FULL PAPER *Internal Medicine*

Cardiovascular and Renal Effects of Carvedilol in Dogs with Heart Failure

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ABSTRACT. To determine the acute effects of carvedilol (β-blocker) on cardiovascular and renal function and its pharmacokinetics in dogs. Fifteen mature mongrel dogs (7–15 kg) of both sexes were used in these experiments. Eight dogs served as controls, and seven dogs served as iatrogenic mitral regurgitation (MR) experimental animals. Carvedilol (0.2, 0.4, and 0.8 mg/kg, P.O.) was administered, and the blood carvedilol concentration was analyzed by reverse-phase high-performance liquid chromatography. The response to isoproterenol or phenylephrine was also evaluated. Isoproterenol (0.025 μg/kg/min) was infused via the saphenous vein for 5 min, and phenylephrine (5 μg/kg) was injected with carvedilol (0.2, 0.4 mg/kg) or placebo for 4 days. The heart rate and arterial blood pressure were measured, and LV fractional shortening was measured by echocardiography. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were measured by intravenous infusion of sodium thiosulfate and sodium para-aminohippurate. Carvedilol (0.2 mg/kg) decreased the heart rate, whereas renal function, arterial blood pressure, and left ventricular contractile function were not affected. Carvedilol (0.4 mg/kg) decreased heart rate, blood pressure, and renal function. The tachycardic response to isoproterenol was significantly diminished for 36 hr by 0.4 mg/kg carvedilol. Carvedilol 0.2 mg/kg inhibited this effect for 24 hr. Thus, it is necessary to titrate the dosage of carvedilol, it should be initiated at less than 0.2 mg/kg and titrated up to 0.4 mg/kg for heart failure dogs.

KEY WORDS: β-blocker, heart failure, hemodynamics, mitral regurgitation, renal function.

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Chronic activation of the sympathetic nervous system significantly contributes to the progression of left ventricular dysfunction to end-stage heart failure [33]. The deleterious effects of prolonged sympathetic stimulation are diminished by β-adrenergic blocking agents [1, 16, 17]. Carvedilol (β-blocker) is a unique multiple-action drug, a nonselective β-blocker with vasodilatory properties due to α_1-blockade [37], and a scavenger of oxygen free radicals [9, 22, 40]. Long-term therapy with carvedilol improves the resting left ventricular performance and patients' functional class [4, 12, 21]. Carvedilol therapy may also increase survival in patients with chronic heart failure [2, 4, 20, 21]. Clinical studies on human subjects have shown that carvedilol improves both idiopathic and ischemic congestive heart failure [5, 31]. Furthermore, recent studies have shown a significant improvement in mortality (65%) after carvedilol therapy when it is combined with conventional treatments for congestive heart failure [32]. Additionally, carvedilol is beneficial in renal dysfunction [27] and significantly limits infarct size in dogs [35].

Sawangkoon *et al.* [36] explored the acute cardiovascular effects and pharmacokinetics of intravenous carvedilol administration in healthy dogs. However, there have been no comprehensive studies of the effects of oral carvedilol administration on cardiovascular and renal function in dogs. Additionally, there are no data available on the efficacy of carvedilol in dogs with heart failure. In this study, we report the cardiovascular and renal functional effects and pharmacokinetics of oral carvedilol in dogs.

MATERIALS AND METHODS

The Institutional Laboratory Animal Care and Use Committee of The School of Veterinary Medicine and Animal Science of Kitasato University approved this study. Fifteen mature mongrel dogs (7–15 kg) of either sex were used in these experiments. Eight dogs served as controls, and seven dogs served as iatrogenic mitral regurgitation (MR) experimental animals. Dogs were given commercial dry food (Hill's Colgate Japan, Tokyo, Japan). Before the experiments began, all dogs were allowed to become accustomed to the experimental room and handling and were able to remain quiet while on the examination table.

Iatrogenic mitral regurgitation: Before surgery to induce experimental MR, 7 dogs were tranquilized with butorphanol (0.1–0.2 mg/kg) and diazepam (5 mg/kg), anesthetized with Ketamine (5–10 mg/kg) and isoflurane, and intubated. During surgery, the dogs were anesthetized with isoflurane (1.5–2.0%) with 100% O_2. Body temperature was measured with an anal probe and maintained at 37–38°C with a heating pad. The heart rate was also monitored.

The chest was opened at the fifth intercostals space under sterile conditions. The pericardium was opened, and a cutting hook was inserted through the apex to cut the mitral chordae. The end point of mitral chordae disruption was determined by color Doppler echocardiography as the point at which the regurgitation jet encompassed two thirds of the left atrial area. The chest was closed in layers and evacuated by standard procedures. The dogs were placed on an antibiotic regimen (ampicillin sodium) for 5 days postoperatively. Follow-up care included daily monitoring of the heart rate, respiratory rate, and temperature. The heart and lungs were

auscultated on a daily basis. Serial chest X-rays were performed in each dog to identify the onset of pulmonary venous congestion. The experiments were performed 3–4 months after the induction of MR.

Instrumentation: All dogs were anesthetized as described above. To measure arterial blood pressure, Tygon catheters (Norton elastic and synthetic division, Akron, OH) were implanted in the abdominal aorta through the femoral artery. The catheter was exteriorized between the scapulae. The dogs were allowed to recover for 4 days after the implantation procedure. The catheter was flushed with heparin every other day and was covered with a jacket.

Plasma carvedilol concentration measurements: Five control dogs were used to determine plasma carvedilol concentrations. Carvedilol (Artist, Daiichi Pharmaceutical Co., Ltd., Tokyo, 0.2, 0.4, and 0.8 mg/kg, P.O.) was administered at 9:00 AM each day. Blood for measuring the blood carvedilol concentration was drawn from the jugular vein before the final administration and 1, 2, 4, 8, 12, and 24 hr after the final administration. Plasma samples were analyzed by on-line, solid-phase extraction followed by reverse-phase high-performance liquid chromatography (Nanospace SI-2, Shiseido, Tokyo).

Cardiovascular effects of carvedilol: Five control dogs and five MR dogs were used to investigate the cardiovascular effects of carvedilol. To determine the efficacy of α- and β-receptor blockade with carvedilol, the responses to isoproterenol (Protanol-L, Nikken Chemicals Co., Ltd., Tokyo) and phenylephrine (Neo-Synesin, Kowa, Nagoya) were evaluated. Isoproterenol (0.025 μg/kg/min) was infused via the saphenous vein for 5 min. Phenylephrine (5 μg/kg) was also injected via the saphenous vein. Either placebo or carvedilol at 0.2 mg/kg or 0.4 mg/kg was administered. Isoproterenol or phenylephrine was administered at before and 3, 12, 24, 36, and 48 hr after carvedilol administration. Heart rate was calculated from an ECG. Arterial blood pressure was measured via the femoral artery catheter, which was connected a fluid-filled transducer (Nihon Kohden Corporation, Tokyo, Japan). ECG and arterial pressure were analyzed by a computer program (Notocord, Croissy, France). As an index of left ventricular (LV) contractility, LV fractional shortening was measured by echocardiography (SONOS 5500, Hewlett Packard, Tokyo, Japan). All measurements were performed after the dogs were sedated with 0.1 mg/kg of butorphanol.

Renal functional effects of carvedilol: Either placebo or carvedilol at 0.2 mg/kg or 0.4 mg/kg was administered. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were measured by intravenous infusion of sodium thiosulfate (Wako Pure Chemical Industries, Ltd., Osaka) and sodium para-aminohippurate (PAH) (Daiichi Pharmaceutical Co., Ltd., Tokyo). Six control and six MR dogs emptied their bladders at 8:00 AM. Studies of renal function began at 8:30 AM after an overnight fast under constant environmental conditions. Clearance studies were performed during a state of water diuresis to assess the tubular function of the proximal nephron. The dogs were given a loading dose of water (10 m*l*/kg body weight) over a 60-min period. At 9:30AM, blood was drawn from the jugular vein to measure basal PAH and sodium thiosulfate. Subsequently, a priming bolus of PAH (6.0 mg/kg) and sodium thiosulfate (50 mg/kg) diluted in 0.7 m*l*/kg of 5% glucose was given over a 5-min period. The bolus was followed by a constant infusion (0.02 m*l*/kg/min) of a solution containing 0.0625 m*l* of 20% PAH and 1.0 m*l* 10% sodium thiosulfate diluted in 500 m*l* of 5% glucose. This rate of infusion resulted in PAH and sodium thiosulfate plasma concentrations of 19.8 ± 2.5 and 2.7 ± 0.4 mg/d*l*, respectively. After a 45-min equilibration period, urine was taken from the bladder. Three 30-min clearance periods were observed, and urine and blood samples were collected at each interval.

Data analysis: All data are expressed as the mean ± SEM. Data were analyzed by ANOVA followed by post hoc testing (Dunnett's test). A value of $p<0.05$ was considered statistically significant.

RESULTS

Plasma carvedilol concentration in dogs: The lowest measurable concentration of carvedilol for which this test is accurate is 0.5 *n*g/m*l*. The plasma concentrations of carvedilol after oral administration increased 1 to 4 hr after administration. The peak concentration for each dosage appeared 1 to 4 hr after administration, and the plasma concentrations returned to near baseline after 8 hr (Fig. 1). At the 0.2 mg/kg dose, the plasma carvedilol concentration was less than 0.5 *n*g/m*l* after 4 hr.

Cardiovascular effects of carvedilol in control dogs: The heart rate decreased significantly compared to placebo 3 hr after the administration of 0.2 mg/kg carvedilol ($p<0.05$) and 0.4 mg/kg carvedilol ($p<0.05$). Systolic and mean blood pressure decreased only with the administration of 0.4 mg/kg carvedilol. The fractional shortening did not differ from the baseline in either dogs given either 0.2 mg/kg or 0.4 mg/kg carvedilol (Table 1).

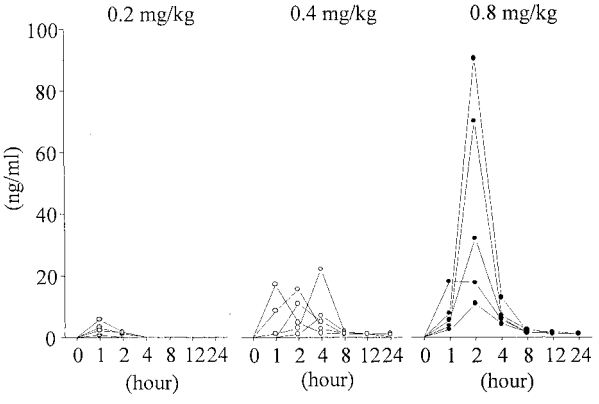

Fig. 1. Plasma carvedilol concentrations measured after oral administration of incremental doses of carvedilol to healthy dogs.

Table 1. Baseline hemodynamics in control dogs with carvedilol

		n	Baseline	3 hr	24 hr	36 hr	48 hr
Heart rate	placebo	5	71 ± 2	75 ± 3	72 ± 5	73 ± 4	76 ± 8
(beats/min)	Carvedilol 0.2	5	73 ± 2	66 ± 2*	68 ± 4	70 ± 3	68 ± 2
	Carvedilol 0.4	5	75 ± 1	66 ± 2*	73 ± 5	68 ± 2	65 ± 5
Systolic pressure	placebo	5	126 ± 3	122 ± 1	127 ± 2	127 ± 1	134 ± 3
(mmHg)	Carvedilol 0.2	5	127 ± 4	122 ± 5	124 ± 2	124 ± 4	125 ± 5
	Carvedilol 0.4	5	127 ± 3	123 ± 1	122 ± 2*	120 ± 2*	128 ± 3
Mean pressure	placebo	5	83 ± 2	82 ± 2	84 ± 1	84 ± 2	86 ± 4
(mmHg)	Carvedilol 0.2	5	85 ± 3	82 ± 3	82 ± 2	82 ± 2	80 ± 3
	Carvedilol 0.4	5	85 ± 3	78 ± 1*	78 ± 2*	78 ± 2*	80 ± 4
Diastolic pressure	placebo	5	59 ± 4	57 ± 3	58 ± 2	58 ± 2	60 ± 5
(mmHg)	Carvedilol 0.2	5	60 ± 4	59 ± 3	56 ± 3	57 ± 3	54 ± 5
	Carvedilol 0.4	5	59 ± 3	55 ± 3	55 ± 3	51 ± 3	56 ± 5
FS (%)	palcebo	5	37 ± 2	36 ± 2	40 ± 3	38 ± 2	37 ± 2
	Carvedilol 0.2	5	39 ± 3	38 ± 1	37 ± 1	35 ± 2	40 ± 2
	Carvedilol 0.4	5	36 ± 3	35 ± 2	39 ± 1	37 ± 1	38 ± 1

Data are mean ± SEM., HR, heart rate; FS, fractional shortening. * is compared with placebo ($p<0.05$).

Table 2. Cardiovascular effects of carvedilol in MR dogs

		n	Baseline	3 hr	6 hr	12 hr	24 hr
Heart rate	placebo	5	115 ± 13	103 ± 12	111 ± 15	109 ± 12	110 ± 11
(beats/min)	Carvedilol 0.2	5	116 ± 14	103 ± 12*	102 ± 13*	99 ± 13*	107 ± 13
	Carvedilol 0.4	5	109 ± 10	85 ± 9*	84 ± 9*	88 ± 9*	89 ± 10*
Systolic pressure	placebo	5	151 ± 3	146 ± 5	148 ± 4	148 ± 4	134 ± 11
(mmHg)	Carvedilol 0.2	5	149 ± 4	143 ± 5	145 ± 2	152 ± 2	145 ± 2
	Carvedilol 0.4	5	146 ± 5	139 ± 8	142 ± 5	141 ± 6	143 ± 3
Mean pressure	placebo	5	105 ± 2	101 ± 4	105 ± 4	106 ± 2	97 ± 7
(mmHg)	Carvedilol 0.2	5	103 ± 3	98 ± 1	103 ± 4	107 ± 3	104 ± 2
	Carvedilol 0.4	5	103 ± 4	99 ± 7	95 ± 4	99 ± 6	99 ± 3
Diastolic pressure	placebo	5	78 ± 4	74 ± 4	78 ± 5	80 ± 2	75 ± 3
(mmHg)	Carvedilol 0.2	5	77 ± 4	73 ± 2	78 ± 4	80 ± 4	79 ± 3
	Carvedilol 0.4	5	76 ± 5	72 ± 7	70 ± 3	75 ± 5	74 ± 4
FS (%)	placebo	5	40 ± 2	36 ± 2	37 ± 2	38 ± 1	36 ± 3
	Carvedilol 0.2	5	37 ± 3	34 ± 2	37 ± 3	34 ± 2	33 ± 3
	Carvedilol 0.4	5	37 ± 2	36 ± 1	36 ± 1	34 ± 1	35 ± 1

Data are mean ± SEM., HR, heart rate; FS, fractional shortening. * is compared with placebo ($p<0.05$).

Cardiovascular effects of carvedilol in MR dogs: The heart rate decreased significantly for 12 hr in dogs given 0.2 mg/kg carvedilol and for 24 hr in dogs given 0.4 mg/kg carvedilol. However, systolic, mean, and diastolic blood pressure and fractional shortening did not differ from the baseline in either dogs given either 0.2 mg/kg or 0.4 mg/kg carvedilol (Table 2).

Response to Isoproterenol and phenylephrine with carvedilol in control dogs: The response of the heart rate to isoproterenol in dogs given 0.4 mg/kg carvedilol was significantly suppressed for 36 hr (Fig. 2). The chronotropic effects of isoproterenol were also suppressed after 3 hr in dogs given 0.2 mg/kg carvedilol. The fractional shortening response to isoproterenol in dogs given either 0.2 or 0.4 mg/kg carvedilol did not differ from that in placebo dogs. No differences in heart rate or systolic pressure in response to phenylephrine were observed in any of the groups (Fig. 3).

Renal function in control and MR dogs: The RPF decreased more in MR dogs (75 ± 9 ml/min) than in placebo dogs (88 ± 7 ml/min). The RPF of the dogs given 0.2 mg/kg carvedilol (81 ± 9 ml/min) was not different from that of untreated MR dogs. The RPF of dogs given 0.4 mg/kg carvedilol revealed 64 ± 4 ml/min. The GFR decreased significantly in the untreated MR dogs (29 ± 2 ml/min) and in dogs given 0.4 mg/kg carvedilol (29 ± 4 ml/min) compared with placebo dogs (44 ± 6 ml/min). However, the GFR remained higher in dogs given 0.2 mg/kg carvedilol (37 ± 5 ml/min) (Fig. 4).

DISCUSSION

Carvedilol was rapidly absorbed following oral administration. The maximum plasma concentration occurred within 1 to 4 hr in dogs. In humans, the median termination half-life of oral carvedilol ranged from 4 to 7 hr [24]. The plasma protein binding of carvedilol is independent of con-

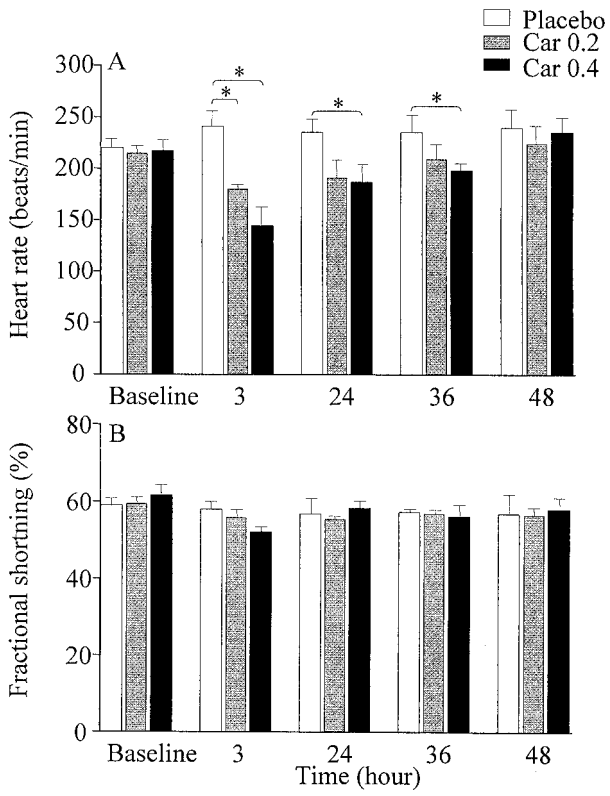

Fig. 2. Heart rate (A) and fractional shortening (B) response to isoproterenol (0.025 μg/kg/min, i.v., 5 min) after carvedilol administration. Car 0.2 is carvedilol 0.2 mg/kg. Car 0.4 is carvedilol 0.4 mg/kg. Baseline is before administration. * Significantly (P<0.05) different from placebo.

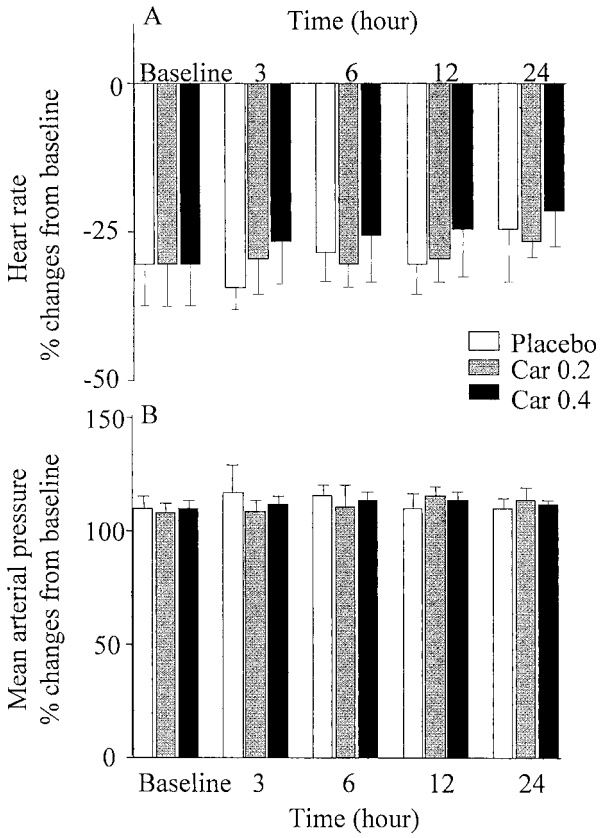

Fig. 3. Heart rate (A) and mean arterial pressure (B) response to phenylephrine (5 μg/kg, i.v.) after carvedilol administration. Car 0.2 is carvedilol 0.2 mg/kg. Car 0.4 is carvedilol 0.4 mg/kg. Baseline is before administration.

centration at concentrations from 50–1,000 ng/m*l* [35]. Food decreases the absorption rate of carvedilol and, therefore, prolongs the time to peak plasma concentrations. However, dose does not alter the extent of absorption of the drug [23]. Carvedilol is extensively metabolized, and less than 2% of a dose is excreted unchanged in the urine. The major route of elimination of these metabolites is biliary excretion [28].

In control dogs, tachycardia induced by isoproterenol was significantly diminished for 36 hr with 0.4 mg/kg carvedilol, and 0.2 mg/kg carvedilol inhibited this effect for 24 hr. LV contractile function (fractional shortening) was not changed by either 0.2 or 0.4 mg/kg carvedilol. The bradycardic effect of oral carvedilol was dose-dependent and continued for 3 to 24 hr in dogs with iatrogenic mitral regurgitation. Thus, the clinically useful dosage of carvedilol may be in the range of 0.2 to 0.4 mg/kg given once daily.

Carvedilol inhibits the β_1-adrenoceptor-mediated positive chronotropic effect of isoproterenol. Carvedilol's β_2-adrenergic antagonism also inhibits the β_2-receptor-mediated relaxation induced by isoproterenol in guinea pig trachea in a concentration-dependent manner [29]. An *in vivo* study in rats has shown that intravenous carvedilol significantly inhibits the β_2-adrenoceptor-mediated vasodilator response to salbutamol and suggests that carvedilol inhibits β_1- and β_2-adrenoceptors to a similar degree *in vivo* [30]. The α_1-blocking activity of carvedilol plays a role in reducing blood pressure [14]. However, the α-blocking activity of carvedilol in dogs is 3.8 times less potent than the β-blocking activity [19]. In the isolated rabbit aorta, carvedilol is approximately ten-fold less potent as an α_1-antagonist than as a β-antagonist [29]. In this study, the response of the heart rate to isoproterenol was diminished by 40% with 0.4 mg/kg carvedilol. However, in agreement with previous studies, carvedilol failed to diminish the hypertensive response to 5 μg/kg phenylephrine, an α-adrenergic agonist.

Elevated catecholamines are acutely beneficial in the restoration of cardiac compensation in heart failure [13, 15, 38]. However, long-term activation of the sympathetic nervous system is deleterious [3, 16, 17, 39]. Therefore, α-adrenergic receptor blockade provides substantial benefits to many patients with heart failure [2, 4, 12, 21]. Nagatsu *et al.* [26] suggested that slowing the heart rate is a key mechanism by which β-blockade ameliorates myocardial dysfunction in chronic experimental mitral regurgitation. In this

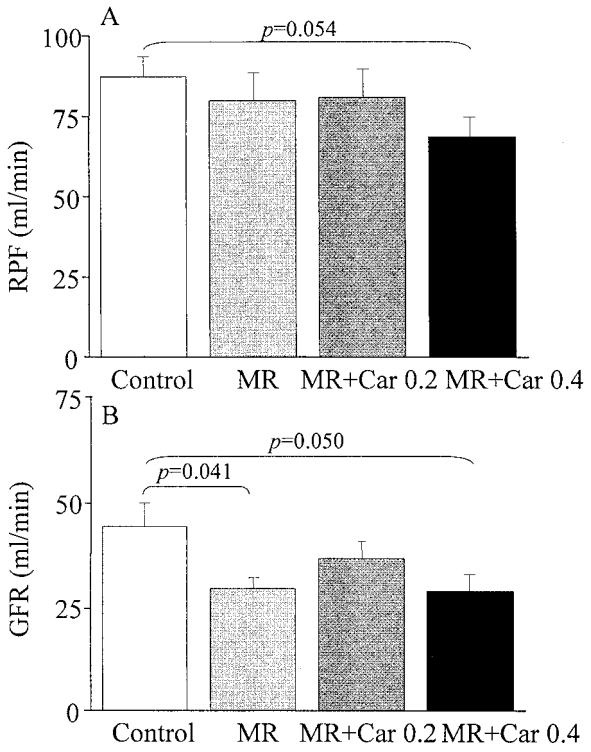

Fig. 4. Comparison of renal plasma flow (RPF) (A) and glomerular filtration rate (GFR) (B) in control and mitral regurgitation (MR) dogs with and without Carvedilol. Car 0.2 is carvedilol 0.2 mg/kg. Car 0.4 is carvedilol 0.4 mg/kg.

study, we found that heart rate and mean arterial pressure in dogs with iatrogenic mitral regurgitation decreased for 24 hr after carvedilol administration, but fractional shortening was not affected. However, Nagatsu *et al.* [25] have shown that the acute administration of β-blockers worsens hemodynamics and contractile function in dogs with experimental MR. In addition, several papers have described human patients with heart failure who could not tolerate β-blockade therapy because of the diminished contractile function, as evidenced by the heart rate, blood pressure, and pulmonary wedge pressure at the initiation of β-blockade therapy [8]. However, since it is difficult to monitor hemodynamics and ventricular contractile function in conscious dogs, using 0.2 mg/kg of carvedilol at the initiation of therapy to preserve contractile function and decrease heart rate and loading may be beneficial in initial treating animals with heart failure.

Chronic overload decreases the contractility of both right and left ventricles and is associated with oxidative stress in both ventricles. *In vitro* studies on rat cardiac myocytes [18] and papillary muscles [11] have demonstrated that oxygen-derived free radicals lead to a decrease in systolic cell shortening and force of contraction and to an increase in diastolic tension. Vitamin E prevents the chronic volume overload-induced decrease in cardiac contractility in dogs [34]. This suggests that oxygen free radicals are involved in the chronic volume overload induced cardiac depression. Flesch *et al.* [10] presents carvedilol and its metabolite partly prevent OH•-induced contractile dysfunction. Therefore, anti-oxidant activity of carvedilol may preserve cardiac contractility on chronic overload in dogs depressed by oxygen-derived free radicals.

Renal plasma flow and GFR tended to reveal low values with 0.4 mg/kg carvedilol, whereas 0.2 mg/kg carvedilol preserved renal plasma flow and slightly increased GFR. Dupont *et al.* [6] examined renal hemodynamics after carvedilol treatment in a randomized, double-blind, placebo-controlled study. Renal blood flow remained constant, but renal vascular resistance was significantly decreased by carvedilol both acutely and after four weeks of treatment. The GFR was slightly reduced acutely but returned to baseline levels after four weeks of treatment. Plasma rennin activity and aldosterone levels were significantly reduced after four weeks. Additionally, Zitta *et al.* [41] have also shown that an acute protein load in essential hypertension induces a decrease in GFR that normalizes after antihypertensive treatment with carvedilol. These suggest that the chronic administration of carvedilol preserves renal function, that is, renal blood flow and GFR.

In conclusion, although the chronic administration of β-blockers improves hemodynamic, contractile function, and renal function, acute β-blockade may be a source of concern. Therefore, the strategy for administering β-blockade in the setting of chronic heart failure is to start at a very low dose and increase it gradually, usually at one- or two-week intervals [7]. Carvedilol 0.2 mg/kg decreased heart rate, but did not affect renal function, arterial blood pressure, or left ventricular contractile function. Carvedilol 0.4 mg/kg decreased heart rate as well as blood pressure and renal plasma flow. Thus, it is necessary to titrate the dosage of carvedilol, it should be initiated at less than 0.2 mg/kg and titrated up to 0.4 mg/kg for heart failure dogs. Since it is difficult to monitor hemodynamics and ventricular contractile function in clinical cases, using less than 0.2 mg/kg of carvedilol at the initiation of therapy to preserve contractile function and decrease heart rate and loading may be beneficial in treating dogs with heart failure.

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