

Improved Post-Myocardial Infarction Survival With Probucol in Rats: Effects on Left Ventricular Function, Morphology, Cardiac Oxidative Stress and Cytokine Expression

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OBJECTIVES	The goal of this study was to evaluate whether reducing the potentially deleterious effects of oxidative stress with the potent anti-oxidant probucol improves prognosis after myocardial infarction (MI) in rats.
BACKGROUND	Oxidative stress has been documented in patients early and late after MI, particularly when it is associated with congestive heart failure.
METHODS	Rats surviving acute MIs for 24 h (n = 247) were assigned to vehicle or probucol (61 mg/kg, daily) for four weeks, at which time cardiac hemodynamic, morphologic and molecular measurements were done.
RESULTS	In rats with large MIs, probucol improved survival (87.9%) when compared with vehicle (50.6%) (p < 0.001). Probucol also partially preserved left ventricular (LV) systolic but not diastolic function. Probucol increased scar thickness and decreased cardiac fibrosis but did not modify LV hypertrophy or dilation. Finally, probucol decreased cardiac oxidative stress, as assessed by measuring cardiac malondialdehydes, and decreased the cardiac expression of the pro-inflammatory cytokines interleukin (IL)-1 β and IL-6 but did not modify fetal gene re-expression in rats with large MIs.
CONCLUSIONS	This study indicates that the anti-oxidant probucol markedly improves post-MI survival in rats despite few demonstrable effects on cardiac remodeling or hemodynamics. Its beneficial effects may, however, be associated with reduced cardiac fibrosis, oxidative stress and expression of pro-inflammatory cytokines. (J Am Coll Cardiol 2002;39:148–56) © 2002 by the American College of Cardiology

Morbidity and mortality after large myocardial infarction (MI) remain elevated and are associated with progressive post-MI ventricular remodeling and dysfunction (1). Why the heart remodels and finally fails in some patients after a large MI remains incompletely understood, but evidence from experimental and clinical trials would suggest that hemodynamic and neurohumoral factors are involved (1). More recently, interest has begun to focus on more local myocardial factors that are influenced by hemodynamic changes and neurohumoral activation. These include pro-inflammatory cytokines, reactive oxygen species and peptide growth factors, which are all upregulated after MI and can lead to further adverse ventricular remodeling and dysfunction (2).

Oxidative stress may contribute to poor post-MI prognosis via numerous mechanisms, of which direct cytotoxic, negative inotropic, cytokine stimulating and apoptotic effects are but a few (3–5). Singal et al. (6) have shown that the occurrence of oxidative stress coincides with the appear-

ance of hemodynamic abnormalities after MI in rats (6). In that post-MI model, therapy with the anti-oxidant vitamin E helped prevent progressive myocardial dysfunction, a finding that was related to the maintenance of a more normal endogenous anti-oxidant status of the heart (7). More recently, Kinugawa et al. (8) demonstrated that the powerful anti-oxidant, dimethylthiourea (DMTU) started 4 h after MI preserved ventricular function, attenuated ventricular dilation, reduced cardiac fibrosis and attenuated the increase in myocardial matrix metalloproteinase-2 activity that normally occurs after MI. Although this is provocative in its implications, more information as to what mechanisms are involved and whether these promising findings lead to improved survival are necessary.

Probucol is a cholesterol-lowering agent with potent anti-oxidant properties (9). It has been shown to prevent the development of adriamycin cardiomyopathy and ventricular dysfunction in rats (10). These beneficial effects of probucol are thought to be the result of improved myocardial anti-oxidant activity and are accompanied by a decrease in cardiac oxidative stress (10). Whether it has beneficial post-MI effects remains to be tested.

In this study we hypothesized that the anti-oxidant probucol would lead to improved post-MI survival and that this would be associated with beneficial effects on left

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Abbreviations and Acronyms

ANF	= atrial natriuretic factor
BW	= body weight
cDNA	= complementary DNA
DMTU	= dimethylthiourea
GAPDH	= glyceraldehyde 3-phosphate dehydrogenase
HR	= heart rate
IL	= interleukin
LV	= left ventricle or left ventricular
LVEDP	= left ventricular end-diastolic pressure
LVSP	= left ventricular systolic pressure
LVW	= left ventricle weight
MHC	= myosin heavy chain
MI	= myocardial infarction
mRNA	= messenger RNA
NE	= norepinephrine
RV	= right ventricle or right ventricular
RVSP	= right ventricular systolic pressure
RVW	= right ventricle weight
SkACT	= skeletal actin
TNF	= tumor necrosis factor
+dP/dt	= maximum rate of pressure rise
-dP/dt	= maximum rate of pressure decline

ventricular (LV) remodeling and function, reduced oxidative stress and reduced cardiac expression of pro-inflammatory cytokines.

METHODS

Animal care. Male Wistar rats weighing from 200 to 250 g were obtained from Charles Rivers Breeding Laboratories

(Saint-Constant, Quebec, Canada). All care was in accordance with the “Canadian Council for Animal Care” and the “Animal Care Committee Guidelines” of the Montreal Heart Institute.

MI operative procedure. Myocardial infarction was induced in rats by ligating the left anterior coronary artery as described previously (11). There was a high early mortality rate (51% within 24 h), the survivors being randomized into different treatment groups. At the time they were euthanized, rats were classified as having either a large MI, an LV scar of >45% of the LV circumference or a scar-to-body-weight ratio >0.2 g/kg, or no or small MI, LV scar between 0% and 20% of circumference or a scar-to-body-weight ratio between 0.0 g/kg to 0.1 g/kg (11). Other rats were not further considered. Rats dying between 24 h and 72 h after MI were assumed to have had a large MI, and those dying later, but before 28 days, had morphologic studies.

Pharmacologic interventions. Rats surviving 24 h after MI were randomized into two groups as follows: 1) vehicle (soya bean oil); and 2) probucol (61 mg/kg/day) (10) (Fig. 1). Soya bean oil was used as the vehicle because probucol is lipid soluble. All drugs were given by gavage. All rats were treated for 28 to 30 days.

Long-term cardiac hemodynamic studies. After four weeks of treatment, the rats were initially anesthetized with 3% halothane mixed with 100% O₂. The halothane percentage was reduced to 1% 3 min before hemodynamic recordings. Left ventricular and right ventricular (RV) hemodynamics were measured as previously described (11).

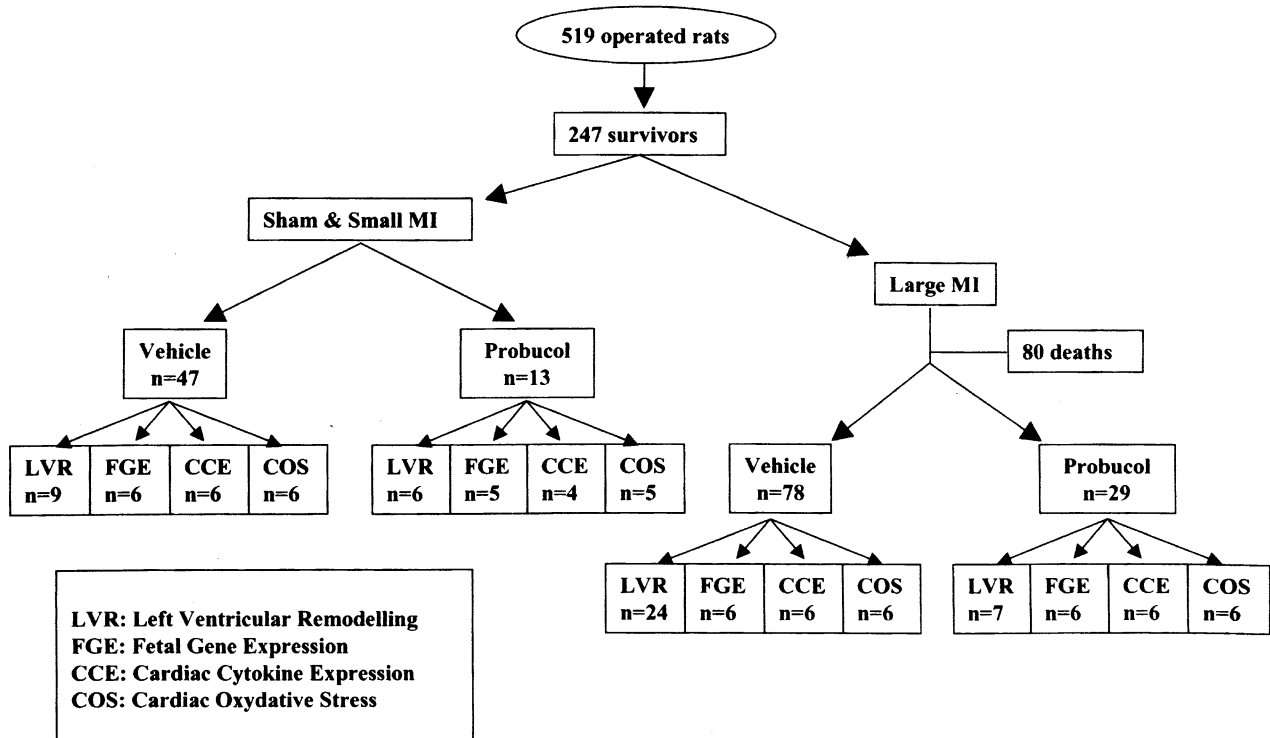


Figure 1. Flow diagram of various groups of rats according to myocardial infarction (MI) size and treatment group.

Passive pressure-volume relationship. After completing the cardiac hemodynamic measurements, 46 rats had their hearts stopped in diastole by an intravenous injection of a saturated potassium chloride solution. The passive pressure-volume relationship of each heart was then assessed as previously described (11).

Cardiac remodeling. Once the pressure-volume curve was completed, the LV was filled with saline solution to a pressure of 15 mm Hg, sealed and fixed in its distended form in 10% formalin phosphate buffer for 24 h. Two cross-sections were obtained at 1-mm intervals midway between the base and the apex of the LV for morphometry as previously described (11). The average scar thickness was obtained directly by planimetry as well. The LV surface area at the endocardium (cavity) and epicardium (cavity and ventricular wall = ventricular area) were numerically summed separately. For both cross-sectional levels, dilation index was expressed as the ratio of LV cavity area/LV area.

Cardiac fibrosis assessment. This procedure consisted of using samples from both cross-sections stained with Sirius red F3BA as a 0.1% solution in saturated aqueous picric acid as described previously (12). Collagen volume density fraction was then determined by measuring the area of stained tissue within a given field and expressing that area as a proportion of the total area under observation. The collagen-rich border zone of vessels was not included in the calculations. Ten fields were analyzed in the subendocardial layer and 10 fields in the subepicardial layer in each LV. The collagen-rich border of the scar was not included.

Cardiac and lung weights. A total of 168 rats were used to assess cardiac hypertrophy once the hemodynamic protocol was completed, as previously described (11). The scarred area was pinned on a paper, and its surface was determined by planimetry. Each tissue was then weighed individually, frozen in liquid nitrogen, and stored at -80°C . The lungs were also weighed and frozen.

Plasma norepinephrine (NE) measurements. Plasma NE was measured by methods described previously (11).

Quantification of fetal gene expression in LV myocardium. Myocardial fetal gene expression, as assessed by measuring messenger RNA (mRNA) for atrial natriuretic factor, beta myosin heavy chain (βMHC), alpha myosin heavy chain (αMHC) and skeleton actin (skACT) was evaluated in myocardium from the LV of six hearts from each group and normalized for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression as previously described (13).

Ribonuclease protection assay for cardiac inflammatory cytokine panel. To determine the gene expression of cytokines from the affected myocardium, ribonuclease protection assay was performed to quantitate the mRNA levels of tumor necrosis factor (TNF)- α , interleukin (IL)- 1β , -5 and -6. The complementary DNA (cDNA) probes specific for the rat target cytokines were prepared from a commercially available kit based on known coding sequences (RiboQuant, PharMingen; Wilmington, Delaware) according to the manufacturer's instructions. Specifically, the cDNA tem-

plates of the target cytokines were amplified using polymerase chain reaction and labeled with $\alpha^{33}\text{P}$ -UTP (Dupont, New England Nuclear; San Diego, California). Commercially available cDNA probes for housekeeping genes L32 and GAPDH were also amplified and labeled to act as standardization controls.

The total RNA from the myocardial samples was extracted and purified according to techniques previously published from our laboratory (14), taking particular care to ensure RNase-free reaction conditions.

Cardiac oxidative stress. Cardiac oxidative stress was assessed by measuring cardiac aldehydes. The four groups of cardiac aldehydes were: 1) straight chained aldehydes; 2) branch chained aldehydes; 3) unsaturated aldehydes; and 4) malondialdehydes. Samples were analyzed by gas chromatography mass spectrometry using the previously described method from Luo et al. (15).

Statistical analyses. All values are expressed as mean \pm SEM. Results were analyzed by using a two-tailed Student *t* test for unpaired data and by analysis of variance (ANOVA) for multiple comparisons followed by a two-sided Dunnett's test or Student-Newman-Keuls test, when appropriate. The pressure-volume relationships of the different groups were compared by repeated ANOVA measurements and the interaction term for the effect of probucol tested. Kaplan-Meier survival curves over the follow-up period were constructed and analyzed by the generalized Savage (Mantel-Cox) test. Gels were analyzed by densitometry, and the results were presented as mean (arbitrary units) \pm SEM. Statistical significance was assumed at $p < 0.05$.

RESULTS

Survival. The total number of rats used in the study was 519. Of these, 272 died within 24 h after coronary ligation and, thus, were excluded from the study (Fig. 2). The remaining 247 rats were treated randomly with soya bean oil ($n = 201$) or probucol ($n = 46$). Regardless of treatment group, the survival rate of rats with sham-to-small MIs was excellent over the four weeks of follow-up.

The early (<72 h) post-MI mortality of rats with large MIs treated with vehicle (soya bean oil) was 16.9% (26 deaths). Rats with large MIs treated with probucol had a strong tendency toward lower post-MI (<72 h) mortality (6.1%) (2 deaths, $p = 0.055$ vs. vehicle). The overall survival rates (28 days) of rats with large MIs were 50.6% for vehicle and 87.9% for probucol (vehicle vs. probucol, $p < 0.001$) (Fig. 2).

Hemodynamic measurements. In rats with sham-to-small MIs, probucol treatment increased heart rate (HR), LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP) and maximum rate of pressure rise and decline ($\text{LV} \pm \text{dP/dt}$) compared with the vehicle group, suggesting increased adrenergic tone (Table 1).

Vehicle rats with large MIs had a decrease in HR, LVSP, $\text{LV} \pm \text{dP/dt}$ and an increase in LVEDP. This was accom-

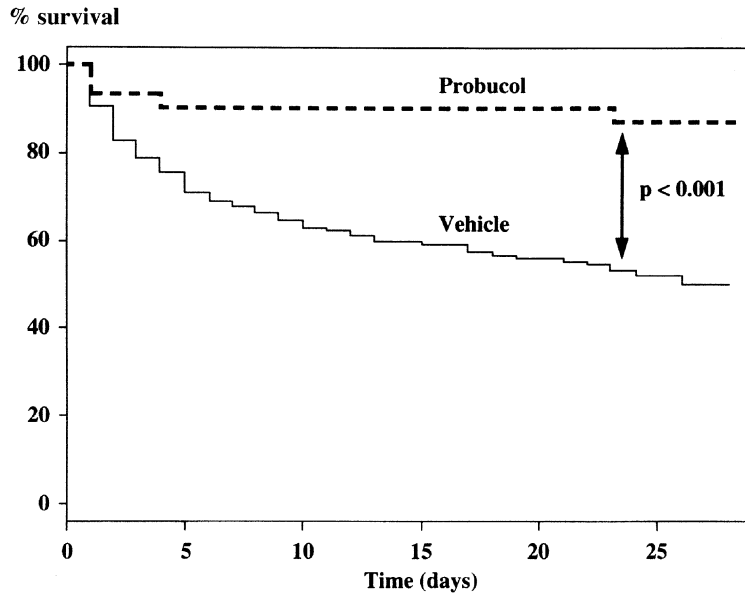


Figure 2. Comparison of survival of rats with large myocardial infarctions during the study period. Survival with probucol (88%) was much better than it was with vehicle (51%).

panied by an increase in both RV systolic and end-diastolic pressures (RVSP and RVEDP) and in RV +dP/dt. Compared with vehicle rats with large MIs, probucol treatment resulted in an increase in HR, LVSP, LVEDP and LV ±dP/dt. Right ventricular hemodynamic parameters were not modified by probucol.

Morphologic assessment. In the sham-to-small-MI groups, probucol resulted in a slight reduction in the LV weight/body weight (LVW/BW) ratio (Tables 2 and 3, Fig. 3). The two large-MI groups had a significant decrease in BW, this decrease being less in the probucol group. The scar weight and scar weight/BW ratios were similar in both large-MI groups. Left ventricular weight/BW was reduced only in the vehicle large-MI group. Atria weight/BW ratio increased in both MI groups, but RV weight/BW (RVW/BW) increased less in the probucol group, and the increase in lung weight/BW ratio also tended to be less in the probucol group, suggesting less pulmonary congestion. When not corrected for BW, LVW was greater in the MI probucol group than in the MI vehicle group, and RVW was no longer significantly different between MI groups.

In the sham-to-small-MI group, the morphologic characteristics of mid-LV cross-sections were similar in both groups, except for cardiac fibrosis, which was less in the probucol group. In rats with large MIs, LV endocardial and epicardial circumferences, scar surface, cardiac fibrosis and dilation index increased (Fig. 3, Table 3). Probucol treatment resulted in increased scar thickness and a marked decrease in cardiac fibrosis, compared with vehicle-treated large MIs.

Passive pressure-volume relationship. In rats with sham-to-small MIs, pressure-volume relationships were similar regardless of treatment group (Fig. 4). A large MI caused a similar rightward shift of this relationship regardless of treatment; no interaction with probucol was found ($p = 0.264$).

Plasma NE four weeks after infarction. In sham-to-small MIs, plasma NE levels were similar in the two groups: 505 ± 62 pg/ml for vehicle and 312 ± 91 pg/ml for probucol. In the large-MI vehicle group, NE increased to $1,183 \pm 211$ pg/ml, $p < 0.01$, and probucol did not significantly modify this increase (846 ± 123 pg/ml).

Fetal gene expression 28 days after MI in the LV. In sham-to-small MIs, expression of skACT, atrial natriuretic

Table 1. Hemodynamic Monitoring Four Weeks After MI

	HR (beats/min)	RVSP (mm Hg)	RVEDP (mm Hg)	RV +dP/dt (mm Hg/s)	LVSP (mm Hg)	LVEDP (mm Hg)	LV +dP/dt (mm Hg/s)	LV -dP/dt (mm Hg/s)
Sham-to-small MI vehicle (n = 46)	386 ± 7	25.7 ± 0.7	0.9 ± 0.6	1,304 ± 45	100.6 ± 2.1	1.9 ± 0.8	5,015 ± 170	-4,829 ± 178
Probucol (n = 13)	426 ± 13*	28.2 ± 0.7	0.9 ± 0.2	1,027 ± 86*	123.6 ± 3.6*	6.3 ± 1.0*	6,104 ± 333*	-6,312 ± 332*
Large MI vehicle (n = 46)	368 ± 8†	45.1 ± 1.7†	8.7 ± 0.9†	1,459 ± 56†	88.7 ± 1.5†	23.4 ± 1.1†	3,308 ± 144†	-2,453 ± 122†
Probucol (n = 29)	406 ± 9*	44.2 ± 2.1†	7.7 ± 1.1†	1,458 ± 59	104.4 ± 1.8*†	28.3 ± 1.5*†	4,051 ± 156*†	-3,091 ± 134*†

* $p < 0.05$ vs. vehicle; † $p < 0.05$ vs. sham or small MI.

HR = heart rate; LV +dp/dt = left ventricular maximum rate of pressure rise; LVEDP = left ventricular end-diastolic pressure; LVSP = left ventricular systolic pressure; MI = myocardial infarction; RV +dP/dt = right ventricular maximum rate of pressure rise; RVEDP = right ventricular end-diastolic pressure; RVSP = right ventricular systolic pressure.

Table 2. Heart and Lung Weight Four Weeks After MI

	Sham-to-Small MI		Large MI	
	Vehicle (n = 47)	Probuco (n = 13)	Vehicle (n = 78)	Probuco (n = 29)
BW (g)	405 ± 5	420 ± 8	376 ± 4*	394 ± 6*†
SW (g)	0.03 ± 0.00	0.02 ± 0.00	0.11 ± 0.00*	0.11 ± 0.00
SW/BW (g/kg)	0.07 ± 0.01	0.05 ± 0.01	0.30 ± 0.01*	0.26 ± 0.01*
LVW/BW (g/kg)	2.10 ± 0.03	1.98 ± 0.05†	1.97 ± 0.03*	2.03 ± 0.06
LVW (g)	0.82 ± 0.01	0.83 ± 0.02	0.63 ± 0.02*	0.71 ± 0.02*†
RVW/BW (g/kg)	0.59 ± 0.02	0.58 ± 0.05	1.08 ± 0.05*	0.94 ± 0.04*†
RVW (g)	0.24 ± 0.01	0.25 ± 0.01	0.41 ± 0.02*	0.37 ± 0.02*
AW/BW (g/kg)	0.21 ± 0.01	0.19 ± 0.01	0.51 ± 0.02*	0.44 ± 0.03*
Lung weight/BW (g/kg)	4.24 ± 0.12	3.99 ± 0.17	8.02 ± 0.30*	7.20 ± 0.41*
Lung weight (g)	1.73 ± 0.05	1.68 ± 0.07	3.06 ± 0.18*	2.80 ± 0.16*

*p < 0.05 vs. sham or small MI; †p < 0.05 vs. vehicle.

AW = atria weight; BW = body weight; LVW = left ventricular weight; MI = myocardial infarction; RVW = right ventricular weight; SW = scar weight.

factor (ANF) and β MHC were barely detectable in LV tissue, whereas α MHC showed marked expression (Table 3). In large MIs, a five- to six-fold induction of skACT, ANF and β MHC were observed, whereas α MHC mRNA levels were reduced to about 50% regardless of treatment. The GAPDH steady state mRNA levels served as a control.

Cardiac inflammatory cytokine gene expression. Cardiac expression of cytokines was similar in the two sham-to-small-MI groups, except for IL-6, which was decreased in the probuco group (Fig. 5). The large-MI vehicle group had an increase only in IL-1 β cardiac expression. Probuco treatment in large MIs prevented the increase in cardiac expression of IL-1 β and reduced IL-6 levels to levels lower than those of sham-to-small-MI controls. It also tended to reduce the expression of IL-5 compared with the large-MI vehicle group (p = 0.12).

Cardiac oxidative stress. Myocardial oxidative stress markers were similar in the sham-to-small-MI groups (Fig. 5). A large MI increased cardiac malondialdehydes only in the vehicle group, this increase being prevented by probuco.

DISCUSSION

This study indicates that the anti-oxidant probuco, when started 24 h after MI, markedly reduces both early and late post-MI mortality in rats. This is accompanied by partial preservation of LV systolic but not diastolic function. This does not appear to be the result of prevention of LV dilation or prevention of LV fetal gene expression but may be related to reduced cardiac fibrosis, reduced cardiac oxidative stress and reduced expression of cardiac pro-inflammatory cytokines. Taken together, these results suggest that excessive oxidative stress occurs after MI, that it is detrimental, and that preventing it with a powerful anti-oxidant such as probuco improves prognosis.

Probuco is a powerful anti-oxidant agent that was first introduced in clinical practice as a cholesterol-reducing agent. It has now been pulled off the market because it was found to exert its cholesterol-lowering effects by reducing the so-called good cholesterol, high-density lipoprotein, and because it was found to prolong the QT interval in some patients. In rats, probuco has been shown to attenuate the development of adriamycin cardiomyopathy, a finding as-

Table 3. Morphologic Characteristics of Mid Left Ventricular Cross-Sections and Fetal Gene Expression Four Weeks After MI

	Sham-to-Small MI		Large MI	
	Vehicle (n = 9)	Probuco (n = 6)	Vehicle (n = 24)	Probuco (n = 7)
Infarction size (% of circumference)	—	—	51.57 ± 1.61‡	49.72 ± 1.5‡
Mean scar thickness (mm)	—	—	0.33 ± 0.04‡	0.64 ± 0.07‡§
Scar surface (cm ²)	0.21 ± 0.03	0.25 ± 0.00	1.26 ± 0.04	1.16 ± 0.07‡
Mean myocardial wall thickness (mm)	1.84 ± 0.13	1.66 ± 0.11	1.74 ± 0.07	1.617 ± 0.11
Dilation index*	0.38 ± 0.04	0.42 ± 0.03	0.61 ± 0.02‡	0.60 ± 0.02‡
Cardiac fibrosis†	2.99 ± 0.18	2.26 ± 0.11§	6.64 ± 0.58‡	2.68 ± 0.08‡§
ANF	2.67 ± 0.76	2.49 ± 0.56	16.69 ± 1.85‡	22.05 ± 1.65‡
β MHC	2.09 ± 0.69	1.76 ± 1.11	13.68 ± 1.05‡	10.18 ± 5.07‡
α MHC	14.36 ± 1.08	15.87 ± 2.17	6.99 ± 0.85‡	6.87 ± 1.08‡
SkACT	3.67 ± 0.69	5.48 ± 1.06	14.81 ± 0.93‡	14.72 ± 1.22‡

*Ventricular cavity area on midleft ventricular cross-section/ventricular area on midleft ventricular cross-section; †collagen volume density percent; ‡p < 0.05 vs. sham or small MI; §p < 0.05 vs. vehicle.

ANF = atrial natriuretic factor; MHC = myosin heavy chain; MI = myocardial infarction; SkACT = skeletal actin.

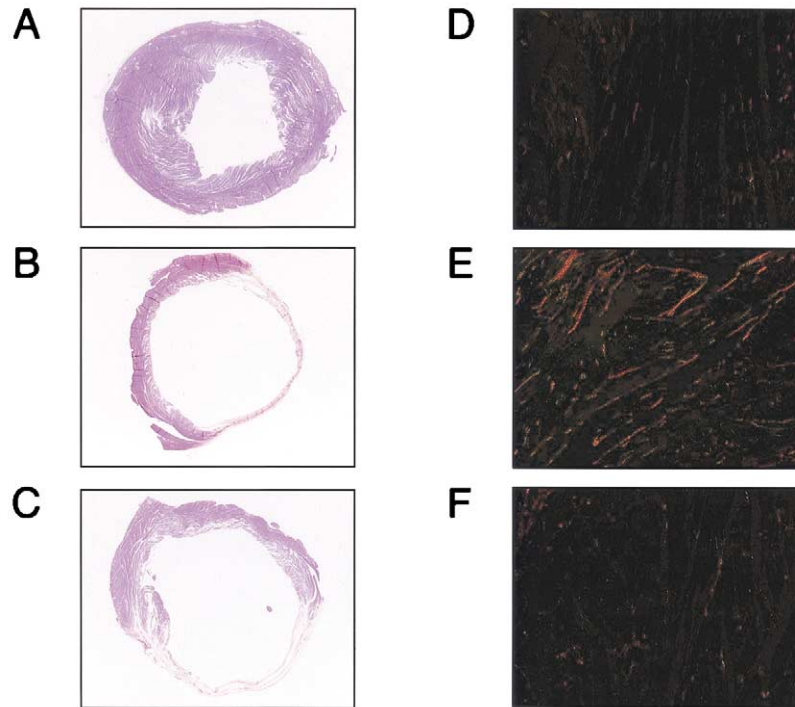


Figure 3. Representative left ventricle midventricular cross-sections (5.3×) of a sham-operated rat (A) and rats with large myocardial infarctions (MIs) treated with vehicle (B) or probucol (C); representative slides of the collage network stained with Sirius red (400×) for a sham-operated rat (D); and rats with large MIs treated with vehicle (E) or probucol (F).

sociated with preservation of the anti-oxidant capacity of the heart (10). In the present study, probucol was given in doses previously shown to be effective as an anti-oxidant

(10) and was demonstrated to normalize the measurement of cardiac oxidative stress (malondialdehydes) in hearts with large MIs.

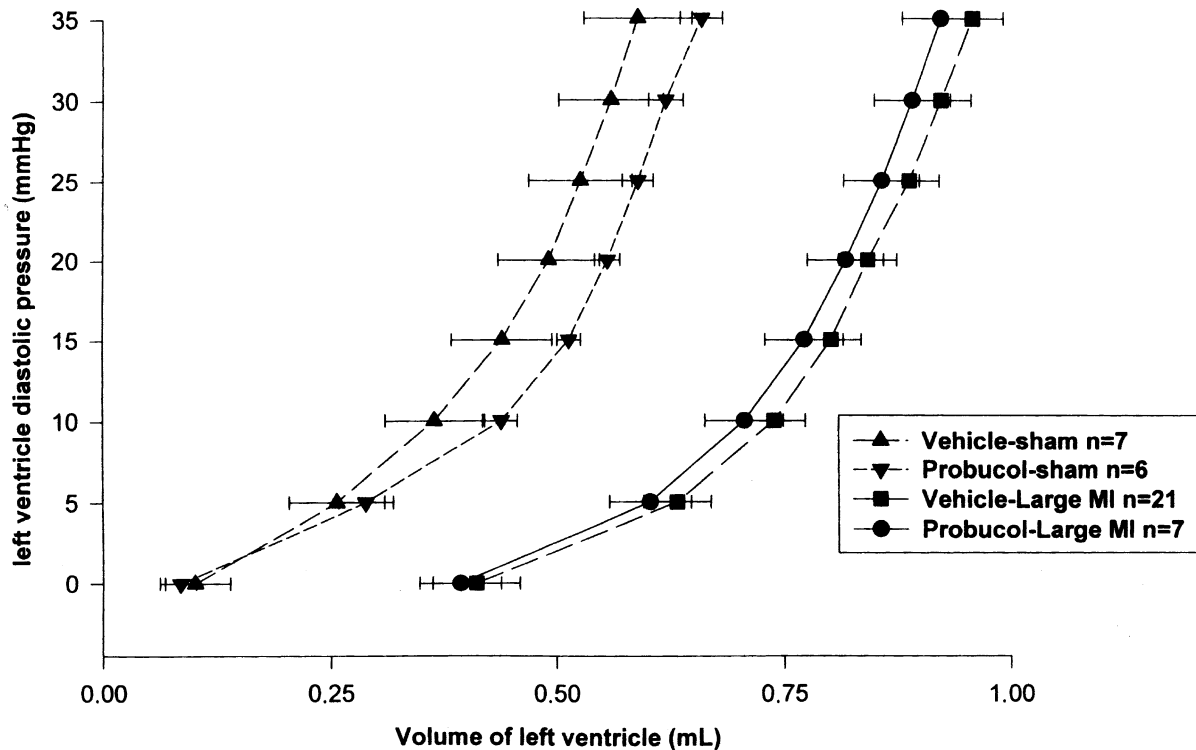
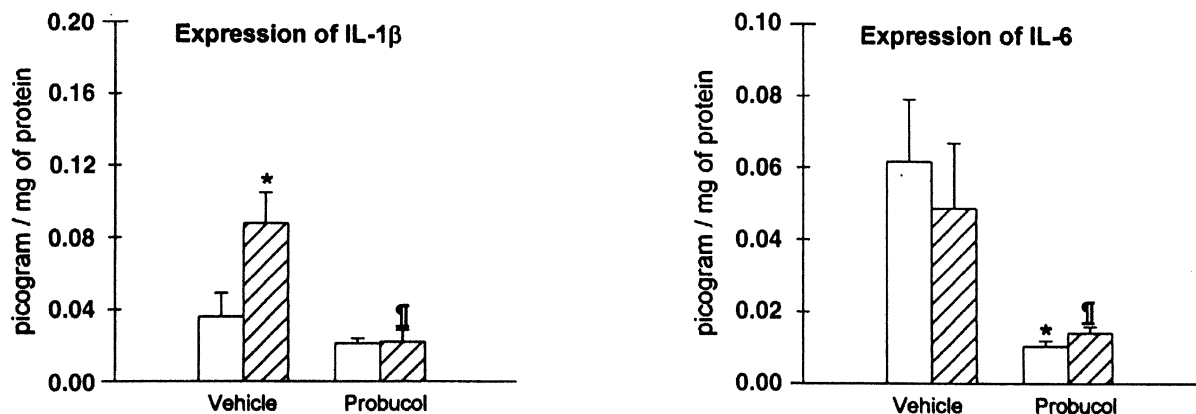


Figure 4. Passive left ventricle pressure-volume relationships of rats with sham-to-small myocardial infarctions (MIs) and large MIs according to treatment group.

Cardiac Cytokine Expression



Cardiac Oxidative Stress Marker Malondialdehydes

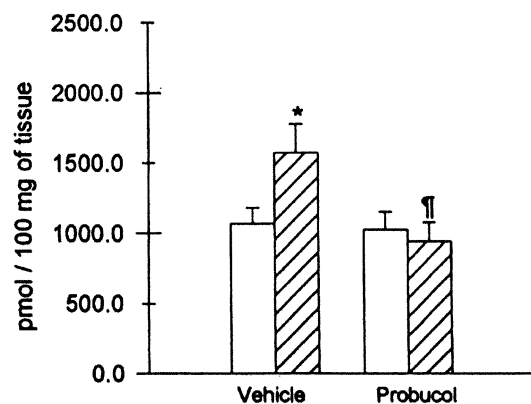


Figure 5. Cardiac expression of inflammatory cytokines (A) and cardiac oxidative stress (B). IL = interleukin.

Survival, cardiac oxidative stress and cytokine expression. We found that probucol resulted in marked improvement in post-MI survival. This improvement was much greater than any we have documented in our previous studies of multiple interventions in this model. Why this occurred remains speculative but most likely results from the powerful anti-oxidant properties of probucol (10). In this post-MI rat model, Singal et al. (16) have shown that an increase in oxidative stress coincides with the appearance of hemodynamic abnormalities, and Kinugawa et al. (8) demonstrated that the anti-oxidant DMTU preserved post-MI LV function. Congestive heart failure itself is known to be associated with an increase in oxidative stress, which is known to exert a number of deleterious effects that can lead to further progression of heart failure and an increased risk of death (3,4). The deleterious effects associated with oxidative stress include direct cellular toxicity, degradation of collagen matrix and decreased myocardial contractility (3-5).

In addition, oxidative stress is a powerful stimulant for the increased expression of pro-inflammatory cytokines (4),

which of themselves can result in further myocardial depression, adverse ventricular remodeling, direct cytotoxicity and further oxidative stress (16). In this study, we documented a lack of increase in TNF- α but a persistent increase in IL-1 β four weeks after MI in the vehicle group, a finding consistent with the results of Ono et al. (17) eight weeks after MI. Probucol reduced the cardiac expression of two inflammatory cytokines (IL-1 β and IL-6) that are known to be cardiotoxic and to have a negative inotropic effect (16). Because of the dynamic nature of post-MI cardiac cytokine activation, particularly early after MI, it is also possible that a probucol-induced reduction in cardiac cytokine expression early after MI contributed to its early beneficial effects. Finally, both oxidative stress and cytokines are powerful stimuli for apoptosis, which can result in further cardiac compromise as post-MI LV dysfunction develops (16,17).

Probucol may have improved post-MI survival by reducing ventricular arrhythmias. Early and late post-MI mortality in this model have been shown to be largely the result of ventricular arrhythmias (18). Global LV remodeling was not different in probucol-treated rats after MI, but probucol

may have had indirect anti-arrhythmic effects by reducing cardiac fibrosis, which has been associated with an increased susceptibility to ventricular arrhythmias (19). In a rabbit ischemia-reperfusion model, probucol has been shown to reduce ventricular arrhythmias and myocardial stunning (20). Judging from the results of this study, the QT interval prolonging effects of probucol did not appear to adversely affect survival (21).

Cardiac function. Long-term treatment with probucol resulted in improved systolic LV function. Reduced cardiac expression of the pro-inflammatory cytokines IL-1 β and IL-6 may have contributed to improved LV systolic function because both of these cytokines are known to reduce cardiac contractility. Prevention of excessive cardiac fibrosis may also have helped because an increase in cardiac fibrosis is associated with both systolic and diastolic dysfunction (22). Finally, the improvement of in vivo systolic ventricular function with probucol could have at least partially resulted from probucol-induced preservation or even enhancement of cardiac β -adrenergic responsiveness, HR having been preserved or even increased with probucol despite a large MI. Oxygen free radicals are known to induce β -adrenergic receptor down-regulation and post-receptor abnormalities that are reversed by anti-oxidants (23). One mechanism that did not contribute to improved LV function is a modification of fetal gene expression. This lack of effect also suggests that oxidative stress is not involved in the process that translates the genotypic characteristics to the functional state of the myocardium.

The apparent probucol-induced increase in LVEDP in vivo is hard to explain. This is particularly true, considering the reduction in cardiac fibrosis with probucol and the lack of difference in the passive diastolic pressure-volume relationship between the two MI groups. Part of the explanation could be related to the greater HR and, thus, decreased ventricular filling time and/or the greater afterload of these hearts. Another possibility is greater LV hypertrophy with probucol, although this difference in LVW did not persist when adjusted for BW. In addition, a direct or indirect effect of probucol on cardiac relaxation or fluid retention cannot be ruled out.

Comparison with DMTU. Significant differences between the effects of the anti-oxidant probucol and the reported (8) effects of the hydroxyl radical scavenger DMTU on ventricular remodeling were noted. In the Kunigawa et al. study, DMTU attenuated ventricular dilation, reduced cardiac hypertrophy, reduced cardiac fibrosis, but did not modify scar thickness; while in our study, probucol did not modify ventricular dilation, did not reduce cardiac hypertrophy (and may even have increased it), but reduced cardiac fibrosis and increased scar thickness. This resulted in better preservation of LV function with DMTU, a decrease in LVEDP rather than the increase with probucol, and a reduction in lung weight—a finding not found with probucol. Why this difference between these two agents should occur is speculative but may result from the earlier post-MI introduction

of DMTU (6 h vs. 24 h for probucol), a period when large amounts of free radicals are known to be present. Alternatively, the difference may be the result of more effective suppression of oxygen free radicals with DMTU. In any case, these two studies taken together strongly support an important deleterious role for post-MI oxidative stress and suggest that further exploration of the post-MI therapeutic use of these agents is warranted.

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