Modification of Ischemia-Reperfusion Induced Changes in Cardiac Performance and TNF-α by Pentoxifylline in Rat Heart

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By

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of

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of

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Dedicated to:

My parents, Zhenxiao and Guiqin,

My husband, Wei

&

My sister's and my uncle's family.

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ABBREVIATIONS

5-HT: Serotonin

AP-1: the activator protein 1

ATP: adenosine triphosphate

cAMP: cyclic adenosin monophosphate

CAT: catalase

CaMK: calcium/calmodulin-dependent protein kinase

CBF: cerebral blood flow

cGMP: cyclic guanosine monophosphate

CR3: complement receptor 3

CT-1: cardiotropin-1

DAG: diacylglycerol

+dP/dt: rate of contraction

-dP/dt: rate of relaxation

ELISA: Enzyme-linked immunosorbent assay

ERK: extracellular signal-regulated protein kinase

gp 130 : glycoprotein 130

GSH: glutathione

GSSG: oxidized glutathione

HSP: heat shock protein

ICAM: intracellular adhesion molecule

IκB: inhibitory κ B

IL: interleukin

iNOS: intracellular nitric oxide synthase

I/R: ischemia/reperfusion

KH: Krebs Henseleit

LPC: lysophospatidylcholine

LPS: lipopolysaccharide

LVEDP: left ventricular end diastolic pressure

LVDP: left ventricular developed pressure

MAPK: mitogen activated protein kinase

NAC: N- acetyl-L-cysteine

NFκB: nuclear factor kappa B

NHE: Na^+-H^+ exchanger

NMR: nuclear magnetic resonance

NO: nitric oxide

PAF: platelet activating factor

PDE: phosphodiesterase

PKA: protein kinase A

PKC: protein kinase C

PLB: phospholamban

PLA: phospholipase A

PLC: phospholipase C

PLD: phospholipase D

PP: phoshatase

PTXF: pentoxifylline

PVDF: polyvinylidene difluoride membrane

RBCs: red blood cells

RyR: ryanodine receptor

SERCA2a: sarcoplasmic reticulum calcium pump ATPase

SDS-PAGE: SDS-polyacrylamide gel electrophoresis

SL: sarcolemma

SOD: superoxide dismutase

SR: sarcoplasmic reticulum

TACE: tumour necrosis factor α converting enzyme

TGF-β: transforming growth factor β

TNF-α: tumour necrosis factor α

TRADD: tumour necrosis factor α-receptor death domain

ABSTRACT

Pentoxifylline (PTXF), a methylxanthine phosphodiesterase inhibitor, is commonly used for the treatment of peripheral vascular disease due to its potent hemorrheologic properties. It also has been clinically prescribed to prevent ischemic heart injury during cardiopulmonary bypass and cardiac transplant surgery. However, the mechanisms of its cardioprotective effect are not fully understood. In this study we examined whether PTXF protected the heart from ischemia-reperfusion injury due to its effect on TNF-α synthesis and NFκB activation in the myocardium. For this purpose, isolated rat hearts were subjected to global ischemia for 30 min, followed by reperfusion for 30 min. Reperfusion of the ischemic heart resulted in impaired cardiac performance as indicated by a 9 fold decrease in LVDP, a 30 fold increase in LVEDP and a 10 fold decrease in ± dP/dt at 30 minutes of reperfusion. These alterations in cardiac function were attenuated by treatment of the heart with 100 μM PTXF. A 2.2 fold increase in TNF-α protein levels and a significant activation of NFκB in the ischemia-reperfused hearts were also prevented by PTXF treatment. These results indicate that the cardioprotective effect of PTXF against ischemia reperfusion injury may be due to depression of TNF-α synthesis and attenuation of NFκB activation.

To determine if the beneficial effects of PTXF are associated with the prevention of intracellular Ca^{2+}-overload, we examined the actions of this agent on Ca^{2+}-paradox in the heart, which is an appropriate model for studying the effects of intracellular Ca^{2+}-overload. Isolated rat hearts were subjected to perfusion with Ca^{2+}-free medium for 5 min followed by perfusion with normal medium containing 1.25 μM Ca^{2+} for 30 min. Ca^{2+}

depleted/repleted hearts showed impaired functional recovery, increased TNF-α synthesis and altered NFκB distribution. These changes in the Ca^{2+}-paradox hearts were significantly attenuated by PTXF treatment. PTXF produced 41% recovery of LVDP, 35% recovery of ± dP/dt, and a significant decrease in LVEDP in the Ca^{2+}-parodox heart. Furthermore, a 2-fold decrease in the TNF-α protein level as well as a 1.5 fold reversal of NFκB distribution to the particulate fraction, were observed in the PTXF-treated Ca^{2+}-paradox hearts.

These results suggest that increased formation of TNF-α and activation of NFκB in the Ca^{2+}-parodox heart may be due to the occurrence of intracellular Ca^{2+}-overload. Furthermore, PTXF may exert cardioprotection in the Ca^{2+}-paradox heart by mechanisms, which are similar to those for the ischemia-reperfusion hearts.

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I. LITERATURE REVIEW

1. Ischemia-Reperfusion Injury in the Heart

Ischemia due to little or no blood flow results in a decrease in the supply of oxygen and nutrients to the heart, and a build up of metabolic wastes locally (1). In fact, myocardial ischemia has been reported to produce a decrease in the rate of cellular oxidation and energy production (2). Myocardial ischemia has been characterized by rapid accumulation of protons, cessation of oxidative metabolism, cessation of electron transport, and the initiation of the inefficient processes of anaerobic metabolism. On the other hand, reperfusion injury is a major complication induced by restoration of flow to a previously ischemic heart (3). Ischemia-reperfusion (I/R) injury is one of the most common cardiovascular problems. This injury damages vascular cells and cardiomyocytes (4). Many factors have been shown to cause ischemic heart disease; major ones include arteriosclerosis of the coronary arteries and coronary artery spasm (5).

In spite of the variable incidence of ischemic heart disease, it has become one of the most significant lethal medical problems and a major economic health-care concern. It has been reported that almost 45% of all deaths in Northern European countries during the past decade were due to cardiovascular disease (6). In addition, 200,000 Americans under 65 die each year from ischemic heart disease and 25 times as many suffer from symptoms related to the disease (7). A similar situation exists in Canada, where cardiovascular disease causes more deaths than any other disease and

more than 58% of these deaths were attributed to ischemic lesions in 1990 (7). In economic terms, the direct and indirect costs of heart attack and stroke per year were about $259 billion in America alone (8). In Canada, cardiovascular disease contributed to 21% of total disease expenditures in 1986, and has become the most expensive disease with direct costs of $5.2 billion and indirect costs of $11.6 billion (5). Consequently, it is crucial to find the pathogenic mechanisms of I/R injury and discover ways to block events associated with irreversible ischemic injury. Many studies have been designed to determine the possibility of amplifying the beneficial effects of reperfusion and diminishing the harmful effects of I/R through pharmacological intervention (9). The following sections review the pathogenic alterations and pharmacological interventions in I/R-induced injury in the heart.

2. Role of Ca^{2+}-Overload in I/R Injury in the Heart

a. Ca^{2+} and heart function: It is well accepted that the Ca^{2+} ion is a major regulator of cardiac excitation-contraction coupling. Some of the functions of Ca^{2+} in cardiac myocytes, which have been identified, include: mediation of systolic contraction and diastolic relaxation, modulation of enzymatic activity, and participation in mitochondrial function. Additionally, Ca^{2+} is an important ion for maintaining cellular integrity, cell proliferation, cell growth and regulation of metabolism. The L-type Ca^{2+} channel is considered the most significant Ca^{2+} channel in the human heart. The small amount of Ca^{2+} entering the cytosol through this channel triggers the release of additional Ca^{2+} from the sarcoplasmic reticulum (SR)

(10). Normally, Ca^{2+} is bound to a negatively charged protein called calsequestrin that is located in the SR. When the release of Ca^{2+} from SR is triggered by depolarization, Ca^{2+} is discharged through the Ca^{2+}-release channel [ryanodine (RyR) receptor]. During the diastolic period, most of the Ca^{2+} is restored by the SR Ca^{2+}-pump ATPase (SERCA2a). A small amount of Ca^{2+} is extruded by the sarcolemmal (SL) Na^{+}/Ca^{2+}-exchanger and SL Ca^{2+}-pump ATPase. Moreover, some regulatory proteins such as Ca^{2+}/calmodulin-dependent protein kinase (CaMK) and cAMP dependent protein kinase A (PKA) are involved in the regulation of Ca^{2+} movements. These proteins mediate cellular activity in response to different stimuli, which ultimately lead to the alteration of Ca^{2+} homeostasis in cardiomyocytes via the phosphorylation and dephosphorylation cycle. It has also been established that CaMK phosphorylates SERCA2a, ryanodine receptor (RyR) and phospholamban (PLB), while PKA phosphorylates PLB and RyR (11-13). Since the role of Ca^{2+} is of paramount significance, the regulatory mechanisms for Ca^{2+} movement may serve as useful sites for improving the therapy of heart disease.

b. Intracellular Ca^{2+}-overload: Reperfusion-induced intracellular Ca^{2+}-overload has been thoroughly documented for the past 30 years (14). Several factors that attribute to this phenomenon of intracellular Ca^{2+} increase include the lack of ATP involved in the Ca^{2+} handling mechanisms (15), an increase in the intracellular concentration of Na^{+} (16-18), an increase in membrane permeability due to the accumulation of long chain fatty acids (19,20), enhanced adrenergic stimulation (21), and degradation of SERCA2a and RyR (22,23). Recent studies have demonstrated

that intracellular Ca^{2+}-overload was a major cause of myocardial cell damage and cardiac dysfunction in ischemic heart disease. The Ca^{2+}-overload evoked by post-ischemic reperfusion is associated with irreversible injury such as ultrastructure damage, enzyme leakage, membrane damage, reduced capacity of the mitochondria to regenerate ATP, and increased infarct size (14). The role of Ca^{2+} in cardiac dysfunction may be further extended to ischemia-induced arrhythmias, especially ventricular tachycardia and ventricular fibrillation, which are known as the primary cause of sudden cardiac death. Since studying Ca^{2+}-overload is so important in the development of ischemic heart injury, a Ca^{2+}-paradox rat heart involving Ca^{2+}-depletion and Ca^{2+}-repletion has been established as an experimental model to understand the correlation between the disturbance of Ca^{2+} homeostasis and other variations that are involved in the pathogenesis of I/R heart injury.

3. Role of Cytokines in I/R Injury in the Heart

a. Overview of cytokines function in the heart: Although there are many mechanisms involved in I/R injury, cytokines have been considered to play an important role in its pathogenesis. Cytokines are produced by different types of cells, and these molecules modify the biological responses of the same or other cells. Cytokines include transforming growth factor (TGF)-β, tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-12 to name a few (24-26). Present evidence has suggested that the basic functions of cytokines include: (i) activation or attraction of specific types of leukocytes, (ii) regulation of cell division,

(iii) modulation of the production and activity of other cytokines, (iv) promotion of inflammation, (v) induction certain cells to produce a multitude of proteins, and (vi) influence on the production of cellular or humoral immunity (26). Since cytokines have played a key role in physiological and pathophysiological conditions, a great deal of work has been carried out to investigate the role of cytokines in various diseases. It has been demonstrated that cytokines are an important mediator of cardiovascular diseases. For example, myocardial I/R event prompts the release of cytokines and other inflammatory mediators to cause coronary vascular injury. The specific target of such mediators has appeared to be the endothelium as well as the neutrophils. Inflammatory cytokines act on neutrophils to adhere to the vascular endothelium. This activity induces the obstruction of capillary beds to cause a no-reflow phenomenon during reperfusion. Moreover, accumulation of these inflammatory cytokines within ischemic tissue directly damages the tissue and leads to the release of oxygen free radicals that results in further injury to the endothelium (27). TNF-α, TGF-β_1, IL-1, IL-6 and IL-8 have been implicated in cardiovascular diseases, especially in ischemic heart disease (28,29).

b. Role of TNF-α in I/R injury: Among so many cytokines, TNF-α is considered to be the most important mediator in cardiovascular disease. Although TNF-α is produced mainly by lymphocytes and macrocytes, other cells such as cardiomyocytes and vascular smooth muscle cells have also been shown to produce TNF-α. This cytokine is synthesized as a 26-kDa propeptide (pro-TNF-α) in the cytosol, and then pro-TNF-α is cleaved to its 17-kDa active form by TNF-α-

converting enzyme (TACE); this cleavage occurs as the propeptide passes through the cell membrane. The activated form of TNF-α binds to the receptors on the surface of cell membrane and triggers alteration of cytosolic protein synthesis and activation of different kinases (30,31). Similar to the pathways for other protein synthesis, there are two levels involved in the regulation of TNF-α gene expression: transcription and translocation. At the transcriptional level, there are many transcription factors implicated including activator protein-1 (AP-1), AP-2, Egr-1 and nuclear factor kappa B (NFκB). In fact, NFκB is believed to be essential for the transcription of TNF-α. It has been shown that inhibition of NFκB signalling blocks 80% of TNF-α production induced by lipopolysaccharide (LPS). At the translocation level, the TACE plays an important role in converting pro-TNF-α to TNF-α and thus inhibition of the TACE activity is effective in controlling the synthesis of TNF-α. Although LPS has been viewed as the important trigger for the production of TNF-α, several studies have demonstrated that TNF-α is formed and released in myocardium in rat and human after I/R injury (31-35). The pharmacological inhibition of TNF-α synthesis significantly improves the recovery of myocardial dysfunction induced by I/R (24,36-42). Although LPS-induced TNF-α expression has been studied extensively, the mechanisms of I/R-induced TNF-α synthesis in the myocardium are not fully understood, and myocardial alterations due to TNF-α remain unclear.

Most studies have demonstrated that TNF-α directly decreases contractile function in hamsters, dogs and humans (24,43). The acute negative inotropic effect of TNF-α appears to be due to changes in Ca^{2+} homeostasis. TNF-α is considered to

disrupt the excitation-contraction coupling and desensitize the β-adrenoceptor mechanisms (40). It has been shown that the initial contractile depression induced by TNF-α is mediated by sphingosine, an endogenous second messenger (44). In addition, TNF-α induces the production of nitric oxide (NO) and thereby depresses the myofilament sensitivity to Ca^{2+} that in turn mediates the late contractile dysfunction (45). Another mechanism of cardiac depression provoked by TNF-α is the induction of apoptosis in cardiomyocytes. It has been reported that the apoptosis was mainly induced via a TNF-α receptor-1 that includes a death domain (TRADD). In turn, the receptor-interacting protein communicates a signal between the TRADD and a kinase domain that induces DNA fragmentation and eventual cell apoptosis. Moreover, this process also appeared to be mediated by sphingosine and NO (37,46,47). Additionally, TNF-α was indicated as an initiator of a cytokine cascade, which elucidated the subsequent production of IL-6, IL-1, and IL-8, to ultimately worsen the deleterious alteration induced by I/R (32,34,48,49). The studies mentioned above have indicated that anti-TNF-α therapy may be valuable in I/R injury.

A body of evidence has accumulated to provide essential intervention by blocking the TNF-α synthesis in the ischemic heart. P38 MAPK and NFκB inhibitors appeared to depress the synthesis of this pro-inflammatory factor (50). Adenosine, as an effective agent for protecting the heart from I/R injury, was also shown to reduce the cardiac TNF-α production in humans (24,40,41). Moreover, Meakawa et al (51) have reported reduced infarct size, decreased occurrence of arrhythmia, and improved cardiac function in TNF-α knockout mice compared with wild type mice upon

subjecting to I/R injury. Furthermore, the TNF-α antibody and the soluble TNF-α receptor were used in the study of the deleterious effects of TNF-α in I/R injury, in rats (42). Other interventions have also been employed in clinical and experimental trials, including the glycoprotein 130 (gp130), an inhibitor of TACE (50).

In contrast to the reports showing adverse effects of TNF-α, different investigators have observed that TNF-α may have protective effects during I/R (51-56). Lecours et al (56) have demonstrated that TNF-α evoked preconditioning, which is an effective intervention for the protection of I/R injury. Nelson et al (55) have indicated that pre-treatment with TNF-α 24 hr before I/R, resulted in improved cardiac contractile functional in rabbit. Several studies have also suggested that TNF-α knockout mice may display larger infarct sizes than normal mice, after undergoing coronary ligation (52-54). These conflicting results may be due to varying time periods for which the heart is exposed to different concentrations of TNF-α. Although it has been indicated that the conflicting effects of TNF-α are probably dependent on the absolute levels of TNF-α during I/R period (30), the effects of TNF-α and the mechanism of these effects still remain a matter of debate. Hence, further research is needed to elucidate the role of TNF-α in the pathogenesis of numerous heart diseases.

c. Role of NFκB in I/R injury: NFκB is a transcription factor that plays a key role in the production of most cytokines. It has been shown that NFκB exists in an inactive state in the cytoplasm of unstimulated cells because of binding to the inhibitory protein IκB. Numerous subunits have been discovered in mammals such as

50-kDa and 65-kDa (RelA) c-Rel, 52-kDa and RelB. Multiple subfamilies of IκB that also exist include IκBα, β, and γ. Most of the research work is focused on the p50/p65 dimer that is associated with IκBα. This complex is phosphorylated in many cells under numerous conditions. It has been reported that multiple regulatory steps are involved in the activity of NFκB, and four phases have been defined: nuclear translocation, phosphorylation of Rel family protein, interaction with the basal transcription complex, and redox regulation (57,58). Blocking of any phase is likely to prevent the activation of NFκB resulting in a subsequent increase in NFκB regulated gene expression in a broad range of physiological and pathophysiological processes. Recent evidence has indicated that NFκB is activated and is followed by pathogenesic effects in the ischemic myocardium upon the initiation of reperfusion (59-61). The activation of NFκB has been proven to stimulate the production of cytokines such as TNF-α, IL-1β, IL-6 and IL-8 (28,62,63). Furthermore, intracellular adhesion molecule-1 (ICAM-1) protein expression and inducible NO synthesis are also increased due to the effect of NFκB activation following the I/R injury in canine heart (63). Since locally produced cytokines in myocardium are activated by NFκB, these changes form a positive feedback loop that further augments the local pathogenesic response. Another important deleterious effect of NFκB included the promotion of cell death, namely apoptosis, which could have possibly induced irreversible myocardial damage or amplified the infarct size in myocardial infarction (64). Hence, better understanding about the relation between the activation of NFκB and I/R injury will provide essential information for the pathogenesis of ischemic

heart disease.

Oxidative stress is considered to be a major trigger for the activation of NFκB following I/R injury (57). It has been reported that numerous antioxidants like N-acetyl-L-cysteine (NAC), α-lipoic acid and vitamin E inhibit the activation of NFκB in different types of cells (65-67). Cargnoni et al (65) have demonstrated that NAC failed to activate NFκB by preventing a decrease in intracellular thio levels such as glutathione (GSH) and oxidized glutathion (GSSG). Additionally, the 20S proteasome inhibitor, PS-519, was found to depress the activation of NFκB due to I/R injury in myocardium (68). Pentoxifylline and nonanticoagulant heparin were also reported to inhibit NFκB activation in cultured vascular smooth muscle cells (69-71). Although several studies have identified the negative effect of NFκB in cardiovascular disease, some studies have reported the protective effect of NFκB in I/R injury. It has been suggested that NFκB may be a key mediator of the beneficial effect of preconditioning against I/R injury. For instance, Morgan et al (72,73) have reported that ischemic preconditioning protected the heart and this required the activation of NFκB. It also has been suggested that NFκB stimulates the production of cytoprotective genes like that for the heat shock proteins (HSP) (74) and NO (75). These cytoprotective genes may inhibit NFκB activation induced by the overwhelming oxidative stress during I/R, and in turn can lead to the inhibitory effect on the production of inflammatory genes (76). Furthermore, Bach et al (77) also suggested that NFκB mediates numerous gene expressions of proteins that inhibit cell death, in particular, bcl family, zinc finger protein, endogenous antioxidants

manganese superoxide dismutase and hemeoxygenase-1. Thus it appears that the role of NFκB in I/R injury remains controversial, and the pathway mediating all these effects of NFκB activation still remains unknown.

4. Role of TGF-β and Interleukins in I/R Injury in the Heart

a. TGF-β: Transforming growth factor (TGF) is a family of peptides that exists in many mammalian tissues. Recently TGF-β has received considerable attention for its multiple functions in controlling cell growth and responding to extracellular environmental changes (78). The most common form of TGF is TGF-β_1, which modulates various biological actions. Normally, TGF-β_1 binds to the TGF-β_1 receptor and initiates numerous signalling pathways, including the smad family, protein kinase C (PKC) and MAP kinases (79). Thus, different studies regarding TGF-β_1 have provided essential information for the therapeutic advancements in various diseases. It should be noted that TGF-β_1 has been identified as a powerful cardioprotective agent (79-81). It has been reported that TGF-β_1 administration during coronary blockade reduced the infarct size due to myocardial infarction in the cat heart. The protective effect was assumed to be due to the inhibition of endothelial/neutrophil interactions, as well as anti-inflammatory actions by reducing the production of TNF-α (79,82). Moreover, Baxter et al (83) also observed a significant limitation of infarct size when TGF-β was persent during the early reperfusion in rat. In this study, it was reported that the protective effect of TGF-β_1 is

associated with its anti-apoptosic actions. Furthermore, it was noted that p42/p44 MAP kinases signalling pathway may be involved in this anti-apoptosic action, which was detected upon expanding the infarct size with p42/p44 MAP kinase inhibitor PD98059 treatment, and the increase of p42/p44 MAP kinase phosphorylation with TGF-β_1 in a rat model of myocardial infarction (83). It was also reported that bcl-2 may be involved in this effect of TGF-β_1 during I/R injury in rat cardiac allografts (84,85). Additionally, it has been demonstrated that the modulation of TGF-β_1 in I/R injury was associated with the activation of PKC and inducible nitric oxide synthase (iNOS) (86). Moreover, the expression of matrix metalloproteinases was inhibited by TGF-β_1, resulting in significant improvement of cardiac function on I/R disease (87). Essentially, TGF-β_1 has cardioprotective effect caused by the inhibition of TNF-α release, resulting in the improvement of endothelium-dependent relaxation and prevention of apoptosis.

b. IL-1: IL-1 is as an important mediator of the inflammatory reaction. There are two forms of IL-1, namely IL-1α and IL-1β. Since IL-1β is easily detected in the blood flow, it has been the main focus in the experimental research field. Several studies have shown the negative inotropic effect of IL-1 in both intact heart and isolated cardiac cells (88-90). IL-1 was also discovered to induce the expression of iNOS at both mRNA and protein levels, and is thus involved in the p38 and p42/p44 MAP kinase-signalling pathways (25). It has been observed that IL-1β and TNF-α have similar pathways causing negative inotropic effects in myocardium (91). Moreover, the decrease of Ca^{2+} regulatory genes has been involved in the deleterious

effect of IL-1, which may be implicated in arrhythmogenesis after I/R injury (92-96). Furthermore, it also has been noted that IL-1, like other cytokines, has an effect, which leads to apoptosis in neonatal cardiac myocytes (97). Although most of the data have suggested a primary negative role of IL-1 in the heart, there are some investigations that have indicated a cardioprotective effect of IL-1 as well. A number of studies have reported the reduction in myocardial injury after I/R with IL-1 pre-treatment (95,98,99).

c. IL-6: Interleukine-6 (IL-6) was originally identified as a T-cell derived cytokine but now it has been documented as a multiple functional cytokine produced by different cells types. The IL-6 family is comprised of interleukine-11 (IL-11), leukemia inhibitory factor, onconstatin M and cardiotropin-1 (CT-1). All cytokines in this family regulate the signal via binding to receptors with gp130 subunit (91,100). IL-6 is reported to be involved in a variety of diseases, as elevated IL-6 levels have been detected in patients with myocardial infarction, especially during the period of reperfusion (101). Other experimental studies have also reported an elevation of IL-6 mRNA and protein contents of IL-6 in canine myocardium subjected to I/R injury (102-104). Hence, it can be seen that IL-6 may play a role in the pathogenesis of ischemic heart disease. An increasing number of investigations have demonstrated that IL-6 serves as a direct cardiodepressant as it has been reported to inhibit contractility in the hamster myocardium (43). It also has been indicated that IL-6 reduces the peak systolic Ca^{2+} transient and contractility via increasing the production of NO and subsequent cGMP-mediated decrease in L-type Ca^{2+} channel current.

Furthermore, it has been suggested that IL-6 induces iNOS in cardiomyocytes, which subsequently causes a sustained depression of myocardial contractility (43,105,106). The induction of IL-6 also has involved in the expression of ICAM-1, which leads to inflammatory injury in the dog ischemic heart (103,107). Conversely, CT-1, a member of the IL-6 family, has been shown to have different effects on cardiac cells. Stephanou et al (108) suggested that pre-treatment with CT-1 was apparently protecting cultured cardiomyocytes against stimulated ischemia/hypoxia, which may have been mediated via enhancing the protein level of HSP70 and HSP90. Furthermore, Latchman et al (109) also demonstrated that CT-1 protected the cultured cardiac cells and/or isolated rat heart from I/R injury via regulating the p^{42}/p^{44} MAPK pathway to inhibit apoptosis in heart. All in all, the involvement of IL-6 in I/R damage has been clearly supported.

d. IL-8: Like other cytokines, IL-8 is also expressed at both mRNA and protein levels in the myocardium subjected to I/R injury. Subsequently, different reports have suggested that IL-8 is important in the development of myocardial injury in human myocardial infarction (110,111). Different cells such as neutrophils, monocytes, lymphocytes-T and endothelial cells can produce IL-8 (112). Presently, no information is available to suggest that cardiomyocytes produce IL-8. IL-8 has been considered as a potent participant of granule enzymatic release and oxidative burst in neutrophils, which in turn lead to further damage in the ischemic heart. Boyle et al (113) have shown that neutralities of IL-8 protected the heart from I/R injury in rabbit; however, the role of IL-8 in the interaction of neutrophil and

endothelial cells has been controversial. Some results have shown inhibitory effects (114-116), as others have indicated the stimulatory effects on the migration of neutrophils via the swollen endothelium (112,117-119). Although the effect of IL-8 on neutrophils is conflicting, it has been reported that IL-8 is not involved in the regulation of myocardial contractility after I/R (91). Hence, IL-8 may be a minor factor for mediating the inflammatory effect in hearts subjected to I/R.

5. Cardioprotection Against I/R Injury

Since the pathophysiology of I/R injury is becoming clear gradually, therapeutic strategies for treatment of I/R injury have provided new insights in the field of cardioprotection. The following sections will focus on the effects of different interventions on I/R injury in the heart; these include preconditioning, antioxidants, Ca^{2+} channel blockers, phospholipase A_2 inhibitors, Na^+-H^+ exchange inhibitors, P38 MAP kinase inhibitors, phosphatase inhibitors, and 5-HT receptor antagonists.

a. Preconditioning: Myocardial ischemic preconditioning is a phenomenon which is produced by brief episodes of cardiac ischemia and reperfusion leading to a decrease in the rate of progression of the ischemia-induced myocardial injury and development of resistance to subsequent ischemic attacks (120-122). The potential therapeutic benefits of this adaptive mechanism have generated much attention that has revolutionized our understanding of the signal transduction and subsequent intracellular events that mediate the I/R injury (123). A variety of intracellular signalling pathways have been implicated in the protective mechanisms of ischemic

preconditioning including the activation of G protein-linked phospholipase C-coupled receptors, adenosine receptor, bradykinin receptor, opioid receptor, tyrosine kinase pathways and PKC (122,124-126). Several mechanisms explain the role of preconditioning in I/R injury. Ischemic preconditioning appears to render protection against the I/R-induced damage to the myocardium by improving SR function. This is attributed to decreased ryanodine-sensitive SR Ca^{2+}-release and the regulation of SR phosphorylation by endogenous CaMK (127,128). Some studies have reported that ischemic preconditioning triggers phospholipase D (PLD) signalling in the ischemic myocardium, which produces beneficial effects on the heart because of the production of phosphatidic acid and diacylglycerol and subsequent activation of PKC (129).

In addition, the ATP-sensitive potassium channel is a strong candidate for mediation of the preconditioning protection. Pharmacological and electrophysiological evidence favourably implicate the involvement of mitoK_{ATP} rather than surfaceK_{ATP} as the relevant mediator of preconditioning (124). Opening of mitoK_{ATP} has been shown to block apoptosis in cardiac myocytes via PKC-ε activation (130). Preconditioning protects against cell necrosis and possibly also against stunning (131). The mechanism of such protection is still unclear, yet it has been demonstrated that the ATP content of the myocardium is reduced following ischemic preconditioning. However, during prolonged coronary occlusion, the rate of decline in ATP is initially slower in preconditioned myocardium as compared to a non-preconditioned one (121). Thus an alteration in the energy supply-demand relationship may be involved (132). Preconditioning may further protect against

necrosis through its action on TNF-α. It has been shown to reduce myocardial TNF-α production and TNF-α induced myocardial injury (50).

Preconditioning may also induce protection against other aspects of I/R injury such as coronary endothelial damage or arrhythmias (123,133). It has been reported that the incidence of ventricular fibrillation decreased from 90% in the control hearts to 20% in the preconditioned heart. It has also been reported that ischemic preconditioning exerted its protective effect primarily by maintaining the function of the forward mode of the Na^{+}-Ca^{2+}-exchanger and limiting the development of intracellular acidosis. This reduces the occurrence of intracellular Ca^{2+}-overload, thus protecting the heart against arrhythmias (134,135). It has also been suggested that the antiarrhythmic benefit of ischemic preconditioning is mediated through the activation of endothelium bradykinin receptor-1 (126). Defily et al. (133) also reported that ischemic preconditioning significantly preserved endothelium-dependent dilation. Therefore, the preservation of endothelial function may be one of the mechanisms by which preconditioning reduced the amount of tissue necrosis during reperfusion. In conclusion, the mechanisms modulating ischemic preconditioning include alterations in antioxidant defence (136), stimulation of adenosine A_1 receptors, activation of PKC, activation of phospholipase D, induction of HSP, reduction in TNF-α production, limiting the development of intracellular acidosis and prevention of Ca^{2+}-overload (50,128,129). The above evidence therefore strongly favours preconditioning as an effective intervention for the treatment of I/R injury.

b. Antioxidants: Reactive oxygen species, including the superoxide anion,

hydrogen peroxide, and the hydroxyl radical, are derivatives of many biologic systems and, in high concentrations are associated with oxidative stress and subsequent cardiovascular tissue injury (121); the superoxide anion is a key entity in the production of the hydroxyl radical. It has been demonstrated that superoxide radicals and hydrogen peroxide exert their deleterious effect on cells through the subsequent generation of the highly reactive hydroxyl radicals and are therefore not toxic directly (9). One major site of oxygen free radical production and cell injury is endothelial cells. In culture, endothelial cells have been shown to possess a system which is capable of generating oxygen radicals (137). Normally oxygen-derived free radicals interact with cellular constituents, including lipids, proteins and nucleic acids. In turn, they can disrupt membrane integrity, ion channels and enzymatic function. Such adverse effects of toxic oxygen metabolites have been associated with dysfunction of sarcoplasmic reticulum, mitochondria, and creatine kinase reperfusion of the ischemic heart (125).

The view that reactive oxygen species are implicated in I/R inury is further substantiated when considering the effects of antioxidants on hearts subjected to I/R. The antioxidant capacity of the cell can be divided into two categories. The first line of cellular defence against oxidative injury are the free radical scavenging enzymes including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). The second line antioxidant is a non-enzymatic scavenger such as alpha-tocopherol (vitamin E), beta-carotene, vitamin A, ascorbate and sulfhydryl-containing compounds (137). Since the endogenous antioxidants are depleted by ischemia, it

predisposes the myocardium to oxidant injury (125). In fact, a direct correlation between the myocardial dysfunction induced by I/R and the magnitude of free radical generation by exogenously administered oxidants has been previously demonstrated; this indicates the major role of antioxidant treatment for I/R injury. It has been previously shown that SOD plus CAT treatment prevents changes in sarcoplasmic reticulum protein phosphorylation and phosphocreatine in the I/R heart (127). Other agents shown to have beneficial effects which act as antioxidants are: N-2 mercaptopropionyl glycine and N-acetylcysteine, melatonin (138), allopurinol, oxypurinol and desferrioxamine (125,138-140). New antioxidant interventions are currently being suggested. Recently certain amino acids, such as taurine, were used for the purpose of maintaining membrane stabilization. In vitro and in vivo studies indicate that taurine has the ability to scavenge HOCl and thereby prevent I/R-induced membrane damage by attenuating lipid peroxidation (141). Although anti-free radical interventions may reduce the severity of reperfusion injury, some negative reports (142,143) indicate that I/R injury is a complex phenomenon and further research is needed to better elucidate this dynamic process.

c. Ca^{2+} channel blockers: Since a great deal of research demonstrates that intracellular Ca^{2+}-overload is a major cause of myocardial cell damage and cardiac dysfunction in ischemic heart disease, the L-type Ca^{2+} channels are considered as one of primary channels of Ca^{2+}-influx for the occurrence of intracellular Ca^{2+}-overload. In this regard, it is pointed out that the L-type Ca^{2+} channel blockers were used in the treatment of ischemic heart disease more than three decades ago. These Ca^{2+}

antagonists function by reducing the contractility of the myocardium, decreasing the contraction of smooth muscle in the vasculature and altering the conducting system of the heart (144). Generally Ca^{2+} channel blockers are classified as dihydropyridines and non-dihydropyridines. The dihydropyridines act primarily by relaxation of the vascular smooth muscle with less effect on cardiac contractility and conduction; nifedipine is the most commonly used representative of dihydropyridines. Non-dihydropyridines such as verapamil and diltiazem act primarily on myocardial and cardiac conducting tissues with less effect on the vascular smooth muscle. At present nifedipine, diltiazem and verapamil are the three most common clinically used calcium channel blockers (145). Other Ca^{2+} channel blockers are also applied in experimental research such as felodipine, S-2150, lacidipine, anipamil and benidipine (146-150). Several mechanisms which describe the protective effect of Ca^{2+} antagonists on the myocardium with I/R injury include: coronary vasodilation (150-152), an energy-sparing effect which results in a lower rate of adenosine triphosphate depletion and slowed loss of adenosine precursors (146,147,153), decreased release of degradative lysosomal proteases (154,155), protection of the sarcolemmal integrity (156,157), attenuation of the I/R-induced mobilization of noradrenaline (158), lower endothelial permeability (159), protection of mitochondrial function (160), attenuation of ischemia-induced acidosis (161,162), retardation of the early rise in cytosolic Ca^{2+} (163), protection of lipid-containing membranes against lipid peroxidation caused by free radicals (148) and antiarrhythmic effect believed to be related to their inhibitory action on the phosphatidylethanolamine N-methylation

activity (164). It appears that Ca^{2+} channel blockers are an effective treatment of I/R injury at different experimental levels: cellular pharmacology, molecular biology, in vitro and in vivo animal pharmacology, clinical pharmacology and clinical efficacy studies (165).

d. Phospholipase A_2 inhibitors: The membranes of living cells consist of phospholipids, cholesterol and proteins. The integrity of the membrane is important for proper cell function. Phospholipids, the major constituents of the cellular membrane, provide the principal structural framework of the membrane and undergo a continued turnover process. The cell is able to synthesize required phospholipids and regulate the fatty acyl composition of the phospholipids. An integral enzyme involved in the hydrolysis of phospholipid is phospholipase A (PLA), with two isozymes, phospholipase A_1 (PLA_1) and phospholipase A_2 (PLA_2) (10). Of at least three different types of PLA_2 in the human heart, the group II PLA_2 has been cloned and been well studied (166,167). The regulation of the group II PLA_2 activity occurs through multiple entities, such as cytokines (TNF-α, IL-1, IL-6) and Ca^{2+} concentration. Phospholipid homeostasis is disturbed during myocardial ischemia. Several studies indicate that the degradation of membrane phospholipid is associated with enhanced phospholipase A_2 activity stimulated by Ca^{2+}-overload and increase in cytokines (10). This activation leads to increased phospholipid catabolism and subsequently the liberation of lysophosphatidylcholine (LPC). LPC has been reported to induce major changes in membrane function. It has been shown to increase the intracellular Na^+ concentration by inhibiting myocardial Na^+-K^+ ATPase and

increasing the burst of the Na^+ influx, in turn producing Ca^{2+}-overload via the Na^+/Ca^{2+}-exchanger. It has also been reported that LPC may directly increase sarcolemmal permeability to Ca^{2+} and increase non-selective cation currents including Na^+, and K^+. All of these effects demonstrate that LPC is an arrhythmogenic substance. In addition, LPC accumulation in cardiac myocytes augments the activity of phospholipase A_2 via a positive feedback mechanism (168-171).

In view of the above results, any pharmaceutical agent possessing anti-phospholipase activity would render protection against I/R damage. Manoalide, a phospholipase A_2 inhibitor, has been shown to protect the heart from the injury by I/R by partially inhibiting the degree of LPC-induced increase in Ca^{2+} (168). Mepacrine, another phospholipase inhibitor, while decreasing the level of phospholipid degradation, displayed a negative inotropic effect and appeared to interfere with calcium currents across the sarcolemma (172). Chlorpromazine and MR-256 (an oligomer of prostaglandin E_1) are two unrelated drugs, both of which were shown to have a protective effect on ischemia-reperfused heart due to their ability to inhibit PLA_2 (173). It has been further reported that coenzyme Q_{10} may inhibit PLA_2 on inner membranes of myocardial mitochondria or dipalmitoyl phosphatidylcholine, and in turn prevent the development of mitochondrial dysfunction and mitochondria phospholipid hydrolysis by phospholipase (174). Despite the encouraging results shown by phospholipase inhibitors, many of their mechanisms of action are still unknown.

e. Na^+-H^+ exchange inhibitors: The sarcolemmal Na^+/H^+ exchanger

(NHE) is an electroneutral exchanger that extrudes one proton in exchange for one Na^+ under normal condition (175). At least five different isoforms of NHE are known to exist. NHE1, the most widely distributed type, is predominant in cardiac tissue. It is thought to mediate a number of physiological functions in various cell types including maintenance of the intracellular pH and cell volume. Additionally, it controls cell growth and proliferation by mediating the action of a number of mitogen and growth factors (176,177). The acidosis induced by a shift to anaerobic metabolism during I/R can activate the NHE. Accordingly, NHE activity correlates with internal pH where the exchanger will be maximally active at low intracellular pH ($pH<6.5$) (177). The intracellular Na^+ level will be elevated by the activation of NHE, thus activating the Na^+-K^+ ATPase. The increase in Na^+ concentration will also lead to the occurrence of Ca^{2+}-overload via Na^+-Ca^{2+} exchanger (178) leading to cell injury via necrosis and/or apoptosis; ventricular arrhythmias will also occur. In chronic responses, I/R injury will stimulate NHE expression and improve NHE synthesis; finally it will induce cell remodelling, ventricular remodelling and heart failure (176). Although not fully understood, the above mechanisms indicate that an inhibitor of NHE may play a key role in protection against I/R injury.

A great deal of research is focused on studying the inhibitor of NHE as a potential effective treatment of I/R injury (179,180). NHE inhibitors are investigated via Na^+ nuclear magnetic resonance (NMR) and many inhibitors of NHE have been used in clinical settings including amiloride and its derivative (EIPA, DMA, MIBA, HMA), or non-amiloride structure inhibitor, cariporide, and (HOE 642) (179,181).

These are commonly known as selective NHE 1 inhibitors. Since the early 1990s, it has been demonstrated that amiloride and its derivatives reduce Na^+-overload in cardiac I/R injury, and consequently influence Ca^{2+} accumulation (182-184). Similarly, cariporide, a non-amiloride structure inhibitor, has no effect on the decline in cytosolic pH while preventing the accumulation of intracellular sodium. A reduction in infarct size, enzyme release, edema formation, arrhythmias and induction of apoptosis in a reperfused myocardium were also observed by NHE inhibitors (185). Some NHE inhibitors such as SM-20550 were reported to reduce the Ca^{2+} and Na^+ levels at the end-stage of ischemia in guinea pig heart; these protective effects may be modulated at the mitochondrial level. Similarly, HOE 694, another inhibitor of NHE, prevented clumping of Ca^{2+} aggregates on mitochondria. Clearly, the mitochondria may play a major role in the regulation of both physiological and pathological cell death in myocytes (186). Future research on this subject should focus on the usage of NHE inhibitors in clinical settings and provide an effective intervention for I/R myocardial injury.

f. P38 MAP kinase inhibitors: MAP (mitogen-activated protein) kinases are recognized as regulators of cell growth and proliferation. They are activated upon binding of peptide growth factors to their tyrosine kinase receptors. Three pathways are currently described that ultimately lead to MAP kinase activation. These are adhesion molecules, G protein-coupled receptors, and stress-activated MAP kinase pathways. The stress-activated MAP kinase pathway plays an important role in response to I/R in the heart since this phenomenon presents a real pathological stress

(187). The MAP kinases involved in this pathway include c-jun amino-terminal kinase, that phosphorylates the transcription factor c-jun and P38 MAP kinase. An increase in hydrogen peroxide concentration in the heart during ischemia and/or reperfusion could activate P38 MAPK (188,189). Normally mitogenic MAP kinases stimulate protein synthesis, cell proliferation and inhibit apoptosis. However, stress-activated pathways promote apoptosis and cytokine production, as in P38 MAP kinase induction of hypertrophy and expression of the fatal gene program in the heart. P38 MAPK appears to be a key factor in the signal transduction cascade of myocardial apoptosis proceeding ischemia and reperfusion (190-194). In addition, P38 MAPK has been implied to phosphorylate 72-kDa heat shock protein or 27-kDa heat shock protein which provides cytoprotection by stabilizing the actin cytoskeleton (195,196).

Extensive research is being directed towards the inhibition of P38 MAP kinase pathway as an intervention for the treatment of I/R injury (197,198). Currently, SB 203580 is the most effective inhibitor of P38 MAP kinase. An important finding from some animal models is that treatment with SB 203580 significantly decreases the levels of myocardial apoptosis, and equally significant is the improvement in cardiac function recovery after reperfusion (199,200). Selective blocking of P38 MAPK activation by SB 203580 and inhibition of the critical component in the signal transduction pathway leading to apoptotic cell death explains these findings. Thus, SB 203580 has the capability to attenuate post-ischemic myocardial injury and improve heart function recovery. Ma et al (198) reported that administration of SB

203580 significantly attenuated postischemic myocardial necrotic injury due to its ability to reduce early apoptosis in I/R heart. Although numerous studies support the notion that P38 MAP inhibition is protective (198,201,202), the benefits of inhibiting this kinase continue to be subject to controversy (203).

g. Protein phosphatase inhibitors: Protein kinases have been studied for many years because of their important role in the regulation of heart function; however, it has now been demonstrated that protein phosphatases play an equally important role (204). Protein phosphatases are currently classified into two groups: type 1 phosphatase (PP1) and type 2 phosphatase (PP2). Type 2 phosphatase is further subdivided into PP2A, PP2B and PP2C; however, three major protein phosphatases control cell function and these are PP1, PP2A and PP2B. They comprise more than 90% of the phosphatase activity in mammalian cells. These phosphatases provide the cell with the ability to rapidly change proteins from their phosphorylated to dephosphorylated form in order to meet different physiological needs such as cell cycle regulation, gene transcription, carbohydrate and lipid metabolism, organization of cytoskeleton, cholesterol and protein biosynthesis (205). It has been postulated that protein dephosphorylation during ischemia could result in damage to the cytoskeletal integrity and lead to cell death. It is believed that the heart is protected by inhibiting the dephosphorylation rate or stimulation of kinase activity to maintain protein phosphorylation, which could be preserved by ATP utilization under physiological conditions (206,207).

Many phosphatase inhibitors have been widely applied in experimental

research and clinical settings. Fostriecin is a highly selective inhibitor for PP2A and is used as an anti-tumor agent (208). Although there is no evidence to show that fostriecin is effective when applied to ischemic heart patients, several research reports have suggested the beneficial effect of fostriecin in ischemic heart disease in experimental animals (207,209,210). In different experimental models, this drug has been reported to protect the heart from myocardial infarction before or after the onset of ischemia. Weinbrenner et al (206) have suggested that fostriecin may inhibit dephosphorylation of PKC-specific substrates, thus protecting the heart during ischemia. In another study it was reported that fostriecin had similar cardioprotective effects as preconditioning in both rabbit and pig models. This protection may occur via the same effectors mechanism that preserves cytoskeletal phosphorylation and integrity of cell plasma membrane (207,211). Moreover, vanadate as a protein tyrosine phosphatases inhibitor has been identified to inhibit the dephosphorylation of the αB-crystallin, which is translocated to intercalated disks, and Z line to stabilize the myofibrils during ischemia in rats (212). It was also demonstrated that this drug presents other beneficial effect on isolated perfused rat heart such as attenuation of acidosis and changing glucose utilization (213). Other PP inhibitors such as okadaic acid, calyculin A and cantharidin have facilitated the study of protein phosphatase function (214,215) and have been shown to protect ischemic rat and rabbit cardiomyocytes (210,216).

h. 5-HT receptor antagonists: Serotonin (5-HT) is stored in platelets and released during platelet aggregation. It is present in large quantities within the heart

and is able to stimulate it directly via specific receptors. Those receptors are classified as 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4 (217). It has been reported that 5-HT plays a role as a mediator of inflammation, since neutrophil uptake of 5-HT results in release of a vasoconstrictive substance. 5-HT similarly affects the function of other leukocytes such as macrophages. These effects of 5-HT suggest a potential therapeutic value on the process of reperfusion injury after experimental ischemia (218). In addition 5-HT has been found to provoke contraction of isolated coronary arteries in various species and may be a major component in eliciting artery vasospasm, thus indirectly contributing to arrhythmias. Those findings suggest that 5-HT may play a pathologic role in a variety of low blood flow conditions. It was believed that 5-HT is released during certain types of myocardial ischemia, particularly when thrombosis persists. The role of 5-HT is to amplify the occlusive event via activation of 5-HT_2 receptor. 5-HT is also implicated in platelet-vessel wall interactions inducing vascular smooth muscle cell proliferation, vasospasms and arterial thrombosis. Therefore, 5-HT receptor antagonists may offer possible treatments for ischemic heart disease (219). Previous studies have shown the contractile effect of serotonin or 5-HT_2 receptor agonists on isolated rat intramyocardial coronary artery, while 5-HT_{1A} or 5-HT_3 receptor agonists showed no contraction (220). It is suggested that 5-HT_1 receptor mediates vasodilatation (221), while 5-HT_2 receptor mediates vasoconstriction. In the reperfused heart there is marked impairment of endothelium-dependent relaxation of the coronary arteries after global ischemia (222). The vasoconstriction of 5-HT_2 occurs due to a defect in the counter regulation of vasorelaxation by normal

endothelial cells.

Since 5-HT_2 receptors play a functional role in platelet aggregation, thrombus formation and in the impairment of endothelin-dependent relaxation of arteries (223), many 5-HT_2 receptor antagonists were studied for their effects on I/R injury; these include MDL28, 133A, LY53857, DV-7028, ICS 205-930, cinanserin, mianserin, ketanserin and yohimbine (224-227). Undoubtedly, in vivo studies of 5-HT_2 receptor antagonists exhibit inhibition of 5-HT-induced platelet aggregation, decreased lysis time and delayed reocclusion. In vitro studies have reported that 5-HT_2 receptor antagonists increased the time to contracture in isolated globally ischemic rat heart (228). It was further suggested that 5-HT might be implicated in the genesis and determination of severity of ventricular arrhythmias induced by acute myocardial ischemia, especially via 5-HT_2 receptor. Hence, 5-HT_2 receptor antagonists may be useful therapeutic agents for these arrhythmias. The mechanism of 5-HT_2-mediated effects may occur through activation of phospholipase C (PLC) and accumulation of inositol phosphates causing the release of Ca^{2+} from intracellular pools (229), yet the exact mechanism of 5-HT_2 receptor antagonists that attenuate ischemic injury are still unknown. Although pharmacological approaches to protect the heart from the I/R injury have been investigated, these still need to be well established by future research.

6. Effect of Pentoxifylline on Cardiovascular Disease

a. General information about pentoxifylline: PTXF, a synthetic methylxanthine, was approved in 1984 to prevent intermittent claudication in chronic occlusive arterial disease (230,231). Like other methylxanthine derivatives, PTXF is not only prescribed for peripheral vascular and cerebrovascular diseases but is also indicated for the treatment of asthma (232). PTXF is used to improve the effectiveness of microcirculation, increase red blood cell (RBC) deformability, decrease platelet aggregation and lower plasma viscosity (232-234). PTXF has also been shown to modify the immune system. For example, this drug improves leukocyte deformability and chemotaxis, depresses neutrophil degranulation, decreases endothelial leukocyte adhesion and lowers the sensitivity of leukocyte to cytokines (235-242). It has also been reported that PTXF can inhibit the production of inflammatory cytokines (27) and thus reduces neutrophil adhesiveness to endothelial cells, enhances chemotaxis and lowers the production of free radicals (243). In other studies, PTXF has been shown to augment the production of prostacyclins and a vasodilator, ecidosanoid (244,245). Since, PTXF is known to inhibit phosphodiesterase (PDE), an enzyme that breaks down cAMP, it elevates the level of intracellular cAMP and thus lowers platelet aggregation (246) and depresses the production of TNF-α (247).

PTXF has received much attention for its pharmacological effects on hemorrheology and immune response; hence its metabolism has been examined extensively. This drug is clinically effective when administered either orally or

intravenously. It is metabolized by erythrocytes and the liver, and is excreted by the kidney with a half-life of 3.4 hr. There are seven metabolites of PTXF; metabolite V is considered as the major urinary metabolite of PTFX in humans (232). Although the effects of these metabolites on cardiovascular disease have not been fully examined, some investigations of metabolites I and V indicate that these have hemorrheologic properties similar to those of PTXF. Moreover, metabolite I, known as lisofylline or BL-149, was found to be the most active metabolite of PTXF for the treatment of intermittent claudication in humans (248). Although lysofylline can be useful in the treatment of cardiovascular disease, PTXF is required clinically due to its diverse effects on hemorrheology and inflammation with fewer side effects. Conclusively, PTXF promotes the oxygenation of ischemic areas and lowers the amount of metabolic derangements associated with I/R injury. In view of a wide variety of effects of PTXF and potent hemorrheologic properties, it is proposed to discuss the pharmacological actions of this agent for a clear understanding of its therapeutic potentials for the treatment of cardiovascular disease.

b. Effect of pentoxifylline on I/R injury: I/R injury in various organs has become a major global health concern, as it is the third leading cause of death in North America and one of the primary factors that disables the elderly. Therefore, searching for an effective pharmacological intervention of ischemic disease has become very important (249). Many investigators have discovered that the hemorrheologic properties of PTXF allow this drug to reduce blood viscosity, improve red blood cell flexibility and inhibit platelet aggregation in ischemic cerebral

disease. These modifications cause an increase in capillary perfusion and regional cerebral blood flow (CBF) (250,251). Several in vitro studies of rats also demonstrate that PTXF extensively enhances red cell elasticity by increasing the amount of ATP in RBCs (232). Similar findings were reported in humans (252). Bowton et al (1984) also found that this change in ATP level, via oral administration of a single sustained release capsule of PTXF, increases global and regional CBF in patients with cerebrovascular disease (253). Moreover, current studies of PTXF indicate that this drug can be beneficial to those with cerebrovascular disease. It can inhibit brain edema by reducing disturbances of brain cell membrane permeability. PTXF can also remove mechanical obstacles in microcirculation (238,254,255). Therefore, PTXF has a broad range of therapeutic effects on patients with cerebrovascular disorders. It can be used to treat transient ischemic attacks, cerebral thrombosis and hemorrhage, and chronic cerebrovascular insufficiency (232). PTXF is potentially a very useful drug in combatting ischemic brain injury.

Meanwhile, it has been discovered that PTXF is a very important agent in establishing a treatment for I/R injuries to skeletal muscle. This agent has been reported to inhibit neutrophil adhesion by blocking the effects of completment receptor (CR) 3 up-modulation, preventing degranulation of myeloperoxidase and lysozyme which are found in the granules of neutrophil, and modulating cytoskeletal interactions at the adenosine A_2 receptor (238,256). PTXF also prevents adherence of neutrophils stimulated by TNF-α (237). Furthermore, it has been shown that PTXF interferes with the leukocyte-signalling pathway by activating phosphatidylinositol-3-

kinase and PLD via a number of different agonists. Eventually, actin polymerization and superoxide production are inhibited by PTXF treatment (257). PTXF treatment blocks the response of granulocytes to platelet-activating factor (PAF) (258,259) and subsequently decreases the level of PAF in the venous blood. This potent lipid mediator, produced by ischemic skeletal muscle during periods of reperfusion, would otherwise result in increased binding of neutrophils to endothelial cells (258,260,261). It also was shown that the administration of PTXF at a high dose decreased the degree of muscle necrosis (260). Adams et al (1995) reported that using 25 mg of PTXF per kg, immediately before reperfusion, extensively diminished the degree of muscle necrosis and PAF levels in the venous effluents of isolated canine gracilis (258). As well, Hanazawa et al (262) reported that PTXF treatment prevented leukocyte adhesion after reperfusion in the rat cremaster muscle venous blood. The administration of PTXF also significantly decreases PAF levels and neutrophil adhesion in the ischemic skeletal muscle (237,258,262). Thus PTXF prescription in the clinic and basic research as the therapeutic strategy of I/R skeletal smooth muscle has provided further information for the treatment of other vital organ I/R injury such as ischemic heart injury. In fact, the I/R heart injury has become a major economic and health-care concern. There are a number of factors to consider before we can successfully treat these injuries. Many modifications have been used in the treatment of this problem as we mentioned in the previous section. Meanwhile, various experimental and pharmacodynamic studies also demonstrated the beneficial effect of PTXF on myocardial vascular disorder (243,263-267). In one study, 40 ischemic

heart disease patients treated with PTXF (600 mg per day for 25 to 30 days) showed lowered level of glyceryl trinitrate consumption, greater ability to exercise and reduced tachycardia (232).

The primary pharmacodynamic actions of PTXF, such as increased RBC deformability and decreased blood viscosity are considered as an important mechanism to protect the heart from I/R injury (232). Dauber et al (268) demonstrated that PTXF attenuated the coronary microvascular protein leak and decrement in endothelium-dependent relaxation in the coronary epicardial arteries after ischemia and reperfusion. In addition, the increase in neutrophil cyclic AMP induced by PTXF also diminished the superoxide production and adherence of neutrophils to vascular endothelium, as well as a reduction in the response of neutrophil to PAF (269-271). PTXF was also reported to decrease myeloperoxidase, an index of tissue leukocyte accumulation in ischemia myocardium. This demonstrates that PTXF modification significantly reduced leukocyte adhesion (263). In addition, PTXF is an effective hydroxyl radical scavenger preventing endothelial injury by reactive oxygen species (243,253).

Additionally, reduction in TNF-α production has been shown to be an important mechanism of PTXF when it was prescribed in the treatment of mild-to-moderate heart failure and advanced heart failure, as well idiopathic dilated cardiomyopathy (272,273). PTXF also have been prescribed to protect heart and lung from cardiopulmonary bypass surgery due to the anti-TNF-α synthesis effect (274,275). According to some investigators, this effect has been shown to occur in

vitro and/or in vivo. There is evidence, which demonstrates that PTXF decreases TNF-α synthesis via two mechanisms. Firstly, one of its metabolites, lisofylline inhibits the lysophosphatidic acid acyl transferase that converts lysophosphatidic acid to phosphatidic acid. This induces a rise in Ca^{2+} concentration and increase in the synthesis of TNF-α (276). Secondly, PTXF acts as an inhibitor of phosphodiesterase and induces prolonged cyclic AMP activity resulting in activation of PKA, which serves to block NFκB activation, in turn inhibiting TNF-α mRNA transcription (277). This indicates that this phosphodiesterase inhibitor blocks TNF-α gene transcription and protein production (278). However, although the anti-TNF-α effect of PTXF has been studied in heart failure and cardiomyopathy, this anti-TNF-α effect of PTXF on I/R heart still remains inconsistent.

In summary, PTXF with limited side effects and favourable activity in hemorrheologic properties has received more attention for its beneficial effect on ischemic heart disease presently. PTXF has gained widespread interest and is widely considered as an effective intervention for I/R-induced damage. Numrous in vitro and in vivo studies have revealed that the hemorrheologic and anti-inflammatory activities of PTXF were responsible for the therapeutic effects of this drug. PTXF can immensely increase the recovery of all kinds of I/R diseases in various organs, including the brain, heart, intestines, testis and skeletal muscle (234,263,263,274,279-282). Based on the cumulative action of all of these effects, PTXF has been recognized as an effective therapeutic strategy for dealing with various cardiovascular diseases. However, its mechanism for alleviating cardiovascular dysfunction and the

optimum dosage for therapy have not been clearly identified. Hence, a great deal of research and appropriate clinical trials still need to be conducted.

II. STATEMENTS OF THE PROBLEM AND HYPOTHESIS TO BE TESTED

Ischemic injury occurs immediately upon cessation of blood flow to the heart and persists even after restoration of coronary perfusion. These alterations in cardiac performance have been partially attributed to the occurrence of intracellular Ca^{2+}-overload in the ischemic and I/R heart. On the other hand, several studies have indicated that the production of TNF-α may participate in cardiac dysfunction in different types of heart diseases such as myocardial infarction, heart failure and various cardiomyopathies. Although PTXF, a phosphodiesterase inhibitor, has been reported to protect the heart during cardiopulmonary bypass surgery, the exact mechanism of its action still remains unclear. Several factors such as increased RBC deformability, decreased platelet aggregation and a reduction in plasma viscosity have been assumed to mediate the beneficial effect of PTXF. Furthermore, PTXF has been reported to inhibit the production of TNF-α and NFκB activity in cardiomyopathy and heart failure. This study was designed to examine if the depression of TNF-α synthesis and inhibition of NFκB activation are involved in the cardioprotective effect of PTXF against I/R-induced injury. To control potential hematological contributions in the beneficial effects of PTXF on I/R injury, this study was conducted in the isolated, crystalloid-perfused rat heart. Since Ca^{2+}-paradox heart is considered to be an appropriate experimental model for studying the effects of intracellular Ca^{2+}-overload, this study also examined the effects of PTXF in hearts

subjected to Ca^{2+}-depletion and Ca^{2+}-repletion. The role of intracellular Ca^{2+}-overload in TNF-α formation and NFκB activation was also investigated by employing the Ca^{2+}-paradox heart with or without PTXF treatment. This research was therefore undertaken to test the hypothesis that improvement in cardiac function by PTXF in both I/R and Ca^{2+}-paradox hearts is associated with inhibition of TNF-α synthesis and NFκB activation.

III. MATERIALS AND METHODS

All experimental protocols for animal studies were approved by the Animal Care Committee of the University of Manitoba, following the guidelines established by the Canadian Council on Animal Care.

1. Perfusion of Isolated Rat Hearts

The perfusion procedure for the isolated heart was similar to previous studies from our laboratory (283,284). Male Sprague-Dawley rats (280-350 g) were anesthetized by injecting a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg). The hearts were rapidly excised and perfused using the Langendorff technique. Each heart was stabilized with perfusion at a constant flow rate of 10 ml/min for 20 min with normal Krebs-Henseleit (KH) buffer containing in mM: 120 NaCl, 4.8 KCl, 1.35 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$ and 11 glucose (pH 7.4). All of these chemicals were purchased from Sigma Aldrich in USA. The perfusion solution was maintained at 37°C and gassed continuously with a mixture of 95% O_2 and 5% CO_2. The hearts were stimulated electrically at 300 beats/min by using 611 Stimulator (Phipps & Bird, Richmond, USA). A water-filled plastic balloon was inserted into the left ventricle and the left ventricular end diastolic pressure (LVEDP) was adjusted at 10 mm Hg at the beginning of the experiment. The left ventricular developed pressure (LVDP), LVEDP, rate of pressure development (+dP/dt) and rate of pressure decay (-dP/dt) were measured by using AcqKnowledge 3.5 for Windows 3.0 (Biopac System Inc., Goleta, CA, USA). Data was recorded online through an analogue-digital

interface (MP 100, Biopac System Inc., Goleta, CA, USA).

2. Experimental Protocol for I/R

The isolated rat hearts were perfused for 20 min for stabilization. For the control group, the hearts were perfused with KH buffer for 60 min and for the I/R group, the hearts underwent 30 min of global ischemia followed by 30 min of reperfusion with normal KH medium. For the PTXF treatment group, PTXF was given for 10 min before inducing ischemia during the stabilization period, as well as during the 30 min reperfusion period. The drug was infused into the perfusion medium via a side arm close to the cannula. The experimental protocol is depicted in Figure 1A. Different concentrations (50, 100, 125 and 150 μM) of PTXF were used in this study. The left ventricular function was measured by recording the LVDP, LVEDP, +dP/dt and -dP/dt and the hearts were stored in -70°C after 30 min reperfusion.

3. Experimental Protocol for Ca^{2+}-Paradox

The Ca^{2+}-paradox study was induced in hearts as in previous studies from our laboratory (20,285). After 20 min of stabilization by using normal KH solution, the hearts were divided into three groups. For the control group, the hearts were perfused with oxygenated KH medium for 35 min. For the Ca^{2+}-paradox group, the hearts were perfused with Ca^{2+}-free KH medium for 5 min followed by 30 min perfusion with normal KH buffer containing 1.25 mM Ca^{2+}. For the PTXF treatment group, PTXF

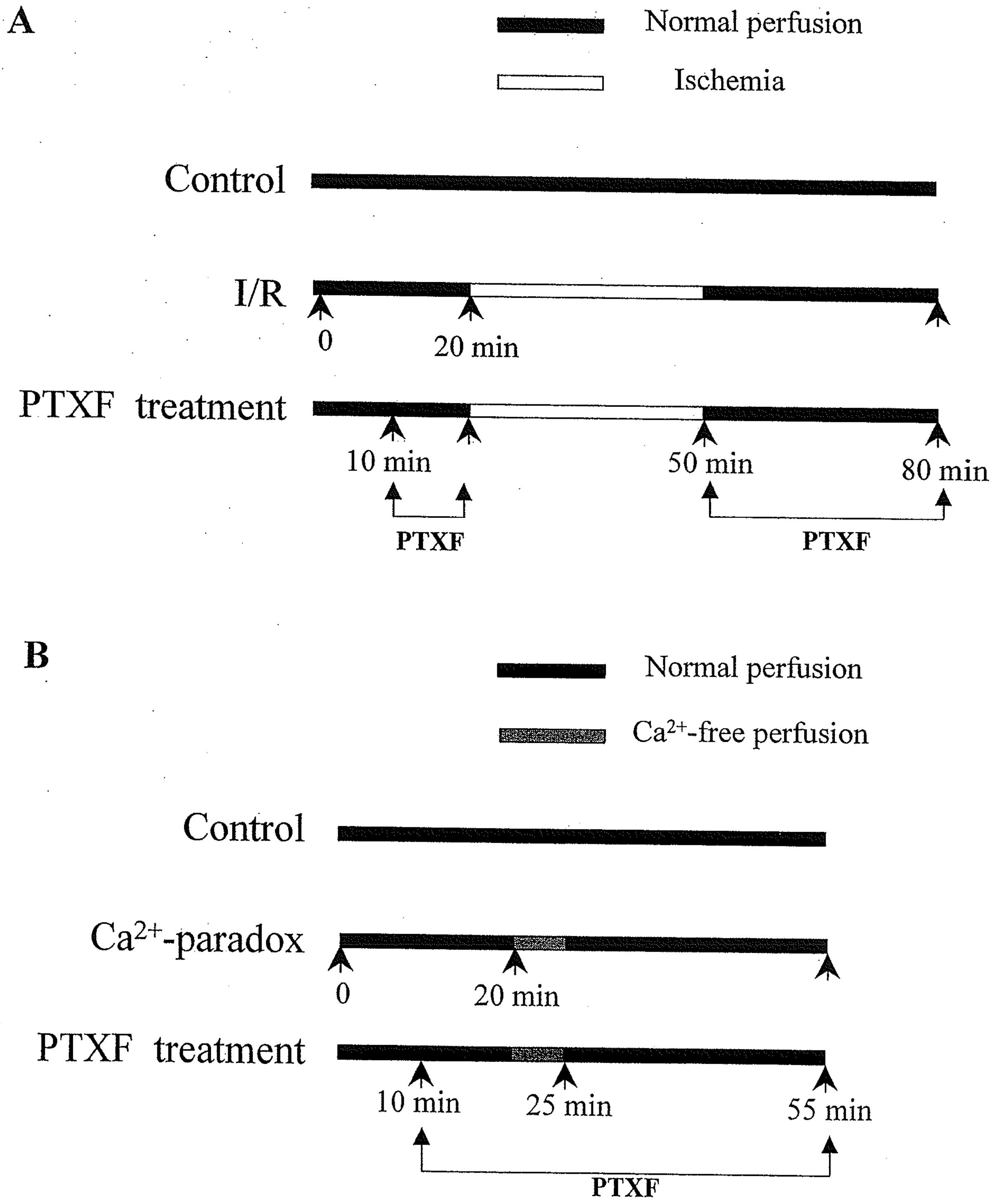

Figure 1: Experimental protocols in I/R model (A), and Ca^{2+}-paradox model (B). All hearts were perfused for 20 min for stabilization before 30 min global ischemia or 5 min Ca^{2+}-free perfusion as this was followed by 30 min reperfusion or 30 min Ca^{2+}-repletion.

infusion was started 10 min before Ca^{2+}-depletion and was carried out throughout the Ca^{2+}-depletion and Ca^{2+}-repletion periods. The experimental protocol is shown in Figure 1B. The left ventricular function was measured by recording the LVDP, LVEDP, +dP/dt and -dP/dt. The hearts were stored in -70°C after 30 min of Ca^{2+}-repletion.

4. Measurement of TNF-α

Ventricular tissue was homogenized in 10 volumes of phosphate-buffered saline (PBS), which contained 1% Triton-100 along with protease inhibitor cocktail (Roche, Laval, Quebec, Canada) (24). The homogenate was centrifuged at 2500 g for 20 min at 4°C. The supernatant was collected and the TNF-α level was measured using a sandwich ELISA kit for rat TNF-α with a 12.5 pg/ml detection limit (R&D Systems, Inc., Minneapolis, USA). The assay was performed according to the manufacturer's instructions. Absorbance of standards and samples were determined spectrophotometrically (SPECTRAmax® PLUS384, Molecular Devices, Sunnyvale, CA, USA) at 450 nm. Results were calculated from the standard curve and were reported as pg/g protein.

5. Preparation of Tissue Extract for NFκB Determination

Ventricular tissue (50 mg) was homogenized on ice at setting 8 for 2 × 30 s with 30 s interval in between (Polytron PT 3000, Brinkmann Instruments; Mississauga, ON, Canada) in 1 ml of buffer A containing: 50 mM Tris-HCl, 0.25 M sucrose, 10 mM EGTA, 4 mM EDTA and protease inhibitor cocktail (Roche, Laval,

Quebec, Canada), pH 7.5. The suspension was sonicated for 2 × 15 s with 30 s interval in between and then centrifuged at 100,000 g for 60 min in an ultracentrifuge (Model L70, Beckman Instruments, Fullerton, CA, USA). The supernatant was collected as cytosolic fraction. The pellet was resuspended in 1 ml of buffer B (buffer A + 1% Triton X-100) and incubated on ice for 60 min, then centrifuged at 100,000 g for 60 min in an ultracentrifuge. The supernatant containing dissolved membrane protein was labelled as particulate fraction. Another piece of 50 mg ventricular tissue was suspended in buffer B, homogenized and sonicated as above. The homogenate was incubated on ice for 60 min, and then centrifuged at 100,000 g for 60 min in an ultracentrifuge. The supernatant obtained was labelled as homogenate fraction. This method for preparing tissue extract is the same as described elsewhere (286).

6. Determination of Protein Concentration

Protein concentration of tissue extract was estimated by the microassay procedure of Bradford. Tissue samples were diluted to a final volume of 1600 µl, and treated identical to standards. A standard curve was obtained by taking different samples of BSA solution containing 2.5, 5, 7.5, 10 µg/ml in total volume of 1600 µl. The colour reagent (400 µl) was added to each tube and the mixture incubated for 15 min at 22°C. The colour intensity was measured in a spectrophotometer at 595 nm.

7. Analysis of NFκB Protein Content

The immunoblotting analysis of NFκB was performed by separation of 20 µg protein from homogenate, cytosolic and particulate fractions on a 10%

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by immunostaining by Western blot assay. The concentration of protein in these samples was adjusted to 1 mg/ml with the buffer A or buffer B and 4 X SDS-PAGE loading buffer containing 250 mM Tris-HCl (pH 6.8@25°C), 8% w/v SDS, 40% Glycerol, 200 mM β-Mercaptoethanol and 0.4% w/v Bromophenol Blue. The electrophoresis was carried out first at 100 volts for 10 min followed by 200 volts for 40-45 min. The proteins separated by SDS-PAGE were then electroblotted at 110 volts for 1 hr at 4°C to polyvinylidene difluoride membrane (PVDF) by employing a transfer buffer containing 25 mM Tris-HCl, 192 mM glycine and 20% methanol (v/v) for the determination of relative protein content with immunoblotting analysis. The transferred membranes were incubated overnight in blocking buffer, TBST (10 mM Tris-HCl, 150 mM NaCl and 0.1% Tween-20) containing 5% non-fat milk powder at 4°C. The membranes were placed at room temperature for 30 min and incubated for 2 hr with anti-P65 NFκB primary antibody (1:1000) (Cell Signaling Technology, Ontario, Canada) in 10 ml blocking buffer with gentle agitation. The membranes were washed 3 times for 10 min each with 15 ml of TBST and then incubated with second antibody (1:10000 Goat Anti-Rabbit IgG Horseradish Peroxidase Conjugate, diluted in TBST containing 1% fat-free milk) at room temperature for 1 hr. The membranes were again washed with TBST as described above. Antigen - antibody complexes in all membranes were detected by the chemiluminescence ECLplus kit (Amersham-Pharmacia Biotech, Baie d'Urfe, Quebec, Canada). An Imaging Densitometer (GS-800, Bio-Rad, Mississauga, Ontario, Canada) was used to scan the protein bands and

quantified using the Image Analysis Software Version 1.0. Protein loading was checked in every experiment by staining the membrane with ponceau S staining before immunoblotting (69).

8. Statistical Analysis

The data were expressed as mean ± SE. Differences between the two groups were analyzed by using an unpaired Student t-test. $P<0.05$ was considered the threshold for statistical significance between the control and the experimental groups.

IV. RESULTS

1. Effect of PTXF on I/R Injury

a. Alteration of cardiac function in I/R-induced injury: Thirty min of ischemia and 30 min reperfusion caused a marked depression of cardiac function. Figure 2 shows alterations in left ventricular pressures as well as pressure development and decay. There was a 9-fold decrease in LVDP and a 30-fold increase in LVEDP after 30 min of reperfusion. A 10-fold decrease in both +dP/dt and -dP/dt in I/R group was also observed (Table 1). The data in Figure 2 and Table 1 also indicate that treatment with PTXF (100 μM) enhanced the cardiac function significantly; there was about 75% recovery of LVDP and about 60% recovery of both +dP/dt and -dP/dt after I/R. Although a significant decrease in LVEDP was seen after PTXF treatment, LVEDP still remained 10-fold higher than pro-I/R value. The dose-response for the beneficial effects of PTXF against I/R injury shows that 50 μM PTXF did not exert any significant action whereas maximal effects are seen with 125 μM PTXF (Figure 3).

b. Myocardial TNF-α production in I/R-induced injury: Myocardial TNF-α contents in ischemic and ischemic-reperfused ventricular tissues are shown in Figure 4. TNF-α levels were decreased in the ischemic heart; however, this change was not affected by treatment with PTXF (100 μM). On the other hand, a significant increase was detected (from 409 ± 32 to 906 ± 92 pg/g protein) at 2 min of reperfusion but thereafter TNF-α levels decreased towards control level. Treatment

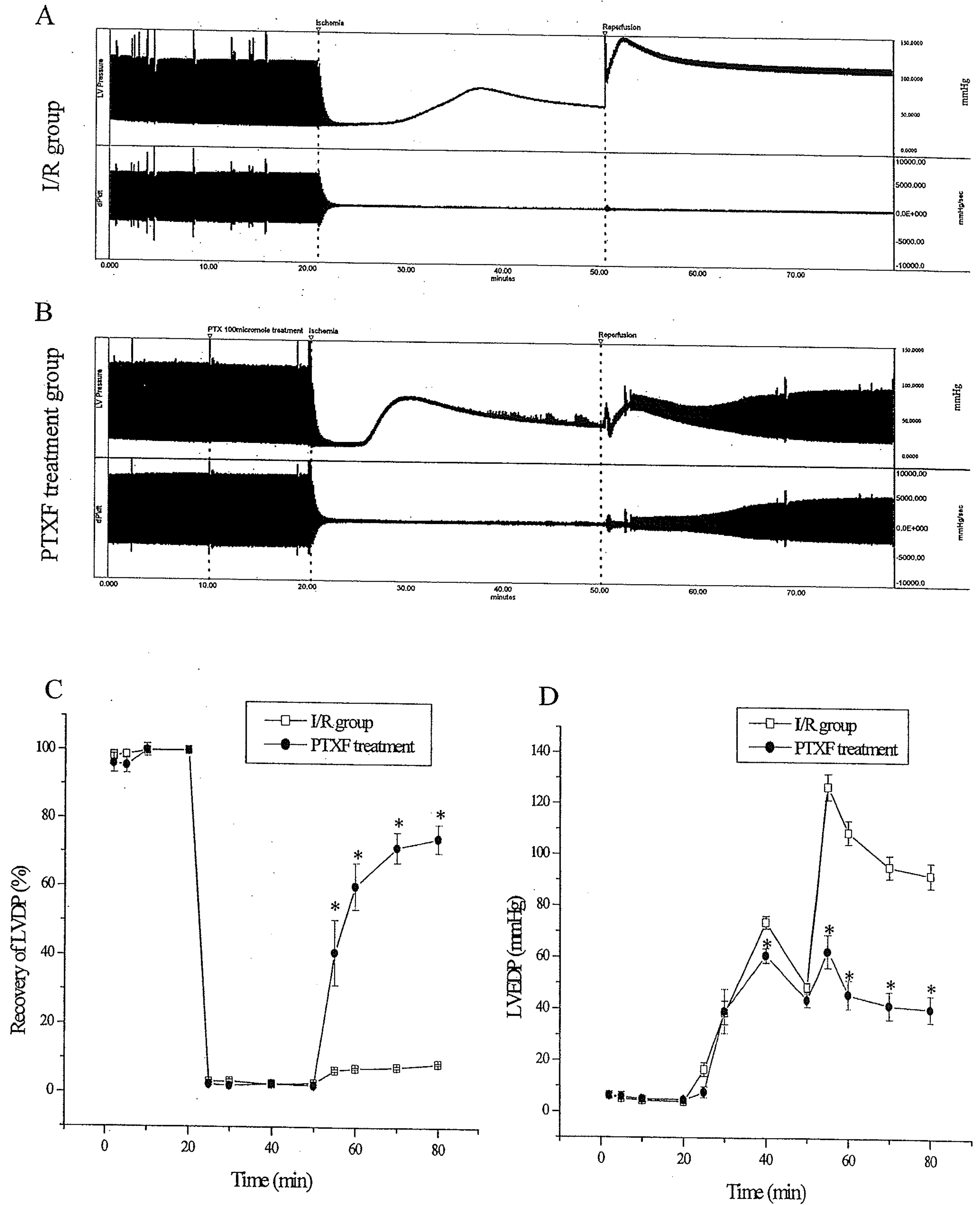

Figure 2: Tracings represent recording from the heart under I/R without (A) and with (B) PTXF treatment. C and D show the effect of PTXF (100 μM) on the alterations of LVDP and LVEDP at the different time point during I/R. Each group consists of 6 experiments. *$p<0.05$ vs. I/R group.

Table 1. Effect of PTXF (100μM) on cardiac performance in I/R heart

Group	LVDP	LVEDP	+dP/dt	-dP/dt
	mmHg		mmHg/sec	
Control	118 ± 5.6	4.0 ± 0.8	6618 ± 268	4024 ± 203
Control with PTXF	103 ± 9.7	4.7 ± 0.8	5883 ± 786	3608 ± 284
30' I	3.1 ± 0.5*	48.6 ± 1.7*	107 ± 16.4*	118 ± 17.6*
30' I with PTXF	2.2 ± 0.8*	43.7 ± 2.5*	97 ± 22.7*	97 ± 25.6*
I/R	15.3 ± 2.9*	92.3 ± 4.8*	568 ± 150*	397 ± 78.1*
I/R with PTXF	75 ± 6.1*#	40.5 ± 5.1*#	4328 ± 328*#	2490 ± 128*#

30' I for 30 min ischemia, I/R for 30 min ischemia and 30 min reperfusion. Values are Mean ± SE of 6 separated experiments *$p<0.05$ vs. control group, #$p<0.05$ vs. ischemia reperfusion group

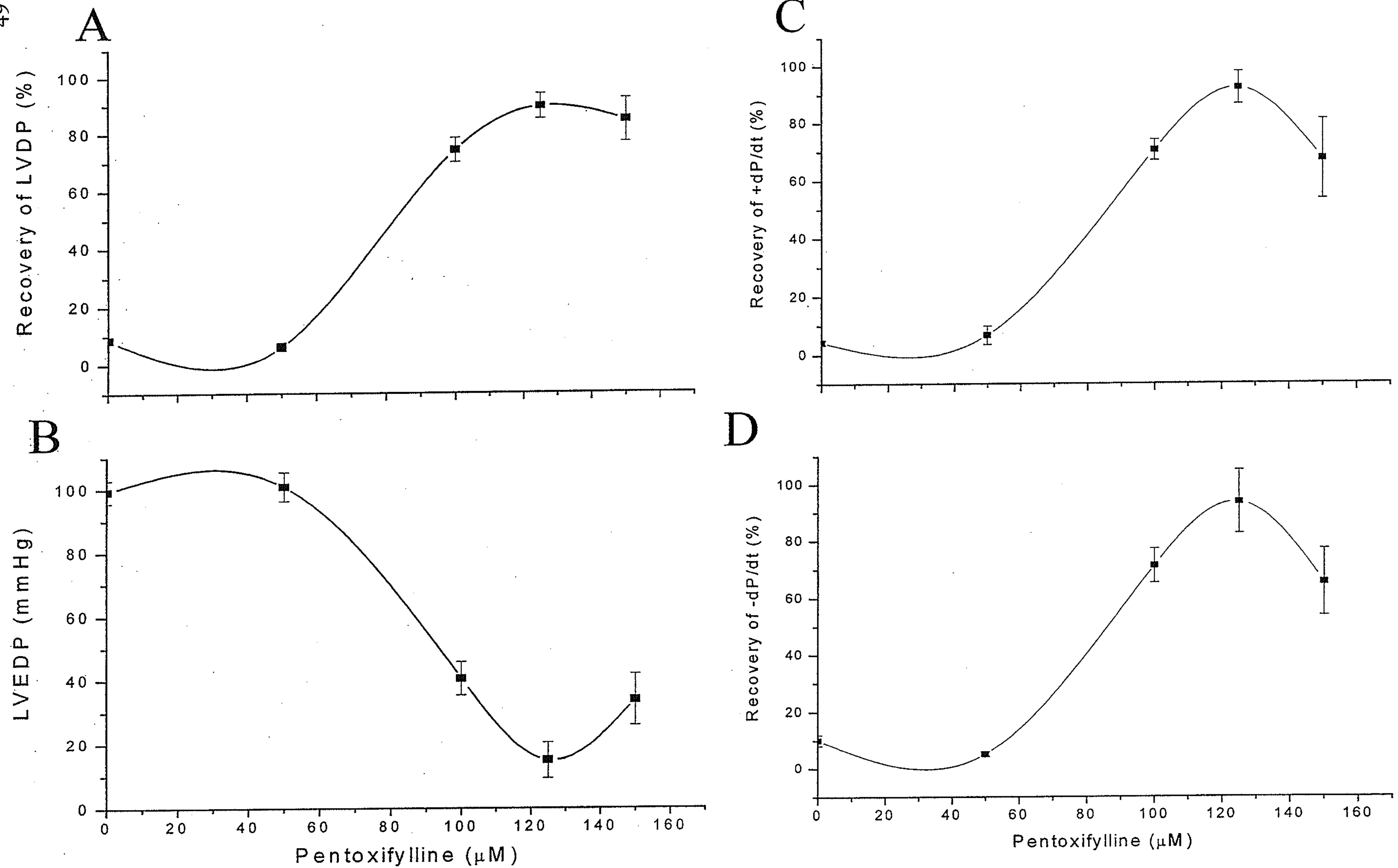

Figure 3: Dose response of PTXF on cardiac performance under I/R. The hearts were exposed to PTXF at 0 μM, 50 μM, 100 μM, 125 μM and 150 μM and subjected to 30 min Ischemia and 30 min Reperfusion. Alterations of LVDP (A), LVEDP (B), +dP/dt (C), and –dP/dt (D) at different dose were presented. Values are mean ± SEM of 6 separated experiments.

of the heart with PTXF attenuated the initial increase in TNF-α content due to reperfusion (at 2 min). However the TNF-α content remained at the same level in the PTXF treated group and in fact, TNF-α level at 10 and 30 min of reperfusion were higher than the untreated hearts.

c. Myocardial NFκB protein content in I/R-induced injury: Figure 5 shows the representative Western blots for NFκB and the analysis of protein contents of NFκB in homogenate, cytosolic and particulate fractions. It is observed that the relative protein content of NFκB in homogenate decreased by about 13% of the control in 30 min reperfused hearts following 30 min of global ischemia. The cytosolic NFκB content was reduced by about 85% of the control (Figure 5B) whereas the particulate content was decreased by about 50% of the control in I/R hearts (Figure 5C). On the other hand, the NFκB protein content in the homogenate fraction (Figure 5A) or in the particulate fraction (Figure 5C) did not change significantly in the PTXF treated I/R group hearts. However, the NFκB content in the cytosolic fraction (Figure 5B) decreased by about 46% in the PTXF treated I/R hearts. It is pointed out that the ratio of particulate to cytosolic ratio for the NFκB protein content in the untreated I/R heart was about 3.3, whereas that in the PTXF I/R hearts was about 1.8. This indicates that redistribution of NFκB in the I/R hearts is attenuated by PTXF treatment.

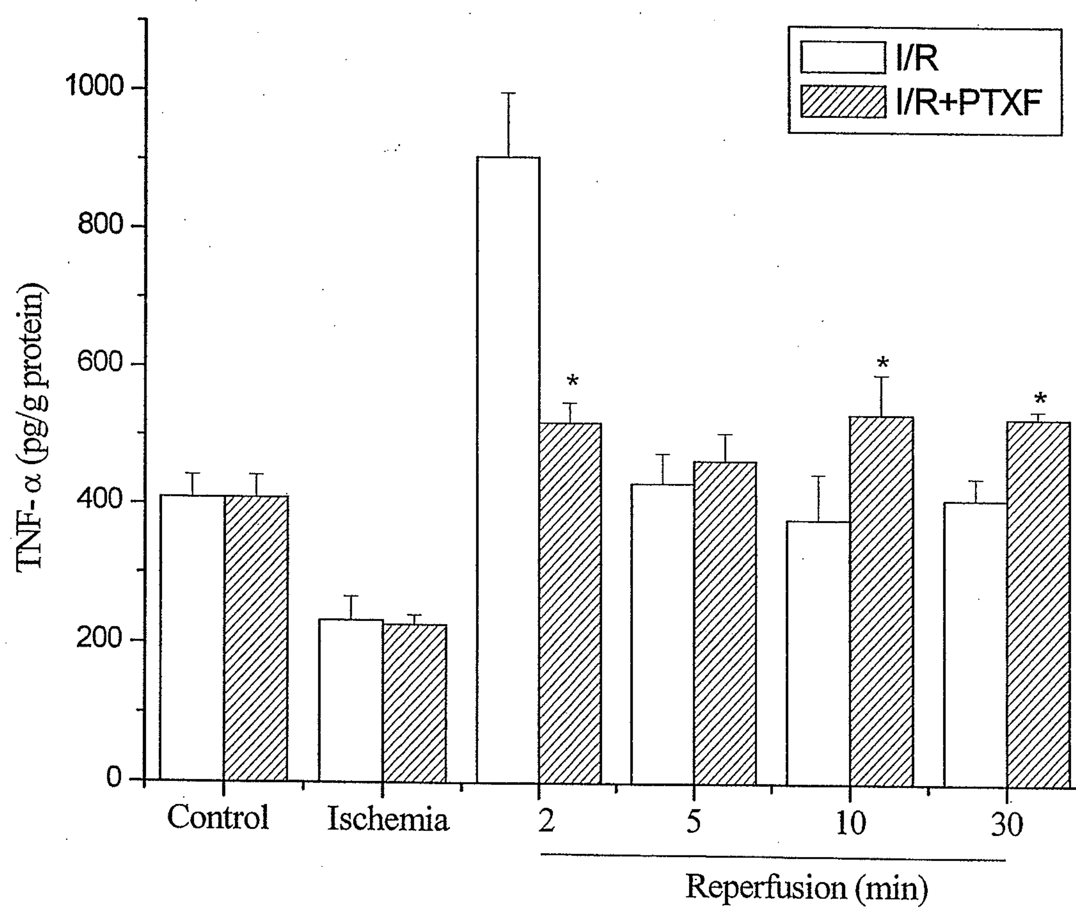

Figure 4: TNF-α protein level in myocardium subjected I/R with or without PTXF treatment. Each value represents 6 separate experiments. *$p<0.05$ vs. I/R group.

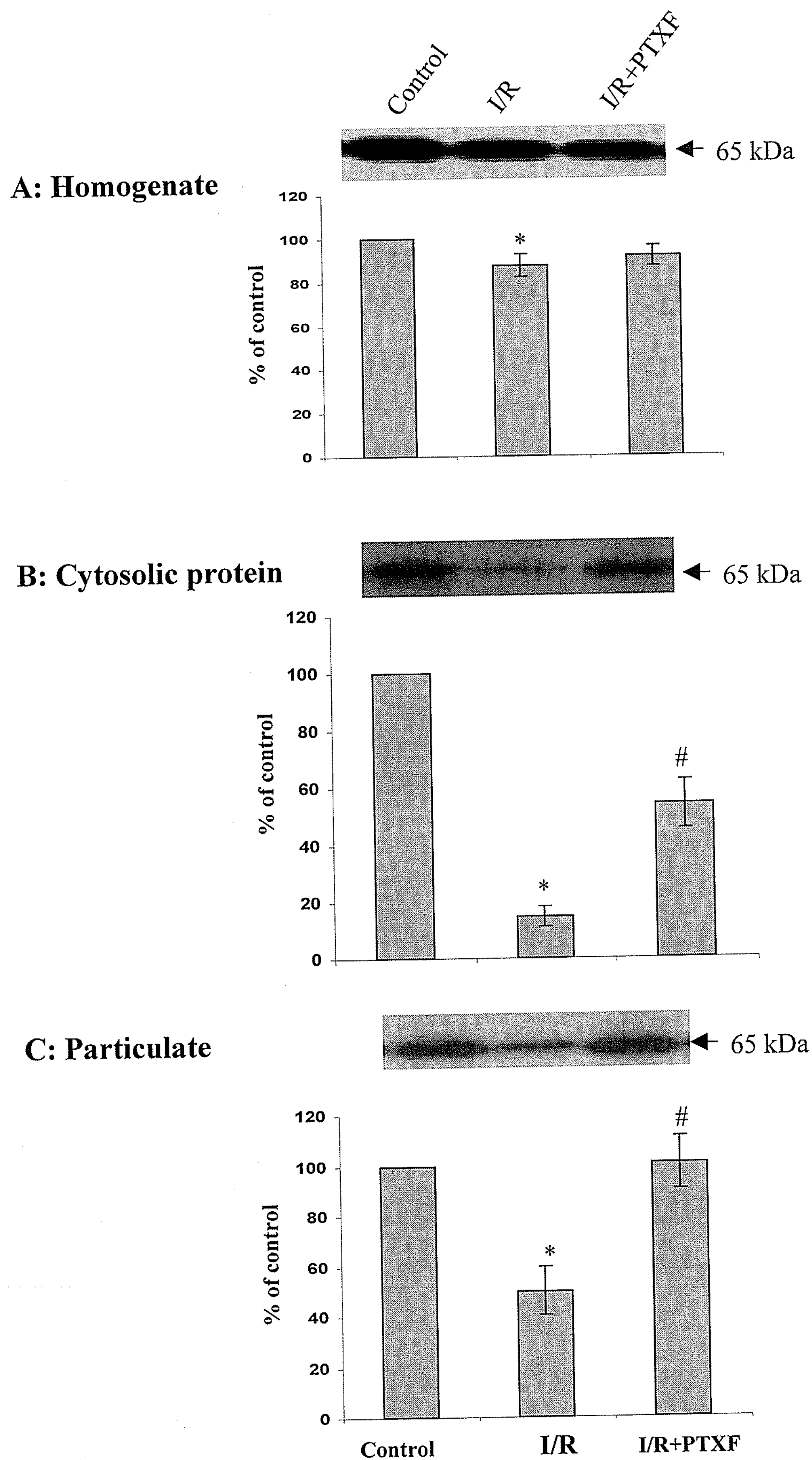

Figure 5: Western blot analysis showing the alteration in the protein content of NFκB at homogenate fraction (A), cytosolic fraction (B), and particulate fraction (C) of I/R heart with or without PTXF (100μM) treatment. *p<0.05 vs. control group, #p<0.05 vs. I/R group.

2. Effect of PTXF on Ca^{2+}-Paradox Heart

a. Cardiac dysfunction in Ca^{2+}-paradox injury: Five min of Ca^{2+}-free perfusion followed by 30 min of Ca^{2+}-repletion caused a dramatic impairment in cardiac performance. This change was demonstrated by a 10-fold decrease in LVDP and about 20-fold increase in LVEDP at 30 min of Ca^{2+}-repletion (Figures 6C and 6D). A 20-fold decrease in ± dP/dt in the Ca^{2+}-paradox heart was also detected (Table 2). To determine if PTXF attenuated cardiac dysfunction caused by Ca^{2+}-paradox, hearts were pretreated with PTXF (100 μM) before the Ca^{2+}-free perfusion. As depicted in Figure 6 and Table 2, PTXF treatment significantly improved cardiac function as demonstrated by 41% recovery of LVDP, 35% recovery of ± dP/dt, and a significant decrease of LVEDP but still 10-fold higher than control group.

b. Ca^{2+}-paradox induced TNF-α production in the heart: As shown in Figure 7, a dramatic increase in TNF-α protein levels (from 409 ± 32 to 1833 ± 180 pg/g protein) was detected in the myocardium after 30 min Ca^{2+}-repletion, whereas there was no increase of TNF-α protein level after 5 min Ca^{2+}-depletion. However, a significant depression in TNF-α level (784 ± 170 pg/g protein) was detected in the PTXF treatment group (Figure 7).

c. Ca^{2+}-paradox induced alterations in NFκB protein content: In order to investigate the possible mechanism of Ca^{2+}-paradox induced injury, the protein content of NFκB in homogenate, cytosolic and particulate fractions from control, Ca^{2+}-paradox and PTXF treated hearts were measured. Figure 8 shows the

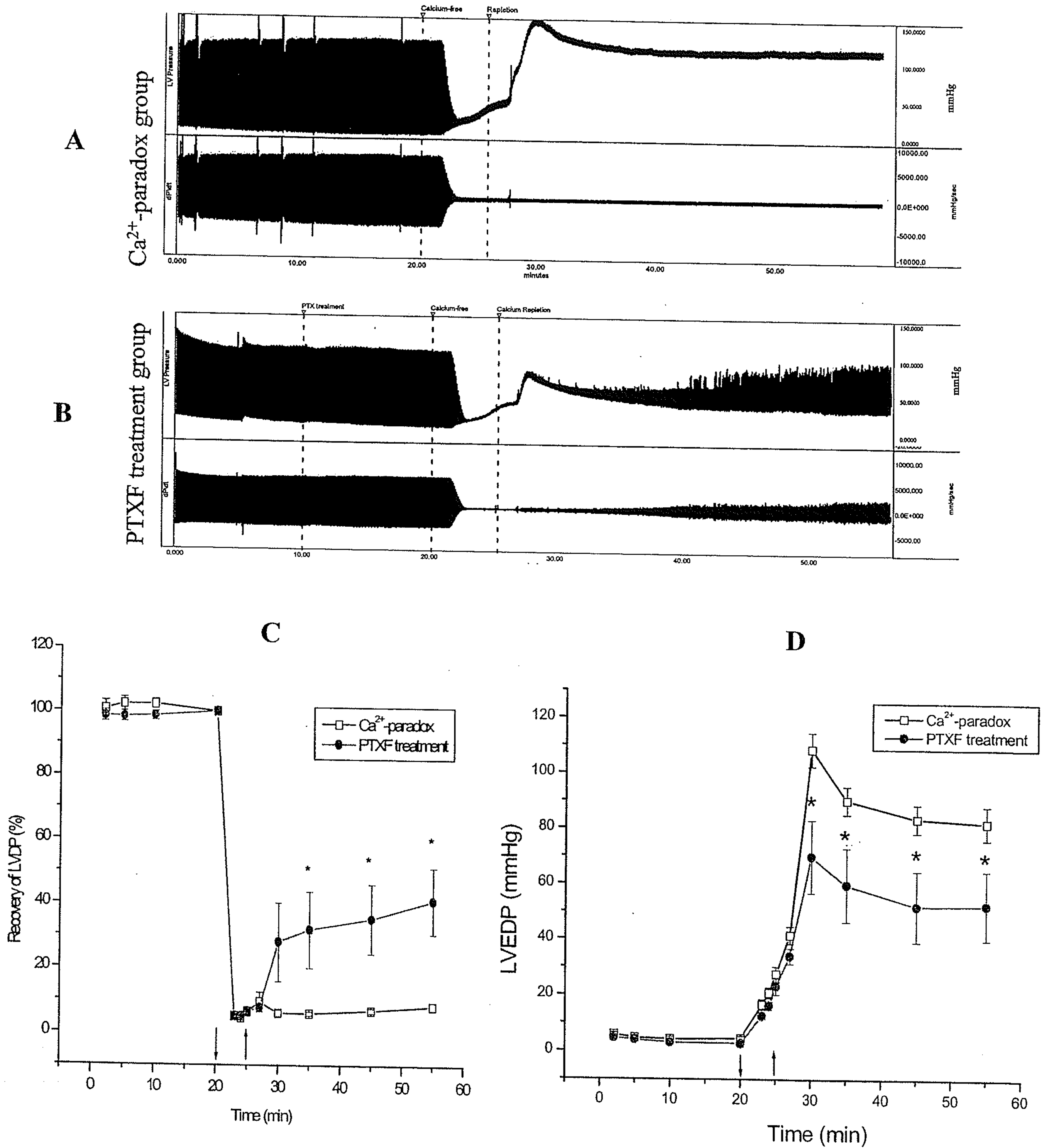

Figure 6: Tracings represent recording from the heart under Ca^{2+}-paradox without PTXF treatment (A) and with PTXF treatment (B). C and D show the effect of PTXF on alterations in LVDP and LVEDP at the different time points during Ca^{2+}-depletion and Ca^{2+}-repletion. Each group consists of 6 experiments. *$p<0.05$ vs. Ca^{2+}-paradox group.

Table 2. Effect of PTXF (100μM) on cardiac performance of Ca^{2+}-paradox heart

Group	LVDP	LVEDP	+dP/dt	-dP/dt
	mmHg		mmHg/sec	
Control	111 ± 4.1	4.5 ± 0.8	5938 ± 320	3821 ± 137
Control with PTXF	106 ± 5.5	3.1 ± 0.3	5666 ± 318	3419 ± 193
Ca^{2+} -depletion	7.3 ±1.0*	27.5 ± 2.6*	156 ± 29.4*	150 ± 32.0*
Ca^{2+}-depletion with PTXF	6.6 ±1.0*	23.3 ± 3.1*	163 ± 42.2*	158 ± 35.7*
Ca^{2+}-paradox	9.6 ± 1.5*	82.3 ± 6.0*	264 ± 53.7*	236 ± 39.6*
Ca^{2+}-paradox with PTXF	44.3 ± 11.9*#	52.5 ± 12.4*#	1912 ± 656.0*#	1259 ± 383*#

Values are mean ± SE of 6 separated experiments *$p<0.05$ vs. control group, #$p<0.05$ vs. Ca^{2+} paradox group

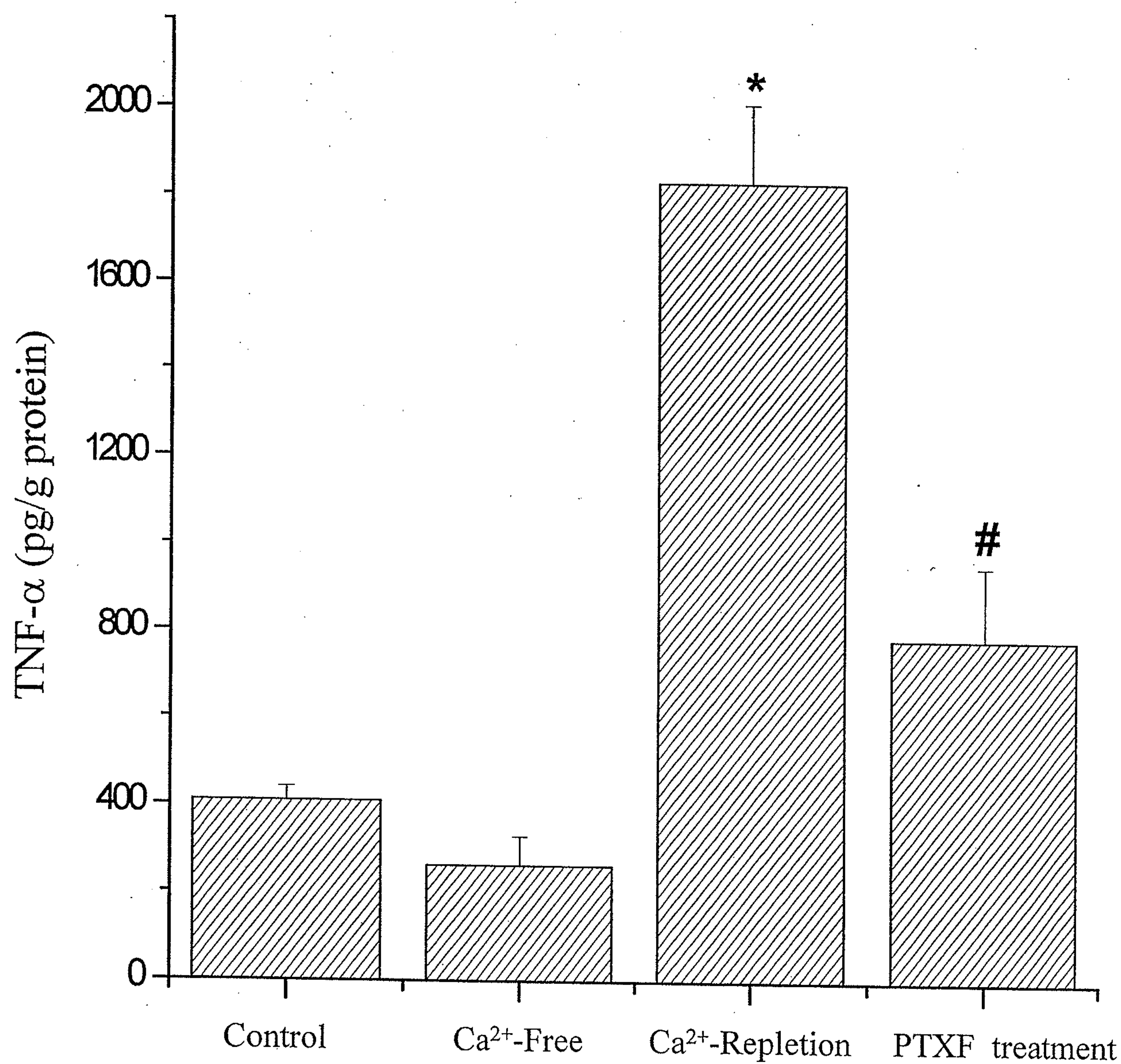

Figure 7: TNF-α protein level in myocardium subjected Ca^{2+}-paradox with or without PTXF (100μM) treatment. Ca^{2+}-Free: data from heart subjected 5 min Ca^{2+}-depletion. Data represent 6 separated experiments in each group. *$p<0.05$ vs. the control group, #$p<0.05$ vs. Ca^{2+}-paradox group.

representative Western blots of NFκB protein content and analysis of the data. It can be seen in the homogenate fraction (Figure 8A), that NFκB protein contents were significantly reduced (about 18%) in Ca^{2+}-paradox group when compared to the control group without any significant changes to PTXF treatment group. The NFκB content in the cytosolic fraction from Ca^{2+}-paradox heart were decreased by about 66% (Figure 8B), whereas that in the particulate fraction was decreased by about 25% (Figure 8C) compared to the control group. PTXF treatment significantly reversed these changes in both cytosolic and particulate fractions. The ratio of NFκB protein content in particulate fraction to cytosolic fraction in the untreated Ca^{2+}-paradox heart was about 2.2, whereas that of the PTXF treated Ca^{2+}-paradox heart was about 1.6. This indicated that PTXF prevented the redistribution of NFκB protein in the Ca^{2+}-paradox heart.

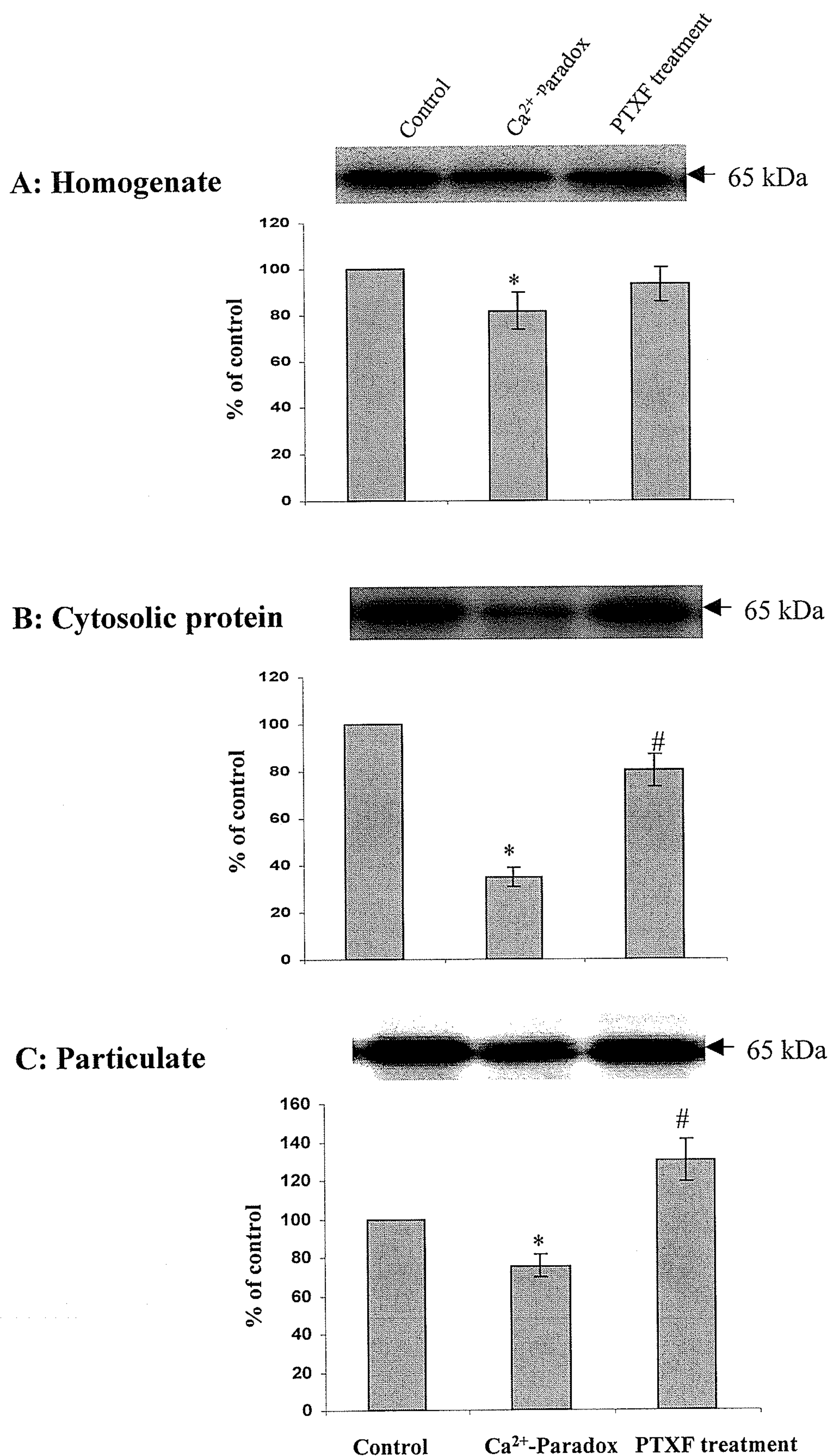

Figure 8: Western blot analysis showing alterations in the protein content of NFκB in homogenate fraction (A), cytosolic fraction (B) and particulate fraction (C) upon Ca^{2+}-paradox in heart with or without PTXF (100μM) treatment. *$p<0.05$ vs. control group, #$p<0.05$ vs. Ca^{2+}-paradox group.

V. DISCUSSION

1. Effect of PTXF on I/R-Induced Injury in the Heart

Depressed cardiac function, as a consequence of I/R injury, has been reported clinically and experimentally. Our results showing depression in cardiac function in I/R hearts are in agreement with previous reports (283). Although depressed cardiac performance due to I/R is considered to be a consequence of intracellular Ca^{2+}-overload and free radical generation, it has been suggested that the production of some cytokines, such as TNF-α, may be an important factor involved in I/R injury (24,40). On the other hand, different investigators have demonstrated that TNF-α may produce cardioprotection by enhancing myocardial tolerance to ischemia (52-54). Such conflicting effects of TNF-α in I/R are probably dependent on the absolute levels of TNF-α during I/R period, as high levels of TNF-α may produce deleterious alterations caused by I/R, thereby decreasing contractile function and inducing apoptosis (30). Other studies have also demonstrated that TNF-α may serve as an initiator for the cytokine cascade for the production of IL-6 and IL-8. They have demonstrated that as TNF-α was washed out from coronary vessels, the newly produced cytokines further reduced the peak systolic Ca^{2+} transients and contractility by increasing the production of NO, as well as a subsequent cGMP-mediated decrease in L-type Ca^{2+} channel current (32,34,48,49). Consistent with previous reports from Gurevitch et al (35), our results demonstrated that TNF-α was synthesized during reperfusion; there was a dramatic elevation at 2 min of

reperfusion. Such an increase in TNF-α may trigger the synthesis of other deleterious cytokine in the heart (48), and in turn, may worsen the I/R-induced myocardium injury. The decrease in TNF-α levels in the I/R heart after 2 min of reperfusion may be due to coronary washing out as a result of cell necrosis and lesions in cell membrane. This view is in agreement with others who have reported a marked increase in creatine kinases and other proteins in the perfusate during the reperfusion period (35). Since TNF-α is known to depress contractile function in isolated hamster trabeculae, as well as dogs and humans (24,43), it is possible that the observed increase in TNF-α protein levels at 2 min of reperfusion may play an important role in the depression of cardiac function in I/R heart.

PTXF is considered to produce beneficial effects in heart failure as well as in idiopathic dilated cardiomyopathy due to its inhibitory effect on TNF-α synthesis (272,273). Furthermore, it has been documented that PTXF protected heart and lungs during cardiopulmonary bypass surgery and protected the skeletal muscle from ischemia (274). PTXF also exhibits hemorrheologic properties (275) as it improved RBC deformability, decreased RBC aggregation and inhibited neutrophil adhesion. Treatment with PTXF was shown to render protection against cardiac dysfunction and provide an overall increase in survival (232). Although the exact mechanism for the cardioprotective action of this agent is not yet fully understood, PTXF is considered to exert beneficial effects on the ischemic heart by promoting oxygenation of the ischemic areas and lowering the amount of metabolic derangements associated with I/R injury. In this study, treatment with PTXF protected the heart from I/R injury

because it showed improved cardiac performance as compared to the I/R group. These results are consistent with a previous study with similar effects of PTXF on I/R injury (265). Since the PTXF treatment depressed of TNF-α synthesis at 2 min of reperfusion, it might be assumed that PTXF has depressed the occurrence of the first peak of TNF-α synthesis, and in turn might have probably blocked the direct deleterious effect of TNF-α in addition to subsequent production of other cytokines according to the current investigations about initiatory effect of TNF-α on deleterious cytokines synthesis (48,49). From the present study, we also found that TNF-α protein level maintained in lower level in the PTXF treatment group but higher than I/R group at 10 min and 30 min reperfusion when TNF-α might have been washed out from I/R heart due to cell necrosis and membrane lesions. Since the previous study (287) have shown that PTXF reduces capillary membrane injury and subsequent protein leakage, it is likely that PTXF treatment might block TNF-α washout after 2 min of reperfusion. Meanwhile, since the effects of TNF-α are considered to be dependent on the absolute levels of TNF-α during I/R period, it is possible that PTXF prevented the first peak of TNF-α synthesis and maintained lower levels of TNF-α, and thereby prevented the deleterious effects of this cytokine by protecting the heart from I/R injury. In addition, it also might be pointed out the PTXf may also have some TNF-α independent effect in I/R heart injury after 10 min and 30 min reperfusion.

Since NFκB inhibition is generally associated with depression of pro-

inflammatory factors gene expression and cell apoptosis (50), it is possible that interference of the activation of NFκB may be implicated in the I/R-induced heart injury. It was documented that PTXF blocked the translocation of NFκB in vascular smooth muscle cells (288). In the present study we have also obtained a similar effect of PTXF on the activation of NFκB in I/R heart. Our data have shown marked alterations of NFκB protein levels in the cytosolic and particulate fractions in the myocardium subjected to I/R injury. In view of the fact that NFκB is phosphorylated in response to diverse stimuli, as this process activates NFκB to then translocate to the nucleus and bind to a promoter that leads the gene expression of pro-inflammatory cytokines. Thus, it is possible that this distribution change of NFκB in the I/R heart may indicate NFκB activation and translocation. It may also be noted that PTXF treatment partially reversed this redistribution of NFκB protein content in particulate fraction and cytosolic fraction as compared to the I/R group. This suggests there were dramatic redistribution changes between cytosolic and particulate parts in the I/R myocardium, and PTXF treatment partially reversed this alteration, even though the total NFκB protein levels in both groups were decreased when compared to control group. This effect of PTXF on this NFκB redistribution suggests that PTXF inhibits the NFκB activation in response to I/R injury. Although PTXF has been reported to inhibit NFκB activity in the tumour cell (289), intestinal epithelial cells (290), T lymphocytes and hepatic satellite cells (291,292), the data presented here show for the first time that PTXF blocks the activation of NFκB in myocardium when subjected to I/R injury. Therefore, it is proposed that NFκB may be an important

factor involved in the mechanism of the beneficial effects of PTXF on the improvement of cardiac performance in I/R heart injury.

2. Effect of PTXF on Ca^{2+}-Paradox Induced Injury

Recent studies have demonstrated that intracellular Ca^{2+}-overload is a major cause of myocardial cell damage and cardiac dysfunction in ischemic heart diseases. The intracellular Ca^{2+}-overload evoked by post-ischemic reperfusion is associated with irreversible injury such as ultrastructural changes, enzyme leakage, membrane damage, and reduction in ATP production by mitochondria, and increased infarct size (14). The role of intracellular Ca^{2+}-overload in cardiac dysfunction may be further extended to I/R-induced arrhythmias, especially ventricular tachycardia and ventricular fibrillation, which are the major cause of sudden cardiac death. Therefore, in view of the important role played by intracellular Ca^{2+}-overload in the development of ischemic injury, the Ca^{2+}-paradox heart has been used as an experimental model for understanding the correlation between the disturbance of Ca^{2+} homeostasis and other pathogenic variations that are involved in the pathogenesis of I/R injury. Several studies have been conducted to understand the mechanism of this disturbance in Ca^{2+} homeostasis, and some therapeutic strategies have been developed to determine whether the Ca^{2+}-related mechanisms were involved in their protective effect on I/R injury (283,285,293,294). To gain some information regarding the effect of PTXF on disturbance of Ca^{2+} homeostasis in the heart, we investigated the effect of PTXF in the heart subjected to Ca^{2+}-paradox. The results showed that (i) the synthesis

of TNF-α was increased and NFκB was activated in Ca^{2+}-paradox induced heart injury; (ii) PTXF improved cardiac function in Ca^{2+}-paradox by blocking the activation of NFκB and depressing the synthesis of TNF-α in myocardium.

It is pointed out that Ca^{2+}-paradox was associated with a loss of contractile function, which is in agreement with previous studies (283,285,293,294). Furthermore, PTXF (100 μM) was observed to partially improve the heart function in the Ca^{2+}-paradox heart. These beneficial effects on Ca^{2+}-paradox heart injury support the view that PTXF not only protects the heart from I/R injury, but is also a general cardioprotective agent in terms of preventing the occurrence of intracellular Ca^{2+}-overload. Accordingly, it is suggested that Ca^{2+}-related mechanisms may play a crucial role in the beneficial effects of PTXF against I/R injury.

It is now well known that the myocardium possesses molecular mechanisms to produce increased amounts of TNF-α proteins under certain conditions such as heart failure, cardiomyopathy and cardiopulmonary bypass surgery (272,273,295). The results from the I/R model have indicated that I/R in isolated rat heart induces TNF-α production after 2 min of reperfusion. Some investigators have reported that TNF-α production is closely related to oxidative stress (31-35) for causing negative inotropic effects by influencing Ca^{2+} homeostasis (24,43,50); there also occurs a disruption in the excitation-contraction coupling and desensitization of the β-receptors (40). It has been documented that early contractile depression, induced by TNF-α is mediated by sphingosine, which is an endogenous second messenger (44). Furthermore, TNF-α is also known to promote the production of nitric oxide (NO), which reduces the

myofilament sensitivity to Ca^{2+} and mediates the late contractile dysfunction (45). Although it has been reported that TNF-α produces negative inotropic effects by influencing Ca^{2+} homeostasis, very little information is available regarding the disturbance of Ca^{2+} homeostasis and its contribution to the production of TNF-α in cardiomyocytes. In the present study, we reported that TNF-α was produced by the myocardium subjected to I/R. Interestingly, our results also demonstrated that TNF-α was produced after 5 min Ca^{2+}-depletion and during 30 min Ca^{2+}-repletion period. While no changes in TNF-α content were seen due to Ca^{2+}- depletions, a marked increase in the synthesis of TNF-α was seen upon induction of intracellular Ca^{2+}-overload. Thus, it is proposed that intracellular Ca^{2+}-overload in I/R may play an important role in the production of TNF-α in addition to oxidative stress which is considered as a major trigger for the synthesis of TNF-α following I/R injury (57).

NFκB, a mediator for the production of TNF-α, is considered to be activated by different pathways that are induced by I/R, especially oxidative stress (57,59-61). Since the level of TNF-α was increased following the disturbance of Ca^{2+} homeostasis in Ca^{2+}- paradox heart, it is likely that NFκB may be involved in this pathophysiological condition. In the present study, it was found that Ca^{2+}-paradox stimulated the distribution of NFκB from cytosolic to particulate fraction and this may be a mechanism that increased the production of TNF-α in the heart subjected to Ca^{2+}-paradox. Although Ca^{2+} has been shown to play an important role in the activation of some transcription factors like NFκB in kidney and lymphocyte (296-298), the present study is first to report the redistribution of NFκB due to intracellular

Ca^{2+}-overload in heart. Since PTXF treatment significantly reversed the activation of NFκB induced by Ca^{2+}-paradox, it appears the anti-TNF-α effect of PTXF in Ca^{2+}-paradox heart may also be related to blocking the activation of NFκB.

VI. CONCLUSIONS

The data presented in this study have demonstrated the protective effect of PTXF in the isolated crystalloid-perfused rat heart subjected to I/R and Ca^{2+}-paradox. In view of the depression of TNF-α production and inhibition of NFκB activation by PTXF treatment, it may be assumed that this protective effect has resulted from the inhibition of the activation of NFκB, and the reduction of the production of TNF-α in the I/R heart at 2 min reperfusion. Furthermore, our study is the first to demonstrate that the beneficial effects of PTXF may be Ca^{2+}-related, which plays an important role in modulating the recovery of cardiac performance in I/R heart. There also appears a close correlation between Ca^{2+} homeostasis as well as TNF-α synthesis and NFκB activation. Our results therefore suggest that the administration of PTXF during surgical interventions may prove useful in the clinical management of cardiovascular diseases.

The following conclusions are drawn from this study:

(i) Both I/R and Ca^{2+}-paradox increase TNF-α synthesis and produce NFκB activation in rat heart.

(ii) PTXF attenuates I/R induced cardiac dysfunction, possibly by depressing the occurrence of the first peak TNF-α synthesis and by blocking the activation of NFκB.

(iii) PTXF attenuate Ca^{2+}-paradox induced heart injury likely due to depressing the synthesis of TNF-α and blocking the redistribution of

NFκB.

(iv) Intracellular Ca^{2+}-overload may play a role in TNF-α production and NFκB activation in both I/R and Ca^{2+}-paradox hearts.

VII. REFERENCES

1. Opie, L. H. Myocardial Reperfusion: Calcium and Free Radicals. The Heart Physiology and Metabolism. New York: Raven Press, 469-484, 1992.

2. Ferrari, R., C. Ceconi, S. Curello, O. Alfieri, and O. Visioli. Myocardial damage during ischaemia and reperfusion. *Eur. Heart J.* 14 (Suppl G): 25-30, 1993.

3. Brennan, J. Reperfusion injury of cardiac myocytes: mechanisms, treatment, and implications for advanced practice nursing. *AACN Clin. Issues* 11: 252-260, 2000.

4. Piper, H. M., D. Garcia-Dorado, and M. Ovize. A fresh look at reperfusion injury. *Cardiovasc. Res.* 38: 291-300, 1998.

5. Pierce, G. N. and M. P. Czubryt. The contribution of ionic imbalance to ischemia/reperfusion-induced injury. *J. Mol. Cell. Cardiol.* 27: 53-63, 1995.

6. Ytrehus, K. The ischemic heart--experimental models. *Pharmacol. Res.* 42: 193-203, 2000.

7. Canadian Task Force for Cardiovascular Science. A Joint Initiative of the Heart and Stroke Foundation of Canada and the Canadian Cardiovascular Society. *Can. J. Cardiol.* 9: 699-735, 1993.

8. Egan, B. M. and D. T. Lackland. Strategies for cardiovascular disease prevention: importance of public and community health programs. *Ethn. Dis.* 8: 228-239, 1998.

9. Chagas, A. C., F. Pileggi, E. A. Lopes, and P. L. Da Luz. Myocardial protection in the occlusion/reperfusion dog model: the role of ischemic necrosis vs reperfusion injury. *Braz. J. Med. Biol. Res.* 28: 951-959, 1995.

10. Katz, A. M. Excitation-contraction coupling: Calcium and other ion fluxes across the plasma membrane. In: M. Laplante, ed. Physiology of the Heart. Philadelphia: Lippincott Williams & Wilkins, 216-240, 2001.

11. Baltas, L. G., P. Karczewski, and E. G. Krause. The cardiac sarcoplasmic reticulum phospholamban kinase is a distinct delta-CaM kinase isozyme. *FEBS Lett.* 373: 71-75, 1995.

12. Hawkins, C., A. Xu, and N. Narayanan. Sarcoplasmic reticulum calcium pump in cardiac and slow twitch skeletal muscle but not fast twitch skeletal muscle undergoes phosphorylation by endogenous and exogenous Ca^{2+}/calmodulin-dependent protein kinase. Characterization of optimal conditions for calcium pump phosphorylation. *J. Biol. Chem.* 269: 31198-31206, 1994.

13. Xu, A., C. Hawkins, and N. Narayanan. Phosphorylation and activation of the Ca^{2+}-pumping ATPase of cardiac sarcoplasmic reticulum by Ca^{2+}/calmodulin-dependent protein kinase. *J. Biol. Chem.* 268: 8394-8397, 1993.

14. Nayler, W. G. Basic mechanisms involved in the protection of the ischaemic myocardium. The role of calcium antagonists. *Drugs* 42 (Suppl 2): 21-27, 1991.

15. Tani, M. Mechanisms of Ca^{2+} overload in reperfused ischemic myocardium. *Annu. Rev. Physiol.* 52: 543-559, 1990.

16. Schumacher, C. A., A. Baartscheer, R. Coronel, and J. W. Fiolet. Energy-dependent transport of calcium to the extracellular space during acute ischemia of the rat heart. *J. Mol. Cell. Cardiol.* 30: 1631-1642, 1998.

17. van Echteld, C. J., J. H. Kirkels, M. H. Eijgelshoven, M. P. van der, and T. J. Ruigrok. Intracellular sodium during ischemia and calcium-free perfusion: a 23Na NMR study. *J. Mol. Cell. Cardiol.* 23: 297-307, 1991.

18. Marban, E., Y. Koretsune, M. Corretti, V. P. Chacko, and H. Kusuoka. Calcium and its role in myocardial cell injury during ischemia and reperfusion. *Circulation* 80: IV17-IV22, 1989.

19. Wu, J. and P. B. Corr. Influence of long-chain acylcarnitines on voltage-dependent calcium current in adult ventricular myocytes. *Am. J. Physiol. Heart Circ. Physiol.* 263: H410-H417, 1992.

20. Dhalla, N. S., V. Elimban, and H. Rupp. Paradoxical role of lipid metabolism in heart function and dysfunction. *Mol. Cell. Biochem.* 116: 3-9, 1992.

21. Mukherjee, A., T. M. Wong, L. M. Buja, R. J. Lefkowitz, and J. T. Willerson. Beta adrenergic and muscarinic cholinergic receptors in canine myocardium. Effects of ischemia. *J. Clin. Invest.* 64: 1423-1428, 1979.

22. Yoshida, Y., T. Shiga, and S. Imai. Degradation of sarcoplasmic reticulum calcium-pumping ATPase in ischemic-reperfused myocardium: role of calcium-activated neutral protease. *Basic Res. Cardiol.* 85: 495-507, 1990.

23. Rardon, D. P., D. C. Cefali, R. D. Mitchell, S. M. Seiler, D. R. Hathaway, and L. R. Jones. Digestion of cardiac and skeletal muscle junctional sarcoplasmic

reticulum vesicles with calpain II. Effects on the Ca^{2+} release channel. *Circ. Res.* 67: 84-96, 1990.

24. Cain, B. S., D. R. Meldrum, C. A. Dinarello, X. Meng, A. Banerjee, and A. H. Harken. Adenosine reduces cardiac TNF-alpha production and human myocardial injury following ischemia-reperfusion. *J. Surg. Res.* 76: 117-123, 1998.

25. Guillen, I., M. Blanes, M. J. Gomez-Lechon, and J. V. Castell. Cytokine signaling during myocardial infarction: sequential appearance of IL-1 beta and IL-6. *Am. J. Physiol.* 269: R229-R235, 1995.

26. Goldman, A. S. and Prabhakar, B. S. Immunology overview. In: S. Baron, ed. Medical Microbiology. Galveston: The University of Texas Medical Branch at Galveston, 35-37, 1996.

27. Dhote-Burger, P., A. Vuilleminot, T. Lecompte, C. Pasquier, L. Bara, P. Julia, C. Chardigny, and J. N. Fabiani. Neutrophil degranulation related to the reperfusion of ischemic human heart during cardiopulmonary bypass. *J. Cardiovasc. Pharmacol.* 25 (Suppl 2): S124-S129, 1995.

28. Herskowitz, A., S. Choi, A. A. Ansari, and S. Wesselingh. Cytokine mRNA expression in postischemic/reperfused myocardium. *Am. J. Pathol.* 146: 419-428, 1995.

29. Marx, N., F. J. Neumann, I. Ott, M. Gawaz, W. Koch, T. Pinkau, and A. Schomig. Induction of cytokine expression in leukocytes in acute myocardial infarction. *J. Am. Coll. Cardiol.* 30: 165-170, 1997.

30. Sack, M. Tumor necrosis factor-alpha in cardiovascular biology and the potential role for anti-tumor necrosis factor-alpha therapy in heart disease. *Pharmacol. Ther.* 94: 123-135, 2002.

31. Torre-Amione, G., S. Kapadia, J. Lee, R. D. Bies, R. Lebovitz, and D. L. Mann. Expression and functional significance of tumor necrosis factor receptors in human myocardium. *Circulation* 92: 1487-1493, 1995.

32. Meldrum, D. R., J. C. Cleveland, Jr., B. S. Cain, X. Meng, and A. H. Harken. Increased myocardial tumor necrosis factor-alpha in a crystalloid-perfused model of cardiac ischemia-reperfusion injury. *Ann. Thorac. Surg.* 65: 439-443, 1998.

33. Shames, B. D., H. H. Barton, L. L. Reznikov, C. B. Cairns, A. Banerjee, A. H. Harken, and X. Meng. Ischemia alone is sufficient to induce TNF-alpha mRNA and peptide in the myocardium. *Shock* 17: 114-119, 2002.

34. Meldrum, D. R., X. Meng, C. A. Dinarello, A. Ayala, B. S. Cain, B. D. Shames, L. Ao, A. Banerjee, and A. H. Harken. Human myocardial tissue TNFalpha expression following acute global ischemia in vivo. *J. Mol. Cell. Cardiol.* 30: 1683-1689, 1998.

35. Gurevitch, J., I. Frolkis, Y. Yuhas, Y. Paz, M. Matsa, R. Mohr, and V. Yakirevich. Tumor necrosis factor-alpha is released from the isolated heart undergoing ischemia and reperfusion. *J. Am. Coll. Cardiol.* 28: 247-252, 1996.

36. Cain, B. S., A. H. Harken, and D. R. Meldrum. Therapeutic strategies to reduce TNF-alpha mediated cardiac contractile depression following ischemia

and reperfusion. *J. Mol. Cell. Cardiol.* 31: 931-947, 1999.

37. Bergman, M. R. and B. J. Holycross. Pharmacological modulation of myocardial tumor necrosis factor alpha production by phosphodiesterase inhibitors. *J. Pharmacol. Exp. Ther.* 279: 247-254, 1996.

38. Wagner, D. R., C. McTiernan, V. J. Sanders, and A. M. Feldman. Adenosine inhibits lipopolysaccharide-induced secretion of tumor necrosis factor-alpha in the failing human heart. *Circulation* 97: 521-524, 1998.

39. Peng, J., J. Xiao, F. Ye, H. W. Deng, and Y. J. Li. Inhibition of cardiac tumor necrosis factor-alpha production by calcitonin gene-related peptide-mediated ischemic preconditioning in isolated rat hearts. *Eur. J. Pharmacol.* 407: 303-308, 2000.

40. Meldrum, D. R., C. A. Dinarello, B. D. Shames, J. C. Cleveland, Jr., B. S. Cain, A. Banerjee, X. Meng, and A. H. Harken. Ischemic preconditioning decreases postischemic myocardial tumor necrosis factor-alpha production. Potential ultimate effector mechanism of preconditioning. *Circulation* 98: II214-II218, 1998.

41. Cain, B. S., D. R. Meldrum, X. Meng, C. A. Dinarello, B. D. Shames, A. Banerjee, and A. H. Harken. p38 MAPK inhibition decreases TNF-alpha production and enhances postischemic human myocardial function. *J. Surg. Res.* 83: 7-12, 1999.

42. Gurevitch, J., I. Frolkis, Y. Yuhas, B. Lifschitz-Mercer, E. Berger, Y. Paz, M. Matsa, A. Kramer, and R. Mohr. Anti-tumor necrosis factor-alpha improves

myocardial recovery after ischemia and reperfusion. *J. Am. Coll. Cardiol.* 30: 1554-1561, 1997.

43. Finkel, M. S., C. V. Oddis, T. D. Jacob, S. C. Watkins, B. G. Hattler, and R. L. Simmons. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science* 257: 387-389, 1992.

44. Mathias, S., K. A. Dressler, and R. N. Kolesnick. Characterization of a ceramide-activated protein kinase: stimulation by tumor necrosis factor alpha. *Proc. Natl. Acad. Sci. USA* 88: 10009-10013, 1991.

45. Goldhaber, J. I., K. H. Kim, P. D. Natterson, T. Lawrence, P. Yang, and J. N. Weiss. Effects of TNF-alpha on $[Ca^{2+}]_i$ and contractility in isolated adult rabbit ventricular myocytes. *Am. J. Physiol. Heart Circ. Physiol.* 271: H1449-H1455, 1996.

46. Krown, K. A., M. T. Page, C. Nguyen, D. Zechner, V. Gutierrez, K. L. Comstock, C. C. Glembotski, P. J. Quintana, and R. A. Sabbadini. Tumor necrosis factor alpha-induced apoptosis in cardiac myocytes. Involvement of the sphingolipid signaling cascade in cardiac cell death. *J. Clin. Invest.* 98: 2854-2865, 1996.

47. Vandenabeele, P., W. Declercq, B. Vanhaesebroeck, J. Grooten, and W. Fiers. Both TNF receptors are required for TNF-mediated induction of apoptosis in PC60 cells. *J. Immunol.* 154: 2904-2913, 1995.

48. Wan, S., J. M. DeSmet, L. Barvais, M. Goldstein, J. L. Vincent, and J. L. LeClerc. Myocardium is a major source of proinflammatory cytokines in

patients undergoing cardiopulmonary bypass. *J. Thorac. Cardiovasc. Surg.* 112: 806-811, 1996.

49. Hennein, H. A., H. Ebba, J. L. Rodriguez, S. H. Merrick, F. M. Keith, M. H. Bronstein, J. M. Leung, D. T. Mangano, L. J. Greenfield, and J. S. Rankin. Relationship of the proinflammatory cytokines to myocardial ischemia and dysfunction after uncomplicated coronary revascularization. *J. Thorac. Cardiovasc. Surg.* 108: 626-635, 1994.

50. Meldrum, D. R. Tumor necrosis factor in the heart. *Am. J. Physiol* 274: R577-R595, 1998.

51. Maekawa, N., H. Wada, T. Kanda, T. Niwa, Y. Yamada, K. Saito, H. Fujiwara, K. Sekikawa, and M. Seishima. Improved myocardial ischemia/reperfusion injury in mice lacking tumor necrosis factor-alpha. *J. Am. Coll. Cardiol.* 39: 1229-1235, 2002.

52. Zhao, T. C., M. M. Taher, K. C. Valerie, and R. C. Kukreja. p38 Triggers late preconditioning elicited by anisomycin in heart: involvement of NF-kappaB and iNOS. *Circ. Res.* 89: 915-922, 2001.

53. Sumeray, M. S., D. D. Rees, and D. M. Yellon. Infarct size and nitric oxide synthase in murine myocardium. *J. Mol. Cell. Cardiol.* 32: 35-42, 2000.

54. Smith, R. M., N. Suleman, J. McCarthy, and M. N. Sack. Classic ischemic but not pharmacologic preconditioning is abrogated following genetic ablation of the TNFalpha gene. *Cardiovasc. Res.* 55: 553-560, 2002.

55. Nelson, S. K., G. H. Wong, and J. M. McCord. Leukemia inhibitory factor and

tumor necrosis factor induce manganese superoxide dismutase and protect rabbit hearts from reperfusion injury. *J. Mol. Cell. Cardiol.* 27: 223-229, 1995.

56. Lecour, S., R. M. Smith, B. Woodward, L. H. Opie, L. Rochette, and M. N. Sack. Identification of a novel role for sphingolipid signaling in TNF alpha and ischemic preconditioning mediated cardioprotection. *J. Mol. Cell. Cardiol.* 34: 509-518, 2002.

57. Bowie, A. and L. A. O'Neill. Oxidative stress and nuclear factor-kappaB activation: a reassessment of the evidence in the light of recent discoveries. *Biochem. Pharmacol.* 59: 13-23, 2000.

58. Valen, G., Z. Q. Yan, and G. K. Hansson. Nuclear factor kappa-B and the heart. *J. Am. Coll. Cardiol.* 38: 307-314, 2001.

59. Li, C., W. Browder, and R. L. Kao. Early activation of transcription factor NF-kappaB during ischemia in perfused rat heart. *Am. J. Physiol. Heart Circ. Physiol.* 276: H543-H552, 1999.

60. Chandrasekar, B. and G. L. Freeman. Induction of nuclear factor kappaB and activation protein 1 in postischemic myocardium. *FEBS Lett.* 401: 30-34, 1997.

61. Shimizu, N., M. Yoshiyama, T. Omura, A. Hanatani, S. Kim, K. Takeuchi, H. Iwao, and J. Yoshikawa. Activation of mitogen-activated protein kinases and activator protein-1 in myocardial infarction in rats. *Cardiovasc. Res.* 38: 116-124, 1998.

62. Mann, D. L. Stress activated cytokines and the heart. *Cytokine Growth Factor*

Rev. 7: 341-354, 1996.

63. Fan, H., B. Sun, Q. Gu, A. Lafond-Walker, S. Cao, and L. C. Becker. Oxygen radicals trigger activation of NF-kappaB and AP-1 and upregulation of ICAM-1 in reperfused canine heart. *Am. J. Physiol. Heart Circ. Physiol.* 282: H1778-H1786, 2002.

64. Yang, J., J. J. Marden, C. Fan, S. Sanlioglu, R. M. Weiss, T. C. Ritchie, R. L. Davisson, and J. F. Engelhardt. Genetic redox preconditioning differentially modulates AP-1 and NFkappaB responses following cardiac ischemia/reperfusion injury and protects against necrosis and apoptosis. *Mol. Ther.* 7: 341-353, 2003.

65. Cargnoni, A., C. Ceconi, G. Gaia, L. Agnoletti, and R. Ferrari. Cellular thiols redox status: a switch for NF-kappaB activation during myocardial post-ischaemic reperfusion. *J. Mol. Cell. Cardiol.* 34: 997-1005, 2002.

66. Suzuki, Y. J. and L. Packer. Inhibition of NF-kappa B DNA binding activity by alpha-tocopheryl succinate. *Biochem. Mol. Biol. Int.* 31: 693-700, 1993.

67. Suzuki, Y. J., B. B. Aggarwal, and L. Packer. Alpha-lipoic acid is a potent inhibitor of NF-kappa B activation in human T cells. *Biochem. Biophys. Res. Commun.* 189: 1709-1715, 1992.

68. Pye, J., F. Ardeshirpour, A. McCain, D. A. Bellinger, E. Merricks, J. Adams, P. J. Elliott, C. Pien, T. H. Fischer, A. S. Baldwin, Jr., and T. C. Nichols. Proteasome inhibition ablates activation of NF-kappa B in myocardial reperfusion and reduces reperfusion injury. *Am. J. Physiol. Heart Circ.*

Physiol. 284: H919-H926, 2003.

69. Bretschneider, E., M. Wittpoth, A. A. Weber, E. Glusa, and K. Schror. Activation of NFkappaB is essential but not sufficient to stimulate mitogenesis of vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.* 235: 365-368, 1997.
70. Bellas, R. E., J. S. Lee, and G. E. Sonenshein. Expression of a constitutive NF-kappa B-like activity is essential for proliferation of cultured bovine vascular smooth muscle cells. *J. Clin. Invest.* 96: 2521-2527, 1995.
71. Thourani, V. H., S. S. Brar, T. P. Kennedy, L. R. Thornton, J. A. Watts, R. S. Ronson, Z. Q. Zhao, A. L. Sturrock, J. R. Hoidal, and J. Vinten-Johansen. Nonanticoagulant heparin inhibits NF-kappaB activation and attenuates myocardial reperfusion injury. *Am. J. Physiol. Heart Circ. Physiol.* 278: H2084-H2093, 2000.
72. Xuan, Y. T., X. L. Tang, S. Banerjee, H. Takano, R. C. Li, H. Han, Y. Qiu, J. J. Li, and R. Bolli. Nuclear factor-kappaB plays an essential role in the late phase of ischemic preconditioning in conscious rabbits. *Circ. Res.* 84: 1095-1109, 1999.
73. Maulik, N., M. Sato, B. D. Price, and D. K. Das. An essential role of NFkappaB in tyrosine kinase signaling of p38 MAP kinase regulation of myocardial adaptation to ischemia. *FEBS Lett.* 429: 365-369, 1998.
74. Marber, M. S., D. S. Latchman, J. M. Walker, and D. M. Yellon. Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated

with resistance to myocardial infarction. *Circulation* 88: 1264-1272, 1993.

75. Zhao, T., L. Xi, J. Chelliah, J. E. Levasseur, and R. C. Kukreja. Inducible nitric oxide synthase mediates delayed myocardial protection induced by activation of adenosine A(1) receptors: evidence from gene-knockout mice. *Circulation* 102: 902-907, 2000.

76. Morgan, E. N., E. M. Boyle, Jr., W. Yun, J. M. Griscavage-Ennis, A. L. Farr, T. G. Canty, Jr., T. H. Pohlman, and E. D. Verrier. An essential role for NF-kappaB in the cardioadaptive response to ischemia. *Ann. Thorac. Surg.* 68: 377-382, 1999.

77. Bach, F. H., W. W. Hancock, and C. Ferran. Protective genes expressed in endothelial cells: a regulatory response to injury. *Immunol. Today* 18: 483-486, 1997.

78. Roberts, A. B. and M. B. Sporn. Physiological actions and clinical applications of transforming growth factor-beta (TGF-beta). *Growth Factors* 8: 1-9, 1993.

79. Yellon, D. M. and G. F. Baxter. Reperfusion injury revisited: is there a role for growth factor signaling in limiting lethal reperfusion injury? *Trends Cardiovasc. Med.* 9: 245-249, 1999.

80. Mehta, J. L., B. C. Yang, B. S. Strates, and P. Mehta. Role of TGF-beta1 in platelet-mediated cardioprotection during ischemia-reperfusion in isolated rat hearts. *Growth Factors* 16: 179-190, 1999.

81. Lefer, A. M., P. Tsao, N. Aoki, and M. A. Palladino, Jr. Mediation of cardioprotection by transforming growth factor-beta. *Science* 249: 61-64,

1990.

82. Lefer, A. M., X. L. Ma, A. S. Weyrich, and R. Scalia. Mechanism of the cardioprotective effect of transforming growth factor beta 1 in feline myocardial ischemia and reperfusion. *Proc. Natl. Acad. Sci. USA* 90: 1018-1022, 1993.

83. Baxter, G. F., M. M. Mocanu, B. K. Brar, D. S. Latchman, and D. M. Yellon. Cardioprotective effects of transforming growth factor-beta1 during early reoxygenation or reperfusion are mediated by p42/p44 MAPK. *J. Cardiovasc. Pharmacol.* 38: 930-939, 2001.

84. Chen, H., D. Li, T. Saldeen, and J. L. Mehta. TGF-$beta_1$ modulates NOS expression and phosphorylation of Akt/PKB in rat myocytes exposed to hypoxia-reoxygenation. *Am. J. Physiol. Heart Circ. Physiol.* 281: H1035-H1039, 2001.

85. Grunenfelder, J., D. N. Miniati, S. Murata, V. Falk, E. G. Hoyt, and R. C. Robbins. Up-regulation of Bcl-2 through hyperbaric pressure transfection of TGF-beta1 ameliorates ischemia-reperfusion injury in rat cardiac allografts. *J. Heart Lung Transplant.* 21: 244-250, 2002.

86. Mehta, J. L., H. J. Chen, and D. Y. Li. Protection of myocytes from hypoxia-reoxygenation injury by nitric oxide is mediated by modulation of transforming growth factor-beta1. *Circulation* 105: 2206-2211, 2002.

87. Chen, H., D. Li, T. Saldeen, and J. L. Mehta. TGF-beta 1 attenuates myocardial ischemia-reperfusion injury via inhibition of upregulation of

MMP-1. *Am. J. Physiol. Heart Circ. Physiol.* 284: H1612-H1617, 2003.

88. Weisensee, D., J. Bereiter-Hahn, W. Schoeppe, and I. Low-Friedrich. Effects of cytokines on the contractility of cultured cardiac myocytes. *Int. J. Immunopharmacol.* 15: 581-587, 1993.

89. Evans, H. G., M. J. Lewis, and A. M. Shah. Interleukin-1 beta modulates myocardial contraction via dexamethasone sensitive production of nitric oxide. *Cardiovasc. Res.* 27: 1486-1490, 1993.

90. Oyama, J., H. Shimokawa, H. Momii, X. Cheng, N. Fukuyama, Y. Arai, K. Egashira, H. Nakazawa, and A. Takeshita. Role of nitric oxide and peroxynitrite in the cytokine-induced sustained myocardial dysfunction in dogs in vivo. *J. Clin. Invest.* 101: 2207-2214, 1998.

91. Stangl, V., G. Baumann, K. Stangl, and S. B. Felix. Negative inotropic mediators released from the heart after myocardial ischaemia-reperfusion. *Cardiovasc. Res.* 53: 12-30, 2002.

92. LaPointe, M. C. and E. Isenovic. Interleukin-1beta regulation of inducible nitric oxide synthase and cyclooxygenase-2 involves the p42/44 and p38 MAPK signaling pathways in cardiac myocytes. *Hypertension* 33: 276-282, 1999.

93. Schulz, R., E. Nava, and S. Moncada. Induction and potential biological relevance of a Ca^{2+}-independent nitric oxide synthase in the myocardium. *Br. J. Pharmacol.* 105: 575-580, 1992.

94. Pinsky, D. J., B. Cai, X. Yang, C. Rodriguez, R. R. Sciacca, and P. J. Cannon.

The lethal effects of cytokine-induced nitric oxide on cardiac myocytes are blocked by nitric oxide synthase antagonism or transforming growth factor beta. *J. Clin. Invest.* 95: 677-685, 1995.

95. Brown, J. M., C. W. White, L. S. Terada, M. A. Grosso, P. F. Shanley, D. W. Mulvin, A. Banerjee, G. J. Whitman, A. H. Harken, and J. E. Repine. Interleukin 1 pretreatment decreases ischemia/reperfusion injury. *Proc. Natl. Acad. Sci. USA* 87: 5026-5030, 1990.
96. Thaik, C. M., A. Calderone, N. Takahashi, and W. S. Colucci. Interleukin-1 beta modulates the growth and phenotype of neonatal rat cardiac myocytes. *J. Clin. Invest.* 96: 1093-1099, 1995.
97. McTiernan, C. F., B. H. Lemster, C. Frye, S. Brooks, A. Combes, and A. M. Feldman. Interleukin-1 beta inhibits phospholamban gene expression in cultured cardiomyocytes. *Circ. Res.* 81: 493-503, 1997.
98. Maulik, N., R. M. Engelman, Z. Wei, D. Lu, J. A. Rousou, and D. K. Das. Interleukin-1 alpha preconditioning reduces myocardial ischemia reperfusion injury. *Circulation* 88: II387-II394, 1993.
99. Nogae, C., N. Makino, T. Hata, I. Nogae, S. Takahashi, K. Suzuki, N. Taniguchi, and T. Yanaga. Interleukin 1 alpha-induced expression of manganous superoxide dismutase reduces myocardial reperfusion injury in the rat. *J. Mol. Cell Cardiol.* 27: 2091-2099, 1995.
100. Wollert, K. C. and H. Drexler. The role of interleukin-6 in the failing heart. *Heart Fail. Rev.* 6: 95-103, 2001.

101. Miyao, Y., H. Yasue, H. Ogawa, I. Misumi, T. Masuda, T. Sakamoto, and E. Morita. Elevated plasma interleukin-6 levels in patients with acute myocardial infarction. *Am. Heart J.* 126: 1299-1304, 1993.

102. Chandrasekar, B., D. H. Mitchell, J. T. Colston, and G. L. Freeman. Regulation of CCAAT/Enhancer binding protein, interleukin-6, interleukin-6 receptor, and gp130 expression during myocardial ischemia/reperfusion. *Circulation* 99: 427-433, 1999.

103. Gwechenberger, M., L. H. Mendoza, K. A. Youker, N. G. Frangogiannis, C. W. Smith, L. H. Michael, and M. L. Entman. Cardiac myocytes produce interleukin-6 in culture and in viable border zone of reperfused infarctions. *Circulation* 99: 546-551, 1999.

104. Kukielka, G. L., K. A. Youker, L. H. Michael, A. G. Kumar, C. M. Ballantyne, C. W. Smith, and M. L. Entman. Role of early reperfusion in the induction of adhesion molecules and cytokines in previously ischemic myocardium. *Mol. Cell. Biochem.* 147: 5-12, 1995.

105. Finkel, M. S., R. A. Hoffman, L. Shen, C. V. Oddis, R. L. Simmons, and B. G. Hattler. Interleukin-6 (IL-6) as a mediator of stunned myocardium. *Am. J. Cardiol.* 71: 1231-1232, 1993.

106. Kinugawa, K., T. Takahashi, O. Kohmoto, A. Yao, T. Aoyagi, S. Momomura, Y. Hirata, and T. Serizawa. Nitric oxide-mediated effects of interleukin-6 on $[Ca^{2+}]_i$ and cell contraction in cultured chick ventricular myocytes. *Circ. Res.* 75: 285-295, 1994.

107. Kukielka, G. L., C. W. Smith, A. M. Manning, K. A. Youker, L. H. Michael, and M. L. Entman. Induction of interleukin-6 synthesis in the myocardium. Potential role in postreperfusion inflammatory injury. *Circulation* 92: 1866-1875, 1995.

108. Stephanou, A., B. Brar, R. Heads, R. D. Knight, M. S. Marber, D. Pennica, and D. S. Latchman. Cardiotrophin-1 induces heat shock protein accumulation in cultured cardiac cells and protects them from stressful stimuli. *J. Mol. Cell. Cardiol.* 30: 849-855, 1998.

109. Latchman, D. S. Cardiotrophin-1 (CT-1): a novel hypertrophic and cardioprotective agent. *Int. J. Exp. Pathol.* 80: 189-196, 1999.

110. Oz, M. C., H. Liao, Y. Naka, A. Seldomridge, D. N. Becker, R. E. Michler, C. R. Smith, E. A. Rose, D. M. Stern, and D. J. Pinsky. Ischemia-induced interleukin-8 release after human heart transplantation. A potential role for endothelial cells. *Circulation* 92: II428-II432, 1995.

111. Abe, Y., M. Kawakami, M. Kuroki, T. Yamamoto, M. Fujii, H. Kobayashi, T. Yaginuma, A. Kashii, M. Saito, and K. Matsushima. Transient rise in serum interleukin-8 concentration during acute myocardial infarction. *Br. Heart J.* 70: 132-134, 1993.

112. Huber, A. R., S. L. Kunkel, R. F. Todd, III, and S. J. Weiss. Regulation of transendothelial neutrophil migration by endogenous interleukin-8. *Science* 254: 99-102, 1991.

113. Isbir, C. S., R. Dogan, M. Demircin, I. Yaylim, and I. Pasaoglu. Aprotinin

reduces the IL-8 after coronary artery bypass grafting. *Cardiovasc. Surg.* 9: 403-406, 2001.

114. Moser, R., L. Olgiati, M. Patarroyo, and J. Fehr. Chemotaxins inhibit neutrophil adherence to and transmigration across cytokine-activated endothelium: correlation to the expression of L-selectin. *Eur. J. Immunol.* 23: 1481-1487, 1993.

115. Luscinskas, F. W., J. M. Kiely, H. Ding, M. S. Obin, C. A. Hebert, J. B. Baker, and M. A. Gimbrone, Jr. In vitro inhibitory effect of IL-8 and other chemoattractants on neutrophil-endothelial adhesive interactions. *J. Immunol.* 149: 2163-2171, 1992.

116. Hebert, C. A., F. W. Luscinskas, J. M. Kiely, E. A. Luis, W. C. Darbonne, G. L. Bennett, C. C. Liu, M. S. Obin, M. A. Gimbrone, Jr., and J. B. Baker. Endothelial and leukocyte forms of IL-8. Conversion by thrombin and interactions with neutrophils. *J. Immunol.* 145: 3033-3040, 1990.

117. Smart, S. J. and T. B. Casale. TNF-alpha-induced transendothelial neutrophil migration is IL-8 dependent. *Am. J. Physiol.* 266: L238-L245, 1994.

118. Smith, W. B., J. R. Gamble, I. Clark-Lewis, and M. A. Vadas. Interleukin-8 induces neutrophil transendothelial migration. *Immunology* 72: 65-72, 1991.

119. Neumann, F. J., I. Ott, M. Gawaz, G. Richardt, H. Holzapfel, M. Jochum, and A. Schomig. Cardiac release of cytokines and inflammatory responses in acute myocardial infarction. *Circulation* 92: 748-755, 1995.

120. Murry, C. E., R. B. Jennings, and K. A. Reimer. Preconditioning with

ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 74: 1124-1136, 1986.

121. Buja, L. M. Modulation of the myocardial response to ischemia. *Lab. Invest.* 78: 1345-1373, 1998.

122. Baines, C. P., M. V. Cohen, and J. M. Downey. Signal transduction in ischemic preconditioning: the role of kinases and mitochondrial K^+ (ATP) channels. *J. Cardiovasc. Electrophysiol.* 10: 741-754, 1999.

123. Richard, V., N. Kaeffer, and C. Thuillez. Delayed protection of the ischemic heart--from pathophysiology to therapeutic applications. *Fundam. Clin. Pharmacol.* 10: 409-415, 1996.

124. O'Rourke, B. Myocardial K_{ATP} channels in preconditioning. *Circ. Res.* 87: 845-855, 2000.

125. Mitchell, M. B., C. B. Winter, A. Banerjee, and A. H. Harken. The relationship between ischemia-reperfusion injury, myocardial stunning and cardiac preconditioning. *Surg. Gynecol. Obstet.* 177: 97-114, 1993.

126. Bouchard, J. F., J. Chouinard, and D. Lamontagne. Role of kinins in the endothelial protective effect of ischaemic preconditioning. *Br. J. Pharmacol.* 123: 413-420, 1998.

127. Netticadan, T., R. Temsah, M. Osada, and N. S. Dhalla. Status of Ca^{2+}/calmodulin protein kinase phosphorylation of cardiac SR proteins in ischemia-reperfusion. *Am. J. Physiol. Cell Physiol.* 277: C384-C391, 1999.

128. Osada, M., T. Netticadan, K. Tamura, and N. S. Dhalla. Modification of

ischemia-reperfusion-induced changes in cardiac sarcoplasmic reticulum by preconditioning. *Am. J. Physiol. Heart Circ. Physiol.* 274: H2025-H2034, 1998.

129. Tosaki, A., N. Maulik, G. Cordis, O. C. Trifan, L. M. Popescu, and D. K. Das. Ischemic preconditioning triggers phospholipase D signaling in rat heart. *Am. J. Physiol. Heart Circ. Physiol.* 273: H1860-H1866, 1997.

130. Liu, H., H. Y. Zhang, X. Zhu, Z. Shao, and Z. Yao. Preconditioning blocks cardiocyte apoptosis: role of K_{ATP} channels and PKC-epsilon. *Am. J. Physiol. Heart Circ. Physiol.* 282: H1380-H1386, 2002.

131. Theroux, P. Protection of the myocardial cell during ischemia. *Am. J. Cardiol.* 20;83: 3G-9G, 1999.

132. Jennings, R. B., C. Murry, and K. A. Reimer. Myocardial effects of brief periods of ischemia followed by reperfusion. *Adv. Cardiol.* 37: 7-31, 1990.

133. DeFily, D. V. and W. M. Chilian. Preconditioning protects coronary arteriolar endothelium from ischemia-reperfusion injury. *Am. J. Physiol. Heart Circ. Physiol.* 265: H700-H706, 1993.

134. Seki S., H. Okumura, T. Izumi, F. Okazaki, S. Takeda, M. Taniguchui, I. Taniguchi, and S. Mochizuki. Effects of ischemic preconditioning on Na^+-Ca^{2+} exchanger activity and ion regulation in isolated perfused rat hearts. In: N. S. Dhalla, S. Mochizuki, M. Nagano, and N. Takeda, eds. The Ischemic Heart. Boston: Kluwer Academic Publishers, 305-326, 1998.

135. Zhu, J. and G. R. Ferrier. Ischemic preconditioning: antiarrhythmic effects and

electrophysiological mechanisms in isolated ventricle. *Am. J. Physiol. Heart Circ. Physiol.* 274: H66-H75, 1998.

136. Zhou, X., X. Zhai, and M. Ashraf. Direct evidence that initial oxidative stress triggered by preconditioning contributes to second window of protection by endogenous antioxidant enzyme in myocytes. *Circulation* 93: 1177-1184, 1996.

137. Flaherty, J. T. Myocardial injury mediated by oxygen free radicals. *Am. J. Med.* 91: 79S-85S, 1991.

138. Lee, Y. M., H. R. Chen, G. Hsiao, J. R. Sheu, J. J. Wang, and M. H. Yen. Protective effects of melatonin on myocardial ischemia/reperfusion injury in vivo. *J. Pineal Res.* 33: 72-80, 2002.

139. Kambara, N., K. Takagi, T. Satake, S. Sugiyama, and T. Ozawa. Mechanism responsible for endotoxin-induced lung microsomal dysfunction in rats. *Lung* 161: 361-368, 1983.

140. Nagai, S., Y. Miyazaki, K. Ogawa, T. Satake, S. Sugiyama, and T. Ozawa. The effect of Coenzyme Q10 on reperfusion injury in canine myocardium. *J. Mol. Cell. Cardiol.* 17: 873-884, 1985.

141. Chahine, R. and J. Feng. Protective effects of taurine against reperfusion-induced arrhythmias in isolated ischemic rat heart. *Arzneimittel forschung.* 48: 360-364, 1998.

142. Arnaud, C., M. Joyeux, C. Garrel, D. Godin-Ribuot, P. Demenge, and C. Ribuot. Free-radical production triggered by hyperthermia contributes to heat

stress-induced cardioprotection in isolated rat hearts. *Br. J. Pharmacol.* 135: 1776-1782, 2002.

143. Coombes, J. S., S. K. Powers, H. A. Demirel, K. L. Hamilton, J. Jessup, H. K. Vincent, and R. A. Shanely. Vitamin E deficiency fails to affect myocardial performance during in vivo ischemia-reperfusion. *Int. J. Vitam. Nutr. Res.* 70: 293-300, 2000.

144. Poole-Wilson, P. A. Calcium channels in the heart. *Postgrad. Med. J.* 67 (Suppl 3): S16-S19, 1991.

145. Sleight, P. Calcium antagonists during and after myocardial infarction. *Drugs* 51: 216-225, 1996.

146. Kimoto, S., M. Haruna, E. Matsuura, O. Uno, M. Ishii, S. Hirono, K. Yoshimura, M. Ueda, and K. Iwaki. Pharmacological studies on a new antihypertensive agent, S-2150, a benzothiazepine derivative: 3. Hypotensive and antimyocardial-stunning effects in dogs. *J. Cardiovasc. Pharmacol.* 29: 180-187, 1997.

147. Ferrari, R., A. Cargnoni, P. Bernocchi, G. Gaia, M. Benigno, E. Pasini, P. Pedersini, and C. Ceconi. Effects of felodipine on the ischemic heart: insight into the mechanism of cytoprotection. *Cardiovasc. Drugs Ther.* 10: 425-437, 1996.

148. Boraso, A., A. Cargnoni, L. Comini, G. Gaia, P. Bernocchi, and R. Ferrari. Effect of lacidipine on ischaemic and reperfused isolated rabbit hearts. *Mol. Cell. Biochem.* 125: 73-86, 1993.

149. Kirkels, J. H., T. J. Ruigrok, C. J. van Echteld, C. Ceconi, and R. Ferrari. Possible mechanisms of the protective effect of pretreatment with anipamil in ischemic-reperfused isolated rat hearts. *Cardioscience* 3: 27-34, 1992.

150. Kitakaze, M., H. Asanuma, S. Takashima, T. Minamino, Y. Ueda, Y. Sakata, M. Asakura, S. Sanada, T. Kuzuya, and M. Hori. Nifedipine-induced coronary vasodilation in ischemic hearts is attributable to bradykinin- and NO-dependent mechanisms in dogs. *Circulation* 101: 311-317, 2000.

151. Kitakaze, M., K. Node, T. Minamino, H. Asanuma, T. Kuzuya, and M. Hori. A Ca channel blocker, benidipine, increases coronary blood flow and attenuates the severity of myocardial ischemia via NO-dependent mechanisms in dogs. *J. Am. Coll. Cardiol.* 33: 242-249, 1999.

152. Ordonez, F. A., F. A. Hernandez, J. M. Borrego Dominguez, C. E. Gutierrez, G. J. Munoz, M. F. Prieto Rodriguez, and M. M. Viloria Penas. Coronary vasomotor disorders during hypoxia-reoxygenation: do calcium channel blockers play a protective role? *Res. Exp. Med. (Berl)* 199: 319-331, 2000.

153. Ferrari, R. and O. Visioli. Calcium channel blockers and ischaemic heart disease: theoretical expectations and clinical experience. *Eur. Heart J.* 12 (Suppl F): 18-24, 1991.

154. Ichihara, K., T. Haneda, S. Onodera, and Y. Abiko. Inhibition of ischemia-induced subcellular redistribution of lysosomal enzymes in the perfused rat heart by the calcium entry blocker, diltiazem. *J. Pharmacol. Exp. Ther.* 242: 1109-1113, 1987.

155. Okamatsu, S. and A. M. Lefer. The protective effects of nifedipine in the isolated cat heart. *J. Surg. Res.* 35: 35-40, 1983.

156. Rah, B. J., H. O. Kim, and Y. W. Park. Effect of pretreatment with diltiazem on left ventricular function and intracellular calcium distribution in postischemic reperfused guinea-pig hearts. *Coron. Artery Dis.* 5: 415-423, 1994.

157. Takahashi, K. and K. J. Kako. The effect of a calcium channel antagonist, Nisoldipine, on the ischemia-induced change of canine sarcolemmal membrane. *Basic Res.Cardiol.* 78: 326-337, 1983.

158. Nayler, W. G., J. S. Dillon, W. J. Sturrock, and D. J. Buckley. Effect of chronic verapamil therapy on cardiac norepinephrine and beta-adrenoceptor density. *J. Cardiovasc. Pharmacol.* 12: 629-636, 1988.

159. McDonagh, P. F. and D. J. Roberts. Prevention of transcoronary macromolecular leakage after ischemia-reperfusion by the calcium entry blocker nisoldipine. Direct observations in isolated rat hearts. *Circ. Res.* 58: 127-136, 1986.

160. Kroner, A., R. Seitelberger, J. Schirnhofer, O. Bernecker, R. Mallinger, S. Hallstrom, M. Ploner, and B. K. Podesser. Diltiazem during reperfusion preserves high energy phosphates by protection of mitochondrial integrity. *Eur. J. Cardiothorac. Surg.* 21: 224-231, 2002.

161. Nayler, W. G. Amlodipine pretreatment and the ischemic heart. *Am. J. Cardiol.* 64: 65I-69I, 1989.

162. Bratus', V. V., A. G. Bezus'ko, S. S. Shabaran, and V. V. Filonenko. Mechanisms of verapamil prevention of disorders of cardiac contractile function during ischemia and reperfusion. *Patol. Fiziol. Eksp. Ter.* 26-29, 1989.

163. Watts, J. A., T. A. Norris, R. E. London, C. Steenbergen, and E. Murphy. Effects of diltiazem on lactate, ATP, and cytosolic free calcium levels in ischemic hearts. *J. Cardiovasc. Pharmacol.* 15: 44-49, 1990.

164. Tappia, P. S., K. Okumura, K. Kawabata, K. R. Shah, M. S. Nijjar, V. Panagia, and N. S. Dhalla. Ca^{2+}-antagonists inhibit the N-methyltransferase-dependent synthesis of phosphatidylcholine in the heart. *Mol. Cell. Biochem.* 221: 89-98, 2001.

165. Bogaert, M. G. How do calcium channel blockers prevent cardiovascular events. Are they all alike? *Drugs* 52 Suppl 4: 3-7, 1996.

166. De Windt, L. J., J. Willems, T. H. Roemen, W. A. Coumans, R. S. Reneman, G. J. Van Der Vusse, and M. Van Bilsen. Ischemic-reperfused isolated working mouse hearts: membrane damage and type IIA phospholipase A2. *Am. J. Physiol. Heart Circ. Physiol.* 280: H2572-H2580, 2001.

167. De Windt, L. J., P. H. Willemsen, S. Popping, G. J. Van Der Vusse, R. S. Reneman, and M. Van Bilsen. Cloning and cellular distribution of a group II phospholipase A2 expressed in the heart. *J. Mol. Cell. Cardiol.* 29: 2095-2106, 1997.

168. Hashizume, H., A. N. Hoque, K. Magishi, A. Hara, and Y. Abiko. A new

approach to the development of anti-ischemic drugs. Substances that counteract the deleterious effect of lysophosphatidylcholine on the heart. *Jpn. Heart J.* 38: 11-25, 1997.

169. Ver, D. L., G. Verellen, H. Geerts, and M. Borgers. Lysophosphatidylcholine-induced Ca^{2+}-overload in isolated cardiomyocytes and effect of cytoprotective drugs. *J. Mol. Cell. Cardiol.* 24: 977-988, 1992.

170. Burnashev, N. A., A. I. Undrovinas, I. A. Fleidervish, J. C. Makielski, and L. V. Rosenshtraukh. Modulation of cardiac sodium channel gating by lysophosphatidylcholine. *J. Mol. Cell. Cardiol.* 23 (Suppl 1): 23-30, 1991.

171. Sargent, C. A., O. Vesterqvist, M. L. Ogletree, and G. J. Grover. Effects of endogenous and exogenous lysophosphatidylcholine in isolated perfused rat hearts. *J. Mol. Cell. Cardiol.* 25: 905-913, 1993.

172. De Windt, L. J., R. S. Reneman, G. J. Van Der Vusse, and M. Van Bilsen. Phospholipase A2-mediated hydrolysis of cardiac phospholipids: the use of molecular and transgenic techniques. *Mol. Cell. Biochem.* 180: 65-73, 1998.

173. Katsuoka, M. and S. T. Ohnishi. Pharmacologic protection of perfused rat heart against global ischemia. *Prostaglandins Leukot. Essent. Fatty Acids* 38: 151-156, 1989.

174. Koyama, T., W. Keatisuwan, M. Kinjo, and H. Saito. Suppressive effect of coenzyme Q10 on phospholipase A2 activation in cardiac cells after prolonged swimming. *Life Sci.* 51: 1113-1118, 1992.

175. Avkiran, M. Sodium-hydrogen exchange in myocardial ischemia and

reperfusion: a critical determinant of injury? *EXS* 76: 299-311, 1996.

176. Avkiran, M. and A. K. Snabaitis. Regulation of cardiac sarcolemmal Na^+/H^+ exchanger activity: potential pathophysiological significance of endogenous mediators and oxidant stress. *J. Thromb. Thrombolysis* 8: 25-32, 1999.

177. Fliegel, L. and H. Wang. Regulation of the Na^+/H^+ exchanger in the mammalian myocardium. *J. Mol. Cell. Cardiol.* 29: 1991-1999, 1997.

178. Ramasamy, R. and S. Schaefer. Inhibition of Na^+-H^+ exchanger protects diabetic and non-diabetic hearts from ischemic injury: insight into altered susceptibility of diabetic hearts to ischemic injury. *J. Mol. Cell. Cardiol.* 31: 785-797, 1999.

179. Karmazyn, M. The role of the myocardial sodium-hydrogen exchanger in mediating ischemic and reperfusion injury. From amiloride to cariporide. *Ann. N.Y. Acad. Sci.* 874: 326-334, 1999.

180. Avkiran, M. and R. S. Haworth. Regulation of cardiac sarcolemmal Na^+/H^+ exchanger activity by endogenous ligands. Relevance to ischemia. *Ann. N.Y. Acad. Sci.* 874: 335-345, 1999.

181. Scholz, W., U. Albus, L. Counillon, H. Gogelein, H. J. Lang, W. Linz, A. Weichert, and B. A. Scholkens. Protective effects of HOE642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. *Cardiovasc. Res.* 29: 260-268, 1995.

182. Pike, M. M., C. S. Luo, M. D. Clark, K. A. Kirk, M. Kitakaze, M. C. Madden, E. J. Cragoe, Jr., and G. M. Pohost. NMR measurements of Na^+ and cellular

energy in ischemic rat heart: role of Na^+-H^+ exchange. *Am. J. Physiol. Heart Circ. Physiol.* 265: H2017-H2026, 1993.

183. Navon, G., J. G. Werrmann, R. Maron, and S. M. Cohen. 31P NMR and triple quantum filtered 23Na NMR studies of the effects of inhibition of Na^+/H^+ exchange on intracellular sodium and pH in working and ischemic hearts. *Magn. Reson. Med.* 32: 556-564, 1994.

184. Murphy, E., M. Perlman, R. E. London, and C. Steenbergen. Amiloride delays the ischemia-induced rise in cytosolic free calcium. *Circ. Res.* 68: 1250-1258, 1991.

185. Hartmann, M. and U. K. Decking. Blocking Na^+-H^+ exchange by cariporide reduces Na^+-overload in ischemia and is cardioprotective. *J. Mol. Cell. Cardiol.* 31: 1985-1995, 1999.

186. Hotta, Y., N. Ishikawa, N. Ohashi, and K. Matsui. Effects of SM-20550, a selective Na^+-H^+ exchange inhibitor, on the ion transport of myocardial mitochondria. *Mol. Cell. Biochem.* 219: 83-90, 2001.

187. Katz A. M. Proltferative signaling: Regulation of gene expression, protein synthesis, cell growth and proliferation, apoptosis. In: M. Laplante, ed. Physiology of the Heart. Philadelphia: Lippincott Williams & Wilkins, 312-368, 2001.

188. Wei, S., E. C. Rothstein, L. Fliegel, L. J. Dell'Italia, and P. A. Lucchesi. Differential MAP kinase activation and Na^+/H^+ exchanger phosphorylation by H_2O_2 in rat cardiac myocytes. *Am. J. Physiol. Cell Physiol.* 281: C1542-

C1550, 2001.

189. Clerk, A., S. J. Fuller, A. Michael, and P. H. Sugden. Stimulation of "stress-regulated" mitogen-activated protein kinases (stress-activated protein kinases/c-Jun N-terminal kinases and p38-mitogen-activated protein kinases) in perfused rat hearts by oxidative and other stresses. *J. Biol. Chem.* 273: 7228-7234, 1998.

190. Sato, M., G. A. Cordis, N. Maulik, and D. K. Das. SAPKs regulation of ischemic preconditioning. *Am. J. Physiol. Heart Circ. Physiol.* 279: H901-H907, 2000.

191. Nakano, A., C. P. Baines, S. O. Kim, S. L. Pelech, J. M. Downey, M. V. Cohen, and S. D. Critz. Ischemic preconditioning activates MAPKAPK2 in the isolated rabbit heart: evidence for involvement of p38 MAPK. *Circ. Res.* 86: 144-151, 2000.

192. Weinbrenner, C., G. S. Liu, M. V. Cohen, and J. M. Downey. Phosphorylation of tyrosine 182 of p38 mitogen-activated protein kinase correlates with the protection of preconditioning in the rabbit heart. *J. Mol. Cell. Cardiol.* 29: 2383-2391, 1997.

193. Luss, H., J. Neumann, W. Schmitz, R. Schulz, and G. Heusch. The stress-responsive MAP kinase p38 is activated by low-flow ischemia in the in situ porcine heart. *J. Mol. Cell. Cardiol.* 32: 1787-1794, 2000.

194. Fryer, R. M., A. K. Hsu, and G. J. Gross. ERK and p38 MAP kinase activation are components of opioid-induced delayed cardioprotection. *Basic Res.*

Cardiol. 96: 136-142, 2001.

195. Sakamoto, K., T. Urushidani, and T. Nagao. Translocation of HSP27 to sarcomere induced by ischemic preconditioning in isolated rat hearts. *Biochem. Biophys. Res. Commun.* 269: 137-142, 2000.

196. Tekin, D., L. Xi, T. Zhao, M. I. Tejero-Taldo, S. Atluri, and R. C. Kukreja. Mitogen-activated protein kinases mediate heat shock-induced delayed protection in mouse heart. *Am. J. Physiol. Heart Circ. Physiol.* 281: H523-H532, 2001.

197. Lazou, A., P. H. Sugden, and A. Clerk. Activation of mitogen-activated protein kinases (p38-MAPKs, SAPKs/JNKs and ERKs) by the G-protein-coupled receptor agonist phenylephrine in the perfused rat heart. *Biochem. J.* 332: 459-465, 1998.

198. Ma, X. L., S. Kumar, F. Gao, C. S. Louden, B. L. Lopez, T. A. Christopher, C. Wang, J. C. Lee, G. Z. Feuerstein, and T. L. Yue. Inhibition of p38 mitogen-activated protein kinase decreases cardiomyocyte apoptosis and improves cardiac function after myocardial ischemia and reperfusion. *Circulation* 99: 1685-1691, 1999.

199. Yin, T., G. Sandhu, C. D. Wolfgang, A. Burrier, R. L. Webb, D. F. Rigel, T. Hai, and J. Whelan. Tissue-specific pattern of stress kinase activation in ischemic/reperfused heart and kidney. *J. Biol. Chem.* 272: 19943-19950, 1997.

200. Bogoyevitch, M. A., J. Gillespie-Brown, A. J. Ketterman, S. J. Fuller, R. Ben Levy, A. Ashworth, C. J. Marshall, and P. H. Sugden. Stimulation of the

stress-activated mitogen-activated protein kinase subfamilies in perfused heart. p38/RK mitogen-activated protein kinases and c-Jun N-terminal kinases are activated by ischemia/reperfusion. *Circ. Res.* 79: 162-173, 1996.

201. Joyeux, M., A. Boumendjel, R. Carroll, C. Ribuot, D. Godin-Ribuot, and D. M. Yellon. SB 203580, a mitogen-activated protein kinase inhibitor, abolishes resistance to myocardial infarction induced by heat stress. *Cardiovasc. Drugs Ther.* 14: 337-343, 2000.

202. Rakhit, R. D., A. N. Kabir, J. W. Mockridge, A. Saurin, and M. S. Marber. Role of G proteins and modulation of p38 MAPK activation in the protection by nitric oxide against ischemia-reoxygenation injury. *Biochem. Biophys. Res. Commun.* 286: 995-1002, 2001.

203. Schulz, R., S. Belosjorow, P. Gres, J. Jansen, M. Michel, and G. Heusch. p38 MAP kinase is a mediator of ischemic preconditioning in pigs. *Cardiovasc. Res.* 55: 690, 2002.

204. Opie, L. H. Receptors and signal transduction. In: R. W. Weinberg, ed. The Heart Physiology: From Cell to Circulation. New York: Lippincott-Raven Press, 173-209, 1998.

205. Oliver, C. J. and S. Shenolikar. Physiologic importance of protein phosphatase inhibitors. *Front. Biosci.* 3: D961-D972, 1998.

206. Weinbrenner, C., C. P. Baines, G. S. Liu, S. C. Armstrong, C. E. Ganote, A. H. Walsh, R. E. Honkanen, M. V. Cohen, and J. M. Downey. Fostriecin, an inhibitor of protein phosphatase 2A, limits myocardial infarct size even when

administered after onset of ischemia. *Circulation* 98: 899-905, 1998.

207. Armstrong, S. C., R. Kao, W. Gao, L. C. Shivell, J. M. Downey, R. E. Honkanen, and C. E. Ganote. Comparison of in vitro preconditioning responses of isolated pig and rabbit cardiomyocytes: effects of a protein phosphatase inhibitor, fostriecin. *J. Mol. Cell. Cardiol.* 29: 3009-3024, 1997.

208. Walsh, A. H., A. Cheng, and R. E. Honkanen. Fostriecin, an antitumor antibiotic with inhibitory activity against serine/threonine protein phosphatases types 1 (PP1) and 2A (PP2A), is highly selective for PP2A. *FEBS Lett.* 416: 230-234, 1997.

209. Armstrong, S. C., W. Gao, J. R. Lane, and C. E. Ganote. Protein phosphatase inhibitors calyculin A and fostriecin protect rabbit cardiomyocytes in late ischemia. *J. Mol. Cell. Cardiol.* 30: 61-73, 1998.

210. Armstrong, S. C., D. B. Hoover, M. H. Delacey, and C. E. Ganote. Translocation of PKC, protein phosphatase inhibition and preconditioning of rabbit cardiomyocytes. *J. Mol. Cell. Cardiol.* 28: 1479-1492, 1996.

211. Armstrong, S. C. and C. E. Ganote. Effects of 2,3-butanedione monoxime (BDM) on contracture and injury of isolated rat myocytes following metabolic inhibition and ischemia. *J. Mol. Cell. Cardiol.* 23: 1001-1014, 1991.

212. Golenhofen, N., W. Ness, R. Koob, P. Htun, W. Schaper, and D. Drenckhahn. Ischemia-induced phosphorylation and translocation of stress protein alpha B-crystallin to Z lines of myocardium. *Am. J. Physiol. Heart Circ. Physiol.* 274: H1457-H1464, 1998.

213. Geraldes, C. F., M. M. Castro, A. D. Sherry, and R. Ramasamy. Influence of vanadate on glycolysis, intracellular sodium, and pH in perfused rat hearts. *Mol. Cell. Biochem.* 170: 53-63, 1997.

214. Knapp, J., P. Boknik, S. Huke, I. Gombosova, B. Linck, H. Luss, F. U. Muller, T. Muller, P. Nacke, W. Schmitz, U. Vahlensieck, and J. Neumann. Contractility and inhibition of protein phosphatases by cantharidin. *Gen. Pharmacol.* 31: 729-733, 1998.

215. De Windt, L. J., H. W. Lim, T. Taigen, D. Wencker, G. Condorelli, G. W. Dorn, R. N. Kitsis, and J. D. Molkentin. Calcineurin-mediated hypertrophy protects cardiomyocytes from apoptosis in vitro and in vivo: An apoptosis-independent model of dilated heart failure. *Circ. Res.* 86: 255-263, 2000.

216. Armstrong, S. C. and C. E. Ganote. Effects of the protein phosphatase inhibitors okadaic acid and calyculin A on metabolically inhibited and ischaemic isolated myocytes. *J. Mol. Cell. Cardiol.* 24: 869-884, 1992.

217. Curtis, M. J., M. K. Pugsley, and M. J. Walker. Endogenous chemical mediators of ventricular arrhythmias in ischaemic heart disease. *Cardiovasc. Res.* 27: 703-719, 1993.

218. Simpson, P. J., J. A. Schelm, J. A. Jakubowski, and J. K. Smallwood. The role of serotonin (5HT2) receptor blockade in myocardial reperfusion injury: effects of LY53857 in a canine model of myocardial infarction. *J. Pharmacol. Exp. Ther.* 258: 979-985, 1991.

219. De Clerck, F. and P. A. Janssen. 5-Hydroxytryptamine and thromboxane A2 in

ischaemic heart disease. *Blood Coagul. Fibrinolysis* 1: 201-209, 1990.

220. Deptuch, T. W., M. Kurenko-Deptuch, A. Witanowska, G. Ciurzynska, M. Gajewski, M. Jozwicka, J. Dzierzkowska, and S. Maslinski. Influence of selective 5-HT agonists and myocardial preconditioning on ischaemia/reperfusion induced changes in isolated heart of the rat. *Inflamm. Res.* 48 (Suppl 1): S96-S97, 1999.

221. Frishman, W. H., S. Huberfeld, S. Okin, Y. H. Wang, A. Kumar, and B. Shareef. Serotonin and serotonin antagonism in cardiovascular and non-cardiovascular disease. *J. Clin. Pharmacol.* 35: 541-572, 1995.

222. Perrault, L. P., C. Nickner, N. Desjardins, E. Dumont, P. Thai, and M. Carrier. Improved preservation of coronary endothelial function with Celsior compared with blood and crystalloid solutions in heart transplantation. *J. Heart Lung Transplant.* 20: 549-558, 2001.

223. Hsieh, C. P., K. Sakai, G. C. Bruns, and R. C. Dage. Effects of MDL 28,133A, a 5-HT2 receptor antagonist, on platelet aggregation and coronary thrombosis in dogs. *J. Cardiovasc. Pharmacol.* 24: 761-772, 1994.

224. McAuliffe, S. J., H. M. Snow, B. Cox, C. C. Smith, and M. I. Noble. Interaction between the effects of 5-hydroxytryptamine and adrenaline on the growth of platelet thrombi in the coronary artery of the anaesthetized dog. *Br. J. Pharmacol.* 109: 405-410, 1993.

225. Morishima, Y., T. Tanaka, K. Watanabe, T. Igarashi, M. Yasuoka, and T. Shibano. Prevention by DV-7028, a selective 5-HT2 receptor antagonist, of the

formation of coronary thrombi in dogs. *Cardiovasc. Res.* 25: 727-730, 1991.

226. Bush, L. R., W. B. Campbell, K. Kern, G. D. Tilton, P. Apprill, J. Ashton, J. Schmitz, L. M. Buja, and J. T. Willerson. The effects of alpha 2-adrenergic and serotonergic receptor antagonists on cyclic blood flow alterations in stenosed canine coronary arteries. *Circ. Res.* 55: 642-652, 1984.

227. Coker, S. J., H. G. Dean, K. A. Kane, and J. R. Parratt. The effects of ICS 205-930, a 5-HT antagonist, on arrhythmias and catecholamine release during canine myocardial ischaemia and reperfusion. *Eur. J. Pharmacol.* 127: 211-218, 1986.

228. Grover, G. J., C. A. Sargent, S. Dzwonczyk, D. E. Normandin, and M. J. Antonaccio. Protective effect of serotonin (5-HT2) receptor antagonists in ischemic rat hearts. *J. Cardiovasc. Pharmacol.* 22: 664-672, 1993.

229. el Mahdy, S. A. 5-Hydroxytryptamine (serotonin) enhances ventricular arrhythmias induced by acute coronary artery ligation in rats. *Res. Commun. Chem. Pathol. Pharmacol.* 68: 383-386, 1990.

230. Clark, W. G., D. C. Brater, and A. R. Jonson. Central nervous system stimulants. In: K. Kist, ed. Goth's Medical Pharmacology. St Louis: Mosby-Year Book, 300-307, 1992.

231. Rall, T. W. Drug used in the treatment of asthma. In: A. G. Gilman, T. W. Rall, A. S. Nies, and P. Taylor, eds. The Pharmacological Basis of Therapeutics. New York: Pergamon Press, 618-638, 1990.

232. Ward, A. and S. P. Clissold. Pentoxifylline. A review of its pharmacodynamic

and pharmacokinetic properties, and its therapeutic efficacy. *Drugs* 34: 50-97, 1987.

233. Muller, R. Hemorheology and peripheral vascular diseases: a new therapeutic approach. *J. Med.* 12: 209-235, 1981.

234. Eun, B. L., X. H. Liu, and J. D. Barks. Pentoxifylline attenuates hypoxic-ischemic brain injury in immature rats. *Pediatr. Res.* 47: 73-78, 2000.

235. Weiss, D. J. and O. A. Evanson. Evaluation of lipopolysaccharide-induced activation of equine neutrophils. *Am. J. Vet. Res.* 63: 811-815, 2002.

236. Schmalzer, E. A. and S. Chien. Filterability of subpopulations of leukocytes: effect of pentoxifylline. *Blood* 64: 542-546, 1984.

237. Samlaska, C. P. and W. D. James. Superficial thrombophlebitis. I. Primary hypercoagulable states. *J. Am. Acad. Dermatol.* 22: 975-989, 1990.

238. Samlaska, C. P. and E. A. Winfield. Pentoxifylline. *J. Am. Acad. Dermatol.* 30: 603-621, 1994.

239. Salyer, J. L., J. F. Bohnsack, W. A. Knape, A. O. Shigeoka, E. R. Ashwood, and H. R. Hill. Mechanisms of tumor necrosis factor-alpha alteration of PMN adhesion and migration. *Am. J. Pathol.* 136: 831-841, 1990.

240. Klinzing, S., T. Lesser, H. Schubert, M. Bartel, and U. Klein. May pentoxifylline improve lung function after one-lung flooding? *Res. Exp. Med. (Berl)* 200: 69-76, 2001.

241. Fossat, C., D. Fabre, Y. Alimi, J. Bienvenu, M. F. Aillaud, M. Lenoble, I. Juhan-Vague, and C. Juhan. Leukocyte activation study during occlusive

arterial disease of the lower limb: effect of pentoxifylline infusion. *J. Cardiovasc. Pharmacol.* 25 (Suppl 2): S96-S100, 1995.

242. Betticher, D. C., H. Keller, F. E. Maly, and W. H. Reinhart. The effect of endotoxin and tumour necrosis factor on erythrocyte and leucocyte deformability in vitro. *Br. J. Haematol.* 83: 130-137, 1993.

243. Horton, J. W. and D. J. White. Free radical scavengers prevent intestinal ischemia-reperfusion-mediated cardiac dysfunction. *J. Surg. Res.* 55: 282-289, 1993.

244. Schermuly, R. T., A. Roehl, N. Weissmann, H. A. Ghofrani, H. Leuchte, F. Grimminger, W. Seeger, and D. Walmrath. Combination of nonspecific PDE inhibitors with inhaled prostacyclin in experimental pulmonary hypertension. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 281: L1361-L1368, 2001.

245. Myers, S. I., J. W. Horton, R. Hernandez, P. B. Walker, and W. G. Vaughan. Pentoxifylline protects splanchnic prostacyclin synthesis during mesenteric ischemia/reperfusion. *Prostaglandins* 47: 137-150, 1994.

246. Manrique, R. V. and V. Manrique. Platelet resistance to prostacyclin. Enhancement of the antiaggregatory effect of prostacyclin by pentoxifylline. *Angiology* 38: 101-108, 1987.

247. Strieter, R. M., D. G. Remick, P. A. Ward, R. N. Spengler, J. P. Lynch, III, J. Larrick, and S. L. Kunkel. Cellular and molecular regulation of tumor necrosis factor-alpha production by pentoxifylline. *Biochem. Biophys. Res. Commun.* 155: 1230-1236, 1988.

248. Lillibridge, J. A., T. F. Kalhorn, and J. T. Slattery. Metabolism of lisofylline and pentoxifylline in human liver microsomes and cytosol. *Drug Metab. Dispos.* 24: 1174-1179, 1996.

249. Rogers, S. J. and D. G. Sherman. Pathophysiology and treatment of acute ischemic stroke. *Clin. Pharm.* 12: 359-376, 1993.

250. Frampton, J. E. and R. N. Brogden. Pentoxifylline (oxpentifylline). A review of its therapeutic efficacy in the management of peripheral vascular and cerebrovascular disorders. *Drugs Aging* 7: 480-503, 1995.

251. Marcel, G. A. and C. George. Pentoxifylline and cerebrovascular diseases. *Eur. Neurol.* 22 (Suppl 1): 89-97, 1983.

252. Schubotz, R. and O. Muhlfellner. The effect of pentoxifylline on erythrocyte deformability and on phosphatide fatty acid distribution in the erythrocyte membrane. *Curr. Med. Res. Opin.* 4: 609-617, 1977.

253. Bowton, D. L., D. A. Stump, D. S. Prough, J. F. Toole, D. S. Lefkowitz, and L. Coker. Pentoxifylline increases cerebral blood flow in patients with cerebrovascular disease. *Stroke* 20: 1662-1666, 1989.

254. Muller, R. and R. Schroer. Cerebrovascular circulatory disorders: new aspects of pathophysiology and therapy. *J. Med.* 10: 347-364, 1979.

255. Ganser, V. and I. Boksay. Effect of pentoxifylline on cerebral edema in cats. *Neurology* 24: 487-493, 1974.

256. Currie, M. S., K. M. Rao, J. Padmanabhan, A. Jones, J. Crawford, and H. J. Cohen. Stimulus-specific effects of pentoxifylline on neutrophil CR3

expression, degranulation, and superoxide production. *J. Leukoc. Biol.* 47: 244-250, 1990.

257. McCarty, M. F. Nitric oxide deficiency, leukocyte activation, and resultant ischemia are crucial to the pathogenesis of diabetic retinopathy/neuropathy--preventive potential of antioxidants, essential fatty acids, chromium, ginkgolides, and pentoxifylline. *Med. Hypotheses* 50: 435-449, 1998.
258. Adams, J. G., Jr., A. Dhar, S. D. Shukla, and D. Silver. Effect of pentoxifylline on tissue injury and platelet-activating factor production during ischemia-reperfusion injury. *J. Vasc. Surg.* 21: 742-748, 1995.
259. Hammerschmidt, D. E., D. Kotasek, T. McCarthy, P. W. Huh, G. Freyburger, and G. M. Vercellotti. Pentoxifylline inhibits granulocyte and platelet function, including granulocyte priming by platelet activating factor. *J. Lab. Clin. Med.* 112: 254-263, 1988.
260. Silver, D., A. Dhar, M. Slocum, J. G. Adams, Jr., and S. Shukla. Role of platelet-activating factor in skeletal muscle ischemia-reperfusion injury. *Adv. Exp. Med. Biol.* 416: 217-221, 1996.
261. Lorant, D. E., K. D. Patel, T. M. McIntyre, R. P. McEver, S. M. Prescott, and G. A. Zimmerman. Coexpression of GMP-140 and PAF by endothelium stimulated by histamine or thrombin: a juxtacrine system for adhesion and activation of neutrophils. *J. Cell. Biol.* 115: 223-234, 1991.
262. Hanazawa, S., R. L. Prewitt, and J. K. Terzis. The effect of pentoxifylline on ischemia and reperfusion injury in the rat cremaster muscle. *J. Reconstr.*

Microsurg. 10: 21-26, 1994.

263. Gale, S. C., J. Y. Hokama, L. S. Ritter, G. D. Gorman, J. G. Copeland, and P. F. McDonagh. Pentoxifylline reduces coronary leukocyte accumulation early in reperfusion after cold ischemia. *Ann. Thorac. Surg.* 71: 1305-1311, 2001.

264. Ulus, A. T., A. Aksoyek, S. F. Katircioglu, P. Gokce, and B. Koc. Preservation of myocardial functions by pentoxyphylline cardioplegia during and after cardiopulmonary bypass. *Panminerva Med.* 42: 253-256, 2000.

265. Lechleitner, P., N. Genser, J. Mair, J. Maier, M. Herold, H. Beimpold, B. Foger, F. Dienstl, B. Puschendorf, and H. Tilg. Pentoxifylline influences acute-phase response in acute myocardial infarction. *Clin. Investig.* 70: 755, 1992.

266. Barnett, J. C. and R. C. Touchon. Therapy of ischemic cardiomyopathy with pentoxifylline. *Angiology* 41: 1048-1052, 1990.

267. Insel, J., A. A. Halle, and D. M. Mirvis. Efficacy of pentoxifylline in patients with stable angina pectoris. *Angiology* 39: 514-519, 1988.

268. Dauber, I. M., E. J. Lesnefsky, R. C. Ashmore, D. M. Martel, F. M. Sheridan, J. V. Weil, and L. D. Horwitz. Coronary vascular injury due to ischemia-reperfusion is reduced by pentoxifylline. *J. Pharmacol. Exp. Ther.* 260: 1250-1256, 1992.

269. Hou, X., N. Baudry, M. Lenoble, and E. Vicaut. Leukocyte adherence in an ischemic muscle perfused by a collateral circulation. *J. Cardiovasc. Pharmacol.* 25 (Suppl 2): S119-S123, 1995.

270. Bessler, H., R. Gilgal, M. Djaldetti, and I. Zahavi. Effect of pentoxifylline on the phagocytic activity, cAMP levels, and superoxide anion production by monocytes and polymorphonuclear cells. *J. Leukoc. Biol.* 40: 747-754, 1986.

271. Semmler, J., H. Wachtel, and S. Endres. The specific type IV phosphodiesterase inhibitor rolipram suppresses tumor necrosis factor-alpha production by human mononuclear cells. *Int. J. Immunopharmacol.* 15: 409-413, 1993.

272. Sliwa, K., A. Woodiwiss, G. Candy, D. Badenhorst, C. Libhaber, G. Norton, D. Skudicky, and P. Sareli. Effects of pentoxifylline on cytokine profiles and left ventricular performance in patients with decompensated congestive heart failure secondary to idiopathic dilated cardiomyopathy. *Am. J. Cardiol.* 90: 1118-1122, 2002.

273. Skudicky, D., A. Bergemann, K. Sliwa, G. Candy, and P. Sareli. Beneficial effects of pentoxifylline in patients with idiopathic dilated cardiomyopathy treated with angiotensin-converting enzyme inhibitors and carvedilol: results of a randomized study. *Circulation* 103: 1083-1088, 2001.

274. Kishi, M., H. Tanaka, A. Seiyama, M. Takaoka, T. Matsuoka, T. Yoshioka, and H. Sugimoto. Pentoxifylline attenuates reperfusion injury in skeletal muscle after partial ischemia. *Am. J. Physiol.* 274: H1435-H1442, 1998.

275. Chapelier, A., J. Reignier, M. Mazmanian, H. Detruit, P. Dartevelle, F. Parquin, J. Cerrina, L. F. Le Roy, and P. Herve. Pentoxifylline and lung ischemia-reperfusion injury: application to lung transplantation. Universite

Paris-Sud Lung Transplant Group. *J. Cardiovasc. Pharmacol.* 25 (Suppl 2): S130-S133, 1995.

276. Abraham, E., S. Bursten, R. Shenkar, J. Allbee, R. Tuder, P. Woodson, D. M. Guidot, G. Rice, J. W. Singer, and J. E. Repine. Phosphatidic acid signaling mediates lung cytokine expression and lung inflammatory injury after hemorrhage in mice. *J. Exp. Med.* 181: 569-575, 1995.
277. Semmler, J., U. Gebert, T. Eisenhut, J. Moeller, M. M. Schonharting, A. Allera, and S. Endres. Xanthine derivatives: comparison between suppression of tumour necrosis factor-alpha production and inhibition of cAMP phosphodiesterase activity. *Immunology* 78: 520-525, 1993.
278. Molnar-Kimber, K., L. Yonno, R. Heaslip, and B. Weichman. Modulation of TNF alpha and IL-1 beta from endotoxin-stimulated monocytes by selective PDE isozyme inhibitors. *Agents Actions* 39: C77-C79, 1993.
279. Bath, P. M., F. J. Bath, and K. Asplund. Pentoxifylline, propentofylline and pentifylline for acute ischaemic stroke. *Cochrane Database Syst. Rev.* CD000162, 2000.
280. Savas, C., H. Dindar, T. Aras, and S. Yucesan. Pentoxifylline improves blood flow to both testes in testicular torsion. *Int. Urol. Nephrol.* 33: 81-85, 2002.
281. Savas, C., H. Dindar, A. Bilgehan, O. Ataoglu, and S. Yucesan. Pentoxifylline attenuates reperfusion injury in testicular torsion. *Scand. J. Urol. Nephrol.* 36: 65-70, 2002.
282. Savas, C., T. Aras, M. Cakmak, A. Bilgehan, O. Ataoglu, N. Turkozkan, F.

Ozguner, S. Yucesan, and H. Dindar. Pentoxifylline inhibits overflow and reduces intestinal reperfusion injury. *J. Pediatr. Surg.* 32: 905-910, 1997.

283. Temsah, R. M., K. Kawabata, D. Chapman, and N. S. Dhalla. Preconditioning prevents alterations in cardiac SR gene expression due to ischemia-reperfusion. *Am. J. Physiol. Heart Circ. Physiol.* 282: H1461-H1466, 2002.

284. Osada, M., T. Netticadan, K. Kawabata, K. Tamura, and N. S. Dhalla. Ischemic preconditioning prevents I/R-induced alterations in SR calcium-calmodulin protein kinase II. *Am. J. Physiol. Heart Circ. Physiol.* 278: H1791-H1798, 2000.

285. Kawabata, K., T. Netticadan, M. Osada, K. Tamura, and N. S. Dhalla. Mechanisms of ischemic preconditioning effects on Ca^{2+} paradox-induced changes in heart. *Am. J. Physiol. Heart Circ. Physiol.* 278: H1008-H1015, 2000.

286. Wang, J., X. Liu, E. Sentex, N. Takeda, and N. S. Dhalla. Increased expression of protein kinase C isoforms in heart failure due to myocardial infarction. *Am. J. Physiol. Heart Circ. Physiol.* 284: H2277-H2287, 2003.

287. Carter, M. B., M. A. Wilson, W. B. Wead, and R. N. Garrison. Pentoxifylline attenuates pulmonary macromolecular leakage after intestinal ischemia-reperfusion. *Arch. Surg.* 130: 1337-1344, 1995.

288. Chen, Y. M., C. J. Tu, K. Y. Hung, K. D. Wu, T. J. Tsai, and B. S. Hsieh. Inhibition by pentoxifylline of TNF-alpha-stimulated fractalkine production in vascular smooth muscle cells: evidence for mediation by NF-kappa B down-

regulation. *Br. J. Pharmacol.* 138: 950-958, 2003.

289. Andela, V. B., B. I. Gingold, M. D. Souza, R. J. O'Keefe, E. J. Puzas, E. M. Schwarz, and R. N. Rosier. Clinical relevance of increased retinoid and cAMP transcriptional programs in tumor cells rendered non-malignant by dominant negative inhibition of NFkappaB. *Cancer Lett.* 194: 37-43, 2003.

290. Seno, H., M. Sawada, H. Fukuzawa, Y. Morita-Fujisawa, S. Takaishi, H. Hiai, and T. Chiba. Involvement of tumor necrosis factor alpha in intestinal epithelial cell proliferation following Paneth cell destruction. *Scand. J. Gastroenterol.* 37: 154-160, 2002.

291. Jimenez, J. L., C. Punzon, J. Navarro, M. A. Munoz-Fernandez, and M. Fresno. Phosphodiesterase 4 inhibitors prevent cytokine secretion by T lymphocytes by inhibiting nuclear factor-kappaB and nuclear factor of activated T cells activation. *J. Pharmacol. Exp. Ther.* 299: 753-759, 2001.

292. Lee, K. S., H. B. Cottam, K. Houglum, D. B. Wasson, D. Carson, and M. Chojkier. Pentoxifylline blocks hepatic stellate cell activation independently of phosphodiesterase inhibitory activity. *Am. J. Physiol.* 273: G1094-G1100, 1997.

293. Lazou, A., S. Seraskeris, V. Tsiona, and G. Drossos. Oxidative status and anti-oxidant enzyme activity during calcium paradox in the rat isolated heart. *Clin. Exp. Pharmacol. Physiol.* 27: 160-166, 2000.

294. Suleiman, J. and M. Ashraf. Adenosine attenuates calcium paradox injury: role of adenosine A1 receptor. *Am. J. Physiol. Cell Physiol.* 268: C838-C845, 1995.

295. Ustunsoy, H., M. C. Sivrikoz, K. Bakir, Senkaya, B. Erbagci, R. Ucak, and F. Nurozler. The inhibition of pro-inflammatory cytokines with pentoxifylline in the cardiopulmunary bypass lung. *Respir. Med.* 96: 275-279, 2002.

296. Dolmetsch, R. E., R. S. Lewis, C. C. Goodnow, and J. I. Healy. Differential activation of transcription factors induced by Ca^{2+} response amplitude and duration. *Nature* 386: 855-858, 1997.

297. Dolmetsch, R. E., K. Xu, and R. S. Lewis. Calcium oscillations increase the efficiency and specificity of gene expression. *Nature* 392: 933-936, 1998.

298. Hu, Q., S. Deshpande, K. Irani, and R. C. Ziegelstein. $[Ca^{2+}]_i$ oscillation frequency regulates agonist-stimulated NF-kappaB transcriptional activity. *J. Biol. Chem.* 274: 33995-33998, 1999.