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Comparison of metoprolol and carvedilol pharmacology and cardioprotection in 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 carvedilol cardioprotection efficacy, 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, metoprolol. Carvedilol (1 mg/kg) or metoprolol (1 mg/kg or 1 mg/kg + 0.5 mg/kg 90 min later) was given intravenously 5 min before reperfusion. In vehicle-treated rabbits, ischemia (60 min) and reperfusion (180 min) resulted in significant increments in left ventricular end diastolic pressure, large infarcts ($59 \pm 2.6\%$ of area-at-risk) and marked increase in myeloperoxidase activity (0.59 ± 0.09 U/100 mg tissue). Carvedilol treatment resulted in sustained reduction of pressure-rate-index and significantly smaller infarcts ($22.0 \pm 2.5\%$, $P < 0.01$ vs. vehicle) as well as decreased myeloperoxidase activity (0.186 ± 0.056 U/100 mg tissue, $P < 0.01$ vs. vehicle). The highest dose of metoprolol, 1 mg/kg + 0.5 mg/kg, that resulted in pressure-rate-index comparable to that of 1.0 mg/kg carvedilol, failed to reduce myeloperoxidase activity in the ischemic myocardial tissue, and the infarct size ($35 \pm 3.1\%$) was significantly larger than in carvedilol-treated animals. Taken together, this study suggests that the superior cardioprotection of carvedilol over metoprolol is not a consequence of hemodynamic variances but possibly the result of the additional pharmacological properties of carvedilol such as the antioxidant and anti-neutrophil effects.

Keywords: Myocardial infarction; β-adrenoceptor antagonist; Metoprolol; Carvedilol; Leukocyte

1. Introduction

Carvedilol (Fig. 1) is a multiple action, non-selective β-adrenoceptor and α_1-adrenoceptor antagonist and potent antioxidant (Nichols et al., 1989; Yue et al., 1992) marketed for the treatment of mild to moderate hypertension, angina and heart failure. Carvedilol produces its antihypertensive effect partly by reducing total peripheral resistance by blocking α_1-adrenoceptors and by preventing β-adrenoceptor-mediated cardiac compensatory mechanisms (Nichols et al., 1989). Carvedilol is the first of the β-adrenoceptor antagonist class that has been approved for treatment of heart failure as two independent, large scale clinical trials demonstrated efficacy of carvedilol in reducing morbidity and mortality and reduction in hospitalization of heart failure patients (Packer et al., 1996; Australian New Zealand heart failure research collaborative group, 1995; Bristow et al., 1996). The clinical results thus concur with many preclinical studies demonstrating remarkable cardioprotection in animal models of cardiac ischemia, with or without reperfusion (Feuerstein et al., 1995). The cardioprotective and anti-ischemic properties of carvedilol have been demonstrated in five different animal species and in numerous experimental paradigms (Feuerstein et al., 1995). Most notably, carvedilol has demonstrated consistently superior acute anti-ischemic properties as compared to propranolol, a 'first generation', non-selective β-adrenoceptor antagonist (Feuerstein et al., 1995;

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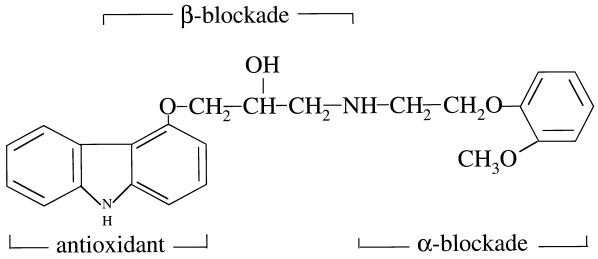

Fig. 1. Chemical structure of carvedilol.

Bril et al., 1992; Hamburger et al., 1991). Moreover, comparison of carvedilol to celiprolol, another vasodilating β-adrenoceptor antagonist (or 'third generation'), demonstrated that at dosing regimens that produce equal pressure rate product changes, celiprolol failed to provide equal cardioprotection as in ischemic conditions (Feuerstein and Ruffolo, 1994). However, celiprolol produces vasodilatation by a different mechanism (α_2-adrenoceptor blockade and β_2-adrenoceptor partial agonist action)(Dunn and Spencer, 1995) than carvedilol, suggesting that agents that may have been categorized by traditional functional hemodynamic features (e.g., vasodilating β-adrenoceptor antagonists) may reveal substantial differences in their discrete pharmacological effects on certain organs and conditions. In this light, we have chosen to explore the relative contribution of the β_1-adrenergic blocking action of carvedilol in mediation of its anti-ischemic/cardioprotective action in experimental models. We have chosen the well characterized rabbit model of cardiac ischemia and reperfusion injury where carvedilol demonstrated significant and consistent efficacy in preserving viable cardiac tissue, reduction of leukocyte accumulation and improving hemodynamic variables (Ma et al., 1996). The effects of carvedilol were compared to doses of metoprolol which resulted in comparable hemodynamic responses so that valid comparison of the cardioprotective action can be made between these two drugs. In addition, we explored the receptor pharmacology of metoprolol in comparison to carvedilol and propranolol to better discern their similarities and differences in interacting with human recombinant adrenergic receptors. Furthermore, we have studied for the first time potential differences in the redox properties of these two compounds to better delineate the uniqueness of carvedilol in this respect.

2. Materials and methods

2.1. Surgical preparations

Adult male New Zealand white rabbits (3.0–3.5 kg) were anesthetized with sodium pentobarbital (30 mg/kg body weight) intravenously. An intratracheal tube was inserted through a midline incision and all rabbits were given intermittent positive pressure ventilation with O_2-enriched room air using a Harvard small animal respirator (Harvard Apparatus, S. Natick, MA). 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 mmHg and 35–45 mmHg, 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 to maintain a surgical plane of anesthesia. An additional polyethylene catheter was inserted through the left femoral artery and positioned in the abdominal aorta for measurement of arterial blood pressure via a Statham P23AC pressure transducer (Spectromed, Critical Care Division, Oxnard, CA). A midline thoracotomy was performed, the pericardium was opened and the heart was exposed. A 3-0 silk ligature was carefully placed around the major marginal branch of the left circumflex coronary artery located on the dorsal surface of the heart, 10–12 mm from its origin. A Millar-tip catheter transducer (1.8 F) was inserted into the left ventricular cavity through the apex, and the left ventricular pressure was obtained. Left ventricular pressure, mean arterial blood pressure and electrocardiogram (ECG) from standard lead II of the scalar electrocardiogram were digitized at 500 Hz using a 12-bit analog-to-digital converter (Data translation devices) and a Dell 486-66 computer. Left ventricular pressure, arterial blood pressure and ECG were continuously monitored during the entire experimental period.

After a 30 min period of stabilization following thoracotomy, myocardial ischemia was initiated by complete ligation of the marginal coronary artery. This was designated as time 0. After 1.0 h of ischemia, the ligature was untied and the ischemic myocardium was reperfused for 3.0 h. Five minutes before reperfusion, vehicle (10% dimethyl sulfoxide, DMSO), carvedilol (1 mg/kg, MW = 406.5, plasma half life $\approx$ 7 h) or metoprolol (1 mg/kg) was given intravenously over a period of 1 min. Eight to 10 rabbits were studied in each group.

2.2. Assessment of myocardial injury

Left ventricular pressure, arterial blood pressure and ECG were continuously displayed on monitor during the entire experimental period, and recorded on hard disk for 10 s at the following time points: immediately before coronary occlusion (0 min); 20, 40 and 60 min after coronary occlusion; 20, 40, 60, 120 and 180 min after reperfusion. The left ventricular systolic pressure, left ventricular end diastolic pressure, first derivative of the left ventricular pressure (dp/dt), and heart rate were obtained using computer algorithms and an interactive videographics program. The pressure-rate-index, calculated as the product of mean arterial blood pressure and heart rate

divided by 1000 was employed as an approximation of myocardial oxygen demand.

At the end of the 3 h reperfusion period, the ligature around the marginal coronary artery was retightened. Thirty milliliters of 5% 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 coenzyme and dehydrogenase. The necrotic 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–necrotic) was weighed. Area-at-risk as a percentage of total left ventricular mass (area-at-risk/total left ventricular mass × 100%), necrotic area as a percentage of area-at-risk (necrotic/area-at-risk × 100%), and necrotic area as a percentage of total left ventricular mass (necrotic/total left ventricular mass) was calculated (Ma et al., 1996).

2.3. 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 Chemical, St. Louis, MO) dissolved in 50 mmol/l potassium phosphate buffer at pH 6 using a PRO 200 homogenizer (PRO Scientific, Monroe, CT). Homogenates were then centrifuged at 36 000 × *g* at 4°C for 30 min. 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 MPO 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.

2.4. Human adrenergic receptor expression and radioligand binding assays

Stable cell lines expressing the recombinant human adrenoceptors were prepared in Chinese hamster ovary (CHO) cells as previously described (Hieble and Ruffolo, 1997). Affinity for these receptors was measured as the K_i value for inhibition of the binding of [^{3}H]prazosin (α_1-adrenoceptors), [^{3}H]rauwolscine (α_2-adrenoceptors), or [^{125}I]iodocyanopindolol (β-adrenoceptors). Membranes were incubated with test antagonists at concentrations ranging from 0.1 nM to 100 μM. Assays were initiated by the addition of membrane protein, and incubated at 25°C for 30 min ([^{3}H]rauwolscine) or 45 min ([^{3}H]prazosin), or at 37°C for 60 min ([^{125}I]iodocyanopindolol). A total of 50 mM Tris–EDTA buffer, pH 7.4, was used for [^{3}H]rauwolscine, 50 mM Tris–HCl, pH 7.4, was used for [^{3}H]prazosin and 50 mM Tris, 12.5 μM $MgCl_2$, 2 mM EDTA, pH 7.4, was used for [^{125}I]iodocyanopindolol. Radioligand was present at a concentration near its own dissociation constant. Nonspecific binding was defined using 10 μM phentolamine for α-adrenoceptors and 100 μM (−) propranolol for β-adrenoceptors. Incubations were terminated by the addition of 2 ml of ice-cold buffer, and the membranes were collected by rapid filtration through Whatman GF/C filters using a Brandell Cell Harvester.

2.5. Determination of redox potential, pK_a and octanol/water log P

The redox potentials for the selected compounds were determined in CH_3CN containing 0.1 M tetraethylammonium perchlorate. A BAS-100 Electrochemical Analyzer (Bioanalytical Systems, West Lafayette, IN) and a glassy carbon electrode were used for the measurements. All Eps reported are vs. an aqueous Ag/AgC1 electrode.

The pK_a (acid ionization constant) and octanol/water partition coefficient were determined using a Sirius model PCA-101 Potentiometric Titration (Sirius Analytical Instruments, East Sussex, England). All aqueous phase contained 0.1 M KCl. The pH–Metric log *P* method for determining ion-pair octanol/water partition coefficients has been described in detail previously (Slater et al., 1994). A vender-supplied PC-based software package, PkaLogP, was used for calculating the log *D* vs. pH profiles.

2.6. Statistical analysis

All values in the text, table and figures are presented as means ± standard errors (S.E.M.) of the mean of *n* independent experiments. All data were subjected to analysis of variance (ANOVA) followed by the Scheffe's correction for post-hoc *t*-test comparison. Probabilities of 0.05 or less were considered to be statistically significant.

3. Results

3.1. Effect of metoprolol and carvedilol on heart rate

A stable heart rate was sustained in the sham control group and in I/R group received only vehicle. Carvedilol

administration resulted in a significant drop in heart rate shortly after its administration (from 236 ± 8 bpm before drug administration to 193 ± 6 bpm 5 min after drug administration, $P < 0.001$). Heart rate in the carvedilol-treated group-remained stable throughout the post-carvedilol administration period. Metoprolol administered as a single dose (D1) of 1 mg/kg produced similar early reduction in heart rate: from 238 ± 5 bpm before drug administration to 194 ± 5 bpm after drug administration, ($P < 0.001$), but this initial effect diminished after 120 min. When an additional (D2) dose of metoprolol (0.5 mg/kg) was given 90 min after the first dose, a comparable persistent heart rate reduction produced by carvedilol was achieved.

3.2. Effect of metoprolol and carvedilol on pressure-rate-index

Fig. 2 indicates a stable pressure-rate-index in the sham control group, and only a transient reduction of pressure-rate-index in the MI + vehicle group (pressure-rate-index was reduced from 22.9 ± 1.1 to 20.2 ± 1.3, 20 min into the ischemic period; $P < 0.05$), which then recovered to match the sham control group throughout the reperfusion phase. In the carvedilol treatment group, pressure-rate-index was further reduced shortly after drug administration (D1) to a nadir of 12.5 ± 1.6 ($P < 0.001$ vs. control period). Pressure-rate-index was maintained at a stable level throughout the reperfusion period. Metoprolol administration (D1) resulted in a similar acute effect on pressure-rate-index as carvedilol with a nadir of 12.8 ± 1.7 ($P < 0.001$ vs. control). However, single dose metoprolol treatment failed to sustain pressure-rate-index levels at a constant level as metoprolol D1 effect diminished during the reperfusion period. In the metoprolol group that included an additional drug injection (D2) 90 min after the first dose, pressure-rate-index was maintained at the same level as in the carvedilol group throughout the duration of the experiment.

3.3. Effect of metoprolol and carvedilol on left ventricular end diastolic pressure

Fig. 3 illustrates a stable left ventricular end diastolic pressure in the sham group throughout the experimental period and, as expected, marked elevation of left ventricular end diastolic pressure in the MI + vehicle group. In fact, peak increase in left ventricular end diastolic pressure was monitored at 40 min past onset of ischemia (left ventricular end diastolic pressure: 7.8 ± 0.5 mmHg vs.

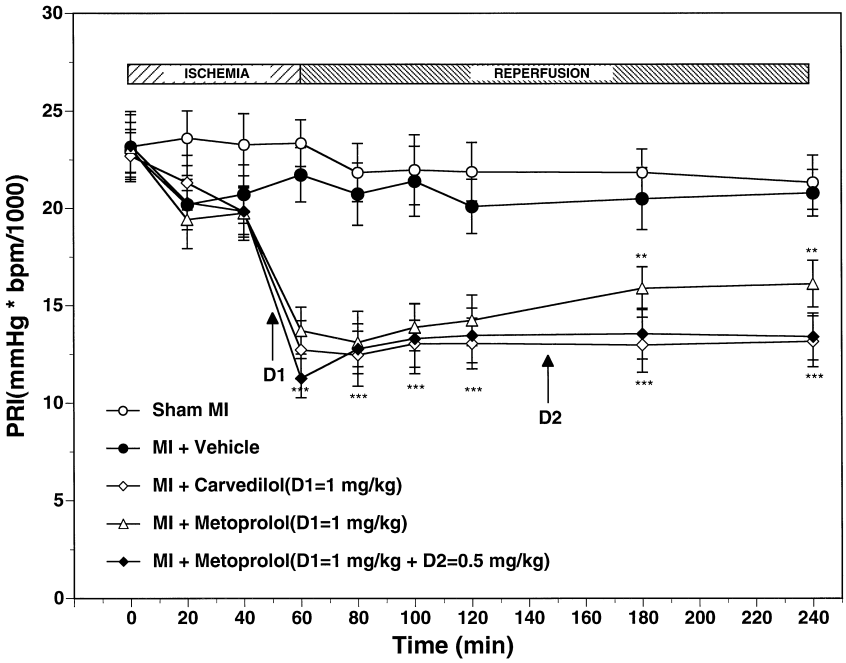

Fig. 2. Time-course of pressure-rate-index in rabbits subjected to sham ischemia/reperfusion, ischemia/reperfusion + vehicle, ischemia/reperfusion + carvedilol (1 mg/kg, single bolus administered intravenously 5 min before reperfusion), or ischemia/reperfusion + metoprolol (1 mg/kg single bolus or 1 mg/kg bolus followed by a second dose of 0.5 mg/kg 90 min later). All values are mean ± S.E.M. of eight to 10 rabbits. $**P < 0.01$, $***P < 0.005$ vs. the vehicle-treated rabbits.

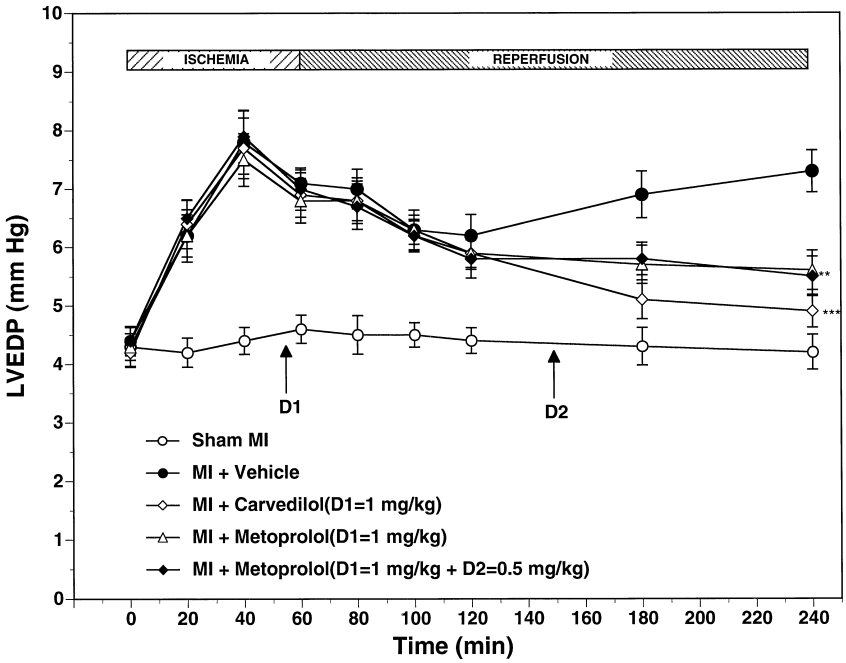

Fig. 3. Time-course of left ventricular end diastolic pressure in rabbits subjected to sham ischemia/reperfusion, ischemia/reperfusion + vehicle, ischemia/reperfusion + carvedilol (1 mg/kg, single bolus administered intravenously 5 min before reperfusion), or ischemia/reperfusion + metoprolol (1 mg/kg single bolus or 1 mg/kg bolus followed by a second dose of 0.5 mg/kg 90 min later). All values are mean ± S.E.M. of eight to 10 rabbits. $* P < 0.05$, $** P < 0.01$ vs. the vehicle-treated rabbits.

4.4 ± 0.24 mmHg in the control period; $P < 0.001$). Left ventricular end diastolic pressure was reduced at the onset of the reperfusion but during the later part, significant secondary increments were observed. Carvedilol administration (D1) was followed by a decline in left ventricular end diastolic pressure which was similar to the vehicle-treated group for about the first hour into the reperfusion; however, over the rest of the reperfusion period (2 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 levels similar to sham controls (carvedilol: 4.9 ± 0.3 mmHg vs. sham control 4.2 ± 0.36 mmHg; $P > 0.05$). Metoprolol treatments (either D1 or D1 + D2 dosing) prevented in part the secondary deterioration of left ventricular end diastolic pressure.

Table 1
Effect of carvedilol and metoprolol on LVSP and dp/dt_{max} in rabbit ischemic-reperfused hearts

	Control	I-20	I-40	I-60	R-20	R-40	R-60	R-120	R-180
LVSP (mmHg)									
Sham MI	117 ± 6	117 ± 7	116 ± 5	113 ± 6	113 ± 5	111 ± 5	112 ± 7	107 ± 5	104 ± 6
MI + Vehicle	119 ± 6	92 ± 6	95 ± 5	98 ± 6	93 ± 7	96 ± 7	95 ± 5	91 ± 6	89 ± 6
MI + CV	116 ± 7	90 ± 7	94 ± 5	75 ± 6[b]	74 ± 6[a]	78 ± 5[a]	77 ± 6[a]	76 ± 5[a]	75 ± 5
MI + MT(D1)	117 ± 5	94 ± 6	96 ± 5	77 ± 5[b]	77 ± 6[a]	78 ± 5[a]	79 ± 6[a]	84 ± 6	83 ± 7
MI + MT(D1 + D2)	118 ± 4	93 ± 6	95 ± 5	76 ± 6[b]	77 ± 6[a]	77 ± 5[a]	79 ± 4[a]	75 ± 5[a]	74 ± 6
dp/dt_{max} (mmHg/s)									
Sham MI	3985 ± 84	4012 ± 92	3854 ± 88	3900 ± 97	3832 ± 89	3724 ± 86	3710 ± 96	3610 ± 97	3518 ± 91
MI + Vehicle	4012 ± 78	2815 ± 88	3111 ± 87	3214 ± 96	3014 ± 97	3118 ± 96	3428 ± 86	3314 ± 91	3218 ± 85
MI + CV	3887 ± 65	2799 ± 84	2997 ± 85	2559 ± 88[b]	2548 ± 97[a]	2664 ± 92[a]	2899 ± 104[a]	2910 ± 95	2885 ± 88
MI + MT(D1)	3911 ± 89	2874 ± 99	2915 ± 88	2600 ± 97[b]	2612 ± 81[a]	2706 ± 88[a]	2911 ± 89[a]	3228 ± 88	3158 ± 91
MI + MT(D1 + D2)	3887 ± 69	2699 ± 93	2914 ± 96	2622 ± 88[b]	2634 ± 94[a]	2771 ± 86[a]	2893 ± 89[a]	2812 ± 91	2910 ± 95

[a] $P < 0.05$.
[b] $P < 0.01$ vs. vehicle group.
LVSP = left ventricular systolic pressure.
dp/dt_{max} = maximal value of first derivative of the left ventricular pressure.
MI = myocardial ischemia. I, Ischemia; R, reperfusion; CV, carvedilol; MT, metoprolol.

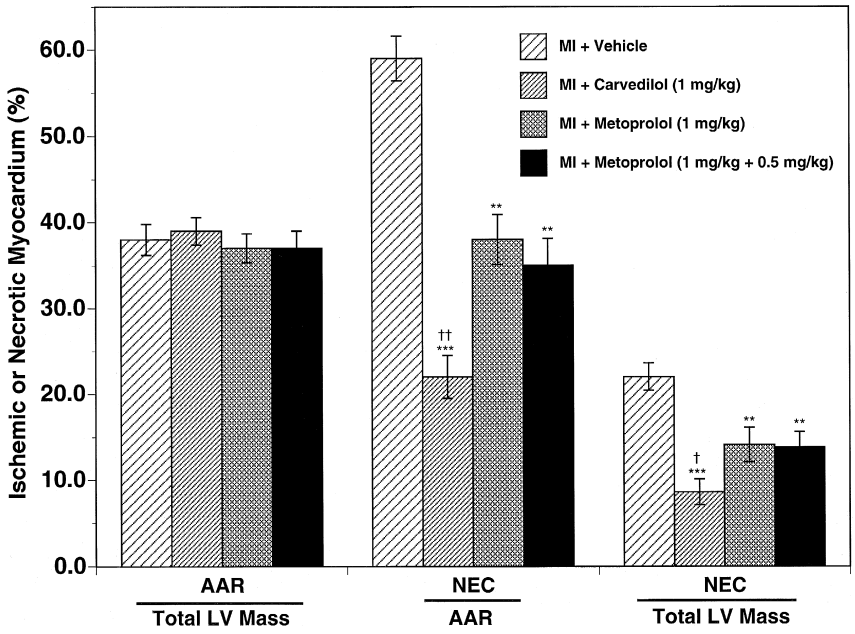

Fig. 4. Tissue wet weight of area-at-risk as a percentage of the total left ventricular wet weight, and of necrotic tissue as a percentage of area-at-risk and of the total left ventricle for the four groups. Height of bars are means; brackets represent ± S.E.M. $**P < 0.01$, $***P < 0.005$ vs. the vehicle-treated rabbits. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs. metoprolol-treated rabbits. AAR, area-at-risk; LV, left ventricle; NEC, necrotic.

3.4. Effect of metoprolol and carvedilol on left ventricular systolic pressure and dp/dt_{max}

The effects of metoprolol or carvedilol on changes in left ventricular systolic pressure and dp/dt_{max} after myocardial ischemia and reperfusion were summarized in Table 1. In sham myocardial ischemia rabbits, both left ventricular systolic pressure and dp/dt_{max} maintained at a relatively stable level throughout the 4 h experimental period, and the spontaneous decline was $< 3\%$ per hour. Coronary occlusion caused a significant decrease in left ventricular systolic pressure and dp/dt_{max} in all four myocardial ischemia groups. Administration of carvedilol or metoprolol (both D1 and D1 + D2) resulted in a further

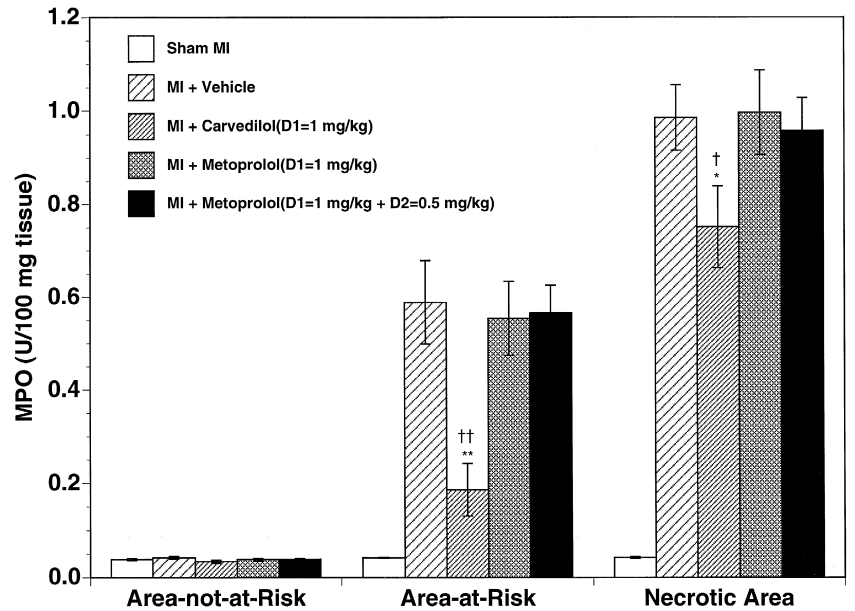

Fig. 5. Tissue myeloperoxidase activity in area-not-at-risk, area-at-risk and necrotic area in U/100 mg tissue wet weight for the five groups. Height of bars are means; brackets represent ± S.E.M. $*P < 0.05$, $**P < 0.01$ vs. vehicle-treated rabbits, $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs. metoprolol-treated group.

Table 2
Receptor binding characteristics of carvedilol, propranolol and metoprolol to human recombinant adrenergic receptors

Compound	α_{1A}	α_{1B}	α_{1D}	α_{2A}	α_{2B}	α_{2C}	β_1	β_2	β_3
Carvedilol	2.8 (7)	0.9 (2.3)	1.1 (2.8)	26 (65)	27 (68)	11 (28)	0.4	0.1 (0.3)	35 (88)
Propranolol	> 5000 (> 1000)	ND	ND	> 5000 (> 1000)	> 10 000 (> 1000)	> 10 000 (> 1000)	2.5	0.4 (0.2)	88 (35)
Metoprolol	> 10 000 (> 100)	> 10 000 (> 100)	> 10 000 (> 100)	> 10 000 (> 100)	> 10 000 (> 100)	> 10 000 (> 100)	96	587 (6.1)	> 10 000 (> 100)

Affinities (K_i, nM) for recombinant human adrenoceptors.
Numbers in parentheses denote relative affinity, assigning $\beta_1 = 1.0$.

decrease in both left ventricular systolic pressure and dp/dt_{max} and there was no significant difference in the initial decrease in left ventricular systolic pressure and dp/dt_{max} among the three groups. However, the effects of metoprolol on left ventricular systolic pressure and dp/dt_{max} diminished after 120 min in the single dose treatment group. When an additional (D2) dose of metoprolol (0.5 mg/kg) was given 90 min after first dose, a comparable reduction of left ventricular systolic pressure and dp/dt_{max} produced by carvedilol was achieved.

3.5. Effect of metoprolol and carvedilol on myocardial necrosis

Fig. 4 illustrates the extent of cardiac necrosis as defined by nitroblue tetrazolium staining (see Section 2). It is important to note that the ischemic area, calculated as 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 necrotic area which encompassed $59.0 \pm 2.6\%$ of the ischemic myocardium; these results are in accord with previous reports (Ma et al., 1996). Carvedilol treatment reduced the necrotic myocardium to $22.0 \pm 2.5\%$ ($P < 0.001$). Metoprolol, at either treatment regimen, reduced the necrotic area to virtually the same extent (35–37%) which was significantly less than carvedilol (metoprolol D1 + D2 vs. carvedilol; $P < 0.05$). The same conclusion was reached by calculating necrotic myocardium directly as percent of individual left ventricular mass.

3.6. Effect of metoprolol and carvedilol on myeloperoxidase activity in the ischemic myocardium

Fig. 5 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. None of the metoprolol treatment groups displayed a significant change in myeloperoxidase levels at either the area of risk or the necrotic zone. In contrast, carvedilol treatment reduced myeloperoxidase levels at both the area-at-risk and necrotic zone ($P < 0.01$).

3.7. Affinity of metoprolol and carvedilol for recombinant adrenoceptors

Carvedilol has high affinity (0.1–2.8 nM) for β_1- and β_2-adrenoceptors and for the three α_1-adrenoceptor subtypes (Table 2). The affinity for the three α_2-adrenoceptor subtypes, as well as for the β_3-adrenoceptor, was lower (11–35 nM). Consistent with data from functional assays, metoprolol shows 6-fold selectivity for the β_1- vs. the β_2-adrenoceptor, and virtually no affinity for the β_3-adrenoceptor or any of the α-adrenoceptor subtypes.

Functional and radioligand binding studies using isolated tissues from a variety of species show metoprolol to have a β_1 vs. β_2 selectivity ratio ranging from 13 to > 1000. This β_1 selectivity was confirmed by our results with human recombinant receptors (Table 2). Although we observed only a 6-fold selectivity for β_1 vs. β_2 receptors, this assay appears to show higher relative β_2 affinity than predicted by functional assays, since both carvedilol and propranolol, which have equivalent functional affinities for β_1- and β_2-adrenoceptors show 4- to 6-fold higher affinity for the recombinant β_2-adrenoceptor than for the β_1 (Table 2). This apparent β_2 selectivity in assays using recombinant β-adrenoceptors has been previously observed for propranolol (Marullo et al., 1990; Blin et al., 1993). Hence, our data (Table 2) would suggest that the pharmacological activity of metoprolol results predominately from β_1-adrenoceptor blockade.

Table 2 confirms the high affinity of carvedilol for the α_1-adrenoceptor. No pharmacologically significant selectivity between α_1-adrenoceptor subtypes is observed. While it is not known which subtype(s) are most important in the control of vascular resistance, it is likely that both the α_{1B} and α_{1D} make an important contribution (Leonardi et al., 1997). Since the adrenoceptor affinities as determined by these radioligand binding assays are somewhat higher than those in functional experiments, it is unlikely that interaction of carvedilol with the α_2-adrenoceptor will contribute to its in vivo pharmacology, since affinity for α_2-adrenoceptors is at least 10-fold lower than for either α_{1B}- or α_{1D}-adrenoceptors (Table 2).

3.8. The redox potentials, pK_a and octanol/water partition coefficient

The redox potentials, octanol/water partition coefficients and acid ionization constants were determined for carvedilol, propranolol and metoprolol. The results are shown in Table 3. Essentially all three compounds have approximately the same oxidation peak potential at ~ 1.1–

Table 3
Physical chemical properties for selective, β-adrenoceptor antagonist

	Ep (V)	pK_a	log P (log $P+$)	log D 7.4
Carvedilol	1.10	7.8	4.1 (1.9)	3.5
Propranolol	1.15	9.5	3.5 (1.0)	1.4
Metoprolol	1.05	9.5	2.1 (0.5)	0.8

Ep voltammetric peak potential in CH_3Cn vias. a Ag/AgCl reference electrode.
pK_a aqueous acid dissociation constant obtained by extrapolating apparent pK_a values determined in different ratios of water/methanol mixed solvent to 0% methanol.
log P octanol/water partition coefficient for the neutral species.
log $P+$ partition coefficient for the ion-paired species.
log D 7.4 distribution coefficient (or apparent partition coefficient) at pH 7.4.

1.2 V vs. an Ag/AgC1 electrode. Interestingly, carvedilol has the highest log P and lowest pK_a value among the three selected β-adrenoceptor antagonists. The combined effect is that at pH 7.4, carvedilol is 2 to 3 orders of magnitude more lipophilic than propranolol and metoprolol, as indicated by the log D values.

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., 1995) and clinical (Basu et al., 1997) studies. Since carvedilol is a multiple action drug, several different and complementary mechanisms have been suggested to contribute to its cardioprotective actions. First, via its potent β-blocking shared by similar agents of the β-blocking agent class, carvedilol reduces cardiac workload and oxygen consumption. Second, α_1-adrenergic blockade may aid the anti-ischemic properties or β-blockade as demonstrated in several experimental models (Brunvand et al., 1996a,b). Finally, as a potent antioxidant, carvedilol may provide additional cardioprotection, especially in situations where ischemia and reperfusion paradigms are exercised since oxygen radicals have been implicated in reperfusion injury (Zweier et al., 1987).

The present study was undertaken to further dissect out the relative contribution of the various pharmacological properties of carvedilol by comparing its cardiac protection efficacy to an agent of high selectivity to the β_1-adrenoceptor. Metoprolol was chosen as such a comparative agent because of its wide clinical use and selectivity towards the human β_1-adrenergic receptors. This latter property has been confirmed by assessing the K_i of metoprolol vs. carvedilol and propranolol at the human recombinant adrenergic receptors (see Table 3). Our data indicate that metoprolol is unlikely to have any pharmacologically significant interaction with any of the adrenergic receptors other than the β_1-adrenergic receptor. In clinical studies of acute myocardial infarction, metoprolol has been shown to provide significant reduction in morbidity and mortality (Hjalmarson et al., 1981). In our model, where metoprolol was not studied beforehand, metoprolol dose-dependently reduced pressure-rate-index and reduced, in part, the increased left ventricular end diastolic pressure and myocardial necrosis induced by ischemia and reperfusion. However, the extent of the cardiac protection by metoprolol was $35.9 \pm 1.8\%$ and $37.3 \pm 2.1\%$ for the respective dosing regimens (D1 and D1 + D2). Furthermore, metoprolol had no effect on the myeloperoxidase (and hence leukocyte) levels in the area of risk or the area of necrosis. Carvedilol, at a dosing regimen that produced precisely the same hemodynamic effects (heart rate, left ventricular systolic pressure, dp/dt_{max} and pressure-rate-index) as the double dosing (D1 + D2) of metoprolol, had superior cardioprotective action as evidenced by the smaller necrotic area ($22 \pm 2.5\%$ of area-at-risk vs. $35 \pm 3.1\%$ in the double dose metoprolol, $P < 0.05$). In addition, carvedilol-treated animals had somewhat lower left ventricular end diastolic pressure, although this trend did not achieve statistical significance. In marked contrast to the failure of metoprolol to reduce myeloperoxidase levels in the ischemic/reperfused myocardium, carvedilol significantly reduced myeloperoxidase levels and especially at the area-at-risk where myeloperoxidase levels were 68.4% lower than the vehicle group. The capacity of carvedilol to reduce leukocyte infiltration into the ischemic myocardium demonstrated in this study is in accord with previous studies with carvedilol where ischemia and reperfusion of swine myocardium was studied (Bril et al., 1992).

The most plausible explanation for the superior cardioprotection of carvedilol over metoprolol demonstrated in this study may reside in the antioxidant action of carvedilol and some of its metabolites. Although we did not directly measure free radical generation and its scavenging by carvedilol in this particular model used in the present study, two lines of evidence strongly suggest that carvedilol may exert myocardial protective effects via its free radical scavenging property. First, it is well documented that ischemia followed by reperfusion is associated with a burst of oxygen-derived free radical generation at the onset of reperfusion, and these highly reactive molecules play a pivotal role in the pathogenesis of post-ischemic reperfusion injury in the heart (Zweier et al., 1987). Second, by utilizing electron paramagnetic resonance technique, it has been previously demonstrated that carvedilol scavenges both superoxide anion and hydroxyl radical (Feuerstein et al., 1997) in both aqueous and lipid environments. Taken together, it can be reasonably speculated that carvedilol may also scavenge superoxide and hydroxyl radical generated after myocardial ischemia and reperfusion and thus protect myocardium from reperfusion injury.

Another likely mechanism which may also contribute to the dramatic protection afforded by carvedilol in this ischemic-reperfusion model is its selective α_1-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.

It should be indicated that reperfusion time in the present study was relatively short. However, it has been previously demonstrated that carvedilol significantly decreases infarct size after 30 (rat) or 60 min (dog) of ischemia and 24 h of reperfusion (Feuerstein et al., 1993). Moreover, we have used an enzyme-dependent staining method to detect infarct size. Utilizing a more sensitive method to detect infarct size may provide more precise information in cardioprotection of carvedilol and metoprolol.

Taken together, the data presented in this report provide further support to the claim that carvedilol, via its multiple

action pharmacology (which results from its unique chemical structure), exerted additional cardioprotection in ischemic heart conditions as compared to selective or non-selective β-adrenoceptor antagonists. Therefore, it is reasonable to suggest that the remarkable reduction in morbidity and mortality, 65%, observed in the US multicenter heart failure trials (Packer et al., 1996), on top of current therapy with diuretics, digitalis and angiotensin-converting enzyme inhibitors, could be the result of multiple pharmacology unique to carvedilol.

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