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Pharmacological Preconditioning in Coronary Artery Bypass Surgery

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ACADEMIC DISSERTATION
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III. Wei M, Wang X, Kuukasjarvi P, Laurikka J, Rinne T, Honkonen EL, Tarkka M. Bradykinin preconditioning in coronary artery bypass grafting. Ann Thorac Surg. 2004 Aug; 78(2):492-7.

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V. Wang X, Jarvinen O, Kuukasjarvi P, Laurikka J, Wei M, Rinne T, Honkonen EL, Tarkka M. Isoflurane produces only minor preconditioning in coronary artery bypass grafting. Scand Cardiovasc J. 2004 Oct; 38(5):287-92.

ABBREVIATIONS

5-HD	5-hydroxydecanoate
ACE	Angiotension-converting enzyme
Bax	Bcl-2 Associated X protein
Bcl-2	B-Cell Lymphoma 2
BK	Bradykinin
CABG	Coronary Artery Bypass grafting
CI	Cardiac index
CK-MB	Creatine Kinase cardiac isoenzyme
CO	Cardiac outputf
COX	Cyclooxygenase
CPB	Cardiopulmonary bypass
CVP	Central venous pressure
dUTP	Deoxyuridine triphosphate
DZX	Diazoxide
Gi-coupled	Gi protein coupled
HR	Heart rate
HSP	Heat stress protein
IAP-1	Inhibitor of apoptosis protein-1
IL	Interleukins
IκB	Inhibitory kappa B
iNOS	Inducilble nitric oxide synthase
IP	Ischemic preconditioning
I/R	Ischemia and reperfusion
ISO	Isoflurane
LVSWI	Left ventricular stroke work index
MAC	Monitored anesthesia care
MAPKs	Mitogen-activated protein kinases
mitoK_{ATP} channel	ATP sensitive mitochondrial potassium channel
NO	Nitric oxide
NFκB	Nuclear factor kappa B
PCWP	Pulmonary capillary wedge pressure
PI-3 Kinase	Phosphatidylinositol 3-Kinase
PKC	Protein kinase C
PPC	Pharmacological preconditioning
PTCA	Percutanerous transluminal coronary angioplasty
PVR	Pulmonary vascular resistance
ROS	Reactive oxygen species
RVSWI	Right ventricular stroke work index
sarcK_{ATP} channel	ATP sensitive sarcolemmal potassium channel
SOD	Superoxide dismutase
SVR	Systemic vascular resistance

SWOP	Second window of preconditioning
TnI	Troponin I
TNF	Tumor necrosis factor
TUNEL	Terminal deoxynucleotidyl transferase-labled dUTP nick end labeling
XIAP	X-linked inhibitor of apoptosis protein

INTRODUCTION

The optimal myocardial protection against ischemic injury during cardiac surgery remains to be pursued. Ischemic preconditioning (IP) is one alternative among effective cardioprotective strategies. However, majority of the cardiac surgeons are reluctant to accept single or repeated clamping of the aorta required for mechanical ischemic preconditioning because of the risk of atheroemboli. (Vaage & Valen 2003) Thus, extensive research has been aimed at identifying pharmacological agents to mimic ischemic preconditioning in hope of achieving clinical benefit.

Several myocardial stresses occurring during cardiac surgery, including ischemia reperfusion, inflammatory response, operative trauma and oxidative stress have been reported to trigger the myocardial injury. The main aspects of the injury after the cardiac surgery include: (1). Necrosis and apoptosis are two types of the cell death, which could both happen during and after cardiopulmonary bypass (CPB); (2) stunned myocardium can pose a significant problem after CPB (Kloner et al. 1994b). (3) A growing body of evidence suggests that CPB is believed to trigger a whole body inflammatory response, which may be responsible for some of the major postoperative complications following open heart surgery (Wan et al. 1997a).

Ischemic preconditioning has been proved a potent cardioprotective strategy. Although the mechanism of IP has been studied for almost two decades, it remains unclear. Investigations into the mechanisms of IP lead to the observation that various pharmacological agents may elicit preconditioning-like effects in experimental animals (Nakano et al. 2000b). Actually, any pharmacological agents that could activate the preconditioning signaling cascades at various levels can evoke preconditioning–like protection. Thus, pharmacological preconditioning (PPC) could provide a safer way than ischemia for inducing cardioprotection in humans.

Among these pharmacological agents, diazoxide, a selected ATP sensitive mitochandrial potassium channel opener, has been proved capable of conferring cardioprotection in all species animal models (Garlid et al. 1997, Baines et al. 1999) of myocardial infarction and in vitro cultured human cardiomyocytes (Ghosh et al. 2000) subjected to ischemia-reperfusion stimulus. Another mitoK_{ATP} channel opener isoflurane and bradykinin are also demonstrated by numerous studies in different animal models that could mimic the effect of ischemic preconditioning to reduce myocardial infarct size. (Jin & Chen 1998, Kostitprapa et al. 2001, Cope et al. 1997, Kersten et al. 1997b). However, the effect of exogenous administration of these three pharcological agents in cardiac surgery is not known yet. The present series of studies was designed to investigate the effect of pharmcologic pretreatment in patients undergoing elective coronary artery bypass grafting (CABG).

REVIEW OF THE LITRATURE

1. Cardiac Surgery and Myocardial Protection Strategies

Since Bigelow (Bigelow et al. 1950) firstly brought the notion of myocardial protection into the cardiac surgery in 1950, highly effective methods of myocardial protection during coronary artery surgery have been dramatically developed including hypothermia, chemical cardioplegia, and cross-clamp ventricular fibrillation. However, with increasing number of operations on older and higher risk patients, there is always a need for improved protection. Recently, preconditioning has been suggested a novel effective myocardial protective strategy.

1.1. Hypothermia, CPB and Cardioplegia

Bigelow (Bigelow et al. 1950) reported that hypothermia could benefit the myocardium in cardiac surgery by decreasing oxygen demand. Thereafter, Melrose (Melrose et al. 1955) described the use of electromechanical cardiac arrest induced by potassium infusion, permitting cardiac surgery to be performed on a non-beating flaccid heart. The combination of both of these techniques has been the 'cornerstone' in myocardial protection during surgery until now, allowing surgery with excellent clinical outcome (Hendry et al. 1994). Shumway's group began using topical hypothermia with simultaneous aortic cross-clamping in 1959(Shumway & Lower 1959). Intermittent cross-clamping was used to confer myocardial protection in cardiac surgery in the mid-1970s, and so far it is still proved a safe, effective established non-cardioplegic myocardial protection, especially in CABG (Sunderdiek et al. 2000, Boething et al. 2004).

However, CPB is the most commonly used method for myocardial protection today in cardiac surgery. Gibbon performed the first successful open heart surgery with total CPB support in 1953 (Gibbon 1954). One year later, Kirklin and colleagues reported a series intracardiac operation using a pump-oxygenator (Kirklin et al. 1955). Since then, the use of pumps and oxygenators began the new era of open heart surgery. Over the past five decades, many modifications based on this strategy, which focus on cardioplegic components, cardioplegic delivery techniques and cardioplegic temperature, have constantly improved the myocardial protection. However, current

cardioprotective techniques remain a suboptimal protection especially in high-risk patients.

1.2. Beating heart surgery

CPB and cross-clamping of the aorta can cause global ischemia, reperfusion injury, detrimental systemic inflammatory response and myocardial edema. Beating heart surgery was design to avoid such damage, especially in CABG. It is suitable in patients for whom CPB, hypothermia or cannulation is not desirable (Tasdemir et al. 1998). Less blood transfusion and lower mortality in high-risk groups have been reported to be superior in such a method (Magovern et al. 1998).

1.3. Preconditioning

Ischemic preconditioning (IP) is a powerful protective endogenous adaptive response of the myocardium against a lethal ischemia. Recent experimental and clinical studies have suggested a possible role for IP in myocardial protection in open-heart surgery. However, the application of ischemic preconditioning in clinical setting as a myocardial protective strategy is difficult to perform due to a number of questions (Vaage & Valen 2003): (1) Most surgeons are reluctant to use intermittent cross-clamping as myocardial protection, which have a psychological antagonism against repeated episodes of cross-clamping the aorta, both due to the “unprotected” ischemia and the possible chances of embolism from the ascending aorta. (2) Ischemic preconditioning may prolong the surgical procedure by 15 to 30 minutes, which will offset the possible beneficial effect. (3) Because several of the drugs used for premedication, CPB with hypothermia or cardioplegia and anesthesia may in themselves induce a preconditioning response, it is assumed that there is “nothing more to gain.” (4) The ideal model of ischemic preconditioning in humans, namely the length and number of the preconditioning cycles, length of reperfusion, and so forth, is unknown. Therefore, a method to avoid these potential problems associated with the clinical application of ischemic preconditioning may be the administration of pharmacological means simulating preconditioning or the manipulation of the signaling pathway involved in the protection, which named pharmacological preconditioning (PPC). Now, more and more evidences from both experiments and clinical trials proved PPC a potential effective safe myocardial protective method. This review will discuss this issue in detail.

2. Myocardial Injury of CPB

Several myocardial stresses occurring during cardiac surgery, including ischemia reperfusion, inflammatory response, operative trauma and oxidative stress have been reported to trigger the myocardial injury.

2.1. Myocardial Necrosis and Apoptosis

Necrosis and apoptosis are two types of the cell death, which could both happen during and after CPB. Myocytes undergoing necrosis and apoptosis show characteristic morphological and biological distinct features. During ischemia, a reduced blood flow to the myocardium will cause limited supply of oxygen and metabolic substrates. Reduced energy production results in depletion of the immediate cellular energy stores in few minutes, which is associated with an accumulation of catabolites and byproducts, to acidosis, increased osmotic load, production of reactive oxygen species (ROS), and activation of various Ca^{2+}-sensitive enzymes. Reperfusion, however, adds further strain onto the cell. Mechanical disintegration of the cellular entity may occur. Thereby, these changes ultimately cause rupture of the cell membrane. This process, which cause early cell death, is called necrosis. Thus, necrosis is a violent, irreversible and non-regulated process of cell killing in consequence of profound disengagement of cell homeostasis. Necrosis entails plasma membrane rupture, thus leading to local inflammation, endothelial activation, monocyte chemoattraction and infiltration (Reimer et al. 1977). It has been well documented in animal studies (Reimer et al. 1977, Matsumura et al. 1998) that myocardial necrosis rapidly develops after ischemia as well as reperfusion.

Apoptosis, in contrast, is an energy-dependent process occurring after some delay and represents a transcriptionally regulated response to moderate cell injury or to the influence of various cytokines. It is characterised by shrinkage of the cell, condensation of chromatin, fragmentation into membrane-bound apoptotic bodies and rapid phagocytosis by neighbouring cells without induction of inflammatory response (Narula et al. 2000, Zhao & Vinten-Johansen 2002). A wealth of the investigations has demonstrated this programmed cell death phenomenon. Membrane signaling pathways, mitochondrial release of mediators, balance of pro-apoptotic Bax and antiapoptotic

Bcl-2 proteins expression, and caspase 8, 9 and 3 activation degree are involved in underlying mechanism accounting for the apoptosis (Zhao & Vinten-Johansen 2002).

2.2. Myocardial Stunning

If the period of ischemia is transient, myocardium can tolerate. Although no cell death results from the ischemia, the myocytes are damaged. Stunning refers to a loss of contractility that immediately follows a sublethal ischemic insult but salvaged by reperfusion. Unlike the infarcted heart, the stunned myocardium recovers fully in a day or two (Braunwald & Kloner 1982). In most species an ischemic insult of 15 min or less stuns the heart but does not cause infarction (Kloner & Jennings 2001). The length of time for function to return is dependent on a number of parameters, including the duration of the original ischemic insult, the severity of ischemia during the original insult, and the adequacy of the return of the arterial flow (Kloner et al. 1883). An important aspect of stunned myocardium is that there is a flow-function mismatch. At a time when coronary blood flow has been restored to normal or near normal and ischemia is resolved, the myocardium still does not contract. Another important aspect of the biology of stunned myocardium is that stunned myocardium is able to contract when exposed to inotropic stimuli (Becker et al. 1986, Patel et al. 1988). But the recovery of the stunned myocardium is independent on the inotropic stimuli (Arnold et al. 1985). The clinical situations related to stunning include the reversible suppressed heart function following angina, unstable angina, coronary vasospasm, and transient ischemia induced by inflation of an angioplasty balloon in the coronary arteries(Carlson et al. 1987, Sheiban et al. 1995). Post-cardiopulmonary bypass is one clinical area in which stunned myocardium can pose a significant problem (Kloner et al. 1994b). It was shown (Bolli et al. 1990) that a decrease in left ventricular wall thickening in patients after cardiac surgery requiring 24 to 48 hours for recovery. Postoperative stunning is a common clinical occurrence, and patients often require inotropes for the first hours to days after surgery until the stunning resolves.

Actually, like reperfusion injury in cell death (necrosis and apoptosis), 50%-70% of the stunning effect is due to a burst of O_2-derived free radicals liberated during the first few minutes of reperfusion with arterial blood (Bolli et al. 1989). Other study groups also provided the evidence that much of the stunning effect could be prevented by pretreatment of the animals with

intravenous infusion of enzymes that scavenge O_2-derived free radicals, SOD and catalase (Przyklenk & Kloner 1986, Murry et al. 1991). All these results lead to the oxyradical hypothesis of the stunning. But the exact change or changes that lead to the failure of contraction in stunning are unknown. Any alterations in the availability of Ca^{2+} and the sensitivity of the contractile apparatus to Ca^{2+} are all the possibilities (Kloner & Jennings 2001).

2.3. Arrhythmias

Like stunning, the arrhythmia is another manifestation of the injured myocardium. Ischemic heart disease carries an increased risk of malignant arrhythmia, myocardial infarction, and sudden cardiac death (Steinberg et al. 1999). Postoperative arrhythmias have been considered to be a manifestation of ischemia–reperfusion injury and have been used as a variable to compare strategies for myocardial protection during cardiac operations.

2.4. Systemic Inflammatory Response

A growing body of evidence suggests that cardiopulmonary bypass (CPB) is believed to trigger a whole body inflammatory response, which may be responsible for some of the major postoperative complications following open heart surgery (Wan et al. 1997a). The inflammatory response is the complex humoral and cellular interaction with numerous pathways contributing to inflammation including activation, generation, or expression of thrombin, complement, cytokines, neutrophils, adhesion molecules, and other multiple inflammatory mediators (Walport 2001a, b). This process has been previously studied in great detail, but its immunoregulatory characteristics remain uncertain. Among the complicated inflammatory mediators, cytokines, which include main proinflammatory cytokines TNF-a, IL-1, IL-6 and IL-8 as well as anti-inflammatory cytokine IL-10, have attracted more spotlights, it has been proved play a pivotal role in inflammatory reactions, in particular inducing myocardial injury after CPB (Wan et al. 1997c, Sawa et al. 1998). Numerous studies have revealed an increase in plasma levels of TNF-a (Menasche et al. 1994 , Teoh et al. 1995), IL-1 (Haeffner-Cavaillon et al. 1989), IL-6(Kawamura et al. 1993), IL-8(Finn et al. 1993), and IL-10 (Seghaye et al. 1996)during CPB. It as been suggested (Haeffner-Cavaillon et al. 1989) that ischemia-reperfusion and complement activation contribute to the release of cytokines, and the levels of cytokine release are associated with the duration of CPB time.

Furthermore, human studies demonstrated that myocardium is a major source of TNF-α, IL-6 and IL-8 (Oz et al. 1995, Wan et al. 1996a), while IL-10 is mainly released from the liver after cardiac surgery (Wan et al. 1997a).

The proinflammatory cytokines can significantly injure myocardial contractility. Also the TNF-α may be involved in the post-I/R myocardial stunning. However, the precise mechanisms of cyokine-induced myocardial contractile depression remain incompletely understood. TNF-α likely induces its acute negative inotropic effects via multiple mechanisms such as interfering with Ca^{2+} homeostasis, inducing direct cytotoxicity, disrupting excitation-contraction coupling, and desensitizing the β-receptor, as well as feedback inducing other myocardial depressants such as IL-1bβ (Meldrum 1998). Mechanistically, TNF-α, IL-1 and IL-6 activate inducilble nitric oxide synthase (iNOS), which induces large concentration of nitric oxide (NO). Studies have shown that NO attenuated contractility of myocardium (Brady et al. 1993), NO desensitizes the myofilament to Ca^{2+} (Goldhaber et al. 1996), resulting in decreased contractility.Thus, there are several ways that proinflammatory cytokines could induce myocardial functional depression.

2.4.1 TNF-α

Many studies have reported elevated systemic levels of TNF-α during and after CPB (Wan et al. 1997b). TNF-α has been recently suggested to be a critical factor in initiating the cytokine cascade responsible for myocyte intracellular adhesion molecule-1 induction and subsequent neutrophil-induced injury (Frangogiannis et al. 2002). Moreover, the studies indicated that TNF-α contributes to myocardial dysfunction and hemodynamic instability following CPB (Hennein et al. 1994, Menasche et al. 1994). TNF-α may depress adrenergic responsiveness of myocyte in an NO-independent manner (Muller-Werdan et al. 1998) in the early phase; it may also induce myocardial dysfunction through an NO-dependent mechanism in the delayed phase (Meldrum 1998). Meanwhile, TNF-α may induce myocardial necrosis, apoptosis and stunning.

2.4.2 IL-6

IL-6 is a sensitive marker of acute inflammatory response and is thought to be related to the extent of tissue injury induced by CPB (Sakai et al. 1993). It derives mainly from the myocardium in

cardiac surgery (Wan et al. 1996a). In general, IL-6 plasma levels increase during and after surgery and CPB (Ozawa et al. 2000) and are related to the duration of aortic clamp time (Kawamura et al. 1997, Ozawa et al. 2000). The raised levels of IL-6 have been proofed link to cardiac dysfunction after CPB (Hennein et al. 1994, Lefer et al. 1998). Hence, IL-6 seems to be responsible for much of the morbidity associated with the inflammatory response to CPB.

2.4.3 IL-8

IL-8 is a potent polymorphonuclear leukocytes (PMN) chemoattactant. Animal models investigations have proved the essential involvement of IL-8 in mediating acute inflammation in different organs post-CBP. Clinical evidence has also documented that the myocardium is a major source of IL-8 during reperfusion after longer duration of ischemia (Wan et al. 1997c). It has been suggested that the degree of myocardial injury may be related to IL-8 production in CABG (Wan et al. 1999).

2.4.4 IL-10

IL-10 was first identified as a cytokine synthesis inhibiting factor which inhibits the production of the proinflammatory cytokines, such as IL-1, IL-6, IL-8 and TNF-α (Frangogiannis et al. 2002, Welborn et al. 2003), and neutrophiles (Wang et al. 1994). In animal studies, it has been demonstrated that the endogenous production of IL-10 appears to be critical in diminishing myocardial injuries after myocardial IR. Multiple studies have demonstrated elevated release of IL-10 in the plasma from patients following acute myocardial infarction or during CPB (Seghaye et al. 1996, Wan et al. 1996b). This leads us to postulate that IL-10 play a pivotal role of immunosuppression and anti-inflammation.

2.4.5 Precisely autoregulatory system

Innate immunity consists of an exquisitely controlled auto-regulatory network of cytokines. Cytokines act both individually and within a network of interrelated and interacting signals. The proinflammatory response is always accompanied by a compensatory anti-inflammatory reaction. For instance, IL-10 production is often proportional to the release of IL-8 during clinical CPB (Wan et al. 1999). Recently, IL-10, which is produced during the induction of lung injury, is

reported capable of powerful inhibiting of NFκB activation (Lentsch et al. 1997), thereby inhibit proinflammatory cytokines production. Thus, keeping a balance between pro- and anti-inflammatory reactions, instead of blocking some individual mediators, may be more crucial in determining the extent of the inflammatory response and the clinical outcome.

3. Pharmacological Preconditioning

3.1. Natural History of Ischemic Preconditioning

Phenomenon of IP was first described in 1986 (Murry et al. 1986) in the canine myocardium. They observed that the size of an infarct resulting from a 40-min occlusion of a branch of a dog's coronary artery could be greatly reduced if the heart were subjected to 4 brief periods of 5 min of ischemia and 5 min of reperfusion prior to sustained ischemia. They found that the heart adapted itself within minutes to become resistant to ischemia-induced infarction. This phenomenon is called classic ischemic preconditioning, also termed early preconditioning. Subsequent studies demonstrated that this phenomenon exist in other species including rat (Liu & Downey 1992), rabbit (Liu et al. 1991), pig (Sack et al. 1993), chicken (Liang & Gross 1999) and sheep (Burns et al. 1995). The classic preconditioning is very transient, which lasts for only 1-2 hours (Murry et al. 1991, Van Winkle et al. 1991, Sack et al. 1993), and is lost somewhere between 2 and 4 hours in animal experiments (Burckhartt et al. 1995). In 1993 two study groups found that classic preconditioning is followed by a second window of protection that is less potent, but lasts for several days (Kuzuya et al. 1993, Marber et al. 1993). This is also termed as delayed or late preconditioning, or second window of preconditioning (SWOP) (Fig.1).

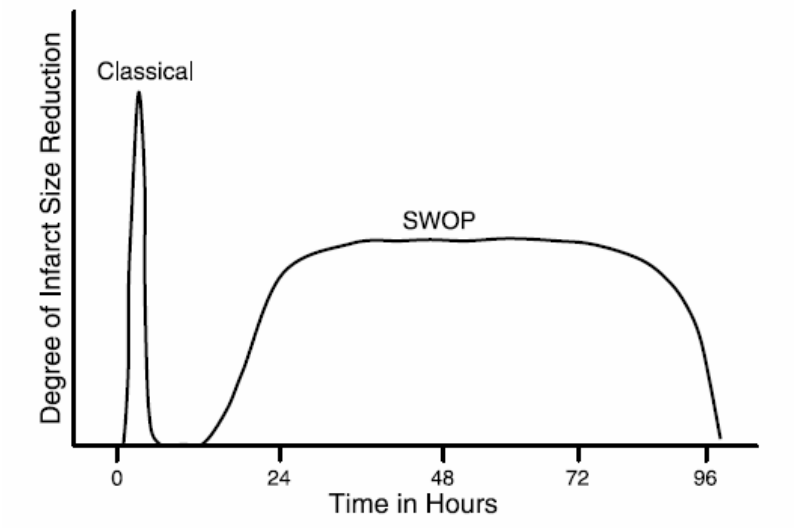

Figure 1. Diagrammatic representation of the temporal nature of the two windows of preconditioning. The classical preconditioning provided a transient but strong protection , and the second window of preconditioning (SWOP) provide a long-lasting but weak protection.(Yellon & Downey 2003)

Although it is difficult to directly examine whether human hearts can be preconditioned against infarction, there is strong circumstantial evidence that preconditioning exists in the human heart (Speechly-Dick et al. 1995, Ikonomidis et al. 1997).

3.2. Mechanism of Preconditioning

Although the mechanism of IP has been studied for almost two decades, it remains unclear. The first breakthrough came when it was demonstrated that protection was receptor-mediated (Liu et al. 1991). The mechanisms of preconditioning probably vary between acute and delayed models. The mode of acute preconditioning does not require protein synthesis (Meldrum et al. 1997), whereas delayed preconditioning is dependent on new protein production (Rowland et al. 1996). But both types of preconditioning share similarities in initial phase that the preconditioning provokes the release of several autacoids that trigger protection by occupying cell surface receptors. Receptor occupancy activates complex signaling cascades that during the lethal ischemia converge on one or more end-effectors to mediate the protection. The end-effectors so far remain elusive, although a

number have been proposed.

Investigations into the mechanisms of IPC lead to the observation that various pharmacological agents may elicit preconditioning-like effects in experimental animals (Nakano et al. 2000b). Acutely, any pharmacological agents that could activate the preconditioning signaling cascades at various levels can evoke preconditioning–like protection. Thus, pharmacological preconditioning (PPC) could provide a safer way than ischemia for inducing cardioprotection in humans.

3.2.1. Classic Preconditioning

3.2.1.1.Triggers

Triggers can be subdivided into receptors-mediated and nonreceptors-mediated form. The former includes adenosine, bradykinin, opioids, prostaglandins and cathecholamines. We now know that any Gi-coupled receptor can trigger the preconditioned state. The latter includes free radicals, NO, Ca^{2+} and cytokines (TNF-α).

Adenosine

Downey and colleagues first found that IP is receptor mediated (Liu et al. 1991). In their study, it showed that the adenosine A1 receptor acts to trigger ischemic preconditioning-like protection in the rabbit heart. Block of the A1 receptor could abolish the protection. And other studies thereafter approved this situation in other species (Auchampach & Gross 1993, Walker et al. 1995).

Bradykinin

Studies showed that intracoronary administration of BK reduces myocardial infarct size after reperfusion in canine, rabbit and pig hearts (Jin & Chen 1998, Kostitprapa et al. 2001, Shigematsu et al. 2001). That BK exerts such powerful protective effects in I/R is surprising because of its well-known proinflammatory actions (Calixto et al. 2000). In addition, there are studies demonstrating that HOE 140, a specific B2 receptor antagonist, abolished the protective effect of BK during I/R injury (Li & Sato 2001). The latter studies suggest that BK is released during I/R and acts to protect the tissue from injury.

Opioids

Mayfield and D'Alecy (Mayfield & D'Alecy 1992) showed that several intermittent hypoxic periods induced an acute adaptation to a subsequent hypoxic challenge in mice, and that the δ_1-opioid receptor mediated this increased tolerance to hypoxia. In 1995, Schultz demonstrated in anesthetized rats that the cardioprotective effect of PC was blocked by the nonselective opioid receptor antagonist naloxone at doses that had no effect on infarct size in nonpreconditioned animals (Schultz et al. 1995). Some studies report opioid-induced preconditioning effects, which are independent of direct receptor stimulation and are mediated solely by free radical formation (Patel et al. 2001).

NO

The role of NO has not been identified. Bolli and colleagues proposed that NO generated in preconditioning act as both a trigger as well as distal mediator in late preconditioning (Bolli et al. 1998). But its role in classic preconditioning remains controversial (Lu et al. 1995, Post et al. 2000). Nakano and colleagues found that exogenous rather than endogenous nitric oxide can trigger a classic preconditioned state through a free radical mechanism (Nakano et al. 2000c). Bell postulated that NO may lower the threshold for the protection observed, even though in itself it may not be a direct trigger of early preconditioning (Bell & Yellon 2001).

Free Radicals

During hypoxia or I/R, the impairment of mitochondrial respiration results in an increased production of ROS and free radicals (Vanden Hoek et al. 1998). Also, activated neutrophils can produce large quantities of free radicals. Several studies have demonstrated that treatment with a free radical scavenger can raise the threshold of preconditioning, and a free radical generator can trigger a preconditioned state (Baines et al. 1997). Subsequent studies propose that the free radicals can directly activate protein kinases (Gopalakrishna & Anderson 1989). In species whose hearts are rich in xanthine oxidase, the free radicals may derive directly from xanthine oxidase's action on purine catabolism

Other Triggers

In 1993, norepinephrine was reported to trigger preconditioning through α-receptors in the rat heart (Banerjee et al. 1993). Ashraf's group revealed that a short period of elevated Ca^{2+} in the coronary perfusate will put the isolated rat heart into a protected state that appears to be the same as that from ischemic preconditioning as it is PKC dependent (Miyawaki et al. 1996). And calcium channel blocker verapamil could block Ca^{2+}-induced preconditioning in the rat heart (Miyawaki & Ashraf 1997). Exogenous TNFα is demonstrated to elicit classic preconditioning (Lecour et al. 1999) as well as in late preconditioning (Nelson et al. 1995). Furthermore, neutralizing antibodies directed against both TNFα and the cytokine IL-1b abrogate exercise induced preconditioning-like cardioprotection in the rat (Yamashita et al. 1999).

Threshold of Preconditioning

Liu and colleagues demonstrated that there were no differences in cardiac protection between 1, 6, and 12 cycles of 5-min coronary occlusions as the preconditioning stimuli in the dog (Li et al. 1990). Another report confirmed the similar results (Van Winkle et al. 1991). Thus preconditioning was originally speculated to be "all or none" phenomenon. But subsequent researches revealed that the resulting infarct size varied with the strength of the preconditioning stimuli in rats (Barbosa et al. 1996) and pigs (Schulz et al. 1998). So it is supposed that preconditioning is more likely follow a very steep dose-response curve (Yellon & Downey 2003). Once the threshold of the preconditioning is reached, further stimulation appears no additional effect, giving an impression of the "all or none" phenomenon.

During a preconditioning ischemia, the heart appears to release many metabolites, by-products and agonists such as adenosine, bradykinin, opioids and norepinephrine. Goto and colleagues found that blockade of the bradykinin receptor in the rabbit blocks protection from a single cycle of preconditioning but not from multiple cycles (Goto et al. 1995b). Thus blockade of a single receptor type acts only to raise the ischemic threshold required to trigger protection rather than completely block it (Fig. 2).

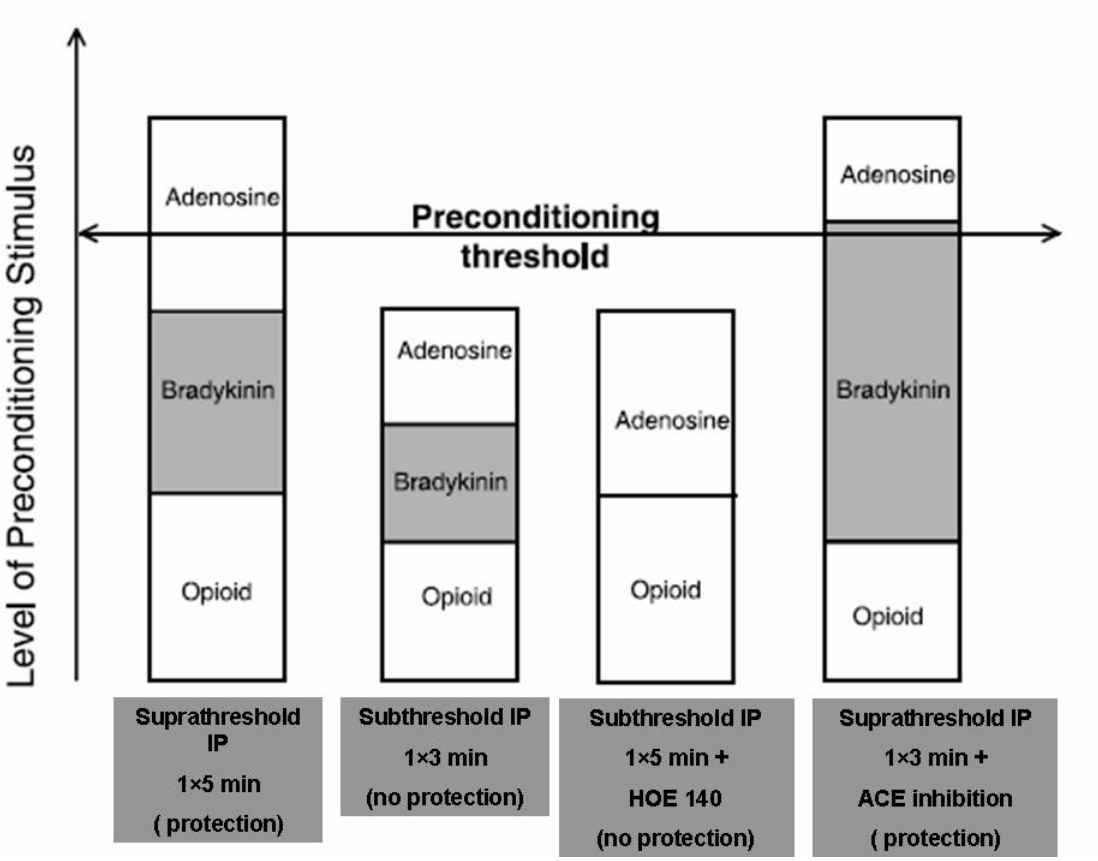

Figure 2. An example of how multiple receptors act in parallel in ischemic preconditioning. In the first panel, 5 min of ischemia reaches the threshold for protection, but in the second panel, 3 min of ischemia does not. In the third panel, blocking the bradykinin B2 receptor with HOE 140 (a bradykinin B_2 receptor antagonist) causes a 5-min ischemic period to become nonprotective because it can no longer reach the threshold. Conversely, augmenting bradykinin's contribution with an angiotensin converting enzyme (ACE) inhibitor, which prevents bradykinin breakdown, allows 3 min of ischemia to reach a protective threshold. (Goto et al. 1995b)

3.2.1.2.Modulators

Modulators in the signaling cascades refer to the protein kinases. G_i-coupled receptor's activation will thereafter activate these protein kinases, which will transfer the signals to the subsequent end-effectors. These modulators include protein kinase C (PKC), tyrosine kinase and the mitogen-activated protein kinases (MAPKs).

PKC

The G-protein coupled receptors' activation subsequently activate the phospholipases and liberation of the second messenger diacyglycerol for directly targeting the PKC (Bolli 2000, Schulz et al. 2001, Zaugg et al. 2003). PKC represents a key-signaling molecule, which, by phosphorylation, may activate the mitoK_{ATP} channels in classic PC as well as transcription factors including NFκB in the delayed PC. PKC can also be directly activated by NO from extracelullar sources or by ROS arising from mitochondria ether during preconditioning ischemia or reperfusion episode.

Tyrosine Kinase and Mitogen-activated Protein Kinases

Maulik and colleagues (Maulik et al. 1998) found that a tyrosine kinase inhibitor named genistein could block protection from ischemic preconditioning and proposed that at least one tyrosine kinase is in the overall pathway. Evidence was (Baines et al. 1998) provided that a tyrosine kinase was downstream of PKC; however, several other studies suggest that it may be in parallel with PKC in both pig (Vahlhaus et al. 1998) and rat (Fryer et al. 1999b).Potential downstream targets of PKC and tyrosine kinases are MAPKs. Each subfamily of the MAPK family, ERK, JNK and p38, has been suggested to play a role in the cardioprotection achieved by ischemic preconditioning (Ping & Murphy 2000). And most attention has focused on the p38 MAPK cascade. Following ischemic preconditioning, phosphorylation of p38 during the index ischemia is increased in isolated rat and rabbit hearts (Weinbrenner et al. 1997, Nakano et al. 2000a), unaltered in pig hearts in vivo (Behrends et al. 2000), or even decreased in isolated rat hearts (Marais et al. 2001) and dog hearts in vivo(Sanada et al. 2001). The importance of p38 activation for cardioprotection thus remains controversial.

Phosphatidylinositol 3-Kinase

Recent studies (Tong et al. 2000) showed that PI 3-kinase inhibitor could block protection from preconditioning using contractile dysfunction as the end point. Others subsequently confirmed the similar results (Mocanu et al. 2002) using an infarct size model. Evidence has implicated PI 3-kinase in the signaling of classical preconditioning.

3.2.1.3.Possible End-Effectors

Mitochondrial K_{ATP} Channel

There are two distinct populations of potassium channels existing in cardiomyocytes:the sarcolemmal and mitochondrial channels, which have different reactivities and different properties. K_{ATP} channels have been shown over the last 10 years to be an important mediator of cardioprotection, and their role in ischemic preconditioning has been demonstrated in all species of animals, isolated hearts, and cardiac myocytes. It was initially assumed that the sarcolemmal K_{ATP} channel was the end-effector of preconditioning's protection. But some study groups provided convincing evidence in a recovery of function models and a cardiomyocyte model that it was not the surface but the mitoK_{ATP} channel that was responsible for the protection (Garlid et al. 1997, Liu et al. 1998). They found that diazoxide, a selective mitoK_{ATP} channel opener which has been shown to be 1,000 times more potent in opening mitoK_{ATP} channels than sarcolemmal K_{ATP} channels (Garlid et al. 1996), can elicit preconditioning-like protection. This diazoxide-induced cardioprotection has been demonstrated in the micromolar range without any action potential duration shortening, excluding sarcolemmal K_{ATP} channel involvement.

Now there is general consensus that K_{ATP} channel plays a key role in preconditioning. A substantial studies have proved that not only do K_{ATP} channel openers mimic preconditioning, but that blockers abolish the ischemic preconditioning's protection (Alonzo et al. 1992, Gross & Auchampach 1992, Van Winkle et al. 1994). However, how the opening the mitoK_{ATP} channel would protect the ischemic heart remains an enigma. Several theories have been conceived to explain the issue. Firstly, Terzic and colleagues found (Holmuhamedov et al. 1999) that K_{ATP} openers make the mitochondria more resistant to calcium overload. The second hypothesis suggested by Garlid and colleagues (Garlid 2000) that mitochondrial swelling subsequent to potassium entry causes preservation of the functional coupling between mitochondrial creatine kinase and adenine nucleotide translocase on the outer membrane through which ADP traditionally enters the intermembrane space. Finally, more recent evidence suggested (Javadov et al. 2000) that opening mitoK_{ATP} channels prevent opening the mitochondrial transition pore during the lethal ischemia. ROS generation at reperfusion and calcium entering the cell could open a large diameter

pore with the mitochondrial membranes. And the transition pore disrupts mitochondrial function and allow foreign substances into the matrix, which destroys the mitochondria (Halestrap et al. 1998). Yellon's group recently found that protection from diazoxide or IP could be blocked by actractyloside, an opener of the transition pore. Also diazoxide could inhibit calcium-induced pore opening in isolated mitochondria. This persuasive evidence supports that transition pore could be end-effector of preconditioning. However, some arguing points were provided by other group (Dos Santos et al. 2002).

It is still a matter of debate on the question of whether activation of the mitoKATP channel is only an end-effector of preconditioning's protection (Gross & Fryer 2000, Pain et al. 2000). Its trigger role has been tested by different study groups (Sato et al. 1998, Pain et al. 2000, Wang et al. 2001a). Forbes and colleagues found that diazoxide's protection could be blocked by a free radical scavenger, *N*-acetylcysteine (Forbes et al. 2001). Further proofs come from that diazoxide increased free radical production in isolated cardiomyocytes (Forbes et al. 2001) in a human atrial-derived cell line (Carroll et al. 2001) and in vascular smooth muscle cells(Krenz et al. 2002). In all cases the increase in radical production could be blocked by 5-HD. These observations led to propose a free radicals hypothesis (Pain et al. 2000) that receptor occupancy leads to mitoKATP channel opening, which then causes the mitochondria to produce reactive oxygen species (ROS). The free radicals would then activate the downstream kinases that ultimately modulate the end-effector.

Osmotic Swelling and the Sodium-Proton exchanger

The theory of sodium/proton exchanger to be end-effector was proposed by Xiao and Allen (Xiao & Allen 2000). They found HOE 642, a highly selective blocker of the sodium/proton exchanger, shortly before reperfusion to a nonpreconditioned heart preserved postischemic function by an amount equal to that achieved by ischemic preconditioning. And HOE 642 had no additive effect when combined with ischemic preconditioning, further suggesting a common mechanism. The similar result was confirmed by others (Sato et al. 1997). Neither kinase inhibitors nor 5-HD could abolish amiloride's protection, suggesting that the sodium/proton exchanger must be protective as an end-effector. However, the postulation has not yet been proved directly.

Cells are in osmotic equilibrium and cannot tolerate an osmotic imbalance. During ischemia ATP is broken down to AMP and two inorganic phosphates, thus tripling the osmotic pull of the nucleotides. Similarly, failure of the sodium-potassium pumps leads to sodium leak into the cell and thus a collapse of the vital sodium gradient. Thus the osmotic swelling was proposed the cause of membrane failure and cell death in reperfused myocardium (Whalen et al. 1974). Preconditioning makes cardiomyocytes very resistant to membrane failure when they are challenged with hypotonic media (Armstrong et al. 1994). In ischemically preconditioned rat (Kevelaitis et al. 1999) and pig (Sanz et al. 2002) hearts, the extent of myocardial edema formation, along with infarct size, is reduced.

Cytoskeleton

Oxygenated myocytes could withstand a hyposmotic shock; those subjected to an ischemic insult could not. This lead to (Vanderheide & Ganote 1987) suggest that ischemic myocytes might be more susceptible to osmotic rupture. While they attributed this to cytoskeletal lesions accompanying ischemia (Ganote & Vander Heide 1987), possibly due to loss of phosphorylation of key cytoskeletal proteins (Armstrong & Ganote 1992). Preconditioned myocytes also seemed to be protected against the development of osmotic fragility during simulated ischemia (Armstrong et al. 1994). This theory relates to the p38 MAPK hypothesis of preconditioning. Activation of p38 through MAPKs causes phosphorylation of the small heat shock proteins hsp27 and its smaller isoform, which in turn causes actin filament assembly in the cytoskeleton (Guay et al. 1997) and is very protective in other cell types (Huot et al. 1996).

Others

TNFα increased during ischemia (Meldrum et al. 1998, Belosjorow et al. 1999). In patients, monoclonal antibodies against TNFα reduce the extent of irreversible tissue damage during acute myocardial infarction, suggesting a deleterious role of TNFα in the scenario of ischemia reperfusion (Li et al. 1999a). Ischemic preconditioning attenuates infarct size and the ischemia-induced increases in the serum and myocardial TNFα-concentration in rabbits in vitro (Meldrum et al. 1998) and in vivo (Belosjorow et al. 1999). Therefore, cardioprotection by

ischemic preconditioning might involve downregulation of TNFα. The detailed mechanism of the anti-inflammatory effect of preconditioning is pictured in 3.3.5. Also the anti-apoptosis effect is supposed to be end-effector of the preconditioning (Piot et al. 1999), the whole scenario of anti-apoptosis effect of preconditioning is depicted in 3.3.2.

It has been known for a long time that infarcts tend to be confluent, suggesting that necrosis spreads from one cell to the next. This spread could occur through gap junctions, the low-resistance channels between adjacent heart muscle cells. An intriguing hypothesis is that preconditioning may act to close gap junctions in the heart. Transgenic mice deficient in connexin43 (a major component of cardiac gap junctions) could no longer be protected by preconditioning (Schwanke et al. 2002). Heptanol, a closer of gap junctions, also blocked the protective effect of preconditioning in isolated mouse hearts (Li et al. 2002).

3.2.2. Second Window of Preconditioning

Since 1993, SWOP has been proved as a potent protective strategy against myocardial infarction (Marber et al. 1993), postischemic myocardial dysfunction (Sun et al. 1995) and ventricular arrhythmias (Vegh et al. 1992). Similar to the classic preconditioning, underlying signaling cascades of SWOP is also divided into triggers, mediators and possible end-effectors. As previously mentioned, SWOP shares the same triggers as classic preconditioning. But the mechanism of SWOP is different, the prolonged interval between the preconditioning stimuli and onset of SWOP allows for the possibility of new protein synthesis, posttranslational protein modification, and a change in the compartmentalization of existing proteins (Yellon & Downey 2003). Among the complex signaling cascades in SWOP, NFκB plays a key role. (Fig.3)

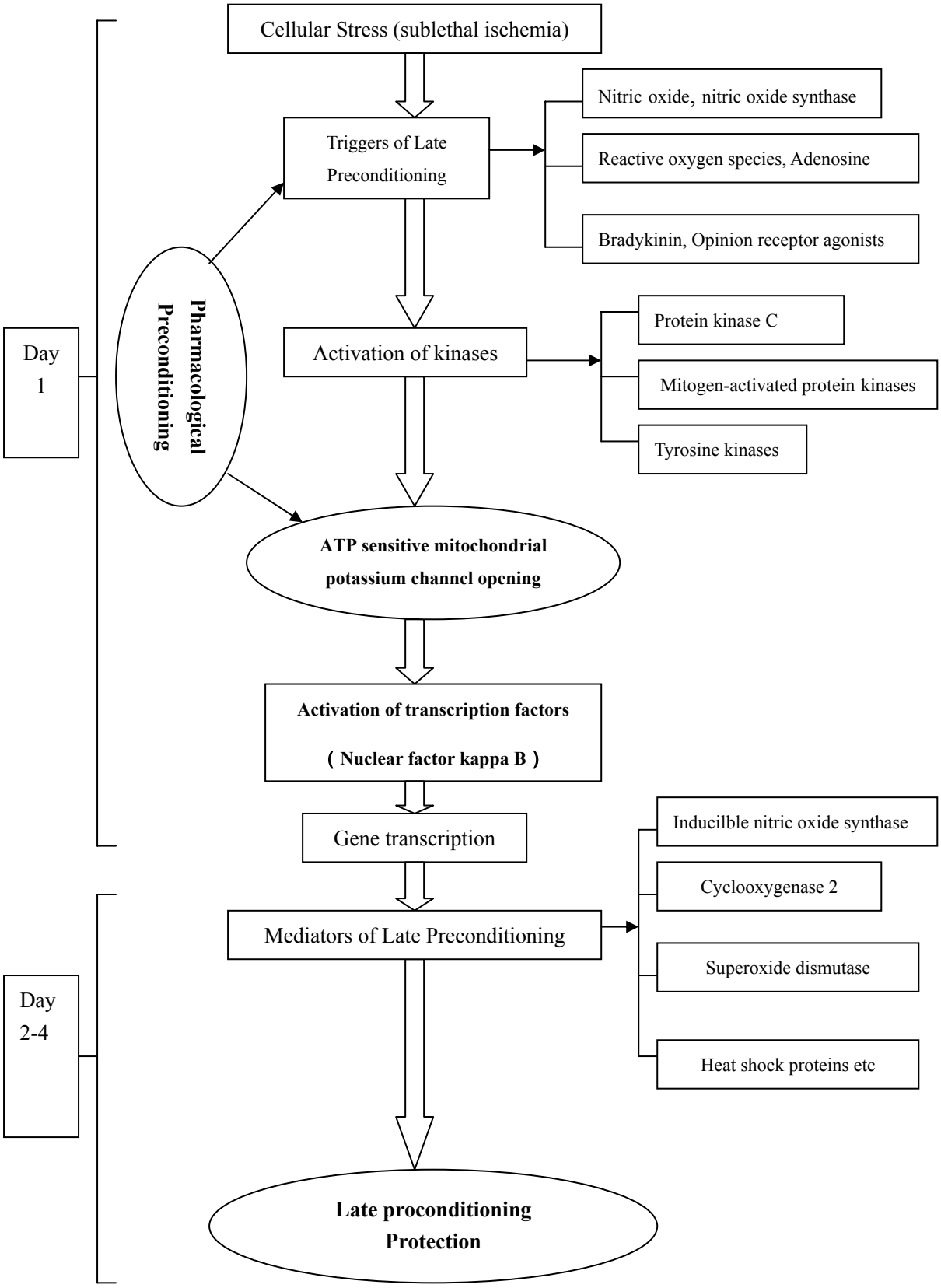
Cellular Stress (sublethal ischemia)
Triggers of Late Preconditioning
Nitric oxide, nitric oxide synthase
Reactive oxygen species, Adenosine
Bradykinin, Opinion receptor agonists
Pharmacological Preconditioning
Activation of kinases
Protein kinase C
Mitogen-activated protein kinases
Tyrosine kinases
Day 1
ATP sensitive mitochondrial potassium channel opening
Activation of transcription factors
(Nuclear factor kappa B)
Gene transcription
Inducilble nitric oxide synthase
Cyclooxygenase 2
Mediators of Late Preconditioning
Superoxide dismutase
Heat shock proteins etc
Day 2-4
Late proconditioning Protection

Figure 3. Schematic representation of the cellular mechanisms underlying late preconditioning. A nonlethal cellular stress causes release of chemical signals (Nitric oxide, Reactive oxygen species, adenosine, and possibly opioid receptor agonists) that serve as triggers for the development of late preconditioning. These substances activate a complex signal transduction cascade that includes protein kinase C (PKC), mitogen-activated protein kinases, and tyrosine kinases. The recruitment of PKC and distal kinases leads to opening of ATP sensitive mitochondrial potassium (mitoK_{ATP}) channel and therefore activation of nuclear factor kappa B and almost certainly other transcription factors, resulting in increased transcription of multiple cardioprotective genes and synthesis of multiple cardioprotective proteins that serve as co-mediators of protection 2 to 4 days after the PC stimulus. A similar activation of PKC and opening the mitoK_{ATP} channel downstream kinases can be elicited pharmacologically by a wide variety of agents. The mediators of late PC identified thus far include inducilble nitric oxide synthase, cyclooxygenase 2, heat shock proteins, and superoxide dismutase.

3.2.2.1. Triggers

The SWOP can be triggered by a spectrum of nonpharmacological and pharmacological stimuli. The former include sublethal ischemia, heat stress, ventricular pacing and exercise. The latter include endotocin, NO donors (Bolli et al. 1997), cytokines, ROS (Sun et al. 1996), adenosine receptor agonists (Baxter et al. 1994, Takano et al. 2001), bradykinin (Ebrahim et al. 2001, Kostitprapa et al. 2001) and opioid receptor agonists (Fryer et al. 1999a). Actually, triggers appear mostly identical to those of acute ischemic preconditioning.

3.2.2.2. Mediators

As like in classic preconditioning, PKC, tyrosine and MARKs are all involved in the signal transduction pathways in SWOP. Yamashita et al. (Yamashita et al. 1994) demonstrated that PKC inhibitor staurosporine could prevent the protection in hypoxically preconditioned myocytes in SWOP. Then a role for PKC in the mechanism of delayed protection from myocardial infarction was seen in the rabbit model (Baxter et al. 1995). Subsequently, Qiu and colleagues (Qiu et al. 1998) demonstrated that, as in classic preconditioning, PKC is also an essential mediator of the second window. Indeed, there have been a number of studies by Bolli's group (Ping et al. 1999a, Ping et al. 1999b, Ping et al. 1999c) demonstrating the importance of PKC in delayed preconditioning against both stunning and infarction. Similarly, Imagawa and colleagues

(Imagawa et al. 1997) demonstrated a role for protein tyrosine kinase in delayed preconditioning. Maulik and colleagues (Maulik et al. 1996) demonstrated that ischemic preconditioning could trigger activation of MAPK in rat hearts. Furthermore, using isolated myocytes, Heads and colleagues (Mockridge et al. 2000) have shown that metabolic inhibition can induce delayed preconditioning, an effect which is PKC dependent but independent of MAPK activation. However, the role of tyrosine and MARKs in kinase cascade of SWOP, which is upstream or downstream, remains an issue of debate (Cohen et al. 2000).

The activation of protein kinases subsequently activates various transcription factors that govern the expression of the cardioprotective genes responsible for late PC. Among them, NFκB was the first and most important transcription modulator. Others, like activating protein 1, may play a similar role in the SWOP effect.

Nuclear Factor Kappa-B

NFκB is a redox-sensitive transcription factor regulating many inflammatory genes. NFkB regulates genes involved in both innate and adaptive immunity, among them proinflammatory cytokines, chemokines, leukocyte adhesion molecules and inflammatory enzymes (Ghosh et al. 1998, Chen et al. 1999). The NFκB dimers in resting cells reside in the cytoplasm in an inactive form bound to inhibitory proteins known as IκB. The phosphorylated IκBs are then ubiquitinated and proteolytically degraded. This process activates NFκB, which translocates to the nucleus and binds to promoter or enhancer regions of specific genes, initiating transcription (Ghosh et al. 1998, Chen et al. 1999). NFκB pathway has been proved to be involved in pathological responses. Activation of NFκB can produce both survival (Beg & Baltimore 1996) and detrimental (Beyaert & Fiers 1994) signals in apoptosis. During the IR, NFκB is activated. Several of the genes that NFκB regulates, such as cytokines and leukocyte adhesion molecules, are implemented in reperfusion injury (Gumina et al. 1997, Cain et al. 1999).

The activation of NFκB plays a pivotal role in SWOP. It may well be a common downstream pathway through which multiple signals generated by ischemia (NO, ROS, and protein kinases) are able to initiate cardiac gene expression. Both ischemic preconditioning and NO donors are able to

induce NFκB activation (Bolli et al. 1998, Xuan et al. 1999). And inhibition of NFκB can abrogate SWOP. Myocardial protection by NFκB activation may be caused by induction of an NFκB-regulated mediator, such as manganese superoxide disumutase(Dana et al. 2000), inducible cyclooxygenase (Shinmura et al. 2000), and inducible NO synthase (Guo et al. 1999), this will be discussed in 3.2.2.3. Myocardial protection by NFκB activation could also be caused by a downregulation of the inflammatory response during reperfusion. It was found that a reduced activation of NFκB after sustained ischemia in hearts that had been subjected to preconditioning (Morgan et al. 1999). HSP70i are upregulated during preconditioning by MARK pathways (Marber et al. 1993). Hsps modulate NFκB DNA binding activity (Vayssier et al. 1998) and might reduce NFκB activation and thereby reduce inflammation during reperfusion. Another possible route of NFκB-mediated cardioprotection is through the antiapoptotic effect of preconditioning. Preconditioning reduces apoptosis during reperfusion, which may be linked to an NFκB-dependent increase of cardiac Bcl-2 (Maulik et al. 1999b).

3.2.2.3. Possible End-Effectors

Unlike the classic preconditioning, new synthesized protein, posttranslational modified proteins, and a change in the compartmentalization of existing proteins should be the end-effectors, which ensure continuous production of NO, and of proteins with antioxidative, cytoprotective and anti-apoptotic potential.

Heat Shock Proteins

The upregulation of HSP 72i has been reported that it could elicit a significant protection against subsequent myocardial infarction 24 hours after the heat shock (Yellon & Latchman 1992). It was known that ischemia was also capable of inducing proteins similar to HSP 72i (Dillmann et al. 1986) as well as causing the rapid and direct expression of heat shock proteins in rabbit (Knowlton et al. 1991). Marber and colleagues (Marber et al. 1993) subsequently demonstrated that in rabbits that 24 hours after an ischemic preconditioning stimulus, there was an increase in HSP72i as well as a significant reduction in MI. They provided further evidence of protection using transgenic mice overexpressing HSP72 (Marber et al. 1995). Other studies in which the human HSP72 gene was transfected directly into cardiac-derived cell lines (Heads et al. 1994) and myotubules (Bluhm

et al. 1998) also demonstrated protection against subsequent hypoxic injury. The smaller heat shock proteins, i.e., HSP27, have also been implicated in the second window and could act via the cytoskeletal mechanism described above. Thus the relative importance of the heat stress proteins in delayed preconditioning are unresolved and more work needs to be undertaken to define their precise role.

Antioxidant Enzyme Systems

Another group of proteins that show either increased transcription or functional upregulation is antioxidant enzyme systems, superoxide dismutase (SOD). The SODs are a family of metalloenzymes responsible for the dismutation of free radicals. Hoshida et al. (Hoshida et al. 1993) were the first to demonstrate an increase in MnSOD activity (mitochondrial origin) in ischemic versus nonischemic myocardium immediately following preconditioning in the canine heart. MnSOD content was increased at 12 and 24 h after preconditioning, but not at less than 6 h. They further demonstrated (Kuzuya et al. 1993), in dog myocardium, that following short periods of ischemia there was a delayed protective effect against a subsequent lethal ischemic insult 24 h later, an effect which they attribute to the upregulation of antioxidant enzymes such as MnSOD (Hoshida et al. 1993). They subsequently showed the same effect in rats (Yamashita et al. 1994). Dana and colleagues (Dana et al. 2000) showed that MnSOD activity was increased 24 h after treatment with the adenosine A1 receptor agonist, which was then attenuated by prior administration of PKC and protein tyrosine kinase inhibitors. But not all the species show the similar scenario (Tang et al. 2002).

Cyclooxygenase

Increased COX-2 expression was found 24 h after an ischemic preconditioning protocol in rabbits (Shinmura et al. 2000). This cardioprotective effect was blocked following the administration of COX-2-selective inhibitors at 24 h after the ischemic preconditioning protocol. In addition, a COX-2 inhibitor was also found to abolish delayed preconditioning against infarction in the mouse heart (Gou et al. 2000). Furthermore, confirmation of the potential importance of COX has recently been shown using COX-1 and -2 knock-out mice in which a decrease in postischemic recovery of left ventricular diastolic pressure was observed (Carmitta et al. 2001). All these results

lead to hypothesize the important role of COX-2 in the SWOP. But How COX-2 mediates protection is not clear.

The mitoK_{ATP} Channel

Bernado and colleagues (Bernado et al. 1999) reported that delayed preconditioning in rabbit was abolished when either glibenclamide or 5-HD was administered just before the index ischemic insult. Takano and colleagues (Takano et al. 1998b) have recently confirmed that 5-HD given before index ischemia abolished delayed preconditioning against infarction but did not modify the delayed antistunning effect of preconditioning. Thus, Pharmacological evidence with inhibitors of K_{ATP} channel opening suggests that these channels play a key role in conferring protection by delayed preconditioning. But the precise molecular mechanism is not clear. Indeed, Takashi and colleagues (Takashi et al. 1999) have reported that diazoxide can trigger a delayed protection via a PKC-dependent mechanism. And other studies came out the similar results (Ockaili et al. 1999).

NO

NO has been proposed as both a trigger as well as end-effector of SWOP (Bolli et al. 1998)**.** Vegh and colleagues (Vegh et al. 1994) demonstrated that delayed preconditioning against arrhythmias, induced by rapid pacing, was due to the inhibition of NOS and cyclooxygenase induction using the nonspecific agent dexamethasone. They proposed that this effect was due to an upregulation of iNOS. Bollis' group then provided strong evidence using pharmacological studies (Takano et al. 1998a) as well as studies in iNOS knock-out mice (Guo et al. 1998) which demonstrated a role for iNOS in mediating delayed preconditioning against myocardial infarction in rabbits. Yellon's group (Imagawa et al. 1997) had earlier demonstrated that dexamethasone given to rabbits before ischemic preconditioning abrogated protection against infarction 48 h later. They also showed that aminoguanidine, a selective inhibitor of iNOS activity, before the sustained ischemic insult, also abolished the protection. But these results were not shown in all forms of delayed preconditioning (Zhao et al. 2000).

3.2.3. Remote Preconditioning

In 1993 Przyklenk and colleagues (Przyklenk et al. 1993) reported that preconditioning one region

of the dog heart caused protection in a remote region. They hypothesized that a circulating humoral or perhaps a neural reflex triggered protection in the remote region. Subsequent animal studies have shown that ischemia of many different organs such as kidney, mesenterium (Verdouw et al. 1996), intestine (Tang et al. 1999) and brain can also protect the heart (Tokuno et al. 2002). At the same time all organs tested demonstrate the presence of the preconditioning response. However, the remote preconditioning has been poorly understood so far. It was postulated that the apparent general effect of ischemic preconditioning might be mediated by humoral or neurogenic factors or through substances in blood released from the preconditioned organ (Vaage & Valen 2003).

3.3. Preconditioning Effects

3.3.1. Myocardial Salvage

Since Murry firstly reported that IP reduced MI size, myocardial infarction size has still being a gold standard for evaluating the effects of preconditioning. Murry and colleagues (Murry et al. 1986) reported that early IP persistently reduced infarct size after 4 days of reperfusion, suggesting a permanent reduction in necrosis. Thereafter, a number of studies (Fliss & Gattinger 1996, Piot et al. 1997) (Maulik et al. 1999a) demonstrated that early IP could also attenuate apoptosis. In addition, they found that IP decreased the expression of the pro-apoptotic protein, Bax. And the analysis from these studies showed that attenuation of apoptosis by IP is associated with a reduction in acute necrosis early after reperfusion. However, it is unknown whether this represents a reduction in overall infarction (necrosis plus apoptosis) or a reduction in the conversion of apoptosis to necrosis. Compared with numerous studies focusing on the anti-myocardial infarction by early preconditioning, few studies were designed to determine the effect of SWOP on apoptosis. Furthermore, inconsistent results regarding the effect of SWOP on infarct size have been reported (Kuzuya et al. 1993, Yang et al. 1995).

As previously mentioned in the mechanism of preconditioning, a sophisticated signal cascade is activated by preconditioning stimuli, and ultimately the end-effectors could salvage the myocardium in early PC. In SWOP, activation of NFkB leads to a gene expression of antiapoptotic

protein, in particular the inhibitor of apoptosis protein-1 (IAP-1) (Liston et al. 1996) and X-linked inhibitor of apoptosis protein (XIAP) (Stehlik et al. 1998). Thus the protein product inhibits several of the caspase enzymes involved in the cell-death program. It was found that preconditioning significantly reduced apoptosis by upregulating expression of the mitochondrial antiapoptotic factor, Bcl-2 and downregulating expression of p53, an intermediate effector of apoptosis which activats the transcription of death proteins (Bax) or suppressing the transcription of Bcl-2 (Maulik et al. 1999b). And preconditioning inhibits inflammatory response (Discussed in detail in 3.3.5). Taken together, all these underlying mechanisms contribute to the preconditioning's antiapoptotic effect.

3.3.2. Anti-Stunning- Recovery of Mechanical Function

Postischemic recovery of contractile function is a commonly used end point for ischemic preconditioning in experiments. However, recovery of mechanical function after an ischemic insult is influenced by both a combination of the number of surviving myocytes and the degree to which they have been stunned. This will complicate the evaluation of the antistunning effect of the preconditioning. Anyway, there have been a number of studies demonstrated that preconditioning have a antistunning effect in rabbits (Bolli et al. 1997), pigs (Tang et al. 1996), sheep (Landymore et al. 1998) and rats (Gelpi et al. 2002). And free radicals have been shown to strongly contribute to stunning of reperfused myocardium (Bolli & Marban 1999). It was proposed that during reperfusion, hypoxanthine is oxidized by xanthine oxidase producing injurious free radicals that stun the heart (Chandrasekar et al. 1987, Brown et al. 1988). Further evidence came from Gelpi's group that the xanthine oxidase inhibitor allopurinol improved postischemic function and greatly attenuated the improvement from preconditioning in the rat model. Preconditioning ultimately elicits the production of the protective protein such as SOD, which is responsible for attenuating the free radicals' stunning effect.

3.3.3. Anti-Arrhythmias

Free radicals are known to lead to the genesis of arrhythmias in the heart (Kusama et al. 1990). It is postulated that preconditioning could confer antiarrhythmic effect by attenuating purine release, therefore decreasing the production of free radical (Yellon & Downey 2003). However, the studies

showed the mixed results. Ischemic preconditioning has also been reported to decrease the ischemia/reperfusion-induced arrhythmias in dogs (Vegh et al. 1990) and rats (Shiki & Hearse 1987), while in other species preconditioning has been shown either has little effect on arrhythmias or actually exacerbates it (Ovize et al. 1995).

3.3.4. Anti-Inflammatory Response

As discussed above, NFκB regulates genes involved in both innate and adaptive immunity, among them proinflammatory cytokines, chemokines, leukocyte adhesion molecules and inflammatory enzymes. Transcription factor NFκB plays a common pathway in the SWOP, NFκB-regulated mediator, such as MnSOD, iNOD, Hsp and COX are all the possible end-effecors which might significantly influence the inflammatory response. Thus preconditioning has been postulated to down-regulate the inflammatory response by NFκB activation. Morgan and colleagues (Morgan et al. 1999) found a reduced activation of NFκB after sustained ischemia in hearts that had been subjected to preconditioning. Similarly, reduced activation of NFκB was found during ischemia and reperfusion in hearts of rats that had been pretreated with hyperoxia, which probably secondary to an increase of IκBa in hyperoxic hearts with induced protection (Tahepold et al. 2001). In human umbilical vein endothelial cells preconditioned by hydrogen peroxide, reduced upregulation of cytokines and leukocyte adhesion molecules after subsequent stimulation with TNF-α was found (Zahler et al. 2000). Heat shock proteins modulate NFκB DNA binding activity (Vayssier et al. 1998) and might reduce NFκB activation and thereby reduce inflammation during reperfusion. However, rare study has focus on the influence on the inflammatory response by IP or pharmacological preconditioning. Wei found (Wei et al. 2001) that although adenosine pretreatment could confer cardioprotection, it failed to regulate inflammatory response. The further investigations are warranted to spot the mechanism of this scenario.

3.4.Preconditioning In Human Myocardium

The power of the preconditioning has been shown by the wealth of animal-based evidence. Although it is assumed that preconditioning's mechanism is common to all species, there have been obvious mechanistic discrepancies among the various reports, and some of them could well be related to species differences. Some of these differences are obvious as discussed above, but it

should be noted that any reported mechanism of preconditioning may be species specific and more importantly may not be relevant to human heart. Furthermore, if it can, could it be a therapeutic strategy to prophylactically benefit the human heart?

3.4.1. In Vitro studies

Cultured human ventricular myocytes (Ikonomidis et al. 1994, Arstall et al. 1998) have been used to demonstrated that ischemic preconditioning confer both early and delayed protection, which adenosine and PKC are shown involved in triggering and signaling the preconditioning (Ikonomidis et al. 1997). Inconsistent results by using endothelial cells are also shown associated with preconditioning (Shirai et al. 1998, Zhu et al. 2001).

Yellon's group has done a series of investigations by using human atrial muscle obtained from patients undergoing coronary artery bypass surgery. They were able to precondition human muscle using postischemic recovery of mechanical function as the end point (Walker et al. 1994). They also demonstrated that adenosine A1 and A3 as well as δ-opioid receptor activation all could trigger this protection (Carr et al. 1997b, Bell et al. 2000) and that both PKC and the K_{ATP} channel appear to be involved in mediating the protection in human muscle (Speechly-Dick et al. 1995, Carr & Yellon 1996, Carr et al. 1997a). These results have been confirmed by Cleveland and colleague (Cleveland et al. 1997b). Other studies, using creatine kinase release from human right atrial muscle as endpoint, have demonstrated that it is possible to early and delayed precondition human muscle by both simulated ischemia as well as pharmacological means (Ghosh et al. 2000).

3.4.2. In Vivo studies

The obvious ethical restrictions associated with studying preconditioning in humans have hampered the acquisition of the direct evidence. The classic endpoint in experimental studies is infarct size, which is not applicable to the cardiac surgery setting. Some surrogate end points have been designed to evaluate the effects of preconditioning. However, one should keep in mind that all such endpoints could be influenced by numerous factors without certainty, and any conclusion based on them should be explained cautiously.

3.4.2.1. Angina

In clinical setting, it is common for patients suffering brief episodes ischemia (i.e. angina) before an acute MI. A number of more recent studies (Iwasaka et al. 1994, Anzai et al. 1995, Ottani et al. 1995) revealed that the presence of preinfarct angina was associated with smaller infarct size based on peak and total creatine kinase release, improved left ventricular function with reduced incidence of congestive heart failure and shock, and reduced mortality. Furthermore, studies indicate (Yamagishi et al. 2000) that pre-infarction angina is only protective if it occurs within 24–72 h of MI, a time course that closely resembles that of the delayed phase of myocardial protection following ischemic preconditioning in animal models. Other studies suggested that patients who experience angina before acute MI seem to have reduced occurrence of life-threatening ventricular arrhythmias associated with reperfusion (Anzai et al. 1995, Tamura et al. 1997) and a lower in-hospital, 1- and 5-year cardiac mortality rate (Kloner et al. 1998b). Although it is theoretically possible that this preinfarct angina has the potential to precondition the myocardium, thereby reducing infarct size and improving survival, it remains a subject of debate.

3.4.2.2. Coronary Angioplasty

Percutanerous transluminal coronary angioplasty (PTCA) usually involves repeated intracoronary balloon inflations with intervening periods of perfusion, and in theory the first period of ischemia may enhance the myocardial tolerance to subsequent balloon inflations via classic ischemic preconditioning. In this respect, several studies (Cribier et al. 1992, Airaksinen & Huikuri 1997) have shown that if the duration of the first balloon inflation is longer than a “threshold” of 60–90 s, all indicators of myocardial ischemia, including clinical, electrocardiographic, metabolic, and hemodynamic measurements, are attenuated during subsequent balloon inflations, providing evidence for myocardial adaptation induced by the first period of ischemia. It has been (Tomai et al. 1994) reported that such beneficial effects could be abolished by blockade of K_{ATP} channels. Investigation into the mechanisms underlying this rapid protection of the myocardium during PTCA has provided further support for a preconditioning-like effect.

Furthermore, the pharmacological agents have also been demonstrated to confer preconditioning -like protection in PTCA. An important role has been demonstrated for adenosine in mediating

myocardial adaptation during coronary angioplasty (Leesar et al. 1997). Inhibition of adenosine receptors by bamiphylline (Tomai et al. 1996) or aminophylline (Claeys et al. 1996) abolishes myocardial adaptation during the second balloon inflation. Two other reports have suggested a role for both opioid (Tomai et al. 1999a) and bradykinin (Leesar et al. 1999) receptors in mediating myocardial adaptation during PTCA. Also opening of K_{ATP} channels with nicorandil were shown to reduce the electrocardiographic indices of ischemia during coronary angioplasty.

3.4.2.3. Cardiac Surgery

Ischemic Preconditioning

During the cardiac surgery, the heart suffers a period of global ischemia induced by aortic cross-clamping and a subsequent reperfusion injury. Thus, cardiac surgery could be the most usable surgical model to test a preconditioning's protection. In this respect, ischemic preconditioning has been widely studied (Yellon et al. 1993, Perrault et al. 1996, Cremer et al. 1997, Jenkins et al. 1997, Kaukoranta et al. 1997, Lu et al. 1997, Illes & Sowyer 1998, Szmagala et al. 1998, Li et al. 1999b, Wu et al. 2000, Wu et al. 2001b, Teoh et al. 2002, Wu et al. 2002, Ghosh & Galinanes 2003).

The inconsistent results have been shown in Table.1, which may relate to difference between the protocols, methods for measurement of the end points and the backgrounds of the clinical setting.
These divergent results have led to the hypothesis that in the setting of cardiac surgery, the additional protection conferred by ischemic preconditioning may only be demonstrable where a potential for suboptimal myocardial protection increases the risk of perioperative infarction (Perrault & Menasche 1999, Vaage & Valen 2003). This hypothesis was supported by Ghosh's finding that ischemia preconditioning only confer cardioprotection in off-pump groups, and in on-pump groups (intermittent crossclamp fibrillation group and cardioplegia arrest group) IP could not provide further protection, which CPB per se induces preconditioning (Ghosh & Galinanes 2003).

Table. 1 Ischemic preconditioning in cardiac surgery

Reference	IP protocol	Results
Yellon, et al (1993)	2 cycles, 3mI/2mR	Preserve ATP levels
Illes, et al (1998)	1 cycle, 1mI/5mR	Improve heart performance, decrease in the need for inotropic support
Lu, et al (1997)	2 cycles, 2mI/3mR	Preserve high energy phosphate, improve heart performance
Szmagala, et al (1998)	1 cycle, 4mI/6mR	Decrease cTnI release
Jenkin, et al (1997)	2 cycles, 2mI/3mR	Decrease cTnI release
Li, et al (1999)	2 cycles, 3mI/2mR	Decrease CK-MB release, better hemodynamic performance, increase SOD/ malondialdehyde activity
Perrault, et al (1996)	1 cycle, 3mI/2mR	Increased CK-MB and lactate release
Kaukoranta, et al (1997)	1 cycle, 5mI/5mR	No better effect of IP
Cremer, et al (1997)	2 cycles, 5mI/10mR	Worse finding of inotropic use
Wu, et al (2000,2001,2002)	2 cycles, 2mI/3mR	Better hemodynamic recovery Anti-arrhythmia effect
Teoh, et al (2002)	2 cycles, 3mI/2mR	Less cTnI release,
Ghosh, et al (2003)	1 cycle, 5mI/5mR	Less cTnI release (only in beating heart group, not in the on-pump groups)

Note: IP: ischemic preconditioning, m: minute, I: ischemia, R: reperfusion

Adenosine

More recently, pharmacological preconditioning has attracted more and more sights. Adenosine was the most invested agent to confer PPC. The mixed results provided by several study groups (Lee et al. 1995, Mentzer et al. 1997, Belhomme et al. 1999, Mentzer et al. 1999, Wei et al. 2001) came out as shown in Table. 2. And in 2002, Teoh and colleagues (Teoh et al. 2002) showed that an adenosine A1 receptor agonist, GR79123x, although statistically unable to demonstrate protection compared with preconditioning with ischemia, did appear to offer some benefit. Two possible reasons have proposed contributing to the inconsistency between the clinical and animals study results. A certain threshold of stimulation has to be reached to elicit the preconditioning response (Goto et al. 1995b, Morris & Yellon 1997). In addition, the duration of ischemia required to elicit preconditioning is known to vary between species (Lawson & Downey 1993). Thus it could be argued that the dose of the adenosine (Belhomme et al. 2000) used may have been insufficient to reach the threshold to trigger the myocardial preconditioning signaling pathway in

humans. Another is that adenosine A1 receptor alone is inadequate to precondition the human heart in this setting, stimulation of both A1 and A3 adenosine receptors may be required to precondition the human heart in vivo (Yellon & Downey 2003).

Table. 2. Adenosine or Its Agonist in CABG

Reference	Protocol	Results
Lee, et al (1995)	2450 μg/kg, before CPB	Better heart performance, less CK-MB release
Mentzer, et al (1997)	1400 μg/kg, after AC	Decrease dopamine need
	0.1-2 mM/L in cardioplegia	Decrease dopamine need
Mentzer, et al (1999)	2000μg/kg after AC + 3000μg/kg after DC	Decrease postoperative complications
Belhomme, et al (2000)	700 μg/kg before AC	No effect
Wei, et al (2001)	650 μg/kg before AC	Better heart performance, less CK-MB release
Teoh, et al(2001)	GR79236X 10μg/kg before CPB	No effect

Anesthetics

Two groups of the anesthetics, opioids and volatile anesthetics that are used as routine anesthetics in cardiac surgery, have been proved the capability of activation of preconditioning. According to the literature, preconditioning by opioids has not yet been investigated in the cardiac surgery. In the animal studies, volatile anesthetics can trigger an acute early preconditioning that lasts beyond their elimination (Cason et al. 1997, Cope et al. 1997, Kersten et al. 1997b). This effect has been abolished by adenosine A1 receptor antagonists (Roscoe et al. 2000), G protein inhibitors (Toller et al. 2000b), reactive oxygen species scavengers (Kevin et al. 2003) (Mullenheim et al. 2002, Tanaka et al. 2002), PKC inhibitors (Cope et al. 1997, Novalija et al. 2003, Uecker et al. 2003), K_{ATP} channel blockers (Kersten et al. 1997b, Piriou et al. 2000, Roscoe et al. 2000) (Toller et al. 2000a), and cyclooxygenase type 2 inhibitors (Mraovic et al. 2003). However, the exact signaling pathway is not yet fully understood. Although the volatile anesthetics are commonly used in clinical setting, only a few studies (Belhomme et al. 1999, Penta de Peppo et al. 1999, Tomai et al. 1999b, Haroun-Bizri et al. 2001, De Hert et al. 2002, Julier et al. 2003, Van Der Linden et al. 2003) to date have investigated its preconditioning effects (Table.3). The results, differed due to different protocols, suggested that volatile anesthetics could benefit the human heart to a limited extent.

Table.3. Volatile Anesthetics in Cardiac Surgery

Reference	Protocol	Results
Tomai, et al.	Isoflurane: 1.5% 15m, 10 m washout before CPB	Less TnI/CK-MB release (only patients with LVEF<50%)
Belhomme, et al.	Isoflurane: 2.5 (2.7%) MAC (membrane oxygenator) 5m, 10m washout before AC	Tendency of less TnI/CK-MB release
Haroun-Bizri, et al.	Isoflurane: 0.5-2% (ventilator) whole pre-CPB time, washout until AC	Better heart performance; less ST change
Penta de Peppo, et al.	Enflurane: 0.5-2% (ventilator) 5m before CPB, 1.5-1.8m washout before AC	Better heart performance
De Hert, et al.	Sevoflurane: 0.5-2% throughout the surgery	Better LV function
Van der Linden, et al.	Sevoflurane: 0.5-2% throughout the surgery	Less TnI level, less inotropic need, less chance of low output
Julier, et al.	Sevoflurane: 2 MAC 10m after CPB	Less NT-proBNP release, less plasma cystain C concentration

Note: NT-proBNP: N-terminal pro brain natriuretic peptide

3.4.3. Therapeutic Implications

The ultimate goal of which understand the potential mechanism associated with the preconditioning phenomenon is to design a preconditioning strategy prophylacticly protecting human from all kinds of ischemia syndromes. These circumstances include two categories, the unpredictable naturally occuring ischemic syndromes such as angina, and the planned procedures that involved a potentially injurious ischemia-reperfusion insults such as cardiac surgery and PTCA. In the former issues, the use of brief antecedent ischemia as a means of prophylactic induction of this protection is not desirable or feasible in most circumstances. On the other hand, the use of pharmacological agents capable of mimicking the protective effects of preconditioning, in lieu of brief ischemia, may provide a more benign approach for eliciting cardioprotection. Studies (Group 2002) have demonstrated that the patients with stable angina pretreated with the K_{ATP} channel opener nicorandil had an improved outcome due to a reduction in major coronary events. Also evidence has (Patel et al. 1999) shown that opening of K_{ATP} channels with nicorandil,

in addition to standard aggressive medical therapy for unstable angina, results in a significant reduction in the incidence of myocardial ischemic episodes and tachyarrhythmias. Although the exact underlying mechanism responsible for the explanations remains elusive, the authors suggested that it might at least partially be due to a preconditioning-like effect.

In the latter predictable clinical setting, the human obviously could get more beneficial protection from preconditioning strategy. The current ischemic preconditioning and pharmacological preconditioning protocol in cardiac surgery and PTCA have been discussed in section 3.4.2. However, from a clinical point, the ischemia as a preconditioning stimulus would not be acceptable by most of the surgeon, both due to the possible chances of embolism from the aorta and the prolonged surgical procedure as discussed in section 1.3. Hence, the pharmacological agents also play a dominant role in preconditioning. Other potential candidates, which have been shown great benefit to myocardium, such as bradykinin or its analogs and other mitoK_{ATP} channel openers like diazoxide, warrant further investigation in clinical setting.

AIMS OF THE PRESENT STUDY

Based on the findings in literature, bradykinin and diazoxide, one highly selective ATP sensitive mitochondrial potassium channel opener that was mostly tested in animal experiments, could protect myocardium in animal models and in vitro human studies. However, to date, there is no evidence that such cardioprotection elicited by diazoxide or bradykinin is involved in the open-heart operation. Also rare study has focused on the relationship between the preconditioning and inflammatory response in cardiac surgery. Isoflurane is now one of the most widely used volatile anesthetics in the CABG. Although it has been shown to be a powerful cardioprotective agent in animal models, the clinical evidence of its benefit to patients is rare. Therefore, the purpose in this series was designed to:

1. Investigate the myocardial protective effect of diazoxide in patients undergoing CABG.
2. Define the relationship of inflammatory response and diazoxide in CABG.
3. Investigate the myocardial protective effect of bradykinin in patients undergoing CABG.
4. Define the relationship of inflammatory response and bradykinin in CABG.
5. Investigate the myocardial protective effect of isoflurane in patients undergoing CABG.

PATIENTS AND METHODS

The study was approved by the Ethics Committee of Tampere University Hospital, Finland, and written informed consent was obtained from each patient. The study was carried out from September 2002 to September 2003 in the Division of Cardiothoracic Surgery, the Department of Surgery, the Department of Anesthesia and Intensive Care, Tampere University Hospital, the Immunopharmacological Research Group, University of Tampere University Hospital, Finland. The study consisted of two parts.

1. Part One (StudyⅠ- Ⅳ)

1.1. Patient Selection

Sixty-one patients with stable angina were scheduled for isolated elective CABG operations and were randomized into the Control group (n=20), DZX group (n=20) and BK group (n=21). Patients were excluded for any of the following reasons: (1) diabetes mellitus treated with sulfonylurea drugs, (2) unstable angina, (3) recent myocardial infarction (<1 month) (4) prior CABG surgery, (5) with hepatic, renal or pulmonary disease, (6) patients on corticosteroid medication.

1.2. Designs

The studies were designed as prospective, randomized and controlled clinical trial. Study I and Ⅲ were carried out on the patients in Control group and DZX group to investigate if diazoxide could precondition myocardium. Study II and IV were carried out on the patients in Control group and BK group to investigate if bradykinine could precondition myocardium.

1.3. Preoperative Data and Perioperative Course

The patients' demographic data and perioperative data are presented in Table 4. Preoperative patient characteristics for the three groups were similar. There were no significant inter-group differences with regard to age, sex, New York Heart Association class, ejection fraction, diseased vessel, preoperative medication, number of grafted vessels, mechanical ventilation time and

inotropic support. The diazoxide group patients had slightly longer cardiopulmonary bypass and cross-clamping times, but the differences to the controls were not statistically significant (p=0.087 and p=0.139, respectively).

1.4. Study Protocol

1.4.1. DZX group

DZX group patients received diazoxide (Hypertonalum, ESSEX Pharma German) infusion prior to the initiation of CPB. The dose of diazoxide (1.5mg/kg) was diluted in 20 ml of 0.9% sodium chloride. This was administered via central venous port of a Swan-Ganz catheter as a 5-minute period, followed by 5-minute washout period. Thereafter CPB was started.

Table 4. Patient Characteristics

Variable	Diazoxide (n=20)	Control (n=20)	Bradykinin (n=21)
Age (years)	64.7±8.9	68.4±8.7	65.6±7.3
Gender (Male/Female)	19/1	18/2	19/2
Weight (kg)	83.0±17.4	82.0±18.6	82.5±10.5
Preoperative ejection fraction	59.3±12.9	55.2±11.4	54.6±15.3
Previous myocardial infarction	11	10	10
New York Heart Association Class (II/III/IV)	8/11/1	8/9/3	1/16/4
Diabetes mellitus	0	2	6
Preoperative medication (yes)			
ACE inhibitor	6	8	7
Calcium channel antagonist	3	7	4
Antihyperlipidemia drug	19	17	17
β-receptor blocker	20	19	20
Nitroglycerine	14	12	17
Coronary artery stenosis percentage			
LAD (%)	78.5±13.4	83.8±15.0	80.6±16.8
LCX (%)	75.5±16.4	83.2±14.2	82.8±13.4
RCA (%)	82.9±20.8	79.9±17.2	87.3±16.1
Vessels bypassed (n)	3.20±0.95	3.40±0.68	3.20±1.03
Cross-clamping time (min)	72.55±23.81	71.60±19.18	76.14±17.68
Cardiopulmonary bypass time (min)	90.45±27.28	88.80±19.05	97.08±21.22
Cardioversion (%)	45	55	40
Mechanical ventilation time (h)	13.7±3.5	14.1±2.3	15.7±2.9
24 hours bleeding (ml)	849.5±586.8	734.3±435.5	803.3±399.6
Inotropic support (yes)	11	15	14

Numerical data are presented as absolute number or mean±standard deviation.

ACE = angiotensin-converting enzyme; LAD = left anterior descending coronary artery

LCX = left circumflex coronary artery; RCA = right coronary artery

1.4.2 BK group

BK group patients received an infusion of bradykinin (Clinalfa, Switzerland) prior to initiation of CPB. This was administered via central venous port of a Swan-Ganz catheter. The initial infusion rate was with an 1 μg/min increment to the full dose of 4μg/min at the second minute, where after the infusion lasted for 6 minutes. Three minutes after the completion of bradykinin infusion. Thereafter the CPB was started.

1.4.3 Control group

Control patients underwent a time-matched (10 minutes) period of placebo (0.9% sodium chloride) infusion.

1.4.4. Cutout Criteria

To avoid hypotension during the infusion of the study medicines, central venous and pulmonary diastolic pressure were maintained at a level no less than the baseline values obtained before the induction of anesthesia. Whether the systolic blood pressure decreased more than 20% compared with pre-infusion value, or the absolute value decreased below 70 mmHg, CPB was commenced immediately to avoid hemodynamic deterioration. Should this happen, the study infusion was completed during the CPB.

1.5. Anesthesia and Surgical Methodology

1.5.1. Anesthesia

A radial artery line and a pulmonary artery catheter were inserted for hemodynamic monitoring. Anesthesia was induced with propofol (0.5-1.0 mg/kg), sufentanil (0.8-1.0 μg/kg) and rocuronium. Propofol infusion was continued with a rate of 50-80 mg/kg/min and sufentanil with

0.03-0.05μg/kg/min. Additional midazolam boluses were given as necessary. Halogenated anesthetic gases were restricted completely due to possible influence on the K_{ATP}-channels. Occasional hypertension was controlled with nitroglycerine or labetalol.

1.5.2. Cardioplegia

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 200 ml/min. No magnesium was used in cardioplegia until rewarming, when10 mmol was given intravenously.

1.5.3. Surgical technique

The surgical techniques were standardized in all cases. A median sternotomy was performed. CPB was established with regular cannulation technique using mild hypothermia (34°C). A retrograde coronary sinus cannula was inserted transatrially for cardioplegia infusions and study sampling. The first cardioplegia infusion was given antegradely for two minutes, and then retrograde cardioplegia were given for another two minutes. Subsequent one-minute retrograde cardioplegia infusion was administered after each distal anastomosis, and final warm retrograde cardioplegia (37°C) was given for 3 minutes before aortic declamping. The proximal anastomoses were constructed during a single cross-clamping period.

1.6.Measurement and Data Acquisition

1.6.1. Hemodynamic data

Hemodynamic data was serially collected at the following five time points: (1) before the induction of anesthesia as the baseline, (2) one hour after CPB, (3) 6 hours after CPB, (4) 12 hours after CPB, (5) on the first postoperative morning. Heart rate (HR), mean artery pressure (MAP), mean pulmonary artery pressure (MPAP), pulmonary capillary wedge pressure (PCWP), central venous pressure (CVP), and cardiac output (CO) were measured. Derived cardiovascular variables, including cardiac index (CI), left ventricular stroke work index (LVSWI), right ventricular stroke work index (RVSWI), systemic vascular resistance (SVR) and pulmonary vascular resistance

(PVR) were calculated using standard formulas.

1.6.2. Biochemical markers

Blood levels of CK-MB and TnI, two biochemical markers of cellular necrosis, were serially measured. Blood samples were taken from peripheral vessels after the induction of anesthesia (baseline), at 6 hours, at 12 hours after CPB, and on the 1st and 2nd days after surgery. All samples were collected and cooled in 4°C immediately, then centrifuged. Serum sample were measured with a Chiron ACS180 analyzer. (ACS: 180; Chiron/Diagnostics, Emeryville, CA) using a direct chemiluminescence method.

Lactate. Peripheral artery and coronary sinus blood samples were taken simultaneously for measurement of lactate content at three time points: (1) before CPB (2) 5 minutes after declamping (3) 20 minutes after declamping. The blood samples were measured with DNA/Protein/Enzyme Analyzer (Biospec-1601E, SHIMADZU) using a commercial kit (Lactic acid, No.826-UV, Sigma Chemical Co. US). The lactate extraction ratio was calculated as follows:

(Arterial lactate concentration- coronary venous lactate concentration)/ arterial lactate concentration×100.

1.6.3. Cytokines

Blood samples for cytokine measurements were collected from the radial artery after the induction of anesthesia (baseline), at 1hour, at 6 hours, at 12 hours after CPB, and on the first postoperative morning. All samples were anticoagulated with ethylenediaminetetraacetic acid, immediately cooled in ice, and centrifuged within 30 minutes (4000 g for 10 min); plasma was transferred to polypropylene test tubes and stored at -70°C until assay. Interleukin (IL)-6, IL-8 and IL-10 levels in the plasma were measured by means of a commercially available enzyme-linked immunosorbent assay (IL-6 and IL-10: PeliPair ELISA, Sanquin, Amsterdam, Netherland; IL-8: DuoSet ELISA, R&D Systems Europe Ltd, Abindgon, UK). The detection limits were 0.95, 3.9, 1.85 pg/ml for IL-6, IL-8 and IL-10, respectively.

1.7.Hemodynamic control and postoperative care

After weaning the CPB, inotropes (noradrenaline and dopexamine) were used to maintain hemodynamic performance if the CI was less than 2.0 L/min/m^2. They were used at time-points when hemodynamic data were recorded and continued for at least 6 hours.

1.8.Statistical methods

Statistical analysis was performed using SPSS (SPSS for Windows, Version 10.1; Chicago IL) software package. With a minimum sample size of 18 cases per group the study could have achieved 90% power at alpha error level 0.05 to detected change of cardiac index at least 0.8 L/min/m^2. The student's t test or Mann-Whitney U test was used to distinguish differences in demographic parameters between the groups. Continuous variables were analyzed by analysis of variance for repeated measures. Logarithmic transformation was used when the variables were not normally distributed. The preoperative values were taken as covariates, and changes with respect to preoperative values were assessed. Correlation between different variables was assessed by Spearman's coefficient. Statistical significance was attributed to *p* values lower than 0.05. Cytokine results are expressed as median and 25% and 75% quartiles. Statistical significance was attributed to *p* values lower than 0.05. All results are expressed as mean ± standard deviation (SD).

2. Part Two (Study Ⅴ)

2.1. Patient Selection

Thirty-four patients with stable angina who were scheduled for isolated elective CABG operations were randomized into the Control group (n=16) and ISO group (n=18). Criteria for patients included were the same as Part One. (1.1)

2.2. Preoperative Data and Perioperative Course

The patients' demographic data and perioperative data are presented in Table 5. Preoperative patient characteristics for the two groups were similar. There were no significant inter-group differences with regard to age, sex, New York Heart Association class, ejection fraction, diseased vessel, preoperative medication, number of grafted vessels, mechanical ventilation time and

inotropic support.

Table 5. Patient Characteristics

Variable	Isoflurane (n=18)	Control (n=16)	p
Age (years)	61.7±8.0	63.6±9.6	0.546
Gender (male/female)	18/0	15/1	0.289
Weight (kg)	90.1±11.7	86.3±12.4	0.365
Preoperative ejection fraction	64.2±14.6	61.3±14.5	0.558
Previous myocardial infarction	6	8	0.186
New York Heart Association Class (II/III)	1/17	2/14	0.483
Diabetes mellitus	0	2	0.128
Preoperative medication (yes)			
ACE inhibitor	5	5	0.827
Calcium channel antagonist	4	2	0.465
Antihyperlipidemia drug	17	16	0.346
β-receptor blocker	18	15	0.289
Nitroglycerine	16	13	0.536
EURO score	2.6±2.3	2.8±2.7	0.758
Vessels bypassed (n)	3.11±0.83	2.81±0.66	0.258
Cross-clamping time (min)	72.89±23.78	69.06±21.55	0.628
Cardiopulmonary bypass time (min)	94.67±25.39	91.81±28.82	0.761
Cardioversion (n)	8	8	0.750
Mechanical ventilation time (h)	12.7±2.6	13.2±3.2	0.666
24 hours bleeding (ml)	753.9±322.2	568.1±295.3	0.091
Inotropic support (yes)	7	5	0.647

Numerical data are presented as absolute number or mean±standard deviation.

ACE = angiotensin-converting enzyme

2.3. Study Protocol

In the ISO group, a five-minute ISO exposure period was commenced after cannulation of aorta and right atrium was finished. End-tidal Isoflurane concentration was targeted to one minimum alveolar concentration (MAC) during the first minute. Age-corrected MAC values were calculated according to Mapleson (Mapleson 1996). After the ISO exposure, another five minutes wash out period was given, using the same ventilation pattern as during the exposure. The control group did

not receive isoflurane. Thereafter CPB was started. To avoid hypotension during the inhalation of the ISO, central venous and pulmonary diastolic pressure was maintained at a level of the baseline values obtained before the induction of anesthesia. Whether the systolic blood pressure decreased more than 20% compared with pre-infusion value, or the absolute value decreased below 70 mmHg, CPB was commenced immediately to avoid hemodynamic deterioration.

2.4. Anesthesia and Surgical Methodology

2.4.1. Anesthesia

A radial artery line and a pulmonary artery catheter were inserted for hemodynamic monitoring. Anesthesia was induced with propofol (0.5-1.0 mg/kg), and sufentanil (0.8-1.0 μg/kg). Propofol infusion was continued with a rate of 4-8 mg/kg/h and sufentanil with 0.03-0.05 μg/kg/min. Additional midazolam boluses were given as necessary. Neuromuscular blockade was provided with rocuronium during the induction of anesthesia. The infusions were adjusted according to clinical demands in the operating room, and during the postoperative care analgesia and sedation were provided with the same infusions. Halogenated anesthetic gases were restricted except for the exposure period in the isoflurane group, and completely in the control group. Occasional hypertension was controlled with labetalol or nitroglyserine.

The Rest of the Part Two

The rest of the methods which includ cardioplegia, surgical technique, the measurement and data acquisition, hemodynamic control, postoperative care and Statistical methods are the same as in part one.

RESULTS

1. PART ONE

Twenty patients were given diazoxide, twenty-one patients were given bradykinin, and the other twenty served as controls. All the patients in DZX group and in control group fulfilled the study protocol without earlier CPB than planned. Twenty patients who were treated with diazoxide suffered a mild hypertension with a decrease in systolic blood pressure 14.8 ± 4.5% compared with pre-infusion value. In BK group, three patients could not tolerate the bradykinin infusion as a profound hypotension happened at the first minute, and had to finish the protocol with CPB. Two of the others who received bradykinin developed profound hypotension during infusion at the third and fourth minute, respectively, and CPB had to be started to finish the full infusion. Bradykinin infusion caused acute decrease of blood pressure in most of the cases and the mean minimum mean arterial pressure (MAP) during bradykinin infusion was 72.8% of the original MAP level (74.7 ± 7.9 vs. 54.4 ± 12.1 mmHg). There were no adverse effects related to diazoxide and bradykinin. There was no major postoperative complication in any of the 61 patients who completed the study. All patients survived the operation and were discharged from the hospital.

1.1. Hemodynamic data

1.1.1. HR, Blood Pressure, and Loading Parameters

The preoperative baseline data in MAP, MPAP, CVP, PVR and SVR were similar between the groups. Slower preoperative baseline HR was found in BK group when compared with control group (p=0.01). In DZX group, the preoperative baseline PCWP level was lower than control group (p=0.04). There were no statistically significant differences in the change of HR (p=0.75, ANOVA for repeated measures), MAP (p=0.80, ANOVA for repeated measures), MPAP (p=0.89, ANOVA for repeated measures), PCWP (p=0.64, ANOVA for repeated measures), CVP (p=0.77, ANOVA for repeated measures), SVR (p=0.09, ANOVA for repeated measures) and PVR (p=0.15, ANOVA for repeated measures) between the groups. (Table. 6)

1.1.2. CI

The baseline values for CI were similar between groups (BK, 2.59±0.47; DZX, 2.67±0.46; CONT, 2.68±0.52 l/min/m^2; *p*=0.77). CI increased over time compared with baseline in both groups except one hour after CPB in the control group (Fig. 4). In the DZX group, the increase of CI was greater than that in the control group (*p*=0.001, ANOVA for repeated measures). There was no significant difference between BK group and control group in CI (p=0.840, ANOVA for repeated measures).

Table 6 Hemodynamic data

	Baseline	I hour after CPB	6 hours after CPB	12 hours after CPB	First POD
HR (beats/min)					
Control	60.7 ±9.2	71.2 ±13.0	78.4 ±12.0	87.5 ±10.6	83.3±7.7
Diazoxide	56.1 ±9.3	78.1 ±21.2	83.9 ±13.9	87.6 ±17.4	78.4 ±10.7
Bradykinin	53.5 ±8.4*	76.3 ±19.3	81.0 ±17.6	84.8 ±16.2	79.2 ±8.3
MAP (mmHg)					
Control	86.3 ±18.1	69.7 ±9.5	68.4 ±8.2	72.4 ±10.8	75.7 ±8.7
Diazoxide	82.4 ±16.5	65.4 ±11.6	69.0 ±11.4	73.0 ±9.5	77.3 ±12.7
Bradykinin	77.1 ±11.9	70.4 ±9.0	68.9 ±9.7	73.0 ±9.3	74.9 ±10.3
MPAP (mmHg)					
Control	19.3 ±4.1	21.2 ±5.6	21.0 ±5.1	20.3 ±4.6	20.0 ±4.6
Diazoxide	17.4 ±4.0	19.9 ±4.7	22.5 ±4.8	20.8 ±4.4	20.1 ±3.8
Bradykinin	18.3 ±5.3	20.7 ±3.1	22.1 ±4.0	21.3 ±3.6	19.0 ±5.1
PCWP (mmHg)					
Control	13.5 ±4.7	13.9 ±4.8	11.3 ±3.4	10.6 ±3.1	10.5 ±3.8
Diazoxide	10.8 ±3.0*	11.8 ±3.4	12.3 ±4.1	10.9 ±3.9	11.7 ±4.3
Bradykinin	10.9 ±3.6	12.0 ±3.1	12.0 ±4.2	11.2 ±2.4	9.8 ±3.4
CVP (mmHg)					
Control	7.7 ±3.3	11.3 ±2.4	9.7 ±3.1	8.4 ±2.8	7.7 ±3.2
Diazoxide	7.5 ±2.8	9.9 ±2.2	10.9 ±3.2	8.7 ±2.9	9.7 ±2.8
Bradykinin	7.8 ±2.7	10.2 ±2.0	9.6 ±2.3	9.1 ±3.2	8.0 ±3.5
PVR(dyn.s/cm^5)					
Control	92.8 ±49.7	115.7 ±53.9	134.8 ±53.1	115.5 ±41.4	105.9 ±38.6
Diazoxide	100.3 ±40.2	109.1 ±45.9	129.7 ±55.6	101.9 ±46.0	98.7 ±37.9
Bradykinin	114.7 ±44.6	115.8 ±46.0	142.2 ±61.2	127.5 ±53.3	129.3 ±61.7
SVR (dyn.s/cm^5)					
Control	1174.1 ±353.1	911.4 ±213.7	837.5 ±244.0	786.8 ±206.8	898.3 ±240.0
Diazoxide	1161.4 ±377.9	705.1 ±230.7	712.8 ±213.8	665.6 ±205.8	794.1 ±309.0
Bradykinin	1108.2 ±269.3	884.0 ±196.7	861.4 ±274.1	824.8 ±202.3	912.3 ±280.5

Data are presented as mean ± SD, no statistically significant differences were found between the groups.
HR = heart rate; MAP = mean arterial pressure; MPAP = mean pulmonary arterial pressure;
PCWP = pulmonary capillary wedge pressure; CVP = central venous pressure
PVR = pulmonary vascular resistance; SVR = systemic vascular resistance
*: $p<0.05$, Significance between study group and control group.

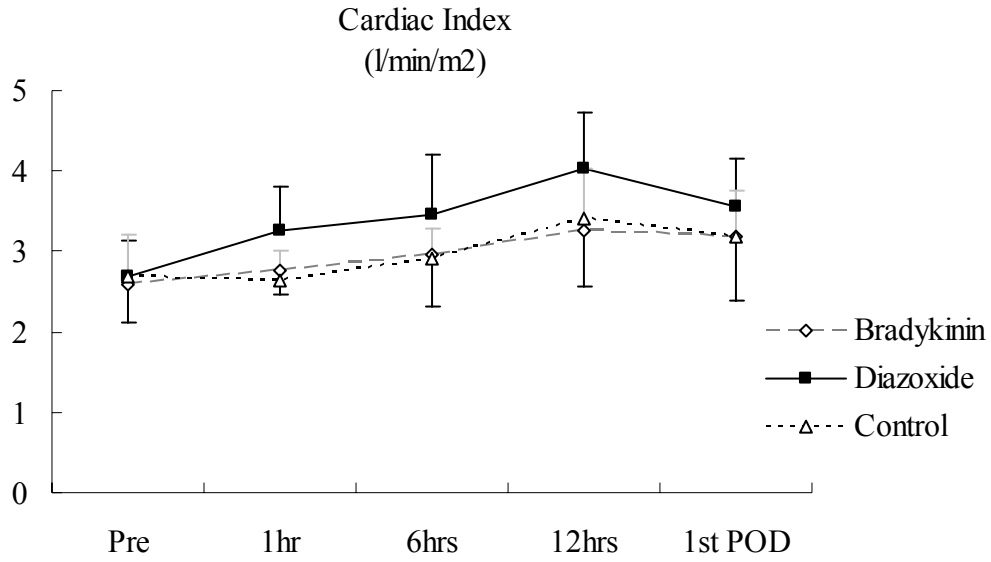

Figure 4. Cardiac Index (CI) prior to the CABG (pre) and at time points after declamping of the aorta. Values are given as mean ± SD. The increase of CI in diazoxide group was greater than that in the control group (p=0.001, ANOVA for repeated measures). There was no significant difference between BK group and control group in CI.

1.1.3. LVSWI

The baseline values for LVSWI were similar between groups (BK, 47.40±11.99; DZX, 51.92±15.48; CONT, 49.85±16.19 g.m/m^2; p=0.63). LVSWI decreased in both groups (Fig. 5), but it recovered much faster in the DZX group (p=0.027, ANOVA for repeated measures). There was no significant difference between BK group and control group in LVSWI (p=0.872, ANOVA for repeated measures).

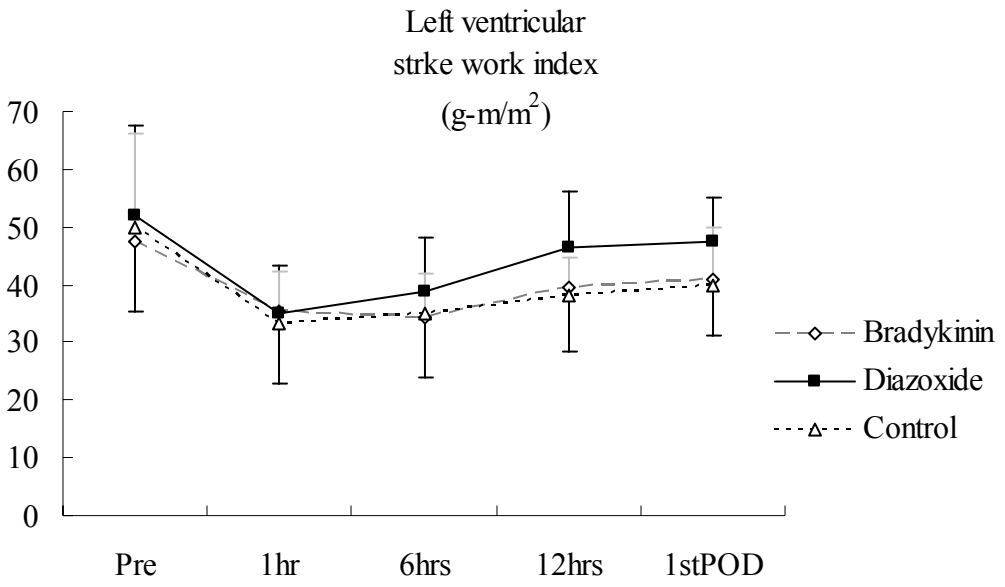

Figure 5. The Left ventricular stroke work index (LVSWI) prior to the CABG (pre) and at time points after declamping of the aorta. Values are given as mean±SD. LVSWI recovered much faster in the DZX group than that in control group (p=0.027, ANOVA for repeated measures). There was no significant difference between BK group and control group.

1.1.4. RVSWI

The baseline values for RVSWI were similar between groups (BK, 9.07±3.60; DZX, 9.13±4.23; CONT, 9.75±3.73 g.m/m^2; p=0.83). RVSWI decreased 1 hour after CPB in the controls and BK group, whereas in the DZX group it increased (Fig. 6), Subgroup analysis showed that the improvement of RVSWI in DZX group was statistically significant higher than that in control group (p=0.049, ANOVA for repeated measures). There was no significant difference between BK group and control group in RVSWI (p=0.903, ANOVA for repeated measures).

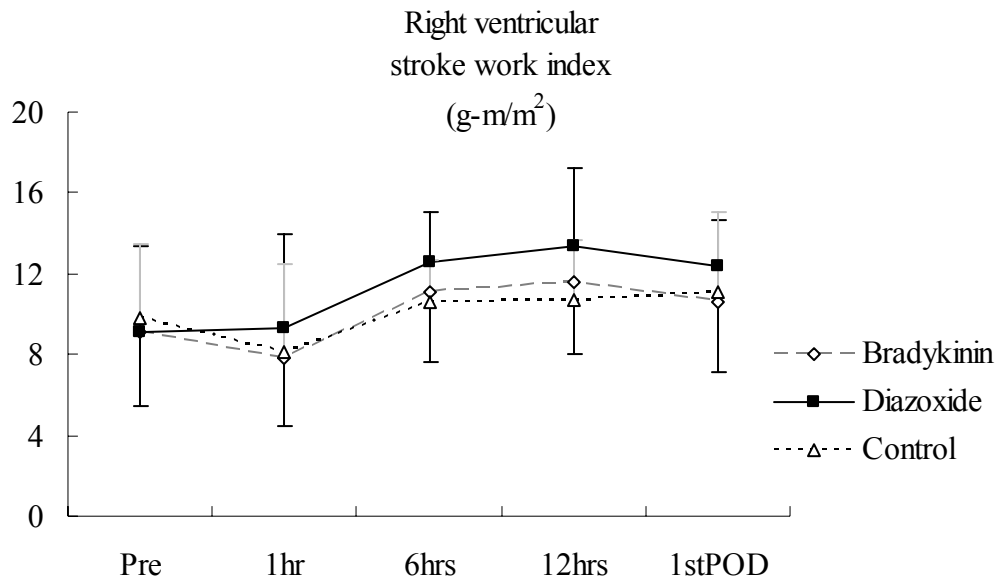

Figure 6. The right ventricular stroke work index (RVSWI) prior to the CABG (pre) and at time points after declamping of the aorta. Values are given as mean±SD. the improvement of RVSWI in DZX group was statistically significant higher than that in control group (p=0.049, ANOVA for repeated measures). There was no significant difference between BK group and control group

1.2. Cellular Viability

1.2.1. CK-MB

No significant difference was found in preoperative baseline CK-MB (BK, 5.59 ± 4.90; DZX, 5.57 ± 2.58; control, 5.80 ± 5.57 U/L, p=0.479) levels between groups. Subgroup analysis showed that CK-MB reached peak value on the first postoperative day. BK patients released significantly less CK-MB than the controls postoperatively (p = 0.036, ANOVA for repeated measurement, Fig 7). ACE inhibitor medication used before the operation (as a covariate) had no effect on the results (p=0.28). DZX patients released less CK-MB than controls postoperatively, but the difference was not significant (p=0.09, ANOVA for repeated measurement, Fig 7).

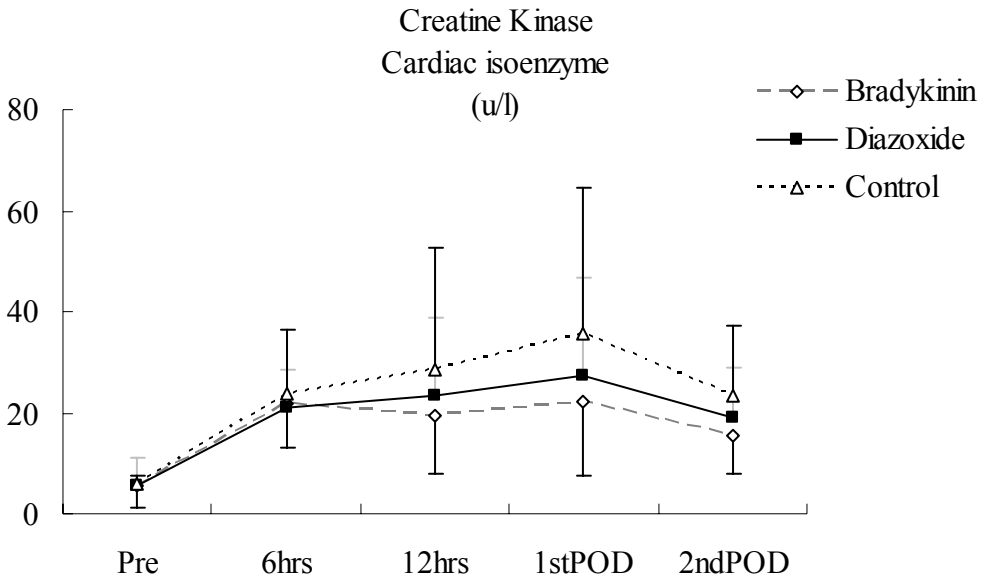

Figure 7. The creatine kinase cardiac isoenzyme (CK-MB) prior to the CABG (pre) and at time points after declamping of the aorta. POD: post operative day. BK patients released significantly less CK-MB than the controls postoperatively (p = 0.036, ANOVA for repeated measurement).

1.2.2 TnI

No significant difference was found in preoperative baseline TnI (BK, 0.21 ± 0.02; DZX, 0.20 ± 0.01; control, 0.24 ± 0.08 U/L, p=0.479) levels between groups. Postoperative cTnI levels were lower than 10 ng/ml in most of the cases in both groups (18 in BK group, 14 in DZX group and 15 in controls). The levels were not different between the groups (p = 0.81, BK group compare with Controls; p=0.54, DZX group compare with Controls, ANOVA for repeated measurement, Table 6).

Table 7. Cardiac Troponin I

	Pre	6 hrs	12 hrs	1st POD	2nd POD
BK (ng/ml)	0.21 ±0.02	5.07 ±3.70	6.12 ±4.65	5.53 ±5.74	3.08 ±4.55
DZX (ng/ml)	0.20 ±0.01	5.00 ±4.46	6.01 ±5.75	6.96 ±9.58	3.76 ±5.39
CONT(ng/ml)	0.24 ±0.08	4.37 ±3.17	7.18 ±9.01	8.09 ±10.22	4.98 ±6.69

The TnI prior to the CABG (pre) and at time points after declamping of the aorta.
Values are given as mean±SD.

1.2.3 Lactate

For some surgical considerations or technical problems, we failed to get blood samples taken from the retrograde coronary sinus cannula at all time-points in 18 cases, only forty-three patients' coronary sinus blood samples were included (15 in the BK group, 15 in the control group and 13 in the DZX group, Table. 8). There were no different of lactate extraction ratio between BK group and controls (p=0.84, ANOVA for repeated measures) as well as between DZX group and controls (p=0.81, ANOVA for repeated measures).

Table 8. Lactate Production

		BK (n=15)	DZX (n=13)	CONT (n=15)
Pre	A	1.05 ± 0.26	0.95 ± 0.20	0.98 ± 0.18
	CS	0.88 ± 0.32	0.79 ±0.22	0.93 ± 0.35
5 mins	A	1.42 ± 0.27	1.22 ± 0.15	1.30 ± 0.26
	CS	1.81 ± 0.31	1.55 ± 0.26	1.64 ± 0.36
20 mins	A	1.44 ± 0.26	1.26 ± 0.27	1.42 ±0.35
	CS	1.62 ± 0.36	1.51 ± 0.34	1.52 ±0.34

The Lactate blood concentration prior to the CABG (pre) and at time points after declamping of the aorta. A: peripheral artery blood sample; CS: coronary sinus blood sample. Values are given as mean±SD.

1.3 Cytokines

Six patients' peripheral blood samples (One in control group, two in BK group and three in DZX group) couldn't be measured in all time points. These six cases were excluded from the analysis.

1.3.1. IL-6

The preoperative baseline levels of IL-6 were similar in three groups (BK, 3.49±3.89; DZX, 2.67±1.48, CONT, 2.49±1.52 pg/ml, p=0.438). In comparison with the baseline values, plasma levels of IL-6 increased significantly after reperfusion and this lasted during the study period in all groups (p<0.05). Concentrations of IL-6 (Fig.8) reached the peak value at 6 hours after CPB. There was no significant difference between groups.

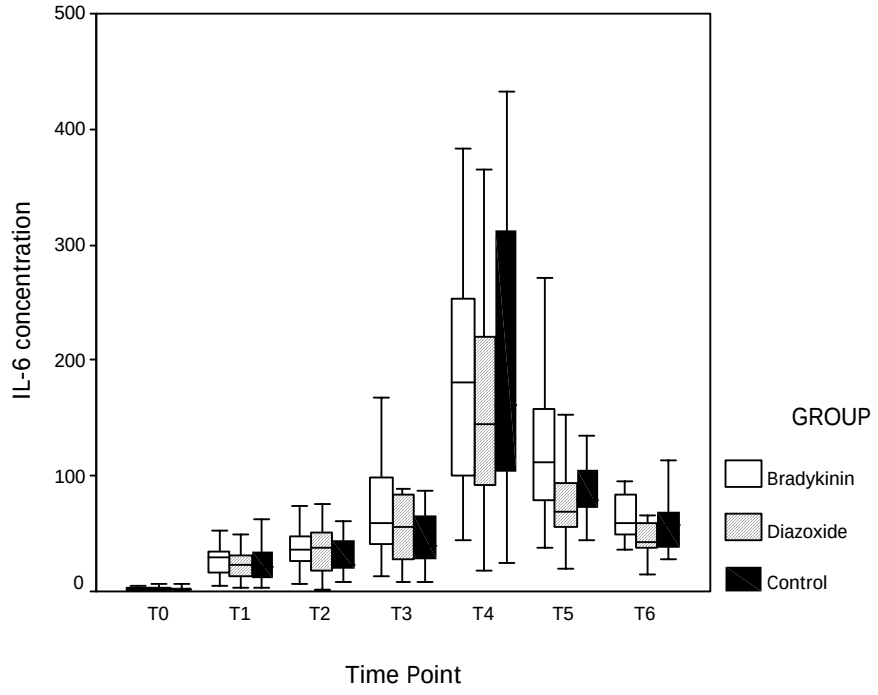

Figure 8. Perioperative systemic plasma IL-6 concentration of cytokines in CABG patients. T0=baseline level, T1=5 min after reperfusion, T2=20 min after reperfusion, T3=1 hour after CPB, T4=6 hours after CPB, T5=12 hours after CPB, T6= first post operative day. Data are presented as mean and 25% and 75% quartiles. (error bars refer to 10% and 90% range)

1.3.2. IL-8

The preoperative baseline levels of IL-8 were similar in three groups (BK, 6.31±5.83; DZX, 7.19±12.57, CONT, 5.59±4.27 pg/ml, p=0.842). In comparison with the baseline values, plasma levels of IL-8 increased significantly after reperfusion and this lasted during the study period in all groups (p<0.05). Concentrations of IL-8 (Fig.9) reached the peak value at 6 hours after CPB. There was no significant difference between groups.

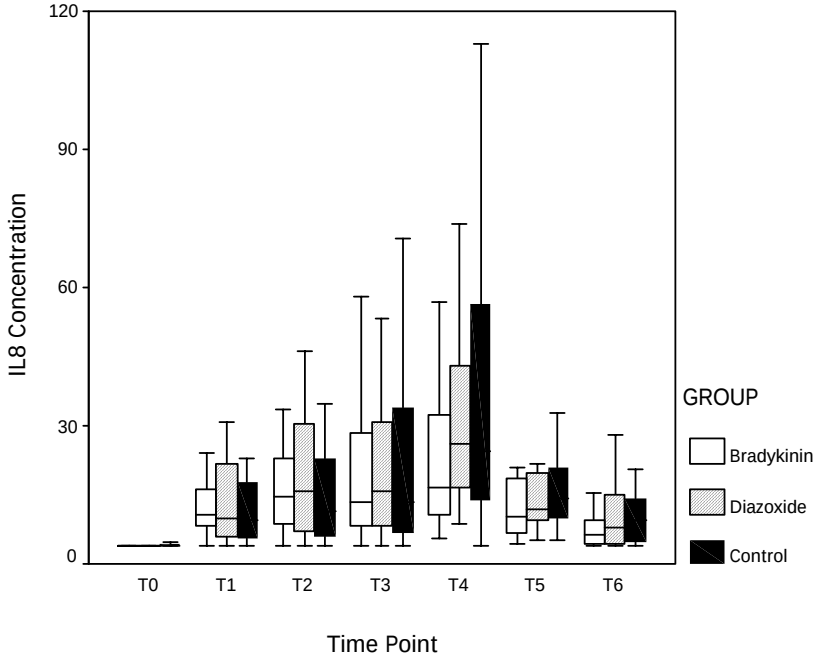

Figure 9. Perioperative systemic plasma IL-8 concentration of cytokines in CABG patients. T0=baseline level, T1=5 min after reperfusion, T2=20 min after reperfusion, T3=1 hour after CPB, T4=6 hours after CPB, T5=12 hours after CPB, T6= first post operative day. Data are presented as mean and 25% and 75% quartiles. (error bars refer to 10% and 90% range)

1.3.3. IL-10

The preoperative baseline levels of IL-10 were similar in three groups (BK, 4.22±9.37; DZX, 5.19±10.74, CONT, 2.01±0.55 pg/ml, p=0.480). In comparison with the baseline values, plasma levels of IL-10 increased significantly after reperfusion and this lasted during the study period in all groups ($p<0.05$). Plasma level of IL-10 (Fig.10) reached the peak value at 20 minutes after reperfusion in both groups. There was significantly higher IL-10 concentration in DZX group than that in controls (p=0.015, ANOVA for repeated measures). There was a tendency of higher IL-10 in BK groups when compare with the controls (p=0.10, ANOVA for repeated measures).

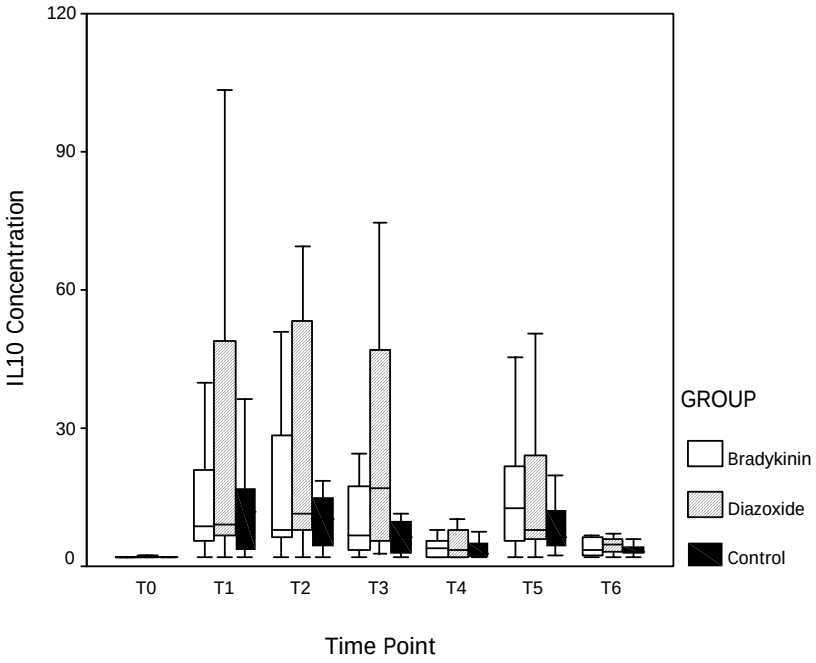

Figure 10. Perioperative systemic plasma IL-10 concentration of cytokines in CABG patients. T0=baseline level, T1=5 min after reperfusion, T2=20 min after reperfusion, T3=1 hour after CPB, T4=6 hours after CPB, T5=12 hours after CPB, T6= first post operative day. Data are presented as mean and 25% and 75% quartiles. (error bars refer to 10% and 90% range)

*: p=0.015 ANOVA for repeated measures

1.3.4. IL-6 to IL-10 Ratio and IL-8 to IL-10 Ratio

The ratios of IL-6 to IL-10 and IL-8 to IL-10 were calculated at each time point (Fig. 11. and Fig 12.). Subgroup analysis showed that both ratios were significantly lower in DZX groups than that in the controls (p=0.025 and p=0.041 for each respectively, ANOVA for repeated measures). The ratio of peak levels of IL-6 to the peak levels of IL-10 were 11.57 ± 3.9 in DZX group and 20.87 ± 3.9 in control group, p=0.10. The corresponding ratio of peak levels of IL-8 to IL-10 were 1.28± 0.3 in DZX group and 3.06 ± 0.5 in the controls, p=0.01 And in the BK group, the ratios of IL-6 to

IL-10 were similar with the controls, but the ratios of IL-8 to IL-10 were significantly lower in BK group than that in the controls (p=0.025, ANOVA for repeated measurements). Taking into account the different appearance interval of pro- and anti-inflammatory mediators, also the ratio of highest IL-6 or IL-8 to the highest IL-10 was calculated in each individual. The ratio of peak levels of IL-6 to the peak levels of IL-10 were 14.37 ± 3.2 in BK group and 20.87 ± 3.9 in control group, p=0.209. The corresponding ratio of peak levels of IL-8 to IL-10 were 1.72± 0.3 in BK group and 3.06 ± 0.5 in the controls, p=0.048 (Wei et al. 2003).

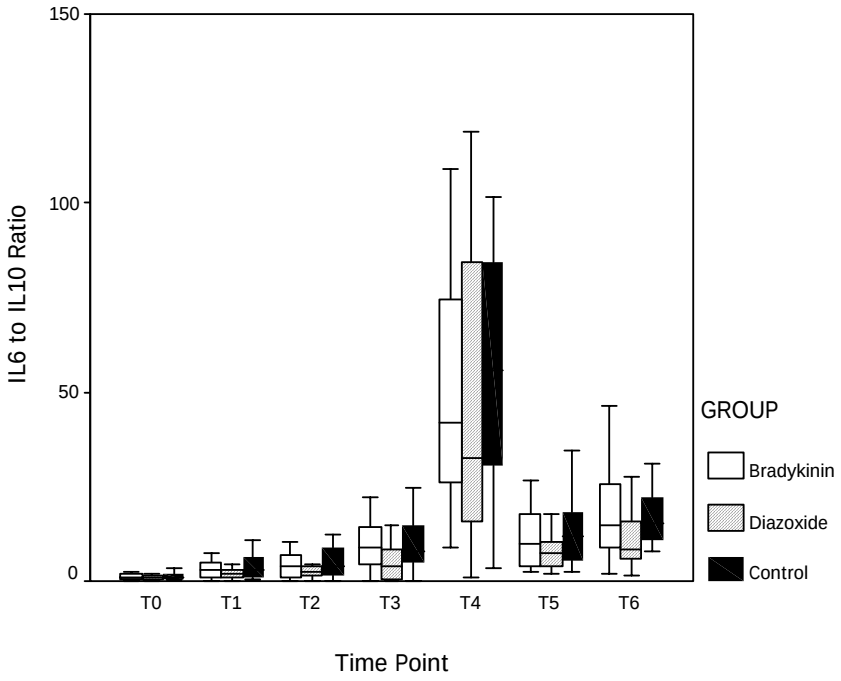

Figure 11. Ratios of IL-6 to IL-10 level. T0=baseline level, T1=5 min after reperfusion, T2=20 min after reperfusion, T3=1 hour after CPB, T4=6 hours after CPB, T5=12 hours after CPB, T6= first post operative day. Data are presented as mean and 25% and 75% quartiles. (error bars refer to 10% and 90% range)

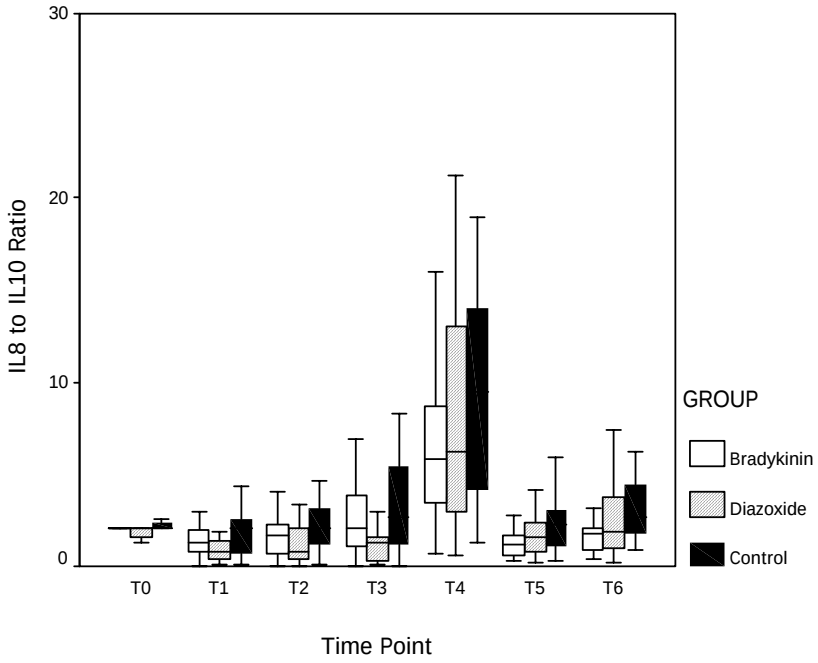

Figure 12. Ratios of IL-8 to IL-10 level. T0=baseline level, T1=5 min after reperfusion, T2=20 min after reperfusion, T3=1 hour after CPB, T4=6 hours after CPB, T5=12 hours after CPB, T6= first post operative day. Data are presented as mean and 25% and 75% quartiles. (error bars refer to 10% and 90% range)

1.3.5. Cytokine Levels in the Coronary Sinus

There were no differences of the baseline levels of IL-6, IL-8 and IL-10 between the groups. The concentrations of IL-6, IL-8 and IL-10 in coronary sinus blood sample increased significantly (p<0.05) in both groups at all measured time points as compared with baseline. The trans-myocardial cytokines levels, as defined by a cytokine level difference in arterial and coronary sinus blood, were shown in Table 8. There were no statistically significant differences between the groups.

Table 9 Change of trans-myocardial cytokines levels

		Pre	5 mins	20 mins
IL-6 (pg/ml)	BK (n=15)	3.9(1.1,5.4)	5.3(0.2,12.5)	4.7(0.6,4.9)
	DZX (n=13)	3.5(0.8,3.3)	3.3(0.1,10.8)	4.8(-1.7,9.1)
	CONT (n=15)	2.7(0.6,3.7)	5.7(-1.4,2.6)	-0.3(-0.5,6.2)
IL-8 (pg/ml)	BK (n=15)	-0.1(-0.6,0)	1.2(-1.4,3.5)	-0.9(-4.2,1.6)
	DZX (n=13)	0.1(0,0)	0.7(-0.4,1.8)	-2.3(-7.5,0)
	CONT (n=15)	0.1(0,0)	1.7(-0.3,2.9)	-0.8(-3.9,0.3)
IL-10 (pg/ml)	BK (n=15)	0.1(0,0)	-0.5(-1.1,0.4)	-0.3(-0.4,0.9)
	DZX (n=13)	0.3(0,0.9)	1.6(-2.6,0.9)	-4.3(-5.2, -0.1)
	CONT (n=15)	0.1(0,0)	0.3(-0.6,1.3)	-1.0(-1.3,0.5)

The trans-myocardial cytokines level = a cytokine level in coronary sinus blood – the cytokine level in arterial blood. Data are presented as mean and 25% and 75% quartiles, no statistically significant differences were found between the groups.
DZX=diazoxide group; CONT=control group; Pre= before the study medicine was given; 5 minutes after reperfusion; 20 minutes after reperfusion.

2. PART TWO

All the thirty-four patients fulfilled the study protocol. The demographic and perioperative data did not differ between the groups (Table 10). Eighteen patients in the ISO group suffered a mild to moderate transient hypotension with a decrease in systolic blood pressure 16.2±9% compared with pre-infusion value. No rescue start of CPB was needed in any of the study patients. There were no adverse effects related to isoflurane. All patients survived the operation and were discharged from the hospital.

2.1. Hemodynamic data

Cardiac index (CI) increased over time compared with baseline in both groups except one hour after CPB in the control group (Fig. 13). There was a tendency of better CI recovery in the ISO group than that in the control group (p=0.054, ANOVA for repeated measures). At 1 hour after

CPB, the change of CI was significantly higher in the ISO group than that in the controls (p=0.001). However, at the other study time points, the changes of CI as compared with the baseline level were not significant different between the two groups. At one hour after CPB, CO was significant higher in the ISO group than that in the control group (p=0.044). There were no statistically significant differences in HR, mean SAP, mean PAP, PAWP, CVP, SVR and PVR between the groups. (Table. 9)

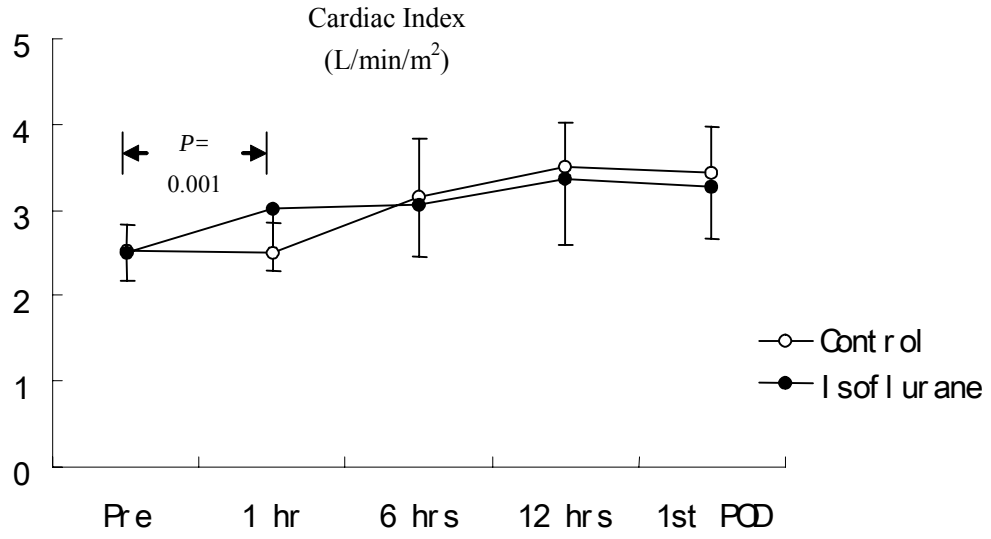

Figure.13 Cardiac Index (CI) prior to the CABG (pre) and at time points after declamping of the aorta. Values are given as mean ±SD. There was a tendency of better CI recovery in the ISO group than that in the control group (p=0.054, ANOVA for repeated measures). At 1 hour after CPB, the change of CI was significantly higher in the ISO group than that in the controls (p=0.001).

Table. 10 Hemodynamic Data

	Baseline	I hour after CPB	6 hours after CPB	12 hours after CPB	First POD
HR (beats/min)					
Control	61.8 ±20.9	68.4 ±10.0	80.2 ±11.8	83.9 ±12.3	78.6 ±10.1
Isoflurane	58.3 ±11.4	73.2 ±12.7	76.4 ±13.0	77.7 ±9.5	75.7 ±8.5
CO (L/min)					
Control	5.61 ±1.2	5.57 ±1.1*	6.45 ±1.2	7.14 ±1.3	6.88 ±1.1
Isoflurane	5.11 ±0.9	6.45 ±1.3*	6.46 ±1.3	7.11 ±1.6	7.38 ±1.3
MAP (mmHg)					
Control	81.8 ±16.7	68.6 ±8.4	71.1 ±11.1	75.6 ±10.3	78.4 ±10.4
Isoflurane	79.6 ±16.3	68.5 ±11.8	70.7 ±11.0	72.7 ±11.3	76.1 ±11.3
MPAP (mmHg)					
Control	19.6 ±4.2	20.0 ±3.9	21.8 ±3.7	21.9 ±4.2	21.8 ±5.6
Isoflurane	18.4 ±3.4	20.9 ±4.2	22.1 ±4.6	21.3 ±5.3	19.2 ±5.8
PCWP (mmHg)					
Control	12.8 ±3.4	12.3 ±3.0	11.8 ±3.3	12.7 ±5.0	13.7 ±5.3
Isoflurane	11.1 ±2.9	12.3 ±3.2	12.2 ±2.7	10.8 ±2.7	12.2 ±4.1
CVP (mmHg)					
Control	9.3 ±2.1	10.4 ±2.6	10.9 ±4.3	10.3 ±4.1	9.4 ±3.4
Isoflurane	9.5 ±1.5	11.6 ±2.6	10.2 ±1.8	8.3 ±2.7	8.1 ±2.3
PVR(dyn.s/cm^5)					
Control	110.3±53.1	115.2±40.1	119.1±41.1	101.0±59.0	91.6 ±51.2
Isoflurane	112.3±44.3	113.8±46.2	125.8±39.1	126.8±64.7	92.6 ±45.5
SVR (dyn.s/cm^5)					
Control	1055.8 ±272.5	876.1 ±268.4	759.4 ±160.5	698.6 ±191.5	768.3 ±156.0
Isoflurane	953.9 ±266.1	757.9 ±272.3	772.5 ±182.7	767.1 ±218.5	828.7 ±229.4

Data are presented as mean ±SD, no statistically significant differences were found between the groups except CO in ISO group at 1 hour after CPB was significant higher than that in Cont group (* p=0.044).

HR = heart rate; CO = cardiac output; MAP = mean arterial pressure; MPAP = mean pulmonary arterial pressure; PCWP = pulmonary capillary wedge pressure; CVP = central venous pressure

PVR = pulmonary vascular resistance; SVR = systemic vascular resistance

2. 2. Biochemical Markers

The baseline levels of CK-MB (6.1±1.26 IU/L and 5.8±1.84 IU/L in the ISO and control groups, respectively p=0.57) and of TnI (0.1±0.0 μg/L in both the ISO and control groups) did not differ. Both TnI and CK-MB reached peak value at 6 hours after CPB. The peak values of CK-MB and TnI were 23.3±6.7 IU/L and 5.4±3.6 μg/L respectively in the ISO group, whereas in the control group they were 26.2±10.1 IU/L and 6.0 ±5.0 μg/L respectively. Patients in the ISO group had slightly less CK-MB release than controls postoperatively, but the difference was not statistically significant (Fig 14, p=0.16 ANOVA for repeated measurements). The release of TnI was similar in two groups (Fig 15, p=0.65 ANOVA for repeated measurements).

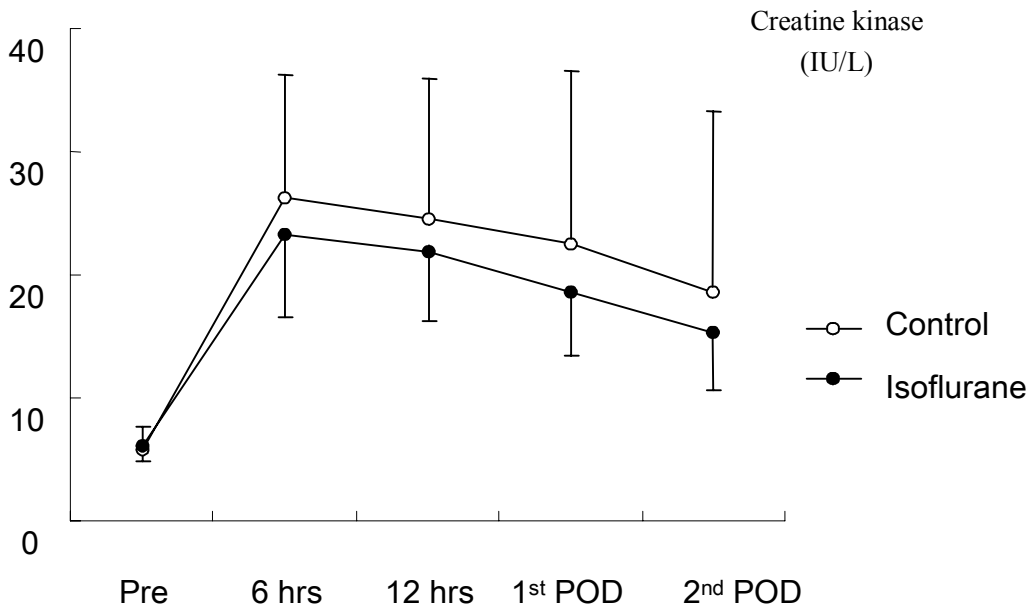

Figure 14. Creatine kinases cardiac isoenzyme (CK-MB) prior to the CABG (pre) and at time points after declamping of the aorta. Values are given as mean ±SD. Patients in the ISO group had slightly less CK-MB release than controls postoperatively, but the difference was not statistically significant (p=0.16 ANOVA for repeated measurements).

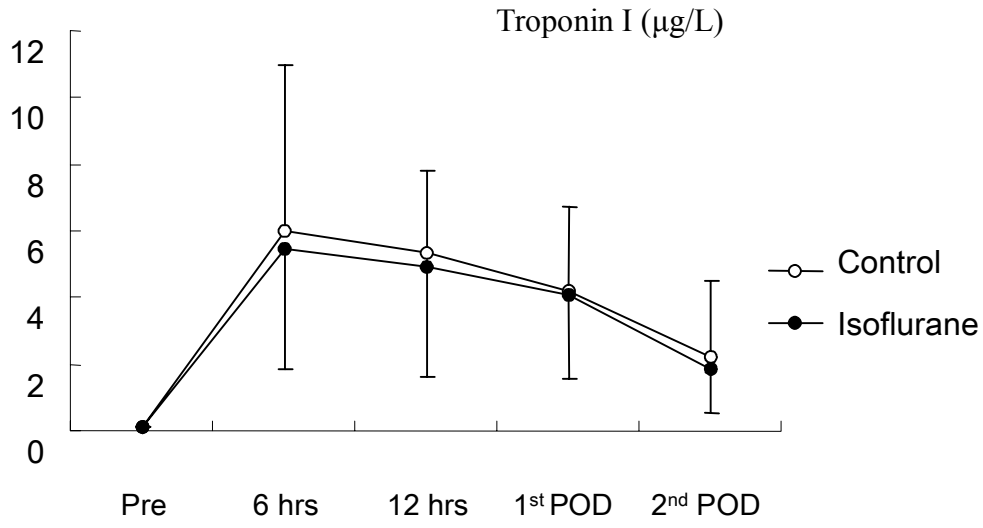

Figure 15. Troponin I (TnI) prior to the CABG (pre) and at time points after declamping of the aorta. Values are given as mean ±SD. The release of TnI was similar in two groups (p=0.65 ANOVA for repeated measurements).

DISCUSSION

1. Methodological Consideration

For logistic and ethical reasons, no clinical study can meet the strict conditions of experimental study on preconditioning. In this respect, the present study was designed with the limitation mainly in three aspects: endpoints, patient selection and protocol design.

1.1. Endpoints

The gold standard of the evaluation of the cellular damage is infarct size. Two mechanisms of cell death, necrosis and apoptosis, are supposed to be tested. The ideal surrogate endpoints to evaluate the apoptosis include (Valen 2003) a). Electron microscopy. b). DNA fragmentation analysis. c) Terminal deoxynucleotidyl transferase-labled dUTP nick end labeling or TUNEL assay. However, some technology may not be available to all laboratories; or the ideal time for biopsy acquisition is impossible. As a result, in the present study we took CK-MB and cardiac TnI as two surrogate endpoints of necrosis. This would lack the sensitivity to apoptosis and underestimate the preconditioning effect.

In the clinical setting, myocardial performance could not tested directly by the maximum rate of LV pressure (dP/dt_{max}) or LVEDP. Some hemodynamic parameters were used as surrogate endpoint. These parameters would more or less be influenced by patients' status of pre- and afterload and many therapeutic interventions thereby biased the results.

The cytokine assays applied have an inherent sensitivity limit. Cytokines are characterized by tight gene control, short duration of action and an autocrine or paracrine rather than an endocrine mode of action. Opposed to hormones, cytokines only affects the immediate environment. Plasma cytokine levels may thus not properly reflect local cytokine production. The present study, based on systemic sampling, may yield a low sensitivity to intracellular cytokine production or cytokine mRNA transcription levels in appropriate target tissues. Other organs may also contribute to the systemic cytokine levels.

1.2. Patients selection

1.2.1 Sulfonylureas

Previous studies have demonstrated that sulfonylureas, the most widely used oral hypoglycemic agents, block K_{ATP} channels and they have possible cardioprotective effects of ischemic preconditioning. In animal models, Yellon and colleagues (Mocanu et al. 2001) were able to demonstrate that glibenclamide blocked the protective effect of preconditioning; this effect was directly attributable to the mitoK_{ATP} channel. Cleveland and colleagues (Cleveland et al. 1997a) have showed that atrial trabeculae obtained from diabetic patients on oral hypoglycemic sulfonylureas, which block K_{ATP} channels, could not be protected by ischemic preconditioning. Furthermore, Tomai and colleagues (Tomai et al. 1994) reported that blockade of K_{ATP} channels with oral glibenclamide before angioplasty abolishes the reduction in ischemic indices observed during subsequent balloon inflations. With regard to this caveat, we excluded the patients who had received sulfonylureas treatment.

1.2.2 Age

There is still a debate of whether preconditioning occurs in the elderly patient. It has been reported that preconditioning of the aged animal may present a problem (Fenton et al. 2000, Schulman et al. 2001). It has also been suggested in several studies in the clinical setting (Kloner et al. 1998a, Jimenez-Navarro et al. 2001, Lee et al. 2002) that it appeared to be impaired preconditioning responses in elderly compared with adult patients. In our clinical setting, it has been also showed that in older patients the preconditioning response was not evident as in younger patients (Wu et al. 2001a). However, we did not selected the limited age as an exclusion criterion in that most of our patients were around 65 to 75 years. Thus, age in the present study would not be ruled out as a factor that blunts the preconditioning like protection.

1.2.3 Unstable Angina

A large number of studies have shown that preinfarction angina could precondition the myocardium (Review of the literature, section 3.4.2.1). Therefore, any patient who suffered from a recent unstable angina or myocardial infarction was excluded.

Taken together, the present series limited the study population to a small number low-risk CABG patient.

1.3. Protocol Design

In the first part of the present study, the protocol was designed to minimize any possible confounding factors by removing isoflurane, so that the control group may not gain extraneous beneficial treatment that could obscure the effect of diazoxide and bradykinin preconditioning in the study group. However, during the whole study pre and intra-operative administration of opioids as anesthetics was inevitable. Also, as triggers of the preconditioning response, δ-opioid agonists have been demonstrated a direct protective effect in both laboratory and clinical settings that appears to be associated with opening of the mitoK_{ATP} channels (Bell et al. 2000, Schultz & Gross 2001). Thus, it may not be possible to completely rule out some protective effect caused by opioids.

2. Dosage and Tolerance

As discussed in the review of the literature, the hypothesis that preconditioning is more likely result of a very steep dose-response curve gives preconditioning an impression of the "all or none" phenomenon. Thus, a minimum duration of ischemia is required to activate endogenous preconditioning response (Yao & Gross 1994), and the duration of ischemia required to elicit preconditioning is known to vary between species (Lawson & Downey 1993).

2.1 Diazoxide

No definitive protocol has been performed to guide the exact diazoxide concentration required for in vivo human preconditioning. Previous studies (Garlid et al. 1997) have shown that in isolated mitochondria diazoxide opens the mitoK_{ATP} channels at low concentrations ($K_{1/2}$ = 0.4 μmol). This is in agreement with the investigations of Garlid and colleagues (Garlid et al. 1996), who have shown that diazoxide decreases cell injury in a dose-dependent manner at concentrations between 1 and 30 μmol/L. Our study suggested that based on a total blood volume of 6-7 L in the human, a circulating diazoxide concentration of approximately 15 μmol/L is adequate to elicit

preconditioning. In the present study, it has been also shown that 1.5 mg/kg diazoxide infusion during 5 minutes prior to CPB didnot elicit severe or prolonged hypotension or hemodynamic instablity (within 10-15% systolic blood pressure decreased). All the DZX group patients had a good tolerance to such a diazoxide concentration.

2.2 Bradykinin

There are no available bradykinin infusion protocols in the cardiac surgery yet. The systemic effect of bradykinin infusion is potent and acute. In the present study, blood pressure decreased dramatically in most of the cases. Our protocol referred to that used in Leesar´s PTCA study (Leesar et al. 1999)., which showed that intracoronary administration of 25 μg bradykinin in 10 minutes appear to be as effective as ischemic preconditioning in patients undergoing PTCA. The BK infusion is well tolerated by patient and bradykinin has no effect on heart rate and blood pressure Our pilot study showed that the maximum bradykinin infusion rate from the superior vena cava is 4 μg /min. Faster infusion rate will lead to profound systemic hypotension (SBP<70mmHg) in most of the CABG patients. In the present study, three patients could not tolerate the infusion even at the starting stage with an infusion rate of 1μg/min only, and had to finish the full protocol with the aid of CPB. It seems to be wise to recommend using the present protocol after the initiation of CPB in patients with unstable hemodynamic status. Further study is warranted to modify the bradykinin protocol to achieve its cardioprotective effect in cardiac surgery. Regional use of the drug might focus the effect on the target organ and therefore avoid the systemic hemodynamic side effect.

2.3 Isoflurane

Previous clinical studies have tested the capability of isoflurane to precondition the myocardium. The results differed as with the different protocols (Table 3. in Review of the literature, section 3.4.2.3). Our protocol that a five-minute ISO (1 MAC) exposure followed by another 5 min washout period showed all patients could tolerate it well, which eighteen patients in the ISO group suffered a mild to moderate transient hypotension with a decrease in systolic blood pressure 16.2±9% compared with pre-infusion value. Furthermore, this protocol could guarantee the patients get beneficial protection from the isoflurane.

Previous studies have indicated that dose-dependent protection was demonstrated in a cellular model of isolated adult rat ventricular myocytes. (Zaugg et al. 2002) Dose-dependent protection by isoflurane was also reported in an in vivo dog model of regional ischemia (Kehl et al. 2002), in which as little as 0.25 MAC isoflurane (0.3 vol/vol %, which is close to 1 MAC_{awake} in humans) significantly reduced infarct size. The maximum preconditioning protection was reached at an isoflurane concentration of approximately 1.5±2 vol/vol % (Kehl et al. 2002, Zaugg et al. 2002). However, the present study was not designed to address this problem, and further investigations are needed to find out an optimal clinical protocol.

3. Cellular Viability

In this series, we took three biological markers, CK-MB, cTnI, and lactate as the surrogate endpoints to evaluate the cellular injury. Our results showed that BK patients released significantly less CK-MB than the controls postoperatively (p = 0.036, ANOVA for repeated measurements); there is a tendency of less CK-MB released in DZX patients than controls postoperatively (p=0.09, ANOVA for repeated measurements); and ISO patients had slightly less CK-MB release than controls postoperatively, but the difference was not significant (p=0.16 ANOVA for repeated measurements). However, as the TnI level, all three study groups showed no superior benefit compared to the controls (p=0.81 BK vs. CONT; p=0.54 DZX vs. CONT; p=0.65 ISO vs. CONT; ANOVA for repeated measurements). Lactate extraction ratio showed no statistical difference between BK group and controls (p=0.84, ANOVA for repeated measures) as well as between DZX group and controls (p=0.81, ANOVA for repeated measures).

A wealth of animal and in vitro human tissue experiments has demonstrated that diazoxide could salvage the myocardium from ischemia-reperfusion injury. Diazoxide is a highly selective $mitoK_{ATP}$ channel opener, which is shown to be 2,000-fold more selective for cardiac $mitoK_{ATP}$ channels as compared with cardiac $sarcK_{ATP}$ channels (Garlid et al. 1997, Sato et al. 1998). It has proved that diazoxide could reduce infarct size or reduce release of CK-MB in rat (Garlid et al. 1997), rabbit (Baines et al. 1999, Sato et al. 2000), dog (Wakiyama et al. 2002) and isolated human myocardial tissue (Ghosh et al. 2000), and this protection could be abolished by using 5-HD, a

selective mitoK$_{ATP}$ channel blocker. More recently, McCully' s group used both Langendorff- and in situ blood– perfused pig models to investigate the role of diazoxide in cardioprotection (Toyoda et al. 2001, Wakiyama et al. 2002). They used 50 μmol/L diazoxide in conjunction with cold blood, magnesium supplemented potassium cardioplegia. This model was used to mimic surgical events in an experimental model that would allow the most relevant comparison. The results indicated that pharmacologic opening of mitoK$_{ATP}$ channels with diazoxide with cardioplegia significantly decreased infarct size ($p < 0.05$ vs DSA) as compared with the effect of cardioplegia alone. Diazoxide is used as antihypertensive drug and has vasodilatory properties; one could attribute this protection to its vasodilator property. However, at a low concentration, it has been shown that the infarct-limiting effects of diazoxide are independent of vasodilatation (Garlid et al. 1996, Garlid et al. 1997, Grover 1997, Toyoda et al. 2001, Wakiyama et al. 2002). Before our study, there had not yet tested the preconditioning-like protection elicited by diazoxide in clinical setting.

Numerous studies in different animal models and in vitro human tissue study have confirmed that exogenously administered bradykinin could mimic the effect of ischemic preconditioning to reduce myocardial infarct size (Goto et al. 1995a, Feng & Rosenkranz 1999, Ebrahim et al. 2001). It has been shown that bradykinin -induced preconditioning is dependent on B2 receptor activation (Goto et al. 1995b) and requires both mitoK$_{ATP}$ channel opening and ROS generation to produce protection (Cohen et al. 2001). The only study that has examined the cardioprotective of bradykinin in vivo in human showed that intracoronary bradykinin infusion appears to be as effective as ischemic preconditioning in PTCA patients (Leesar et al. 1999), in which the only ST segment was used as an end point.

Isoflurane has been demonstrated in animal experiments repeatedly and consistently to decrease myocardial infarction (Kersten et al. 1997b, Kehl et al. 2002). Mechanically, isoflurane induced preconditioning has been proved sharing almost the same steps with ischemic preconditioning (Zaugg et al. 2003). Isoflurane, as a most commonly used anesthetics in CABG, has been studied for its preconditioning in clinical setting. Tomai and colleagues (Tomai et al. 1999b) found that serum levels of TnI and CK-MB at 24 hour after CABG did not differ between isoflurane group

and controls. Then their subgroup analysis showed that only those high-risk patients with preoperative LVEF< 50% exhibited a smaller release of TnI and CK-MB. Belhomme and colleagues (Belhomme et al. 1999) thereafter showed only a tendency of less release of both CK-MB and TnI, but none of the difference in enzyme levels between ISO group and controls reached the threshold of statistical significance. Our results were well consistent with theirs.

In the present study, only bradykinin exhibits the capacity of salvaging the myocardium. The discrepancy between the clinical and experimental results probably is caused by the following reasons. Firstly, in the clinical trial, it is obviously impossible to use infarct size as the endpoint. CK-MB and TnI are supposed to be two sensitive biochemical markers of the myocardial injury, especially the TnI (Birdi et al. 1997). Cardiac troponin I is confined solely to the myocardium, tightly bounding to the contractile apparatus and, therefore, plasma levels are extremely low; and TnI is released within 3 to 5 hours after loss of membrane function (Katus et al. 1991). As we know, necrosis and apoptosis are two mechanisms for cell death. Necrosis is a major form of pathological cell death that rapidly leads to the destruction of myocytes after ischemia. It could also happen in reperfusion. On the contrary, apoptosis is an ATP-dependent precisely programmed and regulated pathway of cell suicide, and pursues a genetically encoded protocol culminating with DNA fragmentation (Zhao & Vinten-Johansen 2002). It happens mainly in reperfusion period. No rupture of plasma membrane occurs and no release of such markers is expected. Therefore, these markers are useful for detection of necrosis related to perioperative myocardial infarction or to subclinical myocyte injury rather than apoptosis. Secondly, in our clinical setting, the myocardium suffered a relatively short time ischemia, and the good distribution of cardioplegia already limited the cellular injury, it was manifested as in most cases that the TnI levels were less than 10 ng/ml, the myocardium would have more suffered from reperfusion injury, which mainly caused myocardial apoptosis and stunning. This would further mask the enzymes-test results. Finally, although there are limitations in the surrogate endpoints, it could still be possible to see the tendency of the less cell damage in the study groups. And this is only a small clinical trial from a selected low-risk population; in order to show the statistical power, we must enroll much more cases or seek a more sensitive and reliable surrogate endpoint.

4. Hemodynamic Function

As discussed in the "Review of the literature", postoperative heart function is determined by the quantity of the myocardial necrosis, apoptosis and stunning. Among the three mechanisms of the cell damage, stunning plays a pivotal role determining the early postoperative hemodynamic performance. Stunned myocardium is characterized by normal blood flow and reversibly diminished contractile efficiency. Myocardium stunning is mostly caused during reperfusion, which is associated with a burst of the O_2-derived free radicals released. Oxyradical and calcium hypotheses, which increased free radical formation could cause cellular Ca^{2+} overload thereby damage the contractile apparatus of the myocytes, are suggested the underlying mechanism of stunning. Previous studies (Gross et al. 1986, Bolli et al. 1989) have demonstrated that much of the stunning effect could be prevented by pretreatment of the animals with SOD and catalase that scavenge O_2-derived free radicals. Much of the stunned myocardium could recover their contractile function within 48 hours (Bolli et al. 1988). The recovery speed may be dependent on the duration of the original ischemic insult, the severity of ischemia during the original insult, and the adequacy of the return of the arterial flow (Kloner et al. 1883). Clinical reports (Kloner et al. 1994a) have demonstrated depressed ventricular function in the initial hours after CABG operations, and found that this dysfunction is usually resolved within 24-48 hours, and that it does not appear to be dependent on alterations in preload and afterload. Our results were well in concordance with these findings. The LVSWI and RVSWI of both the study groups and controls recovered in about 24 hours. One major feature of the stunned myocardium is that it is able to contract when exposed to inotropic stimuli (Kloner & Jennings 2001). In our clinical setting, however, the postoperative control of blood pressure by using inotropes was common, a high percentage of patients received inotropic support. Although there was no significant difference of the inotropic support between groups, this could benefit the stunned myocardium additionally, and mask the possible anti-stunning protection conferred by the studied pharmacological agents.

4.1. Diazoxide

Compared with numerous animal studies that have focused on the infarct-limitation of the preconditioning, few studies were designed to test whether preconditioning really does yield a better heart performance. Garlid and colleagues found that diazoxide preconditioning could

improve the contractile function in Langendorff rat model (Garlid et al. 1997). The similar results also came from other group (Wang et al. 2001b). However, effects of diazoxide elicited preconditioning on postischemic myocardial functional recovery have been shown to vary among species. McCully and colleagues have shown in the isolated rabbit heart as well as in situ blood-perfused pig model that preconditioning the myocardium with diazoxide has no effect on postischemic functional recovery (Toyoda et al. 2001, Wakiyama et al. 2002). So far there is no evidence how diazoxide could protect the postischemic functioning in the in vivo human heart. In the present study, the results have shown that in the control group, CI, LVSWI and RVSWI decreased postoperatively and reached a nadir 1 hour after CPB, CI and RVSWI recovered within 24 hours, and also LVSWI later improved. Compared with the controls, the heart's functional impairment was significantly less in the diazoxide group, as indicated with better CI and faster recovery of RVSWI and LVSWI within 24 hours. In regard to other indicators of the preload and afterload of the ventricles were almost similar between the groups. Therefore the hemodynamic improvement brought by diazoxide was mostly from a better recovery of contractility. Although our study was not a permit to any conclusions of the precise mechanism through which the beneficial effects were seen, the most plausible explanation is that pharmacological preconditioning of the human heart with diazoxide inhibit the myocardial stunning.

4.2. Bradykinin

Previous studies in animal experiments have demonstrated that the administration of bradykinin before regional or global ischemia improved postischemic performance and coronary flow, reduced myocardial enzyme leakage (Brew et al. 1995, Feng & Rosenkranz 1999), attenuated the severity of reperfusion arrhythmias (Linz et al. 1995). These results in animal studies led us to expect the same inspiring results in open-heart surgery. However, our study showed that pretreatment with exogenous bradykinin could not improve the postischemic heart performance. Furthermore, it was reported that angiotension-converting enzyme (ACE) inhibitors could exert cardioprotective effects by inhibiting the breakdown of the bradykinin (Linz & Scholkens 1992). Morris and associates (Morris & Yellon 1997) have found in human atrial trabeculae that the protection afforded by the combination of subthreshold ischemia and ACE inhibitors were abolished by the bradykinin B2 receptor antagonist HOE 140, suggesting that ACE inhibitors

augment ischemic preconditioning by B2 receptor activation. In the present study, some of the patients used ACE inhibitors before the operation. However, statistic analysis showed that an ACE inhibitor used before the operation, taken as a covariate in the analysis, had no effect on the results. The discrepancy between the animal and human study may be due to the species related difference. Further study will be of interest to investigate the effect and the underlying mechanism of the bradykinin preconditioning in human.

4.3 Isoflurane

A number of animal studies (Warltier et al. 1988, Kersten et al. 1997a) have proved that isoflurane could improve the postischemic contractile function and decrease myocardial stunning. There have been a few clinical studies designed to test whether volatile anesthetics could improve postoperative heart function (Belhomme et al. 1999, Tomai et al. 1999b, Haroun-Bizri et al. 2001, Julier et al. 2003, Van Der Linden et al. 2003). The results differed with different anesthetics. Tomai and colleagues (Tomai et al. 1999b) found that isoflurane conferred no improvement on postoperative heart function. While in another group (Haroun-Bizri et al. 2001) it was demonstrated that the CI in the isoflurane group at the weaning of the CPB was significantly higher than that before CPB, whereas no difference was found in the controls. In the present study, the heart function was significantly improved in the ISO group during the first hour as compared with the controls. This result was in concordance with the previous one. However, the change of CI value at 6 and 12 hours after CPB and on the first postoperative day were no longer significantly different as compared to the control group. With regard to the time window, isoflurane may only provide an early preconditioning like protection on the myocardial function. Kehl and colleagues have previously found in an in vivo dog model that isoflurane could not confer a SWOP protection. Their finding further supported our hypothesis.

5. Inflammatory Response

Significant ischemia induces myocardial infarction thereby resulting in an inflammatory response; this response is both accelerated and augmented if the ischemic tissue is reperfused. This IR related inflammation ultimately leads to the myocardial healing and scar formation (Entman & CW. 1994, Frangogiannis et al. 1998). Numerous experimental studies (Frangogiannis et al. 2002) have

demonstrated that various approaches that could inhibit the inflammatory response in myocardial infarcts were also able to reduce ischemia-related injury successfully. Therefore, we postulated that preconditioning, which could effectively mitigate I/R injury, would also suppress the I/R related inflammatory response.

The systemic inflammatory response after CPB is characterized by specific gene expression (Zimmermann et al. 2003) and further release of pro-inflammatory cytokines, such as IL-6 and IL-8, as well as the anti-inflammatory cytokines like IL-10 (Wan et al. 1997b). Our results are well in concordance with these findings. IL-6 is a multifunctional pro-inflammatory cytokine. Recent studies also have shown that IL-6 produces immediate and delayed negative inotropic effects in the heart (Kinugawa et al. 1994), and that IL-6 may be involved in the post-ischemic myocardial stunning after CPB (Finkel et al. 1992). The release of IL-8, a potent chemokine, results in infiltration of neutrophil leucocytes and tissue injury after CPB (Kukielka et al. 1995). IL-6 and IL-8 levels also have been shown to be related with the degree of myocardial injury in cardiac surgery (Kawamura et al. 1993). In the present study, it was showed that plasma levels of IL-6 and IL-8 increased significantly after reperfusion and this lasted during the study period in all groups as in comparison with the baseline values. However, there were no difference between the study groups and the controls.

IL-10 is an important modulator of the inflammation. IL-10 was initially described as an inhibitor of cytokine synthesis, and it inhibits the production of pro-inflammatory cytokines such as IL-1, IL-6, IL-8 and TNF-α (Frangogiannis et al. 2002, Welborn et al. 2003). Recent studies have found that IL-10 could induce powerful inhibition of NFκB activation and thereby inhibit the proinflammatory cytokine production (Lentsch et al. 1997). The mechanism of this inhibition has been linked to the preservation of inhibitory kappa B (IκB). IL-10 has been shown to prevent the DNA binding of NFκB (Schottelius et al. 1999). Utilizing these mechanisms, endogenous production of IL-10 powerfully regulates the inflammatory process, preventing excessive tissue damage through the suppression of acute inflammatory response. Our results well corroborated these findings. In the present study, it has been shown that there was significantly higher IL-10 in DZX group than that in controls, and there was also a tendency of higher IL-10 in BK groups when

compared with the controls. Obviously, diazoxide could augment the postischemic IL-10 production, and thereby suppress the inflammatory response. BK also manifested a tendency of increasing the proliferation of IL-10. This result seems surprising because BK is well known for its proinflammatory actions. However, studies also indicated that BK can exert anti-inflammatory effects and prevent I/R-induced leukocyte rolling, adherence and emigration while maintaining postischemic endothelial barrier function (Shigematsu et al. 2001). And BK acting via B_2 receptors may induce NFκB activation in various cell types (Massoudy et al. 2001).

The innate inflammatory response involves an exquisite autoregulatory mechanism. The proinflammatory response to a biomechanical stress is accompanied by a compensatory anti-inflammatory reaction as indicated by IL-10 production. Changes of the individual cytokine level may blunt the comprehensive reaction of the cytokine network. Therefore, the ratios of pro- to anti- inflammatory cytokines have been used to reflect the response of the intrinsic cytokine network (Taniguchi et al. 1999, Azab et al. 2002), and the ratio was proved to be associated with the outcome (Taniguchi et al. 1999, Loisa et al. 2003). In the present study, it has been shown that both ratios of IL-6 and IL-8 to IL-10 were significantly lower in DZX groups than that in the controls; and in the BK group, the ratios of IL-8 to IL-10 were significantly lower in BK group than that in the controls. Both diazoxide and bradykinin could shift balance of the systemic circulating cytokines to anti-inflammatory direction, although diazoxide showed a more powerful ability.

CONCLUSIONS

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

1. Pharmacological preconditioning with diazoxide prior to commencing CPB in CABG patients with stable angina resulted in significant improvement in hemodynamic performance as well as in tendency of reducing postoperative CK-MB release as compared with the cardioplegia alone.

2. Exogenous bradykinin infusion immediately prior to the initiation of CPB appears to limit myocardial injury after CABG, but the protocol used here failed to show beneficial effect on postoperative hemodynamic parameters.

3. Pharmacological preconditioning with diazoxide prior to commencing CPB in CABG patients can elicit an anti-inflammatory response. It augments the postischemic IL-10 production and shifts the systemic circulating cytokines to anti-inflammatory direction. This was most likely derived from systemic effects.

4. Pharmacological preconditioning with exogenous bradykinin infusion immediately prior to the initiation of CPB may elicit an anti-inflammatory response. It shifts the systemic circulating cytokines to anti-inflammatory direction. Also it manifests a tendency of increasing postischemic IL-10 production.

5. Administration of isoflurane prior to commencing CPB in CABG patients with stable angina may bring a significant early improvement in hemodynamic performance as well as tendency of reducing postoperative CK-MB release as compared with the cardioplegia alone. Isoflurane produces only minor preconditioning in coronary artery bypass grafting.

SUMMARY

Pharmacological preconditioning (PPC) has begun to show its potent ability of myocardial protection. However, studies of PPC effects in cardiac surgery are rare and controversial. The present series of studies were designed to investigate if pharmacological agents, who were mostly tested in the animal experiments, could elicit cardioprotection. The myocardial protective effect and the anti-inflammatory response of the diazoxide, bradykinin and isoflurane were evaluated in on-pump CABG patients.

The finding demonstrated that pharmacological preconditioning with intravenously infused diazoxide (1.5mg/kg), a highly selected mitoK_{ATP} channel opener, prior to commencing CPB in CABG patients with stable angina resulted in significant improvement in hemodynamic performance as well as in tendency of reducing postoperative CK-MB release as compared with the cardioplegia alone. The benefit of this additional protection effect was mostly from the attenuated myocardial stunning and infarction. PPC with diazoxide could also elicit anti-inflammatory response. It augments the postischemic anti-inflammatory cytokine IL-10 production and shifts the systemic circulating cytokines balance (ratio of pro- to anti-inflammatory cytokine, IL-8/IL-10 and IL-6/IL10) to anti-inflammatory direction. This was most likely not due to an effect attributable to the heart.

Exogenous bradykinin (total dose 25 μg) infusion immediately prior to the commencing of CPB appears to limit myocardial injury after CABG. BK patients released significantly less CK-MB than the controls, and the maximum CK-MB was also lower in the BK group. However, the protocol used here failed to show beneficial effect on postoperative hemodynamic parameters. In the BK group, it has been shown that the postoperative IL-10 level was higher than that in the controls, although the difference did not reach the statistical significance. As the results show, the ratios of systemic IL-8/IL-10 in the BK group were significantly lower than that in the control group. This strongly suggests that BK serves as a pharmacological preconditioning stimulus and shifts the cytokine balance towards the anti-inflammatory direction.

The results indicate that administration of isoflurane (1 MAC) prior to commencing CPB in CABG patients with stable angina may bring a significant early improvement in hemodynamic performance 1 hour after CPB as well as tendency of reducing postoperative CK-MB release as compared with the cardioplegia alone. Isoflurane produces only minor preconditioning in coronary artery bypass grafting.

This series of study suggested that beyond the traditional cardioplegia protection, there is still some room left for pharmacological additives, such as bradykinin, diazoxide and isoflurane, to confer extra cardioprotection in coronary artery bypass surgery. The exact underlying mechanism and optimal dose of pharmacological precondition in clinical setting warrants further study.

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