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Comparison of bisoprolol and carvedilol cardioprotection in a rabbit ischemia and reperfusion model

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Abstract

Carvedilol, a selective α_1 and non-selective β-adrenoceptor antagonist and antioxidant, has been shown to provide significant cardiac protection in animal models of myocardial ischemia. To further explore the mechanisms contributing to the efficacy of carvedilol cardioprotection, the effects of carvedilol on hemodynamic variables, infarct size and myeloperoxidase activity (an index of neutrophil accumulation) were compared with a β_1 selective adrenoceptor antagonist, bisoprolol. Carvedilol (1 mg/kg) or bisoprolol (1 mg/kg) was given intravenously 5 min before reperfusion. In vehicle-treated rabbits, ischemia (45 min) and reperfusion (240 min) resulted in significant increases in left ventricular end diastolic pressure, large myocardial infarction ($64.7 \pm 2.6\%$ of area-at-risk) and a marked increase in myeloperoxidase activity (64 ± 14 U/g protein in area-at-risk). Carvedilol treatment resulted in sustained reduction of the pressure-rate-index and significantly smaller infarcts (30 ± 2.9, $P < 0.01$ vs. vehicle) as well as decreased myeloperoxidase activity (26 ± 11 U/g protein in area-at-risk, $P < 0.01$ vs. vehicle). Administration of bisoprolol at 1 mg/kg resulted in a pressure-rate-index comparable to that of carvedilol and also decreased infarct size ($48.4 \pm 2.5\%$, $P < 0.001$ vs. vehicle, $P < 0.05$ vs. carvedilol), although to a significantly lesser extent than that observed with carvedilol. Treatment with bisoprolol failed to reduce myeloperoxidase activity in the ischemic myocardial tissue. In addition, carvedilol, but not bisoprolol, markedly decreased cardiac membrane lipid peroxidation measured by thiobarbituric acid formation. Taken together, this study suggests that the superior cardioprotection of carvedilol over bisoprolol is possibly the result of carvedilol's antioxidant and anti-neutrophil effects, not its hemodynamic properties.

Keywords: Myocardial ischemia; β-Adrenoceptor antagonist; Antioxidant

1. Introduction

Early reperfusion after coronary occlusion is the most effective means of limiting ischemic myocardial injury. However, abundant evidence suggests that reperfusion may cause additional cell death, and the cause of this so termed "reperfusion injury" is apparently multifactorial. Among the many proposed mechanisms, overproduction of reactive oxygen species from vascular endothelial cells and accumulated polymorphonuclear leukocytes, Ca^{2+} overloading in cardiomyocytes, and imbalance between the vasodilators and vasoconstrictors have been demonstrated to play central roles in causing or aggravating post-ischemic myocardial injury (Maxwell and Lip, 1997).

Carvedilol is a multiple action, non-selective β-adrenoceptor, a selective α_1-adrenoceptor antagonist, a potent antioxidant, and a Ca^{2+} channel blocker at higher concentrations (Feuerstein et al., 1994). In previous studies, carvedilol has demonstrated consistently superior acute anti-ischemic properties as compared to the traditional non-selective β-adrenoceptor antagonist, propranolol (Feuerstein et al., 1994; Brunvand et al., 1996). Moreover, comparison of carvedilol to celiprolol, another "third generation" vasodilating β-adrenoceptor antagonist, demonstrated that at dosing regimens that produce equal pressure rate product changes, celiprolol failed to provide equal cardioprotection in ischemic conditions (Feuerstein and Ruffolo, 1994). These results suggest that other properties

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of carvedilol besides its peripheral vasodilating effect are responsible for its superior protective effects against post-ischemic myocardial injury. In a recent study, we compared the cardioprotective effects of carvedilol with metoprolol, a selective β_1-adrenoceptor antagonist, in a rabbit myocardial ischemia/reperfusion model (Feuerstein et al., 1998). However, since different β_1-adrenoceptor antagonists may have its own unique pharmacological properties, it may not be appropriate to make a general conclusion based on a comparison performed with only one compound.

In the present study, we further evaluated the relative contribution of carvedilol's β_1-adrenoceptor blocking effect relative to its anti-ischemic/cardioprotective action by comparing the cardioprotective effects of carvedilol with bisoprolol, another selective β_1-adrenoceptor antagonist, at a dose resulting in comparable β_1-adrenoceptor blockade.

2. Materials and methods

2.1. Materials

Bisoprolol was purchased from Tocris Cookson (Ballwin, MS). All other compounds were purchased from Sigma (St. Louis, MO). A total of 48 adult male New Zealand white rabbits (2.8–3.5 kg) were used. Data from three rabbits (one from each ischemic group treated with vehicle, carvedilol or bisoprolol) were excluded from the final analysis for one or both of the following reasons: (a) sustained hypotension during ischemic or reperfusion phases ($\leq$ 60 mm Hg for $\geq$ 20 min), and (b) greater than two episodes of ventricular fibrillation. The experiments were performed in adherence to NIH Guidelines on the Use of Laboratory Animals and were approved by the Thomas Jefferson University Committee on Animal Care.

2.2. Experimental preparation

Rabbits were anaesthetized with sodium pentobarbital (30 mg/kg, i.v.) and ventilated with a Harvard small animal respirator. Arterial blood gases were measured using a blood gas analyzer (CIBA-CORNINE 288 Blood Gas Analyzer, Ciba-Cornine, Norwood, MA). Arterial PO_2 and PCO_2 were maintained at 100–120 and 35–45 mm Hg, respectively, by adjusting the oxygen flow and ventilatory rates. pH was adjusted to 7.35–7.45 with i.v. sodium bicarbonate as necessary. A polyethylene catheter was inserted into the right external jugular vein for supplemental pentobarbital injection and for administration of test compounds. The arterial blood pressure was measured via a polyethylene catheter cannulating the right femoral artery, and the left ventricular pressure was measured via a Millar Mikro-tip catheter transducer that was inserted into the left ventricular cavity through the left carotid artery.

Following midline thoracotomy, a 4-0 silk ligature was placed around the major marginal branch of the left circumflex coronary artery, 10–12 mm from its origin. After a 20-min stabilization period, myocardial ischemia was initiated by complete ligation of the marginal coronary artery. After 45 min of ischemia, the ligature was untied and the ischemic myocardium was reperfused for 4 h. Sham myocardial ischemia/reperfusion rabbits were subjected to the same surgical procedures performed on myocardial ischemia/reperfusion rabbits, except that the suture was left untied. The rabbits were randomly assigned to one of the following groups: (1) sham myocardial ischemia/reperfusion ($n = 10$); (2) myocardial ischemia/reperfusion + vehicle (10% dimethyl sulfoxide, DMSO, $n = 12$); (3) myocardial ischemia/reperfusion + carvedilol (1 mg/kg, $n = 12$); (4) myocardial ischemia/reperfusion + bisoprolol (1 mg/kg, $n = 11$). Each drug or vehicle was given 5 min prior to reperfusion intravenously over 1 min.

2.3. Myocardial functional injury

Myocardial ischemia/reperfusion-induced cardiac dysfunction was continuously monitored during the entire myocardial ischemia/reperfusion period. The arterial blood pressure and left ventricular pressure were sampled at 250 Hz and digitally processed via a hemodynamic analyzing system (Po-Ne-Mah Physiology Platform P3 Plus, Gould Instrument Systems, Valley View, OH). Mean arterial blood pressure, heart rate and left ventricular end diastolic pressure were derived by computer algorithms.

2.4. Myocardial infarct

At the end of the 4-h reperfusion period, the ligature around the marginal coronary artery was re-tightened. Thirty milliliters of 1% Evans blue dye was injected into the left atrium to stain the area of the myocardium perfused by the patent coronary arteries. The area-at-risk was therefore determined by negative staining. The atria, right ventricle, and major blood vessels were subsequently removed from the heart. The left ventricle was then sliced into sections 3 mm thick parallel to the atrioventricular groove. The unstained portion of myocardium (i.e., the area-at-risk) was separated from the stained portion (i.e., the area-not-at-risk). The unstained portion was again sliced into 1-mm-thick sections and incubated in a 0.1% solution of nitroblue tetrazolium in phosphate buffer at pH 7.4 and 37°C for 15 min to detect the presence of dehydrogenase and NADP. The infarct portion of the myocardium (which does not stain) was separated from the stained portion (i.e., ischemic-viable). Samples from all three portions of left ventricular cardiac tissue (i.e., non-ischemic, ischemic-viable and ischemic-infarcted) were weighed. Area-at-risk as a percentage of total left ventricular mass (area-at-

risk/total left ventricular mass × 100%), infarct area as a percentage of area-at-risk (infarct/area-at-risk × 100%), and infarct area as a percentage of total left ventricular mass (infarct/total left ventricular mass) were calculated (Ma et al., 1996).

2.5. Plasma creatine kinase accumulation

Arterial blood samples (1 ml) were drawn immediately before ligation (0 min), 45 min after ischemia and hourly thereafter. Plasma creatine kinase activity was measured in a blinded manner using a Sigma kit and expressed as IU/g of protein. Protein concentration was determined by using the bicinchoninic acid method (BCA protein assay kit, Pierce, Rockford, IL).

2.6. Measurement of myeloperoxidase activity in cardiac tissue

Myeloperoxidase, an enzyme present in neutrophils, but not cardiomyocytes, was determined in cardiac tissue as described previously (Ma et al., 1996) and was used as an index of neutrophil accumulation. In brief, cardiac tissue samples were homogenized in 0.5% hexadecyltrimethyl ammonium bromide (Sigma) and dissolved in 50 mmol/l potassium phosphate buffer at pH 6.0 using a PRO 200 homogenizer (PRO Scientific, Monroe, CT). Homogenates were then centrifuged at $36{,}000 \times g$ for 30 min at 4°C. The supernatants were then collected and reacted with 0.167 mg/ml of *o*-dianisidine dihydrochloride (Sigma) and 0.0005% H_2O_2 in 50 mmol/l phosphate at pH 6.0. The change in absorbance was measured spectrophotometrically at 460 nm (Beckman DU 640, Fullerton, CA). One unit of myeloperoxidase was defined as that quantity of enzyme hydrolyzing 1 mmol of peroxide per min at 25°C. The assays were performed without knowledge of the group from which each sample originated.

2.7. Measurement of lipid peroxidation in vitro

The rabbit ventricular membrane preparation was prepared as reported previously (Yue et al., 1993). Briefly, rabbit ventricular tissues were homogenized with a polytron homogenizer in cold saline, filtered through four layers of cheese cloth and centrifuged at $1000 \times g$ for 15 min at 4°C. The pellet was discarded, and the supernatant was recentrifuged at $48{,}000 \times g$. The pellet from the second centrifugation was collected and resuspended in saline.

The rates of lipid peroxidation were determined by the formation of thiobarbituric acid reactive substance as previously reported (Braughler et al., 1987). Rabbit ventricular preparations (1 ml) were preincubated at 15°C with 10 μl of a testing compound (vehicle, carvedilol or bisoprolol). Lipid peroxidation was initiated by the addition of 0.1 ml 25 mM $FeCl_2$ and 1 mM ascorbic acid. After 30 min of incubation, the reaction was stopped by adding 0.1 ml of 0.2% butylated hydroxytoluene. Thiobarbituric acid reagent was then added and the mixture was heated for 30 min in a boiling water bath. The thiobarbituric acid was extracted by *n*-butanol and measured at 532 nm. Results were described as percent of vehicle at the carvedilol or bisoprolol concentrations indicated.

2.8. Statistical analysis

Time and group differences were determined by two-way analysis of variance (ANOVA) for repeated measures, correcting for multiple comparisons by Holm's multiple rejective method as appropriate for an overall probability level of 0.05. Non-repetitive data, such as infarct size, were subjected to ANOVA followed by the Scheffe's correction for post-hoc *t*-test comparison. Probabilities of 0.05 or less were considered statistically significant.

3. Results

3.1. Effect of carvedilol and bisoprolol on isoproterenol-induced heart rate increase

The heart rate increase in response to increasing dose of isoproterenol (0.05 to 0.4 μg/kg, i.v.) was determined before and after carvedilol or bisoprolol treatment (1 mg/kg, i.v.). As illustrated in Fig. 1, administration of isoproterenol before carvedilol or bisoprolol treatment resulted in a significant heart rate increase in a dose-dependent fashion. Carvedilol administration resulted in a significant drop in heart rate shortly after its administration, and virtually abolished isoproterenol-induced heart rate increase (after 0.4 μg/kg isoproterenol injection, heart rate increased from 242 ± 5.1 to 281 ± 6.3 bpm before carvedilol treatment and from 216 ± 4.9 to 222 ± 5.6 bpm after carvedilol treatment). Administration of bisoprolol at the same dose produced a comparable persistent heart rate reduction as that produced by carvedilol, and blocked isoproterenol-induced heart rate increase to a level that was comparable to that produced by carvedilol (after 0.4 μg/kg isoproterenol injection, heart rate increased from 243 ± 5.3 to 285 ± 5.8 bpm before bisoprolol treatment and from 218 ± 5.4 to 225 ± 6 bpm after bisoprolol treatment). These results indicate that a comparable level of β-adrenoceptor blockade was achieved by carvedilol and bisoprolol at an equal dose of 1 mg/kg. Therefore, any differences between carvedilol and bisoprolol in the effects on post-ischemic myocardial injury could not be attributed to their different β_1-adrenoceptor blocking property.

3.2. Effect of carvedilol and bisoprolol on left ventricular end diastolic pressure

A stable left ventricular end diastolic pressure was observed in the sham group throughout the experimental

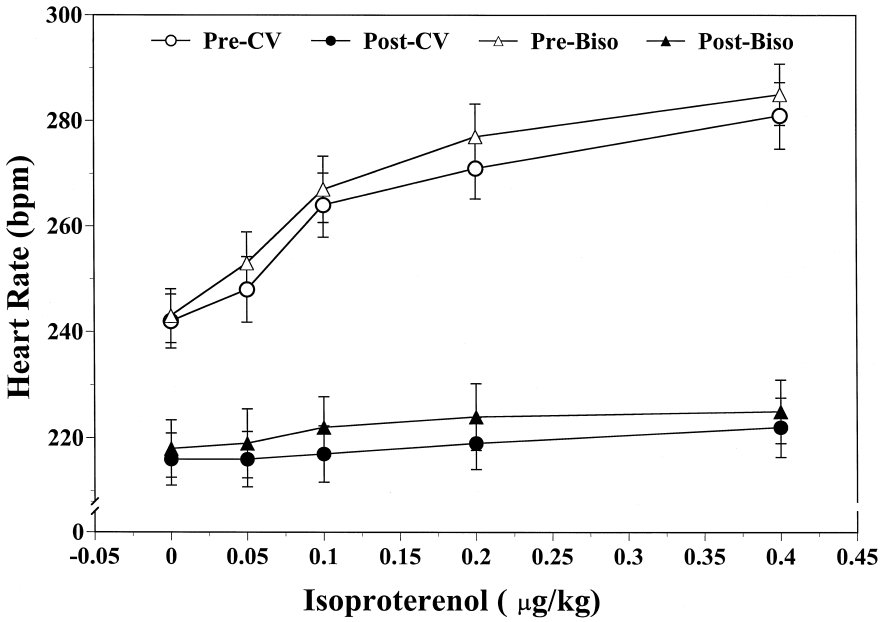

Fig. 1. Dose-dependent increases in heart rate induced by isoproterenol before and after carvedilol (1 mg/kg) or bisoprolol (1 mg/kg) administration. $N = 5$ in each group.

period and, as expected, marked elevation of left ventricular end diastolic pressure occurred in the myocardial ischemia + vehicle group. Left ventricular end diastolic pressure was reduced at the onset of the reperfusion but during the later part, significant secondary increments were observed. In the carvedilol and bisoprolol treatment groups, a left ventricular end diastolic pressure increase comparable to that in the vehicle group was observed during the entire ischemic period and during the first hour of the reperfusion period. However, over the remainder of the reperfusion period (3 h), left ventricular end diastolic pressure in the carvedilol-treated group continued to recover, and at the end of the experiment, left ventricular end diastolic pressure was at a level similar to that in the sham control group. Similarly, treatment with bisoprolol also prevented the secondary elevation of left ventricular end diastolic pressure during the later phase of reperfusion as was observed in the vehicle group, although the degree of protection was less than that observed with carvedilol treatment (Table 1).

Table 1
Comparison of carvedilol and bisoprolol treatment on left ventricular end diastolic pressure (LVEDP) and plasma CK accumulation in rabbit subjected to 45 min of ischemia and 180 min of reperfusion

	LVEDP (mm Hg)				Plasma CK activity (IU/g protein)			
	Sham MI	MI + V	MI + CV	MI + BS	Sham MI	MI + V	MI + CV	MI + BS
Control	4.3 ± 0.33	4.2 ± 0.25	4.3 ± 0.25	4.3 ± 0.23	17 ± 5.4	17 ± 5.2	14 ± 3.6	15 ± 3.9
I20	4.2 ± 0.25	7.2 ± 0.45**	7.1 ± 0.42**	7.3 ± 0.36**				
I45	4.4 ± 0.23	7.1 ± 0.54**	6.8 ± 0.52*	6.9 ± 0.45**				
R20	4.6 ± 0.24	7.3 ± 0.23**	7.1 ± 0.38**	6.8 ± 0.38**				
R40	4.5 ± 0.33	7.2 ± 0.34**	7.2 ± 0.34**	6.8 ± 0.39**				
R60	4.5 ± 0.21	6.2 ± 0.25**	6.9 ± 0.25**	6.5 ± 0.34*				
R120	4.4 ± 0.22	7.3 ± 0.36**	6.4 ± 0.25*	6.5 ± 0.28*				
R180	4.3 ± 0.32	7.5 ± 0.40**	5.2 ± 0.33♣♣,#	6.2 ± 0.33*	19 ± 5.2	183 ± 22**	65 ± 9**,♣♣,#	95 ± 11**,♣♣

I = Ischemia, R = reperfusion, MI = myocardial ischemia, V = vehicle, CV = carvedilol, BS = bisoprolol.
* $P < 0.05$.
** $P < 0.01$ vs. sham MI.
♣♣ $P < 0.01$ vs. MI + vehicle.
# $P < 0.05$ vs. MI + bisoprolol.

3.3. Effect of carvedilol and bisoprolol on plasma creatine kinase accumulation

Plasma creatine kinase accumulation is a reliable index estimating the severity of myocardial injury. As summarized in Table 1, plasma creatine kinase activities were at a range of 14–17 IU/g protein, and there was no significant difference among groups. In the sham myocardial ischemia group, plasma activity remained at a low level at the end of reperfusion, indicating that surgery itself did not result in significant myocardial injury. However, in myocardial ischemia rabbits receiving only vehicle, a 10-fold increase in creatine kinase activity was observed at the end of reperfusion (from 17 ± 2.4 to 183 ± 19.9 IU/g protein, $P < 0.001$). Treatment with bisoprolol significantly attenuated plasma creatine kinase activation ($p < 0.01$). However, plasma creatine kinase activity in the carvedilol-treatment group was not only significantly lower than that in the vehicle group, but was also significantly lower than that in the bisoprolol-treated group ($p < 0.05$).

3.4. Effect of carvedilol and bisoprolol on myocardial infarct size

Fig. 2 illustrates the extent of cardiac infarct as defined by negative nitroblue tetrazolium staining (see Section 2). It is important to note that the ischemic area, calculated as a fraction of total left ventricle mass, was the same in all the experimental groups, and therefore this parameter, a fundamental factor in all calculations of injured tissue, played no differential role in treatment effect. In this model, ischemia/reperfusion resulted in a large infarct size, which encompassed $64.7 \pm 2.6\%$ of the ischemic myocardium; these results are in accordance with previous reports (Ma et al., 1996). Treatment with carvedilol and bisoprolol both reduced infarct size (30 ± 3.1 and $48.4 \pm 2.5\%$, respectively, $P < 0.001$). Bisoprolol, at a dose that produced similar hemodynamic effects, failed to decrease infarct size to a level comparable to that produced by carvedilol. The same conclusion was reached when calculating necrotic myocardium directly as a percent of individual left ventricular mass.

3.5. Effect of carvedilol and bisoprolol on myeloperoxidase activity in the ischemic myocardium

Fig. 3 illustrates the effect of ischemia on myeloperoxidase activity in the ischemic myocardium and its modulation by the various treatments. Myeloperoxidase activity, a marker of leukocyte accumulation, was markedly elevated in the ischemic myocardium of the vehicle-treated animals. Treatment with carvedilol, but not bisoprolol, reduced myeloperoxidase levels at both the area-at-risk and infarct zones ($P < 0.01$).

3.6. Effect of carvedilol and bisoprolol on membrane lipid peroxidation

In order to further elucidate the mechanism underlying the superior cardioprotective effects of carvedilol, an addi-

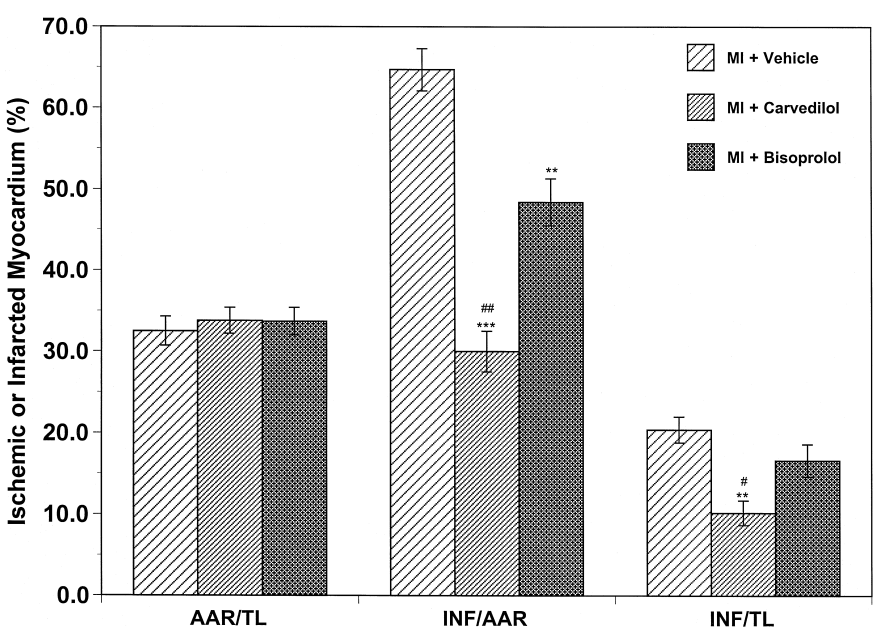

Fig. 2. Tissue wet weight of area-at-risk as a percentage of the total left ventricular wet weight, and of infarct tissue as a percentage of area-at-risk and of the total left ventricle for the three ischemic-reperfused groups. Height of bars are means; brackets represent ± S.E.M. $^{**}P < 0.01$, $^{***}P < 0.005$ vs. vehicle-treated rabbits. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. bisoprolol-treated rabbits. AAR = Area-at-Risk, TL = Total Left Ventricle, INF = Infarct.

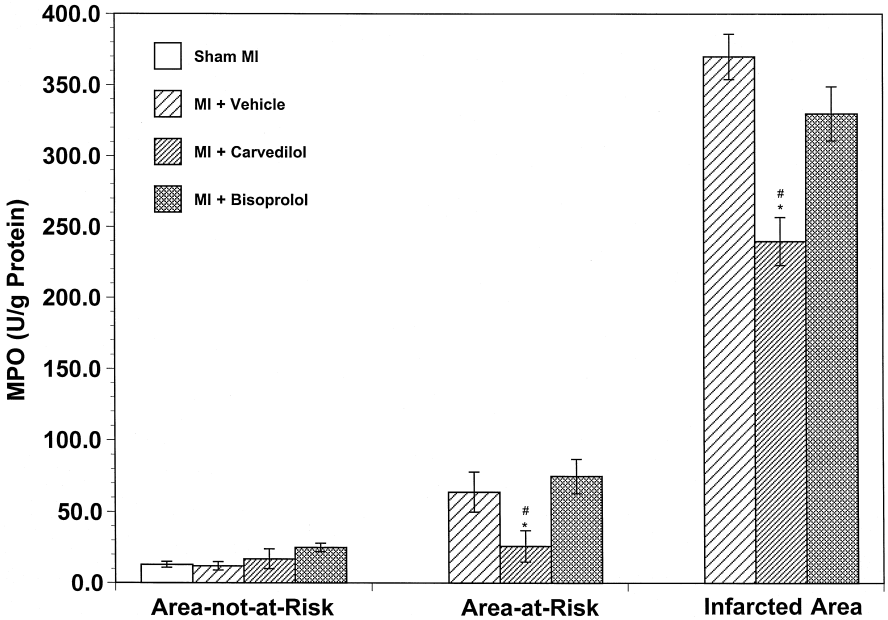

Fig. 3. Tissue myeloperoxidase activity in area-not-at-risk, area-at-risk and infarcted area in U/g protein for the four groups. Height of bars are means; brackets represent ± S.E.M. $^{*}P < 0.05$, vs. vehicle-treated rabbits, $^{\#}P < 0.05$, vs. bisoprolol-treated group.

tional experiment was performed and the effects of carvedilol and bisoprolol on lipid peroxidation of cell membranes obtained from left ventricular tissue was observed. Addition of carvedilol in vitro decreased lipid peroxidation in a dose-dependent manner in the range of 1–30 μM, with an IC_{50} of 3.0×10^{-6} M. When 30 μM of carvedilol was added, the lipid peroxidation was completely blocked (Fig. 4). SB 211475, a main metabolite of carvedilol found in human blood (Feuerstein and Ruffolo, 1994), exerted an even stronger protective effect against lipid peroxidation with an IC_{50} of 1.8×10^{-7} M. In contrast, addition of bisoprolol at a concentration up to 100 μM exerted no significant protective effects against lipid peroxidation. When an even higher concentration of biso-

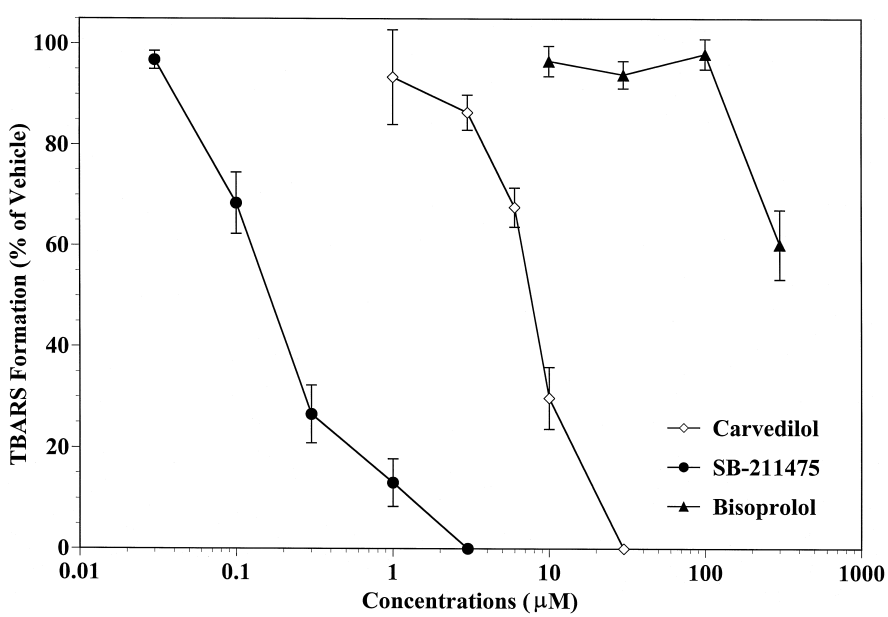

Fig. 4. Concentration-dependent effect of carvedilol, SB 211475 (one of the main metabolites of carvedilol in vivo), and bisoprolol on cardiac cell membrane lipid peroxidation. Thiobarbituric acid formed at the present of vehicle was considered as 100%. Data was from three experiments performed in triplicates.

prolol was added (i.e., 300 μM), lipid peroxidation was only reduced to 60.2 ± 6.9% of vehicle, a level comparable to that observed with 6 μM of carvedilol.

4. Discussion

Carvedilol, a selective α_1- and non-selective β-adrenoceptor antagonist with antioxidant activity, has been shown to improve the outcome of acute myocardial infarction in experimental (Feuerstein et al., 1994) and clinical (Basu et al., 1997) studies. Since carvedilol is a multiple action drug, several different and complementary mechanisms have been suggested to account for its cardioprotective actions. The present study was undertaken to further elucidate the relative contribution of the various pharmacological properties of carvedilol by comparing its cardiac protection efficacy to bisoprolol, an agent of high selectivity to the β_1-adrenoceptor. Consistent with previous reports, our data demonstrated that administration of 1 mg/kg bisoprolol significantly reduced heart rate and mean arterial blood pressure, and abolished isoproterenol-induced heart rate increase (Duncker et al., 1987). Most importantly, we have demonstrated that carvedilol, at a dosing regimen that produced precisely the same hemodynamic and β_1-adreneceptor blocking effects as bisoprolol, had superior cardioprotective action as evidenced by the smaller infarct size (30.0 ± 2.5% vs. 48.4 ± 2.9% of area-at-risk in bisoprolol, $P < 0.01$), less plasma creatine kinase accumulation (65 ± 9 vs. 95 ± 11 IU/g protein at the end of reperfusion, $p < 0.05$), and lower left ventricular end diastolic pressure (5.2 ± 0.33 vs. 6.2 ± 0.34 mm Hg, $p < 0.05$).

The exact mechanisms explaining the marked discrepancy between the two β-adrenoceptor antagonists in their myocardial protective activities are complex. It is unlikely that this difference is due to their different β-adrenoceptor blocking potencies as treatment with carvedilol decreased heart rate and mean arterial blood pressure to the same extent as bisoprolol. The most plausible explanation for the superior cardioprotection of carvedilol over bisoprolol, as demonstrated in this study, may reside in the antioxidant action of carvedilol and some of its metabolites.

In several previous studies, we and others have demonstrated that carvedilol possesses a wide-spectrum free radical scavenging property (Yue et al., 1992, 1993) and exerts marked protection against tissue damage caused by free radicals in vitro (Yue et al., 1994a; Flesch et al., 1999). Moreover, recent pharmacodynamic and pharmacokinetic studies have demonstrated that when administered in vivo, carvedilol is extensively metabolized with less than 2% of the dose excreted unchanged in the urine (Feuerstein et al., 1994). SB 211475 is one of the main metabolites of carvedilol in which an $^{\cdot}OH$ group is introduced at position 3 of its carbazole moiety. In vitro pharmacologic studies have shown that SB 211475 has a very weak β-adrenoceptor blocking effect (about 170 times less than its parent compound carvedilol), but possesses exceptional antioxidant activity (Yue et al., 1994b). Depending upon the in vitro assay used to assess antioxidant potential, SB 211475 has been shown to have IC_{50} values which are 2.7- to 60-fold lower than those of the parent compound. These values are comparable or even lower than EC_{50} values of the newly developed potent antioxidant U78517F. In the present study, we have directly compared the antioxidant property of carvedilol with that of bisoprolol. Consistent with our previous finding, our present data showed that carvedilol, and one of its metabolites, SB 211475, decreased free radical-induced lipid peroxidation in a dose-dependent manner with an IC50 of 3.0 and 0.18 μM, respectively. In contrast, addition of bisoprolol at concentrations up to 100 μM exerted no protective effects against lipid peroxidation in this system. Taken together, it can be reasonably speculated that in addition to its β-adrenoceptor antagonist effect (which is shared by bisoprolol), carvedilol may scavenge superoxide and hydroxyl radical generated after myocardial ischemia and reperfusion (which is lacking by bisoprolol) and thus protect the myocardium from reperfusion injury.

Overproduction of free radicals may not only directly result in tissue oxidative injury, but may also enhance post-ischemic myocardial injury via several indirect mechanisms, such as upregulation of leukocyte adhesion molecules on the endothelial surface, thereby inducing leukocyte accumulation and leukocyte-related tissue injury (Gaboury et al., 1994). By scavenging oxygen free radicals, carvedilol and its metabolites (e.g., SB 211475) may inhibit the upregulation of leukocyte adhesion molecules and thus prevent leukocyte-induced tissue injury. Although we have not directly observed the effects of carvedilol and SB 211475 on adhesion molecule expression after reperfusion in our present study, we have clearly shown that administration of carvedilol, but not bisoprolol, significantly reduced myeloperoxidase activity, a marker of leukocyte accumulation, in ischemic-reperfused tissue.

Another possible mechanism that might contribute to the dramatic protection afforded by carvedilol in ischemic-reperfusion model is its selective α_1-adrenoceptor blocking property, which may cause direct coronary vasodilatation. Such vasodilating effects could increase collateral blood flow to the ischemic region and thus ameliorate ischemic myocardial injury. However, in the present study, carvedilol was not administered until 55 min after ischemia. It is therefore unlikely that the vasodilatation effect exerted by its α_1-adrenoceptor antagonist property would contribute significantly to carvedilol's overall cardioprotective effects observed in the present study.

Taken together, the data presented in this report demonstrated that carvedilol, a chemically unique drug with multiple action pharmacology, exerted additional cardioprotection in ischemic heart conditions when compared to selective β_1-adrenoceptor antagonist. In a recent US multicenter heart failure trial, when carvedilol was added to

current therapy with diuretics, digitalis and angiotensin-converting enzyme inhibitors, a remarkable 65% reduction in human morbidity and mortality was noted (Packer et al., 1996). This may be attributable to the multiple pharmacologic properties unique to carvedilol.

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