응용약물학회지, The Journal of Applied Pharmacology, 9, 167-175(2001)

Down-regulation of TNF-α and IL-6 by Higenamine is Responsible for Reduction of Infarct Size and Myocardial Ischemic Injury in the Rat

Young Soo LEE, Young Jin KANG, Bog Kyu LEE, Young Shin KO, Min Kyu PARK, Han Geuk SEO, Hye Sook Yun-CHOI[1] and Ki Churl CHANG*

Department of Pharmacology, Institute of Hearth Science, College of Medicine, Gyeongsang National University, Jinju, 660-751, Korea; [1]Natural Product Research Institute, Seoul National University, Seoul, 460-100, Korea

(Received August 7, 2001; accepted September 5, 2001)

Abstract – Recent studies have shown that cytokines are capable of modulating cardiovascular function and that some drugs used in the treatment of heart failure variably modulate the production of cytokines. Higenamine, a positive inotropic isoquinoline alkaloid, has been used traditionally as cardiac stimulant, and reported to reduce nitric oxide (NO) and inducible nitric oxide synthase (iNOS) expression in LPS- and/or cytokine-activated cells *in vitro* and *in vivo*. Therefore, we investigated whether higenamine modulates the production of proinflammatory cytokines in myocardial infarction. In addition, effects of higenamine on antioxidant action and antioxidant enzyme expression (MnSOD) were studied. Myocardial infarction (MI) was confirmed by measuring left ventricular (LV) pressure after occlusion of the left anterior descending coronary artery (LAD) for 5 weeks in rats. Treatment of higenamine (10 mg/kg/day) reduced infarct size about 35 %, which accompanied by reduction of production TNF-α, IL-6, but not IFN-γ and IL-1β in the myocardium. The expression of TNF-α mRNA in infracted myocardium was significantly reduced by higenamine. Although iNOS mRNA was not detected, nitrotyrosine staining was significantly increased in myocardium of MI compared to higenamine-treated one, indicating that peroxynitrite-induced damage is evident in MI. Cytochrome c oxidation by peroxynitrite was concentration-dependently reduced by higenamine, an effect which was almost compatible to glutathion. Higenamine treatment did not affect the expression of MnSOD mRNA in myocardial tissues in MI. Taken together, higenamine may be beneficial in oxidative stress conditions such as ischemic-reperfusion injury and MI due to antioxidant action as well as modulation of cytokines.

Key words □ myocardial infarction, cytokine, MnSOD, TNF-α, IL-1β, peroxynitrite

Inducible nitric oxide synthase (iNOS) expression in cardiac myocyte has been greatly increased in experimental model of myocardial ischemia as well as in patients with congestive heart failure (CHF). Several studies have provided strong evidence that enhanced lipid peroxidation and oxygen free radical damage persists in patients with CHF. Although the precise cellular sources of oxygen free radicals and the mechanisms involved in initiating the oxidative stress that occurs in patients with CHF are still unknown, it is conceivable that oxygen free radicals produced by the ischemic failing myocardium contribute to myocardial cellular toxicity and damage and to apoptosis. After myocardial infarction (MI), the heart undergoes a remodeling process that is characterized by hypertrophy of the surviving myocytes and hyperplasia of the nonmyocytes (Anversa *et al.*, 1885, Krimpen *et al.*, 1991, Weber and Brilla, 1991). Although perhaps these responses initially serve an adaptive function, ultimately these alterations are often accompanied by depressed contractile function. During this remodeling process, the expression of several growth factors and cytokines is activated (Hanatani *et al.*, 1995, Herkowitz *et al.*, 1995). For example, increased plasma levels, as well as local myocardial production, of several proinflammatory cytokines, including tumor necrosis factor (TNF)-α interleukin (IL)-1β, IL-6, and IL-8, have been observed in patients early after experiencing acute MI (Guillen *et al.*, 1995, Latini *et al.*, 1994, Neumann *et al.*, 1995, Tashiro *et al.*, 1994). Recently, we reported that higenamine, a positive inotropic benzylisoquinoline alkaloid, inhibited mRNA and protein expression of iNOS in rat aorta and RAW 264.7 cells via inhibition of NF-kB activation (Kang *et al.*, 1999a; 199b). Furthermore, in the process of cardiac remodeling after infarction, nonmyocyte derived cytok-

*To whom correspondence should be addressed.

ines are suggested to play important autocrine and paracrine functions in regulating the alteration in myocardial extracellular matrix and myocyte growth. We, thus, hypothesized that higenamine may decrease myocardial damage in MI by modulating production of proinflammatory cytokines such as TNF-α, IL-1β etc. In the present study, effect of higenamine on the expression of TNF-α, MnSOD mRNA expression, and production of cytokines were investigated in male Sprague-Dawley rats making MI by ligation of the left anterior descending coronary artery (LAD). Here, we provide evidence that higenamine protects myocardial damage by inhibition of TNF-α and IL-6 production without affecting expression of MnSOD mRNA, in which antioxidant action of higenamine, at least in part, plays a role for the reduction of proinflammatory cytokines.

METHODS

Myocardial infarction and Experimental Group

The rat coronary ligation model was used to induce MI and consequently heart failure. Adult male Sprague-Dawley rats 12 to 14 weeks old were anesthetized with ketamine and xylazine, a left thoracotomy was performed. After the pericardium was opened, the heart was briefly exteriorized. The LAD was ligated 1-2 mm from its origin with a 7-0 silk suture. Then the heart was returned to its normal position with the pericardium left open, and the thorax was closed. The same procedure was followed for sham-operated control animals, but the coronary ligature was untied. After the operation, rats were housed in cages, given regular food and water, and subsequently studied 5 weeks later. The animals were divided into 3 groups; (i) MI group (n=7), receiving no therapy but underwent the full protocol of operation, (ii) treatment group (n=8), receiving higenamine (10 mg/kg, i.p) daily after the operation during the entire course of experiment, and (iii) sham-operation group (n =5).

Hemodynamic study

Immediately before being euthanized, the rats were anesthetized with ketamine and xylazine, the left carotid artery was cannulated with pressure transducer (model P23XL. USA) to monitor the blood pressure and heart rate. The analysis was performed with the aids program from Mac Lab computer, to which hemodynamic signals were continually introduced via transducers connected to the rat. After mean arterial blood pressure and heart rates were obtained, the catheters were advanced into the left ventricles (LV), from which we measured the LV pressures.

Myocardial infarct size

On the day of sacrifice, rats were anesthetized with ketamine and the femoral vein was cannulated with a 24-gauge tubing adapter to infuse 1% Evans blue to delineate the "area at risk". The Evans blue dye uniformly distributed to those areas of the myocardium proximal to the ligature; hence, the area of the myocardium that was not stained with Evans blue was defined as the area at risk. The isolated LV sections were incubated in 1% triphenyltetrazolium chloride (TTC) for 30 min. After TTC staining, the area of infarction appears pallid, whereas the viable myocardium appears red. Infarct size can be calculated from the ratio of infarct region weight/left ventricle weight.

Tissue Harvest

Five weeks after the operation, rats were anesthetized as described above, and median stermotomy was performed. The hearts were rapidly excised and placed into cold saline solution. The ventricles were separated from the heart and weighed. The tissues were collected separately from infracted region, peri-infarcted region and contralateral region. Each tissue samples were snap-frozen in liquid nitrogen and stored at -80 until use.

Determination of tissue cytokines content by ELISA Assay

The cytokine contents of infarcted region from each group were compared. The myocardial homogenate was suspended in PBS solution containing protease inhibitors (PMSF14.9 mM, leupeptin 21 nM, and aprotinin 3.1 nM). After centrifugation for 20 minutes at 20,000 g, the supernatant, which contained the non-membrane-bound cytokines, was collected and stored at -70 until use. Contents of TNF-α, IL-1β, INF-γ and IL-6 were quantified by a sandwich ELISA kit (Endogen, USA).

Analysis of messenger RNA levels for TNF-α and MnSOD

Total RNA was extracted by Trizol solution. Levels of gene expression were detected by Northern blot analysis. In brief, total RNA (20 μg) was denatured in formaldehyde, run in 1% agarose-formaldehyde gel, and transferred overnight onto positively charged nylon membrane (Amersham Pharmacia

Biotech, USA). After prehybridization for 30 min at 68°C, filters were hybridized 3 hours at 68°C in Quick Hybridization solution containing the denatured ^{32}P-labeled specific cDNA probes. The filter was sequentially washed for 30 minutes twice with 2×SSC and 0.1% SDS at room temperature, followed by washing in 0.2×SSC and 0.1% SDS at 60°C until the radioactive background was negligible. Messenger RNA of TNF-α and MnSOD was quantitated by densitometry, and normalized to the that of GAPDH expressed. The cDNA probe for TNF-α was generated by polymerase chain reaction (PCR) using mouse TNF-α gene encoding region between 700 bp and 1065 bp. The cDNA probe for MnSOD was also generated by PCR using mouse MnSOD gene encoding from 481 bp to 731 bp.

Serum NOx

Serum nitrite/nitrate contents were increased in HF patients and in experimental animal model of MI. To test whether higenamine reduces serum NOx, serum samples were diluted and the nitrate was reduced to nitrite by addition of nitrite reductase. Then the serum was mixed with an equal volume of Griess reagent (0.1% naphthylethylenediamine dihydrochloride and 1% sulfanilamide in 5% phosphoric acid) and absorbance was measured at 550 nm.

Immunohistochemical localization of nitrotyrosine

Heart tissues were fixed with 10% (v/v) neutral buffered formalin. Paraffin sections cutting into 4 mm thickness were mounted on charged microscope slides (FisherBiotech, PA, USA) and air-dried overnight at 42°C. After deparaffination, slides were incubated in 0.3% hydrogen peroxide diluted in methanol for 5 min at 42°C. Tissues were incubated with anti-nitrotyrosine antibody (Upstate, USA) at 4°C in a humid chamber. Binding of primary antibody was detected by the avidin-biotin complex peroxidase method (ABC Elite, Vector Labs, CA, USA).

Antioxidant effects

The peroxynitrite-dependent oxidation of cytochrome c^{2+} was measured as described (Szabo et al., 1997). Cytochrome c was reduced by sodium dithionite immediately before use and purified by chromatography on Sephadex G-25 using potassium phosphate (100 mM) plus DTPA, pH 7.2 (0.1 mM) as the elution buffer. The concentration of cytochrome c^{2+} was determined spectrophotometrically at 550 nm in the same buffer (ε=21 mM/cm). Cytochrome c^{2+} oxidation (50 μM) yields upon addition of peroxynitrite (25 μM initial concentration after mixing) were assessed by incubation of reaction mixtures in potassium phosphate (100 mM) plus DTPA, pH 7.2 (0.1 mM) at 22°C for 3 min in the absence or presence of higenamine (1 μM-3 mM). Oxidation of cytochrome c^{2+} was followed at 550 nm using a Beckman DU 640 spectrophotometer (Fullerton, CA, USA). In control, experiments we have confirmed that the chemical tested does not reduce cytochrome c^{2+}.

Statistics

Data are expressed as mean±SEM. Statistical analyses were performed using analysis of variance (ANOVA). When significant differences were found, the Student Newman-Keuls test was used to identify the differences between specific groups. The level of significance was defined as $p<0.05$.

RESULTS

Effects of higenamine on hemodynamic parameters of MI

Table 1 shows various parameters between sham-operated group, MI group and MI with higenamine-treated group 5 weeks after the operation. The prominent difference was that left ventricular (LV) pressure and enlargement of myocardium in MI group, where LV pressure was increased to 35% ($p<0.05$) without affecting other parameters such as heart rate, body weight and mean arterial pressure etc. However, the elevated LV pressure in MI group was reduced to normal by higenamine treatment.

Effects of higenamine on infarct size of MI

The ligation of LAD for 5 weeks not only elevated LV

Table 1. Hemodynamic changes 5 weeks after ligation of LAD in rats with and without administration of higenamine

	Sham	MI	MI/Hig
Body wt (g)	460 ± 20	420 ± 26	440 ± 25
LV wt (g)	0.5 ± 0.07	0.9 ± 0.09*	0.6 ± 80.1
LV EDP (mmHg)	18 ± 2	58 ± 4*	22 ± 4
MBP (mmHg)	108 ± 3	90 ± 9	97 ± 9
Heart rate (beats/min)	262 ± 12	26 ± 318	271 ± 10
Number of animals	(3)	(5)	(7)

Results are means ± SE.
LV; left ventricular, EDP; end-diastolic pressure, MBP; mean blood pressure. MI, LAD ligation without treatment MI/Hig, LAD ligation with treatment
*P<0.05

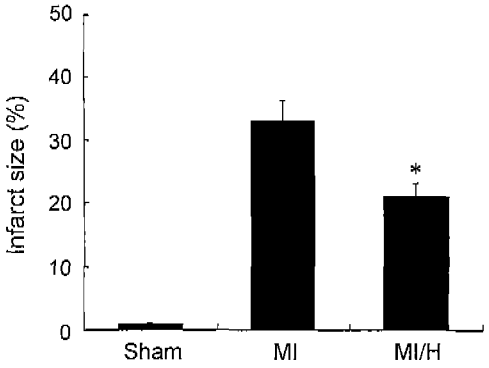

Fig. 1. Effects of higenamine on the size of the myocardial infarct. The size of the area of myocardial infarct (the area without TTC staining) was expressed as a percentage of the area at risk (the area without Evans blue dye). All results are expressed as means±SE of determination of 4 experiments. *P<0.05. MI; LAD ligation group. MI/H; MI+Higenamine (10 mg/kg/day).

pressure but also increased LV weight significantly. For example, the mean LV weight of sham operated group was 0.5±0.07 g, which was significantly increased to 0.9±0.09 g. We can clearly see the LV wall thickening. When we measured infarct size by Evans blue and followed by TTC staining, area at risk was significantly ($p<0.05$) reduced by treatment with higenamine (Fig. 1).

Higenamine modulates tissue cytokine levels in MI

To investigate the beneficial effect of higenamine was due to modulation of production of proinflammatory cytokines, tissue contents of IFN-γ, IL-1β, IL-6 and TNF-α were compared by ELISA. As shown in Fig. 2, tissue contents of IFN-γ and IL-1β were not different in three groups. For example, the contents of IFN-γ, 71±6.3, 71±5.7, 73±5.9 μg/g in sham-operation, MI-induced (MI) and MI with higenamine treated (MI/H) groups, respectively. However, treatment with higenamine significantly reduced both IL-6 and TNF-α contents. In particular, reduction of TNF-α content was stronger than that of IL-6.

Reduced expression of TNF-α mRNA by higenamine

To further confirm that the reduction of tissue content of TNF-α was due to diminished its expression, messenger RNA level of TNF-α was compared between MI-induced group (MI) and higenamine treated MI group (MI/H). Treatment with higenamine significantly diminished the expression of TNF-α mRNA (Fig. 3a). These findings are consistent with that of immunohistochemical staining study (Fig. 3b).

Serum NOx and formation of nitrotyrosine

As measured serum NOx in blood samples from those 3

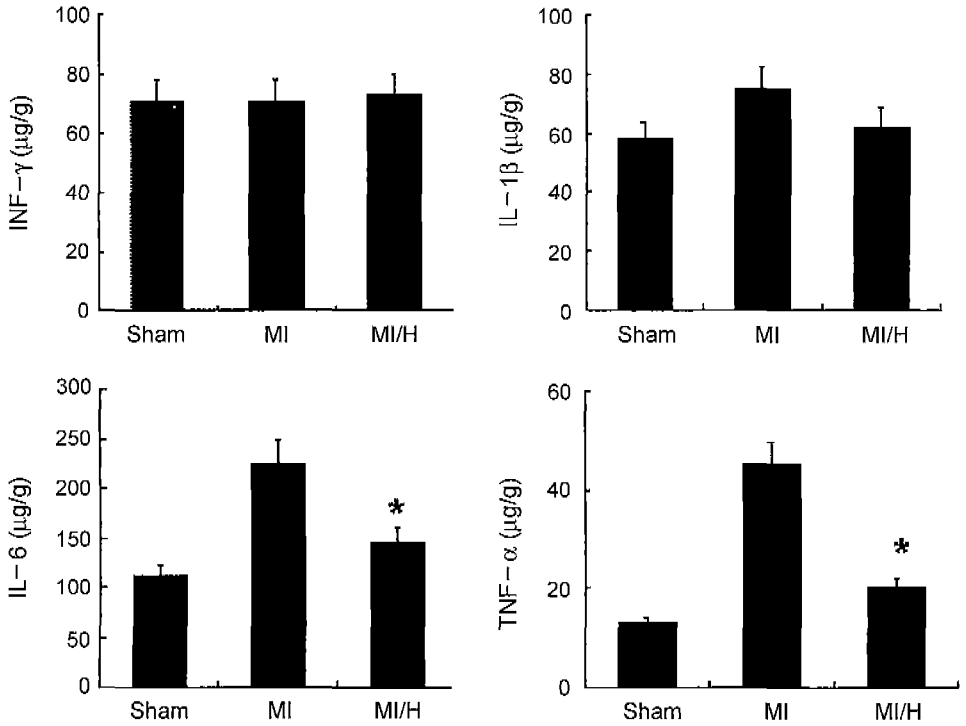

Fig. 2. Detection of cytokines from rat infarcted heart tissue by ELI SA. TNF-α, IL-1β, INF-γ and IL-6 protein were measured in rat heart tissue. All results was expressed as means±SE of determination of 4 experiments. *P<0.05

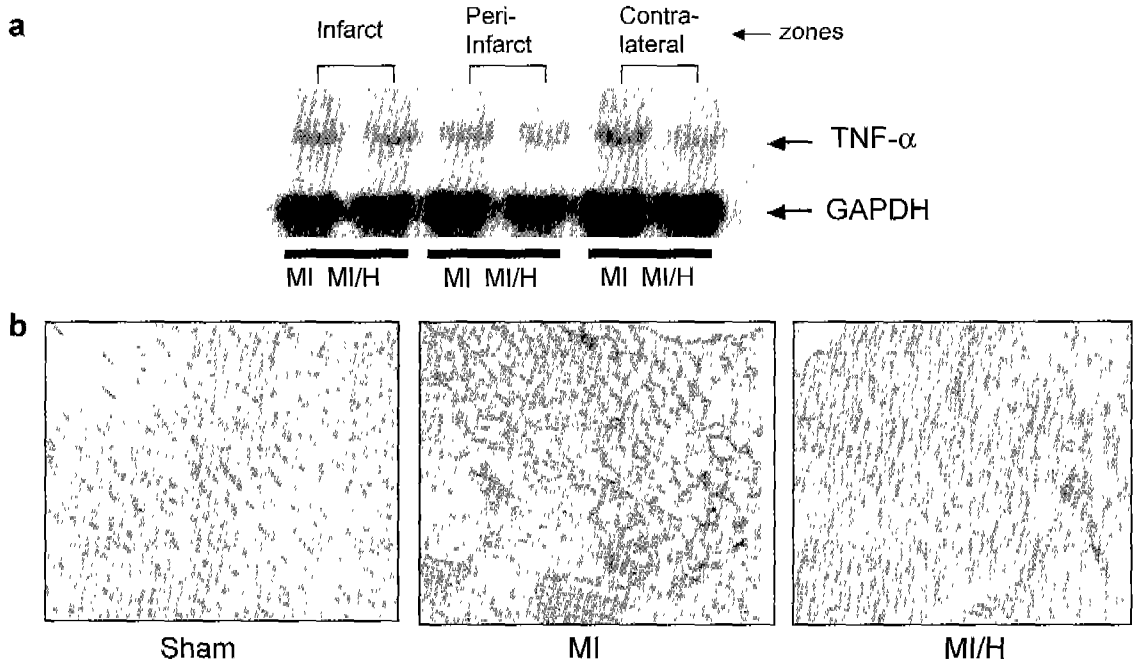

Fig. 3. Northern blot analysis of TNF-α mRNA trancripts in rat heart after LAD ligation. Total tissue RNA was transferred to membrane and hybridized with a TNF-α probe. The same membranes were stripped and rehybridized to a cDNA probe for GAPDH (a). Photomicrographs of immunostaining of TNF-α in rat myocardium. Immunostaining was performed with anti-rat TNF-α antibody (b). Magnification×200. MI; LAD ligation group. MI/H; MI+Higenamine (10 mg/kg/day).

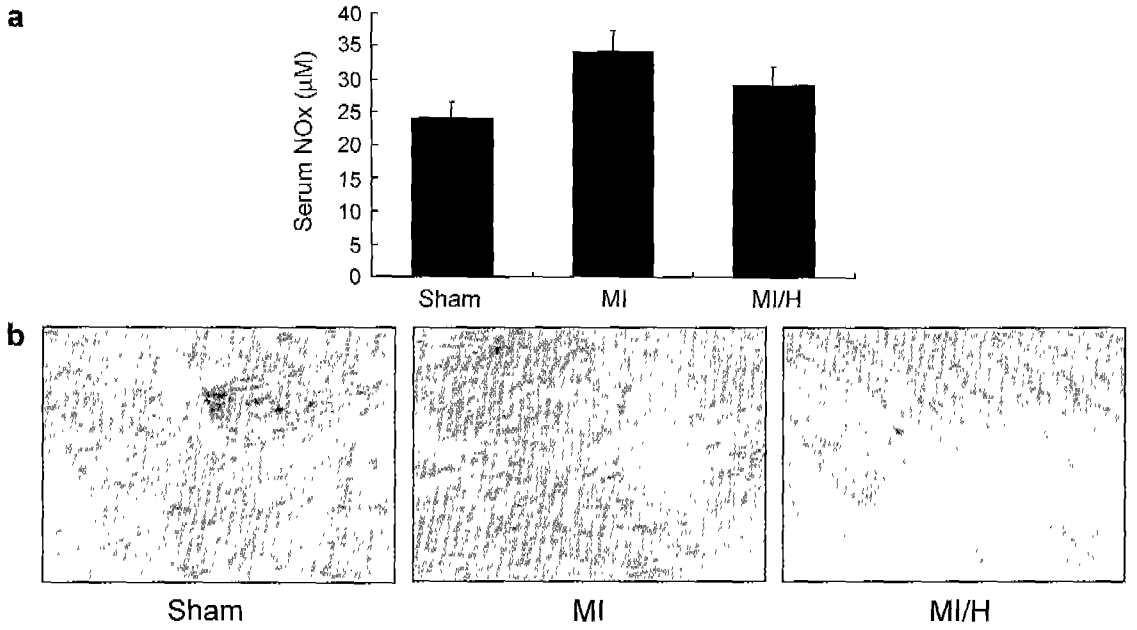

Fig. 4. Nitrate/nitrite concentrations between 3 groups. Blood was collected by cardiac puncture and serum fraction was analyzed for its contents of nitrite/nitrate (a). Data represent mean±S.E. of 4 separate experiments. Photomicrographs of immunostaining of nitrotyrosine in rat myocardium. Immunostaining was performed with anti-rat nitrotyrosine antibody (b). Magnification×200, MI; LAD ligation group. MI/H; MI+Higenamine (10 mg/kg/day).

test groups, no difference of nitrite/nitrate was detected (Fig. 4a). Although serum nitrite/nitrates levels were not much different, we want to know formation of peroxynitrite in cardiac tissues, so nitrotyrosine staining using 3-nitrotyrosine antibody was performed. As shown in Fig. 4b, peroxynitrite staining was significantly increased in myocardium of MI,

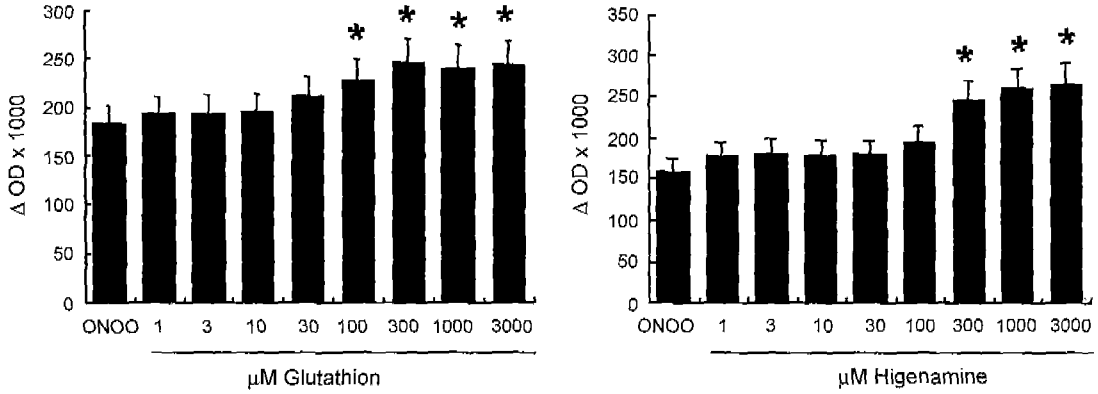

Fig. 5. The oxidation of cytochrome c by peroxynitrite was determined in the presence of various concentrations of glutathion (a) and higenamine (b). The peroxynitrite-induced changes in cytochrome c absorbance is shown. Data are given as mean±S.E of triple experiments. *P<0.05

which was diminished by higenamine treatment.

Antioxidant effect of higenamine

To understand more the mechanism of action of higenamine, antioxidant action was investigated employing the inhibition of cytochrome c oxidation by peroxynitrite. As shown in Fig. 5, antioxidant effect of higenamine was as potent as gluthathione. Higenamine concentration dependently inhibited peroxynitrite-induced oxidation of cytochrome c.

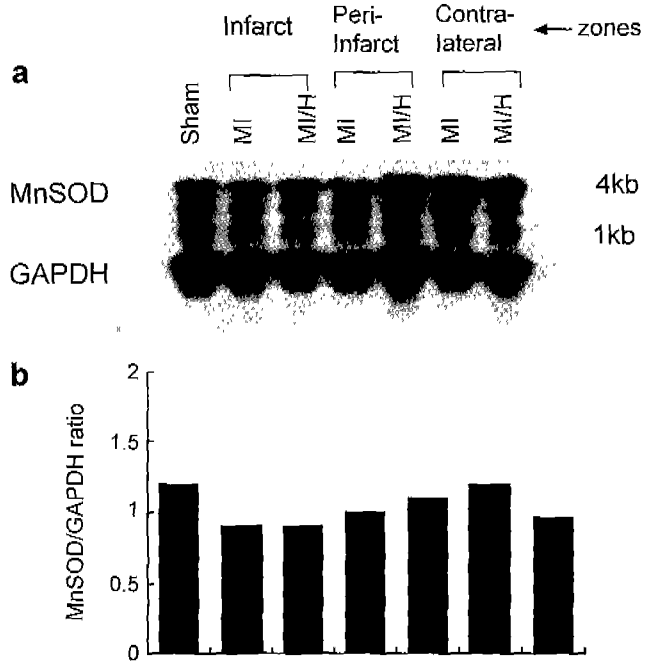

Fig. 6. Expression of MnSOD in rat myocardium. Total tissue RNA was hybridized with MnSOD probe. Same blots were stripped and rehybridized to a cDNA probe for GAPDH (a). Densitometric analysis of the ratio for MnSOD mRNA corrected to GAPDH mRNA. MI; LAD ligation group. MI/H; MI+ Higenamine (10 mg/kg/day).

Effects of higenamine on the expression of MnSOD mRNA

Autoradiogram shown in Fig. 6A indicates that specific cDNA probes for mouse MnSOD recognized 1 kb and 4 kb transcript in all hearts examined. The relative mRNA levels, measured as the ratios of the intensities of mRNA for MnSOD to those of GADPH, are equally expressed between the three study groups as shown in Fig. 6B.

DISCUSSION

In the present study we clearly demonstrated that treatment with higenamine significantly reduced infarct size, thus, improves LV function in the myocardium 5 weeks after the infarction. This finding was consistent with others that myocardial infarction was associated with increment of LV pressure as assessed by hemodynamic measurements. To elucidate the underlying mechanism of action of this beneficial effect of higenamine on MI, proinflammatory cytokine levels such as IFN-γ, IL-1β, IL-6 and TNF-α were measured in the myocardium of MI with or without higenamine treatment. Among these cytokines, TNF-α and IL-6, but not IFN-γ, IL-1β, were significantly decreased by higenamine. We believe that modulation of production of these cytokines by higenamine is associated with reduction of LV pressure and improvement of LV function in the infarcted heart. In fact, increased plasma levels of IL-1β, IL-6, and TNF-α have been reported in patients with acute myocardial infarction (Guillen *et al.*, 1995, Latini *et al.*, 1994, Neumann *et al.*, 1995). Similarly, in rats after coronary ligation, a biphasic induction of mRNAs of

these proinflammatory cytokines was observed in the postischemic myocardium within hours and at 7 days after infarction (Herskowitz *et al.*, 1995). Higenamine inhibited iNOS expression by inhibition of NF-kB activation in RAW 264.7 cells activated by LPS/IFN-γ (Kang *et al.*, 1999a). In the present study, iNOS expression was not detected by Northern and RT-PCR analysis in both infracted and sham operated myocardium (data not shown). Furthermore, plasma NO levels were not different between in MI rats and sham operated rats. At the present time, it is not clear why iNOS expression and serum NO are not elevated in MI rat. In MI models, the expression of iNOS and serum NO changes are controversial; iNOS expression is significantly increased in the myocardium of MI rat (Akiyama *et al.*, 1997) and serum NO is also increased after the ligation of LAD in the rat (Finkel *et al.*, 1992). However, no increase of iNOS and serum NO in MI rats were also reported (Yokoyama *et al.*, 1993). These different results may be related to the duration of MI, severity of MI or methods employed. In our study, the sensitivity of the measurement of NO by Griess reagent seems not to be responsible for the outcome. Although serum NO as well as iNOS expression was not different all three experimental groups, when examined immunostaining of nitrotyrosine, a marker for peroxynitrite, large area of nitrotyrosine staining was found in MI experimental groups. In contrast, those of sham operated and higenamine treated groups were less stained or almost absent, indicating that NO production is increased in myocardial tissue of MI. Our data do not prove that iNOS-related NO contributes to the formation of nitrotyrosine but only demonstrate coincidence of large amount of NO and superoxide ($.O_2-$) are formed in MI. Although the exact source of these two species in MI is not certain, TNF-α may be implicated in reactive oxygen intermediates (ROIs). TNF-α was reported to exert cytotoxic activity on some types of tumor cells, in part via the generation of ROIs (Matthews *et al.*, 1987, Wong *et al.*, 1989, Goossens *et al.*, 1995). ROIs are involved in many biological processes. For example, they play an important role in the defense against microorganisms, or they can cause cell injury directly. In addition, ROIs take part in regulating of the expression of various genes and cell growth. The present experiment does not tell whether ROI stimulates TNF-α production or *vice versa*. It is quite evident, however, that cytokines (TNF-α, IL-6) and ROI ($.O_2-$) are elevated in MI, which higenamine effectively reduced. This conclusion was based on the result of nitrotyrosine staining and mRNA expression of TNF-α. Cardiac hypertrophy induced by TNF-α via generation of ROIs significantly inhibited by antioxidants was reported (Nakamura *et al.*, 1998). Thus, modulation of cytokine or ROI may influence cardiac function. Antioxidant action of higenamine was assessed by inhibition of oxidation of cytochrome c by peroxynitrite. The peroxynitrite scavenging effect of higenamine was as potent as gluthathion, we believe that the reduced nitrotyrosine staining in myocardium of MI in higenamine treated animals is partly responsible for this action. Higenamine was reported to scavenge $.O_2-$ anion in sinovial fluid (Zhang and Chen, 1985), supporting the idea that antioxidant action is one of the main mechanism of action of higenamine. Under physiological conditions, $.O_2-$ has a much shorter half life than NO (less than 50 ms) and a free diffusion path half life of 2 mm (Saran *et al.*, 1994). Thus, the diffusion of NO into areas of high $.O_2-$ production would result in quantitative and localized production of peroxynitrite. It was shown that nitrotyrosine is formed exclusively from peroxynitrite (Ischiropoulos *et al.*, 1992). Peroxynitrite is cytotoxic via a number of independent mechanisms. Its acute cytotoxic effects include initiation of lipid peroxidation, and inactivation of various enzymes such as membrane pumps and mitochondrial respiratory enzymes (Crow and Beckman, 1995). Mitochondria are the primary source of endogenous $.O_2-$ radicals under normal physiological conditions (Richter *et al.*, 1995), and are susceptible to oxidative damage, especially in myocardial tissue (Kaul *et al.*, 1993, Hegstad *et al.*, 1994). Recent studies of MnSOD knock out mice unequivocally demonstrated the importance of MnSOD in the normal heart, since those mice developed a severe cardiomyopathy (Li *et al.*, 1995). These results suggest that free radicals can regulate MnSOD expression in cardiac myocyte. To understand whether higenamine affects MnSOD expression, Northern analysis was performed, but no change was observed in all three groups. Moreover, antioxidant enzymes such as MnSOD, catalase, and glutathione peroxidase are involved in heart failure, in which catalase enzyme has been reported to be up-regulated (Sabime *et al.*, 2000). Although protective action of higenamine in ischemia do not involve MnSOD gene regulation in rat cardiac tissue, the possible modulation of MnSOD activity or protein expression, or catalase activity by higenamine remains to be established. In summary, the protective role of higenamine on myocardial damage was investigated. In MI-myocardium, TNF-α and IL-6 were significantly increased, which was accompanied by increase in nitrotyrosine staining. Higenamine effectively reduced the tissue TNF-α and IL-6 contents and inhibited the

TNF-α expression in MI rat. In addition, higenamine possessed an antioxidant action, which is almost equivalent to gluthathion. We concluded that higenamine can protect oxidative stress-induced inflammatory disorders such as myocardial infarction by modulating of cytokines and scavenging of free radicals. Thus it can be beneficial in ischemic heart diseases.

ACKNOWLEDGMENTS

This work was supported by grant from KOSEF (996-0700-002-2) for Korea-Japan Joint Research Program.

REFERENCES

Akiyama K, Suzuki H, Grant P. and Bing R. (1997). Oxidation products of nitric oxide, NO2 and NO3, in plasma after experimental myocardial infarction. *J. Mol. Cell. Cardiol.* **29**, 1-9.

Anversa P, Beghi C, Kikkawa Y. and Olivetti G (1985). Myocardial response to infarction in the rat. Morphometric measurement of infarct size and myocyte cellular hypertrophy. *Am. J. Pathol.***118**, 484-92.

Finkel M. S, Oddis C. V, Jacob T. D, Watkins S. C, Hattler B. G and Simmons R. L (1992) Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science* **257**, 387-9.

Goossens V, Grooten J, De Vos K. and Fiers W. (1995). Direct evidence for tumor necrosis factor-induced mitochondrial reactive oxygen intermediates and their involvement in cytotoxicity. *Proc. Natl. Acad. Sci. U S A.* **92**, 8115-9.

Guillen I, Blanes M, Gomez-Lechon MJ. and Castell JV. (1995). Cytokine signaling during myocardial infarction: sequential appearance of IL-1 beta and IL-6. *Am. J. Physiol.* **269**, R229-235.

Hanatani A, Yoshiyama M, Kim S, Omura T, Toda I, Akioka K, Teragaki M, Takeuchi K, Iwao H, and Takeda T. (1995). Inhibition by angiotensin II type 1 receptor antagonist of cardiac phenotypic modulation after myocardial infarction. *J. Mol. Cell. Cardiol.* **27**, 1905-1914.

Hegstad A. C, Strand H, and Ytrehus K (1994). Phospholipid peroxidation after 60 min of global ischaemia and 10 min of reperfusion. A study in the isolated rat heart. *J. Mol. Cell. Cardiol.* **26**, 569-578.

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

Ischiropoulos H, Zhu L, and Beckman J. S (1992). Peroxynitrite formation from macrophage-derived nitric oxide. *Arch. Biochem. Biophys.* **298**, 446-451.

Kang Y. J, Koo E. B, Lee Y. S, Yun-Choi H. S, Chang K. C. (1999a). Prevention of the expression of inducible nitric oxide synthase by a novel positive inotropic agent, YS 49, in rat vascular smooth muscle and RAW 264.7 macrophages. *Br. J. Pharmacol.* **128**, 357-364.

Kang YJ, Lee YS, Lee GW, Lee DH, Ryu JC, Yun-Choi HS, Chang KC. (1999b). Inhibition of activation of nuclear factor kappaB is responsible for inhibition of inducible nitric oxide synthase expression by higenamine, an active component of aconite root. *J. Pharmacol. Exp. Ther.* **291**, 314-320.

van Krimpen C, Smits J. F, Cleutjens J. P, Debets J. J, Schoemaker R. G, Struyker Boudier H. A, Bosman F. T, and Daemen M. J. (1991). DNA synthesis in the non-infarcted cardiac interstitium after left coronary artery ligation in the rat: effects of captopril. *J. Mol. Cell. Cardiol.* **23**, 1245-53.

Koyanagi S, Aoki M, Tashiro H, Narabayashi H, Inou T, Takeshita A, Nakamura M, Noma M, Tajimi T, and Kikuchi Y. (1994). Prevalence and pathogenesis of silent myocardial ischemia following myocardial infarction. *Jpn. Circ. J.* **58**, 635-645.

Latini R, Bianchi M, Correale E, Dinarello C. A, Fantuzzi G, Fresco C, Maggioni A. P, Mengozzi M, Romano S, and Shapiro L. (1994). Cytokines in acute myocardial infarction: selective increase in circulating tumor necrosis factor, its soluble receptor, and interleukin-1 receptor antagonist. *J. Cardiovasc. Pharmacol.* **23**, 1-6.

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

Li Y, Huang T. T, Carlson E. J, Melov S, Ursell P. C, Olson J. L, Noble L. J, Yoshimura M. P, Berger C, and Chan P. H, (1995). Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat. Genet.* **11**, 376-381.

Nakamura K, Fushimi K, Kouchi H, Mihara K, Miyazaki M, Ohe T, and Namba M. (1998). Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor-alpha and angiotensin II. *Circulation* **98**, 794-799.

Richter C, Gogvadze V, Laffranchi R, Schlapbach R, Schweizer M, Suter M, Walter P, and Yaffee M. (1995). Oxidants in mitochondria: from physiology to diseases. *Biochim. Biophys. Acta.* **1271**, 67-74.

Smits J. F, Cleutjens J. P, van Krimpen C, Schoemaker R. G, and Daemen M. J. (1991). Cardiac remodeling in hypertension and following myocardial infarction: effects of arteriolar vasodilators. *Basic. Res. Cardiol.* **86**, Suppl 1, 133-139.

Szabo C, Ferrer-Sueta G, Zingarelli B, Southan G. J, Salzman A. L, Radi R.Villanueva F. S, Glasheen W/P, Sklenar J, Kaul S. Assessment of risk area during coronary occlusion and infarct size after reperfusion with myocardial contrast echocardiography using left and right atrial injections of contrast. *Circulation* 1993; **88**, 596-604.

Weber K. T, Brilla C. G. (1991). Pathological hypertrophy and cardiac interstitium. Fibrosis and renin-angiotensin-aldosterone

system. *Circulation* **83,** 1849-1865.

Wong G. H, Elwell J. H, Oberley L. W, and Goeddel D. V. (1989). Manganous superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. *Cell* **58**, 923-931.

Yokoyama T, Vaca L, Rossen R. D, Durante W, Hazarika P, and Mann D. L. (1993). Cellular basis for the negative inotropic effects of tumor necrosis factor-alpha in the adult mammalian heart. *J. Clin. Invest.* **92**, 2303-2312.

Zhang J. J, and Chen W. W. (1985). Protection of the synovial fluid by higenamine *Yao Xue Xue Bao* **20**, 423-426.