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Acute and Chronic Effects of Quinapril on Cardiac Function, Ventricular Remodeling and Cytokine Expression

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Les inhibiteurs de l'enzyme de conversion de l'angiotensine II (IECA) réduisent le taux de mortalité chez les personnes atteintes de défaillance cardiaque congestive (DCC) et/ou victime d'un infarctus du myocarde (IM). Ceci a été démontré aussi bien chez l'humain que dans des modèles animaux. Les mécanismes responsables des effets bénéfiques observés semblent multiples et incluent des effets hémodynamiques, une réduction des effets cellulaires de l'angiotensine II et une augmentation de la production d'oxyde nitrique. Étant donné que les cytokines inflammatoires sont activées en période post IM et en DCC, et que cette augmentation contribue à l'initiation de dysfonctions ventriculaires et au remodelage, nous émettons l'hypothèse que les effets bénéfiques observés par ces agents sont aussi la résultante de leurs capacités à réduire le stress oxidatif et l'expression cardiaque de cytokines.

Un total de 402 rats ont subi un IM par ligature de l'artère coronaire gauche. Afin d'évaluer les effets des IECAs débutés précocement post IM, les rats ont été séparés en trois groupes et suivis durant une période de 28 jours : (1) IM sans traitement; (2) quinapril débuté le premier jour post IM; et (3) quinapril débuté le 25^{ième} jour post IM. De plus, afin d'évaluer les effets des IECAs durant le développement de la DCC (28 jours post IM), trois groupes additionnels ont été suivis durant une période de 84 jours; (1) IM sans traitement; (2) quinapril débuté 28 jours post IM; et (3) quinapril débuté 81 jours post IM. Précocement post IM, quinapril améliore l'hémodynamie cardiaque et prévient l'augmentation de l'expression de cytokines cardiaques (facteur de tumeur nécrosante-α, interleukine-1β, 5 et 10) indépendamment du temps de traitement. Seul le traitement au quinapril appliqué au premier jour post IM résulte en une amélioration du remodelage ventriculaire. Quand débuté plus tardivement post IM (28

jours) le quinapril n'a pas d'effet négatif ou positif sur les paramètres hémodynamiques ou le remodelage ventriculaire. Par contre, le traitement avec le quinapril débuté 28 ou 81 jours post IM, prévient l'augmentation de l'expression de cytokines cardiaques.

Dans ce modèle expérimental de DCC subséquent à un IM, les IECAs ont démontré des effets supérieurs sur les paramètres hémodynamiques ou le remodelage ventriculaire lorsque le traitement est débuté tôt post IM, plutôt que tardivement lorsque la DCC est développée. Par contre, les IECAs atténuent l'augmentation de l'expression cardiaque de cytokines dans les deux situations. Cette étude identifie un nouveau mécanisme par lequel les IECAs peuvent conférer des effets bénéfiques précocement et tardivement post IM.

Summary

ACE inhibitors reduce the mortality rates for congestive heart failure and myocardial infarction. This has been shown to occur both in patients and in animal post-myocardial infarction (MI) models.

Background Inflammatory cytokines have been shown to be activated post-MI and in congestive heart failure (CHF), and to contribute to ventricular dysfunction and remodeling. Angiotensin converting enzyme (ACE) inhibitors have been shown to be beneficial post-MI and in CHF, and we hypothesised that one of the mechanisms by which they exert their beneficial effects is by reducing cardiac expression of cytokines.

Methods and results In order to verify this, 402 rats had a MI created by coronary artery ligation. In order to evaluate the benefit of ACE inhibitors started early post-MI, rats were separated into 3 treatment groups and followed 28 days: (1) MI no therapy; (2) quinapril started day 1; and (3) quinapril started day 25. In order to evalute the effects of ACE inhibitors once CHF had developed, 3 more groups were started and followed for a total of 84 days. Following the initiation of CHF (28 days post-MI), rats were randomised to (1) MI no therapy; (2) quinapril started on day 28; (3) quinapril started on day 81. In the early post-MI group, quinapril improved cardiac hemodynamics and prevented the increase in cardiac cytokine expression (tumor necrosis factor-α [TNF-α], interleukin 1β, 5 and 6), regardless of when it was started. Only when quinapril was started on day 1 did it improve ventricular remodeling. In the CHF group (84 days), quinapril had no effect on cardiac hemodynamics or ventricular remodeling. Quinapril, whether started on day 28 or 81, prevented the rise in cardiac cytokine expression.

Conclusions In this post-MI model of CHF, ACE inhibitors have more beneficial effects on cardiac hemodynamics and ventricular remodeling when started early post-MI, rather than later when chronic CHF has developed. However, ACE inhibitors attenuate the rise in cardiac cytokine expression in both conditions. This study helps to identify a new mechanism by which ACE inhibitors may exert their beneficial effects in the early and late post MI-setting.

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LIST OF ABBREVIATIONS

AII	angiotension II
ACE	angiotensin converting enzyme
AMI	acute myocardial infarction
ANF	atrial natriuretic factor
ANP	atrial natriuretic peptide
AT1	angiotensin II receptor 1
AT2	angiotensin II receptor 2
AT3	angiotensin II receptor 3
AT4	angiotensin II receptor 4
$[Ca^{2+}]_i$	intracellular free calcium concentration
CAD	coronary artery disease
cDNA	cytosine deoxyribonucleic acid
CHF	congestive heart failure
DNA	deoxyribonucleic acid
+dP/dt	maximum rate of pressure rise
-dP/dt	maximum rate of pressure decline
ET	endothelin
g	gram
ICAM-1	intercellular adhesion molecule-1
IgG,	immune globular protein G

IL-1	interleukin-1
IL-1α	interleukin-1 alpha
IL-1β	interleukin-1 beta
IL-5	interleukin-5
IL-6	interleukin-6
INFγ	interferon-γ
Kg.	Kilogram
LV	left ventricle
LVEDP	LV end-diastolic pressure
LVSP	LV systolic pressure
MAP	mitogen activated protein
mg	milligram
MI	myocardial infarction
MI3Q1M	one month MI rats with 3 days quinapril
MI3Q3M	three month MI rats with 3 days quinapril
MIQ1M	one month MI rats with 1 month quinapril
MIQ3M	three month MI rats with last 2 month quinapril
MI1M	one month MI rats without medication
MI3M	three month MI rats without medication
ml	milliliter
mm Hg	millimeter mercury column
mRNA	messenger ribonucleic acid

NADH	dihydronicotinamide adenine dinucleotide
NADPH	dihydronicotinamide adenine dinucleotide phosphate
NO	nitric oxide
NP	natriuretic peptides
PCR	polymerase chain reaction
α^{32}P-UTP	alfar-phosphorus(32)-uridine triphosphate
RAS	renin angiotensin system
RPA	ribonuclease protection assay
RV	right ventricle
RVEDP	RV end-diastolic pressure
RVSP	RV systolic pressure
s1L-2R	soluble interleukin-2 receptor
S3Q1M	one month sham rats with 3 days quinapril
S3Q3M	three month sham rats with 3 days quinapril
SQ1M	one month sham rats with 1 month quinapril
SQ3M	three month sham rats with last 2 months quinapril
S1M	one month sham rats without medication
S3M	three month sham rats without medication
TNF-α	tumor necrosis factor α
tRNA	transfer ribonucleic acid
VIP	vasoactive intestinal polypeptide

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DEDICATION

To my wife, Xiu Ling Qi and my daughter Lan Wei for their endurance and support, without these I cannot achieve my goal.

CHAPTER I:

INTRODUCTION AND LITERATURE REVIEW

Acute and Chronic Effects of Quinapril on Cardiac Function, Ventricular Remodeling and Cytokine Expression

1.1: Angiotensin converting enzyme (ACE) and cardiovascular function

The cardiovascular system consists of the heart and a network of blood vessels that carry blood between the heart and peripheral tissues. Cardiovascular regulation is brought about by circulating substances and by the vasomotor nerves. The renin-angiotensin system (RAS) plays an important role in cardiovascular function and regulation. It is well known that cardiovascular system adjustments are effected by multiple regulatory mechanisms. These include: altering cardiac output via contractility, changing the diameter of the resistance vessels, or altering the amount of blood pooled in the capacitance vessels (the veins). The caliber of the blood vessels is affected by circulating vasoactive substances and by vasomotor nerves. The vasodilator hormones include kinins, vasoactive intestinal polypeptide (VIP), and natriuretic peptides (NP), while circulating vasoconstrictor hormones include vasopressin, norepinephrine, epinephrine, endothelin I and angiotensin II (AII).

Cardiac function is effected by multiple factors which includes neurotransmitters and hormones in the circulator system. Recently, it is increasingly recognized that endothelium can release several vasoactive substances, such as the contractile factors: endothelin, AII, thromboxane A2, and prostaglandins (PGH2); and the relaxing factors: nitric oxide (NO), prostacyclin (PGI2), and natriuretic peptides (NP). The most important factors for the regulation

cardiac function are endothelin, a powerful positive inotropic and vasoconstricting factor and NO, a relaxing factor of cardiac and vascular smooth muscle.

Under normal physiological conditions, the endothelium plays a major role in regulating myocardial contractility, vascular tone and platelet-endothelial interactions. The contractile factor endothelin and the relaxing factors NO, AII and NP are in balance in order to maintain homeostasis of the cardiovascular system. When this balance is altered, this results in pathological situations.

AII, an octapeptide hormone of the RAS, is produced both systemically and locally in the vessel wall. AII produces arteriolar constriction and a rise in systolic and diastolic blood pressure. It is one of the most potent vasoconstrictors known, being 4-8 times as active as norepinephrine on a weight basis in normal individuals (Ganong WF, 1993). AII has multiple sites of action and regulates a wide variety of physiological elements including action of the adrenal cortex, to increase the secretion of aldosterone, facilitation of the release of norepinephrine by a direct action on post ganglionic sympathetic neurons, increasing the secretion of vasopression and also regulation of salt and water balance (Parmley WW, 1998; Ganong WF, 1993).

There are at least 2 types of AII receptors, the AT1 and AT2. The AT1 receptor, which has been cloned, is a serpentine receptor coupled by a G protein to phospholipase C, and its activation increases cytosolic free Ca^{2+}. AT1 receptors, which are found in blood vessel walls, the adrenal cortex, the brain, and many other organs, mediate most of the known effects of AII. The physiological function of AII in the cardiovascular system is mediated mainly through the AII type 1 receptor. The AT2 receptor secondary messenger is not known, although it is not IP3 or cyclic AMP. The role of AT2 receptors is more important during fetal and neonatal life, but

they persist in the brain and other organs in adults. It appears likely that there are AT3 and AT4 receptors as well (Timmerman PB and Smith RD, 1994).

Angiotensin-converting enzyme (ACE) is a dipeptidyl-carboxypeptidase that is located mainly at the cell membrane of the endothelial cells. ACE converts the physiologically inactive angiotensin I into the powerful vasoconstrictor, AII. On the other hand, ACE is also the main pathway for the breakdown of bradykinin into inactive peptides. Most of the converting enzyme that forms AII in the circulation is located in endothelial cells. Much of the conversion occurs as the blood passes through the lungs, but there is also conversion in many other parts of the body.

Because ACE and Kininase II are the same enzyme, there are important interactions between the kinin system and the RAS. Indeed, the vasodilator effects of ACE inhibitors are due in part to their protective effects against the breakdown of locally produced bradykinin. Studies have shown that chronic treatment with an ACE inhibitor not only decreases the ratio of plasma AII to angiotensin I, but also increases the plasma level of bradykinin (Vanhoutte PM et al., 1989; Desta B et al., 1995).

1.2: AII and cardiac remodeling in myocardial infarction and heart failure

Myocardial remodeling is a central feature in the progression of myocardial failure. This process can be stimulated by mechanical stress, angiotensin, and norepinephrine. Several additional mechanisms have been identified that could also be important in mediating myocardial remodeling. These include oxidative stress, inflammatory cytokines, nitric oxide (NO), endothelin (ET), and peptide growth factors. Myocardial remodeling is a normal feature during maturation, and may be a useful adaption to increased demands (e.g., athletic training) in

the adult. However, when this process occurs in response to pathologic stimuli (e.g., abnormal wall stress) the remodeling that ensues, although perhaps adaptive in the short term, is maladaptive in the long term and often eventually leads to further myocardial dysfunction (Katz AM, 1994). The process of myocardial remodeling consists of a number of molecular and cellular changes. In general, pathologic myocardial remodeling involves an increase in myocardial mass associated with hypertrophy of individual myocytes, alterations in gene expression, and changes in both the quantity and composition of the extracellular matrix. Recent work suggests that pathologic remodeling also involves the death of cardiac myocytes by apoptosis, or programmed cell death (Colucci WS, 1997).

The RAS is present in the myocardium, and several components are up-regulated during myocardial remodeling or failure, including ACE activity, the level of angiotensinogen mRNA, and the density of angiotensin receptors (Hirsch AT et al., 1991; Lindpaintner K et al., 1993; Meggs LG et al., 1993). There is evidence that myocyte angiotensin is related to stretch of the cell and can mediate the effects of stretching on myocyte hypertrophy and gene expression (Sadoshima JI et al., 1993). These observations suggest that angiotensin could play an important role in pathologic myocardial remodeling, both as a circulating hormone and as an autocrine/paracrine mediator produced in response to hemodynamic overload. Circulating and locally (cardiac) produced AII may directly stimulate cardiac hypertrophy via induction of protooncogene and growth factor expression.

Recent research on angiotensin and coronary artery disease (CAD) indicates that angiotensin has direct cellular effects on the coronary artery and the myocardium (Dzau VJ, 1998a; Itoh H et al., 1993). Angiotensin is believed to directly affect the pathobiology of cardiovascular diseases through growth promoting activity. Binding of AII to its target, AII type

I receptor, in the vasculature triggers a cascade of secondary messenger systems (Gibbons GH et al.; Dzau VJ, 1998b). A direct outcome is the activation of phospholipase C, which then influences calcium-sensitive protein kinase C and cytoplasmic calcium. In addition, AII binding raises intracellular calcium levels via activation of receptor-operated calcium channels. Calcium mobilization and protein kinase C-mediated protein phosphorylation, activate nuclear elements that affect gene expression, protein synthesis and mitogenesis and/or hypertrophy (Lindpaintner K etal., 1993). AII also activates mitogen activated protein kinase (MAP kinase) that mediates cell growth.

Increased activity of the sympathetic nervous system and the RAS appear to contribute to progressive myocardial failure at least partially by acting directly on the myocardium to cause remodeling, which provides an explanation for the clinical success of agents which inhibit these pathways. Angiotensin increases protein synthesis in cardiac myocytes and DNA synthesis in cardiac fibroblasts (Sadoshima JI and Izumo S, 1993). In addition, angiotensin can cause apoptosis in cardiac myocytes in culture (Kajstura J et al., 1997). Both effects can be blocked by an antagonist selective for the AT1 receptor.

Angiotensin converting enzyme (ACE) inhibitors are now well established drugs in the management of hypertension, heart failure, coronary artery disease, acute myocardial infarction prevention of left ventricular (LV) dysfunction after myocardial infarction and diabetic nephropathy (Schachinger V, 1998; Hamaty M et al., 1998). ACE inhibitors act on the cardiac structure and the vessel wall, displaying cardioprotective and vasculoprotective properties. They influence endothelial function, and the thrombolytic system by acting on platelets and the plasminogen activator inhibitor-1/tissue type plasminogen activator balance. They also modulate ischemia-induced neurohormonal activation.

Similar to AII, plasma endothelin-1 (ET-1) concentrations are raised in patients with heart failure (Ertl G et al., 1982; Westlin W and Mullane K, 1988), ET-1 can be produced by a variety of cells in the myocardium, and both ET-1 and its receptors are up-regulated in remodeled myocardium (Sogaard P et al., 1993). ET-1 could act as an autocrine/paracrine mediator of myocardial remodeling in response to hemodynamic overload (Borghi C and Ambrosioni E, 1996). An interaction exists between these neurohormones with AII stimulating the expression of ET 1 (Ruiz Ortega M, 1997). Moreover, AII increases the production of endothelin in the blood vessel wall that via ET A receptors (Moreau P et al. 1997).

1.3: Cardiac oxidative stress and cytokine production in myocardial infarction and heart failure

Heart failure is a complex cascade of dynamic events controlled and influenced by many factors, including the neurohormonal system, autonomic system, and cytokines. Cytokines are highly potent, pleiotropic, endogenous peptides produced by a variety of cell types (Neta R et al., 1990). The proinflammatory cytokines involved with heart failure and dilated cardiomyopathy include: tumor necrosis factor (TNF-α), interleukin-1 alpha (1L-1α), interleukin-1 beta (1L-1β), interleukin 6 (1L-6), interleukin-2 (1L-2), soluble interleukin-2 receptor (s1L-2R), and interferon-γ (INFγ) (Blum A and Miller H, 1998). Important among these cytokines are TNF-α and 1L-6. They appear to exert deleterious effects on the heart and circulation which may also be involved in the progression of heart failure. Evidence suggests

that proinflammatory cytokines are capable of modulating cardiovascular function through a variety of mechanisms, such as promoting left ventricular (LV) remodeling, (Pagani FD et al., 1992) inducing contractile dysfunction, (Finkel MS et al., 1992) and uncoupling myocardial β-adrenergic receptors (Fowler MB et al., 1986).

Levels of TNF-α, a product of activated macrophages, are increased in the serum of patients with severe congestive heart failure (CHF) (Levine B et al., 1990), TNF-α is also increased in the myocardium of rats during myocardial infarction (MI) (Min W et al., 1999). TNF-α appears to induce apoptosis in cultured cardiac myocytes (Krown KA, 1996) and to play an important role in the regulation of NO metabolism in leukocytes, vascular endothelial cells, and vascular smooth muscle cells (Porber JS and Cortan RS, 1990). TNF-α has been reported to decrease messenger RNA for the constitutive NO synthase in vascular endothelial cells (Yoshizumi M et al., 1993), to increase expression of the inducible form of NO synthase in macrophages, vascular endothelial cells, and vascular smooth muscle cells (Busse R and Mulsch A, 1990) and to increase vascular smooth muscle production of superoxide anion, a substance known to decrease the half-life of NO (Matsubara T and Ziff M, 1986). Studies with animals have shown that TNF-α, 1L-6 and 1L-2 inhibit the contractility of papillary muscles in a concentration-dependent, reversible manner. Part of the negative inotropic effects of cytokines is mediated through the induction of myocardial NO synthesis (Ing DJ et al., 1999). One study demonstrated that patients with acute MI had elevated levels of soluble 1L-2 receptors (s1L-2R) and 1L-1, especially in the presence of CHF and a low ejection fraction (Blum A et al., 1994). The worse the CHF during acute MI, the higher were the levels of the cytokines s1L-2R and 1L-

1. In addition, Interleukin-6 can induce the expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) (Youker K et al., 1992; Ikeda U et al., 1994).

Oxidative stress appears to be increased in the myocardium during the transition from compensated hypertrophy to failure (Dhalla AK and Singal PK, 1994; Dhalla AK et al.,1996). Experiments suggest that mechanical stress on the myocardium can increase tissue oxidative stress and lead to apoptosis of myocytes (Chen W et al., 1995). Another potentially important stimulus for increased oxidative stress in the myocardium is exposure to inflammatory cytokines, such as TNF-α, which are increased in failing myocardium (Levine B et al., 1990; Torre-Amione G et al., 1996) and have the ability to stimulate free radical production (Meier B et al., 1989). A recent study using cardiac myocytes in culture suggests that exposure to free radicals can cause apoptosis and can activate growth pathways associated with increased protein synthesis and fetal gene expression (Ruffolo RR Jr et al., 1998). Oxygen free radicals can induce the expression of immediate early genes associated with myocyte growth and apoptosis (Webster KA et al., 1994), and have also been shown to stimulate fibroblast proliferation (Murrell AC et al., 1990). Observations suggest that increased oxidative stress in the myocardium contributes to myocardial remodeling and failure, and raises the possibility that antioxidants may have a valuable role in the prevention of myocardial failure. Interestingly, the addition of NO-releasing agents attenuates both the generation of reactive oxygen species and apoptosis.

AII is a very powerful stimulus of oxidative stress. When vascular cells are exposed to AII in vitro there is a significant increase in reactive oxygen species production via the activation of NADH/NADPH oxidase (Griendling KH et al., 1994). In vivo infusion of AII results in increased superoxide anion production in the vessel wall (Rajagopalan S et al., 1996).

This increased oxidative stress is associated with an increase in monocyte adhesion and release of cytokines by these monocytes (Hahn AW et al., 1994). It is known that many of the factors that contribute to endothelial dysfunction, such as an imbalance between nitric oxide and reactive oxygen species are mediated by increased AII production. This cycle of events initiates an inflammatory process in the vessel wall.

1.4: The beneficial effects and mechanism of ACE inhibitors in current opinions for myocardial infarction and heart failure

The many actions of ACE inhibitors include vasodilation, inhibition of the renin-angiotensin-aldosterone system, augmentation of the kinin system, an anti-endothelin effect, and inhibition of the growth of myocardial and vascular tissues.

Blockade of the RAS during the acute phase of a MI has been reported to reduce infarct size (Ertl G et al., 1982) and to promote the contractile recovery of stunned myocardium (Westlin W and Mullane K, 1988). More recently, Soogard and coworkers (Sogaard P et al., 1993) demonstrated that captopril exerts an anti-ischemic effect independent of the prevention of left ventricular dysfunction in patients with MI. Neurohormonal activation is a key event in the development and progression of heart failure. In a canine model of myocardial ischemia, the circulating RAS becomes activated only minutes after acute coronary artery occlusion (Ertl G et al., 1983). In patients who develop heart failure, activation of the RAS may last for at least 10 days following MI. It is postulated that ACE inhibitors could reduce myocardial ischemia by inhibiting the increase in vasoconstricting neurohormones during acute myocardial ischemia, and by diminishing inappropriate systemic and coronary vasoconstriction. This would lead to a

reduction in myocardial oxygen demand, while at the same time improving coronary flow. Experimental studies have shown that ACE inhibition improves endothelial function. ACE inhibition stimulates endothelial release of NO and prostacyclin, by a bradykinin-mediated mechanism, thereby enhancing endothelial-dependent vasodilation (Mombouli JV et al., 1992; Groves P et al., 1995). ACE inhibitors are effective in reducing LV mass in animal models as well as in hypertensive subjects (Ferrari R, 1998). ACE inhibitors have been shown to scavenge free radicals in vitro (Suzuki S et al., 1993), and may also exert anti-atherogenic and anti-thrombotic activity (Michael JB et al., 1992; Luskutoff DJ et al., 1989).

In general, the available evidence on the use of ACE inhibitors in a large population of patients with AMI indicates a clear benefit related to their administration early after the onset of symptoms. The clinical advantage can be demonstrated very early (during the first day) and persists during long-term follow-up. Early treatment with an ACE inhibitor can result in some structural or functional benefits, leading to less ventricular dysfunction (Borghi C and Ambrosioni E, 1996). The vast majority of the lives that can be saved with the early use of an ACE inhibitor reside in clinically identifiable populations. Among the high-risk subgroups, early ACE inhibition is particularly beneficial in elderly people, in women and patients with left ventricular dysfunction or heart failure, the treatment of whom results in a significant reduction in both mortality and the occurrence of CHF (The GISSI-3 Investigators, 1994; ISIS-4 Collaborative Group, 1995). However, opportunities for benefit could be lost by unnecessary delays of days or weeks in initiating them (Pfeffer MA, 1998).

There is evidence that at least some of the beneficial effects of ACE inhibitors on ventricular remodeling may be derived from an increase in bradykinin (McDonald KM et al., 1998). Bradykinin has an anti-growth and anti-proliferative effects likely mediated by

stimulation of NO and prostacyclin, which could contribute to the overall effects of ACE inhibition (Scicli AG and Carretero OA, 1995). Moreover, the vasodilator effects of ACE inhibitors might be partially explained by the interaction between angiotensin II and endothelin. AII stimulates the expression of endothelin mRNA (Ruiz-Ortega M, 1997). Moreau et al. Reported that endothelin antagonists may contribute to reduce blood pressure and to prevent vascular structure changes in conditions of increased activity of the renin-angiotensin system (Moreau P et al. 1997). Therefore, even though ACE inhibitors only partially block the production of AII, endothelin transcription and translation may well be suppressed. This implies that the reduced production of endothelin may be a contributory factor in the vasodilator mechanism of ACE inhibition.

1.5: The aims of this thesis

Obviously, ACE inhibitors are beneficial for patients with acute MI and CHF. The mechanisms of these beneficial effects are multiple and quite complex. One potential mechanism is an effect on oxidative stress and cytokine production. However, at this time this relationship remains poorly defined such that more work needs to be performed in this area, especially in clinically relevant in vivo models.

As mentioned above, ACE inhibitors could conceivably exert part of their beneficial effects in CHF by reducing cardiac cytokine expression (Hernandez-Presa MA et al., 1998; Peeters AC et al., 1998). This could be accomplished directly by reducing angiotensin II, and indirectly by increasing nitric oxide production and by improving cardiac hemodynamics (Hartman JC, 1995; Hoshida S et al., 1999; Hu K et al., 1998). In order to test the hypothesis as

to whether the use of ACE inhibitors was associated with a reduction in cardiac cytokine expression in CHF, we studied the cardiac hemodynamic and remodeling changes in the rat post MI model of CHF. We studied the early post-infarction (1 month) period to evaluate what occurred when post-MI ventricular remodeling was involved, and later (3 months) to evaluate this interaction when chronic post-MI heart failure had occurred.

Our hypothesis was that the beneficial effects of the ACE inhibitor, quinapril, on ventricular remodeling and function post-infarction and in chronic CHF were associated with a decrease in cytokine production.

CHAPTER II:

MATERIALS AND METHODS

Animal and MI operations

Male Wistar rats (n=402, Charles River. St-Constant, Qc) weighting 200 to 250g were used for this study. All rats were housed in an environmentally controlled room with 12 hours intervals of light-dark cycles, and free access to normal rat chow. The animals were prepared in accordance with the "Guidelines of the Canadian Council on Animal Care".

Rats were anesthetized with 2% halothane (Wyeth-Ayerst Canada Inc., Montreal, Quebec). A left-side thoracotomy was performed, and the left anterior descending artery was ligated near its origin. The incision was closed with a Mikron wound clip applicator (Clay Adams, Pasipanny, NJ). The sham-operated rats underwent the same procedure except for the ligation of the coronary artery. For the purposes of this study, an MI was defined as a heart with a scar weight > 0.10g (for hearts used for biochemical measurements) or heart with a scar covering > 45% of the circumference of the heart (for hearts used for histologic studies)(Nguyen QT et al., 1998).

Treatment interventions

Protocol A: Early intervention and 28 days follow-up (1 month group):

The goal of this protocol was to evaluate the effects of quinapril when started early post-MI when left ventricular (LV) remodeling is in a very active phase. Rats in this 28 days (1M) protocol were divided into six groups: three groups of sham control: a) rats without medication (S1M); b) rats not treated for 25 days then treated for 3 days with quinapril (S3Q1M); and c) rats treated from day 1 to 28 days with quinapril (SQ1M). and 3 groups with MI: d) MI rats without medication (MI1M); e) MI rats not treated for 25 days then treated 3 days with quinapril

(MI3Q1M); and f) MI rats treated from day 1 to 28 days with quinapril (MIQ1M). The group S3Q1M was included in order to study the effects of quinapril in this setting on cardiac hemodynamics and cardiac cytokine production, independent of its effects on LV remodeling. Quinapril were given in the drinking water at a concentration of 200 mg/L (Ruzicka M and Leenen FHH, 1995).

Protocol B: Late intervention (28 days post-MI) and long term treatment (56 days)(3 months group):

The goal of this protocol was to evaluate the effects of quinapril on chronic post-MI heart failure, once post-MI LV remodeling was no longer a major factor. In this protocol, rats were also divided into six groups. 3 groups of sham control: a) rats without medication (S3M); b) rats not treated for 81 days, then treated for 3 days with quinapril (S3Q3M); and c) rats not treated for 28 days then treated for 56 days with quinapril (SQ3M). and 3 groups of MI: d) MI rats without medication (MI3M), e) MI rats not treated for 81 days then treated for 3 days with quinapril (MI3Q3M), and f) MI rats not treated for 28 days then treated with quinapril for 56 days (MIQ3M). Quinapril was given in the drinking water at the same dose as protocol A.

Cardiac hemodynamic studies

At the time of sacrifice (28 or 84 days post-MI), rats were weighted and anesthetized with a mixture of ketamine (87mg/kg) and rompun xylanine (13mg/kg). The jugular vein and carotid artery then were isolated, and a 2F Millar catheter with a pressure sensor at the tip (model SPR-470, Millar Instrument Inc., Houston, TX) was inserted and advanced into the right and the left ventricles respectively. Left and right ventricular systolic (LVSP) and end-diastolic

(LVEDP) pressures, as well as the maximum rate of pressure increase and decline, were obtained respectively. The LV maximum rate of pressure rise (+dP/dt) and decline (-dP/dt) were derived by active analogue differentiation of the pressure signal. Similarly, for right ventricular pressure measurements, a 2F Millar catheter with the pressure sensor at the tip deviated from the shaft by an angle of about 125^0 was inserted into the right jugular vein. It was then advanced into the right ventricle and ventricular systolic (RVSP), end-diastolic (RVEDP), and RV +dP/dt and –dP/dt were obtained. Hemodynamic measurements were recorded on a Gould 2,600 second recorder. Catheters were then withdrawn and the vessels were ligated. Once the hemodynamic studies were completed (Table 1 and 2), the rats were sacrificed and the hearts randomised to either morphologic or molecular studies. The left and right ventricles, the left and right atria and the lungs were weighted separately.

Passive Pressure-Volume Relationship

After completing the cardiac hemodynamic measurements, 66 rats had their hearts stopped in diastole by an intravenous injection of a saturated potassium chloride solution via the right jugular vein. The heart was then rapidly excised, rinsed with a saline solution, and the right ventricle (RV) was opened to eliminate its influence on LV expansion. Then a double lumen catheter (PE-50 inside PE-200) was inserted 6 mm into the LV via the aorta, the atrio-ventricular groove was ligated, and the LV was emptied of all remaining solution. A negative pressure (-5 mm Hg) was achieved in the LV using a syringe connected to a 3-way stopcock positioned between the LV and the pressure transducer. Physiological saline was infused at 0.68 ml/min via the PE-200. This was done for pressures between –5 and 40 mm Hg. Three pressure-

volume curves were obtained within 10 minutes and an average of these 3 curves was used as the final value ±SEM(Fig 2) (Blum A et al., 1994).

Afterwards, the hearts was filled with saline solution to a pressure of 15 mm Hg, sealed and fixed in 10% formalin phosphate buffer solution for 24 hours. After fixation, the atria and large conduit vessels were dissected, the RV was cut off the heart along its septal insertion and both right and left atria, and ventricular weights were weighed separately. Two hearts were then sectioned midway between the apex and the base and cross-sections slices obtained 1mm apart were analyzed to determine MI size, as previously described (Nguyen QT et al., 1998), or used for immunohistochemical studies.

Cardiac anatomy

After the cardiac hemodynamic studies were finished, 82 hearts were excised and put in liquid nitrogen for biochemiscal studies. Before the isolated hearts were put in liquid nitrogen, Atria, left ventricle, septum, right ventricles and scar were separated and weighted individually. The scarred area surface was determined by planimetry. The lungs also were excised and weighted quickly, all tissues were put in liquid nitrogen immediately and stored at –80°C (Tables 3 and 4).

Immunohistochemistry of TNF-α studies

Expression of TNF-α in cardiac tissues was determined immunohistochemically. Two of the cardiac cross-sections embedded in paraffin were sectioned (6μm) with a microtome and applied on glass slides. The sections were deparaffinized in xylene and ethanol baths, endogenous peroxidase activity quenched in a methanol-hydrogen peroxide solution. Non-specific antibody binding was prevented by pre-incubating the tissues with a 5% horse serum treatment. Sections were exposed to goat polyclonal anti-rat TNF-α IgG (1:250 dilution) (R&D Systems). A purified non-specific goat IgG (1:250 dilution) was used as a primary negative control. The secondary antibodies were biotinylated horse anti-goat IgG (1:400 dilution) (Santa Cruz). Revelation of bound antibodies was achieved with an avidin/peroxidase complex (Vector) and counter-stained in Gill's hematoxylin solution. Positive TNF-α expression was confirmed by a specific brown staining of the cells. Each segment was analyzed with a dedicated 3CCD video microscope adapted to a customized software by an unbiased observer.

Ribonuclease protection assay for cytokine panel

To determine the gene expression of cytokines from the affected myocardium, ribonuclease protection assay (RPA) was performed to quantitate the mRNA levels of TNF-α, interleukin-1, 5 and 6 (IL-1,5,6). The cDNA probes specific for the rat target cytokines were prepared from a commercially available kit based on known coding sequences (RiboQuant, PharMingen), according to manufacturer's instructions. Specifically, the cDNA templates of the target cytokines were amplified using PCR and labelled with α^{32}P-UTP (Dupont, New England Nuclear). Commercially available cDNA probes for housekeeping genes L32 and GAPDH were also amplified and labelled to act as standardization controls.

The total RNA from the myocardial samples were extracted and purified according to techniques previously published from our laboratory (Irwin MW et al., 1999), taking particular care to ensure RNase free reaction conditions. RPA was performed with the co-addition of 10 μg of total RNA, the labelled probes (at least 2 x 10^4cpm) and tRNA to act as background control into prepared hybridization buffer, incubated for 16 hours at 53°C. RNase digestion was then performed with RNase A and Rnase T1 at 30°C for 45 minutes. The reaction was terminated with proteinase K, followed by phenol-chloroform extraction and ethanol precipitation. The protected fragments were then resolved on 5% polyacrylamide gel electrophoresis together with standard size markers. After gel drying and the autoradiography, the probe activities were documented by phosphoimager (Biorad) and the results digitally quantitated and normalized to GAPDH for each sample at each time point observed with NIH image software.

Statistics

All data in this study are express as means ±SEM. When appropriate, results were compared using a 2-tailed student's *t* test for 2 groups of unpaired data. Otherwise, differences between groups were evaluated by ANOVA followed by a Dunnett's test. The differences of survival rate among the different groups of animals were assessed by Rank test (Kaplan – Meier).

Differences were considered significant at $p < 0.05$.

CHAPTER III:

RESULTS

MI operations and survival

Protocol A: 1 month:

A total of 112 rats had their left anterior descending coronary artery ligated and followed for a total of 28 days. Of these, 79 (70.5%) survived the first 24 hours. The 4-week survival rate of the quinapril treated rats was 58.8%, not statistically different from the survival rate of the untreated MI rats (50.0%) (Figure 1A). The rats treated only last 3 days with quinapril are included in the untreated MI survival group for this analysis.

Protocol B: 3 month:

A total of 168 rats had their left anterior descending coronary artery ligated and followed for a total of 84 days. Of these, 97 (57.7%) survived the first 24 hours. The 12-week survival rate of the quinapril treated rats (therapy started after 4 weeks only) was 48.5%, not statistically different from the survival rate of the untreated MI rats (44.0%) (Figure 1B). The rats treated only last 3 days with quinapril are included in the untreated MI survival group for this analysis.

Cardiac hemodynamic study

Protocol A: 1 month:

Quinapril had no hemodynamic effect in the sham operated rats except for a decrease in left ventricular systolic pressure (LVSP) in the 1 month quinapril group (SQIM) (Table 1). As compared to the sham operated rats, the MI no treatment group (MI1M) had widespread evidence of LV dysfunction. There was a decrease in LVSP, in LV maximum rate of pressure rise (LV+dp/dt) and decline (LV-dp/dt) and an increase in LV end diastolic pressure (LVEDP),

in right ventricular systolic (RVSP) and end diastolic pressures (RVEDP), and an increase in RV+dp/dt and in RV-dp/dt. Treatment with quinapril, whether for the full 28 days (MIQ1M) or for only the last 3 days (MI3Q1M), had the same hemodynamic effects. Although as compared to sham-operated rats they had widespread evidence of LV dysfunction, they had less of an increase in LVEDP, in RVSP, in RVEDP, in RV+dp/dt and in RV-dp/dt than their untreated MI counterparts, indicating some improvement in LV function with quinapril.

Protocol B: 3 months:

In the chronic group, quinapril had no hemodynamic effect in the sham operated rats, except for a decrease in LVSP in the 3 month quinapril group (SQ3M) (Table 2). As compared with the sham-operated rats, the MI no treatment group (MI3M) had widespread evidence of LV dysfunction, similar to that of the MI rats followed only 1 month post-infarction. The only exceptions were that the increase in LVEDP, RVSP and RVEDP were less marked. As compared to sham-operated rats, there was a decrease in LVSP, in LV+dp/dt, in LV-dp/dt, and an increase in LVEDP, in RVSP, in RVEDP, and RV+dp/dt. Treatment with quinapril, whether it was for 56 or only 3 days, did not have a significant effect on these changes. This contrasts with the 1-month rats where both 27 days and 3 days of quinapril had significant beneficial effects on cardiac hemodynamics.

Cardiac passive-volume relationship

Protocol A: 1 month:

A total of 41 rats were used to evaluate the passive diastolic pressure-volume relationship of the various 1 month groups (Fig. 2A). Rats treated only 3 days with quinapril were considered together with untreated rats, as their results were similar. Sham-operated

quinapril treated hearts (SQ1M) had a pressure-volume relationship that was shifted to the left as compared with the sham untreated hearts (S1M). The MI untreated hearts (MI1M) had a curve that was significantly shifted to the right, indicating ventricular dilatation. MI quinapril treated hearts (MIQ1M) had a pressure-volume relationship that was significantly shifted leftward as compared to the MI1M group indicating attenuated ventricular dilatation.

Protocol B: 3 months:

A total of 25 rats were used to evaluate the passive diastolic pressure-volume relationship of the various 3 month groups (Fig. 2B). Rats that were sham-operated had similar pressure-volume relationships regardless of the treatment. Rats that had MI had a similar rightward shift in the pressure-volume relationship regardless of treatment group. This rightward shift was greater than that seen in hearts 1-month post-infarction indicating progressive ventricular dilatation over time post-infarction.

Cardiac morphology:

Protocol A: 1 month:

There were no significant differences in the 2 sham-operated 1-month groups, except for a significant decrease in LV weight in the quinapril treated group as compared to the untreated group (Table 3). As rats treated with quinapril for only 3 days were similar to the untreated rats, they were considered together. Rats with MI that were untreated (MI1M) had a significant increase in RV and septal weights and a decrease in LV free wall weight as compared to untreated shams. MI rats treated with quinapril had no increase in RV or septal weights but a decrease in LV free wall weight as compared to untreated or quinapril treated shams. Part of these differences could be explained by the decrease in body weight of the rats. The scar weight

was similar among the 2 MI groups (p=0.248) but lung weight was higher in the MI groups indicating more lung congestion in the group. MI size, as expressed by % of LV circumference, was similar in all 3 MI groups, a mean of 49 to 51% of the circumference being scar.

Protocol B: 3 months:

There were no significant differences in the 2 sham-operated 3-months groups except for a significant decrease in LV and atrial weights in the quinapril treated group as compared to the untreated group (Table 4). As rats treated with quinapril for only 3-days were similar to rats that were untreated they were considered together. Both MI groups had an increase in RV, septal and atrial weights as compared with sham operated rats. Lung and scar weights were also similarly increased in the three MI groups. The only difference between the MI groups was a lower LV free wall weight in the quinapril group. The LV free wall weight was lower in the 2 MI groups as compared to their respective controls. MI size, as expressed as % of LV circumference, was similar in 3 MI groups, a mean of 47 to 51% of the circumference being scar, depending on the group.

Cytokines expression:

Protocols A and B:

In the sham operated hearts, there was only mild expression of the various cytokines measured (Figure 3 and Table 5). Treatment with quinapril had no effect on cytokine expression. The cytokines measured included TNF alpha, interleukin-1 beta (IL-1 beta), IL-5 and IL-6. Hearts with an MI had an increase in cytokine expression, whether it be in the 1 or 3 month groups. Treatment with quinapril, whether it was chronic or for only 3 days, significantly reduced the expression of all of the cytokines measured in both the MI 1 and 3 month groups.

Immunohistochemistry

Protocols A and B:

Very little myocardial staining for TNF-α could be found in the control hearts (Figure 5 and 6). There was more myocardial staining for TNF-α in the untreated MI hearts, particularly in the peri-infarct area. This was true for both the MI1M and MI3M groups. Quinapril, whether it be given chronically or for only 3 days, significantly reduced myocardial staining for TNF-α.

CHAPTER IV:

DISCUSSION AND CONCLUSION

Discussion

The present study demonstrates that early treatment with the ACE inhibitor quinapril post large MI exerts beneficial effects on cardiac hemodynamics, pulmonary congestion, LV remodeling and cardiac expression of various pro-inflammatory cytokines. Long-term administration of quinapril only appears to be necessary for the beneficial effects of quinapril on LV remodeling in this setting. When started later post-infarction, when most of LV remodeling is completed, the effects of 3 days or 56 days of quinapril treatment are much less impressive. Quinapril did not significantly modify cardiac hemodynamics, pulmonary congestion, or LV remodeling. It did, however, reduce cardiac expression of inflammatory cytokines. Taken together, these results suggest that quinapril suppresses cardiac expression of inflammatory cytokines, regardless of time post-infarction but appears to have more important effects on cardiac hemodynamics and LV remodeling early post-infarction. Quinapril does not appear to exert its beneficial hemodynamic effects via reduction of cardiac cytokine expression either earlier (1 month) or later post-infarction (3 months). This study thus provides another mechanism by which ACE inhibitors may exert their beneficial effects in patients post-MI or with CHF.

In this study, treatment with quinapril did not improve survival. These results are compatible with those of Pfeffer et al in rats with large MI treated with captopril (Pfeffer JM et al., 1985). In this study, only rats with a moderate MI had improved survival with captopril treatment, those with the largest MI having ~~the~~ no prolonged survival with captopril. Nevertheless, had the treatment with quinapril been continued longer, it is possible that the beneficial trends in favor of quinapril could have become significant. Although we did not

evaluate whether heart failure had developed at the time when quinapril was started in the long-term post-MI rats, because their MI size was similar to that of the untreated rats at 28 days, and CHF had developed in those rats, it is likely that CHF had developed at the time that quinapril was started.

Hemodynamics:

Earlier post-MI, we and others have documented activation of the renin-angiotensin system, both in humans (Rouleau JL et al., 1994) and in this post-infarction model (Bélichard P et al., 1994). In humans, this activation persists at least 10 days post-MI when there is LV dysfunction (Rouleau JL et al., 1994). Thereafter, there is gradual attenuation of this activation (Vantrimpont P et al., 1998). In some patients there is long-term activation of the system secondary to the simultaneous use of diuretics that are known to chronically maintain activation of the system (Laragh JH, 1986). In this rat post-infarction model, we have documented activation of the renin-angiotensin system until at least 28 days post-infarction (Bélichard P et al., 1994). Thereafter the degree of activation in this model is the subject of speculation. Consistent with activation of the renin-angiotensin system at 28 days post-infarction, we found that the use of the ACE inhibitor quinapril resulted in significant beneficial hemodynamic effects. This beneficial hemodynamic effect appeared to be at least partially independent of its effects on LV remodeling, as it was found to occur whether quinapril was started 1 day post-infarction and continued for 27 days or just 3 days prior to the hemodynamic measurements. When started later post-infarction, once LV remodeling and LV dysfunction were firmly established, quinapril had little hemodynamic effect, whether it was given 56 days or 3 days only. These findings are consistent with less activation of the renin-angiotensin at this stage of

chronic CHF when no diuretics are given (Laragh JH, 1986), and less beneficial effect on LV remodeling when an ACE inhibitor is started so late post-infarction (The RESOLVD Pilot Study Investigators, 1999).

Ventricular remodeling:

ACE inhibitors have been shown to have beneficial effects on LV remodeling when started early post-MI (Pfeffer JM et al., 1985; St John Sutton M et al., 1994). When started once chronic CHF has developed, the effects of ACE inhibitors on LV remodeling are less important, being limited to attenuation of slowly progressive ventricular dilatation (The RESOLVD Pilot Study Investigators, 1999). In this study, quinapril markedly attenuated LV dilatation when started early post-infarction, but had little effect once CHF was well established and post-MI ventricular scar remodeling was finished and the early dynamic phase of myocardial remodeling was over (Pfeffer JM et al., 1985; Pfeffer MA, Braunwald E 1990). Nevertheless, quinapril tended to reduce LV weight regardless of whether an MI had occurred or not, suggesting that quinapril attenuated growth to some extent in all hearts in which it was given. Taken together, these results suggest that the major beneficial effect of ACE inhibitors on LV remodeling post-MI occur early, and once chronic CHF has occured its effects are much less noticeable.

Cardiac cytokines expression:

Some expression of cardiac cytokines was documented in all hearts, even the untreated sham-operated hearts. The hearts that had suffered an MI had an increase in all of the inflammatory cytokines that we measured. This was true both 1 and 3 months post-infarction. It has been observed that CHF involves activation of several cytokine systems, including TNF-α (Torre-Amione G et al.,1996;Ono K et al., 1998; Blum A et al., 1994; Irwin MW et al., 1999; Testa M et al., 1996). In CHF cytokines are thought to be induced by local increases in myocardial wall stress, oxygen free radicals, and local neurohumoral activation as part of the myocyte phenotype change following infarction (Nakamura K et al., 1998; Ikeda U et al., 1995; Yamauchi-Takihara K et al., 1995; Kapadia SR et al., 1997). TNF-α is known to activate matrix metalloproteinases and promote modest hypertrophic growth in cardiac myocytes (Yue P et al., 1998; Yokoyama T et al., 1997; Westermarck J et al., 1995; Dollery CM et al., 1995). It also stimulates the expression of inducible nitric oxide synthase (Balligand JL et al., 1993). One study has shown that TNF-α upregulation occurs very early after myocardial injury and persists in myocytes for a significant period of time (Irwin MW et al., 1999). The persistence of TNF-α into the late stages of the MI may be cardiotoxic not only due to the negative inotropic effects of TNF-α and the other inflammatory cytokines, but also from TNF-α induced apoptosis as part the overall remodeling process (Krown KA et al., 1996; Robaye B et al., 1991).

Quinapril reduced the expression of all measured cytokines at both 1 and 3 months post-infarction. This was true whether the rat was treated with quinapril chronically or for only 3 days and regardless of whether quinapril treatment resulted in significant hemodynamic effects or not. Taken together, these results suggest that the anti-cytokine effects of quinapril

are the result of its direct effects on the heart and not due to its hemodynamic effects nor its effects on ventricular remodeling. Also, because in the chronic post-infarction hearts, quinapril therapy resulted in a significant reduction in cardiac cytokine expression without accompanying hemodynamic changes, it would appear that the increase in cytokine expression in these infarcted hearts did not result in significant hemodynamic changes.

Why quinapril should reduce cardiac cytokine expression remains speculative, but it has been shown to do it in other tissues (Hernandez-Presa MA et al., 1998; Peters AC et al., 1998), and a number of possibilities exist. Quinapril can reduce the tissue levels of angiotensin II, which has multiple effects on the myocyte. Angiotensin II through both G-protein coupled and JAK-STAT pathways can activate a number of transcription factors such as, AP- and NF-kB (Berk BC, Corson MA. 1997). The latter can in turn increase the expression of cytokines within the myocyte. Another important mechanism is the indirect anti-oxidant effect of quinapril. ACE inhibitors can exert anti-oxidant effects by reducing angiotensin II production at the tissue level, by reducing myocardial stretch, and by stimulating NO production via its effect on bradykinin (Nakamura K et al., 1998; Ikeda U et al., 1995; Yamauchi-Takihara K et al., 1995; Kapadia SR et al., 1997; Koh KK et al., 1999; Pepine CJ. 1998). Oxygen free radicals are powerful stimuli for the expression of cytokines (Yamauchi-Takihara K et al., 1995;Satoh M et al., 1997) which in turn further stimulate oxygen free radical production setting up a vicious cycle which further amplifies the activation of both cytokines and oxydative stress (Mohler ER et al., 1997). This effect of quinapril on cardiac cytokine expression could be one of the mechanisms by which it improves endothelial function in large and small vessels in patients with coronary artery disease and LV dysfunction (Drexler H et al., 1995; Qi XL et al., 1999). By increasing oxygen free

radicals, TNF-α may lead to the destruction of the NO produced by the endothelium. TNF-α also leads to down-regulation of constitutive NO synthase and to an increased rate of endothelial-cell apoptosis (Robaye B et al., 1991).

Another potential mechanism by which ACE inhibitors may reduce cytokine production is by reducing angiotensin II induced expression of ET-1. Available data would suggest that ET-1 stimulates the synthesis of the potent cytokines TNF-α and interferon-γ (Ruetten H, Thiemermann C et al., 1997).

This study thus demonstrates the important contribution of angiotensin II related pathways in promoting cytokine production in the myocardium following MI and in CHF. In addition, this study shows for the first time that quinapril, as an ACE inhibitor, can decrease the intrinsic myocardial levels of cytokines, independent of its hemodynamic effects. This suggests that ACE inhibitors likely exert their beneficial hemodymanic effects post-MI and in CHF by mechanism that are not directly related to cytokine production. It would appear that early ACE inhibitor administration is more beneficial than the later application of ACE inhibitors to post-MI treatments.

Conclusion

In this study, we evaluated the effects of the ACE inhibitor quinapril on cardiac cytokine expression in the early (28 days) and late post-infarction (84 days) period, the expression of cardiac cytokines was documented in all hearts. Our results demonstrated that the early quinapril treatment continued for 4 weeks not only improved hemodynamic parameters significantly but also attenuated ventricular dilatation and hypertrophy and reduced pulmonary congestion. However, when started later post-infarction (3 weeks), once heart failure was established and the most acute phase of ventricular remodeling finished, it had few effects on cardiac hemodynamics or ventricular remodeling but reduced cardiac cytokine expression. This was true whether quinapril was given for a period of 56 days or only 3 days prior to sacrifice. These results highlight differential effects of quinapril on cardiac hemodynamics and ventricular remodeling early and late post-infarction and provides a new mechanism by which ACE inhibitors may exert their beneficial effects, though a decrease in cardiac cytokine expression.

CHAPTER V:

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Table 1 **The hemodynamic characteristics of 1 month rat hearts**

Groups	S1M (n=11)	S3Q1M (n=11)	SQ1M (n=12)	MI1M (n=9)	MI3Q1M (n=12)	MIQ1M (n=17)	P value
HR (beats/min)	256±9	251±11	251±6	259±13	255±8	250±6	=0.975
LVSP (mmHg)	118±2.6+	118±6.8+	106±4.4+	96±4.5*	93±4.4*	97±3.6*	<0.001
LVEDP (mmHg)	5.3±0.4+	5.6±0.6+	5.8±1.0+	28.8±1.0*	16.3±2.6*+	19.3±1.9*+	<0.001
LV+dp/dt (mmHg/s)	7014±454+	7182±418+	6729±248+	4300±389*	4475±170*	4547±226*	<0.001
LV-dp/dt (mmHg/s)	5168±317+	5391±347+	4646±272+	1984±321*	2327±186*	2519±128*+	<0.001
RVSP (mmHg)	28.8±0.7+	28.6±1.4+	27.0±0.5+	58.4±4.0*	41.0±4.4*+	37.4±3.4*+	<0.001
RVEDP (mmHg)	2.6±0.2+	2.9±0.3+	2.8±0.4+	9.1±1.5*	6.2±1.0*+	5.8±0.7*+	<0.001
RV+dp/dt (mmHg/s)	1461±90+	1559±107+	1401±62+	2031±164*	1598±133+	1630±144+	=0.038
RV-dp/dt (mmHg/s)	925±49+	1010±81+	832±42+	1422±89*	998±70+	937±97+	<0.001

Values are means ± s.e.m.; HR, heart rate; +dp/dt, rate of pressure rise; -dp/dt, rate of pressure decline;
* $p<0.05$ vs S1M, +$p<0.05$ vs MI1M.

Table 2 **The hemodynamic characteristics of 3 months rat hearts**

Groups	S3M (n=14)	S3Q3M (n=14)	SQ3M (n=16)	MI3M) (n=13)	MI3Q3M (n=12)	MIQ3M (n=14)	P value
HR (beats/min)	261±5	269±10	248±8	252±7	254±8	254±9	=0.496
LVSP (mmHg)	127±7.2+	117±4.6+	112±3.4*+	93±3.3*	98±1.4*	93±5.8*	<0.001
LVEDP (mmHg)	5.6±0.6+	6.5±0.8+	6.1±0.8+	21.0±2.7*	17.7±3.6*	19.7±2.7*	<0.001
LV+dp/dt (mmHg/s)	7293±260+	7029±236+	5891±214*+	4420±267*	4521±125*	4279±394*	<0.001
LV-dp/dt (mmHg/s)	5575±323+	5279±221+	4463±149*+	2439±137*	2943±110*	2313±224*	<0.001
RVSP (mmHg)	29.8±1.5+	26.9±0.8+	25.1±0.6+	39.2±2.6*	38.2±2.9*	44.2±4.3*	<0.001
RVEDP (mmHg)	2.5±0.3+	2.6±0.3+	2.6±0.4+	7.0±1.2*	5.2±0.7*	6.5±1.1*	<0.001
RV+dp/dt (mmHg/s)	1596±92	1329±54+	1194±50*+	1777±113	1433±104+	1664±104	<0.001
RV-dp/dt (mmHg/s)	1073±55	956±48	782±36*+	1139±78*	1005±83	1126±76	<0.001

Values are means ± s.e.m.; HR, heart rate; +dp/dt, rate of pressure rise; -dp/dt, rate of pressure decline;
* $p<0.05$ vs S3M, +$p<0.05$ vs MI3M.

Table 3

The anatomic characteristics of 1 month rat hearts

Groups	RV/W (g/Kg)	LV/W (g/Kg)	Septum/W (g/Kg)	Atria/W (g/Kg)	Scar/W (g/Kg)	Lung/W (g/Kg)	Body Weight (g)
S1M + S3Q1M (n=12)	0.55±0.01+	1.29±0.03+	0.55±0.01+	0.17±0.01+	----	3.71±0.06+	380.2±6.7
SQ1M (n=6)	0.54±0.01+	1.08±0.02*+	0.54±0.02+	0.19±0.01+	----	3.66±0.06+	371.8±10.8
MI1M + MI3Q1M (n=12)	0.89±0.08*	0.81±0.05*	0.75±0.02*	0.40±0.04*	0.33±0.03	5.57±0.64*	382.8±8.1
MIQ1M (n=7)	0.66±0.03+	0.78±0.09*	0.67±0.04*+	0.30±0.03*+	0.38±0.02	4.66±0.49*	337.4±9.5*+
P value	<0.001	<0.001	<0.001	<0.001	=0.248	=0.011	= 0.004

Values are means ± s.e.m.; LV: left ventricle; RV: right ventricle; S1M: sham 1 month; S3Q1M: sham with 3days of quinapril 1 month; SQ1M: sham quinapril treated 27 days; MI: myocardial infarcttion.

* $p<0.05$ vs S1M+S3Q1M, +$p<0.05$ vs MI1M+MI3Q1M.

Table 4

The anatomic characteristics of 3 months rat hearts

Groups	RV/W (g/Kg)	LV/W (g/Kg)	Septum/W (g/Kg)	Atria/W (g/Kg)	Scar/W (g/Kg)	Lung/W (g/Kg)	Body Weight (g)
S3M + S3Q3M (n=15)	0.48±0.01+	1.14±0.02+	0.51±0.01+	0.16±0.01+	----	3.07±0.07+	555.1±12.2+
SQ3M (n=8)	0.47±0.01+	0.87±0.03*	0.46±0.01+	0.15±0.00+	----	3.16±0.09+	471.6±14.7*
MI3M + MI3Q3M (n=14)	0.92±0.09*	0.87±0.04*	0.68±0.03*	0.42±0.05*	0.28±0.02	5.49±0.43*	494.7±20.0*
MIQ3M (n=9)	0.94±0.11*	0.65±0.05*+	0.66±0.02*	0.33±0.03*	0.33±0.03	5.61±0.65*	513.8±13.1*
P value	<0.001	<0.001	<0.001	<0.001	=0.163	<0.001	= 0.004

Values are means ± s.e.m.; LV: left ventricle; RV: right ventricle; S3M: sham 3 month; S3Q3M: sham with 3days of quinapril 3 month; SQ3M: sham quinapril treated 56 days; MI: myocardial infarcttion.

* $p<0.05$ vs S3M+S3Q3M, +$p<0.05$ vs MI3M+MI3Q3M.

Table 5

Cytokines in acute (28 day)myocardial infarction (MI) of rat hearts

Groups (6)	IL-1 β	IL-5	IL-6	TNF-α
SS (n=4)	0.006±0.002*	0.044±0.015	0.057±0.010*	0.093±0.026
S3Q (n=5)	0.009±0.004*	0.035±0.005*	0.047±0.005*	0.076±0.013*
SQ (n=4)	0.008±0.003*	0.036±0.002*	0.052±0.005*	0.087±0.018
MIS (n=4)	0.029±0.012#	0.064±0.005	0.112±0.027#	0.138±0.035
MI3Q (n=6)	0.011±0.002*	0.041±0.008*	0.059±0.007*	0.095±0.023
MIQ (n=6)	0.011±0.004*	0.036±0.005*	0.053±0.014*	0.069±0.014*
P	0.0379	0.0748	0.0092	0.2500

Values are means ± s.e.m.;

SS: sham saline; S3Q: sham treated with quinapril at the last 3 days; SQ: sham treated with quinapril at last 27 days; MIS: MI saline; MI3Q: MI treated with quinapril at the last 3 days; MIQ MI treated with quinapril at last 27 days.

* $p<0.05$ vs. MIS (MI un-treated), # $p<0.05$ vs SS (Sham un-treated).

Table 6

Cytokines in chronic (84 days)myocardial infarction (MI) of rat hearts

Groups (5)	IL-1 β	IL-5	IL-6	TNF-α
SS (n=3)	0.028±0.016	0.025±0.010*	0.017±0.002*	0.035±0.008*
SQ (n=4)	0.020±0.010	0.035±0.014*	0.035±0.022*	0.050±0.019*
MIS (n=3)	0.061±0.037	0.087±0.035	0.108±0.039	0.123±0.045
MI3Q (n=4)	0.020±0.007	0.029±0.006*	0.036±0.009*	0.055±0.007*
MIQ (n=5)	0.022±0.006	0.056±0.005	0.073±0.006	0.076±0.007
P	0.3851	0.0746	0.0323	0.0682

Values are means ± s.e.m.;

SS: sham saline; SQ: sham treated with quinapril at last 56 days; MIS: MI saline; MI3Q: MI treated with quinapril at the last 3 days; MIQ MI treated with quinapril at last 56 days.

* $p<0.05$ vs. MIS (MI untreated).

Fig. 1

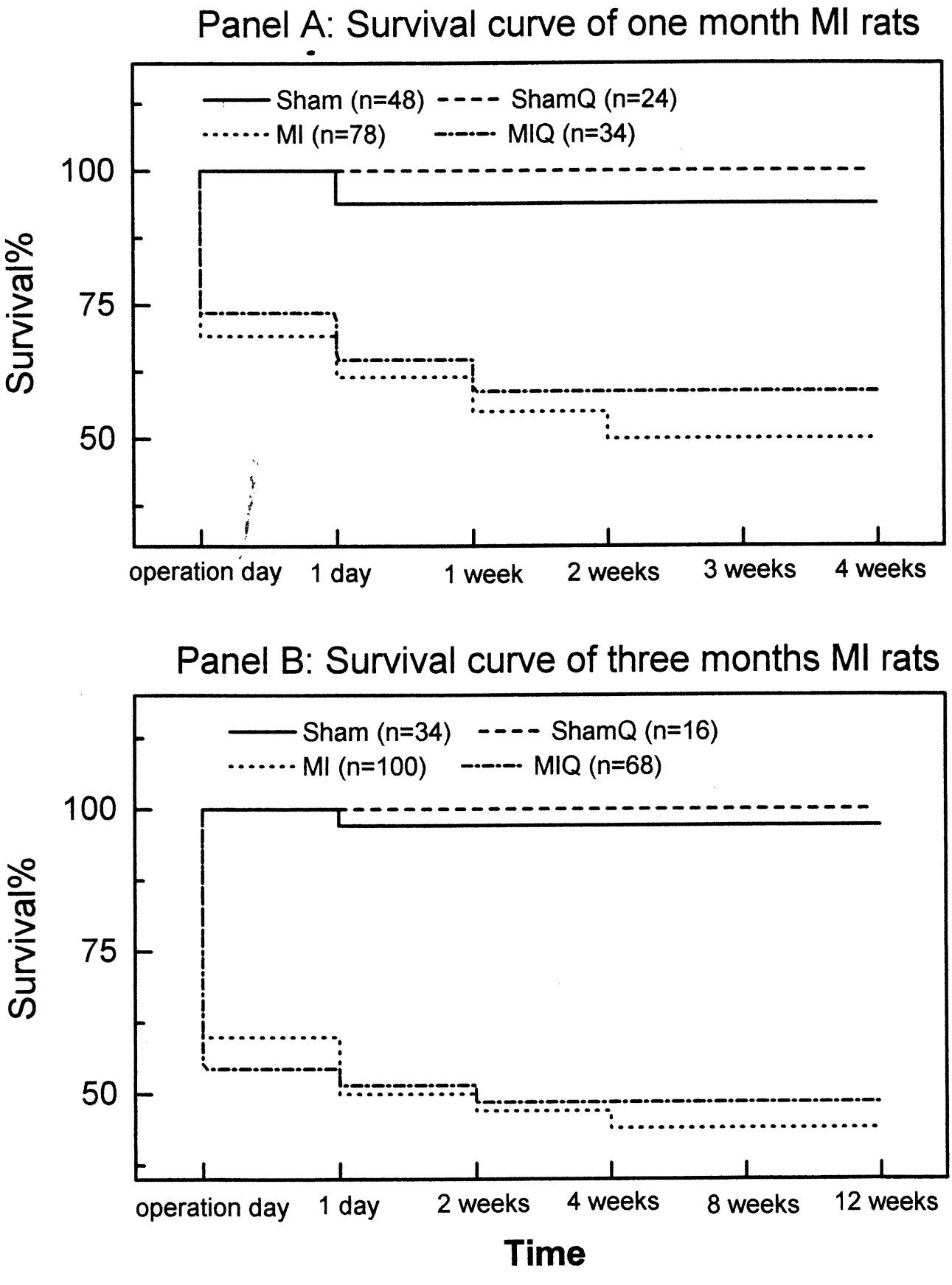
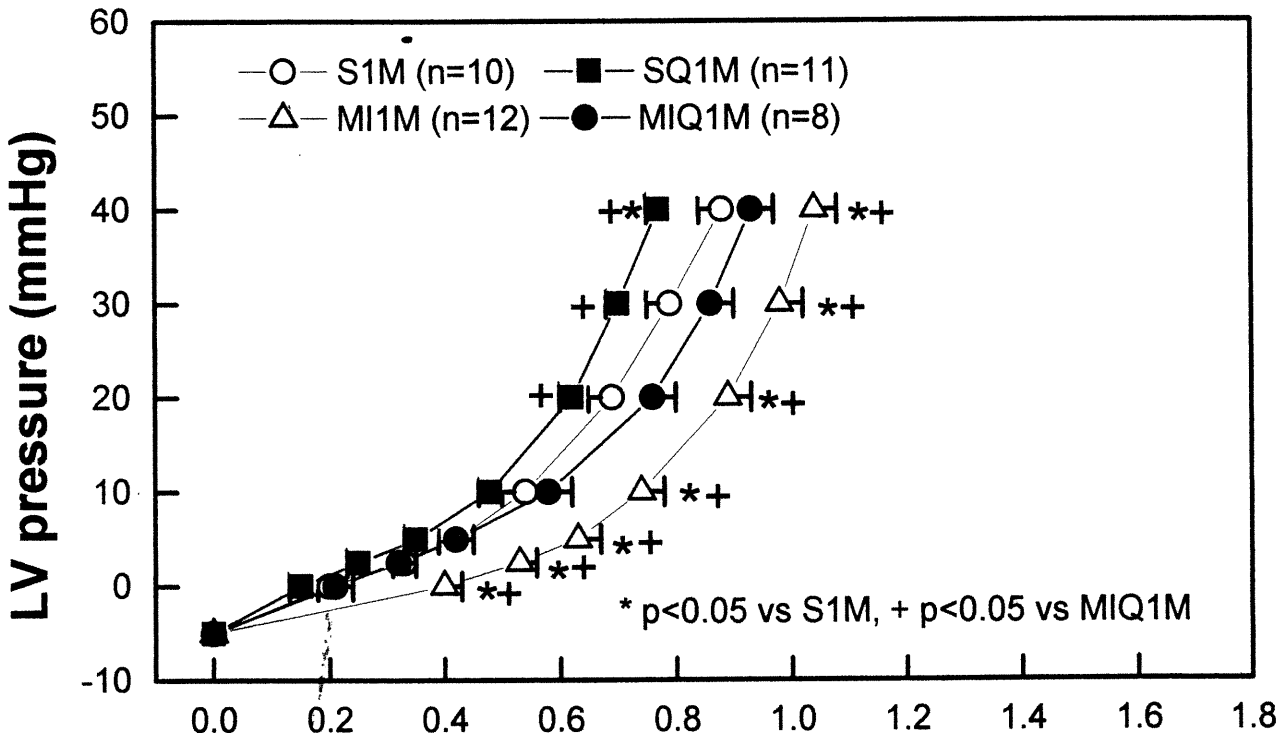

Panel A: Survival curve of one month MI rats
Sham (n=48)
ShamQ (n=24)
MI (n=78)
MIQ (n=34)
Survival%
100
75
50
operation day
1 day
1 week
2 weeks
3 weeks
4 weeks
Panel B: Survival curve of three months MI rats
Sham (n=34)
ShamQ (n=16)
MI (n=100)
MIQ (n=68)
Survival%
100
75
50
operation day
1 day
2 weeks
4 weeks
8 weeks
12 weeks
Time

Fig. 2

Panel A: One month passive diastolic pressure-volume relationship



Panel B: Three months passive diastolic pressure-volume relationship

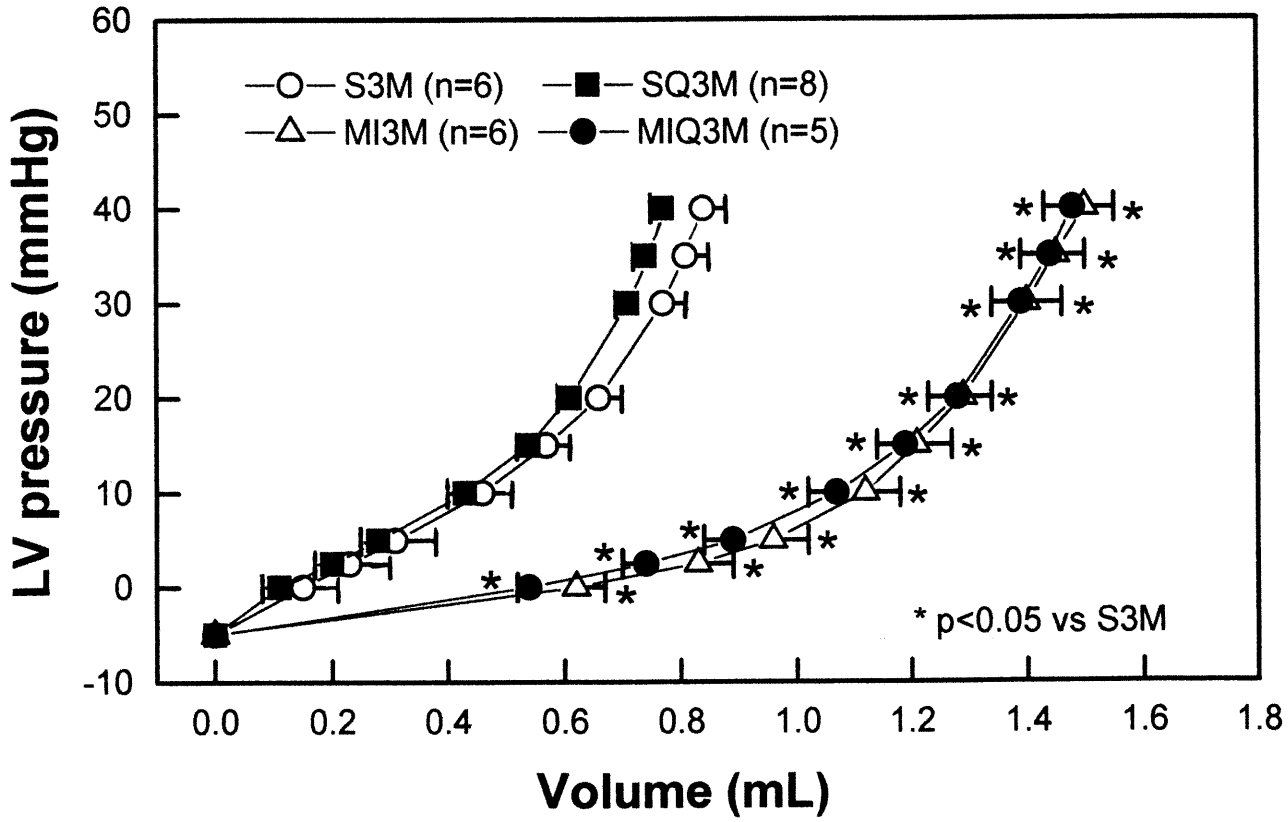
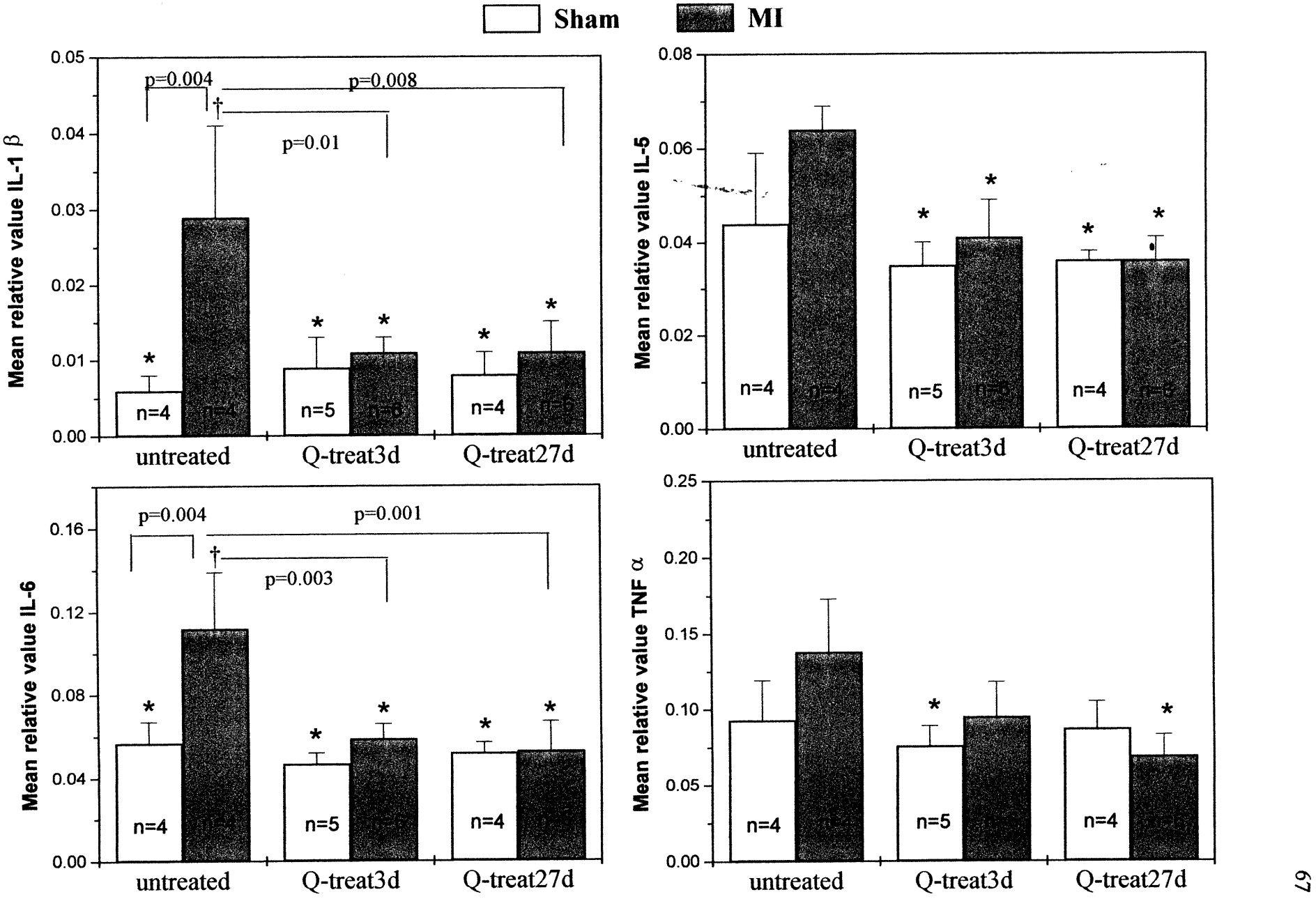


Fig. 3

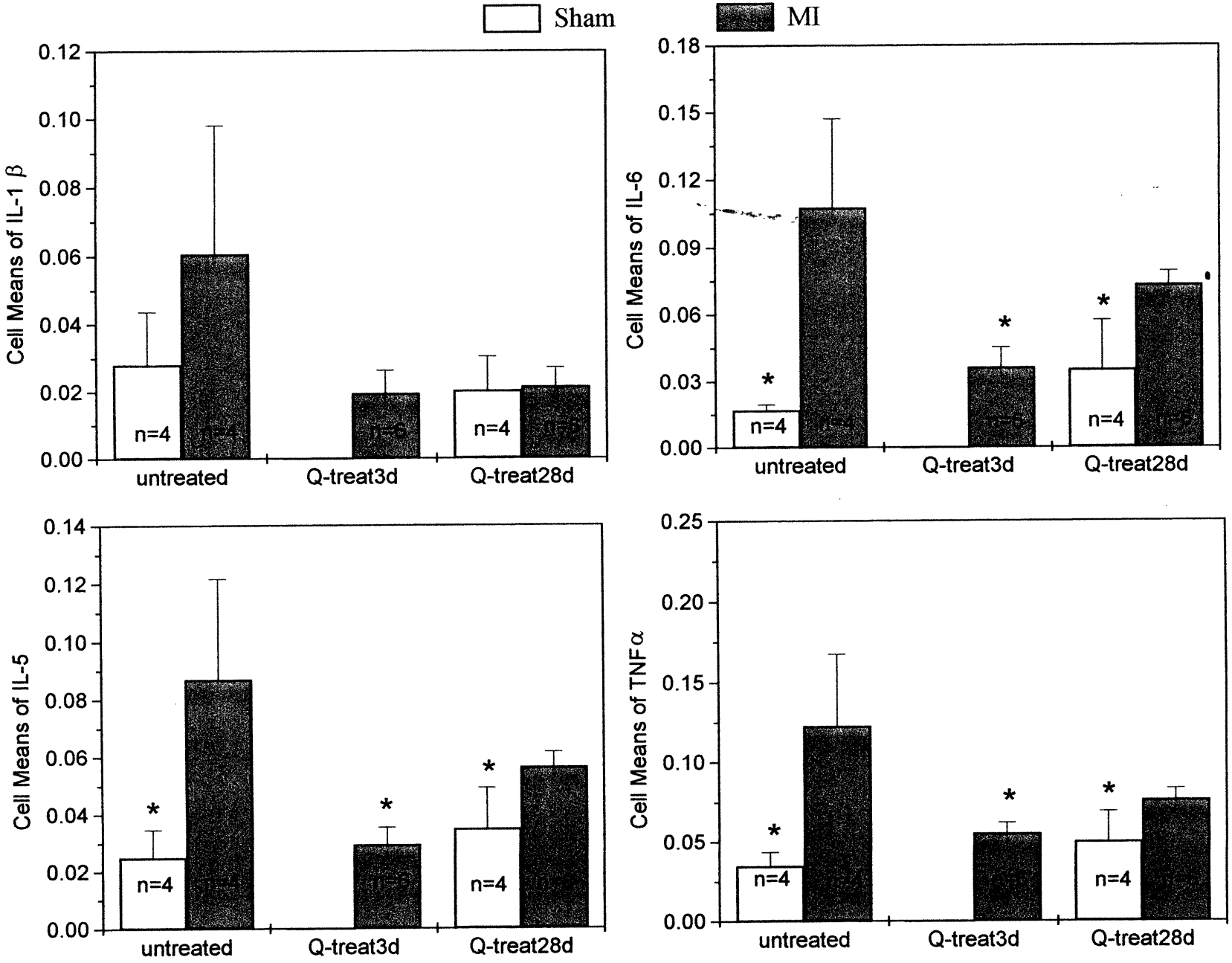
Cardiac cytokines expression in acute (28 days) myocardial infarction (MI) of rat hearts



*p<0.05 vs. MI untreated; #p<0.05 vs sham untreated

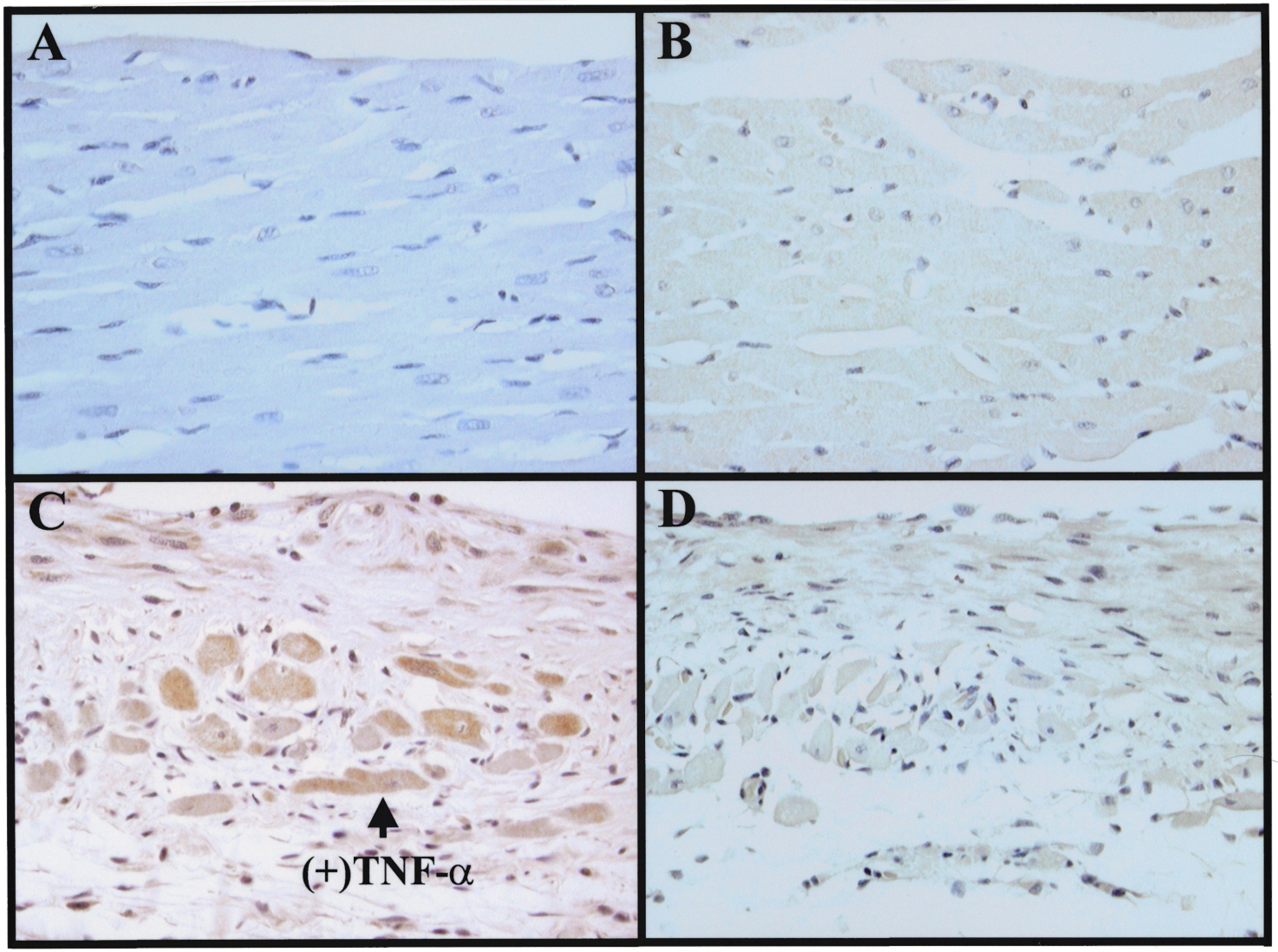
Fig. 4

Cardiac cytokines in chronic (84 day) myocardial infarction (MI) of rat hearts



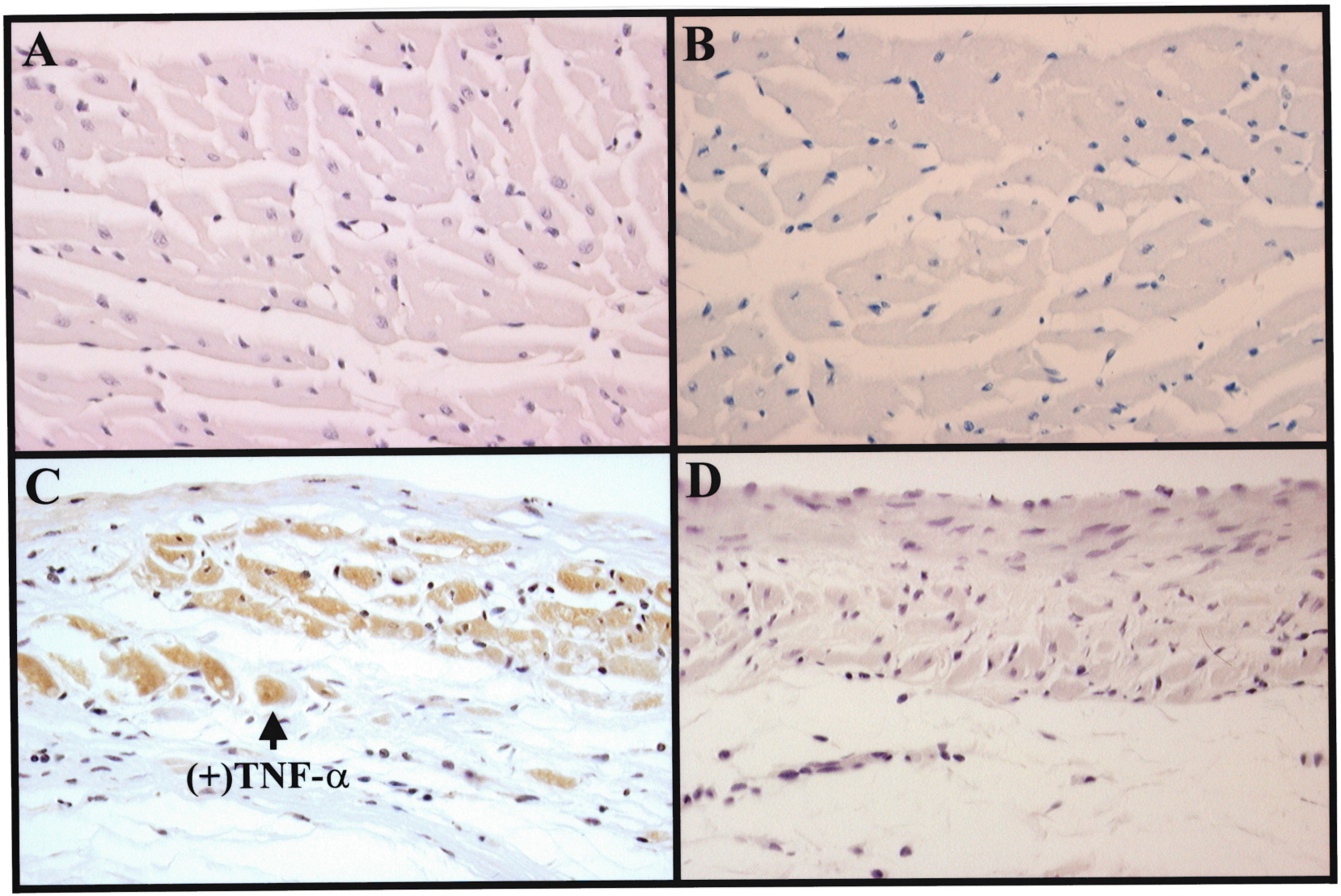
*p<0.05 vs. MI untreated

Fig. 5. Expression of TNF-α in cardiac tissue in one month (28 days) rats



Expression of TNF-α in cardiac tissue in 1 month (28 days) rats as determined immunohistochemically. Sham-operated with no primary antibodies to TNF-α (panel A); sham-operated with primary antibodies to TNF-α, showing no staining (panel B); untreated myocardial infarction (MI) with marked myocyte staining for TNF-α (panel C); and MI treated 3 days with quinapril (Q), showing marked reduction of myocyte staining for TNF-α (panel D).

Fig. 6. Expression of TNF-α in cardiac tissue in three months (84 days) rats



Expression of TNF-α in cardiac tissue in 3 months (84 days) rats as determined immunohistochemically. Sham-operated with no primary antibodies to TNF-α (panel A); sham-operated with primary antibodies to TNF-α, showing no staining (panel B); untreated myocardial infarction (MI) with marked myocyte staining for TNF-α (panel C); and MI treated with quinapril (Q) for 56 days, showing marked reduction in staining for TNF-α (panel D).