reperfusion. Both peaks were lower in the E_2 group than in controls (5 minutes, 634.4 ± 213.1 versus 773.1 ± 209.3 μg/ml; and 4 hours, 305.0 ± 108.0 versus 441.3 ± 191.6 μg/ml) (ANOVA for repeated measures, p = 0.02, Fig. 6).

Levels of IL-6, IL-8 and IL-10 were raised after reperfusion, and similar cytokine responses were seen in both groups. None of the differences reached statistical significance in respect of TNF-α, IL-6, IL-8 and IL-10. No differences were found between the groups in CK-MB levels.

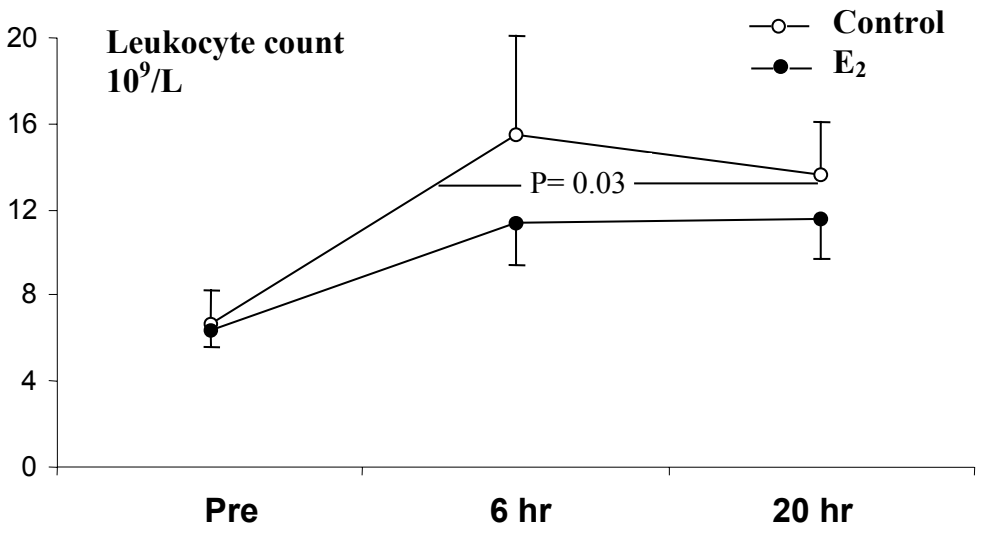

Figure 5 Leukocyte counts after reperfusion in the E_2 group and the controls. Data are mean ± standard deviation. Sampling time: the day before operation (Pre), and 6 and 20 hours after operation.

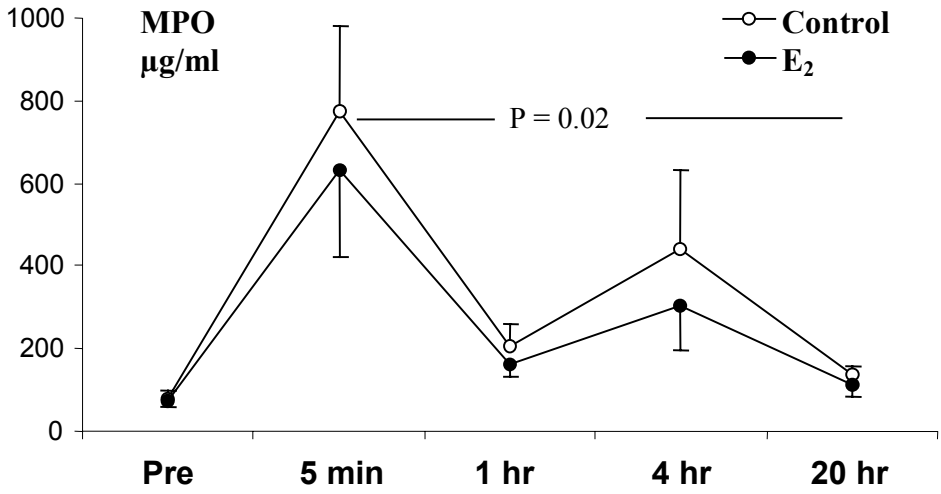

Figure 6 Perioperative plasma myeloperoxidase (MPO) levels in the E_2 group and the controls (Data are mean ± standard deviation). Sampling time: before anesthesia induction (Pre), 5 minutes and 1, 4, and 20 hours after reperfusion.

5. Adenosine pretreatment in CABG (V)

Fifteen patients were pretreated with adenosine, another 15 serving as controls. There was no major complication in any of the 30 patients who completed the study. Four of the 15 patients who received adenosine could not finish the full dosage owing to resultant profound hypotension (SAP < 70 mmHg) during infusion. Their average time for adenosine infusion was 4.63 ± 0.48 minutes, and the total dose in these cases was 412.5 ± 47.87 µg/kg (63.5% of the full dosage).

Adenosine patients released significantly less CK-MB than the controls postoperatively ($p = 0.007$, Fig. 7). Maximum CK-MB was also lower in the adenosine group (41.9 ± 12.3 versus 75.1 ± 50.0 U/L, $p = 0.013$).

Mean systemic arterial pressure decreased and HR increased after the operation. The changes in HR, MAP, CVP, MPAP and PCWP were closely identical in both groups. The recovery of CI was better in the adenosine group than in the controls ($p = 0.039$, Table 13).

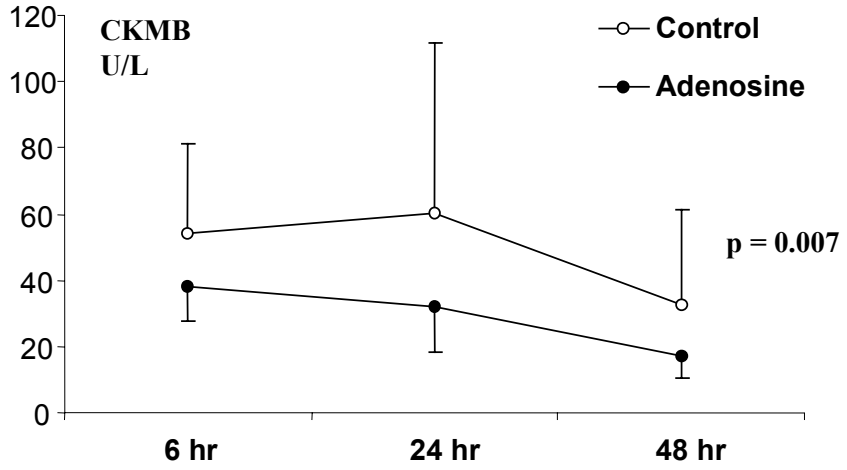

Figure 7 Postoperative CK-MB in CABG patients. Data are mean ± standard deviation. Lower CK-MB was seen in the adenosine group (ANOVA for repeated measurement p = 0.007).

Though leukocyte counts were lower in the adenosine group than in the controls at all sampling time-points, the difference did not reach statistical significance. Transcoronary neutrophil differences were similar in both groups before the start of CPB (0.06 ± 0.19 versus 0.05 ± 0.10 10^9/L). After 1 and 10 minutes of reperfusion the transcoronary leukocyte difference in the controls was higher than that in the adenosine group (0.11 ± 0.35 versus 0.07 ± 0.35 10^9/L at 1 minute, and 0.18 ± 0.55 versus 0.12 ± 0.57 10^9/L at 10 minutes); again, however, the differences were not statistically significant. There was a trend for myocardial leukocyte sequestration to be less in the adenosine group than in the controls.

Plasma levels of IL-6, IL-8 and IL-10 increased after reperfusion. Similar cytokine responses were seen in both groups. None of the differences in IL-6, IL-8 and IL-10 reached statistical significance.

Table 13 Hemodynamic data

	Baseline	15 min after CPB	6 hours after CPB	20 hours after CPB
HR (beats/min)				
Control	59.9 (12.4)	78.8 (8.8)	90.3 (14.6)	84.3 (14.6)
Adenosine	54.7 (8.7)	75.0 (17.7)	87.6 (16.1)	82.3 (11.5)
MAP (mmHg)				
Control	89.4 (8.0)	73.6 (8.0)	79.4 (17.7)	78.4 (15.0)
Adenosine	91.6 (12.5)	72.6 (11.2)	84.0 (13.1)	70.2 (12.4)
MPAP (mmHg)				
Control	18.4 (2.3)	17.6 (3.4)	20.4 (4.5)	20.0 (4.6)
Adenosine	19.1 (4.5)	22.4 (2.9)	22.8 (5.4)	20.8 (4.1)
PCWP (mmHg)				
Control	11.6 (2.6)	10.6 (2.3)	9.0 (3.1)	10.8 (3.1)
Adenosine	12.1 (2.9)	13.3 (2.0)	10.8 (3.0)	10.4 (2.4)
CVP (mmHg)				
Control	7.4 (2.5)	10.1 (2.1)	8.9 (2.2)	8.4 (3.1)
Adenosine	8.3 (2.3)	10.9 (2.1)	9.8 (2.4)	9.1 (2.8)
CI ($L/min/m^2$)				
Control	2.6 (0.5)	2.7 (0.5)	3.2 (0.7)	3.2 (0.8)
Adenosine*	2.4 (0.3)	3.3 (0.8)	3.1 (0.5)	3.8 (0.7)

Data are presented as mean (SD), * p =0.039

DISCUSSION

1. Methodological considerations

In most studies of surgical patients, cytokine measurements are taken from peripheral blood. The present studies used ELISA to measure peripheral plasma cytokines (TNF, IL-6, IL-8, IL-10) in low-risk CABG patients. Only traces TNF-α were detected perioperatively in most of the patients, an observation in keeping with some but not all previous reports. This may in part be due to the different sensitivity and specificity of the assays (Engelberts I et al. 1991). The presence of sTNFR may also affect TNF assays in their ability to detect TNF (Engelberts I et al. 1991). However, failure to detect an increase in the plasma concentration of TNF-α does not imply that it is not activated, as TNF-α is released locally in paracrine manner. TNF-α has a short biological half-life in the circulation (Fong Y and Lowry SF 1990), which may explain why it is difficult to find it after uncomplicated surgery. Compared with TNF-α, sTNFRI and sTNFRII are stable substances with relatively long half-lives (Tracey KJ et al. 1990). Shedding of soluble receptors from the entire cell surface can be induced by binding only 1-5% of cell surface receptors by TNF-α (Curtis GE et al. 1995). The sTNFR response may thus be substantial with minor stimulation. Hence, the plasma concentration of TNF-α would not seem to be an acceptable parameter of activation of this cytokine pathway after low-risk CABG.

The cytokine assays applied have an inherent sensitivity limit. Cytokines are characterized by tight gene control, short duration of action and an autocrine or paracrine rather than an endocrine mode of action, as opposed to hormones, thus affecting only the immediate environment. Plasma cytokine levels may thus not properly reflect local cytokine production. The present study, based on systemic sampling, may have yielded a lower sensitivity of detection than studies examining intracellular cytokine production or cytokine mRNA transcription levels in appropriate target tissues. Regional myocardial cytokine release may be more important in the mechanism involved in postoperative myocardial dysfunction.

The myocardium has been reported to be a major source of cytokines after CABG. Myocardial cytokine release was proved to be significant 1 hour after operation and to last up to 18 hours after (Liebold A et al. 1999). Transcoronary cytokine difference is

one of the important parameters in evaluating the anti-inflammatory effect of different strategies. However, we could not leave a coronary sinus catheter in patients after operations in our hospital; coronary sinus samples could be taken only during a short period after aortic declamping, and previous studies have shown that there are no significant transcoronary cytokine differences shortly after reperfusion (Wan S et al. 1996B, Karube N et al. 1996). Hence, the present studies did not measure transcoronary cytokine differences.

Many factors such as sex, age, medication, health conditions, myocardial ischemic time and CPB temperature may also affect cytokine levels. Gender has been suggested as a risk factor for the CABG operation in some studies, and in our hospital most female patients undergoing CABG use HRT treatment, which has been thought to have some effect on cytokine release. The present study chose only male patients to facilitate comparison in postoperative cytokine release.

It has been reported that cytokine release is associated with the myocardial ischemic time (Wan S et al. 1997B). We therefore used 120 minutes as one of the exclusion criteria to ensure that the ischemic time would be similar between groups. Whenever the ischemic time was over 120 minutes, we stopped the study and excluded the patient. The length of ischemic time has been related to the releases of IL-6 and IL-8. In our study, the ranges of both aortic cross-clamping time and CPB time were narrower than in other studies. For this reason we could not show any significant relationship between cytokine levels and either myocardial ischemic or CPB time.

We have found in a previous study that patients with surgical bleeding who need re-exploration have extremely high plasma interleukin levels during the study period (data not reported) and this would lead to observation bias. Hence, we decided to exclude all patients who needed a re-exploration during the study period. It has been reported that preoperative cardiac dysfunction is associated with higher postoperative cytokine release (Deng MC et al. 1996). We excluded patients with poor cardiac function and anti-inflammatory drugs such as corticosteroids; aprotinin (except in study III) was not used during the study period, and tepid CPB was used in all patients with CPB.

It has been reported that higher circulating endotoxin and TNF concentrations are to be detected in elderly than in younger patients, and myocardial performance in elderly patients after cardiopulmonary bypass is suppressed by TNF (te Velthuis H et

al. 1995). However, a recent study of patients undergoing CABG surgery with CPB and comparable perioperative management evinced no significantly different cytokine responses in the two age groups (Roth-Isigkeit A et al. 1998). In the present study, there was no age difference between groups. The present series limited the study population to male low-risk CABG patients with similar ischemic time, and standard anesthetic and surgical technique would lead to least bias in cytokine comparisons.

Norepinephrine (NE) has been found to inhibit lipopolysaccharide-induced TNF-α and IL-6 production in human whole blood. In acute sepsis, enhanced release of NE may be part of a negative feedback mechanism meant to inhibit ongoing TNF-α and IL-6 production (van der Poll T et al. 1994). β-adrenergic receptor agonists inhibit the production of TNF-α in a number of cell types (Newman WH et al. 2000). However, other studies have shown that NE and adrenalin enhance cytokine release from some other cell types (Liao J et al. 1995). The total effect of NE and adrenalin on the whole body inflammatory response after CPB is not yet clear.

Recently, the use of troponin (cTn) I and T has been suggested to be better markers for myocardial injury. However, there is little differences among cTnI, cTnT and CKMB after CABG to diagnose myocardial damage as assessed by new Q wave on the electrocardiogram (Bonnefoy E et al. 1998).

2. Cytokine responses in CABG and association with myocardial injury

Positive correlations have been thought to prevail between cytokine levels and the incidence of postoperative complications (Table 12). Studies I and II confirmed the previously reported cytokine responses after cardiac surgery, a finding recorded in varying degrees in other studies, and showed that circulating cytokine levels indeed correlated with a postoperative increase in CK-MB. Plasma levels of IL-6 and IL-8 were higher in patients with decreased cardiac index after CPB. This may indicate that the degree of cytokine production is partially related to the degree of myocardial injury during CABG, although a causal relation remains to be established.

Though none of the differences reached statistical significance, study I showed that higher levels of IL-6 and IL-8 were associated with a decrease in CI after CPB. This is in accordance with a previous result obtained when changes in transesophageal echocardiographic wall motion scores (WMS) were used as end point (Hennein HA et al. 1994). Noteworthy was a trend towards higher cytokine levels in patients with

diminshed postoperative myocardial function. In human cardiac tissue IL-6 and TNF-α have a negative inotropic effect (Finkel MS et al. 1992). The correlation between systemic cytokine elevation and myocardial ischemic injury and postoperative cardiac function suggest that proinflammatory cytokines may be among the many variables which affect postoperative cardiac function.

The myocardium has been shown to be a major source of IL-6 and TNF-α after CABG, but systemic cytokine levels are the result of overall cytokine release from different tissues. The plasma cytokine level may thus not properly reflect local cytokine production. The potential effects of myocardial cytokine production on postoperative myocardial dysfunction needs to be verified in further studies. However, an inflammatory response after CABG in patients with a high degree of comorbidity such as advanced lung or kidney disease represents a high risk of postoperative organ dysfunction (Cremer J et al. 1997). Lower cytokine responses and less serious myocardial injury might be of special importance for postoperative recovery in these patients.

It is noteworthy that cytokines are likely to act both individually and within a complex network of interrelated and interacting signals (Leeuwenberg JF et al. 1994). A transient elevation in IL-10 after CABG was observed in patients undergoing the conventional operation. This occurred simutaneously with an elevation in IL-6 soon after reperfusion. IL-10 is regarded as an anti-inflammatory cytokine. The rise in the plasma pro-inflammatory cytokines is balanced by this anti-inflammatory cytokine response and this effect was also evident in our study. The positive relation between IL-10 and maximum CK-MB may have been brought out by the balance of pro- and anti-inflammatory cytokine responses, and this balance may indeed be more important in determining the extent of the inflammatory response and the clinical outcome.

3. Anti-inflammatory strategies

3.1 Off-pump technique

Myocardial injury

The off-pump group here had lower CK-MB levels compared with the CPB group. This would imply that the off-pump technique with sternotomy is associated with lesser injury to the myocardium, since the extent of surgical trauma was similar. As noted above, the myocardium is a major source of cytokine release after CABG, and

the release of IL-6 and IL-8 has been shown to be related to the myocardial ischemic time (Wan S et al. 1997B). The earlier rises in cytokine levels in the CPB group correlated with elevated CK-MB. This earlier increase in cytokines in the CPB group soon after reperfusion might be due to the global myocardial ischemia-reperfusion injury, while only regional myocardial ischemia was observed in off-pump revascularization.

Cytokine responses

Excessive activation of the inflammatory process might be avoided through minimally invasive surgery. Compared with conventional CABG, off-pump revascularization is associated with reduced cytokine responses and less severe myocardial injury. However, the difficulty remains of separating the impact of CPB from the general immune response prompted by anesthetic and surgical trauma. Conventional CABG is accompanied by a longer myocardial ischemia and operation time, in addition to the use of CPB compared to ”off-pump” CABG. A reduction in cytokine response has been found in MIDCAB via minithoracotomy (Gu YJ et al. 1998, Strüber M et al. 1999). The present off-pump patients were operated via a median sternotomy. In agreement with the findings reported by Wan and colleagues (1999), the present results showed a delayed IL-6 release and statistically significantly lower IL-8 and IL-10 levels in the off-pump patients. It seems that IL-6 is not influenced by the use of CPB, while IL-8 and IL-10 response are depended more on the use of CPB. This was also proved by a recent study showing that IL-6 release were not different among CPB, off-pump and MIDCAB groups throughout the entire period (Diegeler A et al. 2000).

Many factors attending the use of CPB, for example surgical trauma, ischemia-reperfusion to the organs, changes in body temperature, release of endotoxin and the exposure of blood to nonphysiologic surfaces and conditions, may be reponsible for the rapid initial rise in cytokines (Cremer J et al. 1997). It has been shown that complement activation occurs in coronary surgical patients operated on without CPB, and it is suggested that clinical consideration of complement inhibition during heart operations should include the contribution of tissue injury in addition to blood-material interaction in the heart-lung machine (Gu YJ et al.1999). It should be noted that apart from CPB itself, the procedures compared differed in the degree of coronary artery revascularization and the duration of the operation. The results might thus be influenced not only by CPB but also by longer operating and anesthesia time, and

cardioplegic arrest. Further studies are warranted focusing on stepwise analysis of the influence of different aspects associated with the CPB procedure.

3.2 Aprotinin

Serine protease inhibitors have the potential to moderate the inflammatory response to CPB by regulating cytokine release and leukocyte activation. (Royston D, 1996). Several studies have indicated that low- (Hill GE et al. 1995) and high-dose aprotinin (Hill GE et al. 1996) has anti-inflammatory effects during and after CPB. However, a recent study has shown that aprotinin in pump prime only fails to modify cytokine response after CPB (Ashraf S et al. 1997). In keeping with this observation, the present results show no differences between the aprotinin and control groups with regard to systemic proinflammatory cytokine levels. This could be due to a dose-dependent phenomenon (Table 7).

A statistically significant early reduction in IL-10 was observed 5 minutes after reperfusion in the aprotinin group when compared with the controls. The exact mechanism involved here is not clear, but the lower mean IL-8 in the aprotinin group at 5 minutes after reperfusion (half of the level in the controls) might be responsible for this reduced early IL-10 response after reperfusion. This also calls for a further more extensive series with more sampling time points to verify the effect of pump prime aprotinin on systemic and myocardial cytokine release after cardiac surgery.

Neutrophil-endothelial adherence is a fundamental (”final common pathway”) step in the inflammatory response after CABG. Recent research has established that the effect of aprotinin is mediated through platelet-leukocyte adhesion or interaction. This particular mechanism may be the key to an understanding of the overall mechanism of action of aprotinin, establishing a link between hemostasis and the anti-inflammatory interaction of this agent (Primack C et al. 1996). Our results showed a significantly lower leukocyte count in the aprotinin group compared with the controls. Seghaye and colleagues found that low-dose aprotinin affects neutrophil mobilization but not white blood cell degranulation related to CPB (Seghaye MC et al. 1996C). Though cytokine production was similar in the groups, this would suggest that pump prime aprotinin might have an anti-inflammatory effect.

Pump prime aprotinin is technically simple to administer and confers substantial blood-saving, equivalent to that achieved with the high-dose protocol. It is favored as

a potentially safer and a more cost-effective alternative to the high-dose regimen. In addition to being effective in reducing post-CPB bleeding and transfusion requirements, pump prime only aprotinin modulates the CPB-induced up-regulation of neutrophil CD11b integrin, an important indicator of the systemic inflammatory response to CPB. Such low-dose regimens can be effective both therapeutically and in terms of cost (Alonso A et al. 1999). Pump prime is convenient and further aprotinin use might be according to the circulating cytokine levels after CPB. However, in cases with predisposing factors for graft occlusion aprotinin should be used with caution.

3.3 17β- estradiol

Anti-inflammatory effect

Though the mechanism involved is as yet unclear, our present results show that 17β-estradiol pretreatment can limit leukocyte activation. Estrogen is believed to possess anti-inflammatory properties. Estrogen treatment has been associated with a decrease in wound elastase levels secondary to reduced neutrophil numbers, and reduced fibronectin degradation (Ashcroft GS et al. 1999). Animal studies have suggested that infiltration of neutrophils in the endotoxin-induced glomerular inflammatory response is under the control of estradiol (Faas MM et al. 1999), and recent research has demonstrated that estradiol reduces LPS-induced IL-6 and TNF-α production. This inhibition of cytokine production could have a profound effect on the immune response during inflammatory reaction (Deshpande R et al. 1997).

A recent study with an animal model has also shown that acute administration of 17β-estradiol protects against myocardial reperfusion injury (Delyani JA et al. 1996). Short-term estrogen has also been seen in an animal model to reduce myocardial infarct size (Snabes MC et al. 1997). 17β-estradiol may act directly on the myocardium, triggering a protective adaptive response independent of its vascular effect (Sbarouni E et al. 1998). It may also play a role in maintaining myocardial protein synthesis.

Recent studies have shown that the possible mechanism by which 17β-estradiol exert its cardioprotective effects consists in limiting the inflammatory response. Results of animal studies suggest that 17β-estradiol, by inhibiting TNF-α production, limits the deleterious intercellular adhesion molecular (ICAM)-1-mediated binding of

leukocytes to the injured myocardium and protects against myocardial ischemia-reperfusion injury (Squadrito F et al. 1997). One study in women with coronary artery disease found a significant increase in all circulating cellular adhesion molecules (cCAMs) in men and postmenopausal women not receiving HRT, as compared to women receiving HRT (Caulin-Glaser T, 1998). Our present clinical data showed that 17β-estradiol pretreatment limited leukocyte activation after CABG. Leukocyte counts and circulating MPO levels were significantly lower in the E_2 group than in controls. Though no difference was found in clinical outcome between groups in the present trial with only low-risk patients, this phenomenon might be more important when patients with higher risk are operated.

Studies in humans have generally supported the conception of an inhibitory effect of estrogens on TNF-α production. After a period of months on HRT, TNF-α, and thromboxane B_2 have been found to be decreased (Aune B et al. 1995). TNF-α inhibition has also been seen in unstimulated monocytes from postmenopausal women on HRT (Ralston SH et al. 1990). Loy and colleagues (1992) demonstrated a dose-related decrease in TNF-α messenger RNA expression in monocytes from premenopausal women. Women pretreated with 17β-estradiol (sublingually) have proved able to exercise longer before signs of angina and myocardial ischemia appear (Rosano GM et al. 1993). Our data, on the otherhand showed no differences in peripheral blood cytokine levels. Further studies are necessary to address the effect of 17β-estradiol pretreatment on leukocyte sequestration and cytokine release in myocardium after CABG.

Hemodynamic effect

Animal and human studies have shown that administration of estrogens leads to a restoration of endothelial function, an increase in cardiac output and arterial flow velocity, and a decrease in vascular resistance (Lang U et al. 1997). Short-term hormone replacement therapy induces modest but significant increases in cardiac output, ejection fraction and left ventricular mass in healthy postmenopausal women (Sites CK et al. 1999). The present results showed lower SVRI and higher cardiac index in the E_2 group after CPB as compared to the preoperative data. The higher cardiac index might be due to the lower systemic vascular resistance in the E_2 group after CPB. The decrease in SVRI and slightly lower MAP in the E_2 group might for its

part be due to the vasodilatory effect of 17β-estradiol. The mechanism by which estrogen induces systemic vasodilatation is still not understood; it may reflect increases in endothelium-derived vasodilators or in the alterations and remodeling of vascular smooth muscle (Magness RR et al. 1998). The vasodilatory effects of estrogen might be mediated by enhanced bioavailability of nitric oxide (Lang U et al. 1997). However, it is difficult to compare the hemodynamic effect of 17β-estradiol in the present study, as the study population was small and some of the patients were given inotropic drugs; these effects may thus warrant further investigation.

Dosage

Unlike many drugs, exogenous estrogens have been used for decades without an understanding of their mechanism of action. Though there are several studies investigate the acute effect of estradiol (Table 8), the appropriate dosage of estradiol for cardioprotective purpose remains unknown. It proved to be safe for men in the present study, and resulted in diminished leukocyte activation in male patients after CABG. Unfortunately, the present study did not measure the circulating estradiol levels perioperatively. These levels during and after CPB may be of interest and importance.

3.4 Adenosine

Cardioprotective effect

Exogenous adenosine reduces infarct size, post-ischemic microvascular injury and time to ischemic contracture in isolated perfused rat hearts (Toombs CF et al. 1992). Blood cardioplegia supplemented with adenosine reduces postischemic left ventricular dysfunction and attenuates postcardioplegia dysfunction in ischemically injured dog hearts (Hudspeth DA et al. 1994). Experimental findings indicate that adenosine is most effective in protecting the reversibly injured heart when administered prior to ischemia. Adenosine pretreatment induces potent endogenous protection against subsequent ischemic stress in the human myocardium (Vinten-Johansen J et al. 1999). Leesar and colleagues have reported that 10-minute intracoronary adenosine preconditioning treatment significantly reduced ST segment changes during percutaneous transluminal coronary angioplasty (Leesar MA et al.

1997). There are also reports that intravenous adenosine administered to patients with acute myocardial infarction is well tolerated and may lead to increased salvage of ischemic tissue (Garratt KN et al. 1998).

Ischemia-reperfusion results in contractile dysfunction, necrosis and vascular injury. Myocardial stunning after CABG is associated with increased morbidity and mortality in patients with severe multivessel disease, and with reduced myocardial function. Adenosine has been shown to reduce experimental myocardial ischemic reperfusion injury in many species (Lasley RD, 1998, Vinten-Johansen J, 1999, Toombs CF et al. 1992). In agreement with other previous reports (Lee HT et al. 1995), the present results showed that ADO-pretreated patients had improved recovery in myocardial performance postoperatively, as indicated by faster recoveries of CI. Since the preload of the heart, manifested as CVP and PCWP, was similar in both groups, the improvement in cardiac performance might result from better recovery of contractility. Even though safety issues limited the dose, the present protocol resulted in improved post-ischemic cardiac performance and less CK-MB release after the operation.

Anti-inflammatory effect

Experimental studies have demonstrated that adenosine attenuates the adherence of neutrophiles to endothelial cells (Jordan JE et al. 1999) and myocytes (Bullough DA et al. 1996). Though the present results failed to show significant differences between the groups, there was a trend towards lower myocardial leukocyte sequestration in the adenosine group than in controls. Adenosine has a broad spectrum of physiological effects which make it suitable as a cardioprotective agent with efficacy in all three windows of opportunity (pretreatment, during ischemia and reperfusion) and against numerous targets, including the neutrophils. Preischemic adenosine treatment reduces experimental myocardial infarct size, but there is additional evidence to suggest that adenosine treatment during reperfusion may also reduce infarct size in that it reduces platelet and neutrophil adherence to the coronary endothelium. Intracoronary administration of adenosine after reperfusion has significantly reduced neutrophil and red blood cell stagnation in capillaries, and was associated with reduced infarct size and improved regional ventricular function in the ischemic zone (Jordan JE et al. 1997). Adenosine has potent antineutrophil effects which reduce lethal postischemic injury when given only at the onset of reperfusion. During reperfusion, endogenous

and exogenous adenosine may effect cardioprotection by inhibiting the neutrophils and endothelium (or their interaction) directly. An additional adenosine infusion during reperfusion could be included in future studies to maximize the inhibitory effects of adenosine on neutrophils, which may contribute to reperfusion injury.

One animal study has shown that adenosine reduces cardiac TNF-α production following ischemia-reperfusion (Cain BS et al. 1998). Adenosine may inhibit the release of the proinflammatory cytokines (IL-6 and IL-8) involved in the response to ischemia and reperfusion (Bouma MG et al. 1996), and enhance IL-10 secretion by stimulated monocytes (Le Moine O et al. 1996). However, the present results failed to demonstrate an effect of adenosine pretreatment on cytokine response after CABG. Possibly experimental findings under ideal and standardized circumstances may be difficult to repeat and observe in the clinical setting. Furthermore, systemic plasma cytokine levels may not properly reflect myocardial cytokine production (as discussed above).

Dosage

Adenosine is a well-known vasodilator, and its infusion causes hypotension. The infusion rate of ADO selected here was based on a pilot study showing that patients tolerate an infusion rate only up to 100 μg/kg/min prior to CPB. Several adenosine protocols have been used in CABG (Table 9). Our present protocol resembles that of Lee, but differed from that of Mentzer in that adenosine infusion was terminated 3 minutes before the initiation of cardiopulmonary bypass — a treatment mode referred to as adenosine preconditioning. Our dosage (650 μg/kg totally) was lower than the recommended safety dosage in patients with coronary artery disease (140 μg/kg/min for 6 minutes, 840 μg/kg totally) (Cerqueira MD, 1994). Belhomme and colleagues (2000) found that the full dosage of 700 μg/kg has no benefit in myocardioprotection. Our dosage is similar to theirs, but without CPB during adenosine infusion. The intravasal concentration in our patients was thus higher than in theirs.

In general, it is assumed that the intravasal concentration of adenosine causes vasodilation, whereas most of the direct protective effects of adenosine are attributable to the interstitial fluid concentration. A “threshold level”/”critical level” for the protective effect of adenosine has been proposed. However, this is difficult to determine as interstitial fluid levels of adenosine do not necessarily follow the intravasal levels. Unless both intravasal and interstitial levels are directly and

simultaneously measured with appropriate techniques, it remains difficult to determine what dosage is suitable for any given patient. In the present study, 4 of the 15 patients who received adenosine could not finish the full dosage. Analysis of these 4 patients showed that their myocardial enzyme release and cardiac performance were similar to those in other adenosine patients, and different from the controls. Adenosine pretreatment was thus still of benefit in these patients, even though fell short of the full dosage.

CONCLUSIONS

Based on the results obtained, the following conclusions were reached:

1. Pro-inflammatory (IL-6, IL-8) and anti-inflammatory (IL-10) cytokines are elevated after CABG. Increased systemic cytokine levels were associated with postoperative myocardial dysfunction.

2. Off-pump CABG is accompanied by less marked cytokine responses and less myocardial injury. Cytokine responses are associated with myocardial injury. Further studies focused on stepwise analysis of the influence of different aspects associated with the CPB procedure are warranted.

3. Pump prime aprotinin failed to limit cytokine response in circulating blood. This could be due to a dose-dependent phenomenon. The effect of aprotinin on leukocyte activation needs further evaluation.

4. 17β-estradiol pretreatment limits leukocyte activation after CABG, but the dose used in this study failed to limit systemic cytokine responses. A vasodilative effect of 17β-estradiol was observed after CABG, and this effect needs further investigation.

5. Adenosine pretreatment reduces myocardial injury and improves cardiac function after CABG, but the dose used in our patients failed to prevent systemic cytokine responses. Further study is warranted to define whether adenosine may attenuate neutrophil accumulation in the myocardium after CABG.

SUMMARY

It is well established that conventional coronary artery bypass grafting with cardiopulmonary bypass induces a systemic inflammatory response, but the mechanism involved is still less full understood. The present series of studies was designed to define the cytokine response and its relationship to myocardial dysfunction. The anti-inflammatory effects of aprotinin, estradiol and adenosine were evaluated in low-risk male CABG patients.

The finding confirmed that peripheral plasma levels of inflammatory cytokines IL-6, IL-8 and IL-10 are elevated after CABG with CPB, while off-pump CABG is accompanied by less marked cytokine responses and lesser myocardial injury. Further studies are warranted focusing on stepwise analysis of the influence of different aspects associated with the CPB procedure. There was a trend towards higher peripheral cytokine levels in patients with impaired postoperative myocardial function. Peripheral cytokine levels were correlated to postoperative myocardial injury. However, regional cytokine levels might be more important in the mechanism involved in myocardial dysfunction.

Pump prime aprotinin fails to limit the systemic cytokine respone after CABG. This could be due to a dose-dependent phenomenon. However, lower leukocyte counts and IL-10 release after reperfusion were found in the aprotinin group compared to the controls. The anti-inflammatory effect of pump prime aprotinin calls for further evaluation.

Pretreatment with two doses of 2 mg 17β-estradiol proved safe for men during CABG in the present study. Leukocyte counts and plasma MPO levels were lower in patients receiving 17β-estradiol pretreatment, which would indicate that estrogen limits leukocyte activation after CABG, while the present dose fails to limit systemic cytokine responses. A vasodilative effect of 17β-estradiol was observed after CABG, and this effect needs further investigation.

The present low-dose adenosine pretreatment protocol (as compared to doses in the literature) reduced myocardial injury and improved cardiac function after CABG, but failed in limiting systemic cytokine responses. However, four of the fifteen patients developed profound hypotension during adenosine administration. This calls for further study to evaluate the safe protocol for adenosine pretreatment. A trend towards

lower myocardial neutrophil sequestration were observed in the adenosine patients, and further study is warranted to define whether adenosine may attenuate neutrophil accumulation in myocardium after CABG.

The systemic inflammatory response after CABG and anti-inflammatory strategies do indeed provide us with a rich source of research and of argument. Doctor Johnson felt no obligation to his colleagues to progress toward an understanding, but the modern cardiovascular surgeon must aspire to it.

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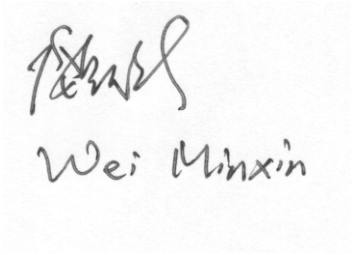

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