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Magnolol reduces myocardial ischemia/reperfusion injury via neutrophil inhibition in rats

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Abstract

The accumulation of oxygen-free radicals and activation of neutrophils are strongly implicated as important pathophysiological mechanisms mediating myocardial ischemia/reperfusion injury. It has been proven that various antioxidants have cardioprotective effects. Magnolol, an active component extracted from the Chinese medicinal herb *Magnolia officinalis*, possesses potent antioxidant and free radical scavenging activities. In this study, the cardioprotective activity of magnolol was evaluated in an open-chest anesthetized rat model of myocardial ischemia/reperfusion injury. The results demonstrated that pretreatment with magnolol (0.2 and 0.5 μg/kg, i.v. bolus) at 10 min before 45 min of left coronary artery occlusion, significantly suppressed the incidence of ventricular fibrillation and mortality when compared with the control group. Magnolol (0.2 and 0.5 μg/kg) also significantly reduced the total duration of ventricular tachycardia and ventricular fibrillation. After 1 h of reperfusion, pretreatment with magnolol (0.2 and 0.5 μg/kg) caused a significant reduction in infarct size. In addition, magnolol (0.2 μg/kg) significantly reduced superoxide anion production and myeloperoxidase activity, an index of neutrophil infiltration in the ischemic myocardium. In addition, pretreatment with magnolol (0.2 and 0.5 μg/kg) suppressed ventricular arrhythmias elicited by reperfusion following 5 min of ischemia. In vitro studies of magnolol (5, 20 and 50 μM) significantly suppressed *N*-formylmethionyl-leucyl-phenylalanine (fMLP; 25 nM)-activated human neutrophil migration in a concentration-dependent manner. It is concluded that magnolol suppresses ischemia- and reperfusion-induced ventricular arrhythmias and reduces the size of the infarct resulting from ischemia/reperfusion injury. This pronounced cardioprotective activity of magnolol may be mediated by its antioxidant activity and by its capacity for neutrophil inhibition in myocardial ischemia/reperfusion. © 2001 Published by Elsevier Science B.V.

Keywords: Magnolol; Antioxidant; Myocardial ischemia; Arrhythmia; Neutrophil

1. Introduction

The oxygen-free radical system has been implicated in the pathogenesis of myocardial ischemia/reperfusion injury. Oxygen-free radicals are mainly produced at the time of reperfusion and can damage the cell membrane and subcellular structures, which contain large amounts of phospholipids and proteins, resulting in phospholipid peroxidation and sequentially, structural and metabolic alterations, leading to cell death and necrosis (Kukreja and Hess, 1992). There are various mechanisms described in the production of free radicals in biological tissues. They include xanthine oxidase (McCord, 1985), activated neutrophils (Werns et al., 1985), leakage of electrons from the electron transport system with mitochondria (Boveris, 1977), catecholamine oxidation (Singal et al., 1982), cyclooxygenase and lipoxygenase enzymes (Rowe et al., 1983; Kukreja et al., 1986). Several approaches for protection against free radical damage have been considered. Among them, the free radical scavengers such as superoxide dismutase and antioxidants, which interrupt peroxidation by a number of different mechanisms, have been used as a protection strategy against reperfusion injury (Kukreja and Hess, 1992).

Ischemic myocardial injury initiates an acute inflammatory response in which polymorphonuclear leukocytes are major participants. Polymorphonuclear leukocytes accumulate in ischemic and reperfused myocardium under the influence of chemoattractants, wherein a substantial body of evidence has shown that polymorphonuclear leukocytes

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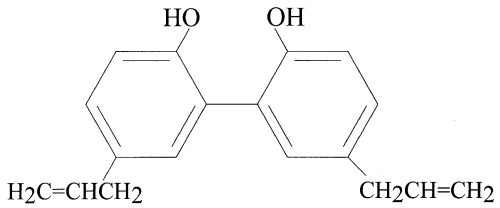

Fig. 1. The chemical structure of magnolol.

are involved in the genesis of myocardial ischemia/reperfusion injury (Hansen, 1995). Polymorphonuclear leukocyte depletion can decrease reperfusion arrhythmias, ameliorate post-ischemic no-reflow (Engler et al., 1986) and attenuate post-ischemic contractile dysfunction (Engler and Covell, 1987) after myocardial ischemia and reperfusion in dogs. Pronounced reduction of experimental infarct size was obtained after perfusion of the coronary circulation with neutrophil-depleted blood using leukopak filters (Engler et al., 1986; Litt et al., 1989) and neutrophil antiserum (Romson et al., 1983). Based on this evidence, a drug capable of polymorphonuclear leukocyte inhibition in myocardial ischemia and reperfusion may be of use in the treatment of patients with myocardial ischemia.

Magnolia officinalis is a commonly used Chinese medicinal herb for the treatment of fever, headache, anxiety, diarrhea and thrombotic stroke. Magnolol (Fig. 1), a principal constituent isolated from the bark of *M. officinalis*, possesses an antioxidant activity which is 1000 times greater than that of α-tocopherol and exhibits free radical scavenging activity but is less potent than α-tocopherol (Lo et al., 1994). In addition, magnolol has been demonstrated to suppress polymorphonuclear leukocyte infiltration in a mouse model of the ionophore A23187-induced pleurisy and to reduce leukotriene B_4 formation in rat isolated peripheral neutrophil suspension (Wang et al., 1992) and in rat basophilic leukemia-2H3 cells (Hamasaki et al., 1999). Magnolol also inhibits platelet-activating factor (PAF) production in human polymorphonuclear leukocytes (Yamazaki et al., 1994). These beneficial effects of magnolol could contribute to the suppression of inflammatory processes elicited by myocardial ischemia/reperfusion. Recently, Hong et al. (1996) reported that magnolol reduced infarct size in a rat model of sustained 4-h myocardial ischemia and suppressed both ischemia- and reperfusion-induced arrhythmias. Therefore, in this study, we further evaluated the cardioprotective effects of magnolol in an open-chest anesthetized rat model of myocardial ischemia/reperfusion injury and its possible mechanisms.

2. Materials and methods

2.1. Animal preparation

Adult Sprague–Dawley rats of either sex weighing 250–300 g were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg). Tracheotomy was performed and an intubating cannula was connected to a rodent ventilator. The animals were ventilated artificially with room air. Respiratory rate was synchronized with the rat's spontaneous rate (60–80 strokes/min, 1 ml/100 g body weight). Arterial blood pH and blood gases were maintained within normal physiological limits (pH 7.35–7.45; P_{CO_2}: 30–35 mm Hg; P_{O_2}: 85–100 mm Hg) by adjusting the respiratory rate and tidal volume. The left femoral artery and vein were cannulated for measurements of arterial blood pressure and heart rate via a Statham pressure tranducer and a Biotechnometer (RS3400, Gould, USA) and for the administration of drugs, respectively. Electrocardiograms were recorded from standard lead II limb leads. An oscilloscope electrocardiogram monitor (DSO 420, Gould, USA) was used to display the electrocardiogram continuously throughout the experiment. All signals, including the electrocardiogram and hemodynamic data, were recorded on chart paper.

After a left-side thoracotomy was performed at the fifth intercostal space, the pericardium was incised and the heart was exteriorized. A ligature (6/0 silk suture) was placed around the left main coronary artery close to its origin. The thread was then made into a knot as an occluder and another thread was tied to the former knot as a releaser. The ends of both threads were brought outside the thoracic cavity. Thus, the occlusion could be tightened or loosened by pulling the thread of the releaser. The rat was then allowed to stabilize for 30 min. During this period, the rats that showed functional instability such as hypotension (systolic blood pressure value less than 100 mm Hg) or occurrence of cardiac arrhythmias were discarded. One to two rats were discarded in each treatment group. The coronary artery was occluded for 45 min followed by 1 h of reperfusion.

The animals were randomly assigned to one of three treatment groups at the beginning of the study. There was an even spread of the sexes among the groups. First group (control): rats received the vehicle, dimethyl sulfoxide (DMSO; 0.1%, i.v., 0.25–0.30 ml) 10 min before occlusion ($n = 12$); second group: magnolol (0.2 μg/kg, i.v. bolus) was given as an intravenous bolus 10 min before occlusion ($n = 6$); third group: magnolol (0.5 μg/kg, i.v. bolus) was administered 10 min before occlusion ($n = 11$). The blood pressure, heart rate and electrocardiograms were continuously monitored throughout the experimental period.

2.2. Measurements

2.2.1. Hemodynamics

Measurements of heart rate and mean arterial blood pressure were performed in all groups at baseline 5, 10 and 30 min after occlusion and 5, 30 and 60 min after reperfusion. An indirect index of myocardial oxygen consumption

was provided by calculation of the product of the systolic blood pressure and heart rate.

2.2.2. Ventricular arrhythmias during ischemic period

Ventricular arrhythmias, which occurred within 30 min of the onset of myocardial ischemia, were assessed by the onset, incidence, total number of ventricular premature contraction and total duration of ventricular tachycardia and ventricular fibrillation. We defined ventricular premature contraction as discrete and identifiable premature QRS complexes (premature in relation to the P wave), ventricular tachycardia as a run of six or more consecutive ventricular premature beats and ventricular fibrillation as signals lacking identifiable individual QRS deflections in which a rate could no longer be determined. The mortality in each group was also evaluated.

2.2.3. Area at risk and infarct

At the end of the experiments, the coronary artery was reoccluded and 0.5-ml methylene blue (3%) was injected intravenously to denote the area at risk. The heart was then excised and the atria were removed. The entire ventricular area was sectioned into four 2–3-mm-thick slices from the apex to the base and incubated in (0.1%) nitroblue tetrazolium chloride (20 min, 37 °C). This solution stained the normal myocardium purple while the infarct portion remained pale. The areas of risk and infarct were traced carefully by hand on transparent paper. The traced areas were then measured by computerized planimetry.

2.2.4. Cardiac activity of myeloperoxidease

In order to quantify myocardial neutrophil infiltration, the cardiac activity of myeloperoxidease, a plentiful enzyme of neutrophils, was assessed using the method modified from that of Mullane et al. (1985). Myeloperoxidase activity was used as a marker for neutrophil content in the heart, since it correlates closely with the number of neutrophils (Weyrich et al., 1992). Myocardial tissue (control and 0.2 μg/kg magnolol-treated group) was taken from the non-ischemic and ischemic regions of the heart and frozen rapidly in liquid nitrogen. The myocardium was homogenized in 0.5% hexadecyltrimethyl ammonium bromide and dissolved in 50 mM potassium phosphate buffer (pH 6.0). Specimens were centrifuged at $12{,}500 \times g$ at 2 °C for 30 min, afterwhich the supernatant fluids were assayed. To assay myeloperoxidase activity, the supernatants were reacted with 0.167 mg/ml *O*-dianisidine dihydrochloride and 0.0005% H_2O_2 in 50-nmol phosphate buffer (pH 6.0). The change in absorbance was measured spectrophotometrically at 460 nm. Myeloperoxidase activity is defined as the quantity of enzyme hydrolyzing 1 mmol peroxide/min at 25 °C and expressed in milliunits per gram tissue.

2.2.5. Superoxide anion production in cardiomyocytes

Superoxide anion production in cardiomyocytes is measured by modified lucigenin-enhanced chemiluminescence. The chemical specificity of this light-yielding reaction for superoxide ion was reported previously (Gyllenhammer, 1987). Briefly, myocardium samples (control and 0.2 μg/kg magnolol-treated group) (2 × 2 mm) taken from the non-ischemic and ischemic regions were placed in 37 °C Krebs–HEPES buffer and allowed to equilibrate for 30 min. Scintillation plates containing Krebs–HEPES buffer with lucigenin (250 μM) were placed into a microplate luminometer (LB96V, EG&G Berthold, Germany). After 20 min, background counts were recorded and then a myocardium sample was added to each well. Counts were then recorded for 15 min for each well and the respective background was subtracted. All samples were dried in a 90 °C oven (16 h) for expressing results on a milligram myocardium dry weight basis. Lucigenin chemiluminescence is calibrated using known rates of superoxide production from 0.5 mU/ml xanthine oxidase plus 100 μM of xanthine, as determined by cytochrome *C* reduction. The results are expressed as relative luminescence unit/mg of dry tissue weight.

2.2.6. Reperfusion-induced arrhythmias

After a 5-min left coronary artery occlusion, ventricular arrhythmias were also elicited by reperfusion. The data for onset, incidence, total number of ventricular premature contractions, total duration of ventricular tachycardia and ventricular fibrillation and mortality were collected from the control and magnolol 0.2 and 0.5 μg/kg groups.

2.2.7. Neutrophil chemotaxis

Chemotactic activity of neutrophils was evaluated, as described previously, using the modified Boyden chamber technique (Falk et al., 1980; Sehmi et al., 1992). To the bottom of Boyden chambers were added the 100-μl stimuli (25 nM *N*-formylmethionyl-leucyl-phenylalanine; fMLP). This was covered with polyvinylpyrrolidone-free membrane (5 μm pore size; 140 μm thickness) (Neuro-Probe, USA), which was soaked in PBS containing Ca^{2+} (1 mM), Mg^{2+} (0.5 mM) and 0.05% bovine serum albumin before use. Two-hundred microliters of a human neutrophil suspension at a concentration of 2.5×10^5/ml, which was pretreated with magnolol 5, 20 or 50 μM for 5 min, was added to each of the top chambers. After incubation for 45 min at 37 °C, the filter was removed, fixed and stained with Hemacolor (Merk, USA). The numbers of cells on the bottom surfaces of the filters were counted in five different fields at a magnification of ×400 for each incubation. The data are expressed as percentages of total migrated cells under fMLP-induced attraction.

2.3. Chemicals

Magnolol, a gift from Dr. Chen of the Department of Medicinal Chemistry, National Research Institute of Chinese Medicine, Taipei, Taiwan was dissolved in 0.1% DMSO.

Table 1
Summary of hemodynamic measurements in myocardial ischemia/reperfusion

Treatment	Baseline	Time for ischemia (min)			Time for reperfusion (min)		
		5	10	30	5	30	60
Mean blood pressure							
Control	86.4 ± 6.2	78.0 ± 3.1	85.8 ± 5.5	87.4 ± 3.1	88.4 ± 4.8	82.1 ± 5.6	83.0 ± 6.7
Magnolol							
0.2 μg/kg	89.7 ± 4.8	83.9 ± 4.0	86.8 ± 5.1	85.3 ± 6.2	86.2 ± 4.8	84.6 ± 3.7	85.1 ± 5.3
0.5 μg/kg	86.4 ± 4.5	82.8 ± 3.1	85.5 ± 5.7	86.1 ± 4.8	87.9 ± 3.0	87.6 ± 4.8	85.6 ± 5.9
Heart rate							
Control	386.0 ± 4.7	383.6 ± 6.2	389.0 ± 7.8	389.6 ± 8.8	388.6 ± 4.7	388.0 ± 4.8	384.6 ± 5.7
Magnolol							
0.2 μg/kg	390.8 ± 5.8	389.0 ± 8.5	401.7 ± 6.5	394.0 ± 6.3	397.0 ± 7.8	408.0 ± 3.4	398.5 ± 5.1
0.5 μg/kg	382.0 ± 7.8	384.7 ± 5.8	387.3 ± 4.7	390.7 ± 3.1	383.8 ± 8.2	387.0 ± 5.1	394.4 ± 6.1
Rate–pressure product (×1000)							
Control	47.3 ± 6.2	46.1 ± 3.8	46.0 ± 4.4	45.6 ± 3.8	46.3 ± 3.6	45.1 ± 4.4	48.4 ± 3.6
Magnolol							
0.2 μg/kg	49.9 ± 4.6	50.2 ± 3.7	49.8 ± 3.2	51.2 ± 4.3	50.2 ± 4.2	48.6 ± 5.1	48.7 ± 5.6
0.5 μg/kg	50.2 ± 1.8	49.5 ± 3.3	51.1 ± 3.5	47.4 ± 2.8	47.2 ± 2.5	46.8 ± 3.8	50.3 ± 4.4

Values are expressed as means ± S.E.M.

2.4. Statistical analysis

The measurements of hemodynamics, total number of ventricular premature contractions, total duration of ventricular tachycardia and ventricular fibrillation, level of superoxide anion production, percentage of neutrophil migration, area at risk and infarct are expressed as group means ± standard error of the mean (S.E.M.). The x^2 test was used to analyze the differences in the incidence of arrhythmias and mortality between the control and magnolol-treated groups. Student's *t*-test was used to test the difference in the activities of superoxide anion production and myeloperoxidase between the control and the magnolol-treated groups. The other parameters were compared by one-factor analysis of variance. If this analysis indicated significant differences among the group means, the control group was compared with each of the treatment groups by means of the Newman–Keuls method. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Hemodynamic measurements

The hemodynamic data including mean blood pressure, heart rate and rate–pressure product are summarized in Table 1. No significant changes in these measurements were observed when the control was compared with each of the three treatment groups throughout the experimental period.

3.2. Ischemia-induced arrhythmias

Ligation of the left coronary artery invariably resulted in ventricular arrhythmias, which commenced within 4–5 min of occlusion, manifesting as ventricular premature contraction, ventricular tachycardia and ventricular fibrillation. Table 2 shows the effects of magnolol on the onset and incidence of arrhythmias following coronary artery occlusion in the rat. All the rats in the control group

Table 2
The protective effect of magnolol on ischemia-induced ventricular arrhythmias in anesthetized open-chest rats

Treatment	*N*	VPC		VT		VF		Mortality (%)
		Onset (min)	Incidence (%)	Onset (min)	Incidence (%)	Onset (min)	Incidence (%)	
Control	12	5.3 ± 0.2	100.0	6.0 ± 0.5	91.7	7.8 ± 0.5	91.7	58.3
Magnolol								
0.2 μg/kg	6	6.6 ± 0.6	83.3	7.7 ± 1.3	50.0	–	0[a]	0[a]
0.5 μg/kg	11	7.0 ± 0.7	90.9	7.5 ± 0.3	54.5	–	0[a]	0[a]

N: number of rats; VPC: ventricular premature contraction; VT: ventricular tachycardia; VF: ventricular fibrillation.Values are expressed as means ± S.E.M.
[a] $P < 0.05$ compared with control group.

developed arrhythmias during the 30-min post-ligation period. Pretreatment with magnolol (0.2 and 0.5 μg/kg) before coronary artery ligation significantly suppressed the occurrence of ventricular fibrillation as compared to the control. There was no mortality in the magnolol-treated groups compared with 58.3% in the control. Furthermore, magnolol at both 0.2 and 0.5 μg/kg caused a pronounced shortening of the total duration of ventricular tachycardia and ventricular fibrillation (Fig. 2).

3.3. Infarct size

All control and treated hearts showed clearly demarcated areas of infarction as a consequence of 45 min of ischemia followed by 1-h reperfusion, as assessed by the *p*-nitro-blue-tetrazolium staining technique. No significant differences in the area at risk, expressed as percentage of the total left ventricle, were noted among the groups (Fig. 3). A significant reduction in infarct size, expressed as percentage of the area at risk was noted with magnolol treatments when compared with the control (magnolol 0.2 μg/kg: 21.0 ± 4.8%, magnolol 0.5 μg/kg: 32.4 ± 3.1% vs. control: 63.9 ± 4.5%) ($P < 0.05$).

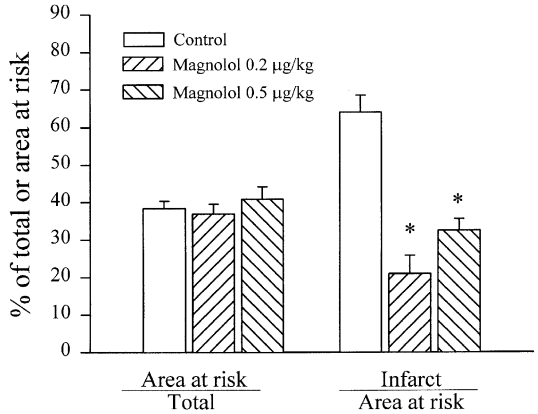

Fig. 3. Effect of magnolol on infarct size, expressed as a percentage of risk region, in rats undergoing 45 min of left coronary occlusion followed by 1 h of reperfusion. Values are expressed as means ± S.E.M., * $P < 0.05$ vs. the control.

3.4. Cardiac activity of myeloperoxidase

Neutrophil infiltration of the ischemic region during reperfusion is considered to be one of the major mechanisms responsible for reperfusion injury. To determine the effects of magnolol on the accumulation of neutrophils into the ischemic myocardium, we examined myocardial myeloperoxidase activity in non-ischemic and ischemic regions in the control and magnolol (0.2 μg/kg)-treated groups after 45-min ischemia/1-h reperfusion (Fig. 4). In the non-ischemic region, it is evident that myeloperoxidase activity is very low in the control and magnolol-treated groups and there is no difference between them. However, in ischemic myocardium, a significant increase in myeloperoxidase activity was observed in the control group ($P < 0.05$). In contrast, the magnolol-group exhibited sig-

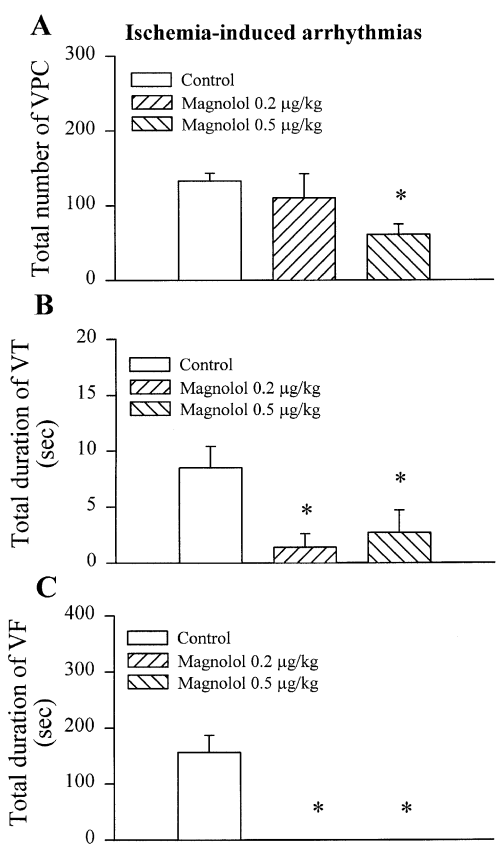

Fig. 2. Effects of pretreatment with magnolol on the total number of ventricular premature contractions (VPC) and the total duration of ventricular tachycardia (VT) and ventricular fibrillation (VF) during a 30-min coronary artery occlusion in anesthetized rats. Values are expressed as means ± S.E.M., * $P < 0.05$ vs. the control.

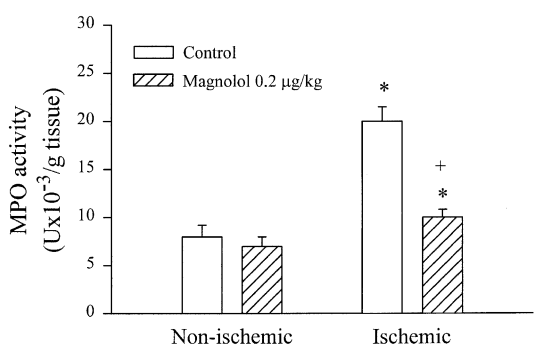

Fig. 4. Effect of magnolol (0.2 μg/kg) on myocardium myeloperoxidase (MPO) activity during 45 min of left coronary occlusion followed by 1 h of reperfusion in rats. Values are expressed as means ± S.E.M. * $P < 0.05$ vs. the non-ischemic region; + $P < 0.05$ vs. the control.

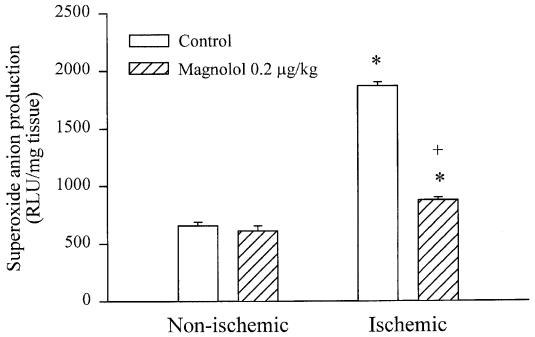

Fig. 5. Bar graph showing suppression by magnolol (0.2 μg/kg) of superoxide anion production in myocardium exposed or not exposed to 45-min ischemia and 1-h reperfusion. RLU: relative luminescence unit; Values are expressed as means ± S.E.M.; * $P < 0.05$ vs. the non-ischemic region; + $P < 0.05$ vs. the control.

nificantly lower myeloperoxidase activity (10.06 ± 0.90 U $\times 10^{-3}$/g tissue weight) in ischemic myocardial tissue as compared with the control (19.37 ± 1.60 U $\times 10^{-3}$/g tissue weight) ($P < 0.05$). These results indicate that neutrophil infiltration in ischemic myocardium was clearly inhibited by magnolol.

3.5. Superoxide anion production

Myocardial superoxide anion production was measured in non-ischemic and ischemic regions of the control and magnolol (0.2 μg/kg)-treated groups after 45-min ischemia/1-h reperfusion. Superoxide anion concentrations in the non-ischemic myocardium were similar in control and treatment groups ($P > 0.05$, Fig. 5). Pretreatment with 0.2 μg/kg magnolol prevented the increase in superoxide anion production in the myocardium after ischemia and reperfusion (magnolol 0.2 μg/kg: 876 ± 23 vs. control: 1868 ± 33 relative luminescence unit/mg tissue weight) ($P < 0.05$).

3.6. Reperfusion-induced arrhythmias

After a 5-min period of ischemia, reperfusion-induced ventricular arrhythmias were also observed and manifested as ventricular premature contraction, ventricular tachycardia and ventricular fibrillation. Pretreatment with magnolol (0.2 and 0.5 μg/kg) before coronary artery ligation significantly suppressed the occurrence of ventricular tachycardia and ventricular fibrillation as compared to the control, with the reduction of total duration of ventricular tachycardia and ventricular fibrillation (Table 3 and Fig. 6). Mortality in the two magnolol-treated groups was zero and significantly lower than that of the control.

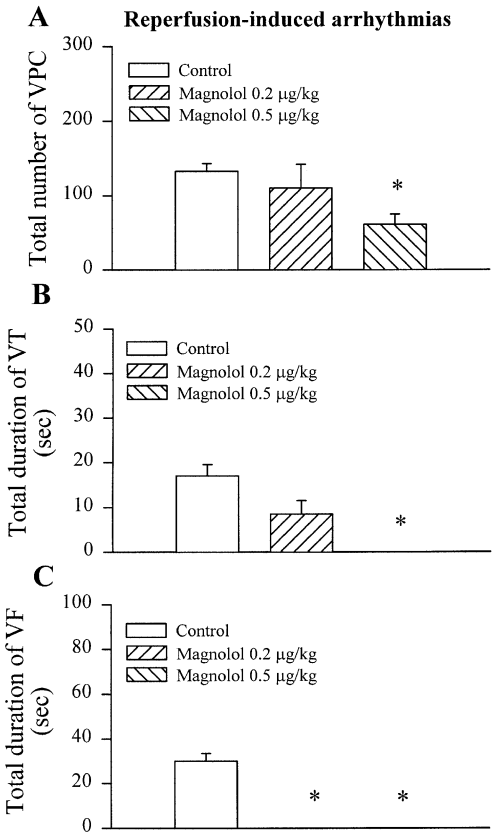

Fig. 6. Effects of pretreatment with magnolol on the total number of ventricular premature contractions (VPC) and the total duration of ventricular tachycardia (VT) and ventricular fibrillation (VF) during reperfusion after a 5-min coronary artery occlusion in anesthetized rats. Values are expressed as means ± S.E.M., * $P < 0.05$ vs. the control value.

Table 3
The protective effect of magnolol on reperfusion-induced ventricular arrhythmias in anesthetized open-chest rats

Treatment	*N*	VPC		VT		VF		Mortality (%)
		Onset (min)	Incidence (%)	Onset (min)	Incidence (%)	Onset (min)	Incidence (%)	
Control	9	0.22 ± 0.07	100	0.20 ± 0.05	89	0.39 ± 0.09	100	58.3
Magnolol								
0.2 μg/kg	6	0.28 ± 0.10	100	1.70 ± 0.80	50	–	0[a]	0[a]
0.5 μg/kg	6	0.30 ± 0.07	100	–	0[a]	–	0[a]	0[a]

N: number of rats; VPC: ventricular premature contraction; VT: ventricular tachycardia; VF: ventricular fibrillation. Values are expressed as means ± S.E.M.
[a] $P < 0.05$ compared with control group.

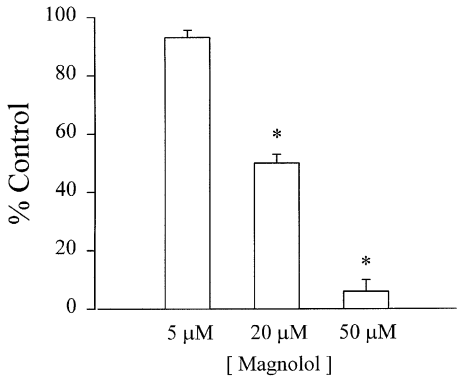

Fig. 7. Inhibition by magnolol of fMLP (25 nM)-induced chemotaxis of human neutrophils. Values are expressed as means ± S.E.M. $^{*}P < 0.05$ vs. the control value.

3.7. Neutrophil chemotaxis

Magnolol was evaluated in the fMLP-induced neutrophil chemotaxis assay using a Boyden chamber with a polyvinylpyrrolidone-free polycarbonate filter. Magnolol (5, 20 and 50 μM) inhibited fMLP-induced chemotaxis in a concentration-dependent manner (Fig. 7). Furthermore, such chemotaxis was almost completely inhibited by magnolol at a concentration of 50 μM.

4. Discussion

The present study was performed to determine the effects of magnolol on ischemic arrhythmias and reperfusion injury in an anaesthetized opened-chest rat model of acute myocardial ischemia. The results demonstrate that magnolol markedly suppressed ventricular arrhythmias elicited by ischemia, reduced infarct size and ventricular arrhythmias due to the reperfusion injury. The pronounced cardioprotective action of magnolol was also characterized by zero mortality. These effects may have been achieved by its antioxidant activity and by its capacity for neutrophil inhibition.

It is well known that oxygen-free radicals are involved in the genesis of reperfusion injury. A substantial body of evidence has shown that interrupting peroxidation by antioxidants through a number of different mechanisms can prevent the production of oxygen-free radicals (Kukreja and Hess, 1992). Among these, the antioxidant, vitamin E (α-tocopherol), has been used by several investigators as a cardioprotective agent in a variety of animal studies (Guarnieri et al., 1978; Massey and Burton, 1989; Janero and Burghardt, 1989). Magnolol has a potent antioxidant activity on isolated rat heart mitochondria lipid peroxidation induced with ADP and ferrous sulfate (Lo et al., 1994), which is 1000 times that of α-tocopherol. Magnolol also exhibits free radical scavenging activity, but is less potent than α-tocopherol (Lo et al., 1994). We also showed that magnolol suppressed superoxide anion production in myocardium exposed to ischemia and reperfusion (Fig. 5). Thus, magnolol may protect against myocardial ischemia/reperfusion injury by preventing the production of free radicals.

Accumulating evidence has indicated that myocardial ischemia elicits an acute inflammatory response that is greatly augmented by reperfusion. Polymorphonuclear leukocytes are integrated into the acute inflammatory response to tissue injury. Polymorphonuclear leukocytes accumulate in ischemic and reperfused myocardium under the influence of chemoattractants, and participate in the myocardial injury after ischemia and reperfusion (Hansen, 1995). Activated polymorphonuclear leukocytes were shown to aggregate and adhere to endothelium, which resulted in capillary plugging and subsequent impairment of coronary blood flow and participation in the development of endothelial cell edema. Moreover, activated polymorphonuclear leukocytes exacerbate ischemic myocardial injury by release of cytotoxic oxygen-free radicals and proteolytic enzymes. It has been reported that superoxide anion and hydrogen peroxide can modulate leukocyte Mac-1 expression and leukocyte endothelial adhesion, which can be diminished by superoxide dismutase and/or catalase pretreatment (Fraticelli et al., 1996; Serrano et al., 1996). Therefore, magnolol can inhibit polymorphonuclear leukocyte activity and suppress superoxide anion production in the ischemic myocardium, indicating that a significant part of the cardioprotection is attributable to these effects.

Over the last several years, it has been demonstrated that nitric oxide (NO) is generated in biological cells and tissues and is important in the regulation of a broad range of important biological functions. The role of NO in myocardial ischemia/reperfusion injury is still controversial. Infusion of NO into the reperfused tissue could increase vascular permeability and augment the tissue injury (Seekamp et al., 1993). The interaction of NO with superoxide anion generates the highly reactive free radicals, peroxynitrite and hydroxy radical, which are cytotoxic in excess (Kukreja and Hess, 1992). In contrast, NO has been shown to be an endogenous inhibitor of platelet aggregation (Moncada et al., 1991), leukocyte chemotaxis (Belenky et al., 1993) and adherence (Kubes et al., 1991). It is conceivable that reduced basal NO release after myocardial reperfusion due to endothelial damage may promote polymorphonuclear leukocytes adherence to endothelium. In fact, maintenance of the NO level during reperfusion by administration of NO donors (Siegfried et al., 1992; Lefer et al., 1993) and L-arginine (Nakanishi et al., 1992) can effectively minimize endothelial and/or myocardial injury associated with reperfusion. Accordingly, the suppression of superoxide anion production by magnolol is expected to: (1) protect the coronary endothelium against free radical damage in the early minutes after reperfusion to sustain NO production by the endothelium; (2) prevent the interac-

tion of superoxide anions with NO, and thereby prolong and amplify the protective effects of NO.

The pronounced cardioprotection by magnolol is unlikely to be mediated through its hemodynamic effects because no significant changes in mean blood pressure, heart rate or rate–pressure product occurred during experimental periods when compared with the control group. Magnolol did not alter the rate–pressure product, an index of myocardial oxygen consumption, indicating that reduced oxygen consumption cannot account for the antiarrhythmic effect. Teng et al. (1990) reported that magnolol produced a vasodilator effect, which resulted in a reduced afterload and improved coronary flow, which may mediate the antiarrhythmic effect. However, the rat heart is deficient in functional collaterals (Johns and Olson, 1954), precluding any blood supply to the ischemic myocardium during the 45-min ligation in this model. Furthermore, the concentration of magnolol required to exert the vasodilator effect in vitro is around 200 μM (Teng et al., 1990). It is highly unlikely that the plasma concentrations of magnolol in rats could reach this range with the low doses (0.2 and 0.5 μg/kg) used in the present study.

The exact mechanism underlying the antiarrhythmic action of magnolol during ischemia is still uncertain. Although a marked increase in oxygen-free radical levels during early reperfusion has been shown, whether oxygen-free radical levels increase during ischemia remains controversial (Goldhaber and Weiss, 1992). Zweier et al. (1987) reported that oxygen-free radical concentrations in isolated rabbit hearts increased about twofold after 10 min of ischemia and over sevenfold within the first few minutes of reperfusion, remaining significantly elevated for more than an hour. Generation of reactive oxygen-free radicals attacks cell membranes, which contain large amounts of polyunsaturated fatty acids, leading to lipid peroxidation and loss of cell integrity and function. Fragmentation of the membrane and increased permeability to Ca^{2+} and other ions leads to irreversible cell destruction (Kukreja and Hess, 1992). This may participate in electrophysiological derangements and malignant arrhythmia. Thus, the antioxidant activity of magnolol is an underlying mechanism of antiarrhythmia. Furthermore, in view of the inhibitory effects of magnolol on platelet aggregation, the potential arrhythmogenic effects of substances (e.g. 5-hydroxytryptamine and thromboxane A_2) derived from platelets (Wainwright and Parratt, 1988; Coker et al., 1981; Coker et al., 1986), it is likely that the antiplatelet effect of magnolol was partly responsible for the observed antiarrhythmic effect. On the other hand, results of studies with several PAF antagonists support a role for PAF as an endogenous mediator of arrhythmogenesis in various pathophysiological circumstances (Curtis et al., 1993). The suppression of PAF production by magnolol (Yamazaki et al., 1994) could be another possible mechanism.

In conclusion, magnolol has pronounced cardioprotective activity in a rat model of myocardial ischemia/reperfusion. We postulated that the cardioprotective effects of magnolol could be attributed to its capacity for neutrophil inhibition and antioxidant property.

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