ZHONG-KAI WU

Ischaemic Preconditioning in Coronary Artery Bypass Surgery

The Protective Effect of Ischaemic Preconditioning on Myocardial Ischaemia-reperfusion Injury

University of Tampere
Tampere 2000

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Acta Universitatis Tamperensis 787

ACADEMIC DISSERTATION
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http://granum.uta.fi

Cover design by
Juha Siro

Printed dissertation
Acta Universitatis Tamperensis 787
ISBN 951-44-4980-0
ISSN 1455-1616

Electronic dissertation
Acta Electronica Universitatis Tamperensis 78
ISBN 951-44-4981-9
ISSN 1456-954X
http://acta.uta.fi

Tampereen yliopistopaino Oy Juvenes Print
Tampere 2000

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ACADEMIC DISSERTATION

To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
for public discussion in the auditorium of Finn-Medi,
Lenkkeilijänkatu 6, Tampere,
on January 12th, 2001, at 12 o'clock.

University of Tampere
Tampere 2000

To Xiaodan and Steve

CONTENT

LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following original communications, referred to in the text by their Roman numerals I-V.

I. Zhong-Kai Wu, Matti R. Tarkka, Erkki Pehkonen, Liisa Kaukinen. Eva L. Honkonen, Seppo Kaukinen. Ischaemic preconditioning has beneficial effects on left ventricular haemodynamic function after a coronary artery bypass grafting operation. Scand Cardiovasc J 2000; 34:247-253.

II. Zhong-Kai Wu, Matti R. Tarkka, Erkki Pehkonen, Liisa Kaukinen. Eva L. Honkonen, Seppo Kaukinen. Beneficial effects of ischemic preconditioning on right ventricular function after coronary artery bypass grafting. Ann Thorac Surg 2000 (in press).

III. Zhong-Kai Wu, Matti R. Tarkka, Jussi Eloranta, Erkki Pehkonen, Liisa Kaukinen, Eva L. Honkonen, Seppo Kaukinen. Effect of ischemic preconditioning (IP) on myocardial protection in CABG patients: Can the free radicals act as a trigger for IP? Chest 2000 (in press).

IV. Zhong-Kai Wu, Erkki Pehkonen, Jari Laurikka, Liisa Kaukinen, Eva L. Honkonen, Seppo Kaukinen, Matti R. Tarkka. Protective effect of unstable angina in coronary artery bypass surgery. Scand Cardiovasc J 2000 (in press).

V. Zhong-Kai Wu, Erkki Pehkonen, Jari Laurikka, Liisa Kaukinen, Eva L. Honkonen, Seppo Kaukinen, Matti R. Tarkka. Ischemic preconditioning protects right ventricular function in coronary artery bypass grafting patients experiencing angina within 48-72 hours. (submitted).

ABBREVIATIONS:

CABG	Coronary artery bypass grafting
CI	Cardiac index
CK-MB	Creatine kinase isoenzyme MB
CPB	Cardiopulmonary bypass
CO	Cardiac output
CTnI	Cardiac troponin I
CVP	Central venous pressure
EF	Ejection fraction
ESR	Electron spin resonance spectroscopy
FR	Free radicals
GLM	General linear model
HR	Heart rate
HSP	Heat shock protein
ICU	Intensive care unit
IP	Ischaemic preconditioning
I/R	Ischaemia-reperfusion
K_{ATP}	ATP-sensitive potassium channels
LAD	Left anterior ascending artery
LCX	Left circumflex artery
LM	Left main coronary artery
LVEDP	Left ventricular end diastolic pressure
LVEF	Left ventricular ejection fraction
LVSWI	Left ventricular stroke work index
MAP	Mean artery pressure
MI	Myocardial infarction
MPAP	Mean pulmonary artery pressure
MS	Myocardial stunning
NFκB	Nuclear factor kappa B
NYHA	New York Heart Association class
PBN	α-phenyl-N-tert-butylnitrone
PC	Preconditioning
PCO	Potassium channel opener
PCWP	Pulmonary capillary wedge pressure
PKC	Protein kinase C
POD	Postoperative day
PVRI	Pulmonary vascular resistance index
RCA	Right coronary artery
RVEF	Right ventricular ejection fraction
RVSWI	Right ventricular stroke work index
SVRI	Systemic vascular resistance index
TNF	Tumour necrosis factor
UAP	Unstable angina pectoris

INTRODUCTION

Even though advances in surgical technique, anaesthesia, myocardial protection and postoperative care have reduced the risk involved in open heart surgery, compromised ventricular function and arrhythmias still occur in CABG patients (Boldt 1989, Christakis 1990, Stein KL 1990, Kloner 1995b, Bril 1996, Ferrari 1999). Cardiac surgeons and anaesthesiologists have sought more optimal methods to protect the myocardium from ischaemia-reperfusion injury. It has been well established that when the heart is diseased, antegrade cardioplegia may fail to give adequate protection to all regions (Gundry SR 1984). Retrograde cardioplegia has been proposed as an alternative or additive to antegrade cardioplegia (Buckberg 1995), but recent studies show that combined delivery of cardioplegia does not provide adequate protection to the ischaemia-reperfusion damaged myocardium in patients with severe coronary stenosis, especially in right ventricular function with RCA stenosis (Savunen 1994, Allen 1995, Winkelmann 1995, Honkonen E 1997ab). Thus consideration of additional strategies in myocardial protection appears necessary.

One or more brief period of myocardial ischaemia following reperfusion increases myocardial tolerance to subsequent long-period ischaemic insult. This paradoxical phenomenon was first described by Murry and colleagues and termed ischaemic preconditioning (IP) (Murry 1986). After over ten years of studies, IP has been so far proved to be the most powerful endogenous myocardial protective mechanism. Evidence also suggests that when the delivery of cardioplegia is impaired, combination of cardioplegia and IP affords superior protection compared with cardioplegia alone (Galinanes 1995). Thus IP might offer protection additional to the current protective strategy in patients with severe coronary stenosis (Cleveland 1996).

REVIEW OF THE LITERATURE

Cardiac surgery traditionally requires a quiescent heart and a bloodless field. The technique commonly used to achieve these surgical environments includes CPB and cardioplegic cardiac arrest during the aortic cross-clamping period. The principles of intraoperative myocardial protection include: the immediate induction of complete electromechanical arrest to prevent useless expenditure of energy stores; adequate hypothermia to reduce metabolic requirements; an appropriate buffer of ischaemically accumulated metabolic acids during aortic cross-clamping; avoidance of intracellular oedema related to cardioplegic solution (Krukenkamp 1996).

1. History of open heart surgery and myocardial protection

1.1. Hypothermia and total circulatory arrest

Bigelow in 1950 introduced the notion that total body hypothermia by surface cooling with total circulatory arrest might be useful in cardiac surgery (Bigelow 1950). Later, in 1953, Lewis and Taufic (Lewis 1953), and Swan and colleagues (Swan 1953) successfully used this technique to perform cardiac operations. In 1958, Sealy and colleagues (Sealy 1958) studied various drugs in the prevention of ventricular fibrillation and used the solution for elective cardiac arrest along with hypothermia. Shumway´s group in California began using topical hypothermia with simultaneous aortic cross-clamping by circulating cold saline solution through the pericardial sac (Shumway 1959). Intermittent cross-clamping was used to reduce the incidence of low output syndrome in the mid-1970s. These non-cardioplegic methods are also in current use, especially in CABG operations (Antunes 1992).

1.2. Cardio-pulmonary bypass (CBP)

Gibbon began his pioneering experimental work on the development of CPB in the 1930s (Gibbon 1939). In 1953, he performed the first successful operation to repair an atrial septal defeat in a young patient with total CPB support (Gibbon 1954). In 1954, Lillihei and colleagues began operations on congenital heart disease using ‘controlled cross-circulation’ (Warden 1954) and Kirklin and colleagues subsequently reported a series of intracardiac operation using a pump-oxygenator (Kirklin 1955). The use of pumps and oxygenators began thereafter with the era of open-heart surgery. In 1958, Sealy and cooperators reported successful clinical cases (Sealy 1958) in which hypothermia was combined with CPB. Heparin-coated circuit (Lazar 1997), circuit treated with surface-modifying additive (SMA) (Gu 1998) and silicone-coated oxygenator (Shimamoto 2000) were recently used to attenuate the inflammatory response of CPB.

1.3. Cardioplegia

Melrose and colleagues (Melrose 1955) used potassium citrate as a means to achieving cardiac arrest in animal experiments. However, the focal myocardial necrosis caused by citrate (Hoelscher 1967) discouraged the use of cardiac arrest infusions. In 1958, Sealy and colleagues (Sealy 1958) studied various drugs in the prevention of ventricular fibrillation. They developed a solution containing potassium, magnesium and neostigmine and used it for elective cardiac arrest along with hypothermia. They were the first to use the term ´cardioplegia` in these studies. Gay and Eber used potassium-induced cardioplegia in conjunction with hypothermia (Gay 1973). Bretschneider and associates studied various chemical additives to cardioplegic solutions and developed Bretschneider´s solution (Bretschneider 1975). Hearse and colleagues developed the St. Thomas solution in 1976 (Hearse 1976). These approaches led to the use of alternative techniques to produce a quiet operative field. Buckberg´s group in the USA reported reduced postischaemic myocardial damage after modification of calcium, potassium, pH, and osmosity in blood cardioplegic mixture (Follette 1978). Fully oxygenated cardioplegia was also used by Engelman´s group (Engelman 1980). Lillihei and colleagues (Lillihei 1956) described retrograde coronary sinus perfusion. A group in Toronto reintroduced the concept as an adjunct to myocardial protection, and developed warm continuous blood cardioplegia (Lichtenstein 1991). Minicardioplegia was recently used in order to achieve better myocardial protection (Gundry 1997, Hayashida 1998). Hyperpolarised arrest achieved by potassium channel opener (PCO) shifts the membrane potential toward more negative values, resulting in minimal transmembrane ion influx including Ca^{++} and reduced energy expenditure. Recently, Jayawant reported that PCO pinacidil cardioplegia was superior to St. Thomas solution in attenuating myocardial stunning in a CPB pig model (Jayawant 1999). PCOs may prevent potassium-evoked calcium influx by its membrane–depolarisation effect.

1.4. Beating heart surgery

CPB and cross-clamping cause global ischaemia and I/R injury as well as detrimental systemic inflammatory effects (Wan 1996). Cross clamping also induces myocardial oedema by the cessation of lymphatic flow. Beating heart surgery was used to avoid such damage, especially in CABG operations. It is suitable in patients for whom CPB, hypothermia or cannulation is not desirable (Tasdemir 1998). Less blood (homologous) transfusion and lower mortality in high-risk groups had been claimed to be superior in such a method (Magovern 1998).

2. Temperature, vehicle and delivery of cardioplegia

The principle in the clinical model of cardioplegia involves: immediate and sustained arrest to lower energy demands and depletion, hypothermia to further reduce energy demands, provision of substrate for energy production, buffering to counteract acidosis, hyperosmolarity to reduce oedema, membrane stabilisation in the form of additives or avoidance of hypocalcaemia, and washout of metabolic inhibitors (Lell 1990).

Hypothermia diminishes the metabolic and oxygen requirement of tissue. The deleterious effects of hypothermic cardioplegia include impairment of mitochondrial energy generation, substrate utilisation and membrane stabilisation. Warm or normothermic blood cardioplegia releases oxygen more easily; avoids adverse effects of hypothermia and is thus more physiological. Thus it offers better protection in high-risk patients (Lichtenstein 1991). Warm blood cardioplegia can also be used as a part of the protection schedule termed controlled reperfusion or 'hot shot' (Teoh 1986). However, warm heart surgery has been found to be associated with increased technical demands and an increased possibility of neurological complication (Buckberg 1995). Currently cold (4-10^{o}C), tepid (32 ^{o}C) and warm (37 ^{o}C) cardioplegia are used. Tepid cardioplegia has the lowest incidence of neurological adverse effects (Engelman 1996).

The advantages of blood cardioplegia are increased oxygen-carrying capacity, better buffering, increased colloid osmotic pressure and a more physiologic composition of substrates and trace elements (Engelman 1980). The advantage of crystalloid cardioplegia has been considered to be reduced viscosity and possibly a more homogeneous distribution (Landymore 1984). Controversy persists as to the relative merits of blood versus crystalloid as the delivery vehicle, but the former is preferred by a majority (Robinson 1995). In critical patients, for instance those with low EF (<40%) or long clamping time, blood cardioplegia has been reported to be better (Teoh 1986). Recent studies suggest that minimal blood dilution tepid cardioplegia reduces the release of lipid peroxidation products during reperfusion, has better lactate extraction and ventricular function as well as reduced incidence of conduct disturbance (Gundry 1997, Hayashida 1998).

It has been well established that when the heart is diseased, antegrade cardioplegia may fail to give adequate protection to all regions (Gundry 1984). Retrograde perfusion of cardioplegia has been proposed as an alternative or additive to antegrade cardioplegia (Buckberg 1995). Despite the absence of coronary stenosis, RV perfusion has proved poor with retrograde delivery (Allen 1995). Although the problem caused by the maldistribution of cardioplegia in the presence of critical

stenosis may be reduced by retrograde perfusion (Gundry 1984), it has been shown that there is less flow to the posterior LV septum and RV free wall (Partington 1989, Winkelmann 1995). Retrograde cardioplegia likewise does not improve the ischaemic or infarcted myocardium in the LAD area (Carrier 1997a,b).

3. Myocardial ischaemia-reperfusion injury

Myocardial I/R injury varies from cardiomyocyte dysfunction manifested as arrhythmias and myocardial stunning (MS) to cell death. The pathogenesis of I/R injury is not totally clear; probably a broad spectrum of factors are involved in its genesis (Vaage 1993). Free radicals and calcium metabolism may play a role (Opie 1991). The severity of reperfusion injury correlates to a certain degree with the duration of ischaemia (Vaage 1993, Bolli 1998). In CABG operations success in avoiding reperfusion injury depends on how myocardial protection has been managed, and especially on whether at the end of the cross-clamp period the heart is allowed to receive controlled reperfusion (Buckberg 1987).

A sudden, massive reduction or total cessation of blood flow in coronary arteries has major consequences: a reduced availability of oxygen and substrate for metabolism and an accumulation of by-products. The amount of endogenous ATP is severely depleted within a few minutes. This ATP decline is associated with morphological changes, including mitochondrial swelling, glycogen granula depletion, sarcoplastic reticulum swelling and dysfunction of the ultimate contraction banding of the filaments and disruption of the sarcolemma (Bretschneider 1975). Cardiac contractility depresses. FR generate during the reperfusion period, leading to damage of cell membrane. Accumulation of intracellular Ca^{++} accelerates the cellular damage (Opie 1991).

During CPB, many events may predispose to myocardial necrosis and prolonged functional impairment. These include myocardial vulnerability, duration of exposure and the inadequacy of protective measures, possible risk factors being inadequate myocardial perfusion, abnormal perfusate composition, persisting ventricular fibrillation, ventricular distension, ventricular collapse, coronary embolism, catecholamines, aortic cross-clamping and reperfusion (Lell 1993).

3.1. Haemodynamic function recovery—myocardial stunning

3.1.1. Definition:

Myocardial stunning (MS) is the mechanical dysfunction which persists after reperfusion despite the absence of irreversible damage and despite restoration of normal or near-normal coronary flow. The most important factors underlying stunning are the severity and duration of flow

deprivation and the myocardial temperature. Other factors include the size of the ischaemic region and the loading condition of the heart (Bolli 1998).

MS is in part a form of reperfusion injury: the reperfusion injury component appears to be more substantial than the ischaemia injury component (Bolli 1998). There is, however, evidence that the magnitude of FR generation after reperfusion is proportional to the magnitude of ischaemia (Bolli 1990a). This would support the important concept that the severity of the reperfusion injury component in MS is proportional to the severity of the ischaemic injury component. Thus any interventions which attenuate the severity of ischaemic injury will attenuate the subsequent reperfusion injury. Hence interventions which alleviate the ischaemic injury (adenosine, Ca^{++} antagonist, K_{ATP} channel opener) are also effective in mitigating MS, even though they have no direct effect on the reperfusion injury component in MS (Bolli 1998).

3.1.2. Mechanism

The mechanism of stunning remains unclear. The calcium and oxyradical hypotheses represent different facets of the same pathophysiological process. The oxyradical hypothesis is widely accepted and proved. It proposes that toxic oxygen radicals elaborate upon reperfusion (Bolli 1990). This is supported by the fact that improved mechanical function is achieved with free radical scavenger administration (Bolli 1989). Depletion of the substrates, accumulation of cellular glycolytic product lactate and attenuated myocardial response to the sympathetic nervous system also contribute to MS (McFalls 1987).

3.1.3. Stunning in cardiac surgery

Breisblatt and cooperators demonstrated a decrease in mean LV and RV ejection fraction from 58% to 37% after CABG, which was associated with a decrease in CI and LVSWI. These were lowest at 4 hours and resolved after 24-48 hours postoperatively (Breisblatt 1990). Mangano and colleagues reported that LVEF and RVEF decreased in all postoperative CABG patients from 81% to 26% (Mangano 1985). Boldt and colleagues showed cardiac function to be decreased after a CPB operation within 1st POD (Boldt 1989). Others have demonstrate that LV wall thickening decrease after surgery in almost every patient, reaching a nadir at 2-6 h (Bolli 1990b). Stunning can profoundly depress LV function and cause haemodynamic instability which requires treatment with inotropes, vasoactive agents and LVAD in high-risk patients. Patients receiving inotropes and afterload-reducing therapy and with elevated plasma catecholamine would mask myocardial stunning (Bolli 1998).

3.1.4. FR and myocardial stunning in clinical setting.

FR are produced in the stunned myocardium (Bolli 1998). One experimental study has suggested that FR have an impact on I/R injury, including myocardial stunning and arrhythmia, after a single or repetitive ischaemic insult (Bolli 1990a, Bolli 1998). There are a few studies assessing the effect of FR on myocardial damage in a clinical setting. Ferrari and associates found reperfusion after aortic clamping to be associated with release of oxidised glutathione (GSSG) in the coronary sinus, the magnitude of the release being inversely related to the values of CI measured in the ensuing hours (Ferrari 1990). Prasad and co-workers found marginally increased malondialdehyde in venous blood following release of aortic cross-clamping (Prasad 1992). Yau and colleagues showed that pretreatment with an antioxidant (alpha-tocopherol) had a beneficial effect on metabolic and functional recovery after cardiac surgery (Yau 1994).

3.2. Myocardial apoptosis and necrosis

The severe damage to cardiomyocytes during ischaemia-reperfusion period includes cellular apoptosis and necrosis. Apoptotic cell death is characterised morphologically by chromatin condensation and biochemically by degradation of DNA into a specific pattern of fragments. Necrosis is a catastrophic metabolic failure resulting in loss of cell membrane integrity and late random digestion of DNA (random DNA fragmentation) (Wang 1999). Apoptosis is a result of an active cellular response (cell suicide) involving a specific cascade of molecular events and can possibly be prevented. In contrast to necrosis, apoptosis requires energy and protein synthesis, maintaining membrane integrity and avoids an inflammatory response. This type of cell death is characterised by internucleosomal DNA fragmentation or laddering (Piot 1997, Wang 1999). Apoptosis is a myocardial I/R injury rather than a consequence of ischaemia alone (Fliss 1996).

3.3. Arrhythmia

Sudden cardiac death remains a major unsolved clinical problem; most cases of this are caused by acute ischaemia or reperfusion-induced ventricular tachycardia or fibrillation (Kloner 1995b, Bril 1996). Incomplete myocardial protection is a major cause of the postoperative rhythm and conduct disturbance (Pehkonen 1993&1995). Cardiac arrhythmia is commonly encountered soon after coronary artery surgery. Supraventricular arrhythmias are more common events than ventricular arrhythmias after CABG and happen later, but as much are less well tolerated (Ormerod 1984).

Atrial fibrillation (AF) is the most frequent complication after CABG. Clinically significant AF which lasts several hours and needs medication occurs in 12-38% (Creswell 1993). Post-CABG

AF can have serious consequences such as systemic embolism and haemodynamic compromise, and at least cost of care.

Conduction disturbances (CD) typically occur immediately after the operation. CD may exist at discharge except perioperative complete heart block (Pehkonen 1993, Gundry 1997). There is evidence that patients with CD have lower CO and need more inotropic medication or other intervention than usual, or even have high mortality (Caspi 1987). Among these, left side lesions have higher incidences of low CO and higher CK release (Caspi 1987).

3.4. Systemic inflammatory changes

Conditions such as CPB can often cause systemic inflammation and dysfunction of major organs. Pulmonary, renal, myocardial and intestinal function may suffer various degrees of impairment during and after cardiac surgery. Systemic inflammation proceeds through activation of serum proteins, activation of leukocytes and endothelial cells, secretion of cytokines, leukocyte-endothelial cell interaction, leukocyte extravasation and tissue damage (Kirklin 1983, Asimakopoulos 1999). During the period of CPB, the tissue perfusion is changed, and these conditions elicit a local production of inflammatory reactants, including cytokines. The lung and the myocardium seem to be the production sites. During the reperfusion period, the mediators produced are washed out into the systemic circulation and provoke a systemic inflammatory response. Cytokines are the key mediators of such acute response (Tonnesen 1996). Proinflammatory cytokines, including TNF-α, IL-6 and IL-8, may contribute to myocardial dysfunction and haemodynamic instability following CPB and postoperative multiple organ failure. The release of the anti-inflammatory cytokine IL-10, in contrast, is potentially protective (Wan 1999). I/R increases the myocardial TNF-α level by cardiac resident macrophages and cardiomyocytes. TNF-α is an autocrine contributor to myocardial dysfunction and cardiomyocyte death in I/R injury (Cain 1998).

3.5. RV function in CABG patients

3.5.1. Importance of RV function in CPB surgery

It has been known that RV function contributes essentially to the maintenance of global heart performance (Polak 1983), and malfunctioning of RV may limit the success of an operation (Rabinovitch 1983). The RV is anatomically bonded to the LV by subepicardial muscle fibers which run from the free wall of the RV to the anterior wall of the LV. They also share the interventricular septum and an overlapping blood supply. RV pump function is important in

preventing LV failure by ensuring delivery of the necessary preload required to subserve LV output. LV abnormalities affect RV function via diastolic volume, systolic function and RV afterload by pressure elevation in the pulmonary circulation (Boldt 1989). Inadequate RV protection may lead to unexpected low postoperative cardiac output despite good preservation of left ventricular function (Mullen 1987).

3.5.2. RV function during CABG surgery

Hines and Barash (Hines 1986) reported that an intraoperative onset of ischaemia was associated with isolated RV dysfunction, manifested in a decrease in RVEF. Boldt and associates (Boldt 1989) showed that acute volume loading after cardiopulmonary bypass (CPB) in patients with severe RCA stenosis and prolonged aortic cross-clamping time led to reducing of RVEF and CO, while in patients without RCA stenosis RVEF and CO increased. The depression of RV systolic function was most severe at 4-6 hours after CPB (Stein 1990, Honkonen 1997ab).

3.5.3. Difficulty in RV protection during CABG surgery

Current myocardial protective methods do not always offer adequate protection of RV (Christakis 1990), especially in conjunction with right coronary artery stenosis (Hilton 1979). Patients with severe RCA stenosis are at increased risk of developing perioperative RV ischaemia; neither antegrade nor retrograde cardiaplegia delivery provides adequate protection of right atrium and ventricle, this manifesting itself in a decrease in RVEF (Boldt 1988).

RV disorder is caused by the deleterious effect of ischaemia-reperfusion injury to the heart and lung, as well as systemic inflammatory response of CPB, which causes pulmonary vasoconstriction and congestion, resulting in increased RV afterload (Kirklin 1983, Byrick 1977). Also the microvascular endothelium in the RV may be more vulnerable than that in the LV to be damaged by cardioplegia and reperfusion (Murphy 1995). Mostly, however, it is due to limitation of the RV myocardial protection.

Protection of the RV myocardium is more difficult with cold blood cardioplegia than that of the LV by reason of the closer contact of RV to RA with warmer systemic circuit blood and anterior position of the right heart, which favours rewarming of that chamber by handling, contact with room air, and exposure to radiant energy from the surroundings (Christakis 1985).

It is well established that when the heart is diseased antegrade cardioplegia may fail to give adequate protection to all its regions (Gundry 1984). Retrograde cardioplegia has been proposed as

an alternative or additive to antegrade cardioplegia. Despite the absence of coronary stenosis, RV perfusion has been proved poor with retrograde delivery (Allen 1995) because the RV free wall drains directly into the lesser venous system (Thebesian veins) and to the RV (Partington 1989). A more important reason is the location of the inflated balloon of the coronary sinus catheter. It may occlude the posterior interventricular vein to which the blood from RV diaphragmatic wall and two thirds of ventricular septum drains (Menasche 1994). Although drains to Thebesian vein help cool the RV, it cannot repay the energy debt. Since the blood reaching the Thebesian veins comes only through veno-venous collaterals so it is non-nutritive (Partington 1989). Only 21% to 25% of all hearts have a small cardiac vein; this vein courses in the sulcus between RA and RV and drains to the dorsal wall of RV, entering the sinus at its origin. Thus it is difficult to perfuse the vein with a balloon-tipped catheter. When the small cardiac vein is missing, the drain of RV is forward to the Thebesian vein, then directly to RV (Gate 1993). Therefore, retrograde perfusion to the RV free wall may be technically impossible to accomplish via the sinus cannula.

This is even more difficult in patients with RCA stenosis, where neither antegrade nor retrograde techniques ensure cardioplegic delivery to the RV (Christakis 1985, Savunen 1994, Winkelmann 1995). Obstructive lesion of the coronary artery results in inconsistent distribution of antegrade cardioplegia and hence in inadequate preservation of the myocardial area subserved by the stenotic vessels (Hilton 1979). Although the problem caused by the maldistribution of cardioplegia in the presence of critical stenosis may be reduced by retrograde perfusion (Gundry 1984), it has been shown that here is less flow to the posterior LV septum and RV free wall (Partington 1989, Winkelmann 1995). Retrograde cardioplegia does not improve the ischaemic or infarcted myocardium in the LAD area either (Carrier 1997a). There is no evidence of better myocardial protection in cardiac output or other clinical aspect by combined delivery of cardioplegia (Carrier 1997b) and this does not protect RV better than the technique alone in patients with right coronary stenosis (Savunen 1994, Allen 1995, Honkonen 1997ab).

3.5.4. Measurement of RV function

RVEF is the most frequently used parameter to quantify RV function. RVEF was reported to be associated with mortality in patients with congestive heart failure associated with coronary artery disease (Polak 1983). The injection-to-injection reproducibility of the thermodilution method has been studied in an in vitro validation model and the coefficient of variation for RVEF has been found to be as low as 4.7% (Ferris 1992). RVEF is dependent on contractility, preload and afterload. Preload-normalised RV stroke work was used to assess RV function. Stroke work is a

function of both contractility and preload; it has also been seen as one of the best measures of the mechanical efficiency of the ventricle (Mangano 1985).

4. Free radicals (FR)

FR are chemical species generated in small amounts during the normal metabolism of cells. FR are transient, with 10^{-6} to 10^{-3} second half-lives and short diffuse radii. The univalent pathway of oxygen reduction is the source of free radicals and postischaemic dysfunction occurs only when FR are not inhibited (Bolli 1989). FR are highly reactive compounds which can oxidate many cellular components and cause damage (Bolli 1998).

4.1. Source of FR

The source of FR remains unclear. Possible sources include xanthine oxidase, catecholamine oxidation, cyclooxygenase, neutrophils and mitochondria (Hess 1995). Activated leukocyte infiltration to the myocardium from the vasculature is one of the sources of FR (Kloner 1989). But evidence from isolated heart's model shows that neutrophils are insignificant. There is evidence of FR generated from xanthine oxidase, but this is conflicting in humans, and no evidence in pigs and unimportant in the rabbit (Muxfeldt 1987). Thus activation of the arachidonate cascade, autoxidation of catecholamine and damage to mitochondrial electron transport chain may be important in humans (Bolli 1998). Among these, oxidative metabolism in the mitochondria appears to be responsible for FR formation (Ambrosio 1993).

4.2. Damage due to FR

FR are highly reactive compounds which as noted above can oxidate many cellular components and cause damage. FR generated after an I/R attack different cellular components and lead to I/R contractile dysfunction (stunning) and arrhythmia (Hess 1995, Bolli 1998). FR attack non-specifically all cellular components: mainly proteins and lipids, leading to protein denaturation and enzyme inactivation, as well as peroxidation of the polyunsaturated fatty acids in cellular membrane (Bolli 1990a, 1998). These injuries include: (a) attack on nucleic acid and nucleotide coenzyme; (b) lipid peroxidation causing substantial disturbances to membrane structure and function, and hence to cellular control mechanism, impairment of selective membrane permeability and interference with the function of various cellular organelles; (c) covalently binding to many substances containing double bonds (Slater 1987). Sarcolemma may be a critical target of FR, since FR result in increased transsarcolemmal Ca^{++} influx and cellular Ca^{++} overload (Bolli 1998). Production of selective damage of contractile proteins such as oxidation of critical thiol group may cause decreased responsibility of myofilaments for Ca^{++} (Bolli 1990a).

4.3. Mechanism to remove FR

Cells are normally equipped with endogenous scavenger systems of two categories: preventive antioxidants which inhibit the production of FR, and chain-breaking antioxidants which interact with the chain-propagating FR. FR are transient with short diffuse radii; therefore, the FR scavenger must reach the right site at the right time and in a suitable concentration (Slater 1987). During reperfusion of postischaemic tissue, large amounts of FR are generated and then overwhelm the cellular defence, thus inducing tissue injury (Becker 1987, Ambrosio 1995).

4.4. Method of measurement

FR are transient with 10^{-6} to 10^{-3} second half-lives and short diffuse radii; these contribute to the difficulty of FR measurement. However, reactive free radical can be trapped by a non-radical species such as PBN to yield a product (adduct) which is a relatively long-lived free radical species (Slater 1987). The relative concentration of spin-trapped radicals is thus determined by the value of the signals of PBN spin adducts.

5. Ischaemic preconditioning (IP)

5.1. Introduction

One or more brief periods of myocardial ischaemia followed by reperfusion increases myocardial tolerance to subsequent long-period ischaemia insult. This paradoxical phenomenon was first described by Murry and colleagues after investigating the effect of repetitive regional ischaemic episodes in anaesthetised dogs. They found that, although myocardial ATP declined during the first 5-min period of ischaemia, it was not depleted further by subsequent similar ischaemic insult and no necrosis result despite a 40 minutes' ischaemic period after four cycles of brief 5-min I/R episodes. This phenomenon of endogenous myocardial protection was termed ischaemic preconditioning (IP) (Murry 1986). From then on, a similar ischaemic preconditioning effect was also found in almost every kind of mammary animals tested, including mice, rats, rabbits, dogs, pigs, cats, gerbils, sheep and baboons (Schott 1990, Liu GS 1991, Przyklenk 1997, Schwarz 1997, Yellon 1998). There was evidence of preconditioning in cultures of human cardiomyocytes (Ikonomidis 1994), human myocardial tissue (Morris 1997) and clinical patients (Muller 1990, Kloner 1995). This myocardial protective effect was also studied in clinical interventions including coronary angioplasty (Deutsche 1990, Tranchesi 1990, Airaksinen 1997) and open-heart surgery (Yellon 1993, Alkhulaifi 1994, Jenkins 1997, Lu 1997, Illes 1998, Szemagala 1998, Li G 1999).

IP does no occur only in young healthy hearts; there is also evidence of IP effects in the neonatal (Overlgonne 1996) and aged myocardium (Burns 1995) and even in the hypertrophied heart

(Speechly-Dick 1994). The presence of a critical coronary artery stenosis does not abolish the protective effect of IP either (Kapadia 1997). Furthermore, when delivery of cardioplegia is impaired, the combination of cardioplegia and IP affords superior protection compared with cardioplegia alone (Galinanes 1995). There are reports that current myocardial protective methods do not provide adequate protection in patients with severe coronary stenosis, especially in the right ventricle (Allen 1995, Winkelmann 1995, Honkonen 1997). Thus IP might give additional reinforcement to the current protective strategy in patients with severe coronary stenosis (Cleveland 1996).

5.2. IP effect

IP has been proved to be a potent endogenous factor in delaying myocardial infarction (Murry 1986, Li YW 1992), suppressing arrhythmias induced by ischaemia and reperfusion (Shiki 1987, Lucas 1997), preserving high-energy phosphate (Murry 1986, Yellon 1993, Alkhulaifi 1994), improving postischaemic functional recovery (Kaplan 1994, Sun 1996, Illes 1997, Lu 1997, Landymore 1998), attenuating myocardial cellular apoptosis (Wang 1999, Nakamura 2000), delaying myocardial ultrastructure damage (Murry 1990), attenuating intracellular acidosis, reducing anaerobic glycolytic end-product lactate (Finegan 1995, McNulty 1996, Minamino 1998), reducing the utilisation of myocardial glycogen (Alkhulaifi 1994) and protecting the coronary endothelium (Kaeffer 1997).

5.2.1. Protection against myocardial stunning

Kaplan and associates demonstrated IP effects in protecting the MS as well as preserving end-ischaemic ATP and improving coronary flow during reperfusion (Kaplan 1994). Mosca and colleagues proved that IP significantly increases the maximal inotropic response and diminishes the contractile dysfunction of early stunning in the isolated perfused rat heart (Mosca 1998). A group under Landymore showed that IP prevented myocardial stunning and preserved high-energy phosphates after cardiac transplantation in experimental sheep (Landymore 1998) and Bolli´s group clearly demonstrated the delayed IP effect on stunning in a pig model (Sun 1995), which effect was dependent on the formation of new proteins in intact animals (Rizvi 1999). Protein kinase C (PKC) plays an essential role in this process (Qiu 1998).

There are also controversial reports. Xi and colleagues (Xi 1998) found that IP is able to reduce myocardial infarct size and intracellular enzyme leakage caused by a sustained I/R in the isolated perfused mouse heart. This anti-necrosis cardioprotection induced by IP was not associated with the amelioration of post-ischaemic ventricular dysfunction. Jahania and co-workers held that IP

does not ameliorate in vivo porcine myocardial stunning (Jahania 1999). Faris and associates suggested that IP was unable to attenuate postcardioplegia stunning of minimally infarcted hearts and questioned its usefulness in clinical cardiac operations (Faris 1997).

5.2.2. Anti-arrhythmias

There is evidence that IP reduces the incidence of VT and VF in anaesthetised rat (Hagar 1991, Li YW 1992). The beneficial effect of IP appears to be very short-lived; it is lost after 1 hour but reoccurs 24 hours later (Li YW 1992). In the anaesthetised pig, IP attenuated the incidence of ventricular extrasystoles (VES) during coronary occlusion but not the incidence of more severe ventricular arrhythmias (Cinca 1997). In the isolated pig ventricle, neither IP protocol (2 min and 5 min) affected the incidence of ventricular tachycardia (VT) in ischaemia. However, the 5 minutes protocol significantly decreased VES and transmural conduction block. IP of 5 minutes, but not 2 minutes significantly reduced reperfusion-induced VT and VES. IP did not affect action potential, but attenuated the depression of transmural conduction by ischaemia and early reperfusion and thereby prevented the conduction delays necessary for transmural reentry (Zhu 1998). Shiki and associates show an inverse relationship; if the duration of the first ischaemic episode increased from 1 to 5 minutes, the second ischaemic episode had lesser VT, VF and premature ventricular complex (PVC) and more normal sinus rhythm (Shiki 1987).

Lucas and colleagues reported that one IP cycle completely protects the rabbit heart against ischaemia-induced arrhythmia (VF) and 2 cycles partially, whereas 3 or 4 cycles adversely increased VF (Lucas 1997). On the other hand, Tosaki and group suggested that multiple cycles have better effects on anti-arrhythmia and reduce FR formation more than a single cycle (Tosaki 1994). The antiarrhythmic effects of IP were more marked with ischaemic arrhythmias than with reperfusion arrhythmias, and increased with duration of IP in one clinical study (Pasceri 1996). Lucas and colleagues also showed that IP is more effective in suppressing ischaemia versus reperfusion-induced arrhythmia in rabbit heart (Lucas 1997). However, the protective effect of IP includes suppression of reperfusion-induced VF (Osada 1994, Tosaki 1994).

In balloon angioplasty where coronary artery was occluded for 111 seconds (mean) separated by 5 minutes, fewer VES and atrial premature beats were found in the second occlusion. Thus IP increases electric stability (Airaksinen 1997). Okishige and colleagues also found that IP reduced the inhomogeneity in ventricular repolarisation and modified the ion channels, resulting in fewer arrhythmias (Okishige 1996). After comparing ST segment changes and complex ventricular arrhythmias (CVAs) frequency, Pasceri and associates found that the IP anti-arrhythmic effect

induced by the previous angina was not related to a reduction in either severity or duration of ischaemia, which suggested that arrhythmic protection was a direct consequence of IP rather than an epiphenomenona of ischaemic protection (Pasceri 1996).

5.2.3. Anti-inflammatory effect

There is evidence that adenosine reduces tumour necrosis factor (TNF) production in lipopolysaccharide (LPS)-challenged mice, isolated human atrial tissue and ventricular tissue from the failing heart. It also reduces LPS-stimulated inducible NO synthase expression and inhibits neutrophil adhesion and injury to cardiac myocytes. Another important IP mediator, norepinephrine, lowers TNF production and macrophage superoxide and NO release (Meldrum 1997, Cain 1998, Wagner 1998).

Heat shock protein (HSP), a protective protein involved in the delayed IP mechanism, protects against injury by reducing macrophage/TNF-mediate tissue injury. HSP binds nuclear factor kappa B (NFkB), a TNF transcription factor in the cytosol, and prevents its translocation to the nucleus. HSP also inhibits TNF production at the post-translational level. Therefore, IP might reduce the inflammatory response (Meldrum 1997). However, there is no study concerning the IP effect on the inflammatory response at the present.

5.3. Theories of mechanism

The precise mechanism of IP remains unknown. One early theory explained that preconditioning was merely a manifestation of increased collateral blood flow. However, this cannot explain the preconditioning effect subjected to global ischaemic stimuli. It was disproved by a study which showed that preconditioning was independent of collateral flow (Murry 1986). Another suggestion was that sublethal preconditioning causes depressed contractility, and then slows metabolic demand. This, again, cannot explain the time course difference, and that the degree of stunning and protection effect do no correlate, as well as the phenomenon that reversing depressed contractility with dopamine does not prevent preconditioning (Murry 1991).

Detecting the biological changes after preconditioning, using analogues or antagonists to mimic or abolish the preconditioning effect in different experimental models, many authors have sought to elucidate the IP mechanism. Findings include adenosine A_1 receptor stimulation (Liu 1991), α_1-adrenergic pathway (Benerjee 1993), bradykinin release and nitrite oxide production (Parratt 1997), FR triggering process (Zhou 1996), glycogen depletion and lactate production (Wolfe 1993, Aresta

1997), heat shock protein (HSP) synthesis (Marber 1993), formation of endogeneous anti-oxidant proteins (Hoshida 1993), G-protein coupled receptor mediation (Cleveland 1996), protein kinase C (PKC) (Downey 1997, Cleveland 1997a) and tyrosine kinase (Das 1999) activation and ATP-sensitive potassium channel opening (Cleveland 1997b).

It is well known that brief episodes of myocardial ischaemia result in the production of substrates such as adenosine, bradykinin and nitrite oxide from coronary vascular endothelial cells or plasma kallikrein. It also stimulates norepinephrine release from noradrenergic termini which present in the mammalian heart. These chemical factors act on one or more types of myocyte receptors: adenosine A_1, muscarinic receptor, bradykinin B_2 and alpha sympathetic receptor, result in translocation and activation of tyrosine kinase and PKC to cellular membrane, working with inhibitory G-protein, subsequently phosphorate ATP-dependent potassium channel for trigger IP response (Burns 1996, Das 1999). If these PKC agonists reach the threshold level of PKC stimuli, a preconditioning effect will result. Increased release of one agonist or stimulation by more cycles of brief ischaemic stimuli can compensate for the lack of another to achieve the protection effect (Downey 1997, Qiu 1998). PKC translocate from the cytosol to the cell membrane after activation, mediating phosphorylation of target proteins, ion channels (including ATP-sensitive potassium channels) and myofilaments to achieve the effect (Cleveland 1996). G-protein is considered to be the message transmitter between the signals (adenosine, noradrenaline, etc) and PKC (Sumeray 1997). The activation of G-proteins leads to the formation of diacylyglycerol which subsequently activates PKC. The opening of ATP-sensitive potassium channels leads to increased potassium conductance and shortening of action-potential duration, which might limit calcium entry and reduce energy metabolism (Alkhulaifi 1996).

The delayed cardiac adaptation is consistent with induced gene transcription and the subsequent translocation of protective molecular proteins (Yellon 1995), including protooncogenes, stress proteins and antioxidant enzyme systems (Marber 1993). NF-κB, a transcription factor of proteins in response to mutagenic, oxidative and hypoxic stress, may play an essential role in this IP effects (Morgan 1999, Das 1999). HSP 70 elevated 24 hours following IP which coincides in time course and degree with delayed IP (Marber 1993). In transgenic mice which overexpress HSP 70i result in significant myocardial protection (Radford 1994). Thus, HSP is proposed as a mediator in induction, transcription and expression of delayed IP. There is also evidence that the activity and content of manganese-superoxides dismutase and catalyse is increased in dog heart 24 hours after multiple brief coronary occlusion (Hoshida 1993). Thus antioxidant enzymes may also contribute to delayed IP.

Other possible triggers include 5´-ectonucleotidase (5-NT), acetycholine, angiotension II, bradykinin and opioids. These triggers vary between species and with respect to the end-points: for example, prostanoids appear not to be involved in infarct size reduction but in attenuation of arrhythmia (Parratt 1997, Przyklenk 1998). Other possible signalling pathway might include phospholipase C and D activation, 5´-neucleodise and calcium (Meldrum 1996, Przyklenk 1998, Yellon 1998).

A metabolism process might also be involved in IP mechanism. During the ischaemic phase of IP, glycogen stores are depleted, lactate and protons accumulated. During the reperfusion phase of IP, accumulated catabolites are rapidly washed out (Van Wylen 1994). Since glycogen synthesis is slow and the glycogen-depleted myocardium is exposed to prolonged ischaemia, lactate accumulation is low. Therefore, the effect of IP on acidosis is more prominent than that on energy depletion (Steenbergen 1993). Complete catabolite washout by effective reperfusion seems to be an essential condition for IP, and the restoration of glycogen after IP is parallel with the loss of anti-infarct effect (Wolfe 1993). Moreover, reduction of proton production inhibits transmembrane Na^{+}-H^{+} exchange, then inhibits Ca^{++} overload (Piper 1996). Myocardial glycogen depletion appears to be necessary for the infarct-limiting IP effect, but not the antiarrhythmic effect in the intact rat heart (McNulty 1996). Therefore it contributes to the anti-infarct effect, in co-operation with other mechanism. Furthermore, evidence suggests that lactate, which accumulates during IP periods, can activate several triggers of preconditioning. However, whether the transient exposure to external lactate improves contractile recovery as shown by IP stimuli is controversial (Doenst 1996, Aresta 1997). It should be noted that the glycogen depletion induced by IP influences postischaemic functional and metabolic recovery, but not the only determinants (Soares 1997). There is also controversial report on whether preischaemic glycogen depletion and subsequent attenuation of ischaemic lactate accumulation play a major role in IP protection against contractile dysfunction (Yabe 1997).

5.3.1. FR

FR are not only detrimental, but also an essential part of normal respiratory function within mitochondria, for example, phagocytic cells destroy foreign material by producing FR (Badwey 1980). Recent studies have shown that FR may have opposite effects when produced during IP (beneficial) or during prolonged I/R (deleterious). This is related to the different amounts of FR produced during IP and I/R, as shown in isolated myocytes (Zhou 1996). One other explanation is that the production of FR during IP is not accompanied by other aspects of the inflammatory

reaction which could contribute to endothelial injury such as complement activation and expression of leukocyte adhesion molecules (Kaeffer 1997).

The relation between FR and IP was first suggested by Murry: administration of oxygen radical scavengers during the first reperfusion period could block the beneficial effect of IP on infarct size in the dog (Murry 1988). He suggested that the generation of low amounts of FR during a short ischaemic episode is not sufficient to cause cell necrosis, but enough to modify cellular activity and induce preconditioning. Brief ischaemia may increase the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase or glutathione peroxidase in isolated myocytes (Zhou 1996) and in vivo (Hoshida 1993). Oxidant stress can induce HSPs in the heart (Kukreja 1994) and lead to delayed increases in the activity of SOD, catalase or glutathione peroxidase in endothelial cells (Das 1999).

In isolated rat myocytes, FR generation during IP activated endogenous antioxidant defence, as demonstrated by increased MnSOD activity 24 hours later (Zhou 1996). In the isolated rabbit heart, a low flux of FR generated by purine/xanthine oxidase (P/XO) significantly improved postischaemic recovery of contractile function and reduced infarct size. This effect was prevented by scavenger SOD and PKC inhibitor polymyxin B (Tritto 1997). FR produced during IP also protect the coronary endothelium from reperfusion injury 24 hours later (Kaeffer 1997).

5.3.2. Mechanism of antiarrhythmic IP effects

The mechanism involved in the antiarrhythmic effect of IP is not known (Pasceri 1996). Change in the ATP dependent potassium channel and sodium channel excitability may be the biocellular basis (Parratt 1994). The higher sinus heart rate at peak ST associated with ischaemic episodes showing cumulative ventricular arrhythmias (CVAs) would suggest a role of an antiadrenegic and/or an increased vagal effect of IP (Miyazaki 1989). It has been suggested that the anti-arrhythmic protection afforded by IP may be mediated by preservation of autonomous function (Miyazaki 1989). Another potential mechanism entails the possibility that the reaction to the repeated ischaemic stimuli is attenuated by modulation of the central nervous system (Parker 1987). However, Lawson and colleagues suggest that this is not important judging from the evidence of a similar result in the isolated and in vivo heart (Lawson 1993). Furthermore, the protective action of IP against ischaemia-induced arrhythmias can be prevented by inhibition of cyclooxygenase and NO synthesis. This suggests a role of prostanoid substances and NO, which do not seem to be involved in the anti-necrosis effect (Parratt 1997). The protective IP effect on

reperfusion-induced VF was abolished by SOD in the rat, suggest that the free radicals may involve in the triggering process of IP effects (Osada 1994).

it is generally accepted that IP effect on antiarrhythmia is different from the effects in protecting against metabolic and functional changes. Although delaying myocardial necrosis reduces ischaemia-induced arrhythmias, it does not change their temporal sequences (Hagar 1991). IP protection was afforded by a reduction in the incidence of ischaemia-induced arrhythmias with no evidence of a shift in the temporal pattern of vulnerability (Lawson 1993). A 2-min occlusion increases electrical stability but does not trigger metabolic and functional improvement (Airaksinen 1997). In unstable angina patients, transient myocardial ischaemia confers protection against arrhythmia during subsequent ischaemia episodes in the absence of any evidence of anti-ischaemic protection, and this effect is not related to a reduction in either severity or duration of ischaemia (Pasceri 1996). Thus the antiarrhythmic effect is not mediated by anti-ischaemic protection other than direct consequence of IP.

5.4. Early, delayed and remote IP

A early preconditioning effect occurs within minutes, has been found to be transient and to last for only a short period, between 1-3 hours among different species, the smaller the species, the faster the decline in preconditioning (Li YW 1992, Meng 1996, Schwarz 1997). The IP in this period is called the early phase of IP or classic IP. Recent animal studies have observed another phenomenon of delayed preconditioning occurring 24 hours after preconditioning stimuli and possibly extending to 72 hours. This is called the second window of protection or delayed IP (Marber 1993).

Early IP can be mimicked by adenosine A_1, α-adrenergic, bradykinin B_2 receptor stimulation, and can also occur through the activation of K_{ATP} channels and PKC isoforms. Delayed IP is induced by transient ischaemia, endotoxaemia, inflammatory mediators, rapid ventricular pacing, α-adrenergic stimulation, adenosine and FR (Meldrum 1997, Przyklenk 1998).

Early IP may be independent of new protein synthesis. Delayed IP requires hours for completing induction, and is sustained by new protein synthesis (Meng 1996). The delayed IP is consistent with induced gene transcription and the subsequent translocation of protective molecular proteins (Yellon 1995), including protooncogenes, stress proteins and antioxidant enzyme systems (Marber 1993). Adenosine, norepinephrine, PKC and K_{ATP} are involved in the signal transduction cascades

of both forms, and thus must share a common mediator early in the signal transduction. It should be also noted that the protection afforded by delayed IP might be less important than that of classic IP. For example, early IP has been found to be much more powerful in limiting infarct size than delayed IP in a rat model (Richard 1996).

A brief left circumflex occlusion can induce tolerance to ischaemia in the myocardium perfused by the left anterior descending coronary artery in canines (Przyklenk 1993). This phenomenon of preconditioning the outlying tissue is termed remote preconditioning. Further studies have found that non-cardiac IP, e.g. renal or peripheral artery IP, is also capable of preconditioning the rat and human heart (Gho 1996, Gunaydin 2000).

5.5. Preconditioning in the human heart

5.5.1. Culture of cardiac myocytes or isolated myocardial tissue

Ikonomidis and colleagues were the first to demonstrate that IP attenuates cellular injury in monolayer culture of human ventricular myocytes (Ikonomidis 1994). Cleveland and colleagues proved that preconditioning can protect the human heart against normothermic ischaemic injury in a series of studies of the isolated right atrial trabeculum (Cleveland 1997a,b,c,d). The IP mechanism has also been investigated in these cultured cardiac myocytes or isolated myocardial tissue. Adenosine receptor and α_1 adrenoceptor stimulation confer protection. The effect is dependent upon the ATP-sensitive potassium channel (Cleveland 1997b) and mediated by PKC (Cleveland 1997a). There was also a close relationship between bradykinin and preconditioned human heart tissue (Muller 1990). These studies suggested that the IP mechanism in the human heart involve stimulation of adenosine A_1, α-adrenaline, bradykinin B_2 receptors, activation of PKC and opening of K_{ATP} channels (Speechly-Dick 1995, Ikonomidis 1997, Morris 1997).

5.5.2. PTCA patients

Deutsch and colleagues were the first to report IP in patients. They compared ST changes on ECG, symptoms, haemodynamics and myocardial lactate production during the first and second balloon inflation during angioplasty, and found evidence of less ischaemia during the second inflation compared with the first (Deutsch 1990). Eltchaninoff and co-workers confirmed the adaptation of myocardial ischaemia to repeated coronary occlusions through measurement of clinical changes, ECG, echocardiography and metabolic parameters (Eltchaninoff 1997). Airaksinen and associates also found the anti-arrhythmic effect of IP during coronary balloon angioplasty (Airaksinen 1997). These effects are mediated partly by adenosine A_1 receptor or α-adrenergic receptor, since they were blocked

by bamiphylline (an adenosine receptor blocker) and phentolamine (Tomai 1996, 1997) and also prevented by glibenclamide, a selective ATP-sensitive potassium channel blocker (Tomai 1994).

5.5.3. Warm-up phenomenon—protective effect of unstable angina

Muller and associates compared the clinical status and short-term outcome of patients with or without preinfarction angina history after thrombolytic therapy and found a less complicated in-hospital course and lower mortality in the preinfarction angina group (Muller 1990). Kloner and colleagues reported their analysis of 350 patients experiencing angina prior to infarction. The infarct size, Q waves, mortality, severe congestive heart failure or shock were less than those without previous history of angina. He contributed this beneficial effect to preconditioning (Kloner 1995a). Clinical data on patients undergoing thrombolysis and PTCA also suggest that episodes of angina immediately prior to acute myocardial infarction are associated with improved contractile recovery, smaller infarct size, lower in-hospital mortality and better 5-year survival (Iwasaka 1994, Ottani 1995, Ishihara 1996, Napoli 1998). A similar phenomenon has been noted by other authors (Tranchesi 1990, Andreotti 1996, Nagao 1997, Kloner 1998, Ottani 1999).

5.5.4. Open heart surgery, with intermittent ischaemic arrest technique

The direct evidence of preconditioning in the human myocardium comes from a study of patients undergoing coronary bypass grafting using the intermittent ischaemic arrest technique. Yellon and colleagues were the first to show the possibility of preconditioning and protecting the human myocardium during an operation. They preconditioned the heart by two periods of 3-minute ischaemia followed by 2-minute reperfusion. ATP in the preconditioned heart was about 50 percent lost but did not change after subsequent 10-minute ischaemic fibrillation. The amount of ATP in the control heart declined significantly (Yellon 1993, Alkhulaifi 1994). With the same preconditioning protocol, they demonstrated that cardiac troponin T (CTnT) decreased in the preconditioning group, especially 72 hours after 33-34 minutes of ischaemia. They concluded that IP might directly delay myocardial necrosis in humans (Jenkins 1997).

5.5.5. CPB operation, with cardioplegia

There has been studies of preconditioning in myocardial protection in open heart surgery. Lu and colleagues found the protective effect of preconditioning with 2 cycles of 2-min ischaemia followed by 3-min reperfusion to be superior to cold crystalloid cardioplegia during valve replacement (Lu 1997). Illes and Swoyer recently reported that ischaemic preconditioning significantly improved heart function and decreased the need for inotropic support in cardiac

operations, with only one cycle of 1-min ischaemia followed by 5-min reperfusion (Illes 1997). They suggested that IP exerts its beneficial effect by preventing stunning. Using 4 minutes cross-clamping followed by 6 min reperfusion before CABG performed in 57 patients, Szmagala and colleagues found the concentration of blood CTnT to be significantly lower one hour after CPB in the IP group (Szmagala P 1998), similar to the findings of Yellon´s group in patients undergoing CABG with intermittent ischaemic arrest technique (Jenkins 1997). They concluded that IP limited myocardial damage during the operative procedure and might afford protection during the postoperative period. They suggested that the long unchanged difference between lower CTnT in IP group and higher in control group may indicate a second window of protection (Szemagala 1998). In double valve replacement patients with cold-blood cardioplegia, IP was found to increase the amount of myocardial superoxide dismutase/malondialdehyde (T-SOD/MDA) during the early reperfusion period, as well as lower release of CK-MB and better haemodynamic outcome with the protocol of two cycles of 3 min of aortic cross-clamping and 2 min of reperfusion (Li 1999).

In view of previous findings, Kloner and Yellon suggested that there is now convincing evidence that human myocardial tissue can be preconditioned (Kloner 1994). However, study of the protective effect of IP in open heart surgery is more complicated, since the pathological changes in the patient may differ in cellular mechanism. The preoperative medication, anaesthetic drugs, operative insult before cross-clamping and CPB might have already preconditioned the myocardium. Different myocardial protective methods, different protocols of preconditioning used and different end points measured might also lead to different conclusions.

5.5.6. Conflicting results from CPB operation

Perrault and colleagues found that CK-MB and lactate increased in their IP group in CABG patients using a single 3min/2min IP protocol. No evidence of IP was found from measuring c-fos and HSP 70 level in atrium biopsy samples. Thus, even though there was no adverse clinical effect, they assumed that pharmacological preconditioning might be more practical in open-heart surgery (Perrault 1996). Kaukoranta and colleagues support this opinion after finding no better IP effect in CABG operation, using a single period of 5-minute ischaemia followed by 5-minute reperfusion (Kaukoranta 1997). Cremer and coworkers also suggested that the beneficial effects of experimental IP seem not to be directly transferable to the clinical setting, after the finding of more inotropic need in CABG patients subjected to 2 cycles of 5 minutes ischaemia and 10 minutes reperfusion (Cremer 1997).

5.6. Clinical factors which may affect IP

5.6.1. Hypothermia and cardioplegia

There are reports regarding IP in conjunction with hypothermia and cardioplegia (Cave 1992, Illes 1994, Kolocassides 1994, Perrault 1996, Landymore 1997, Yellon 1998, Takeshima 1999). Cave's group reported that IP improved functional recovery after hypothermia. Because metabolism and oxygen consumption decreased during hypothermia, the usual period and cycle of ischaemic insult do not stimulate the cellular signal to initiate the IP process. Thus, longer or more cycles of IP are necessary when the myocardium is preconditioned during hypothermia (Cave 1992). Using an isolated rabbit heart perfused on a Langendorff apparatus, Illes and colleagues showed that IP was an effective adjunct to cold crystalloid cardioplegia in both mechanical and biochemical outcomes. Takeshima and colleagues showed that IP was effective with hyperkalaemia and moderate hypothermia, but not in deep hypothermia (Takeshima 1999). In an isolated rat heart model, Galinanes and group found that IP is as effective as cardioplegia, but their combined use does not afford greater protection than the use of either alone. When delivery of cardioplegia was impaired, however, myocardial protection was better served by its use alone (Galinanes 1995). Landymore and associates further proved that IP preceding warm blood cardioplegia arrest reduced myocardial stunning in dogs (Landymore 1997).

5.6.2. CPB

Total CPB triggers a preconditioning response which is as potent as three brief periods of ischaemia as measured by a reduction of infarct size in an ovine model. The loss of atrial and ventricular filling may stimulate a sympathetic-receptor mediated release of local catecholamines, which may act as a trigger for the IP signalling process. Therefore, CPB may by itself elicit the IP effect (Burns 1995). There is a report that direct application of IP stimuli at the beginning of an operation could result in better myocardial protection, this disapproving the assumption that cardio-pulmonary bypass itself may induce IP (Jenkins 1997, Szemagala 1998).

5.6.3. Atherosclosis and coronary stenosis

The existence of coronary stenosis may affect IP induction. The stenosis may cause ischaemia prior to the transient ischaemic episode of IP, and the reperfusion may be restricted by the effect of continuous flow-limiting stenosis (Kloner 1993). A flow-limiting stenosis may precondition the patient's myocardium before the study even begins (Schlaifer 1997). Though IP was achieved by cross-clamping, some blood supply to myocardium may persist because of residual blood in the heart and from collateral flow (Perrault 1996). On the other hand, this may not abolish the induced

IP effects. There is evidence that critical coronary artery stenosis may limit but not abolish the benefit of IP, suggesting that a critical stenosis itself is not enough to induce the IP effect (Kapadia 1997).

Though some authors have suggested that full reperfusion is necessary to elicit IP (Ovize 1992), current evidence suggests that full reperfusion may be less critical than previously believed (Kapadia 1997). Schulz showed that full reperfusion is not necessary in swine: IP effects exist in the situation of coronary flow only 30% of normal (Schulz 1995). It is nonetheless obvious that flow must be re-established between IP and subsequent test occlusion, otherwise the net effect would simply be a more prolonged ischaemic insult. Therefore, even in partial ischaemic model, reperfusion is necessary (Ovize 1992).

Measuring ECG changes and LVEDP in a pacing-induced conscious rabbit preconditioning model, preconditioning effects were lost in a cholesterol-enriched diet in a rat model for 4 weeks. The loss of IP efficacy is correlated with increased serum cholesterol but not with the presence of atherosclerosis. It was re-established after changing to normal diet for 6 weeks (Szilvassy 1995). Therefore, the existence of atherosclerosis and coronary stenosis does not disturb the IP effect.

5.6.4. Age

The immature heart is structurally, functionally and metabolically different from the adult heart. IP fails to work in the Langendorff rat heart under 7 days old (Awad 1998). On the other hand, another report shows that IP effects exist in the isolated cardiomyocytes in 6-day rat (Overlgonne 1996). There are no reports on study of IP in the immature human myocardium.

More and more senescent patients nowadays accept CABG operations. The reports on the IP protective effects on the aged heart are controversial. Animal model studies show that the effect is lost in the senescent (24 month-old) rat heart (Abete 1996) or even middle-aged (50 weeks) rat heart (Tani 1997), whereas the preconditioning response in senescent sheep (5.7 to 8.0 years) is well conserved (Burns 1996). Abete and colleagues found that the angina-induced protection against myocardial infarction was lost in the senescent heart (≥65y). A warm-up phenomenon can be observed in adult but not in older patients (Napoli 1999). A recent report shows that exercise-induced ischaemia protects against successive ischaemia only in adult but not in elderly patients, further confirming the hypothetical age-related reduction of IP in the aging heart (Longobardi

2000). However, Kloner and colleagues showed that the protective effect of preinfarct angina existed in patients 60 years or older (Kloner 1998).

The IP protective effect might also be age-related in studies of IP in open-heart surgery. The patients are relatively young in the reports demonstrating the IP phenomenon in this setting (Table 1). The patients' age in Illes' reports is the only one with mean over 60 years. However, the protocol used in that study to induce the protective effects was much different from the other 3 studies on elderly patients with a mean age over 60 (Yellon 1993, Alkhulaifi 1994, Jenkins 1997, Lu 1997, Illes 1998, Szemagala 1998, Li G 1999).

Table 1. Reports of IP in open-heart surgery

Authors	Age of patients	IP protocol	Results
Yellon and Alkhulaifi	58.0±2.8	2 cycles, 3mI/2mR	Preserve high-energy phosphate
Illes, et al	61.6±1.5	1 cycle, 1mI/5mR	Improve heart performance, decrease in the need for inotropic support
Lu, et al	31.9±3.6	2 cycles, 2mI/3mR	Preserve high-energy phosphate, improve heart performance
Szemagala, et al	56.4±9.2	1 cycle, 4mI/6mR	Decrease in cardiac troponin T release
Jenkin, et al	57±2	2 cycles, 2mI/3mR	Decrease in cardiac troponin T release
Li, et al	32±4	2 cycles, 3mI/2mR	Lower release of CK-MB and better haemodynamic outcome, increase myocardial superoxide dismutase/malondialdehyde activity
Perrault, et al	68±3	1 cycles, 3mI/2mR	CK-MB and lactate increased, no clinically adverse and protective effect
Kaukoranta, et al	63.9±2.1	1 cycles, 5mI/5mR	No better effect of IP
Cremer, et al	62.1± 4.6	2 cycles, 5mI/10mR	Worse finding of inotropic use

Note: IP: ischaemic preconditioning, m: minutes, I: ischaemia, R: reperfusion.

The senescent myocardium has decreased adrenergic responsiveness, an altered coronary microcirculation, impaired calcium transport and impaired excitation-contraction coupling (Burns 1996, Moore 1998). The aged heart is also related to a decrease in the myocardial activity of superoxide dismutase and production of HSP (Tani 1997). Therefore, the diminishing of the IP effect in the senescent heart may be due to the absence of mediators such as norepinephrine, calcium and heat shock protein which trigger this protective mechanism, as well as the decreased in response of effectors (Burns 1996, Abete 1996&1997, Tani 1997). This hypothesis is in agreement with studies showing a reduction in norepinephrine release from cardiac adrenergic terminals after ischaemic reperfusion in older animals (Abete 1997). The IP stimuli could have cumulative effects in the aged myocardium, which is more vulnerable to ischaemia (Tani 1997). Therefore, the IP stimuli needed to induce protective effect at different ages might be different. Further studies are called for to elucidate this hypothesis.

5.6.5. Diabetes

Tosaki and associates suggested that IP might be a healthy heart phenomenon which may not occur in diabetic patients (Tosaki 1996). In isolated human right atrial trabeculae, myocytes from patients with diabetes receiving short-term sulfonylurea hypoglycaemic agents which inhibit K_{ATP} channels can be preconditioned, but not in patients with long-term sulfonylurea hypoglycaemic medication (Cleveland 1997d). Controversy existed in another study; although diabetes induced by streptotocin render the rat heart resistant to infarction, an additional IP effect of reducing infarct size was achieved in the diabetic cohort (Liu Y 1993). Thus the loss of IP effects in Cleveland's study might be due to K_{ATP} channel blocker sulfonylurea hypoglycaemic agents.

5.6.6. Hypertrophy myocardium

Speechly-Dick and colleagues found infarct sizes attenuated in IP group (19% vs. 67% of control) in a DOCA-salt-induced hypertension and hypertrophy rat model (Speechly-Dick 1994). Other authors have described better LV function recovery in spontaneous hypertensive (Boutros 1995) and transgenic hypertensive rat models (Randall 1997). Here again there has been controversy. Pabst and associates found no evidence of IP effect by infarct size (35 % vs. 30%) in a chronic LV hypertrophy model secondary to aortic stenosis in dogs (Pabst 1993).

5.6.7. Medications possibly affecting the study of IP

Some medications in CABG patients might influence the study of IP effects, including angiotensin-converting enzyme (ACE) inhibitor (Dogan 1998), isoflurane (Belhomme 1999), morphine (Liang 1999) and aprotinin (Bukhari 1995).

Angiotensin may act as one of the triggers for IP (Liu Y 1995); thus an ACE inhibitor such as captopril slows the degradation of angiotensin and may mimic the IP effect. There has been evidence that captopril improves post-ischaemic function and decreases myocardial damage (Dogan 1998). Aprotinin, which inhibits NO release from the endothelium, may lead to microvascular vasoconstriction or occlusion, thus increasing ischaemic myocardial damage; this is neutralised by IP (Bukhari 1995). Isoflurane enhances I/R myocardial recovery by activating adenosine A_1 receptors and opening the ATP-dependent potassium channel. PKC may also be involved in the mechanism. Thus isoflurane may mimic the IP effects (Belhomme 1999). Opioid receptor activation mimics the cardioprotective effect of ischaemic preconditioning via mitochondrial K_{ATP} channels activation (Liang 1999).

5.7. IP Protocol

The numbers and duration of cycles of ischaemia required to achieve protection in the human heart are unknown (Galinanes 1995). The repeated cycles and time courses of brief ischaemic insult have varied from 1 to 4 cycles and 1 to 10 minutes in different species (Murry 1986, Schott 1990, Gross 1992, Illes 1998, Przyklenk 1998).

5.7.1. Duration of ischaemia

It is known that IP stimuli cannot be minimised in an effort to limit the detrimental effects of ischaemia without reducing the degree of protection afforded (Cohen 1998). IP may in fact contribute to stunning (Hess 1995); however, it is not necessary for the IP protocol to cause the deleterious effect of myocardial stunning (Schjott 1994). Preconditioning could be produced with ischaemic episodes of less than 5 min, which do not produce stunning in the rat heart. Thus, stunning is probably not needed during IP protocol (Schjott 1994). There is no correlation between the magnitude of stunning and the extent of resultant protection (Miura 1991). In minioperation with no changes in ST-T segment and T-wave during IP protocol, there is still an IP effect of improved contractility (Jacobsohn 1997).

There have been reports that as little as 1.5-2.5 minutes of brief ischaemia provide an effective IP stimulus in dog and rabbit (Yellon 1998). During coronary angioplasty, a balloon inflation time longer than 90 seconds can induce preconditioning (Deutsch 1990, Airaksinen 1997). However, if the inflation time is only 60 seconds, this effect does not occur (Inoue 1996). De Jong and colleagues found that balloon inflation for 60-90 seconds was not enough to induce IP, based on the absence of no changes in lactate and hypoxanthine (De Jong 1993). There is further evidence that balloon inflation with 2 minutes or more improves tolerance to myocardial ischaemia after repetitive coronary

occlusion (Cribier 1992, Tomai 1994 and 1996). During open heart surgery, 2 cycles of 3-minute ischaemia followed by 2-minute reperfusion, 2 cycles of 2-minute ischaemia followed by 3-minute reperfusion or even 1 cycle of 1-minute ischaemia followed by 5-minute reperfusion showed an IP effect (Yellon 1993, Lu 1997, Jenkins 1997, Szemagala 1998, Illes 1998). However, only one 5-minute ischaemia and 5-minute or 10-minute reperfusion could not induce a beneficial protective effect (Kaukoranta 1997, Cremer 1997). It is as yet not known whether this different result is due to the different patients and myocardial protection methods, or merely different IP stimuli.

5.7.2. Cycles

Schlaifer and colleagues believe that one episode of ischaemia is sufficient for protection (Schlaifer 1997), whereas Liu and associates found that 3 cycles are required (Liu Y 1992). Awad and colleagues found that two cycles of 3-min/3-min and 5-min/5-min is the best protocol in rats (Awad 1998). In the rabbit, single 5 min-I 10 min-R conferred the same effect as 4 cycles. The fact that N-2-mercaptopropionyl glycine (MPG), a FR scavenger cannot block 4 cycles but blocks 1 cycle IP suggest that FR act in concert with other triggers such as adenosine and bradykinin via PKC activation (Iwamoto 1991, Tanaka 1994). More cycles of IP might have resulted in greater generation of adenosine and bradykinin, enough to trigger IP by itself even in the absence of FR (Baines 1997). Thus more cycles of IP stimuli are more effective. Lucas and associates have also proved that 2 or 3 cycles, but not 1 or 4, effect on ischaemic contracture, and 1 or 2, not 3 or 4, cycles act on ischaemic anti-arrhythmia in the rabbit (Lucas 1997).

The cycles, or dose of preconditioning needed to achieve an effect may depend on the specific end point (Lucas 1997). It is now clear that there are differences in the degree of dose dependency in different end points. Reducing infarct size is not dose-dependent (Li GC 1990), but both ischaemia and reperfusion-induced arrhythmias and diastolic function are highly dose-dependent (Shiki 1987, Lawson 1993). Repeated cycles lead to a cumulative increase in protection against VF, VT, VPB (Lawson 1993). Sandhu and colleagues showed that 3 cycles IP provide more effective protection against myocardial necrosis than one cycle and are less susceptible to blockage by PKC inhibitor or cAMP agonist (Sandhu 1997). Goto and colleagues postulated that one cycle IP produces a substantial amount of adenosine, bradykinin and norepinephrine, which is enough to stimulate PKC. But if the second component is absent due to bradykinin B_2-receptor blockage with HOE140, extra cycles are needed to produce enough agonist for PKC stimulation and preconditioning (Goto 1995). However, these findings do not mean that more cycles are more effective. In open chest dog, after three 5-min-I/10 min-R cycles, IP effects were lost and recurrent

ischaemic episodes began to have a cumulative effect (Bolli 1995). Beyond 4 cycles, protective effect will diminish with increasing numbers of preconditioning cycles in rabbits (Iliodromitis 1997).

5.7.3. Reperfusion

It is obvious that flow must be re-established between IP and subsequent test occlusion, otherwise the net effect would simply be a more prolonged ischaemic insult; reperfusion is necessary even in a partial ischaemia model (Ovize 1992). Previous studies suggest that the minimum period of reperfusion required to give protection after preconditioning ischaemia lies between 30 seconds and 1 minute (Alkhulaifi 1993). Though some studies suggest that reperfusion is not necessary in coronary stenosis models (Schulz 1995), complete washout of catabolite by effective reperfusion seems to be an essential condition for IP (Wolfe 1993).

5.8. Alternatives to preconditioning stimuli

5.8.1. Adenosine

Adenosine infusion mimics the protective effect of IP on infarct size in rabbits (Liu GS 1991), dogs (Yao 1994), pigs (Van Winkle 1994) and PTCA patients (Kerensky 1995). Cox and associates found a concentration-dependent enhancement of contractile function by adenosine-supplemented cardioplegia in isolated myocytes (Cox 1997). Cohen and colleagues showed that adenosine has most protective effect when administered prior to ischaemia. It preserved ATP and increased lactate production by stimulating glycolysis (Cohen G 1998). Protection is also obtained with agents which indirectly increase tissue adenosine levels, for example acadesine, an adenosine-regulating agent which increases tissue adenosine during ischaemia (Menasche 1995a) and dipyridamole, an adenosine transport inhibitor (Miura 1992).

In a clinical setting, Hudspeth and coworkers added adenosine to blood cardioplegia during an operation in a severely injured heart and showed improvement in myocardial protection (Hudspeth 1994). Leesar and colleagues also mimicked the IP effect in angioplasty by 10 minutes intracoronary adenosine (2mg/min) infusion (Leesar 1997). Repeated intravenous low-dose dipyridamole infusion mimics preconditioning in patients with coronary heart disease (Pasini 1996). However, adenosine has significant vasodilatory and hypotensive effects in clinical patients (Cohen 1998). Thus the use of selective A_1 agonists might have better prospects. For example, R (-)-N-(2-phenylisopropyl)-adenosine (PIA), an adenosine receptor agonist, mimics preconditioning effect in the rabbit (Hale 1993) and isolated atrial trabeculae in humans (Carr 1997).

5.8.2. Potassium channel opener (PCO)

Cromakalim affords additional protection to that provided by cardioplegic arrest and prolonged cold storage using an extracellular solution (Kirsch 1998). Nicorandil provides protective effects similar to IP (Menasche 1995b). PCOs, including pinacidil and aprikalim, are associated with greater coronary flows but greater incidence of reperfusion arrhythmias, compared with St.Thomas solution (Lawton 1996). Lopez and associates suggested that the combined use of potassium cardioplegia with aprikalim (40 μmol/L) or nicorandil (300 μmol/L) can prevent potassium-induced calcium overload and thus might be an effective cardioprotective means (Lopez 1996). Recently, Jayawant and colleagues reported that PCO pinacidil cardioplegia was superior to St. Thomas solution in attenuating myocardial stunning in a CPB pig model (Jayawant 1999). Thus PCOs in cardioplegia provide a useful additive to conventional cardioplegia solution (Dorman 1997) or might replace hyperkalaemic solution as cardioplegia (Cohen NM 1993). However, Dorman and colleagues found that although PCO (SR47063) pretreatment before the induction of cardioplegic arrest provided a protective effect on both LV and myocyte contractility in porcine, it was also associated with severe ventricular arrhythmia in the early reperfusion and rewarming period. The nitrovasodilatory effects and hypotension may limit the clinical use of PCOs (Dorman 1998).

Other pharmacological preconditioning means might include preischaemic administration of $CaCl_2$, norepinephrine, morphine and PKC activator 4 beta-phorbol-12, 13-dibutyrate (PDBu) (Yellon 1998, Przyklenk 1998).

5.8.3. Other preconditioning stimuli

It is now known that stress of almost any kind, including endotoxin, haemorrhage, bacterial lipopolysaccharide, hypoxia, rapid cardiac pacing, acute volume overload and walking exercise, induces endogenous myocardial protection mechanism against ischaemia-reperfusion injury. One form of insult can also induce cross-tolerance to other forms of injury (Meldrum 1997, Przyklenk 1998, Yellon 1998).

5.9. IP in non-cardiac tissue

Skeleton: Pang and colleagues reported that three brief cycles of antecedent ischaemia result in less necrosis in porcine latissimus dorsi flap. This effect was blunted by adenosine acceptor blockade and elicited by A1 receptor activation and K_{ATP} activation (Pang 1995).

Neuronal tissue: A delayed-phase IP effect was found in the rat hippocampus 2-3 days following a short period of sublethal cerebral ischaemia, the effect being associated with HSP_{72} expression (Liu 1992). Adenosine and K_{ATP} are also involved (Heuryeaux 1995).

Lung: In the Langendorff perfused pig lung, 2 cycles of 5-minute I/ 5-minute R have a protective effect on subsequent 3-hour normothermic ischaemia-reperfusion injury (Soncul 1999).

Liver: Preconditioning with prostaglandin I_2 (epoprostenol) infusion intravenously before cold perfusion in human donor liver protects the liver after transplantation (Klein 1999).

5.10. IP and off-pump CABG patients

During minimal invasive CABG operation, Jacobsohn described the protective effect of IP, even no change in ST-T segment and T-wave during IP has an IP effect of improved contractility (Jacobsohn 1997). However, in a pig model resembling an off-pump CABG operation, IP does not improve short-term myocardial function or baseline myocardial perfusion after I/R. Thus the short-term beneficial role of IP in myocardial protection during off-pump bypass surgery may be limited (Tofukuji 1998). Heres and colleagues further proved from a retrospective clinical study that a single 5-minute test LAD occlusion did not protect against subsequent regional ischaemic dysfunction in minimally invasive direct coronary bypass (Heres 1998). Controversial still existed in this field.

5.11. IP and transplantation:

In sheep, 5 minutes of ischaemia followed by10 minutes of reperfusion prevented myocardial stunning of the transplanted heart after 2 hours of crystalloid cardioplegia preservation (Landymore 1998).

AIMS OF THE PRESENT STUDY

1. This series was designed to investigate the IP effect in CABG surgery using combined antegrade and retrograde cold blood cardioplegia on

- haemodynamic function
- right ventricular function
- generation of free radicals
- myocardial cellular necrosis as measured by biochemical markers

2. to study the possible mechanism of IP in open heart surgery by measuring

- free radial production in the myocardium
- lactate production

3. to establish whether unstable angina pectoris before the operation has a protective effect resembling preconditioning.

4. to establish whether a patient with unstable angina pectoris (UAP) can be protected by IP.

PATIENTS AND METHODS

1. Patients and study designs

The study design was accepted by the Ethical Committee of Tampere University Hospital, Finland, and informed consent was obtained from all patients. The study was carried out from October 1998 to June 2000 in the Division of Cardiac Surgery, the Department of Surgery, the Department of Anaesthesiology and Intensive Care, the Centre for Laboratory Medicine, Tampere University Hospital, Tampere, and the Department of Chemistry, University of Jyväskylä, FINLAND.

1.1. Patient selection

Eighty patients with stable and unstable angina and with 3-vessel disease undergoing CABG were randomised equally into 4 groups: group 1: IP group with stable angina patients (IP-s, N_1=20), group 2: control group with stable angina patients (C-s, N_2=20), group 3: IP group with unstable angina patients (IP-u, N_3=20), group 4: control group with unstable angina patients (C-u, N_4=20). All unstable patients had UAP within 3 days before the operation. These were diagnosed by ECG

and treated with nitroglycerine infusion. The period between the most recent ischaemic episode and cross-clamping was registered and divided into subgroups.

The 11 unstable control cases having UAP within 48 hours, including 8 cases who experienced UAP within 24 hours and 3 within 24-48 hours, were merged into the same subgroup (subgroup A) in view of the small number of cases and absence of statistical significance in CI between these patients, but different from the patients having UAP within 48-72 hours (subgroup B) and the stable controls. Similarly, The 7 unstable IP patients having UAP within 48 hours were set as subgroup A and 13 patients having UAP within 48-72 hours as subgroup B.

Patients with low EF (<40%), recent myocardial infarction, additional cardiac diseases, severe noncardiac diseases (such as renal failure, severe chronic hepatic cirrhosis and carcinoma) and calcified or severely dilated ascending aorta were excluded from the study.

1.2. Designs

The studies were designed as prospective, randomised and controlled clinical trial. Studies I-III were carried out on the patients in group 1 and group 2. Study IV compared the results between group 2 and group 4 to investigate whether unstable angina pectoris prior to the operation has a protective effect resembling preconditioning. Study V included the patients in group 3 and group 4 to study the preconditioning phenomenon in unstable patients.

1.3. Preoperative data and peri-operative course

There were no differences in patients' age, sex, severity of stenosed vessels, numbers of cases with LV hypertrophy, numbers of patients with diabetes, preoperative risk factor (Cleveland), and preoperative medication between the groups. More patients in the unstable groups were in NYHA class III and IV and they also had a lower mean value of LVEF. There were also more episodes of previous MI infarction in the unstable group. There were no differences in these variables between the IP and the control groups in stable and unstable angina patients. The numbers of vessels bypassed in the stable IP group was significantly higher ($p = 0.041$), entailing a longer cross-clamping time compared with the stable control group ($p = 0.042$). No differences were found in CPB time and ventricular fibrillation (VF) after declamping. The numbers of cardioversions in unstable controls are significantly less than in stable controls ($p = 0.025$) (Table 2).

Table 2: Preoperative data and peri-operative course.

	IP-s (N=20)	C-s (N=20)	IP-u (N=20)	C-u (N=20)
Age (years)	63.7 ± 10.7	66.6 ± 9.3	64.6 ± 8.6	67.0 ± 8.4
Sex (female/male)	4/16	5/15	5/15	5/15
NYHA (II/III/IV)	4/13/3	1/17/1	0/1/19	0/2/18 ††
LVEF (%)	63.4 ± 9.0	66.8 ± 8.9	56.3 ± 12.0	59.2 ± 10.4 †
LAD (stenosis %)	72.9 ± 22.8	78.6 ± 12.3	88.0 ± 14.5	82.0 ± 25.5
LCX (stenosis %)	85.6 ± 14.5	79.2 ± 15.7	74.4 ± 28.6	71.1 ± 16.8
RCA (stenosis %)	91.8 ± 11.7	82.7 ± 18.3	77.3 ± 25.8	76.1 ± 26.0
LM (>50%, No.)	9	9	7	5
MI history (No.)	7	4	13	13 †
LV hypertrophy (No.)	3	4	5	3
Diabetics (No.)	2	4	6	7
Risk factor (Cleveland) (0/1/2/3/4/5)	4/11/4/0/0/1	4/8/5/2/0/1	4/9/3/3/0/1	5/5/5/2/2/1
ACE inhibitor (No.)	3	3	6	5
Ca^{++} antagonist (No.)	5	6	7	6
Vessels bypassed (3/4/5/6)	2/12/6/0	8/9/1/1	10/8/2/0	10/6/4/0
Vessels bypassed (No.)	4.2 ± 0.6 *	3.7 ± 0.9	3.5 ± 0.8	3.5 ± 0.9
Cross-clamping (minutes)	86.4 ± 12.7 *	77.5 ± 15.1	76.5 ± 21.4	76.2 ± 17.1
CPB time (minutes)	117.5 ± 13.4	107.2 ± 17.0	112.1 ± 25.3	111.3 ± 23.0
VF after declamping (No.)	6	11	7	7
Cardioversion (No.)	6	12	9	5 †

Note: IP-s: IP group in stable angina patients, C-s: control group in stable angina patients, IP-u: IP group in unstable angina patients, C-u: control group in unstable angina patients. NYHA: New York Heart Association class; LVEF: Left ventricular ejection fraction; LAD: Left anterior ascending artery; LCX: left circumflex artery; RCA: right coronary artery; LM: left main coronary artery; stenosis %: mean value of percentage of vessel stenosis in the patients. MI: Myocardial infarction; ACE: angiotensin-converting enzyme; CPB: cardiopulmonary bypass; VF: ventricular fibrillation. Data are presented as mean ± SD. Significance between group 1 and group 2, *: $p<0.05$, Significance between group 2 and group 4, †: $p<0.05$, ††: $p<0.01$.

2. IP protocol:

After establishing CPB and running the pump to empty the heart, the ascending aorta was occluded by cross-clamping for 2 minutes, followed by 3 minutes reperfusion, the procedure being

repeated once. In the control group, the pump had also been run for 10 minutes before the routine operation (Figure I). The temperature was kept normothermic during this period.

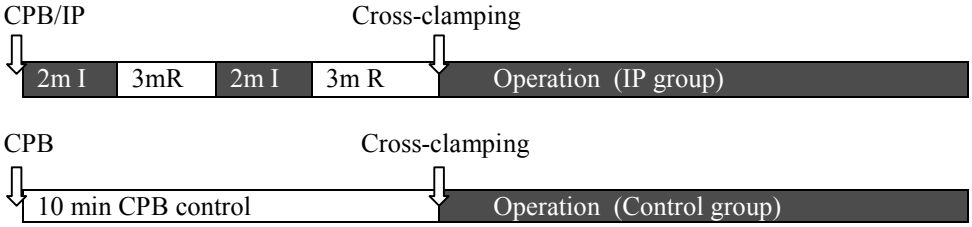

Figure 1: Ischaemic preconditioning protocol. I: ischaemia achieved by aortic cross-clamping; R: reperfusion by releasing cross-clamping.

3. Anaesthesia

A standardised anaesthetic technique was used with sufentanil, midazolam and pancuronium.

4. Cardiopulmonary bypass and surgical technique

Cardiopulmonary bypass with non-pulsatile perfusion flow (2.2-2.4l/min/m2) was conducted using membrane oxygenators with arterial line filtration. Mild systemic hypothermia (32^{o}C) was maintained.

Surgical techniques were the same in all patients. Aortic root and two-stage single venous cannula were used for CPB. A retrograde, self-inflating cardioplegia cannula (RC014, Research Medical Inc., UTAH) with a pressure-monitoring port was guided into the coronary sinus. A nine-gauge cannula was placed in the aortic root for antegrade cardioplegia and for venting (during retrograde delivery). Distal anastomoses were made in the order RCA-CX-LAD. LITA-LAD was used in all patients, venous grafts or left radial artery for the other vessels. The proximal anastomoses were constructed during cross-clamping in reverse order.

5. Cardioplegia delivery schemes

Blood from the pump reservoir was mixed with crystalloid in a ratio of 4:1, yielding a cardioplegia solution with a hematocrit value of 0.21 and 21 mmol/l potassium concentration in the initial dose and 9 mmol/l in subsequent. In antegrade delivery, cardioplegia was administered at a pressure of 80 mmHg and in retrograde 30-40 mmHg, at least with a flow of 200ml/min. The initial high-potassium cardioplegia was given 1.5 minutes antegrade then 2.5 minutes retrograde, with a solution temperature of 6-9 ^{o}C. An additional dose of 1 minute was given retrogradely and also to

RCA and CX area grafts after each distal anastomosis. Warm cardioplegia (37^{o}C) was given retrogradely for 3 min before cross-clamping released. No magnesium was used in cardioplegia until rewarming, when 10 mmol was given intravenously.

6. Monitoring

6.1. Haemodynamic and right ventricular function measurements

Heart rate (HR), mean arterial pressure (MAP), mean pulmonary artery pressure (MPAP), pulmonary capillary wedge pressure (PCWP), cardiac output (CO) and right ventricular ejection fraction (RVEF) were monitored. Derived cardiovascular variables, including cardiac index (CI), right ventricular stroke work index (RVSWI), left ventricular stroke work index (LVSWI), pulmonary vascular resistance index (PVRI), systemic vascular resistance index (SVRI) and right ventricular end diastolic volume index (RVEDVI) were calculated using standard formulas.

RV function was measured using a fast-response volumetric thermister-tipped pulmonary artery catheter (93A-434H-7.5F, Baxter Health Care Corp., CA) and a microprocessor (Explorer^{TM} Baxter Health Care Corp., Edwards Division, CA), which allowed measurement of the diastolic washout plateaus of a thermodilution cardiac output curve using exponential curve analysis. All measurements based on the thermodilution technique were made at end-expirium using ice-cold saline. The mean value of three consecutive measurements at one-time points was registered. Before each measurement of RVEF, the correct position of the catheter and right atrial delivery site were confirmed by analysis of the transduced pressure waveform.

Haemodynamic data were collected at four time-points: (1) baseline: before induction of anaesthesia, (2) 1 hour after declamping; (3) 6 hours after declamping; (4) first postoperative day (1st POD).

6.2. Free radicals

Free radicals (FR) were measured in 40 stable patients (group 1 and 2). Blood samples (1ml) were taken into syringes containing 1 ml of 50 mmol/l solution of α-phenyl-tert-N-butylnitrone (PBN) from the coronary sinus at three time-points: (1) before CPB, (2) at the end of IP protocol or at 10 minutes of CPB, (3)10 minutes after declamping.

The oxygen-centred free radicals are known to react with PBN to form stable spin adduct radicals. The resulting adducts were then extracted into toluene and further analysed by electron spin

resonance (ESR) spectroscopy measurement. The relative spin adduct radical concentration was obtained by determining the first derivative peak height and width of the signal. All the ESR measurements were performed with a Varian E-line X-band ESR spectrometer (Varian, Analytical Instrument Division, Palo Alto, California, USA) using a modulation amplitude of 1 mT. The samples were kept in glass tubes at -70^{o}C after drawing, and during storage, transportation and ESR measurements. The FR concentration before CPB in sinus samples was set as baseline. FR generated after IP and cross-clamping release was calculated according to baseline values.

6.3. Biochemical markers

Lactate: Simultaneous samples of radial and coronary sinus blood were obtained for measurement of lactate content at three time-points: (1) before CPB, (2) at the end of IP protocol or at 10 minutes of CPB immediately prior to cross-clamping, (3) 5 minutes after cross-clamping release. One ml of blood was immediately placed in a test tube containing 2 ml of 0.6 mol/l percholoric acid and gently mixed. After centrifugation, the clear supernatant was removed and analysed using a commercial kit (Lactic acid, No.826-UV, Sigma Chemical Co., St Louis, MO) and DNA/Protein/Enzyme Analyzer (Biospec-1601E, SHIMADZU, JAPAN).

CK-MB, cardiac troponin I (CTnI), myoglobin: Blood samples were collected from the coronary sinus and peripheral vessels: (1) before CPB, (2) at the end of IP protocol or at 10 minutes of CPB, (3) 5 minutes after cross-clamping release, and from the peripheral vessel (4) 6 hours after declamping, (5) 1^{st} POD and (6) 2^{nd} POD. Samples were collected in heparin-coated plastic tubes and centrifuged. Serum samples were measured with a Chiron ACS180^{R} analyzer (ACS 180:Plus, Chiron Diagnostics Corporation, East Waalpole, MA) using a direct chemiluminescence method.

7. Haemodynamic control and postoperative care

Peri-operatively, volume infusion was designed to maintain filling pressures at, at least, preoperative level and optimal for heart performance. Inotropes (dopexamine or adrenaline) were used to maintain the cardiac index (CI) greater than 2.0 L/min/M^2. Amrinone with noradrenaline was used when dopexamine or adrenaline was insufficient to maintain the criteria. They were applied after release of cross-clamping and continued for at least 6 hours. Inotrope was not discontinued at the time-points when haemodynamic data were measured.

After the operation, the patients were attended in ICU, where mechanical ventilation was continued for at least 6 hours. Extubation was performed based on respiratory recovery. Patients were treated in the ICU till stable condition was ensured. Perioperative infarction was diagnosed if

any new Q wave appeared (one third QRS height and >0,04 seconds) or a CK-MB value was higher than 100 μg/L.

8. Statistical methods

Statistical analyses were made using the SPSS/Win (version 9.0) statistical package program; unpaired Student ´s t test (2-tails) and Pearson's X^2 test with Fisher's Exact test correction were used in comparing variables between the two groups. One-way ANOVA was used to compare variables in more than 2 groups, if any significance appeared; unpaired Student ´s t test (2-tails) was then used to compare the difference between the two groups (groups 1 and 2, groups 2 and 4 and groups 3 and 4). Non-parametric test (Mann-Whitney U) was used for skewed distributions. General Linear Model (GLM) analysis with repeated measures of variance was used to test repeated observation variables after the operation. Baseline values were used as a covariate when appropriate in the analysis. Pearson's correlation was used to measure the relation of two variables. Data are presented as mean $\pm$ standard deviation. Significance was assumed when the p value was less than 0.05.

RESULTS

1. Outcome of surgery:

One patient of 79 years with systemic atherosclerosis in the stable control group (group 2) died of a cerebral complication on the 4th POD. There was no evidence of myocardial infarction or poor haemodynamic performance in this patient. There were no other cerebral complications or perioperative myocardial infarctions. No intra-aortic balloon pump was used.

There was a tendency to longer mechanical ventilation period in stable controls, albeit without statistical significance (p = 0.068). The stay in ICU was similar in the groups. More patients in the stable control group needed inotropic support (p = 0.044), and the duration of inotropic medication in this group was also longer (p = 0.008) (Table 3).

Table 3: Postoperative care

	IP-s (N=20)	C-s (N=20)	IP-u (N=20)	C-u (N=20)
Mechanical ventilation (hours)	12.7 ± 3.5	21.2 ± 23.5	11.9 ± 3.3	15.7 ± 8.7
Stay in ICU (hours)	37.0 ± 23.9	46.2 ± 43.3	31.3 ± 27.3	38.3 ± 21.4
Free of inotropes (No.)	7 *	1	8	7 †
Duration of inotropes (hours)	11.7 ± 11.8	26.4 ± 31.8	8.4 ± 9.0	9.7 ± 9.3 †
Dopexamine or adrenaline (No.)	9	15	6	10
Amrinone/noradrenaline (No.)	10	11	10	9

Note: IP-s: IP group in stable angina patients, C-s: control group in stable angina patients, IP-u: IP group in unstable angina patients, C-u: control group in unstable angina patients. Significance between the group 1 and group 2, *: $p<0.05$, Significance between group 2 and group 4, †: $p<0.05$.

2. Haemodynamic and right ventricular function

HR, BP and loading parameters

The preoperative baseline data in HR, MAP, CVP, MPAP, PCWP, RVEDVI, PVRI and SVRI were similar between the groups. There were no statistical significances in HR (p = 0.328), MAP (p = 0.764), CVP (p = 769), MPAP (p = 642), PCWP (p = 0.994), RVEDVI (p = 0.067) and PVRI (p = 0.814) and SVRI (p = 0.077) after the operation. Subgroup analysis of different time of antecedent angina likewise showed no significant differences in these parameters (Table 4).

Table 4. Haemodynamic data

		Baseline	1h declamping	6h declamping	1st POD
HR (beats/min)	IP-s	58.7 ± 11.8	80.7 ± 18.9	92.7 ± 13.9	80.4 ± 8.4
	C-s	61.2 ± 10.6	79.7 ± 11.2	85.8 ± 24.0	82.6 ± 11.7
	IP-u	63.0 ± 5.7	76.2 ± 11.4	92.0 ± 15.7	80.4 ± 13.2
	C-u	67.0 ± 8.4	74.7 ± 15.2	87.4 ± 17.8	81.6 ± 11.9
MAP (mmHg)	IP-s	89.3 ± 12.2	76.8 ± 10.8	80.0 ± 11.9	77.4 ± 10.6
	C-s	94.1 ± 10.4	77.4 ± 11.6	77.9 ± 11.6	82.1 ± 12.7
	IP-u	85.9 ± 14.5	75.7 ± 8.4	76.6 ± 13.6	75.3 ± 10.6
	C-u	88.5 ± 16.9	77.0 ± 12.0	77.9 ± 11.1	77.9 ± 10.3
CVP (mmHg)	IP-s	7.6 ± 3.1	9.8 ± 2.0	10.3 ± 2.9	9.5 ± 2.4
	C-s	8.5 ± 2.0	9.9 ± 2.2	10.1 ± 2.7	8.6 ± 3.4
	IP-u	7.0 ± 2.4	9.6 ± 1.9	10.0 ± 2.4	8.4 ± 3.4
	C-u	7.3 ± 3.1	9.5 ± 3.1	11.5 ± 3.3	7.8 ± 2.5
MPAP (mmHg)	IP-s	20.7 ± 4.3	19.1 ± 2.8	23.8 ± 5.4	21.5 ± 3.3
	C-s	23.0 ± 7.2	21.4 ± 5.3	24.3 ± 5.1	21.9 ± 5.9
	IP-u	20.6 ± 7.6	20.0 ± 8.7	22.5 ± 5.1	20.6 ± 4.2
	C-u	17.8 ± 4.5*	19.4 ± 4.9	22.8 ± 5.7	19.2 ± 3.4
PCWP (mmHg)	IP-s	11.8 ± 2.8	10.5 ± 2.3	10.5 ± 3.4	11.8 ± 1.8
	C-s	14.1 ± 5.7	11.7 ± 2.6	11.6 ± 3.1	11.6 ± 2.9
	IP-u	12.3 ± 4.3	11.1 ± 4.8	11.2 ± 3.2	10.8 ± 2.5
	C-u	11.5 ± 3.6	11.1 ± 3.3	11.1 ± 3.4	10.1 ± 2.0
RVEDVI (ml/M^2)	IP-s	99.8 ± 14.2	79.4 ± 15.6	88.7 ± 17.8	95.7 ± 14.7
	C-s	102.9 ± 14.1	79.0 ± 20.7	84.9 ± 15.4	98.2 ± 26.8
	IP-u	109.6 ± 23.6	85.7 ± 18.3	96.1 ± 20.5	100.0 ± 22.0
	C-u	98.3 ± 20.7	90.3 ± 22.7	101.0 ± 33.0	104.8 ± 21.9
PVRI ($dyn.sec/cm^5/M^2$)	IP-s	252.1 ± 112.6	290.9 ± 68.9	386.8 ± 130.4	287.3 ± 100.5
	C-s	272.2 ± 101.0	347.4 ± 136.1	424.7 ± 137.8	284.2 ± 91.4
	IP-u	293.2 ± 198.8	398.2 ± 454.7	323.7 ± 143.1	283.5 ± 105.2
	C-u	201.2 ± 78.9	275.0 ± 83.5	361.3 ± 197.7	243.0 ± 80.2
SVRI ($dyn.sec/cm^5/M^2$)	IP-s	2871 ± 495	2265 ± 559	1977 ± 430	2036 ± 487
	C-s	2641 ± 492	2436 ± 665	2290 ± 714	2074 ± 472
	IP-u	2705 ± 732	2117 ± 356	1902 ± 636	1908 ± 554
	C-u	2712 ± 655	2257 ± 610	1900 ± 669	1894 ± 390

Note: IP-s: stable angina IP group. C-s: stable angina control group. IP-u: unstable angina IP group. C-u: unstable angina control group. HR: heart rate, MAP: mean artery pressure. CVP: central venous pressure. MPAP: mean pulmonary artery pressure. PCWP: pulmonary capillary wedge pressure. RVEDVI: right ventricular end diastolic volume index. PVRI: pulmonary vascular resistance index. SVRI: systemic vascular resistance index. Data are presented as mean ± SD. Significance between group C-s and group C-u, *: $p<0.05$

CI

The baseline values for CI were similar between the groups (2.35 ± 0.33, 2.53 ± 0.39, 2.51 ± 0.34, 2.54 ± 0.49 L/min/M2, p = 0.336). CI decreased at 1 and 6 hours after declamping in the control group (88% and 97% of baseline) and recovered on the 1st POD (112%). On the other hand, CI increased at all three time-points after the operation in the stable IP group (106%, 129%, 126% respectively), the unstable control group (104%, 122% and 131% of baseline respectively) and unstable IP group (106%, 125% and 124% of baseline respectively).

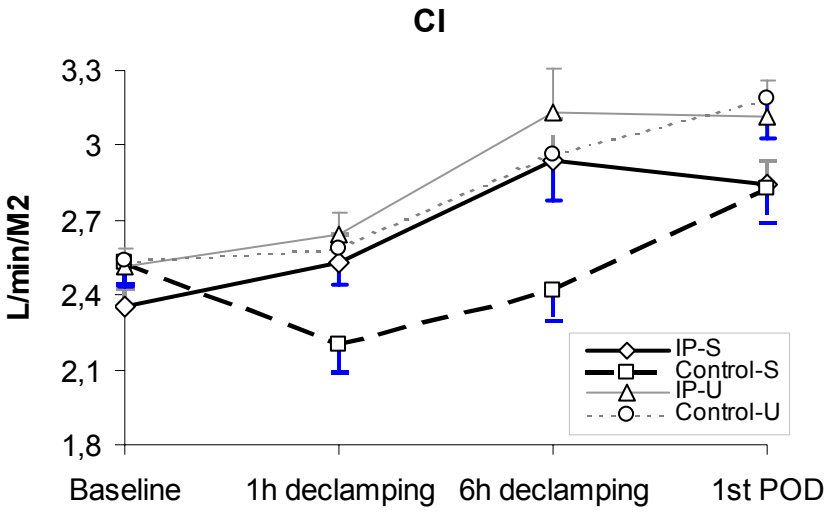

Figure 2: CI after CABG operation in IP and control group in the stable and unstable patients. IP-S: IP group in stable angina patients, Control-S: control group in stable angina patients, IP-U: IP group in unstable angina patients, Control-U: control group in unstable angina patients. CI depressed at 1 and 6 hours after declamping in the stable control group, whereas the CI increased after the operation in the other groups. Data are presented as mean ± SD.

In the stable patients (groups 1 and 2), the values of CI after the operation were significantly higher in the stable IP group than in stable controls (p = 0.013) (Figure 2).

In the stable and unstable control patients (groups 2 and 4), the CI values after the operation in the unstable control patients were better compared to those in stable controls (p = 0.007) (Figure 2). Subgroup analysis showed that the recovery of CI was better in subgroup A unstable control patients (UAP within 48h) than in stable controls (p = 0.005). There was no difference in CI after surgery between subgroup B unstable control patients (UAP within 48-72h) and stable control patients (p = 0.154) (Figure 3).

In the unstable patients (groups 3 and 4), there were no differences in CI after the operation between the unstable IP and control groups (p = 0.717) (Figure 2). Subgroup analysis showed no differences between the IP and controls in CI after the operation in subgroup A unstable patients (UAP within 48h) (p = 0.670). The CI after surgery in subgroup B unstable IP patients (UAP within 48-72h) was higher than in control patients, but without statistically significant difference by analysis of variance for repeated measures (p = 0.142) (Figure 3).

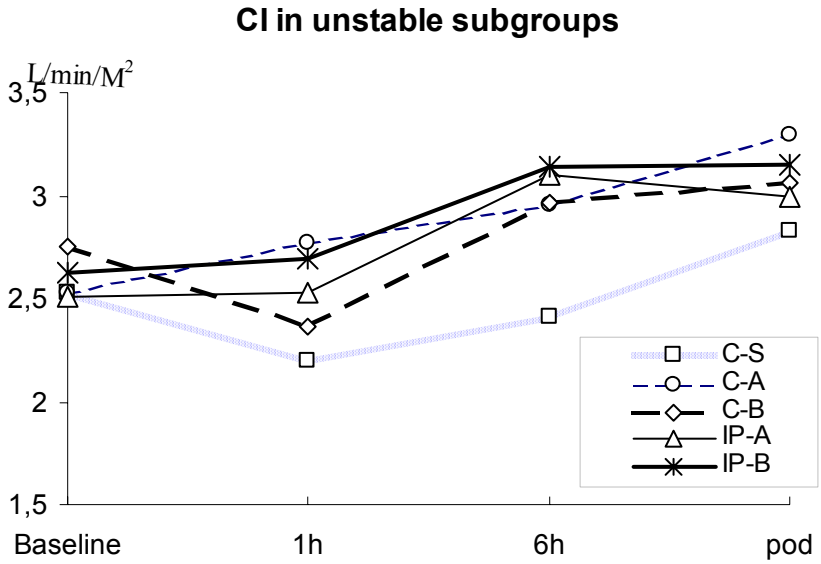

Figure 3: CI in the unstable subgroups and the stable control group. C-S: stable control group, C-A: unstable control subgroup A (UAP within 48h), C-B: unstable control subgroup B (UAP within 48-72h), IP-A: unstable IP subgroup A (UAP within 48h), IP-B: unstable IP subgroup B (UAP within 48-72h). The recovery in CI after the surgery was better in the C-A (p = 0.005) but not in the C-B subgroup (p = 0.154) when compared with the stable controls. IP did not result in better CI outcome in subgroup A patients (UAP within 48h), but in the better CI at 1 hours after declamping in subgroup B patients (UAP within 48-72h) (p = 0.027).

RVEF

The baseline values for RVEF were similar between the groups (40.05 $\pm$ 4.26, 40.85 $\pm$ 6.17, 42.35 $\pm$ 7.34, 43.95 $\pm$ 7.05 %, p = 0.226). RVEF at 1 and 6 hours after declamping and 1st POD was 101%, 90% and 91% of baseline in the stable IP group, 88%, 78% and 89% of baseline in the stable controls, 99%, 87% and 91% in the unstable IP group, 89%, 79% and 86% of baseline in the unstable controls (Figure 4).

In the stable patients (groups 1 and 2), RVEF after the operation was better in the stable IP patients than in stable controls (p = 0.012) (Figure 4).

In the stable and unstable control patients (groups 2 and 4), there was no significant difference in RVEF (p = 0.304) (Figure 4). Subgroup analysis between these groups showed that the recovery of RVEF tended to be better in subgroup A unstable patients (UAP within 48h) than in stable controls, albeit without statistically significant difference (p = 0.097). There was no difference in RVEF after the operation between subgroup B unstable patients (UAP within 48-72h) and stable control patients (p = 0.915) (Figure 5).

In the unstable patients (groups 3 and 4), RVEF was similar between unstable IP and control groups (p = 0.158) (Figure 4). Subgroup analysis showed that there were no differences in RVEF after the operation between the IP and controls in the subgroup A unstable patients (UAP within 48h) (p = 0.773). RVEF after surgery was better in the subgroup B unstable IP patients than in unstable controls (p = 0.030) (Figure 5).

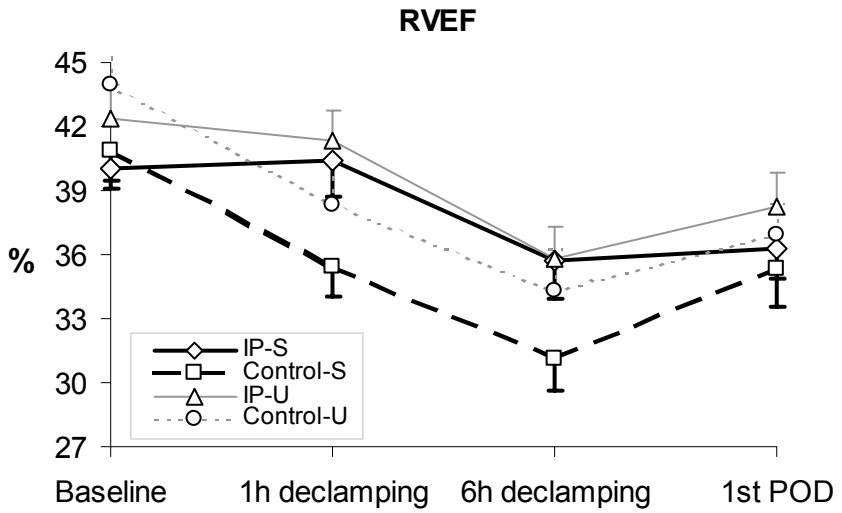

Figure 4: RVEF after CABG operation in IP and control group in the stable and unstable patients. IP-S: IP group in stable angina patients, Control-S: control group in stable angina patients, IP-U: IP group in unstable angina patients, Control-U: control group in unstable angina patients. Data are presented as mean $\pm$ SD.

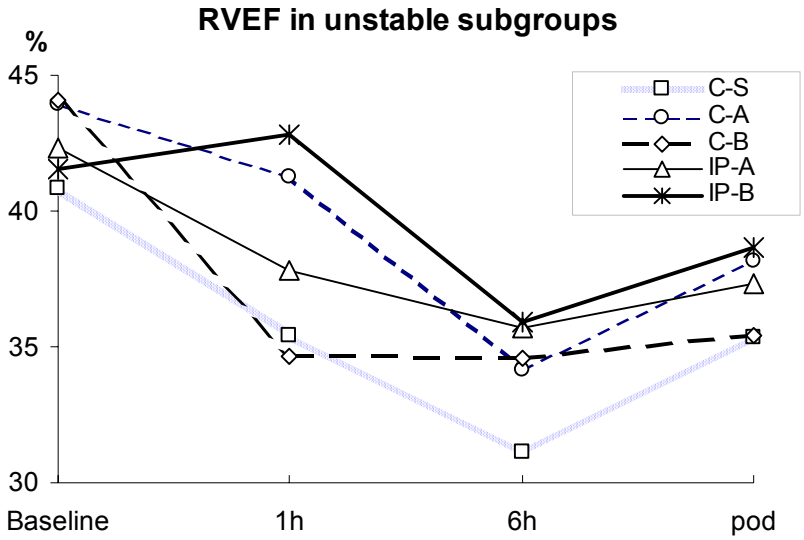

Figure 5: RVEF in the unstable subgroups and the stable control group. C-S: stable control group, C-A: unstable control subgroup A (UAP within 48h), C-B: unstable control subgroup B (UAP within 48-72h), IP-A: unstable IP subgroup A (UAP within 48h), IP-B: unstable IP subgroup B (UAP within 48-72h). The recovery in RVEF after surgery tended to be better in C-A (p = 0.095) when compared with the stable controls. RVEF was similar between the unstable control subgroup B and the stable controls. IP did not result in better RVEF outcome in the subgroup patients (UAP within 48h), but resulted in better RVEF in subgroup B patients (UAP within 48-72h) (p = 0.030).

LVSWI

The baseline values for LVSWI were similar between the groups (44.22 $\pm$ 9.69, 47.07 $\pm$ 12.06, 46.44 $\pm$ 11.42, 45.22 $\pm$ 12.30 (gm-m)/M^2/b, p = 0.828). LVSWI was depressed after the operation and did not recover until the 1st POD in all patients. The values for LVSWI after surgery were significantly higher in the stable IP group than in stable controls (p = 0.027). The values after the operation in the unstable control patients were also better compared to the stable controls (p = 0.027). There were no differences in LVSWI after the operation between the unstable IP and control groups (p = 0.433) (Figure 6).

Subgroup analysis between the stable and unstable control subgroups showed that the recovery of LVSWI was better in the subgroup A unstable control patients (UAP within 48h) than in stable controls (p = 0.019). There was no difference in LVSWI after surgery between the subgroup B unstable control patients (UAP within 48-72h) and the stable control patients (p = 0.142).

Subgroup analysis between the unstable IP and unstable control patients brought out no differences in LVSWI after surgery between the subgroup A unstable patients (UAP within 48h) (p = 0.867), and subgroup B unstable patients (UAP within 48-72h) (p = 0.844).

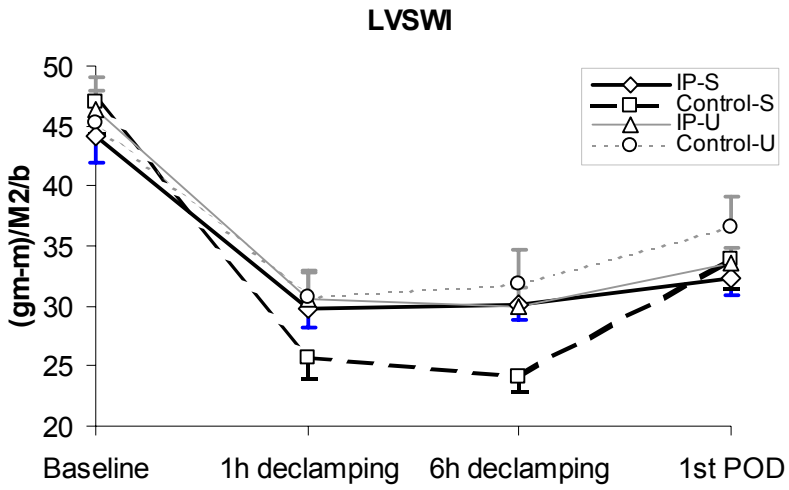

Figure 6: LVSWI after CABG operation in IP and control group in the stable and unstable patients. IP-S: IP group in stable angina patients, Control-S: control group in stable angina patients, IP-U: IP group in unstable angina patients, Control-U: control group in unstable angina patients. Data are presented as mean ± SD.

RVSWI

The baseline values for RVSWI were similar between the groups (6.92 ± 1.72, 7.34 ± 2.88, 8.23 ± 3.03, 6.38 ± 2.26 (gm-m)/M^2/b, p = 0.138). The RVSWI values after the operation were significantly higher in the stable IP group than in stable controls (p=0.002). The postoperative values in the unstable control patients were also better compared to the stable controls (p = 0.002). There were no differences in RVSWI after surgery between unstable IP and control groups (p = 0.897) (Figure 7).

Subgroup analysis between the stable and unstable control patients showed recovery of RVSWI to be better in the subgroup A unstable control patients (UAP within 48h) than in stable controls (p = 0.003). There was no difference in RVSWI after the operation between the subgroup B unstable control patients (UAP within 48-72h) and the stable control patients (p = 0.099).

Subgroup analysis between the unstable IP and control patients showed that there were no differences in RVSWI after the operation in subgroup A unstable patients (UAP within 48h) (p = 0.302), and subgroup B unstable patients (UAP within 48-72h) (p = 0.309).

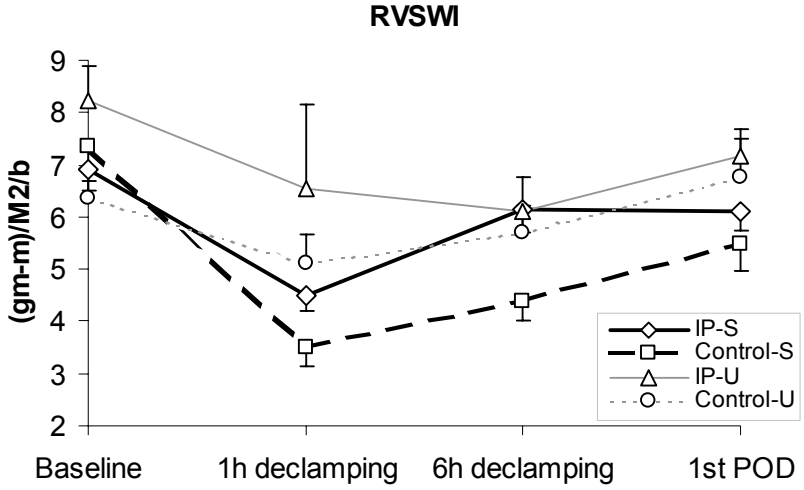

Figure 7: RVSWI after CABG operation in IP and control group in the stable and unstable patients. IP-S: IP group in stable angina patients, Control-S: control group in stable angina patients, IP-U: IP group in unstable angina patients, Control-U: control group in unstable angina patients. Data are presented as mean ± SD.

3. Cellular viability

CK-MB

The baseline values for CK-MB were similar in the 4 groups (1.91 ± 1.68, 1.97 ± 1.21, 1.44 ± 0.63, 1.55 ± 0.0.91 µg/l respectively, $p = 0.470$). Both 10 minutes of CPB or IP protocol induced a significant release of CK-MB ($p < 0.001$). The extent of release after IP protocol and CPB control was similar in stable and unstable patients ($p = 0.364$). A considerable amount of CK-MB was released after the operation. Peak elevation CK-MB was reached at 6 hours after declamping in groups 1, 2 and 4 (30.52, 25.85 and 19.05 µg/l respectively). The peak value in group 3 was 19.93 µg/l on the 1st POD.

There were no statistically significant differences between the IP and control in the stable and unstable patients ($p = 0.226$ between group 1 and 2, and 0.496 between group 3 and 4). The CK-MB values after the operation were insignificantly lower in the unstable control patients than the stable control patients (group 4 and 2, $p = 0.056$). The antecedent angina in different subgroups did not affect these enzyme levels. The CK-MB values between the sinus and arterial samples were similar ($p = 0.114$) and closely correlated with each other ($r = 0.965$) (Figure 8).

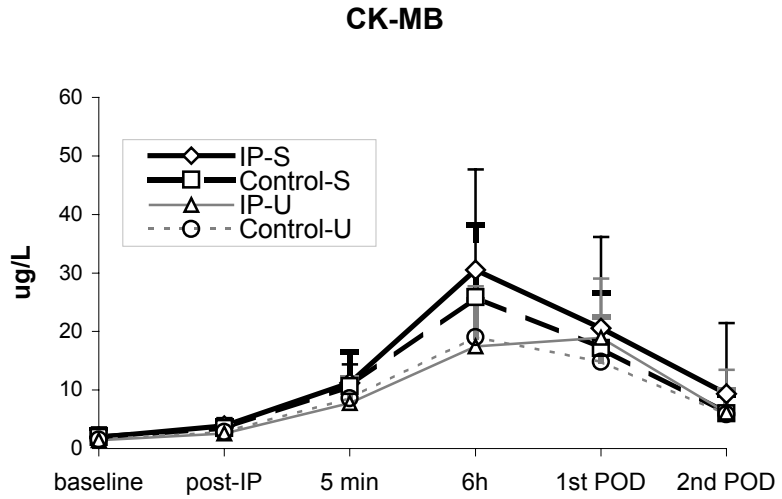

Figure 8: Creatine kinase isoenzyme MB (CK-MB) from peripheral blood samples after CABG operation in IP and control group in the stable and unstable patients. IP-S: IP group in stable angina patients, Control-S: control group in stable angina patients, IP-U: IP group in unstable angina patients, Control-U: control group in unstable angina patients. The CK-MB release after the operation tended to be lower in the unstable patients without statistical significance ($p = 0.056$). IP does not change the values of CK-MB release after a CABG operation. Data are presented as mean $\pm$ SD.

CTnI

The baseline values for CTnI were similar among the 4 groups (0.15 $\pm$ 0.12, 0.16 $\pm$ 0.16, 0.27 $\pm$ 0.38, 0.18 $\pm$ 0.30 µg/l respectively, $p = 0.403$). Both 10 minutes of CPB or IP protocol resulted in a significant release of CTnI ($p < 0.001$). The extent of increasing release after IP protocol was higher than CPB control in stable patients (0.36 $\pm$ 0.24 vs. 0.23 $\pm$ 0.23 µg/l, $p = 0.047$). A considerable amount of CTnI was released after the operation. Peak elevation CTnI was reached at 6 hours after declamping in groups 1 and 2 (23.30 and 17.39 µg/l respectively). The peak values in groups 3 and 4 were 9.81 and 8.29 µg/l on the 1st POD.

There were no statistically significant differences between the IP and control groups in the stable and unstable patients ($p = 0.299$ between groups 1 and 2, and 0.670 between groups 3 and 4). The CTnI values after the operation were significantly lower in the unstable control patients than in the stable control patients (group 4 and 2, $p = 0.006$). The most significant difference was seen at 6 hours after declamping ($p < 0.001$). Antecedent angina in the various subgroups did not affect these enzyme levels. When unstable control subgroups A and B (UAP within 48h and 48-72h) were compared to the stable controls, there was a significant decrease in CTnI at 6 hours after

declamping (5.0 ± 2.5 µg/l in subgroup A, 6.6 ± 3.3 µg/l in subgroup B compared to 17.4 ± 9.6 µg/l in the stable controls; $p < 0.001$ and $p = 0.001$), but no difference was found between the unstable subgroups A and B. The CTnI values in the coronary sinus samples were significantly higher than in arterial samples ($p = 0.034$) but closely correlated with each other ($r = 0.934$) (Figure 9).

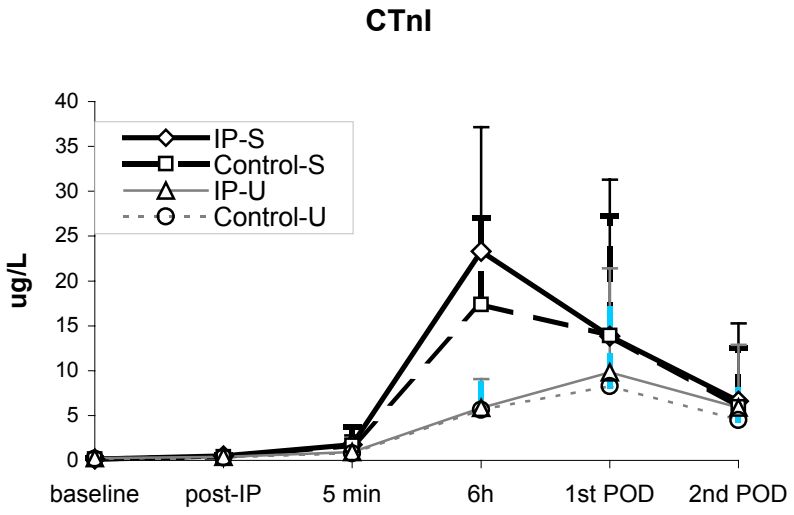

Figure 9: Cardiac troponin I (CTnI) from peripheral blood samples after CABG operation in IP and control group in the stable and unstable patients. IP-S: IP group in stable angina patients, Control-S: control group in stable angina patients, IP-U: IP group in unstable angina patients, Control-U: control group in unstable angina patients. The IP protocol resulted in the higher CTnI values in the stable IP patients ($p = 0.047$). The CTnI release after the operation was lower in the unstable patients ($p = 0.006$). IP does not change the values of CTnI release after a CABG operation. Data are presented as mean ± SD.

Myoglobin

The baseline values for myoglobin were 70.3 ± 14.9, 97.1 ± 31.0, 66.9 ± 22.0, 75.6 ± 39.0 µg/l respectively in group 1 to group 4. The values in group 2 was significantly higher than the others ($p = 0.005$). Both 10 minutes of CPB or IP protocol caused a significant release of myoglobin ($p < 0.001$). The release of myoglobin after this period was similar between IP protocol and CPB in stable and unstable patients ($p = 0.056$ and 0.582). A considerable amount of myoglobin was released after the operation. Peak elevation in myoglobin was reached on the 1st POD in group 1, 2, 3 and 4 (573.7, 671.8, 422.9 and 784.1 µg/l respectively).

There were no statistically significant differences between the IP and control group in the stable and unstable patients (p = 0.462 between groups 1 and 2, and 0.225 between groups 3 and 4). No difference was found in myoglobin after the operation in the stable and unstable control patients (group 2 and 4, p = 0.996). The myoglobin values in the sinus samples were significantly higher than in arterial samples (p = 0.036), but closely correlated with each other (r = 0.964) (Figure 10).

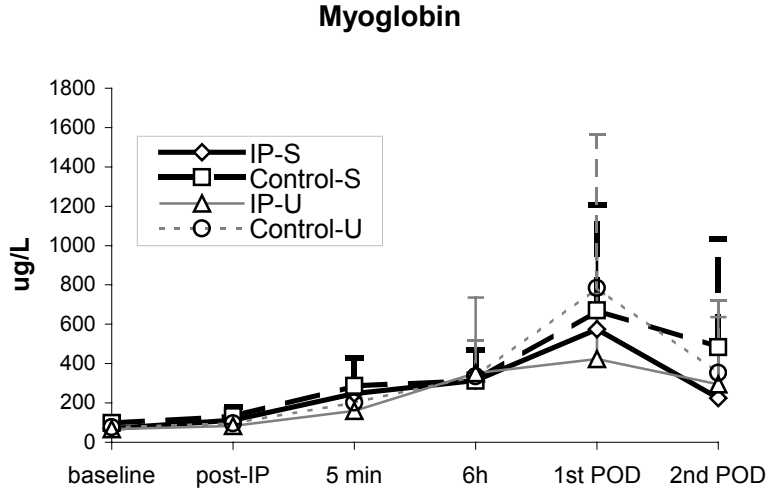

Figure 10: Myoglobin from peripheral blood samples after CABG operation in IP and control group in the stable and unstable patients. IP-S: IP group in stable angina patients, Control-S: control group in stable angina patients, IP-U: IP group in unstable angina patients, Control-U: control group in unstable angina patients. Data are presented as mean ± SD.

Lactate production

Before CPB, the baseline values for lactate production were 11.6 ± 42.5, 20.3 ± 53.3, -7.0 ± 30.4 and -2.9 ± 34.8% from group 1 to group 4 respectively. There were no differences between the IP and control groups in the stable (p = 0.570) and unstable (p = 0.693) patients, or between the unstable subgroup A (p = 0.625) and subgroup B (p = 0.387).

Compared with baseline, lactate production increased significantly after the IP protocol in stable (39.0 ± 21.8%, p = 0.016) and unstable patients (47.5 ± 37.7%, p < 0.001). Ten minutes of CPB did not cause any statistically significant increase in lactate production (29.7 ± 37.3%, p = 0.525 in the stable patients, 19.0 ± 35.7, p = 0.061 in the unstable patients). Comparing between the IP and the control groups, lactate production was more significant after IP protocol than 10 minutes of CPB in the unstable angina patients (47.5 ± 37.7 vs. 19.0 ± 35.7%, p = 0.021) but not in the stable patients (39.0 ± 21.8 vs. 29.7 ± 37.3%, p = 0.342). Subgroup analysis showed that such a

significant difference existed in subgroup A (61.1 ± 47.4% vs. 11.0 ± 19.4%, p = 0.031), but not in subgroup B (40.2 ± 31.0% vs. 28.0 ± 47.8%, p = 0.471).

At 5 minutes after declamping, the lactate production was significantly increased in all patients (p = 0.010 and 0.049 in the IP and controls) compared with the baseline. Similar contents of lactate production were found between the groups (47.5 ± 31.4, 31.7 ± 43.1, 35.4 ± 32.9 and 35.6 ± 62.5% respectively from group 1 to 4, p = 0.690). There were no differences in lactate production after the operation between the IP and controls in the unstable subgroups (p = 0.623 in subgroup A, 0.265 in subgroup B) (Figure 11).

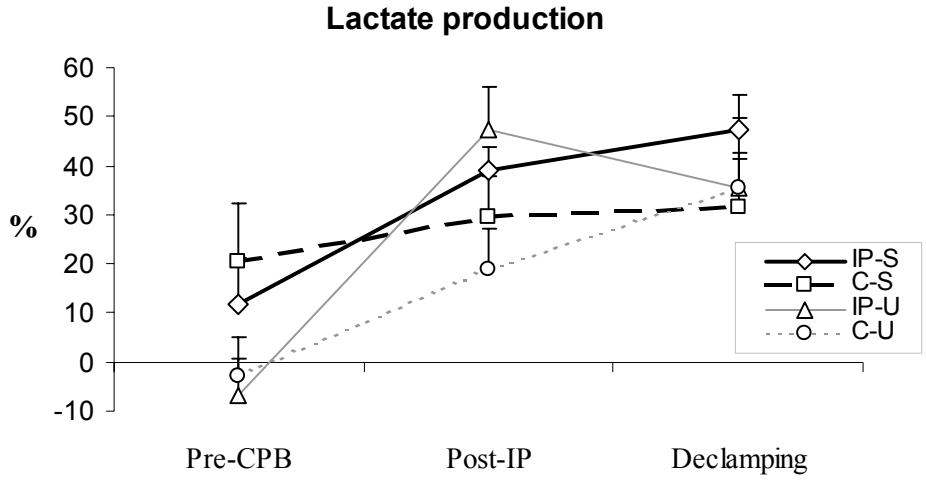

Figure 11: Lactate production after CABG operation in IP and control group in the stable and unstable patients. IP-S: IP group in stable angina patients, Control-S: control group in stable angina patients, IP-U: IP group in unstable angina patients, Control-U: control group in unstable angina patients. IP stimuli resulted in a significant increase in lactate production in the unstable IP patients with antecedent unstable angina within 48 hours prior to surgery. Data are presented as mean ± SEM.

4. Free radicals

A small amount of FR was generated after IP (5.6 % from the baseline) but not in the controls 10 minutes after initiating CPB. Larger amounts were generated 10 minutes after declamping in both groups (8.4 % in IP and 7.7 % in controls) with statistical significance from baseline (p = 0.046 and 0.032). There was no statistically significant difference in FR generation after IP or operation between two groups (Figure 12). The FR generation after the operation correlated with cross-clamping time (r = 0.529). There was no association between FR and CPB time (r = 0.225).

Correlation between sinus FR concentration and haemodynamics

FR generation after declamping correlated with the depression of LVSWI at 1 and 6 hours after declamping in the control group (r = - 0.71 and - 0.59). No such negative correlation could be found in the IP group (r = 0.42 and 0.43). There was a significant positive correlation between FR generation during IP protocol and LVSWI at 1 and 6 hours after declamping (r = 0.56 and 0.54). FR during IP protocol also correlated with CI at 1 and 6 hours after declamping (r = 0.50 and 0.61). FR after 10 minutes of CPB in the controls showed no statistically significant correlation with LVSWI and CI at these time points (r = -0.31 and -0.07 in LVSWI, r = 0.22 and 0.15 in CI respectively).

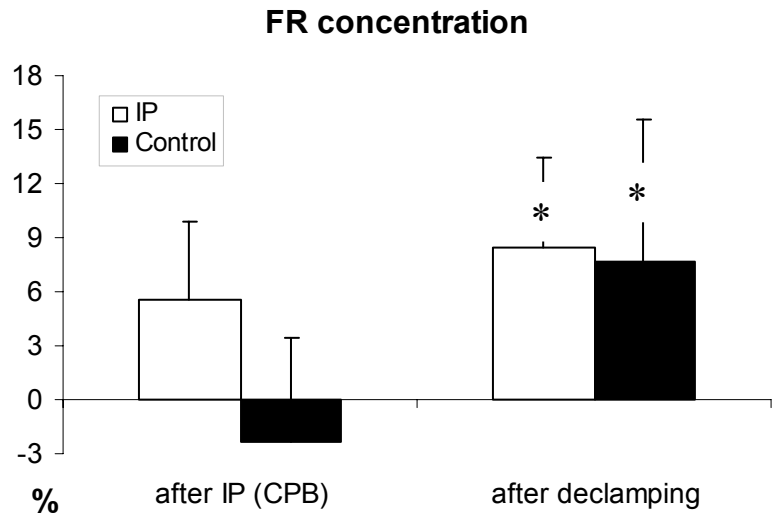

Figure 12: Free radicals (FR) concentration after the IP protocol (10 minutes of CPB) and 10 minutes after declamping. Data are presented as mean ± SD. Significance of increase from the baseline: * $p<0.05$.

5. GLM analysis

General Linear Model analysis with repeated measures showed that CI after the operation was associated with the baseline level (p = 0.003), and negatively with age (p = 0.005), peak value of CTnI (p = 0.005) and lactate production during the IP protocol (p = 0.041). Stratified analysis showed that in the IP patients, IP was negatively associated with age and lactate production (p = 0.002 and 0.026). No association was found between CI after the operation and peak values CTnI in the IP patients. In the control patients, CI was associated negatively with the peak CTnI (p = 0.002). No association was found between age and lactate production after 10 minutes of CPB in the controls. No association was found between CI after the operation and vessels bypassed, cross-clamping and CPB time.

In the stable patients with sinus FR measurement, CI and LVSWI after the operation were associated with FR generated during the IP protocol ($p = 0.035$ and 0.037), but not during 10 minutes of CPB in the controls. Whereas LVSWI was negatively associated with the FR generated after the operation in the control group ($p = 0.022$) but not in the IP group ($p = 0.316$). CK-MB values were positively associated with the cross-clamping time ($p = 0.040$) and CPB time ($p = 0.018$), but without association with the numbers of bypassed vessels ($p = 0.253$). There was a strong association between CK-MB release and FR concentration after the operation ($p = 0.004$).

DISCUSSION

1. Ischaemia-reperfusion injury and myocardial protection

Myocardial I/R injury varies from cardiomyocyte dysfunction, manifested as arrhythmias and myocardial stunning (MS), to cell death. The pathogenesis of I/R injury is not totally clear, probably a broad spectrum of factors are involved (Vaage 1993). Free radical and calcium metabolism may play a role (Opie 1991, Vaage 1993). Systemic inflammatory changes may further intensify the injury (Tonnesen 1996).

Using PBN-spin spectroscopy to measure FR concentration directly, we demonstrated that FR were generated after the ischaemia-reperfusion period during CABG surgery. Our findings in the control group further support the conception that during a CPB operation, the I/R myocardium is sufficient to generate FR, and this generation is detectable in patient's coronary sinus blood by means of ex-vivo spin-trapping (Tortolani 1993, Movahed 1996). The FR generation was correlated with the duration of myocardial ischaemia, as noted in a previous report (Bolli 1998). FR generation was accompanied by contractile dysfunction in LV and RV, manifested by the depressed CI, RVEF, LVSWI and RVSWI after the operation in our control group, and by the significant negative correlation observed between FR generation and LVSWI. The association between FR and CK-MB values implied that FR might be a culprit in cellular necrosis. These clinical findings support the theory that FR plays an important role in myocardial ischaemia reperfusion injury (Bolli 1989, 1998).

Even though FR generation is in fact mainly a phenomenon of reperfusion injury, the severity of FR generated during the reperfusion period is closely correlated with the severity of the ischaemic period (Bolli 1998). Therefore, successful cardioprotective strategies to reduce ischaemia can potentially reduce FR generation and subsequently influence the outcome of CPB surgery.

Administration of cardioplegia has been used as a main myocardial protective strategy in conventional CPB surgery for decades (Gay 1973, Bretschneider 1975, Hearse 1976). When the coronary vessels are stenosed or occluded, antegrade cardioplegia may fail to give adequate protection to all regions, and retrograde cardioplegia has been proposed as an alternative or additive to overcome this limitation (Buckberg 1995). Combined delivery of antegrade/retrograde cardioplegia protects the myocardium in jeopardy of inadequate cardioplegic protection (Partington 1989). If the balloon catheter does not obstruct the terminal tributaries of the coronary

sinus, retrograde delivery of cardioplegia can ensure RV protection with an adequate flow rate (Menasche 1994). However, discrepant reports show that RV perfusion is poor with retrograde delivery with or without RCA stenosis (Partington 1989, Allen 1995). Nor does retrograde cardioplegia improve the ischaemic or infarcted myocardium in the LAD area (Carrier 1997a). Combined delivery of cardioplegia does not protect the RV better than the single technique in patients with right coronary stenosis (Savunen 1994, Allen 1995, Honkonen 1997ab).

Our results in the stable control group were in concordance with such findings. RVEF, RVSWI, LVSWI and CI were decreased after the operation, with a nadir 6 hours after the operation (Breisblatt 1990, Stein 1990, Honkonen 1997ab). RVEF did not recover within 24 hours, while CI recovered. This supported the conception that the right ventricle is more vulnerable to ischaemic reperfusion injury. The functional depression called for the extensive use of inotropes and longer mechanical ventilation. It should be noted that the use of inotropes might elevate haemodynamic measurements, which in part mask the severity of myocardial stunning (Bolli 1998). Our findings support previous conclusions that combined antegrade and retrograde cold blood cardioplegia offers inadequate protection to the heart, especially the right ventricle (Allen 1995, Winkelmann 1995, Honkonen E 1997ab). RV function is known to contribute essentially to the maintenance of global heart performance (Polak 1983, Boldt 1989). Thus consideration of strategies employing IP in myocardial protection against ischaemia and reperfusion injury would appear necessary and worthwhile.

2. Haemodynamic and right ventricular function

2.1. IP effect on haemodynamic function in stable angina patients

Our results proved that, in severe three-vessel diseased patients with stable angina undergoing CABG, IP protected RV and global haemodynamic function. Preservation of haemodynamic function was attributed to myocardial contractility, since SWI correlated with preload and contractility. The preload of RV and LV, manifested as CVP, RVEDVI and PCWP, were kept stable throughout. Thus the improvement in RVSWI and LVSWI was due to the better recovery of contractility. Better functional recovery leads to less inotropic medication and a tendency of shorter mechanical ventilation support. The recovery of CI was not associated with the numbers of vessels bypassed, cross-clamping time and CPB duration, thus the nonparallel selection did not disturb the conclusion.

IP has also been reported to be effective in preserving high-energy phosphate (Yellon 1993, Alkhulaifi 1994, Lu 1997), improving heart performance (Lu 1997, Illes 1998, Li G 1999) and

reducing cardiac troponin T release (Szmagala 1998, Jenkins 1999) during open-heart surgery. However, the exact mechanism is as yet unclear. Studies concerning IP in protect ischaemia-reperfusion (I/R) injury in cardiac surgery are still few and controversial (Perrault 1996, Kaukoranta 1997, Cremer 1998). The difference in IP protocol and study object might be the reason for conflicting results. To our knowledge, this was the first report that IP improved haemodynamic recovery in the patients with three-vessel coronary artery disease and good LV function undergoing elective CABG.

2.2. Recent unstable angina—an IP stimulus

Compared with stable controls, the haemodynamic recovery was much better in unstable control patients, suggesting that the myocardium of patients with recent unstable angina might already be preconditioned by the antecedent ischaemic episodes before cross-clamping. Thus repeated ischaemic episodes prior to surgery might lead to cardioprotective effects in unstable patients. Subgroup analysis showed that the better functional recovery only took place in patients who had experienced antecedent angina within 48 hours. This implied that the delayed IP protection in anti-stunning in the human heart lasts only 48 hours.

Previous evidence demonstrates that pre-infarction angina improves the clinical outcome and reduces infarct size (Muller 1990, Kloner 1995&1998, Ottani 1999). Clinical data on patients undergoing thrombolysis and PTCA also suggest that episodes of angina immediately prior to acute myocardial infarction are associated with improved contractile recovery, smaller infarct size, lower in-hospital mortality and better 5-year survival (Iwasaka 1994, Ottani 1995, Ishihara 1996, Napoli 1998). Antecedent angina may act as a preconditioning stimulus (Muller 1990, Iwasaka T 1994, Kloner 1995a&1998, Ottani 1995, Ishihara 1996, Napoli 1998, Ottani 1999). There are nevertheless conflicting reports as to the outcome of patients with angina preceding an acute MI (Behar 1992, Barbash 1992). Differences in patient selection and study protocols, as well as inconsistency in the definition of antecedent angina, could explain the discordant results (Ottani 1995). Studies carried out in the prethrombolytic era showed that previous angina was associated with a higher risk profile, as well as with an unfavourable clinical outcome. In the thrombolytic era, however, the widespread use of various reperfusion strategies to reopen the occluded arteries has renewed the interest in the possible protective role of preinfarction angina, seen as the clinical indicator of preconditioning (Ottani 1995, Napoli 1998). These findings are not controversial, since IP delays but does not abolish the following prolonged myocardial ischaemic injury. Re-establishment of the coronary blood flow to the ischaemic myocardium is necessary to the induction of the IP effects (Ovize 1992, Wolfe 1993).

Though IP contributes to the protective effect, other mechanisms have also an important role in the warm-up phenomenon. For example, more rapid lysis of the occlusive thrombus within the infarct-related artery, or rapid opening of an intramural collateral not visible at angiography may also be the reasons (Ottani 1999). Since there were more cases with previous infarction and lower preoperative LVEF in the unstable group in the present study, the myocardial viability of the unstable patients could not be better than in the stable controls. The coronary stenosis of the unstable patients might have lasted longer than in the stable controls. Therefore, the protective effects of the recent unstable angina in this group were not due to earlier revascularisation. The protective effects of unstable angina in CABG surgery seem to be a more relevant model to study the IP phenomenon in humans.

2.3. IP in unstable patients

It has been reported that the delayed IP effects are less potent than the early effects (Richard 1996). It might be reasonable to assume that further IP stimuli to induce an early IP effect might result in additional protection in unstable CABG patients who have already been preconditioned. However, our results did not support this hypothesis when judged from haemodynamic parameters between the IP and control group in patients having antecedent angina within 48 hours. On the other hand, the weaning IP effects could be regained by another IP stimulus in humans, as shown in our data where the IP protocol provided better preservation of RVEF in patients with angina-induced stress 48-72 hours before surgery.

The IP effect triggered by antecedent angina might be involved in both early and delayed IP protection. Our study involved patients with recent ischaemic episodes; emergency cases were not included. In most of cases, therefore, the preoperative onset of angina episodes in our unstable CABG patients fits the time course of the delayed IP protection. This result also confirms the previous finding that a delayed IP phenomenon exists in CABG patients (Szmagala 1998, Jenkins 1999).

Animal studies have shown that the early IP effect occurs within minutes and lasts for 1-3 hours. Delayed IP occurs 24 hours after IP stimuli and lasts for even 6 days (Sun 1995, Schwarz 1997). It is not yet known when the IP effects start and how long they last in humans. Previous reports suggest that the early IP effect might last for 2-6 hours, and the delayed IP effect begins after 24 hours and lasts for 48 to 72 hours (Richard 1996, Lim 1997, Nagao 1997). We could not conclude here when the delayed IP started, but the effect of functional improvement waned after 48 hours. This weaning effect could be regained by another IP stimuli, which proved the previous findings in

animal and human studies (Cohen 1994, Lim 1997). Unstable angina frequently evolves toward myocardial infarction, despite full medical therapy. We should therefore be more active to operate unstable patients with 3-vessel disease within 2 days after the episodes.

In a clinical study setting, such as our study, it is difficult to evaluate the duration and frequency of ischaemic episodes, since some of them may be asymptomatic or difficult to be monitored. Our results thus apply only to the time range of the ischaemic stress prior to the operation.

3. Cellular viability

Our data showed that IP was effective in attenuating myocardial stunning but could not evince the effects on cellular necrosis measured by biochemical markers, including CK-MB, CTnI, myoglobin and lactate production. This might be due to the fact that the peak release of these markers occurred 6 hours after ischaemia. The release of biochemical markers after IP stimuli was mainly demonstrated later at the time-points of measurement. The second reason is that the longer clamping and CPB times in the IP patients may also cause higher biochemical marker release. Since both a short period of ischaemic stimuli and as short as 10 minutes of CPB may result in the release of these biochemical markers. This would lead to an observation bias in this variable. However, it is very possible that the IP protocol does not enhance the biochemical marker release. This would indicate that the IP effect on cellular viability and myocardial contractile function could be based on separate IP mechanism.

The peak CTnI value was negatively associated with the CI after the operation in the controls; there was no relationship between them in the IP patients, even similar mean CTnI value was found between the groups. This implied that the better haemodynamic recovery in the IP patients might be independent of the anti-necrosis effect. The IP protocol applied in our study patients had no protective effect on cellular viability.

However, the antecedent angina within 72 hours before surgery showed a protective effect of anti-cellular necrosis, demonstrated by lower values of CTnI. As discussed in the haemodynamic data, this effect was probably achieved by the delayed phase of IP protection. The results implied that the delayed IP effect on cellular viability in CABG patients might last for 3 days, longer than the anti-stunning mechanism. This further supports the hypothesis envisaging independent protective mechanisms involved in the preconditioning process. The protective effect on cellular viability in unstable patients could not be further improved by another applied IP stimulus, indicating that

early IP effects do not provide more powerful protection than the delayed IP. The other possibility was that the IP protocol applied to the heart was not effective in this kind of patients.

Lactate has been used in assessing myocardial viability following coronary bypass surgery (Sharma 1978). There are reports that IP reduces lactate production in animal models (Finegan 1995, McNulty 1996, Minamino 1998). This effect has also been reported in cultures of human cardiomyocytes in IP models (Shirai 1998), as well as in IP patients with crystalloid cardioplegia or without cardioplegia in open-heart surgery or coronary angioplasty (Alkhulaifi 1994, Eltchaninoff 1997, Lu 1997). However, we could not show such effects in the present study. The IP effect of modifying myocardial metabolism was not confirmed here.

The IP effect of reducing lactate production is probably due to the process of preischaemic glycogen depletion (McNulty 1996). Increasing duration of reperfusion prior to prolonged ischaemia is associated with the recovery of tissue glycogen, resulting in the loss of IP capabilities (Wolfe 1993). There is no nutritional cardioplegic perfusion to provide metabolic substrates to produce lactate in the previous models without the use of blood cardioplegia. However, the depletion of glycogen could be restored by merit of the repeated perfusion of blood cardioplegia. Thus myocardial lactate production from glycolysis was not discontinued during cross-clamp in the present study. These might explain the difference in IP effect in suppressing lactate production from the other clinical trial (Alkhulaifi 1994, Eltchaninoff 1997, Lu 1997). Parallel with the finding of lactate production after the operation, there was no IP effect on cellular viability measured by CK-MB and CTnI in the IP patients in the present study. This supports the evidence that postoperative acidosis is a culprit in cellular necrosis (Wolfe 1993). Hence an anti-necrosis IP effect has been demonstrated to be associated with the attenuation of lactate production in previous studies in open-heart surgery (Alkhulaifi 1994, Lu 1997), but not in the present study. On the other hand, our results also proved a concept that the ischaemic lactate accumulation does not play a major role in IP protection against contractile dysfunction (Yabe 1997).

4. Free radicals

The better haemodynamic recovery in the stable IP group showed that IP attenuated myocardial stunning, but did not change FR generation during the early reperfusion period. Previous reports showed that IP activates endogenous antioxidant defence, demonstrated by increase in MnSOD activity in animal study (Zhou 1996, Das 1999) and increase in myocardial superoxide dismutase/malondialdehyde (T-SOD/MDA) during the early reperfusion period in CPB surgery (Li G 1999). The present results implied that the early IP effect resulted in the adaptation of the

heart performance to the following oxidative stress, but did not involve the FR generation during the early reperfusion period. Since the FR generation was associated with the cross-clamping time, the longer clamping time in the stable IP group might partly affect the observation. A more important reason is that the antioxidant enzymes stimulation, transcription and expression need time to accomplish (Meng 1996, Sun 1996, Das 1999). They appear and exert their effect hours later, in the delayed-phase IP.

5. IP mechanism

The precise mechanism of IP remains unclear. Brief episodes of myocardial ischaemia result in the production of adenosine, norepinephrine, free radicals, lactate and bradykinin. These chemical factors act on one or more types of myocyte receptors, and result in translocation and activation of tyrosine kinase and protein kinase C (PKC) to the cellular membrane, working with inhibitory G-protein, subsequently phosphorate ATP-dependent potassium channel to trigger the IP response (Burns 1996, Das 1999). The delayed cardiac adaptation is consistent with induced gene transcription and the subsequent translocation of protective molecular proteins (Yellon 1995). A growing body of evidence shows that FR generated during the IP period act as one of the triggers for the IP effect (Osada 1994, Tanaka 1994, Kaeffer 1997, Kukreja 1997, Tritto 1997, Das 1999). However, whether the lactate which accumulates during IP periods can trigger the IP process is controversial (Doenst 1996, Aresta 1997).

5.1. Free radicals

Our results demonstrated that two cycles of 2-minute ischaemia followed by 3-minute reperfusion generated FR from the heart. This amount of generation was rather low compared with the larger amount of FR generated after declamping. This FR concentration after brief periods of ischaemia and reperfusion in humans is very similar to that in the animal study previously mentioned (Sun 1996). Ten minutes of CPB alone did not produce FR, as seen in the result in the control group. The low concentration of FR during the IP protocol was associated with better haemodynamic recovery in CABG patients. Such evidence supports the theory that the low dose of FR generated during IP is beneficial for the subsequent prolonged I/R myocardial insult (Tritto 1997, Das 1999). We assume that this beneficial effect may be transmitted via triggering signal transduction by FR. The protective mechanism may also involve activation of tyrosine kinase and protein kinase C (PKC), and K_{ATP} channel opening by the mild oxidative conditioning produced during IP (Osada 1994, Tanaka 1994, Kukreja 1997, Tritto 1997, Das 1999).

Though the evidence here showed FR to be very possibly a trigger of IP, it was not the only trigger. Animal studies have found that a single cycle of 5-minute ischaemia followed by 10-minute reperfusion has the same effect as 4 cycles, and MPG can block one cycle IP but cannot block protection by 4 cycles (Iwamoto 1991, Tanaka 1994). This suggests that FR may act in concert with other triggers such as adenosine, norepinephrine and bradykinin. In rabbits, more cycles of IP might have resulted in greater generation of adenosine and bradykinin, sufficient to trigger IP by itself even in the absence of FR (Baines 1997). Therefore, IP with repeated cycles is more effective in inducing protective effects. In view of the complexity of the clinical event, we suggest that multiple cycles may be better in a clinical study. In our present study we could not verify a cause-effect relationship between FR during the IP protocol and myocardial protection after surgery, although the results suggested that FR might possibly be one of the triggers of IP. Further investigations are necessary to elucidate such a relationship.

5.2. Lactate

In the present study, there was an increase in myocardial lactate production during the IP protocol, but not after 10 minutes of CPB. This suggested that there was myocardial ischaemia and anaerobic glycolysis during the IP period. However, this lactate production was negatively associated with CI recovery. It is thus unlikely that lactate acts as one of the triggers for the IP process.

Recent studies have shown that increasing release of one trigger bears a positive association with the IP effects, and can compensate for lack of other triggers (Downey 1997, Cohen 1998). In the present study, subgroup analysis showed that IP induced more significant lactate production than the controls only in the patients with UAP within 48h. This was the only subgroup of patients in which IP did not provide additional protective effects. The functional recovery evinced a decline as lactate production increased. This disproves the hypothesis that lactate was involved in triggering IP effects in CABG surgery.

Nonetheless lactate production can be a parameter of ischaemic insult (Sharma 1978). The different lactate production between IP and controls in different groups (subgroups) implied that the sensitivity to ischaemic stimuli was different in the stable and unstable angina myocardium. The recently unstable patients were more sensitive to ischaemic insult. Thus the effective IP stimuli in stable patients might not be suitable in recently unstable cases.

We know that IP cannot be minimised in an effort to limit the detrimental effects of ischaemia without reducing the degree of protection afforded (Cohen 1998). This does not means that the longer ischaemic insult in the IP protocol results in better protective effect. It is not clear how long an ischaemic is necessary and optimal in humans. Higher lactate production associated with lower CI recovery in the present study implied that the ischaemic insult could not exceed certain limits, otherwise the applied IP protocol does not induce a protective effect but is rather deleterious to the heart. This might be one of the reasons why the IP protocol with 5 minutes ischaemia did not show any beneficial effects in CABG patients in whom myocardial lactate production was significantly higher after IP insult (Kaukoranta 1997, Cremer 1997).

6. Clinical factors in the IP phenomenon in CABG surgery

6.1. Clinical reports

There are reports of the IP phenomenon during coronary balloon angioplasty (Deutsch 1990, Tomai 1994 & 1996, Eltchaninoff 1997, Airaksinen 1997). Better outcome of infarction patients with preinfarction angina also supports the conception of IP effects in clinical cases (Muller 1990, Tranchesi 1990, Kloner 1995&1998, Andreotti 1996, Nagao 1997, Napoli 1998, Ottani 1999). Recently, several studies have confirmed that IP is protective in CPB operations (Alkhulaifi 1994, Lu 1997, Illes 1998, Szmagala 1998, Jenkins 1999, Li G 1999). IP has been found effective in preserving high-energy phosphate, protecting the myocardium against I/R injury and improving postischaemic functional recovery after cardiac surgery (Yellon 1993, Alkhulaifi 1994, Lu 1997, Illes 1998, Szmagala 1998, Jenkins 1999, Li G 1999). Thus Kloner and Yellon suggest that there is now convincing evidence that human myocardial tissue can be preconditioned (Kloner 1994). However, the study of IP in open-heart surgery is more complicated than in animals. Different IP protocols and different end-points measured would also lead to different or even conflicting conclusions. In several studies, the authors suggest that IP is not protective but seems to be possible detrimental (Perrault 1996, Kaukoranta 1997, Cremer 1997) (Table 1).

6.2. CPB, hypothermia and cardioplegia

Unlike most laboratory studies**,** the CPB operation is performed in conditions of hypothermia, with cardioplegia and a CPB machine. Cave and associates reported that IP improved functional recovery after hypothermia (Cave 1992). Because metabolism and oxygen consumption are suppressed during hypothermia, the usual period and cycle of ischaemic insult does not stimulate the cellular signal to initiate the IP process. Thus a longer duration or more cycles of ischaemic episodes are necessary if IP is applied during hypothermia (Dote 1998). Therefore, the temperature

was kept normothermic during IP, and the heart was empty to minimise the residual flow in our study in order to ensure IP stimuli.

Using an isolated rabbit heart perfused on a Langendorff apparatus, Takeshima showed that IP was effective with hyperkalaemia and moderate hypothermia (23^{o}C), but not in deep hypothermia (6-8^{o}C) (Takeshima 1999). In an isolated rat heart model, Galinanes and Kolocassides found IP to be as effective as cardioplegia, but their combined use did not afford greater protection than the use of either alone. When the delivery of cardioplegia was impaired, myocardial protection was better served by its use alone (Galinanes 1995). Landymore further proved that IP preceding warm blood intermittent cardioplegia arrest reduced myocardial stunning in dogs (Landymore 1997). However, IP with warm blood cardioplegia during a CABG operation has yielded conflicting results (Kaukoranta 1997). In the present study, we demonstrated that the myocardium with mild systemic hypothermia and intermittent perfusion of deep hypothermic cardioplegia could be preconditioned.

Total CPB might by itself elicit the IP effects, being as potent a stimulus as three brief periods of ischaemia measured by a reduction of infarct size in an ovine model (Burns 1995). In our control group, all biochemical markers and lactate production increased after 10 minutes of CPB. Nevertheless the stress caused by CPB did not exert the same protective effect as IP. Possible reasons here might be that it was insufficient to induce the IP effect, or our IP protocol yielded more potent protection from subsequent I/R injury, since 10 minutes of CPB did not generate FR which might trigger the IP process.

6.3. IP protocol

The ideal IP protocol should involve the least ischaemic insult and cycles to induce maximal myocardial protective effects and the fewest possible side-effects, as well as minimal inconvenience from the standpoint of the operative procedures. In our protocol, 2 cycles of 2-minute ischaemia followed by 3-minute reperfusion did induce some extent of myocardial ischaemia, manifested by an increase in biochemical markers. Our results proved that the protocol is effective and feasible for a CABG operation. It is not yet known what is the necessary shortest and optimal ischaemic stimulus, our result suggested that 2 minutes ischaemia is long enough to induce ischaemic stimulus. However, this stimulus might be too strong for recently unstable patients, as manifested in the significantly higher myocardial lactate production.

Animal studies suggest that there is no correlation between the magnitude of stunning after the IP protocol and the extent of resultant protection (Miura 1991). However, IP cannot be minimised in

an effort to limit the detrimental effects of ischaemia without reducing the degree of protection afforded (Cohen 1998). A longer duration or more cycles of ischaemic episodes are necessary if IP is applied during hypothermia (Dote 1998). This implies that optimal IP stimuli are different in protecting different objects. Increased release of one agonist or stimulation by more cycles of brief ischaemic stimuli can compensate for the lack of another to achieve the protective effect (Downey 1997). For this reason the double period of ischaemic stimuli was selected in the present study. It is not known how long an ischaemic episode is necessary and optimal in humans. During coronary angioplasty, the balloon inflation time longer than 90 seconds can induce preconditioning (Deutsch 1990, Airaksinen 1997), but if the inflation time is only last 60-90 seconds, this effect does not occur (De Jong 1993, Inoue 1996). Therefore, we selected the IP protocol used during the study, after a pilot study of effective and safety test.

6.4. Age

In the present study, the CI was negatively associated with the age of the patients in the IP group. This suggested that IP might be an age-related phenomenon. As far as we know, the age-related aspects of the IP phenomenon in open-heart surgery have not been studied. The reports on the warm-up phenomenon, a counterpart of IP in the aging heart, are controversial (Abete 1997, Kloner 1998, Napoli 1999). In reports of IP in open-heart surgery, the age of the patients has been relatively younger in those which support the IP phenomenon in open-heart surgery (Yellon 1993, Alkhulaifi 1994, Jenkins 1997, Lu 1997, Illes 1998, Szmagala 1998, Li G 1999, Table 1). The diminution of IP effect in the senescent heart may be due to the absence of mediators such as norepinephrine, calcium and heat shock protein which trigger this protective mechanism, as well as the decreased response to effectors (Burns 1996, Abete 1996&1997, Tani 1997). Further studies are called for to elucidate this hypothesis.

6.5. Other clinical factors

There are controversial reports on the loss of the IP phenomenon in diabetic patients with administration of sulfonylurea hypoglycaemic agents, K_{ATP} channel blockers (Liu Y 1993, Tosaki 1996, Cleveland 1997d). Patients with hyperlipidaemia (Szilvassy 1995) and with angiotensin-converting-enzyme (ACE) inhibitor medication (Liu Y 1995, Dogan 1998) may also affect the observation of IP effects. In our IP patients, we found no association between the IP effects and such factors. It has been reported that the anaesthetic drug isoflurane (Belhomme 1999) and morphine (Wang 1998), may precondition the heart. The standardised anaesthetic method did not include isoflurane in the present study. The use of sufentanyl might possibly already

'precondition' the heart, so as to interfere the applied with the IP effects. Even so, we still demonstrated the effectiveness of the IP protocol in this control study.

7. Perspective

Cross-clamp manipulation has been identified as the most significant cause of particulate emboli release during cardiac surgery. Neurological injury following myocardial revascularisation may result from embolisation of atheromatous debris from the diseased ascending aorta (Reichenspurner 2000). Whereas in patients with no clinical evidence of aortic or cerebro-vascular disease, the incidence of peri-operative cerebral microemboli and post-operative neuropsychological disturbances is similar with multiple and single cross-clamping (Musumeci 1998). The manipulation of the severely calcified or dilated ascending aorta may increase the danger of aortic rupture. Thus we also considered dilated aorta as a contra-indication to IP and excluded such cases.

It is essential to bear in mind the potential damage involved in repeated aortic cross-clamping by way of embolisation and neurological injury. It is probably that we will be able to introduce IP pharmacologically without the need of ischaemia, but with adenosine or selective A_1 agonist and potassium channel openers instead. It is of note that PKC activators would not be safe in humans by reason of their carcinogenic potential. Use of perioperative medication such as angiotensin-converting enzyme (ACE) inhibitor and isoflurane beneficial to IP should be encouraged. However, our understanding of the cellular mechanism is incomplete and the potential for therapeutic use is only now being explored. Practical application of IP in cardiac surgery remains a goal.

8. Methodological consideration

The present series were designed as prospective, randomised and controlled study. The blinded method was adopted in most variable collection. It is impossible to be double-blinded in the study concerning the operators and anaesthesiologists, as well as in the collection of haemodynamic data. However, standardised anaesthetic, operative and data collection methods, as well as limited numbers of surgeons and anaesthesiologists participating in the study would lead to the least bias in observation.

The use of inotropes would mask the value of haemodynamic measurement. Since we did not discontinue inotropes at the time-points of haemodynamic measurement, it is possible that the haemodynamic values were higher in patients who accepted inotropes. This is a study limitation in

a clinical setting. Our criteria for the use of inotropes were wide, so that a high percentage of inotropes was used during the study. The same criteria and double-blinded approach in the ICU team in the use of inotropes may correct the bias. Since fewer IP cases needed inotropic support and for shorter periods, even if the use of inotropes might affect the haemodynamic data, this did not hamper but supported our conclusion.

Both CPB and cross-clamping resulted in the release of biochemical markers. Longer CPB and cross-clamping time in stable IP patients may cause different values for biochemical markers and FR. This would influence the observation of IP effects.

The reactive characteristics of FR contribute to the difficulty of FR measurement. However, free radicals can be trapped by a non-radical species such as PBN to yield a product (adduct) which is a relatively long-lived free radical species (Slater 1987). The PBN-spin FR adducts remains an active specie, however. This again increases the difficulty of FR measurement in a clinical setting. It may be impossible to measure all the FR generated after I/R episodes by ex vivo techniques, since FR may decay before being trapped by PBN. The spin adducts may also be lost during sample-taking, processing, storage, transportation and ESR measurements. The FR concentration measured in the present study may be much lower than the value actually generated. Even so we detected higher signals of FR spin adduct by ESR spectroscopy after cross-clamping release compared with the baseline level.

CONCLUSIONS:

1). The present results show that myocardial stunning is present in severe three-vessel diseased coronary cases with stable angina undergoing CABG, this being manifested as global and RV function depression on the operative day. Functional depression leads to the need of inotropic support and longer mechanical ventilation. MS is probably caused by FR generation during the reperfusion period. This indicates that there is room for IP to improve on the suboptimal protection.

2). Two cycles of 2-minute ischaemia followed by 3-minute reperfusion before aortic cross-clamping constitute a protective IP protocol in open-heart surgery. In patients with three-vessel coronary artery stenosis undergoing an elective CABG operation, this IP protocol has a protective effect on both left and right ventricular haemodynamic functional recovery. IP can be used as an additional protective means in the myocardial protection strategy of combined antegrade and retrograde delivery of cold blood cardioplegia.

3). Recent unstable angina before CABG might act as a preconditioning stimulus and lead to improved myocardial viability and better haemodynamic function. This effect resembles delayed preconditioning, since the heart was better protected in the recent unstable CABG patients with 2-day interval between ischaemic episode and CABG. This finding needs to be verified in a large cohort of unstable patients, and if confirmed, it may guide in the time of the CABG operation in unstable patients and further improve the recovery of the myocardium from the surgically induced ischaemia.

4). IP has a beneficial effect on the right ventricular haemodynamic functional recovery in CABG patients with unstable angina when angina has occurred within 48-72 hours before surgery. However, no additional protective effects of IP were seen in unstable CABG patients when angina episode was within 48 hours prior to the operation. This indicated that the already preconditioned heart could not derive additional protection by another IP protocol unless the time interval was longer than 48 hours.

5). The association of better haemodynamic recovery after CABG with FR generation during the IP period suggests that FR might possibly act as one of the triggers for IP. The present results suggest that lactate production during the IP protocol seems not to be involved in the IP triggering process. Whether free radicals act as the IP trigger as an epiphenomenona or a real mechanism needs further studies to verify.

6). The IP protective effects diminish in senescent patients. Whether IP is an age-related phenomenon or the IP protocol which is effective in the adult myocardium is not suitable for the senescent heart calls for further study.

SUMMARY

Ischaemic preconditioning (IP) has been proved to be the most effective mode of endogenous myocardial protection. However, studies of IP effects in cardiac surgery are rare and controversial. Myocardial protection in severely stenosed three-vessel disease is unsatisfactory in patients with RCA critical stenosis or occlusion. Thus consideration of strategies employing IP in myocardial protection against ischaemia-reperfusion injury appears necessary and useful.

Eighty consecutive patients with stable and unstable angina with 3 main coronary stenosis admitted for a CABG operation were randomised into IP and control groups, each with 20 cases. In the IP group two cycles of 2-minute ischaemia followed by 3-minute reperfusion were induced before cross-clamping. Left and right ventricular haemodynamic data were collected till the 1st POD. RVEF was measured by thermodilution technique using a fast-response volumetric thermister-tipped pulmonary artery catheter. Sinus and arterial samples were collected to measure CTnI, CK-MB, myoglobin and lactate. Sinus FR content was measured directly using PBN-spin spectroscopy in stable patients.

There was a larger amount of FR generated after the operation, which was associated with haemodynamic depression. IP improved haemodynamic functional recovery, manifested in better postoperative CI ($p = 0.013$) and RVEF ($p = 0.012$). IP did not protect against the myocyte necrosis and change the FR values in stable patients. The release of CTnI after the operation was significantly lower in unstable angina patients than in stable controls ($p = 0.006$). There was better recovery of CI ($p = 0.005$) and a tendency to better RVEF ($p = 0.097$) in the unstable patients experiencing antecedent angina within 48 hours than in stable controls. There was no difference in CI and RVEF between the unstable patients experiencing antecedent angina within 48-72 hours and stable controls. The haemodynamics and biochemical markers in the IP and control groups were similar in the unstable patients experienced antecedent angina within 48 hours. The RVEF recovery was significantly better in unstable IP patients experiencing antecedent angina within 48-72 hours ($p = 0.030$). CI after the operation was associated with myocardial FR generation during the IP protocol ($p = 0.035$), and negatively associated with age and lactate production during the protocol in IP patients ($p = 0.002$ and 0.026).

In patients with three-vessel coronary artery stenosis undergoing a CABG operation, IP has a protective effect on both left and right ventricular haemodynamic functional recovery. With recent unstable angina the myocardium has already been preconditioned before the operation. The IP protocol applied did not induce additional protection to the myocardium already preconditioned by antecedent unstable angina. However, the waned IP effect in unstable patients can be regained by the applied IP. The IP protective effects diminish in senescent patients. Lactate seems not to be involved in the IP triggering process. Whether FR acting as an IP trigger is an epiphenomenon or a real mechanism needs further studies.

ACKNOWLEDGEMENTS

The study was carried out in the Division of Cardiac Surgery, the Department of Surgery, the Department of Anaesthesiology and Intense Care in Tampere University Hospital and the Department of Chemistry, Jyväskylä University, during the years 1998-2000.

My sincere thanks are due to Professor of Surgery Markku Järvinen, M.D., Medical School, University of Tampere and Docent Ossi Auvinen, previous Head of the Department of Surgery, and Docent Isto Nordback, present head of the Department of Surgery for providing an opportunity for my postgraduate training and providing working facilities for this study.

I would like to express my deepest gratitude to my supervisor, Docent Matti Tarkka, who introduced me to the world of coronary surgery and clinical investigation. This study would not have been possible without his help. His expert comments and constructive criticism have been valuable in many ways during the study period and more importantly for the future.

I am profoundly indebted to my supervisor, Docent Seppo Kaukinen, who kindly guided me, created facilities and made invaluable suggestions for the manuscripts during the study.

It is a great pleasure to express my sincere thanks to my supervisor Erkki Pehkonen, M.D., for his expertise in guiding me in the field of myocardial protection. His continuous support and kindly help have been crucial to ensure the continuation of the study.

I own my warm gratitude to my co-authors, Docent Liisa Kaukinen and Eva Honkonen, M.D., for their valuable support through all the study. They spend their free time to help me collect the data. I could have done nothing without their help. I am also deeply grateful to my co-author Jari Laurikka, M.D., his optimistic attitude and broad knowledge in clinical study has been valuable in my career.

I also much appreciate the help of Jussi Eloranta, Ph.D., for his assistance and valuable contribution in the field of free radicals study.

I wish to express my special thanks to Pekka Kuusinen, M.D., Pekka Iisalo, M.D., Bernard Negre, M.D., Docent Sisto Tero, Ossi Teerenhovi, M.D. and Pekka Kuukasjärvi, M.D., for teaching me the skill of cardiac surgery.

The clinical trial requires great effort on the part of the personnel in the ward of Cardiac Surgery, Operation Theatre, Intense Care and Clinic Centre Laboratory. Special thanks go to the staffs of these departments, especially for their friendly cooperation and kindly help, even when these efforts were supernumerary to their routine work.

I am very grateful to Reviewers Docent Timo Savunen, and Docent Juhani Rämö, for their constructive criticism and valuable advice at the stage of final revision of the manuscript.

I would like to acknowledge the help of the personnel in the Medical Library of Tampere University Hospital in collecting the literature for the undertaking, and of Robert MacGilleon, M.A., for his careful revision of the language of this thesis.

I address my sincere gratitude to my parents, for their education in encouraging me to be better and better both in personality and study, no matter what happens. I also express my deep thanks to my parents-in-law for their encouragement and help.

My profoundest thanks go to my wife Huang Xiao-Dan for her loving support and patience. This work would not have been possible without her encouragement and her care of the whole family. I apologise for my long absence. I also wish to thank my wonderful son, who is forever my source of pleasure. Here I dedicate my work to them.

The study was financially supported by the Medical Research Foundation of Tampere University Hospital.

Tampere, December 2000

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