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Effects of a hydroxylated metabolite of the β-adrenoreceptor antagonist, carvedilol, on post-ischaemic splanchnic tissue injury

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1 Reactive oxygen species have been demonstrated to play a critical role in post-ischaemic tissue injury. The present experiment was designed to evaluate the effects of SB 211475, a hydroxylated metabolite of the new β-adrenoceptor antagonist, carvedilol, on rat splanchnic ischaemia (SI, 60 min) and reperfusion(R)-induced shock and tissue injury.

2 Administration of SB 211475 two min before R attenuated SI/R injury in a dose-dependent manner. At doses of 0.5 mg kg^{-1} and 1.0 mg kg^{-1}, SB 211475 exerted significant anti-shock and endothelial protective effects, characterized by prolonged survival times, increased survival rates, attenuated increases in tissue myeloperoxidase activity and haematocrits, and preserved endothelium-dependent vasorelaxation.

3 Administration of 1 mg kg^{-1} carvedilol attenuated shock-induced tissue injury and endothelial dysfunction. However, administration of 0.5 mg kg^{-1} carvedilol had no protective effects on post-ischaemic tissue injury.

4 Previous studies have shown that SB 211475 has virtually no β-blocking activity but possesses more potent antioxidant activity than carvedilol. In the present study, SB 211475 exerted more potent protective effects than the parent compound, suggesting that this metabolite of carvedilol is superior to carvedilol with regard to its protection against post-ischaemia tissue injury.

Keywords: Endothelium; leukocytes; reactive oxygen species; antioxidant

Introduction

Splanchnic ischaemia followed by reperfusion results in a severe form of circulatory shock (SI/R shock) characterized by a marked decrease in post-reperfusion systemic arterial blood pressure and an associated high mortality rate (Glenn & Lefer, 1970); Menge & Robinson, 1979). This form of shock involves intestinal mucosal lesions, increased microvascular permeability and local release of lysosomal hydrolases resulting in enhanced proteolysis, intravascular fluid loss and tissue oedema (Haglund & Lundgren, 1978). The cause of SI/R shock is apparently multifactorial. However, numerous experiments have demonstrated that reactive oxygen species (ROS), including superoxide ($\cdot O^-_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$), play a pivotal role in post-splanchnic ischaemic tissue injury (Granger *et al*., 1981; 1986; Chamulitrat *et al*., 1996).

Recent studies have demonstrated that carvedilol, a novel antihypertensive agent with non-selective β-adrenoceptor and selective α_1-adrenoceptor blocking activity, is also a potent antioxidant (Yue *et al*., 1992; Lopez *et al*., 1995; Maggi *et al*., 1996). In a previous study, we demonstrated that administration of carvedilol 5 min before reperfusion exerted significant anti-shock and endothelial protective effects in SI/R shock (Christopher *et al*., 1995). In contrast, administering an equivalent dose of propranolol, a comparable β-blocker lacking free radical scavenger activity, failed to exert significant protective effects in this severe form of circulatory shock. These results suggested that the protective effects of carvedilol against post-ischaemic tissue injury in splanchnic organs appeared to be independent of its β-blocking effect.

SB 211475 (1-[3-hydroxycarbazolyl-(4)-oxy]-3-[(2-methoxyphenoxyethyl) amino]-propanol-(2)), is a main metabolite of carvedilol found in the human blood (Feuerstein & Ruffolo, 1996). *In vitro* pharmacological studies have shown that SB 211475 only has a weak β-adrenoceptor blocking effect (K_B value = 1.7×10^{-7} M vs 1×10^{-9} M for carvedilol), but possesses exceptional antioxidant activity (Yue *et al*., 1994). The present studies were designed to elucidate the effects of SB 211475 on post-ischaemic tissue injury and endothelial dysfunction, and to test further the hypothesis that it is the antioxidant activity of carvedilol, and not its β-adrenoceptor blocking activity, that is primarily responsible for its protective effects against post-ischaemic splanchnic tissue injury.

Methods

Male Sprague-Dawley rats (Ace Animals, Inc.) weighing 175–225 g were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}) via intraperitoneal (i.p.) injection. Then, the trachea was cannulated with PE-240 tubing to insure a patent airway. Polyethylene catheters (PE-50) filled with heparin-treated 0.9% NaCl solution were inserted into the left common carotid artery for recording mean arterial blood pressure (MABP), and into the right external jugular vein for administration of drugs or vehicle. After a midline laparotomy had been performed, the celiac and superior mesenteric arteries were isolated from surrounding connective tissues near their aortic origins. All cannulations and laparotomies were completed within 20 min for each animal. MABP was

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recorded at 5, 10, 20, 40 and 60 min of ischaemia, at 5 and 20 min postreperfusion, and at subsequent 20 min intervals following reperfusion on WindoGraf Recorders (Gould Inc., Valley View, OH) with Cobe CDXIII transducers (Lakewood, Colorado).

SI/R shock was induced by total occlusion of the superior mesenteric artery (SMA) and the celiac artery with nontraumatic clamps for 60 min. Immediately after occlusion, the rats in all groups were given heparin, 500 u kg^{-1} intravenously (i.v.), to prevent coagulation and ensure reperfusion of the arteries 60 min later. After 60 min of ischaemia, the occlusive clamps were removed, first from the SMA and then from the celiac artery. The rats were then observed for an additional 120 min or until the MABP fell to 45 mmHg. Survival time was defined as the time elapsed from the removal of the occlusive clamps to the time the MABP fell to 45 mmHg. Survivors were defined as rats maintaining a MABP above 45 mmHg for 120 min after reperfusion.

Previous pharmacodynamic studies have shown that carvedilol has a relatively long half-life (7 h) (Morgan, 1994), whereas the half-life of SB 211475 in physiological solution is much shorter (0.5 h, SmithKline Beecham Pharmaceuticals, unpublished data). Based on these results, SB 211475 was injected as a bolus followed by a continuous infusion for the remainder of the reperfusion period, whereas carvedilol was only given as a bolus. The animals were randomly assigned to one of six experimental groups: (1) sham shock rats given SB 211475 (SmithKline Beecham Pharmaceuticals, King of Prussia, PA) (i.v. bolus 2 min before reperfusion, 1 mg kg^{-1} in 1 ml 30% DMSO followed by 1 mg kg^{-1} h^{-1} continuous i.v. infusion, final DMSO concentration in blood $<0.5\%$, $n=10$); (2) SI/R shock rats receiving vehicle (1 ml kg^{-1} 30% DMSO followed by 1 ml kg^{-1} h^{-1} continuous i.v. infusion, $n=14$); (3) SI/R shock rats receiving SB 211475 (1 mg kg^{-1}, i.v. bolus followed by 1 mg kg^{-1} h^{-1} continuous i.v. infusion, $n=11$); (4) SI/R shock rats receiving SB 211475 (0.5 mg kg^{-1}, i.v. bolus followed by 0.5 mg kg^{-1} h^{-1} continuous i.v. infusion, $n=14$); (5) SI/R shock rats receiving carvedilol (1.0 mg kg^{-1}, i.v. bolus, $n=12$); and (6) SI/R shock rats receiving carvedilol (0.5 mg kg^{-1}, i.v. bolus, $n=14$). The volumes of drugs or vehicles used did not affect haemodynamic or biochemical properties of the rats. At the end of the experiments, intestinal tissue samples were obtained for myeloperoxidase (MPO) determination. Sham shock rats were subjected to all the surgical procedures performed on SI/R shock rats, including isolation of the SMA and celiac arteries, except that these arteries were not occluded. Additional pentobarbitone was given i.p. to all rat groups 10 min after surgery was completed (10 min before ischaemia), 20 min before reperfusion and as needed during the 120 min reperfusion period to maintain a surgical plane of anaesthesia. At the end of surgery and at the end of the experiments, 0.1 ml of arterial blood was drawn and haematocrits were determined with a microhaematocrit centrifuge (Marathon 6K, Fisher Scientific, Pittsburgh, PA).

Determination of tissue MPO

All SI/R shock rats exhibited areas of intestinal infarction and haemorrhage, but care was taken to avoid taking tissue specimens from these areas. Small intestine MPO, an enzyme occurring almost exclusively in neutrophils, was determined by means of a standard method (Bradley *et al*., 1982; Mullane 1985). Briefly, the small intestine was homogenized in 0.5% hexadecyltrimethyl ammonium bromide (Sigma Chemical Co., St. Louis, MO) and dissolved in 50 mM potassium phosphate buffer at pH 6 with a PRO 200 homogenizer (PRO Scientific Inc, Monroe, CT). Homogenates were centrifuged at 12,500 *g* 4°C for 30 min. The supernatants were then collected and reacted with 0.167 mg ml^{-1} of o-dianisidine dihydrochloride (Sigma) and 0.0005% H_2O_2 in 50 mM 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 hydrolysing 1 mmol of peroxide min^{-1} at 25°C. The assays were performed without knowledge of the group from which each sample originated.

It has been recently shown that high concentrations of haemoglobin or myoglobin interfere with MPO measurements performed with the o-dianisidine method (Xia & Zweier, 1997). To determine whether the MPO assay in the intestinal tissues was affected by these molecules, four additional experiments were performed. The ischaemic-reperfused intestinal tissue was separated after it had been homogenized in 0.5% haxadecyltrimethyl ammonium bromide. One half of the supernatant was directly assayed for MPO activity as described above; the other half was chromatographed on the Sephadex G-75 columns (Pharmacia Biotech Inc., Piscataway, NJ) as described by Xia & Zweier (1997) and the effluents were collected for MPO assay. The intestinal MPO activity in four samples without chromatography was 1.89 ± 0.35 u 100 mg^{-1} tissue vs 1.94 ± 0.28 u 100 mg^{-1} tissue after chromatography. These results indicate that one-step gel filtration chromatography of intestinal tissue extracts did not significantly change MPO activity, as has been shown in myocardial tissue.

Isolated SMA ring studies

The SMA was isolated from at least three rats in each experimental group and placed into ice-cold Krebs-Henseleit (K-H) buffer consisting of (mM): NaCl 118, KCl 4.75, $CaCl_2.2H_20$ 2.54, KJ_2PO_4 1.19, $MgSO_4.7H_20$ 1.19, $NaHCO_3$ 25 and glucose 10.0. SMA segments were carefully cleaned of fat and loose connective tissue, and cut into 9–11 rings of 2–3 mm length. These rings were then mounted on stainless steel hooks, suspended in 37°C and aerated (95% O_2 and 5% CO_2) 7.5 ml K-H tissue baths, and connected to FORT-10 force transducers (WPI, Sarasota, FL) to record changes in tension on WindoGraf Recorders. The rings were then stretched to an optimum preload of 0.5 g of force, determined in previous experiments in this laboratory (Ma *et al*., 1996), and allowed to equilibrate for 60 min. During this period, the K-H buffer in the tissue bath was replaced every 15 min and the tension of vascular rings was adjusted until 0.5 g of preload was maintained.

After equilibration, the rings were then exposed to 50 nM U-46619 (9,11-epoxymethano-prostaglandin; Biomol Research Laboratories, Plymouth Meeting, PA), a thromboxane A_2 (TxA_2) mimetic, to generate approximately 0.5 g of developed force. Once a stable contraction was obtained, acetylcholine (ACh), an endothelium-dependent vasodilator, was added to the bath in cumulative concentrations of 0.001, 0.01, 0.1, 1 and 10 μM to determine endothelial function. After the cumulative response had stabilized, the rings were washed and allowed to equilibrate to baseline. The procedure was then repeated with an endothelium-independent vasodilator, acidified $NaNO_2$ (0.1, 1, 10, and 100 μM), to determine smooth muscle function. $NaNO_2$ was prepared by dissolving the compound in 0.1 N HCl and titrating it to pH 2.0. Titrating distilled water to pH 2.0 and adding aliquots to the bath did not produce any vasorelaxation.

Statistical analysis

Data were analysed with the StatView or SuperANOVA programmes (Abacus Concepts, Inc. Berkeley, CA). MABP data were analysed by two way (time and group) analysis of variance for repeated measures; *post hoc* testing was done with the Tukey-Kramer HSD test. Survival time, HCT, MPO and vasorelaxation data were analysed by one-way ANOVA. *Post-hoc* testing was done with the Bonferroni correction. Survival rate data were assessed by chi square analysis. Probabilities of 0.05 or less were considered to be statistically significant.

Results

Figure 1 illustrates the time course of MABP changes in the six groups of rats observed in this study. The initial MABP in each group ranged from 106–123 mmHg and these were not statistically different. Moreover, the initial MABP of the sham shock rats was 106 mmHg and did not vary significantly over the course of the experiment, suggesting that the surgical procedures performed did not contribute significantly to the severity of the SI/R shock state. All rats subjected to occlusion of their splanchnic arteries developed a rapid rise in MABP of 22–27 mmHg followed by a gradual decrease of 10–24 mmHg during the 60 min of occlusion, so that MABP was close to initial levels by the time of reperfusion.

MABP did not differ significantly between the 5 SI/R groups, either immediately before or during the occlusion of splanchnic arteries, or immediately after the reperfusion. Those rats subjected to SI/R but receiving only vehicle experienced an abrupt decrease (60–70 mmHg) in MABP upon reperfusion of the splanchnic arteries, followed by a partial recovery and then a gradual secondary decline in MAPB. Administration of 0.5 mg kg^{-1} carvedilol had no effects on MAB change following reperfusion, and administration of 1 mg kg^{-1} carvedilol or 0.5 mg kg^{-1} SB 211475 maintained MABP at a slightly high level at the later stage of reperfusion, when compared with vehicle-treated rats. However, treatment with 1 mg kg^{-1} SB 211475 significantly improved post-ischaemic blood pressure recovery, and MABP was significantly higher than the vehicle group at 120 min post-reperfusion (Figure 1).

All ten sham SI/R shock rats survived for the entire 2 h observation period (survival rate 100%). The SI/R shock rats receiving only the vehicle demonstrated a significantly shorter survival time (77±7 min); only 2 of 14 of these rats survived at the end of the 2 h reperfusion period (survival rate 14%). However, a significant improvement in survival time (116±4 min, 113±5 min, respectively) and survival rate (91% and 58%, respectively) was observed in rats treated with either 1 mg kg^{-1} SB 211475 or 1 mg kg^{-1} carvedilol. Treatment with a lower dose of SB 211475 (i.e., 0.5 mg kg^{-1}) also significantly improved the survival time (106±6 min, $P<0.01$ vs vehicle) and increased the survival rate (86%). However, treatment with low dose carvedilol (i.e., 0.5 mg kg^{-1}) did not significantly improve either survival time (88±8 min, $P>0.05$ vs vehicle) or survival rate (27%).

Haemoconcentration, resulting from loss of fluid from the vascular compartment due to altered microvascular function and increased permeability, is another marker of shock severity. Before SI/R or sham SI/R shock, there was no significant difference in haematocrit readings between all the groups of rats studied (ranging from 40% to 48%). The % increase in haematocrit was low in sham SI/R shock rats (8.0±2%), indicating that surgical procedures did not induce a significant increase in vascular permeability. However, SI/R shock rats receiving only vehicle exhibited a 41±5% increase in haematocrit ($P<0.001$ compared to sham SI/R shock). As depicted in Figure 2, although treatment with high dose carvedilol (i.e., 1 mg kg^{-1}) produced less haemoconcentration compared with shock rats treated with vehicle alone ($P<0.05$), treatment with low dose carvedilol (i.e. 0.5 mg kg^{-1}) did not attenuate the

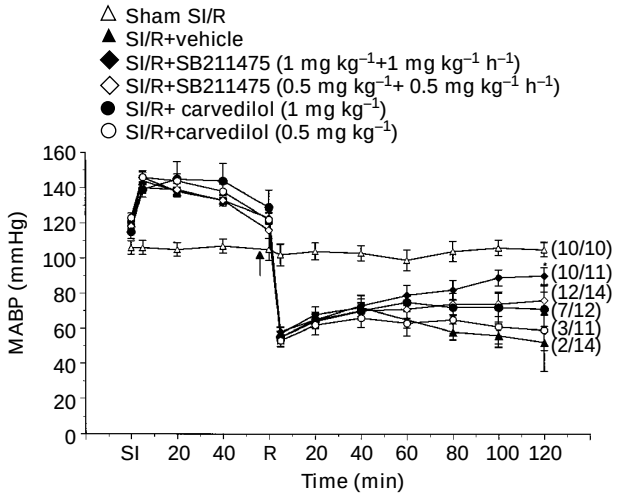

Figure 1 Time course of mean arterial blood pressure in sham SI/R and SI/R shock rats given vehicle, SB 211475 or carvedilol. Drugs were given 2 min before reperfusion. All values are mean and vertical lines show s.e.mean. Number fractions in parentheses indicate the number of surviving animals at the indicated point in time over the total number of animals in each group. SI = splanchnic ischaemia; R = reperfusion; D = drugs. *$P<0.05$ vs vehicle.

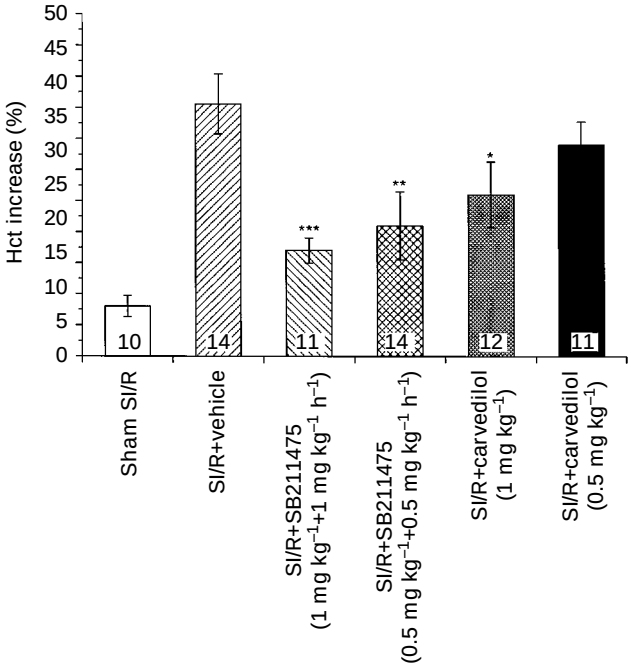

Figure 2 Mean haematocrit (Hct) increase (as %) for sham SI/R and SI/R rats given vehicle, SB 211475 or carvedilol. Columns represent mean values±s.e.; numbers in columns indicate number of rats in each experimental group. *$P<0.05$, **$P<0.01$ vs vehicle.

increase in haematocrit. However, rats receiving SB 211475 (either 1 mg kg^{-1} or 0.5 mg kg^{-1}) demonstrated a statistically significant attenuation of shock-induced increase in haematocrit ($17\pm2\%$ and $21\pm5\%$, respectively, $P<0.01$) compared to SI/R shock rats receiving only vehicle. These findings indicate that, in this model of SI/R shock, SB 211475, to a greater extent than the parent compound carvedilol, curtailed an increase in haematocrit, an indirect measurement of loss of fluid from the vascular compartment.

Figure 3 illustrates MPO activity of ileal tissue isolated from the six experimental groups. All sham SI/R shock rats exhibited apparently normal ileal tissue and low MPO activity (i.e. 0.09 ± 0.04 u 100 mg^{-1} tissue), indicating that there was no significant polymorphonuclear leukocyte (PMN) accumulation in normal intestinal tissue. In contrast, the ileal MPO activity in SI/R shock rats receiving only vehicle was approximately 23 times higher (2.04 ± 0.18 u 100 mg^{-1} tissue) than the MPO activity in sham SI/R shock rat intestine ($P<0.01$). Treatment with SB 211475, at either dose, or the parent compound carvedilol, at high dose (i.e., 1 mg kg^{-1}), resulted in a statistically significance decrease in MPO activity compared with rats treated with vehicle alone. However, treatment with 0.5 mg kg^{-1} carvedilol only slightly decreased MPO activity ($P>0.05$ vs vehicle). These results suggest that

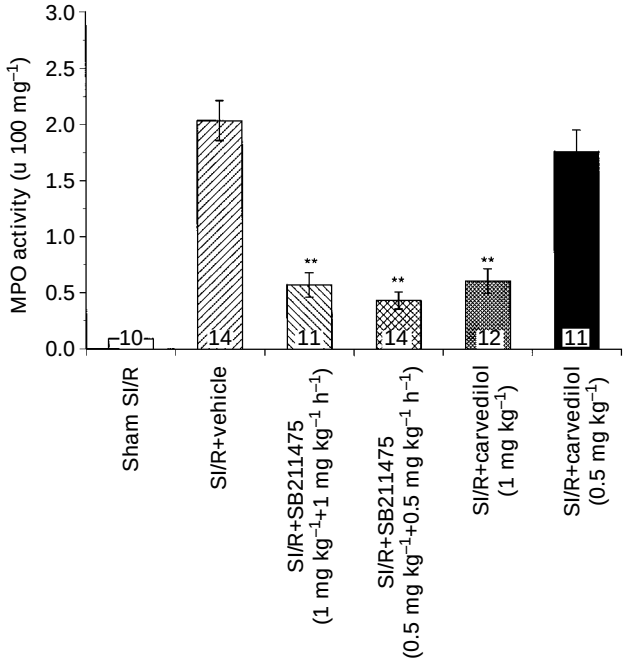

Figure 3 Intestinal MPO activity (u 100 mg^{-1} tissue wet wt.) in sham SI/R and SI/R rats given vehicle, SB 211475 or carvedilol. Columns represent mean values $\pm$s.e.; numbers in columns indicate number of rats in each experimental group. $^{**}P<0.01$ vs vehicle.

the stronger antioxidant metabolite of carvedilol, in both high and low doses, and carvedilol itself in a high dose retarded accumulation of neutrophils in intestinal tissue following SI/R shock.

Table 1 summarizes the change in heart rate within 1 min of vehicle or drug bolus infusion for the five groups of treated SI/R shock rats. Neither vehicle nor a low or high dose of the metabolite SB 211475, with primarily antioxidant effects, significantly changed the heart rates of SI/R shock rats when injected in a bolus towards the end of the period of ischaemia. However, both low and high dose carvedilol, with known β-adrenoceptor blocking effects, significantly decreased heart rates in these shock rats within 1 min of a bolus injection (-32 ± 6 and -25 ± 4 beats min^{-1}, respectively, $P<0.05$). These results further support previous *in vitro* studies which demonstrated that SB 211475 has virtually no β-adrenoceptor blocking activity.

Figure 4 illustrates representative recordings of SMA rings isolated from rats at the end of the experimental period after being subjected to either sham SI/R shock or SI/R shock. SMA rings isolated from sham-operated control rats precontracted with U-46619 relaxed fully to the endothelium-dependent vasodilator, ACh, as well as to the endothelium-independent vasodilator, $NaNO_2$. In contrast, SMA rings isolated from rats subjected to SI/R shock and treated with only vehicle exhibited diminished vasorelaxant responses to ACh, but a normal response to $NaNO_2$, indicating severe endothelial dysfunction but normal vascular smooth muscle responsiveness. SB 211475, at high and low doses, and high dose carvedilol all protected against this shock-induced endothelial dysfunction. However, treatment with 1 mg kg^{-1} SB 211475 exerted the most significant protection. Low dose carvedilol (i.e., 0.5 mg kg^{-1}) exerted no significant protection from shock-induced endothelial dysfunction.

Figure 5 presents a summary of the vasorelaxant responses of isolated SMA rings from rats following SI/R to increasing doses of an endothelial-dependent vasodilator, ACh, or to an endothelium-independent vasodilator, acidified $NaNO_2$. SMA rings from sham shock rats exhibited complete vascular relaxation to both the endothelium-dependent (10 μM ACh) and the endothelium-independent vasodilators (100 μM $NaNO_2$). In contrast, SMA rings from vehicle-treated SI/R rats responded to 10 μM ACh with only a $44\pm4.0\%$ relaxation. However, the response of these rings to the endothelium-independent vasodilator, $NaNO_2$, was normal. SMA rings from SI/R shock rats treated with either dose of SB 211475 (1.0 or 0.5 mg kg^{-1}) or with high dose carvedilol, demonstrated improved endothelium-dependent vasorelaxation responses. The concentration-response curves of these rings to ACh were significantly shifted to the left. In contrast, SMA rings from SI/R shock rats treated with low dose carvedilol exhibited depressed vasorelaxation responses to ACh, similar to those observed in vehicle-treated SI/R shock rats. These findings indicate that SI/R shock results in a vascular endothelial, not smooth muscle, dysfunction which

Table 1 Heart rate change 1 min after drug administration

	Sham SI/R	*SI/R+ vehicle*	*SI/R+ SB 211475* (0.5 mg kg^{-1})	*SI/R+ SB 211475* (1 mg kg^{-1})	*SI/R+ carvedilol* (0.5 mg kg^{-1})	*SI/R+ carvedilol* (1 mg ml^{-1})
HR changes beats min^{-1}	1.3 ± 0.7	1.6 ± 1.6	5 ± 1.7	2.1 ± 1.5	-32.2 ± 1.9**	-25.4 ± 4.4**

HR = heart rate; $^{**}P<0.01$ vs vehicle.

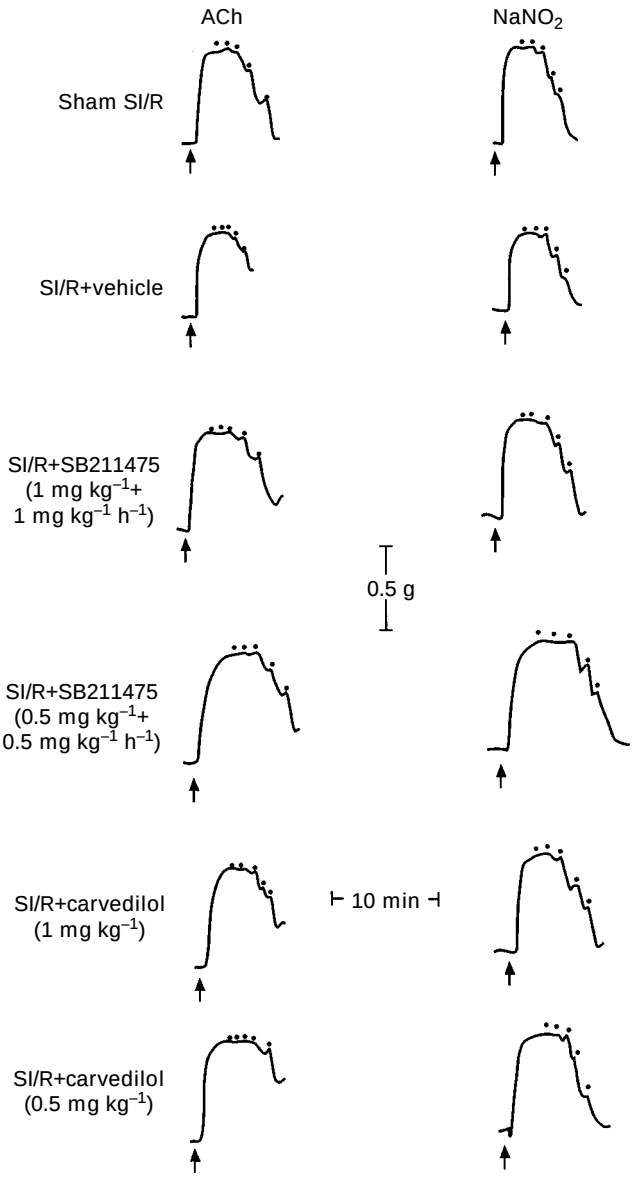

Figure 4 Representative recordings of endothelium-dependent vasodilator, ACh, and endothelium-independent vasodilator, $NaNO_2$-induced relaxation of U-46619 precontracted superior mesenteric arterial (SMA) rings isolated from sham SI/R and SI/R rats given vehicle, SB 211475 or carvedilol. The arrows indicate addition of U-46619 (50 nM); dots on top indicate addition of ACh (0.001 – 10 μM) or $NaNO_2$ (0.1 – 100 μM).

both high and low dose SB 211475, but only high dose carvedilol, can significantly attenuate.

Discussion

Originally marketed as a β-adrenoceptor blocking antihypertensive agent, carvedilol has recently been shown to possess antioxidant effects (Yue *et al*., 1992; Lopez *et al*., 1995; Maggi *et al*., 1996). In a previous study, we have demonstrated that administration of carvedilol 5 min before reperfusion exerts significant anti-shock and endothelial protective effects in SI/R shock (Christopher *et al*., 1995). In contrast, administering an equivalent dose of propranolol, a comparable β-blocker lacking free radical scavenger activity at the dose employed, failed to exert significant protective effects in this severe form of circulatory shock. These results suggest that the protective effects of carvedilol against post-ischaemic tissue injury in splanchnic organs are unrelated to its β-blocking effect.

Recent pharmacodynamic and pharmacokinetic studies have demonstrated that carvedilol is extensively metabolized *in vivo* with less than 2% of the dose excreted unchanged in the urine (Ruffolo *et al*., 1990). SB 211475, (1-[3-hydroxycarbazolyl-(4)-oxy]-3-[(2-methoxyphenoxyethyl)amino]-propanol-(2)), is one of the main metabolites of carvedilol in which an ·OH group is introduced at position 3 of its carbazole moiety. *In vitro* pharmacological 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*., 1994). Specifically, SB 211475 has been shown to quench superoxide anions and protect endothelial cells against xanthine/xanthine oxidase-initiated cytotoxicity. 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 (Yue *et al*., 1994). These values are comparable or even lower than IC_{50} values of the newly developed potent antioxidant U78517F (Yue *et al*., 1994). Taken together, these results suggest that SB 211475 may exert more protective effects against post-ischaemic tissue injury where free radicals play critical roles.

In the present study, we tested the anti-shock and endothelial protective effects of SB 211475 in a rat splanchnic ischaemia and reperfusion model. SB 211475, when administered just before reperfusion, exerted significant protective effects in a dose-dependent manner. Our preliminary experiment showed that administration of 0.1 mg kg^{-1} SB 211475 exerted no significant protection in this lethal model of shock (data not shown). However, administration of 0.5 mg kg^{-1} SB 211475 maintained blood pressure over a sustained period after reperfusion, prolonged survival times, increased survival rates, attenuated neutrophil accumulation, lessened haemoconcentration and preserved endothelial function. Administration of 1 mg kg^{-1} SB 211475 exerted the most significant protective effect of all of the groups. Carvedilol administered at a 1 mg kg^{-1} dose attenuated tissue injury to a comparable degree as did 0.5 mg kg^{-1} SB 211475. At a dose of 0.5 mg kg^{-1}, carvedilol demonstrated no significant protection in this model.

Administration of carvedilol at both doses tested in the present study resulted in a significant decrease in heart rate. In contrast, administration of a major metabolite of carvedilol, SB 211475, at either dose did not result in any significant change in heart rate, but exerted more protective effects against SI/R-induced tissue injury. These results indicate that the antioxidant property, but not the β-adrenoceptor blockade property of carvedilol, play a significant role in protection against SI/R injury. In this connection, we have previously demonstrated that infusion of superoxide dismutase in this model exerted comparable protection against SI/R injury as that obtained with carvedilol (Christopher *et al*., 1995). Besides its potent antioxidant property, SB 211475 possesses selective α_1 adrenoceptor blocking activity, although less potent than its parent compound, carvedilol (K_B value $= 2.6 \times 10^{-8}$ M vs 1.2×10^{-8} M for carvedilol, Yue *et al*., unpublished observations). The role of a potential α_1 adrenoceptor antagonist component of carvedilol and SB 211475 in observed anti-shock effects is perhaps more complicated and cannot be determined precisely in the present study. Vasodilatation resulting from α_1 adrenoceptor blockade may improve the microcirculation and thus attenuate tissue injury. On other

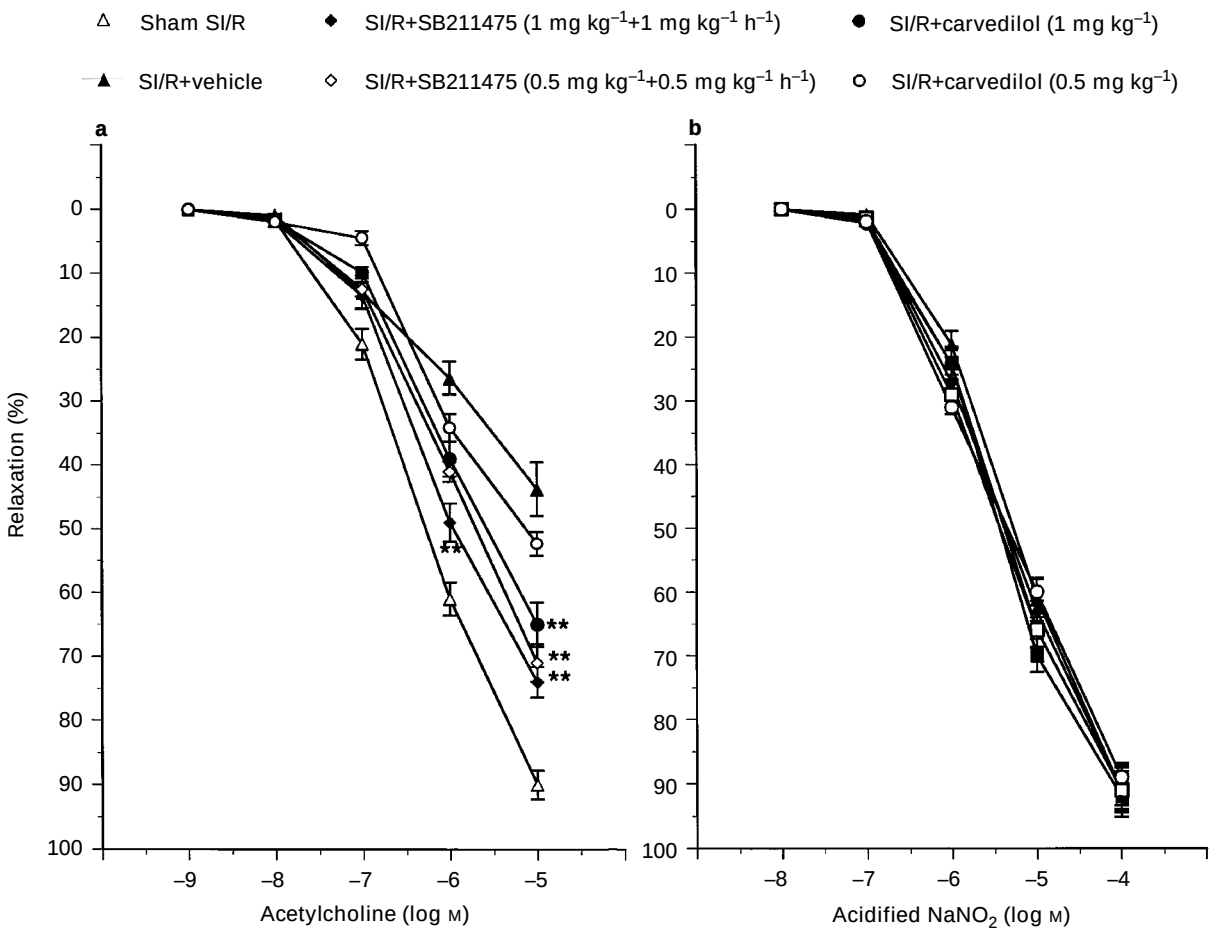

Figure 5 Summary of vasorelaxation responses of sham SI/R and SI/R SMA rings (9 to 11 rings from 3 to 4 rats in each group given vehicle, SB 211475 or carvedilol) to increasing concentrations of (a) ACh and (b) $NaNO_2$ to determine endothelium function. $^{**}P<0.01$ vs rings from SI/R shock rats given vehicle.

hand, a hypotensive response to α_1 adrenergic blockade may aggravate shock status and enhance tissue injury. In the present study, we have demonstrated that SB 211475, a metabolite of carvedilol that possesses a weaker α_1-adrenoceptor blocking activity, exerted more significant anti-shock effects than carvedilol. Specifically, administration of 0.5 mg kg^{-1} SB 211475 exerted similar or more protective effects against post-ischaemic tissue injury compared with the effects seen with 1 mg kg^{-1} carvedilol. Therefore, it is unlikely that α_1-adrenoceptor antagonism contributes significantly to the protective effects of SB 211475.

The antioxidant action of carvedilol and its hydroxylated metabolite, SB 211475, may attenuate post-ischaemic tissue injury via a variety of mechanisms. It is well recognized that superoxide anion plays an important role in post-ischaemic tissue injury (Granger *et al*., 1986). Although superoxide itself has limited reactivity with most biological molecules, it can cause tissue injury by forming more toxic oxidants such as $^{\cdot}OH$ and peroxynitrite ($ONOO^-$) (Bolli, 1991; Beckman, 1994). By scavenging superoxide anion, carvedilol and SB 211475 can prevent the formation of more toxic oxidants, and thus reduce post-ischaemic oxidative tissue injury.

Anther important mechanism by which superoxide and hydrogen peroxide cause tissue injury is by upregulation of PMN adhesion molecules, such as P-selectin, on the endothelial surface, thereby inducing PMN accumulation and PMN-related tissue injury (Gaboury *et al*., 1994). P-selectin, also known as granule membrane protein-140 (GMP-140), is an integral membrane glyprotein located in platelets and in Weibel-Palade bodies of endothelial cells. Upon activation of the endothelium with agonists such as thrombin, histamine and free radicals, the Weibel-Palade bodies fuse with the plasma membrane, and P-selectin is rapidly translocated to the endothelial cell surface, within approximately 5 min, where it tethers circulating PMNs and positions them for activation by endothelial-bound platelet aggregating factor (Patel *et al*., 1991). This P-selectin mediated PMN-endothelial interaction has been demonstrated to be one of the most important steps in the PMN adhesion and migration process (Lasky, 1993). Ischaemia followed by reperfusion is associated with a burst of free radical generation at the onset of reperfusion and may thus stimulate the expression of P-selectin and promote PMN adherence to the endothelial cells (Weyrich *et al*., 1993). By scavenging oxygen free radicals, carvedilol and SB 211475 may inhibit the upregulation of leukocyte adhesion molecules and thus prevent PMN-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 SB 211475 significantly reduced the MPO activity, a marker of PMN accumulation, in the ischaemic-reperfused tissue.

In summary, in this model of murine SI/R shock, we have demonstrated the beneficial effects of a potent antioxidant metabolite of carvedilol, SB 211475, which unlike the parent

compound, has virtually no β-adrenoceptor blocking activity. SB 211475 most likely ameliorates the adverse effects of SI/R shock by quenching superoxide anion, thus preventing the formation of hydroxyl radical and peroxynitrite and reducing the tissue injury induced by these highly toxic molecules. Furthermore, by scavenging ROS, SB 211475 may inhibit PMN accumulation in post-ischaemic tissue and therefore attenuate PMN-induced endothelial dysfunction and tissue injury. Our results provide further evidence that carvedilol exerts its anti-shock and endothelial protective effects primarily through its antioxidant activity.

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