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POTENTIAL FOR CARVEDILOL TO MODIFY DOXORUBICIN CARDIOTOXICITY

DISSERTATION

Presented in Partial Fulfillment of the Requirements for

the Degree Doctor of Philosophy in the Graduate

School of The Ohio State University

By

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*****

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2002

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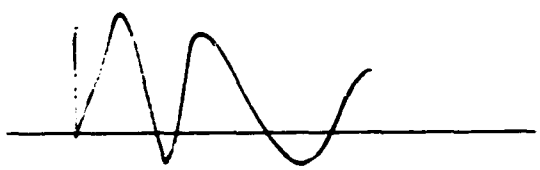

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ABSTRACT

Carvedilol is a beta blocker which also has α_1-adrenergic blocking and free radical scavenging activities. The commercial drug is a racemic mixture of both R(+) and S(-) enantiomers, and is available in an oral preparation. It has been used for treatment of patients with hypertension, cardiac ischemia, and heart failure. Carvedilol is reported to improve left ventricular function, and to prevent the remodeling processes. However, there is little information about the use of carvedilol in veterinary medicine. The objectives of the present study were to study the physiological and pharmacological effects of carvedilol in dogs, and to determine if carvedilol has a protective effect against doxorubicin-induced cardiotoxicity. Results indicate that acute effects of intravenous carvedilol on cardiac function include increase in heart rate and velocity of fiber shortening at zero load (V_{max}), shortening of PQ interval, and decrease in systemic and pulmonary arterial systolic pressures. Pharmacokinetic studies after an intravenous injection of carvedilol at 100 μg/kg showed that the plasma concentration curve was fitted by a two-compartment model. Half lives of distribution and elimination phases were 3.6 and 52 minutes respectively. The mean clearance was 24.3 ml/kg/min, and the mean volume of distribution was 1830 ml/kg. The increase in heart rate, in response to an infusion of 0.512 μg/kg/min of isoproterenol, was decreased approximately 50% by an oral dose of approximately 0.3125 mg/kg of carvedilol at the 1st, 2nd, and 4th hours after

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the last dose of carvedilol. The dose of isoproterenol required to increase heart rate 50% of the increase achieved when no carvedilol was given, increased proportionally with the amount of carvedilol given (0.15625, 0.3125, 0.625, and 1.25 mg/kg). The amount of isoproterenol, required to increase heart rate 50% of the maximal increase, increased to a maximum at the 4^{th} hour after dosing with carvedilol. Protective effects of carvedilol on doxorubicin-induced cardiotoxicity were demonstrated. Dogs given doxorubicin and carvedilol maintained PEP/ET ratios, systemic blood pressures, and V_{max}. Moreover, they also had decreases in tau and minimal QT prolongation, and had higher heart rate variability. The present study suggests that carvedilol protects the heart from doxorubicin by improving left ventricular function, decreasing energy required for contraction and relaxation, and preventing cardiac arrhythmias.

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Dedicated to my parents

Haumong Tieu

Kimlung Lim

Who gave me my life, always encourage my endeavor, and will

support whoever will I be

Dedicated to my teacher

Professor Robert L. Hamlin

Who inspires me and brings me to the world of cardiology

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3. N. Chaiyabutr, S. Komolvanish, **S. Sawangkoon**, S. Preuksagorn, and S. Chanpongsang, "Glucose metabolism is vivo in crossbred Holstein cattle feeding on different types of roughage during late pregnancy and early lactation." *Comp. Biochem. and Physiol. A*, 119(4), 905, (1998).

4. N. Chaiyabutr, S. Komolvanish, **S. Sawangkoon**, S. Preuksagorn, and S. Chanpongsang, "The regulation of body fluids and mammary circulation during late pregnancy and early lactation of crossbred Holstein cattle feeding on different types of roughage." *J. of Anim. Physiol. and Anim. Nutri.*, 77(4-5), 167, (1997).

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CHAPTER 1

INTRODUCTION

CARDIAC ADRENERGIC RECEPTORS

Adrenergic receptors have been investigated for many years by various techniques e.g. physiological and pharmacological responses, radioligand-binding methods, and molecular cloning (Insel, 1996). From physiological responses, adrenergic receptors can be divided into two major types, α and β. From pharmacological responses, α-adrenergic receptors are also subdivided into two subtypes, α_1 and α_2, and β-adrenergic receptors are subdivided into two subtypes, β_1 and β_2. Recently, The α_{1A}, α_{1B}, α_{2A}, and α_{2B} adrenergic receptor subtypes were classified by radioligand binding. The α_{1D}, α_{2C}, and β_3-adrenergic receptor subtypes were found by using molecular cloning techniques. All adrenergic receptors are related to G-proteins. The α_1, α_2 and β-adrenergic receptors are linked to G_q, G_i and G_s proteins respectively (Insel, 1996). The G_q proteins activate phospholipase enzyme systems and exert the cellular response through secondary messengers, inosital 1,4,5-triphosphate and diacylglycerol. The inosital 1,4,5-triphosphate causes calcium releases from the sarcoplasmic reticulum, whereas diacylglycerol stimulates protein kinase C. Furthermore, the G_i protein inhibits adenylyl cyclase while the G_s protein stimulates adenylyl cyclase, and both of them cause the decrease and increase in

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intracellular cAMP level respectively. Finally, the activation of protein kinase A is regulated through the intracellular cAMP level.

β_1, β_2, H_2 and VIP receptors are coupled to the G_s protein and cause the activation of adenylyl cyclase in the heart (Bristow, 1989b); however, the inotropic effect on the heart in vivo comes mostly from β_1 and β_2-adrenergic receptors. Besides, histamine and VIP are rarely found in the heart, and VIP may not act as a circulating hormone.

There are three cardioactive adrenergic receptors: β_1, β_2 and α_1. In the past, it was thought that only the β_1-adrenergic receptor played an important role on the heart, but now it is known that all three adrenergic receptor subtypes have impact on cardiac structure and function. Normally, the inotropic effect results from β_1-adrenergic stimulation; however, β_2-adrenergic receptors, α_1-adrenergic receptors, and histamine receptors also play roles on contractility in the hearts of humans (Bristow, 1989a), rats (Hayes, 1986) and guinea pigs (McNeill, 1972) respectively. Yet, the density of α_1-adrenergic receptors in the human heart is much lower than β-adrenergic receptors, and may contribute less to cardiac contractility in the normal state (Bristow, 1993). There is evidence that norepinephrine mediated through α_1-adrenergic receptors causes an increase in protein synthesis and enlargement of cultured myocardial cells (Simpson, 1983; Knowlton, 1993). Stimulation of α_1-adrenergic receptors also results in the reduction of coronary blood flow and increases in intracellular calcium and oxyradical production (Kern, 1999)—oxidative stress. Moreover, fibroblast stimulation which produces DNA and protein syntheses is also related to β-adrenergic receptors

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(Calderone, 1995), and may lead to the impairment of cardiac relaxation in cardiac hypertrophy with interstitial fibrosis (Conrad, 1995).

Distributions of α-adrenergic receptors may vary among species. The density of cardiac α_1-adrenergic receptors is highest in rats, but cat, calf, dog, and primates have the same densities of cardiac α_1-adrenergic receptors (Fedida, 1993). Distribution of β-adrenergic receptors may also vary among tissues. Change of β-adrenergic receptor density in lymphocytes is related to the change of β-adrenergic receptor density in the heart, but the correlation of receptor densities between both sites is poor. Hence, β-adrenergic receptors in lymphocytes may not serve as a surrogate for myocardial adrenergic receptors density in patients with heart failure (Gilbert, 1993).

There are many factors that regulate adrenergic receptors, e.g. diseases, hormones and drugs (Lefkowitz, 1984). The increase in the ratio of α_1 to β-adrenergic receptors is found in the failing heart (Fedida, 1993). The numbers of β_1 and β_2-adrenergic receptors in the normal human heart are 80% and 20 % respectively, but the numbers of β_1 and β_2-adrenergic receptors may change to 60% and 40% respectively in the failing heart (Bristow, 1997). Increase in number and sensitivity of α_1-adrenergic receptors is also found in canine ischemic heart, and may be related to arrhythmogenesis (Wilber, 1987). Moreover, Weiss et al. report that the elevation of plasma norepinephrine depresses sinus node function, slows atrioventricular conduction, and prolongs atrial and ventricular refractoriness in humans (Weiss, 1998). Hyperthyroidism also affects adrenergic receptor density by increasing cardiac β-adrenergic responsiveness and decreasing the number of α_1-adrenergic receptors (Williams, 1979).

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In the failing heart, β_1-adrenergic receptors are down-regulated (Brodde, 1986), but the G_s protein does not change the functional activity (Bristow, 1993). The β_2-adrenergic receptors are mildly uncoupled, and the β_2-adrenergic receptor density may or may not increase. These changes cause the reduction of adrenergic functions in response to adrenergic agonists. Moreover, down regulation of β-adrenergic receptors may partially result from the high circulating concentrations of norepinephrine and epinephrine in heart failure.

BETA-ADRENERGIC BLOCKING DRUGS

After the first β-adrenergic blocking drug (beta blocker) was synthesized more than 30 years ago, nowadays beta blockers have become among the most important drugs used for treatment of patients with cardiovascular diseases. The first-generation beta blockers, e.g. propranolol and nadolol, possess non-specific β-adrenergic blocking activity, and are used for treatment of arrhythmias, systemic hypertension, angina pectoris, and hypertrophic cardiomyopathy. However, side effects of these beta blockers including negative inotropy, bronchoconstriction, and peripheral vasoconstriction, all of which may cause concerns for clinicians who use these drugs. Therefore, the second-generation beta blockers were synthesized to be more specific to β_1-adrenergic receptors, with the objective to minimize the unwanted effects from β_2-adrenergic blockade. Atenolol, esmolol, acebutolol, and metoprolol are examples of drugs in this second generation. Nevertheless, drugs in both generations still have negative inotropy, and are contraindicated in patients with heart failure.

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To overcome this problem, the third-generation beta blockers were synthesized to have nonspecific β-adrenergic blocking activity and vasodilating properties (Prichard, 1992) either due to blocking α_1-adrenergic receptors e.g. carvedilol and labetalol, or stimulating β_2-adrenergic receptors e.g. celiprolol, or having intrinsic sympathomimetic activity e.g. bucindolol. Beta blockers with vasodilating properties can improve left ventricular function, and may be used in patients with mild and moderate heart failure. By far, carvedilol seems to be the only drug that shows success in clinical trials by reducing both mortality rate and hospitalization rate (Packer, 1996).

CARVEDILOL: A NON-SPECIFIC BETA BLOCKER

Carvedilol is a non-specific beta blocker which also possesses α_1-adrenergic blocking activity and free radical scavenging activity. The generic name is (±)-1-(carbazol-4-yloxy)-3-[{2-(o-methoxyphenoxy) ethyl}amino]-2-propanol. The compound is white crystalline powder with a molecular weight of 406.479. The molecular formula is $C_{24}H_{26}N_2O_4$ as shown in Figure 1.1.

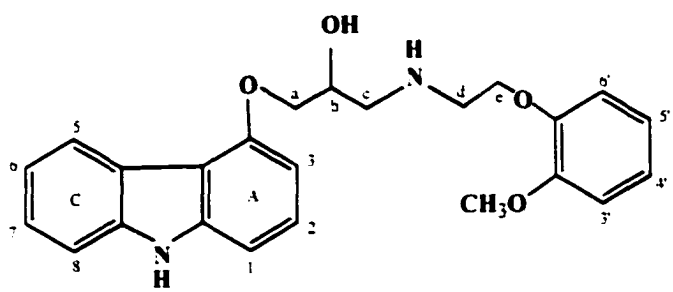

Figure 1.1. Structural formula of carvedilol.

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Carvedilol is a lipophilic drug which has limited solubility in water (less than 30 mg/L at pH 7). The compound which is available for clinical use is a racemic mixture between R (+) and S (-) enantiomers. Carvedilol, studied in vitro, has been shown to have affinity for β_1-adrenergic receptors about 7 times higher than β_2-adrenergic receptors in the heart (Yoshikawa, 1996). The S (-) enantiomer contributes mostly to the β-adrenergic blockade, but both enantiomers have similar blocking effect at α_1-adrenergic receptors (McTavish, 1993). Carvedilol has α_1-adrenergic blocking activities 2 times less than labetalol (at the same β-adrenergic blocking dose) and 2- 50 times less than prazosin (Sponer, 1992). Moreover, carvedilol seems to have a very weak blocking activity on α_2-adrenergic receptors studied in isolated canine mesenteric vascular preparations (Seki, 1988). The membrane-stabilizing activity produced by carvedilol is also minimal. However, the β_1-adrenergic blocking activity of carvedilol has been shown to be 10 times more potent than propranolol. Carvedilol also has calcium channel blocking activity at high doses resulting in vasodilation.

Pharmacokinetics of carvedilol have been extensively studied in both healthy and hypertensive human patients (Neugebauer, 1987; Louis, 1987). In normal subjects, carvedilol is well absorbed from the gastrointestinal tract after oral administration. The maximal time to reach peak plasma concentrations is about 1-2 hours after a single oral dose, but the maximal time may be delayed when carvedilol is mixed with food. Bioavailability of carvedilol is about 20 to 45 % in humans, and first-pass hepatic metabolism may play an important role on the bioavailability. The plasma concentration curves of intravenous injections of carvedilol were also fitted to the two-compartment model with the half-lives of distribution and elimination phases about 0.16 and 3.0 hrs

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respectively. In humans, carvedilol is exclusively metabolized by liver, and its metabolites are eliminated via fecal and renal routes about 60% and 16% respectively (Neugebauer, 1987).

Vasodilatory is produced by carvedilol by two mechanisms, adrenergic and non-adrenergic. The adrenergic vasodilatory mechanism is mainly mediated through α_1-adrenergic receptor blockade (Giannattasio, 1992). The non-adrenergic vasodilatory mechanisms may result from the calcium channel antagonism (Hattori, 1989) which is found at high doses (Sponer, 1992; Hattori, 1989; Strein, 1987). Carvedilol is also shown to inhibit vasoconstriction induced by KCl, serotonin and $PGF_{2\alpha}$. (Hashimoto, 1988).

Unlike other beta blockers, carvedilol does not decrease renal blood flow, glomerular filtration rate, or electrolyte excretion, but it does reduce renal vascular resistance (Gellai, 1990). Takami et al. have shown that carvedilol increases renal blood flow and reduces renal afferent arteriolar resistance at 10 and 20 minutes after intra-renal artery infusion in dogs (Takami, 1988). The urine volume is increased, but the urinary excretion of protein is decreased after carvedilol administered to nephrectomized, spontaneously hypertensive rats (Nakamoto, 1988). These results may imply that carvedilol protects the kidneys from the proteinuria and glomerular sclerosis which are normally found in this model. Moreover, Barone et al. showed that carvedilol reduces the degree of renal damage, plasma renin activity, and aldosterone in hypertensive, stroke-prone rats (Barone, 1996). The preservation of renal function has also been shown in patients with essential hypertension (Tomita, 1992).

Carvedilol was first introduced for treatment of hypertension. The daily single dose shows the anti-hypertensive effect persists day and night without circadial variation

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(Meyer-Sabellek, 1987; Ogihara, 1987; Stienen, 1992). Blood pressure and heart rate are reduced significantly (Heber, 1987) without left ventricular depression. Schnurr et al. report that the use of carvedilol in human hypertensive patients is safe, and side effects were found in only 6.8 % of 154 patients (Schnurr, 1987). The long-term treatment with carvedilol does not interfere with the atrial natriuretic peptide regulation; however, the plasma norepinephrine level may increase during exercise but not during the rest (Omvik, 1992).

THE USE OF BETA BLOCKERS IN HEART FAILURE

In the past, use of beta blockers has been cautioned because of the side effects, e.g. negative inotropy, hypotension, and bronchoconstriction-- especially in patients with heart failure. Until recently, several beta blockers have proven beneficial in medical management of congestive heart failure (Fowler, 1997). However, carvedilol is the first and only beta blocker which is approved by the FDA for use in human patients with the NYHA classes II and III congestive heart failure, and it has been proven useful in management of patients with severe heart failure (Macdonald, 1999; Louis, 2001).

There are many studies which report the benefit of carvedilol in the treatment of patients with heart failure. Bristow et al. report that carvedilol given orally improves left ventricular function and survival rate, and lowers hospitalization rate by 26-28 % (Bristow, 1996). Improvement of the hospitalization rate and the NYHA functional class in patients are also reported in the Metoprolol in Dilated Cardiomyopathy trial, and in the Cardiac Insufficiency Bisoprolol Study. However, the exercise-capacity improvement is found only in the metoprolol trial (Fowler, 1997). Acute administration of metoprolol, a

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β_1-adrenergic antagonist, may cause the elevation of cardiac norepinephrine spillover (Newton, 1996). On the other hand, propranolol, a nonspecific β-adrenergic antagonist, decreases the cardiac norepinephrine spillover, and this result shows the favorable effect of a nonspecific β-adrenergic blockade on cardiac sympathetic activity. Moreover, Krum et al. report that carvedilol reduces the plasma endothelin-1 level in patients with chronic heart failure, and improves left ventricular function and the clinical response (Krum, 1996).

The intravenous administration of carvedilol in patients with congestive heart failure (NYHA class II and III) produces an increase in ejection fraction with little changes in cardiac index or stroke volume index. Intravenous carvedilol also reduces heart rate and blood pressure, but not systemic vascular resistance (DasGupta, 1991). However, significant reduction of systemic vascular resistance is found in the chronic oral administration of carvedilol. Decreases in both left ventricular filling pressure and systemic vascular resistance may explain the improvement of stroke volume and ejection fraction in chronic treatment (DasGupta, 1992). Moreover, carvedilol is also proven to improve cardiac geometry as well as the degree of mitral regurgitation, and reduces the left ventricular mass, in mitral regurgitating patients with chronic heart failure (Lowes, 1999). The attenuation of carvedilol on norepinephrine-induced cardiac hypertrophy is also demonstrated in rats by Nagano et al. (Nagano, 1993).

The free-radical scavenging activity of carvedilol is also favorable for patients with heart failure related to cardiac ischemia. As we know oxygen reactive species or free radicals produced during ischemic-reperfusion may cause damage to the heart.

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FREE RADICALS AND ITS FORMATION IN THE HEART

Oxygen reactive species have been studied for more than three decades. However, the role of oxygen reactive species on the heart is still not entirely understood. As we know, oxygen is important to living organisms in the oxidative phosphorylation process as the final electron acceptor. The complete reduction of oxygen yields energy and water as final products of the electron transport chain. If this process is incomplete or partially reduced, oxygen reactive species, e.g. superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$) are formed. These oxygen radicals have unpaired electrons in their outer orbits, are extremely unstable, and are harmful to membrane and intracellular organelles. The hydroxyl radical is the most harmful, but it has limited diffusion. On the other hand, hydrogen peroxide may spread farther and damage remote targets. Polyunsaturated fatty acids in the membrane are likely to be attacked by these oxygen radicals, and this results in the chain reaction of lipid peroxidation. Sulfhydryl groups of proteins in the cell may also be attacked by these oxygen-derived free radicals, and this results in the dysfunction of these proteins. Normally, cells contain antioxidant enzymes (e.g., superoxide dismutase, glutathione peroxidase, and catalase) and antioxidant substances (e.g., vitamin E, vitamin C, β-carotene, and glutathione) to protect membranes and organelles from reactive oxygen species. However, the increase in reactive oxygen species and the decrease in antioxidants may lead to oxidative stress, which causes cellular damage. These changes are supported by studies of Baumer et al. (Baumer, 2000).

Systems to defended free radicals in the heart, as in other tissues, are composed of enzymes and antioxidant substances. Superoxide dismutase, found in the cytoplasm and

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mitochondria, catalyzes reactions that change superoxide to hydrogen peroxide. However, the level of this enzyme in the heart is much lower than in the liver (Ferrari, 1985). Then, glutathione peroxidase changes hydrogen peroxide to water or alcohol by working together with glutathione, a tripeptide antioxidant. Another enzyme is catalase which also catalyzes the reduction of hydrogen peroxide to water and oxygen. By working together, all these enzymes change the highly reactive molecules to the more stable and non-toxic molecules.

Non-enzymatic antioxidants in the heart are vitamins (e.g., E, C and β-carotene) and biological substances (e.g. glutathione, uric acid and metal proteins). Vitamin E, found in membranes because of high lipid solubility, protects membranes by reacting with free radicals, and produces hydroperoxide which can be reduced by glutathione peroxidase. However, vitamin C, a hydrophilic molecule found in the cytosol, functions as the regenerator of Vitamin E, and also reacts directly on free radicals as does β-carotene. The high levels of glutathione in the heart implies that this tripeptide may play a major role to defend the heart against injury by free-radicals. Together with glutathione peroxidase, glutathione protects membranes from injury by free radical –mediated lipid peroxidation (Singal, 1999a). Moreover, Ames et al. also report that uric acid acts as an antioxidant by interacting directly with hydroxyl radical (Ames, 1981).

In the heart, oxidative stress and oxygen radicals mediated injury have been reported both in vitro and in vivo models related to ischemic-reperfusion (Paradies, 1999), heart failure (Keith, 1998), and drug-induced cardiomyopathy (Singal, 1999a; Herman, 1988). In the ischemic-reperfusion model, the increase in reactive oxygen species like hydrogen peroxide has been reported both during ischemia and early

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reperfusion (Boraso, 1999). Concurrently, the levels of antioxidants also decline. Oxidative stress, which is developed during reperfusion, may induce apoptosis proved by direct fluorescence detection of digoxigenin-labeled genomic DNA (Maulik, 1998).

The presence of oxidative stress in models of heart failure is also reported in many studies (Randhawa, 1992; Prasad, 1996; Keith, 1998). However, oxidative stress is found only during heart failure and not during compensated hypertrophy (Dhalla, 1994). This higher susceptibility of cardiac myocytes in the failing heart may be caused by an intrinsic factor without the reduction of antioxidants (Tsutsui, 2001). Moreover, reactive oxygen species can destroy the sarcoplasmic reticulum membrane, and decrease Ca^{2+}-ATPase activity resulting in elevation of intracellular calcium (Matsubara, 1996). This change affects the calcium homeostasis and may cause prolongation of action potential and early after-depolarizations (Kukreja, 1999). Free radicals can also damage contractile proteins, transporter molecules, and molecules involving signal transduction (Peters, 1997), and decrease phosphorylation of troponin-I, phospholamban (Sulakhe, 1997), and creatine kinase (Genet, 2000).

Oxidative stress is also found in catecholamine-induced cardiomyopathy. The oxidation of catecholamines produces free radicals and causes an increase in lipid peroxides (Singal, 1982; Singal, 1983). Arrhythmia and cardiac dysfunction in this model can be attenuated by pretreatment with Vitamin E. Moreover, cytokines, e.g., TNF-α, angiotensin II (Singal, 1999b), and interleukin-1β (Cheng, 1999b) may be involved in the free radical formation in cardiomyopathic changes.

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DOXORUBICIN-INDUCED CARDIOMYOPATHY

Doxorubicin (adriamycin), an antibiotic classified as an anthracycline, is one of the most effective anticancer drugs for hematologic and solid tumors. It is isolated from *Streptomyces peucetius*. The compound is lipophilic, and has a red color and long half-life. The structure contains aminosugar linked to adriamycinone as shown in figure 1.2 below.

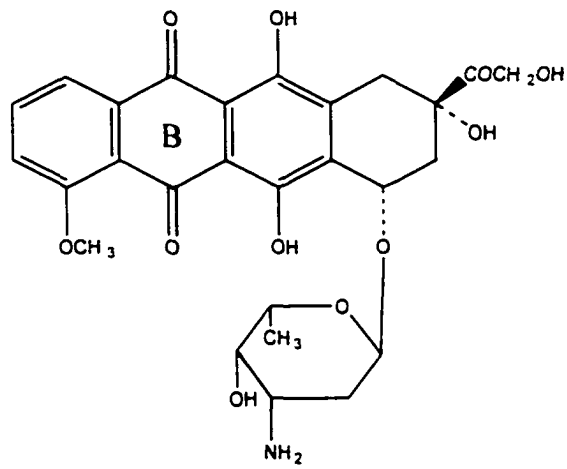

Figure 1.2. Structural formula of doxorubicin.

Doxorubicin-induced cardiotoxicity is related to many effects including the free radical formation (Doroshow, 1983). The doxorubicin molecule contains a quinone (ring B in figure 1.2) which has the potential to form free radicals. When doxorubicin receives an electron and forms a semiquinone, the free radical is relatively stable under anaerobic conditions, but can donate an unpaired electron to an oxygen molecule in aerobic condition (Singal, 2000). Consequently, the superoxide radical can be produced, and the semiquinone is changed to quinone again. This reaction can be produced repeatedly, and results in the formation of many superoxide radicals from a small amount of doxorubicin.

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Doxorubicin-generated free radicals are produced by enzymatic and nonenzymatic pathways (Horenstein, 2000). Since doxorubicin can bind to cardiolipin, a phospholipid found in the inner membrane of mitochondria, it causes malfunction of mitochondria, especially the electron transfer process. The single electron from NADH is interchangeably transferred to doxorubicin, and finally semiquinone is formed. Next, semiquinone can transfer an electron to an oxygen molecule, and then it is oxidized to quinone which can be recycled again. This reaction can be catalyzed by the enzyme NADH dehydrogenase which is found only in the heart.

The nonenzymatic pathway of free radical formation is related to ferric ion (Fe^{3+}) which reacts with doxorubicin and produces the doxorubicin-ferrous iron (Fe^{2+}) free radical complex. This complex can transfer an electron to an oxygen molecule, and generates a superoxide radical and the doxorubicin-Fe^{3+} free radical complex. This secondary free radical complex can rearrange to doxorubicin-Fe^{2+} aldehyde, and then donate another electron to an oxygen molecule. Finally, superoxides are coupled to form hydrogen peroxide, and hydrogen peroxide can be changed to hydroxyl radicals which are among the most reactive oxygen species. Finally, injury to cardiac myocytes causes the release of enzymes e.g. creatine kinase or protein fractions e.g. troponin which are valuable for clinical detection of doxorubicin cardiotoxicity.

In addition to formation of free radicals, doxorubicin also injures genes in rat cardiac myocytes that encode enzymes related to energy production (Jeyaseelan, 1997), and it inhibits fatty acid oxidation. The release of vasoactive substances e.g. histamine, catecholamines, prostaglandins (Bristow, 1980; Rossi, 1994), and plasma natriuretic peptides (Jantunen, 1999) is also reported. Moreover, doxorubicin causes adrenergic

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dysfunction (Shan, 1996), down regulation of β-adrenergic receptors (Bocherens-Gadient, 1992), intracellular calcium overload (Kusuoka, 1991), and decreases in antioxidants (Singal, 1999b).

Doxorubicin also causes changes in electrocardiograms in many models (Doherty, 1990) and patients (Mauldin, 1992). The electrocardiographic changes include: supraventricular arrhythmias, ventricular arrhythmias, nonspecific alterations of R wave and QRS duration, and ST segment slurring.

SPECIFIC GOALS

The aim of the present dissertation is to evaluate the protective effects of carvedilol in doxorubicin-induced cardiotoxicity. Due to limitations in knowledge of the use of carvedilol in the veterinary medicine, we will study the physiological effects, pharmacokinetics and phamacodynamics of carvedilol in dogs.

Currently, doxorubicin is used in cancer patients because of the high efficacy of doxorubicin and other anthracyclines in the treatment of hematologic and solid tumors. However, the use of doxorubicin is still limited by the cardiotoxicity related to the cumulative doses. There are many studies which report the success of using antioxidants to protect the heart from doxorubicin-induced cardiotoxicity. These compounds include enzymes e.g. superoxide dismutase, catalase, glutathione peroxidase, and nonenzymatic substances e.g. glutathione, Vitamin E, probucol, and dexrazoxane. However, none of them has beneficial cardiovascular effects and reduces energy utilization. Moreover, there is a report of using afterload reducers e.g. captopril and prazosin (Khaper, 1997) to

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alleviate the cardiac damage from doxorubicin. However, there is no drug like carvedilol which possesses the ability to both reduce afterload without cardioacceleration and serve as a free radical scavenger.

Carvedilol possesses nonspecific β-adrenergic and α_1-adrenergic blocking activity which reduces the afterload and energy required for the heart to maintain cardiac output, and free radical scavenging activity which shows the successful protective effect as studied in ischemic-reperfusion models (Feuerstein, 1993). Moreover, carvedilol also has an antiarrhythmic activity produced by blocking the delayed rectifier potassium channels and calcium channels (Cheng, 1999a). Therefore, the use of carvedilol to attenuate doxorubicin toxicity in the heart may have three advantages: (1) decrease energy requirement for maintaining cardiac output, (2) reduce cardiac damage from free radicals, (3) prevent the heart from developing potentially harmful arrhythmias.

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CHAPTER 2

PHYSIOLOGICAL EFFECTS OF CARVEDILOL IN HEALTHY DOGS

ABSTRACT

Carvedilol is a nonspecific β-adrenergic blocker which possesses α_1-adrenergic blocking and free radical scavenging activities. To describe the physiological effects of carvedilol, we used 12 healthy beagle hounds for hemodynamic and electrocardiographic studies. Animals were anesthetized with morphine and α-chloralose before catheterization of the aorta, both ventricles and right atrium to record systemic pressure and pulmonary pressure. Cardiac output was measured by thermodilution technique, and leads I, aVF and V_3 electrocardiograms were recorded. All parameters were measured before and after intravenous bolus injections of vehicle (n=6) or carvedilol (n=6) at incremental doses which achieved cumulative doses of 10, 30, 70, 150, 310, and 630 μg/kg of body weight. Carvedilol increased heart rate and velocity of fiber shortening at zero load (V_{max}) at 310 and 10 μg/kg respectively. Aortic and pulmonary systolic pressures were reduced only at high doses. Carvedilol affected neither systemic nor pulmonary vascular resistances. The electrocardiograms showed shortening of the PQ

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interval starting from the dose of 150 μg/kg. The present study suggests that carvedilol improves left ventricular function with minimal effects on electrocardiograms.

INTRODUCTION

Carvedilol is a nonspecific beta blockers which also has α_1-adrenergic blocking (Seki, 1988) and free radical scavenging activities (Feuerstein, 1997). It was recently approved by the FDA (Fisher, 1999), and has showed promise in the treatment of hypertensive (Heber, 1987; Schnurr, 1987; Cournot, 1992; Lund-Johansen, 1992) and congestive heart failure patients (DasGupta, 1991). Unlike other beta blockers, carvedilol decreases both mortality and number of hospitalizations (Sharpe, 1996; Packer, 1997) comparable to ACE inhibitors. Carvedilol improves left ventricular function without tachycardic feedback, and recently it has been shown to prevent cardiac remodeling (Feuerstein, 1997). Carvedilol also reduces endothelin release (Krum, 1996) and decreases cardiac norepinephrine storage without resulting in up-regulation of β-adrenergic receptors (Bohm, 1998). While there are many reports which show the valuable of carvedilol in human medicine, we are aware of no references in the veterinary literature. Therefore, the purpose of this study was to describe the acute effects of graded doses of carvedilol on physiological parameters. This knowledge is essential before starting clinical trials.

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MATERIALS AND METHODS

Animal use:

Twelve, young-mature, healthy beagle hounds of either sex were used. Animals were divided into two groups of equal numbers. Vehicle or carvedilol was given intravenously in the same volume. Carvedilol stock solution (1mg/ml) was prepared by dissolving 10 mg of carvedilol, in 10 ml of vehicle (1 ml of dimethyl formamide plus 10 µl 1M HCl, diluted with normal saline to 10 ml). Each dose was withdrawn from the stock solution, and diluted with normal saline to a final volume of 10 ml before slow intravenous injection over 1 minute. Animals in the second group received carvedilol every 15 minutes for 6 periods with incremental doses of 10, 20, 40, 80, 160, 320 µg/kg. This achieved cumulative doses of 10, 30, 70, 150, 310, 630 µg/kg respectively. Blood samples for measurements of carvedilol plasma concentration were collected at the middle of each period during electrocardiographic and hemodynamic recordings.

Surgical preparation:

Animals were anesthetized with morphine at a dose of 2 mg/kg of body weight given intramuscularly, then 15 minutes later with α-chloralose at the dose of 100 mg/kg given intravenously. Anesthesia was maintained by α-choralose infusion at the dose of 30 mg/kg/hour. Animals were intubated, and were ventilated with room air by a respirator (Harvard apparatus). Samples of arterial blood were collected and measured immediately with a blood gas analyzer (Instrumentation Laboratory), and the respirator was adjusted to maintain $PaCO_2$ between 35 and 40 mmHg. Animals were placed in right lateral

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recumbency, and ECG electrodes were connected. The left femoral artery was surgically exposed and a Millar catheter (Model SPC-350, 5F) was introduced into the left ventricle to record left ventricular pressure. A fluid-filled catheter was inserted through the right femoral artery to measure aortic pressure. A Swan-Ganz catheter was inserted through the jugular vein and advanced so that a port for injecting saline lay in the right atrium and a thermistor bead lay in the pulmonary trunk. After infusion of either vehicle or carvedilol for 5 minutes, cardiac output was measured by thermodilution technique (Baxter, model COM-2), and electrocardiograms (leads I, aVF, and V3), left ventricular pressure, aortic pressure, pulmonary artery pressure, pulmonary wedge pressure and right atrium pressure were recorded on a physiograph. All analog signals were converted to digital signals by using the Biopac system (model MP100) with a sampling rate of 1000/sec, and kept in hard disks for data analysis.

Data analysis:

All data were analyzed by the Acknowledge software (Version 3.5), and transferred to the Microsoft Excel program for further calculations.

Parameters that were calculated are:

1. Electrocardiograms. RR, PQ, QRS, and QT intervals were measured, and corrected QT (QTc) was calculated by the formula below.

   Heart rate = 60,000/RR interval (msec)

   $QTc = QT / RR^{1/3}$

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2. Aortic pressure. Systolic and diastolic pressures were measured, and pulse pressure and mean pressure were calculated by the following formulas.

Pulse pressure = Systolic pressure – Diastolic pressure

Mean pressure = 1/3(pulse pressure) + Diastolic pressure

3. Left ventricular pressure (LVP). End diastolic pressures were measured at the peak of the R wave just before the isovolumic contraction period. Maximal rates of rise (dP/dt_{max}), maximal rates of fall (dP/dt_{min}), Tau (Weiss, 1976), and velocities of fiber shortening at zero load (V_{max}) (Mason, 1970; Katz, 1992) were calculated by the following formulas.

$dP/dt = f_{output}(n) = \Sigma f_{input}(k) + [[f_{input}(n-1) + f_{input}(n)]/2]^* \Delta Ts$
where f is the pressure and ΔTs is the sampling interval

Tau = the time required for left ventricular pressure to fall 63% of the distance between the time when dP/dt min occurs and when ventricular pressure reaches 15 mmHg above end diastolic pressure.

V_{max} = an estimate of the maximal velocity of contractile elements calculated by extrapolating the force-velocity curve to P_0 where the force employed from the isovolumic pressure (P), and velocity(V_{CE}) is dP/dt divided by kP.

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4. Cardiac output. To generate the thermodilution curve, 3 ml. of cold 5% dextrose in water were injected. The cardiac output was calculated by the formula below.

Cadiac output = K*V* ΔT/ area under the curve
Where K is the constant value, V is the injectate volume and ΔT is temperature difference

Cardiac index = Cardiac output/ body surface area

Stroke volume = Cardiac output/ heart rate

Stroke volume index = Stroke volume/ body surface area

5. Vascular resistance.

Pulmonary vascular resistance (PVR)
PVR = Mean pulmonary arterial pressure / Cardiac output

Systemic vascular resistance (SVR)
SVR = Mean aortic pressure / cardiac output

A two-way ANOVA with repeated measures design was used to compare all mean values. Once a significant ($P< 0.05$) F-statistic was achieved, the specific means in the same group at different periods were compared by the contrast of multiple comparisons, and the specific means between two groups at the same period were compared by the unpaired t-test.

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RESULTS

Effects of carvedilol on electrocardiography:

There were no changes in heart rate for dogs in the control group who received vehicle, but heart rates were significantly increased in the carvedilol group starting from the cumulative dose of 30 μg/kg. Heart rate increased in an apparent dose-response manner and achieved significant difference at the cumulative dose of 310 μg/kg. PQ intervals shortened in both groups, but PQ intervals of dogs in the carvedilol group changed from the first dose compared to the third dose in the control group. There were no changes in QRS durations. QT intervals and QTc did not show any differences between both groups except the QT interval in the control group shortened only in the last period as shown in table 2.1.

Effects of carvedilol on cardiac contractility:

Cardiac index (cardiac output/body surface area) did not change in the control group as shown in table 2.2. However, significant increases were observed in the carvedilol group starting from the dose of 70 μg/kg. There were no significant changes in stroke work index. dP/dt_{max} increased in both groups from the dose of 150 μg/kg without any differences between groups. dP/dt_{min} increased (i.e. became more negative) only in the control group starting from the dose of 150 μg/kg, and significantly exceeded the carvedilol group at the dose of 630 μg/kg. Graded increases of V_{max} were found only in the carvedilol group starting from the first dose. Tau of dogs in the carvedilol group tended to be shortened, but this did not reach statistical significance.

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Effect of carvedilol on blood pressures, vascular resistances and plasma concentrations:

Aortic and pulmonary systolic pressures of dogs in the carvedilol group were lower than in the controls from doses of 630 and 310 μg/kg respectively. Carvedilol tended to decrease both systemic vascular resistance and pulmonary vascular resistance. However, the changes did not reach statistical significance as shown in table 2.4. The means of plasma carvedilol concentrations of cumulative doses of 10, 30, 70, 150, 310, and 630 μg/kg were 5.6, 13.4, 33.5, 80.8, 183.7, and 391.6 ng/ml respectively as shown in Figure 2.1.

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Parameter	Unit	Cumulative Doses (μg/kg)						
		baseline	10	30	70	150	310	630
		mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE
Heart rate	bpm							
control		65 ± 3	68 ± 4	67 ± 4	66 ± 4	66 ± 4	68 ± 4	68 ± 3
carvedilol		76 ± 8	90 ± 10	84 ± 6 $	84 ± 4 $$	87 ± 2 $$$	95 ± 4 *, $$$	106 ± 5 ***,$$$
PQ interval	msec							
control		113 ± 7	109 ± 6	108 ± 4	105 ± 3#	106 ± 3 #	100 ± 5###	101 ± 5 ###
carvedilol		111 ± 6	102 ± 6 *	102 ± 4 *	101 ± 4 *	99 ± 4 **	95 ± 4 ***	91 ± 5 ***
QRS	msec							
control		49 ± 2	49 ± 2	49 ± 2	49 ± 2	49 ± 2	49 ± 2	50 ± 2
carvedilol		49 ± 1	49 ± 2	50 ± 1	50 ± 1	49 ± 1	49 ± 2	48 ± 1.0
QT interval	msec							
control		274 ± 10	272 ± 12	274 ± 12	273 ± 10	268 ± 7	267 ± 8	258 ± 7 ##
carvedilol		261 ± 8	250 ± 5	257 ± 6	254 ± 6	251 ± 8	244 ± 10	233 ± 10
QTc	msec							
control		280 ± 3	280 ± 3	281 ± 3	280 ± 3	275 ± 2	277 ± 3	272 ± 2
carvedilol		280 ± 3	284 ± 3	288 ± 4	284 ± 4	284 ± 4	283 ± 4	281 ± 4

Table 2.1: Effects of intravenous carvedilol on heart rate and electrocardiograph.

#p<0.05, ##p<0.01, ###p<0.001 compared doses versus the baseline in the control group(n=6).
*p<0.05,**p<0.01, ***p<0.001 compared doses versus the baseline in the carvedilol group(n=6).
$p<0.05,$$p<0.01, $$$p<0.001 compared between the control and the carvedilol groups at the same period.

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Parameter	Unit	Cumulative doses (μg/kg)						
		baseline	10	30	70	150	310	630
		mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE
Cardiac index	L/min/m^2							
control		2.4 ± 0.2	2.4 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.5 ± 0.2	2.6 ± 0.2
carvedilol		2.6 ± 0.2	2.8 ± 0.2	2.9 ± 0.2	3.0 ± 0.2 *	3.1 ± 0.2 **	3.2 ± 0.3 **	3.2 ± 0.3 **
Stroke vol. index	ml/m^2							
control		37.1 ± 2.3	36.2 ± 1.4	38.2 ± 1.3	37.1 ± 1.6	37.0 ± 1.8	36.8 ± 0.6	38.5 ± 1.2
carvedilol		34.3 ± 2.3	33.0 ± 3.7	35.3 ± 3.3	35.7 ± 3.1	35.6 ± 3.1	33.3 ± 3.0	30.2 ± 2.3
dP/dt $_{max}$	mmHg/sec							
control		1701 ± 78	1739 ± 78	1863 ± 161	1928 ± 115	1949 ± 104 #	2064 ± 205 ##	2138 ± 261 ###
carvedilol		1898 ± 130	2101 ± 182	2157 ± 164	2224 ± 192	2285 ± 196 *	2340 ± 192 **	2316 ± 159 ***
V_{max}	sec^{-1}							
control		74.4 ± 3.0	74.7 ± 3.1	77.2 ± 3.9	74.7 ± 2.8	76.7 ± 4.2	77.6 ± 7.3	78.2 ± 5.4
carvedilol		80.3 ± 3.9	90.7 ± 6.3 **,$	91.3 ± 5.3 **	89.5 ± 4.8 **,$	93.1 ± 4.8 ***,$	95.1 ± 4.7 ***	97.9 ± 5.2 ***,$
dP/dt $_{min}$	mmHg/sec							
control		-2788 ± 125	-2750 ± 91	-2764 ± 108	-2879 ± 135	-3029 ± 170 #	-3155 ± 149 ###	-3299 ± 139 ###
carvedilol		-2624 ± 160	-2586 ± 247	-2800 ± 345	-2821 ± 299	-2833 ± 301	-2708 ± 283	-2511 ± 262 $
Tau	msec							
control		33.5 ± 1.4	33.1 ± 1.5	33.2 ± 1.7	32.9 ± 1.8	33.2 ± 1.9	32.2 ± 2.1	31.6 ± 2.8
carvedilol		30.8 ± 1.5	28.0 ± 1.0	28.9 ± 1.2	28.6 ± 1.5	27.9 ± 1.9	28.0 ± 2.3	28.9 ± 2.3

Table 2.2: Effects of intravenous carvedilol on cardiac functions.

#p<0.05, ##p<0.01, ###p<0.001 compared doses versus the baseline in the control group(n=6).
*p<0.05,**p<0.01, ***p<0.001 compared doses versus the baseline in the carvedilol group(n=6).
$p<0.05 compared between the control and the carvedilol groups at the same period.

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Pressure	Unit	Cumulative Doses (μg/kg)						
		baseline	10	30	70	150	310	630
		mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE
Aortic sys	mmHg							
control		99.2 ± 4.4	101.1 ± 4.5	104.2 ± 5.1	106.6 ± 5.3 ##	109.6 ± 5.7 ##	115.2 ± 7.0 ###	117.8 ± 7.1 ###
carvedilol		98.1 ± 4.5	98.8 ± 5.7	104.2 ± 8.8	103.7 ± 7.2	104.3 ± 7.2	100.3 ± 6.4	94.5 ± 5.4 $
Aortic mean	mmHg							
control		78.7 ± 4.3	79.5 ± 4.0	81.3 ± 3.6	83.2 ± 4.3	87.2 ± 5.1 ###	89.2 ± 5.4 ###	92.0 ± 6.1 ###
carvedilol		76.1 ± 2.8	75.3 ± 3.0	80.7 ± 6.0	80.8 ± 4.7	81.8 ± 5.3	79.8 ± 5.0	74.9 ± 4.4
Aortic dias	mmHg							
control		60.8 ± 4.1	60.9 ± 4.1	61.3 ± 3.6	62.7 ± 4.1	66.4 ± 4.8 #	68.6 ± 5.5 ##	69.4 ± 6.0 ###
carvedilol		56.7 ± 2.2	55.7 ± 0.9	59.6 ± 3.6	60.2 ± 2.5	61.7 ± 3.4	61.5 ± 3.4	58.3 ± 3.6
Pulm sys	mmHg							
control		22.8 ± 1.2	22.3 ± 1.8	23.2 ± 2.0	24.4 ± 2.2 #	24.5 ± 2.0 #	25.5 ± 2.1 ###	26.5 ± 2.2 ###
carvedilol		20.9 ± 1.5	19.3 ± 1.8	20.3 ± 2.1	20.3 ± 1.7	19.0 ± 2.5 *	19.8 ± 1.3 $	17.7 ± 2.4 **,$
Pulm mean	mmHg							
control		14.1 ± 0.4	13.7 ± 0.6	14.0 ± 0.5	14.6 ± 0.7	14.8 ± 0.6	15.1 ± 0.6 #	15.6 ± 0.9 ##
carvedilol		13.8 ± 1.1	13.2 ± 1.2	13.7 ± 1.4	13.9 ± 1.2	13.0 ± 1.7	13.9 ± 0.9	12.5 ± 1.7
Pulm dias	mmHg							
control		8.6 ± 0.4	8.5 ± 0.6	9.2 ± 0.3	9.0 ± 0.9	8.9 ± 0.3	8.9 ± 0.3	9.1 ± 0.4
carvedilol		8.6 ± 1.0	8.4 ± 1.0	8.6 ± 1.2	8.7 ± 0.9	8.0 ± 1.2	9.1 ± 0.7	7.9 ± 1.3

Table 2.3: Effects of intravenous carvedilol on aortic and pulmonary pressures.

#p<0.05, ##p<0.01, ###p<0.001 compared doses versus the baseline in the control group(n=6).
*p<0.05,**p<0.01 compared doses versus the baseline in the carvedilol group(n=6).
$p<0.05 compared between the control and the carvedilol groups at the same period.

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Parameter	Unit	Cumulative Doses (μg/kg)						
		baseline	10	30	70	150	310	630
		mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE	mean ± SE
svr	$dyn.sec.cm^{-5}$							
control		4583 ± 244	4614 ± 332	4502 ± 256	4770 ± 404	5016 ± 408	5034 ± 473	4994 ± 496
carvedilol		4785 ± 269	4323 ± 389	4444 ± 443	4386 ± 384	4289 ± 379	4148 ± 426	3880 ± 479
pvr	$dyn.sec.cm^{-5}$							
control		824 ± 41	791 ± 59	783 ± 59	837 ± 77	857 ± 75	857 ± 69	850 ± 86
carvedilol		866 ± 76	773 ± 115	759 ± 108	759 ± 92	693 ± 120	738 ± 90	645 ± 121

Table 2.4: Effects of intravenous carvedilol on vascular resistances.

svr, systemic vascular resistance; pvr, pulmonary vascular resistance.

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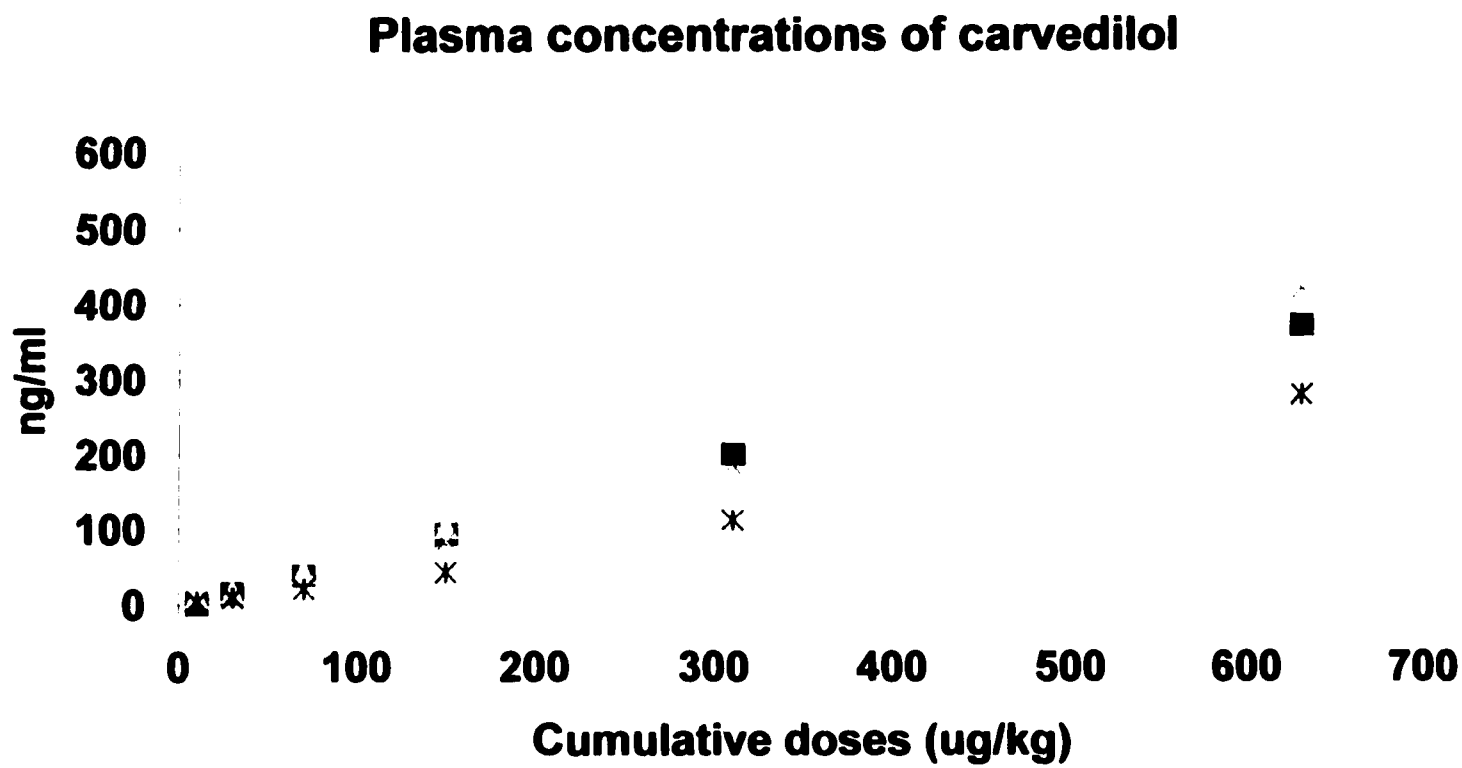

Figure 2.1: Plasma concentrations of carvedilol in anesthetized healthy beagle hounds (n=4). The line represents the means of plasma concentrations in these four dogs.

DISCUSSION

Unlike other beta blockers, carvedilol increased heart rate after acute intravenous injection consistent with observations by Sponer et al. (Sponer, 1992; Sponer, 1987). On the other hand, carvedilol significantly reduced tachycardia induced by isoproterenol (1 μg/kg). The brief increase in heart rate may contribute to a decrease in systemic vascular resistance together with activation of the baroreceptor reflex. PQ interval shortened as a natural consequence of the increasing heart rate. QT interval also tended to shorten in the same manner as PQ interval. These QT changes were normalized by dividing them by the cube root of the RR interval (Fridericia's correction). This has been shown to less overcorrect than the square root of the RR interval (Bazett's correction) in beagle dogs (Spence, 1998). Therefore, although QT may have changed, all of the change is attributable to change in heart rate and not due to a primary effect on ventricular repolarization.

The increase in cardiac index--but not in stroke volume index--in the carvedilol group starting from the dose of 70 μg/kg may be caused by the increase in heart rate together with a reduction in systemic vascular resistance. However, the increase in V_{max}, which is relatively load-independent, may imply that carvedilol may possess a positive inotropic effect as reported elsewhere (DasGupta, 1991; Bristow, 1996). This positive inotropy may occur for several reasons. First, the Bowditch-Treppe phenomenon, found in the normal heart, but not the failing heart (Just, 1996), states that contractility increases proportional with an increase in heart rate. This phenomenon is explained by an increasing intracellular calcium concentration resulting from activation of the sodium-calcium exchanger that results from increased intracellular sodium. The augmented

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intracellular calcium, then, binds to troponin-C, and increases force of contraction. Secondly, there are several beta blockers (e.g., bucindolol, pindolol, and labetalol) which have vasodilating property similar to those of carvedilol (Frishman, 1998; Prichard, 1992), and that also possess intrinsic sympathomimetic activity (i.e. partial agonistic). This would increase myocardial contractility and possibly accelerate heart rate. However, there is no data to support the possibility that carvedilol has partial agonistic activity. Another possibility for the increased contractility may be that carvedilol, at concentrations less than 0.5μM, may block only the rapid component of the delayed rectifier potassium channels (Cheng, 1999a), and prolong phases 2 and 3 of action potential of cardiac myocytes. The prolonged phase 2 may increase the duration of calcium influx into the cell. This calcium binds to the ryanodine receptors, calcium is released from sarcoplasmic reticulum, it binds to troponin-C, and force of contraction is augmented (Fozzard, 2001). However, at higher dose, carvedilol may also block the calcium current, and the prolongation of action potential may be limited.

Moreover, the increases in cardiac index and dP/dt_{max}, which are load-dependent, may partially result from volume expansion resulting from infusion of α-chloralose--about 250 ml and bolus injections of cold saline of about 70-100 ml during cardiac output measurements. Although dP/dt_{min} and Tau in the carvedilol group tended to be lower than in the control group, statistical differences between both groups were not shown in these healthy beagle hounds. However, if carvedilol has a positive lusitropic effect, this may have an advantage in conditions of negative lusitropy like cardiac fibrosis, myocardial ischemia, or negative lusitropic drugs. Recently, carvedilol was shown to decrease the

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remodeling process (Feuerstein, 1997). This may be useful in patients with diseases (e.g. atrial fibrillation, pressure or volume overloads) that lead to remodeling.

From the present study, a dose of carvedilol of 150 μg/kg affects heart rate, cardiac output, arterial blood pressure, afterload, and inotropy. This dose yields a plasma concentration of about 100 ng/ml, which is similar to that reported for humans (McTavish, 1993).

Increases in systemic and pulmonary pressures in the control group may be due to the volume expansion and the decline, with time, of the hypotensive effect of morphine. Aortic and pulmonary systolic pressures were lower, without changing the diastolic pressure, in the carvedilol group compared to the controls at high doses. This may be attributed to the vasodilating properties of carvedilol due to its α_1-adrenergic and calcium-channel blocking properties. However, the acute effect of carvedilol on blood pressure in healthy subjects may not be obvious compared to hypertensive subjects. The reduction of systemic and pulmonary vascular resistances did not reach statistical significance due to high variances.

In conclusion, acute cardiovascular effects of intravenous carvedilol are including increases in heart rate and V_{max}, shortening of PQ intervals, and decreases in systemic and pulmonary systolic pressures.

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CHAPTER 3

PHARMACOKINETICS AND DEGREE OF BETA ADRENERGIC BLOCKADE OF CARVEDILOL IN HEALTHY DOGS

ABSTRACT

Carvedilol, a non-specific beta blocker which possesses α_1-adrenergic blocking and antioxidant activities, is well known in human medicine to be beneficial for the treatment of hypertension and heart failure related to ischemic heart disease and dilated cardiomyopathy. However, there is little data on the pharmacokinetics and degree of β-adrenergic blockade applicable to veterinary medicine. In this study, 11 dogs were given intravenously carvedilol at the dose of 100 μg/kg for a pharmacokinetic study. Blood samples were drawn before and at 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 minutes after injections. Plasma concentrations were measured by on-line, solid-phase extraction followed by reverse-phase HPLC. Four other dogs were given carvedilol at 0.15625, 0.3125, 0.625, 1.25 mg/kg of body weight, twice daily for 3 days, to establish degree of β-adrenergic blockade. To test for degree of β-adrenergic blockade, dogs were given isoproterenol intravenously with incremental doses from 0.001 to 8.192 μg/kg, and heart rate response was measured. The equation, $C_t = Ae^{-\alpha t} + Be^{-\beta t}$, describes the relationship

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between plasma concentration (C_t) and time (t) after administration, which represents a two-compartment model. The mean half-life of distribution is 3.6 minutes, and the mean elimination half-life is 52 minutes. The clearance is 24.3 $\pm$ 4.6(Mean $\pm$ SD) ml/kg/min, and the volume of distribution is 1830 $\pm$ 918 ml/kg. The degree of β-adrenergic blockades was dose-related. The dose of carvedilol which decreased the heart rate response to the highest dose of isoproterenol by 50% (ED_{50}) was determined. The peak depression of the increase in heart rate due to isoproterenol occurred in the 4^{th} hour after the last dose of carvedilol. The ED_{50} of carvedilol at doses of 0.15626, 0.3125, 0.625, and 1.25 mg/kg were 135.6, 237.1, 239.1, 435.6, and 1164.6 ng/kg/min respectively. This study should serve as the basis for clinical use of this compound as an antiarrhythmic and possibly to modify doxorubicin toxicity in dogs.

INTRODUCTION

Beta blockers constitute a group of drugs which play an important role in the treatment of systemic hypertension, cardiac ischemia, and cardiac arrhythmia. Recently a beta-blocker, carvedilol, has been shown to benefit patients with mild to moderate--and possibly even severe--heart failure.

Carvedilol, (±)-1-(carbazol-4-yloxy)-3-[{2-(o-methoxyphenoxy) ethyl}amino]-2-propanol, is a non-specific beta blocker which also has α_1-adrenergic blocking (Giannattasio, 1992) and free-radical scavenging (Feuerstein, 1997) activities. Carvedilol does not possess intrinsic sympathomimetic activity (van Zwieten, 1993). The commercial drug is a combination between the R(+) and S(-) enantiomers at equal

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potencies. Both enantiomers have equal α_1-adrenergic blocking property, but the S(-) enantiomer also contributes to the β-adrenergic blockade. Even though carvedilol is classified as a nonspecific β-adrenergic blocker, the affinity for β_1-adrenergic receptors is higher than for β_2-adrenergic receptors. However, the ratio of β_1/β_2 selectivity is still far from selective (McTavish, 1993). Carvedilol is a lipophilic drug which binds strongly (up to 98%) to proteins (Frishman, 1998). It is well absorbed from the human gastrointestinal tracts, but the bioavailability is less than one third of the oral dose due to the high first-pass hepatic metabolism (Neugebauer, 1992). The bioavailability is also dependent on the dose. There are at least 15 beta blockers which are approved for use in humans. They are used for treatments of hypertension, arrhythmias, heart failure, hypertrophic cardiomyopathy, cardiac ischemia, angina pectoris, mitral valve prolapse, migrane, essential tremor, and glaucoma (Frishman, 1998). Many of them share common structures either an arylethanolamine or an aryloxyisopropanolamine moiety (Schaefer, 1998). However, only a few have been used in veterinary medicine due to the lack of pharmacodynamic and pharmacokinetic studies. Therefore, the present experiments were designed to study pharmacokinetics of intravenous carvedilol and the degree of β-adrenergic blockade in healthy dogs. This should provide important data for clinical studies of carvedilol.

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MATERIALS AND METHODS

1. Experiment I: Pharmacokinetic Study.

1.1. Animal preparation:

Eleven healthy beagle hounds were used in this study. Dogs were fasted for 12 hours, but they had access to water ad libitum before the study began. Both cephalic veins were catheterized with 22-gauge intravenous catheters, and heparinized saline was used to prevent blood clotting inside catheters. Animals were given carvedilol at a dose of 100 μg/kg intravenously into the right cephalic vein, and blood samples (2 ml.) were drawn from the left cephalic vein before and at 1, 2, 4, 8, 16, 32, 64, 128, 256 and 512 minutes after injection. Heparin was used as an anticoagulant. Plasma samples were separated by a refrigerated centrifuge at 1,500 rpm for 15 minutes then kept at –70 ^{0}C until measurements were performed. Plasma concentrations of carvedilol were measured by on-line, solid-phase extraction followed by reverse-phase HPLC. The lowest detectable concentration is 1 ng/ml in 0.1 ml of plasma.

1.2. Data Analysis:

Plasma concentrations of carvedilol were plotted against time. Pharmacokinetic analyses were performed using the nonlinear regression analysis of the SigmaPlot 2000 for windows software (SPSS inc., version 6), and were defined as a two-compartment model ($C_t = Ae^{-\alpha t} + Be^{-\beta t}$), where C_t is the concentration at time t, A and B are coefficients, and α and β are rate constants of the distribution and elimination phases, respectively.

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Half-life of distribution phase was calculate as:

$$t_{½}\text{ (distribution)} = 0.693/\alpha$$

Elimination half-life was calculate as:

$$t_{½}\text{ (elimination)} = 0.693/\beta$$

The area under curve (AUC) was calculated as:

$$AUC = A/\alpha + B/\beta$$

The volume of distribution (V_d) was calculated as:

$$V_d = \text{dose}/(AUC \times \beta)$$

The plasma clearance rate (CL) was calculated as:

$$CL = \text{dose}/AUC$$

2. Experiment II: Degree of β-adrenergic blockade.

2.1. Animal preparation:

Four healthy beagle hounds of either sex, approximately 10 kg of weight, were used in this study. Before starting the experiment, the animals were trained daily, for at least 2 weeks, to stand in a dog sling for 30 minutes with ECG leads attachment, until

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they were familiar with the experimental regimen. Degree of beta blockade was determined as follows. First, without having received carvedilol, the dogs were given, by intravenous infusion, doses of isoproterenol varying from 0.001 μg/kg/m to 8.192 μg/kg/m, at 1, 2, 4, 8, 16, and 24 hours. Each dose of isoproterenol was infused about 2 minutes until heart rate was reached the maximum response. If the dog became apparently uncomfortable as detected by excitement, no higher doses of isoproterenol were given. One week later, dogs received, orally bid, escalating doses of carvedilol, given 3 days at each dose. The initial dose of carvedilol was 0.15625 mg/kg, which was then doubled (0.3125 mg/kg), which was then doubled (0.625 mg/kg) and then finally doubled again (1.25 mg/kg). Isoproterenol challenges were conducted at the prescribed hours after the last of each dose of carvedilol. Dogs received no carvedilol for 3 days between each challenge. Heart rates were recorded continuously during the challenges from ECG leads I and III using a Biopac system (MP100).

2.2. Data Analysis.

Electrocardiograms were analyzed by using the Acqknowledge Software (Biopac System Inc., Version 3.5.7). Heart rate was calculated by divided 60000 ms/m by the RR interval (ms). All data were saved as text files, then transfer to the SigmaPlot Program for creating graphs and fitting curves. Heart rates were plotted against doses of isoproterenol using both linear and semi-logarithmic scales. Each curve represented the mean of heart rates of four dogs, and was fitted to the exponential equation below.

$$y = y_0 + a(1-e^{-bx})$$

Where y is the heart rate and x is an isoproterenol dose

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The doses of isoproterenol, which were required to increase heart rate 50 % of the maximal response (ED_{50}) of each dog, were extrapolated from the curves that were generated. A two-way ANOVA with repeated measured design was used for seeking differences. If a statistically significant ($p< 0.05$) f-statistic was achieved, differences among specific means were sought using the Student-Newman-Keuls post-hoc test requiring a $p<0.05$ for significance.

RESULTS

Pharmacokinetics of intravenous carvedilol:

After intravenous administration, plasma concentrations of carvedilol were highest at the 1-minute sample, then declined rapidly to two phases: distribution, elimination (Figure 3.1). For the distribution phase, mean half-life was 3.6 minutes with a standard deviation (SD) of 2.4 minutes. For the elimination phase, mean half-life was approximately 52 minutes with a SD of 24 minutes. The mean AUC was 70.6 μg.hr/L with a SD of 12.6 μg.hr/L. The mean volume of distribution was 1830 ml/kg with a SD of 918 ml/kg. The mean rate of plasma clearance was 24.3 ml/kg/min with the SD of 4.6 ml/kg/min.

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Degree of β-adrenergic blockade.

Carvedilol shifted the dose-response curves for the isoproterenol challenge to the right, and decreased the maximal heart rate responses at all doses. These changes occurred in a dose-effect relationship. That is, the higher the dose of carvedilol, the more depression of maximal response (as shown in figure 3.2 to 3.7). The shift to the right and depression of maximal response by carvedilol at the dose of 0.15625 mg/kg persisted up to 8 hours after the last given dose of carvedilol, and these changes were found up to 16 hours after the last doses of 0.3125 and 0.625 mg/kg. For the 1.25 mg/kg, suppression of the heart rate response existed for up to 24 hours. At carvedilol doses of 0.625 and 1.25 mg/kg, maximal responses of heart rates were eliminated even though such high doses of isoproterenol (4.096 and 8.192 μg/kg/min respectively) were given that the dogs did not tolerate them during the control period.

The relationship between the doses of isoproterenol which produced a 50 % increase in heart rate (ED_{50}) and the dose of carvedilol are shown (table 3.2). The highest values of ED_{50} were found at the fourth hour after every given dose of carvedilol. The ED_{50} at the first, second, fourth, and eighth hours after giving carvedilol at the dose of 1.25 mg/kg were significantly different ($p<0.05$) from the control and other carvedilol doses.

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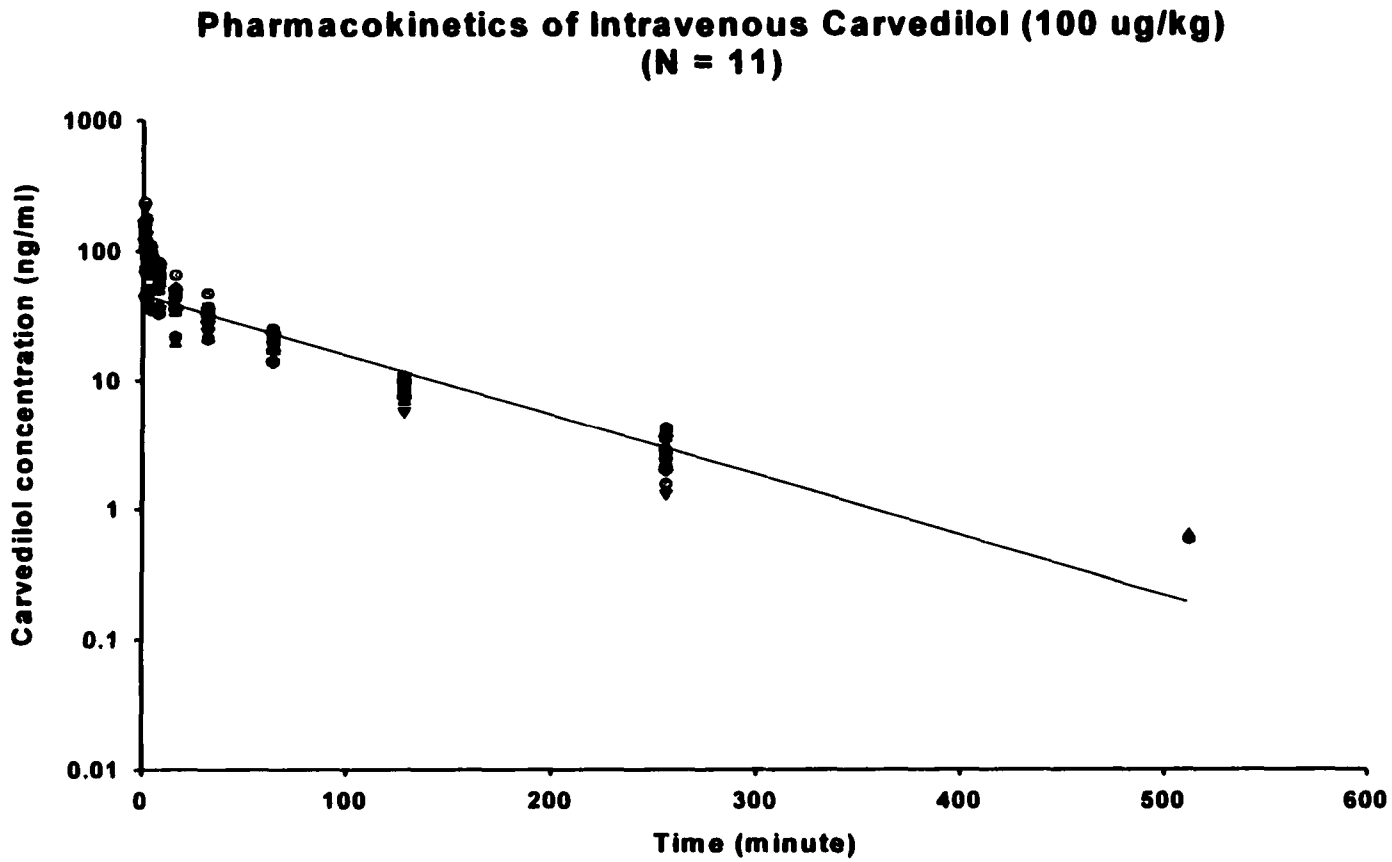

Figure 3.1: The semi-logarithmic curve of plasma concentration of carvedilol against time. Plasma concentrations of carvedilol in 11 healthy adult beagles after intravenous administration of 100 μg of carvedilol/kg of body weight at time 0.

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Dog No.	A	alpha	B	beta	$t_{1/2}$ (alpha)	$t_{1/2}$ (beta)	AUC	Clt	V_d
	ng/ml	1/min	ng/ml	1/min	min	min	µg*hr/l	ml/kg/min	ml/kg
1	192.13	0.2619	52.48	0.0145	2.65	47.79	72.55	22.97	1584.41
2	302.55	0.2586	88.96	0.0198	2.68	35.00	94.39	17.66	891.83
3	300.95	0.3517	57.86	0.0167	1.97	41.50	72.00	23.15	1386.10
4	219.50	0.4120	58.20	0.0250	1.68	27.72	47.68	34.96	1398.30
5	145.01	0.1104	62.12	0.0166	6.28	41.75	84.27	19.78	1191.49
6	169.35	0.2639	60.85	0.0172	2.63	40.29	69.66	23.93	1391.06
7	203.44	0.2184	65.93	0.0171	3.17	40.53	79.79	20.89	1221.59
8	168.54	0.3632	42.07	0.0122	1.91	56.80	65.21	25.56	2094.98
9	124.53	0.1627	42.55	0.0154	4.26	45.00	58.80	28.34	1840.42
10	51.33	0.0710	23.98	0.0070	9.76	99.00	69.15	24.10	3443.29
11	89.25	0.2661	24.71	0.0071	2.60	97.61	63.59	26.21	3691.69
Mean	178.78	0.2491	52.70	0.0153	3.60	52.09	70.64	24.32	1830.47
SD	78.25	0.1050	18.78	0.0052	2.42	23.96	12.60	4.63	918.33

Table 3.1: Pharmacokinetic parameters of carvedilol. Pharmocokinetic parameters are calculated after intravenous injection of carvedilol at a dose of 100 µg/kg body weight in eleven healthy beagle hounds.

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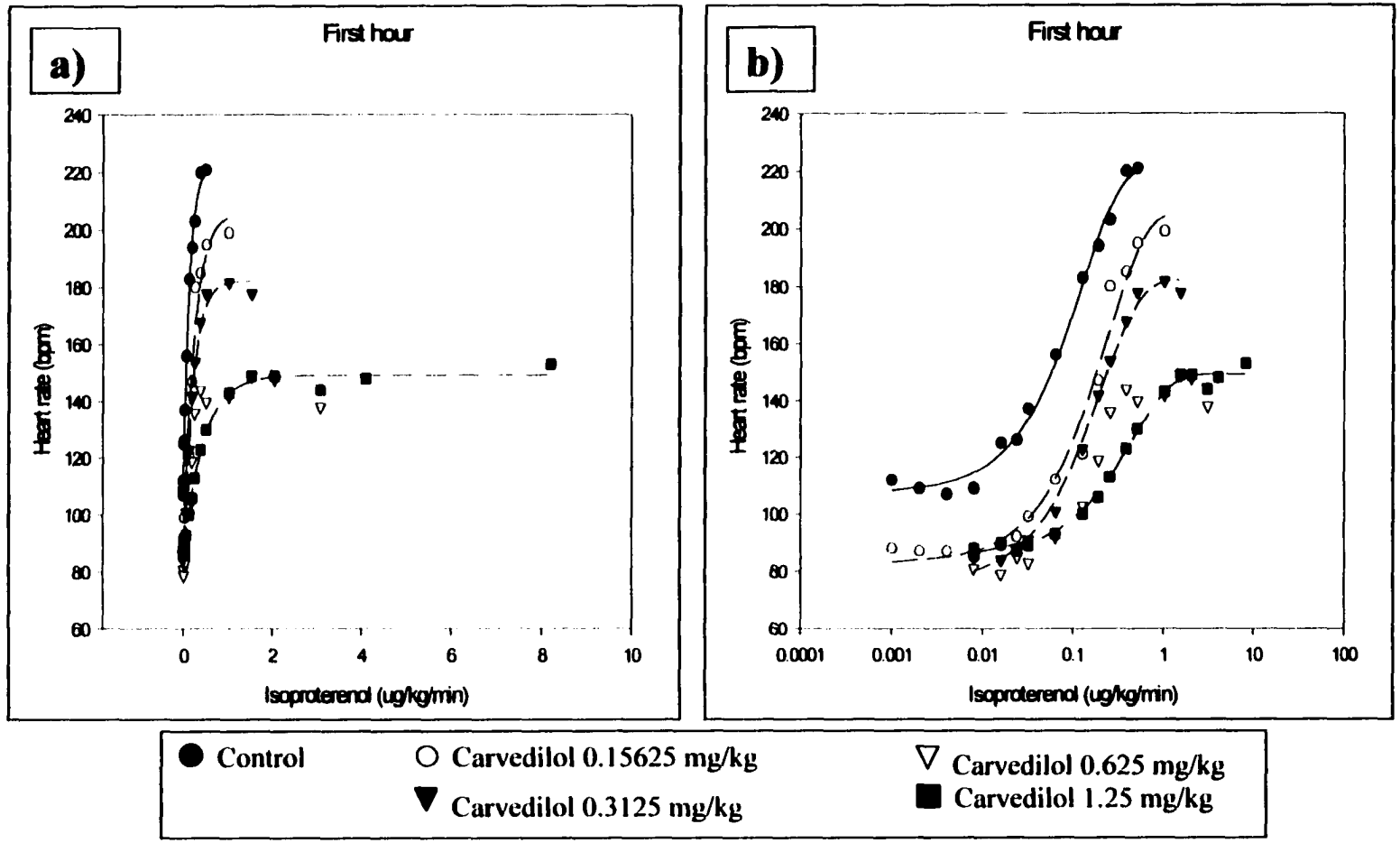

Figure 3.2: Dose-response curves at the first hour plotted in normal scale (a) and semi-logarithmic scale (b). Each point represents the mean of heart rate at a given dose of isoproterenol. Doses of carvedilol are 0.15625, 0.3125, 0.625, and 1.25 mg/kg, twice a day (n=4).

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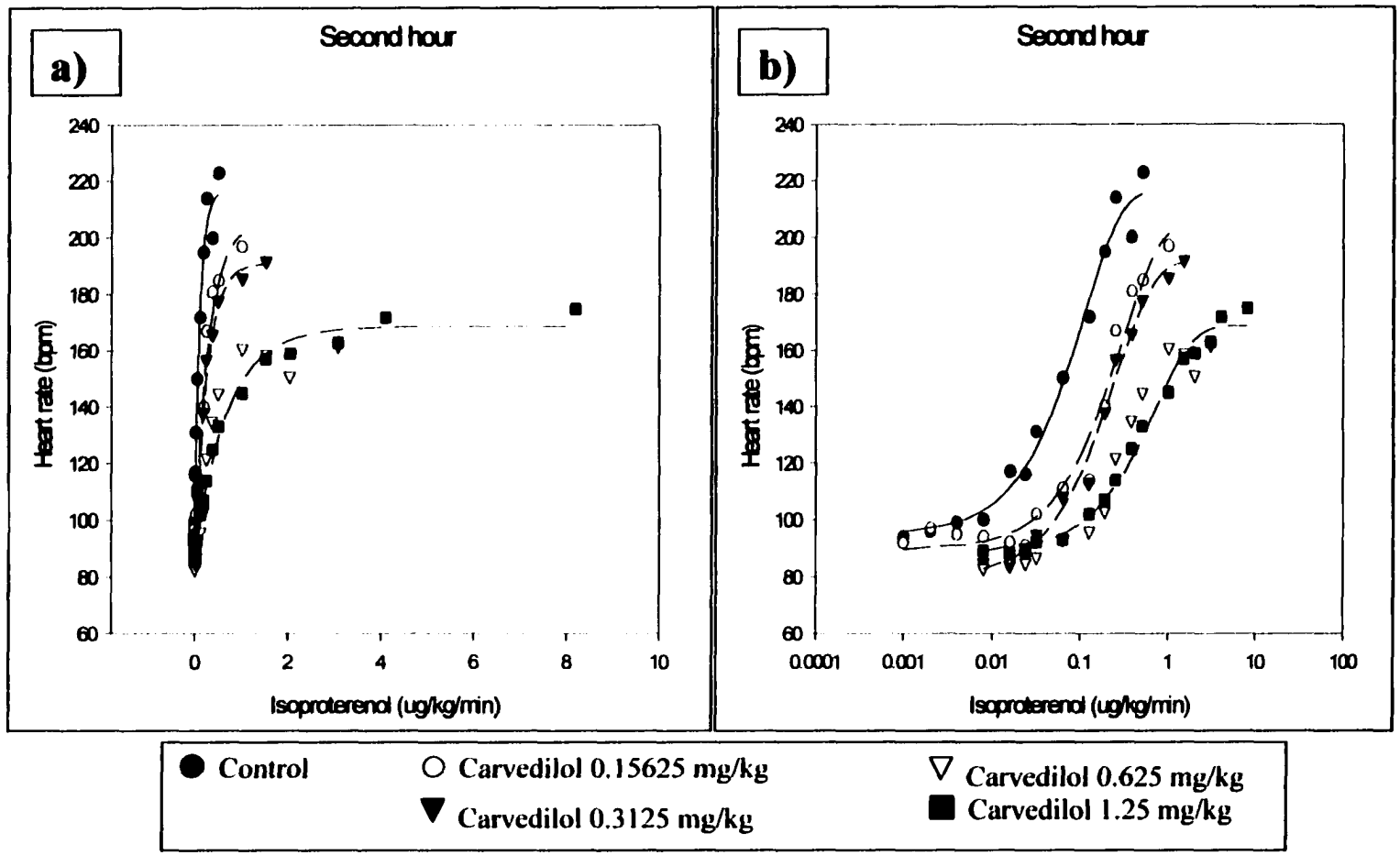

Figure 3.3: Dose-response curves at the second hour plotted in normal scale (a) and semi-logarithmic scale (b). Each point represents the mean of heart rate at a given dose of isoproterenol. Doses of carvedilol are 0.15625, 0.3125, 0.625, and 1.25 mg/kg, twice a day (n=4).

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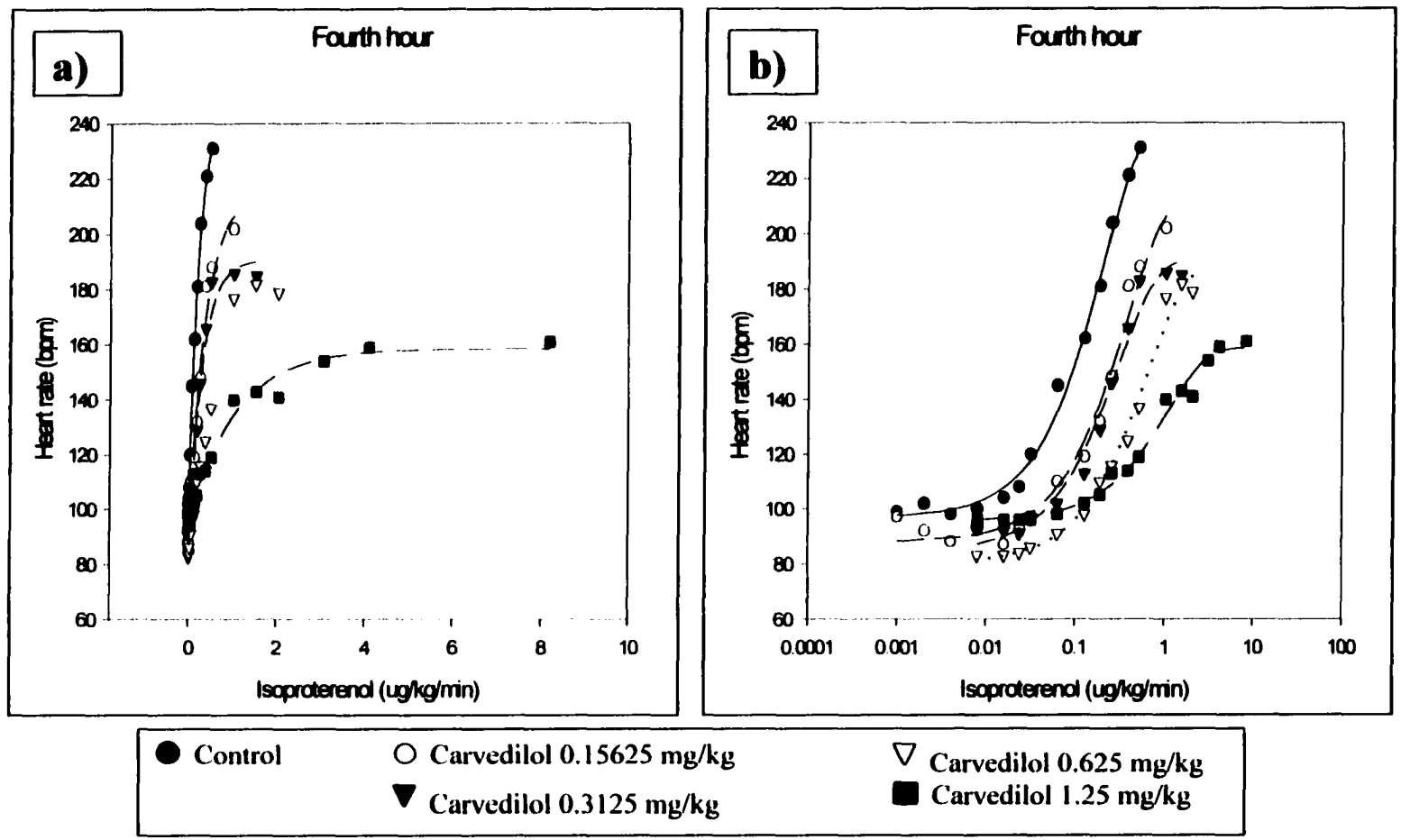

Figure 3.4: Dose-response curves at the fourth hour plotted in normal scale (a) and semi-logarithmic scale (b). Each point represents the mean of heart rate at a given dose of isoproterenol. Doses of carvedilol are 0.15625, 0.3125, 0.625, and 1.25 mg/kg, twice a day (n=4).

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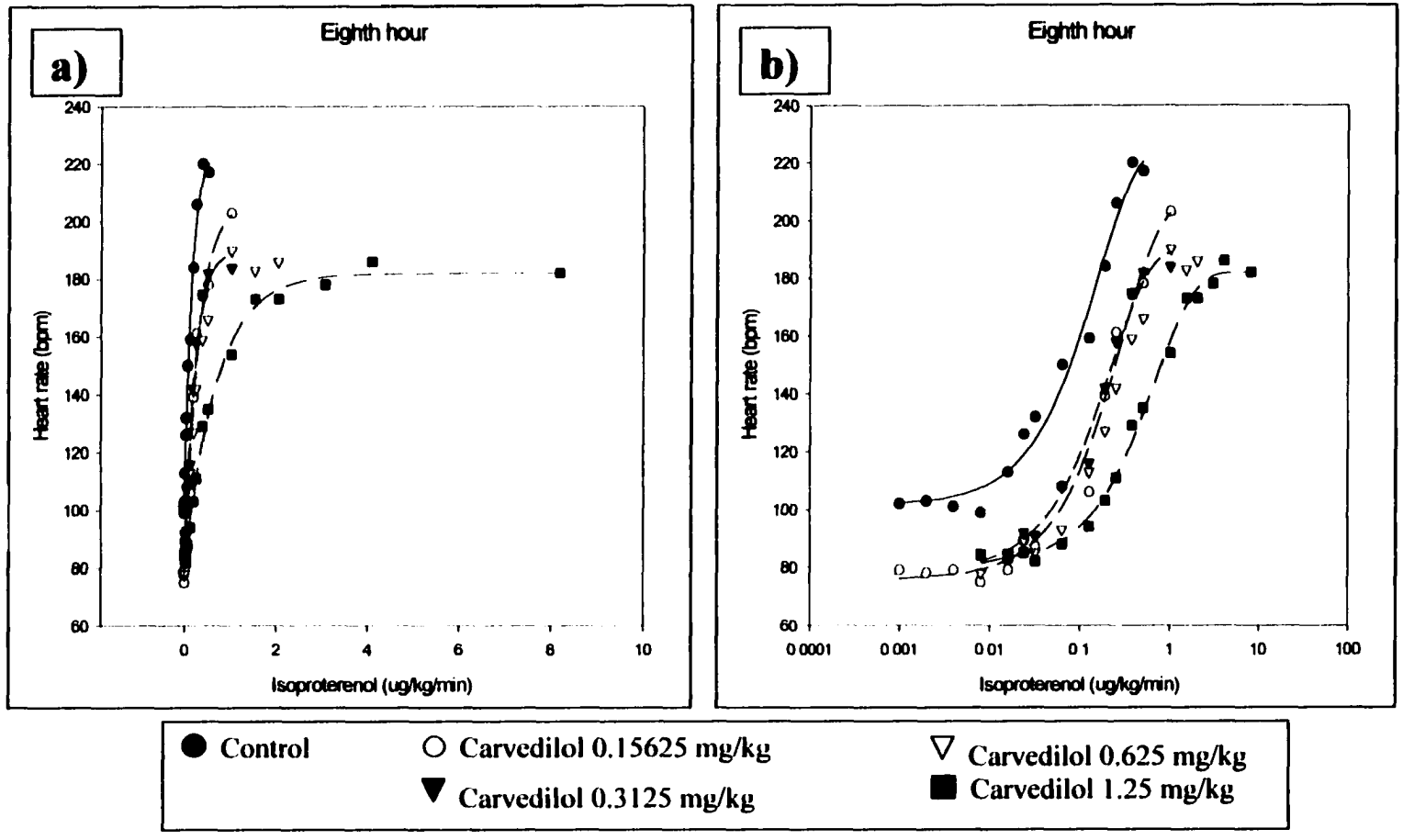

Figure 3.5: Dose-response curves at the eighth hour plotted in normal scale (a) and semi-logarithmic scale (b). Each point represents the mean of heart rate at a given dose of isoproterenol. Doses of carvedilol are 0.15625, 0.3125, 0.625, and 1.25 mg/kg, twice a day (n=4).

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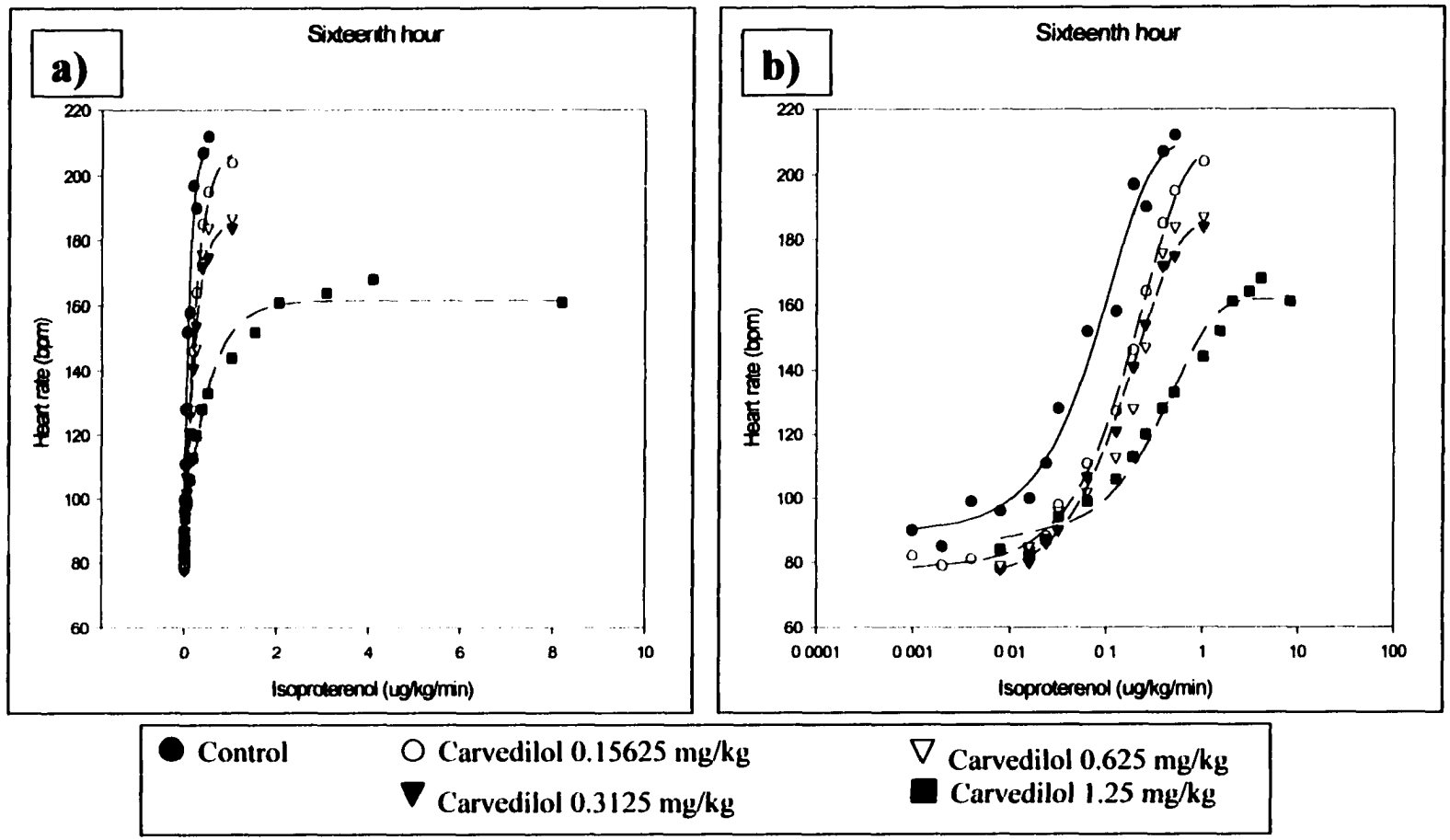

Figure 3.6: Dose-response curves at the sixteenth hour plotted in normal scale (a) and semi-logarithmic scale (b). Each point represents the mean of heart rate at a given dose of isoproterenol. Doses of carvedilol are 0.15625, 0.3125, 0.625, and 1.25 mg/kg, twice a day (n=4).

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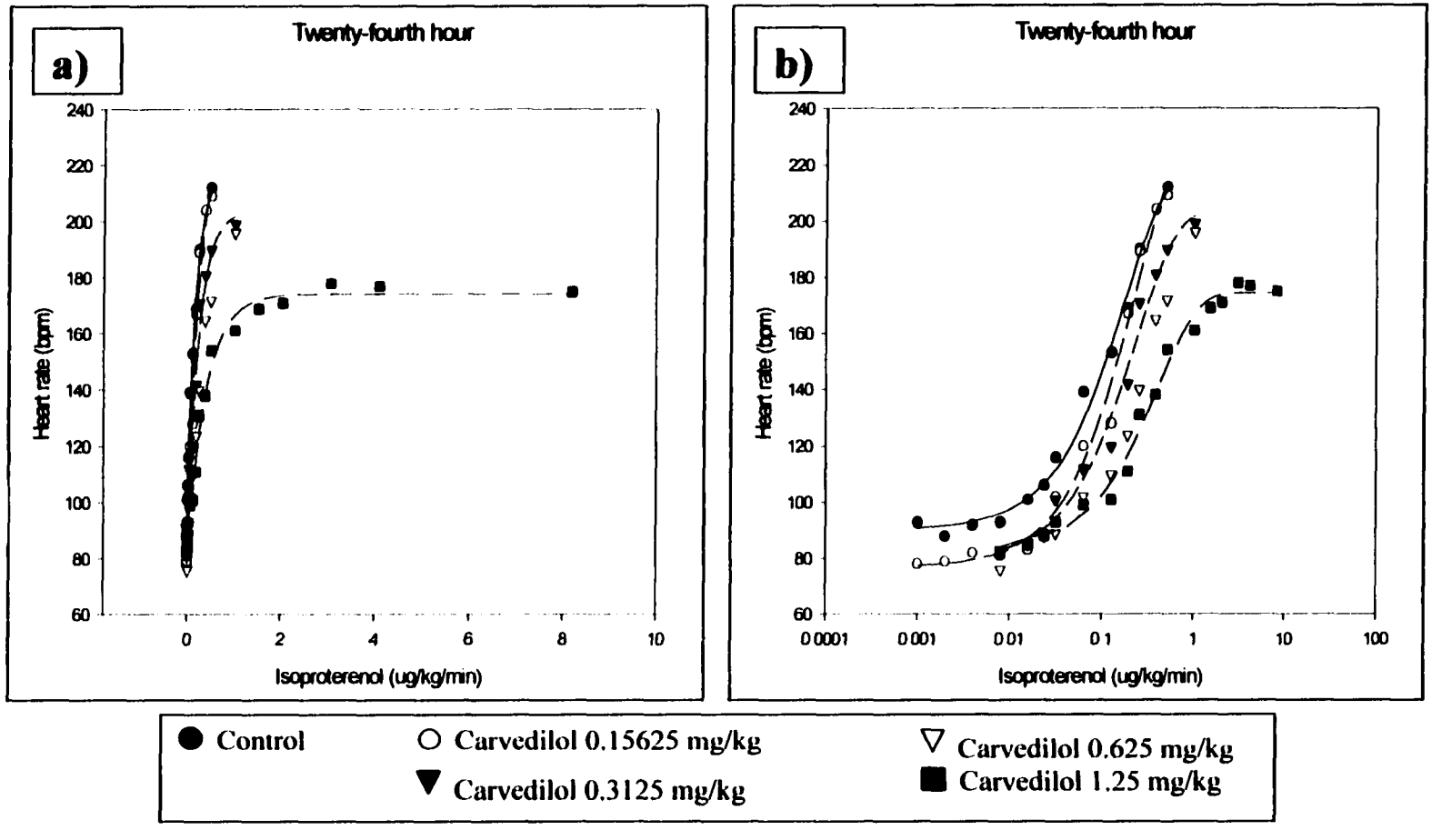

Figure 3.7: Dose-response curves at the twenty-fourth hour plotted in normal scale (a) and semi-logarithmic scale (b). Each point represents the mean of heart rate at a given dose of isoproterenol. Doses of carvedilol are 0.15625, 0.3125, 0.625, and 1.25 mg/kg, twice a day (n=4).

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Carvedilol doses	Doses of isoproterenol which increase heart rate 50 % of maximal response (ng/kg/min)					
	First hour	Second hour	Fourth hour	Eighth hour	Sixteenth hour	Twenty-fourth hour
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Control	94.2 ± 19.1 [a,c]	85.4 ± 10.2 [a,c]	135.6 ± 27.2 [a,c]	94.5 ± 22.4 [a,c]	87.6 ± 23.9 [a,c]	124.8 ± 39.4 [a,c]
0.15625 mg/kg	153.5 ± 18.6 [a,c]	208.5 ± 58.8 [a,c]	237.1 ± 58.5 [a,c]	209.6 ± 48.6 [a,c]	167.2 ± 38.0 [a,c]	126.3 ± 15.5 [a,c]
0.3125 mg/kg	166.0 ± 28.2 [a,c]	225.5 ± 66.5 [a,c]	239.1 ± 50.9 [a,c]	159.1 ± 26.4 [a,c]	163.8 ± 42.2 [a,c]	179.4 ± 37.6 [a,c]
0.625 mg/kg	212.1 ± 55.8 [a,c]	318.3 ± 69.3 [a,c]	435.6 ± 53.5 [a,c]	238.5 ± 59.2 [a,c]	201.3 ± 30.2 [a,c]	294.4 ± 118.0 [a,c]
1.25 mg/kg	423.2 ± 109.9 [b,c]	664.2 ± 183.2 [b,c]	1164.6 ± 315.2 [b,d]	544.2 ± 71.9 [b,c]	392.3 ± 183.2 [a,c]	420.6 ± 161.2 [a,c]

Table 3.2 Doses of isoproterenol which increase heart rate 50 % of maximal response (ED_{50}). Means (±SE) of ED_{50} are shown in the table at the control period and after given carvedilol at doses of 0.15625, 0.3125, 0.625, and 1.25 mg/kg of body weight (n=4).

a = no difference between doses in the same hour
b = significant difference ($p < 0.05$) between doses in the same hour
c = no difference between hours at the same dose
d = significant difference ($p<0.05$) between hours at the same dose

DISCUSSION

Pharmacokinetics of intravenous carvedilol:

The present study shows the AUC of carvedilol in dogs (70 μg.hr/L) is smaller than in humans (354 μg.hr/L) (Neugebauer, 1987). The dose (100 μg/kg) used in the present study is also lower than the doses used in man (135-200 μg/kg), but clearance for dogs (24.3 ml/kg/min) in the present study was higher than for humans (6-10 ml/kg/min). These no doubt contribute to the differences in AUC between species. The elimination half-life in dogs (< 1 hour) is also shorter than in humans (2.38 hrs). Because carvedilol is lipophilic, its volume of distribution should be greater than for a hydrophilic compound. That is it has access to greater extracellular and possibly intracellular spaces. A study of carvedilol metabolism in dogs shows that as much as 87%, when given orally, is excreted in feces within 24 hours, indicating that this compound is extensively eliminated by hepatic metabolism (Schaefer, 1998). Therefore, differences of metabolism and elimination between species may play roles in the different pharmacokinetics.

Carvedilol and its metabolites are recycled via enterohepatic pathways. Metabolites of carvedilol are primarily formed by hydroxylation, then by conjugation (Schaefer, 1998). The glucuronidation of the parent compound is the major metabolic pathway of elimination. There are differences among species in their metabolic pathways. The metabolic pathway in rats may differ from dogs, and shows oxidation as a primary metabolic step. Hydroxylation of the carbazolyl ring is the main pathway in dogs, but hydroxylations of the phenyl ring and the carbazolyl ring are common pathways in humans (Schaefer, 1998). Interestingly, hydroxylation of the carbazolyl ring may produce

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the more potent antioxidant analog, BM-910228 or SB 211475 (Kramer, 1996; Lysko, 1998), and this hydroxylated analog which is conjugated to glucoronide is also found in vivo (Schaefer, 1998). Therefore, it is possible that some metabolites of carvedilol may still posses pharmacological activity.

Carvedilol is highly bound to plasma protein-- especially to albumin, and this binding does not depended on concentration within the therapeutic range. Therefore, the dose which is given to elder patients (Louis, 1987) or to patients with heart failure, renal insufficiency (Masumura, 1992), or hepatic impairment (Neugebauer, 1992), should be monitored due to the different levels of plasma proteins, clearances and volumes of distribution.

Degree of β-adrenergic blockade:

The degree of β-adrenergic blockade of carvedilol was documented by how much the increase in heart rate due to isoproterenol was attenuated. The higher doses of carvedilol increased the ED_{50} and decreased the maximal heart rate response. The commercial drug used in this study is the combination of both stereoisomers. It is thought that the β-adrenergic blockade is mostly the effect of the S(-) enantiomer (Sponer, 1992; McTavish, 1993).

In the present study, maximal heart rate, in response to an infusion of 0.512 μg/kg/min of isoproterenol, was decreased approximately 50% by an oral dose of approximately 0.3125 mg/kg of carvedilol at the 1^{st}, 2^{nd}, and 4^{th} hours after the last dose of carvedilol (Figure 3.8).

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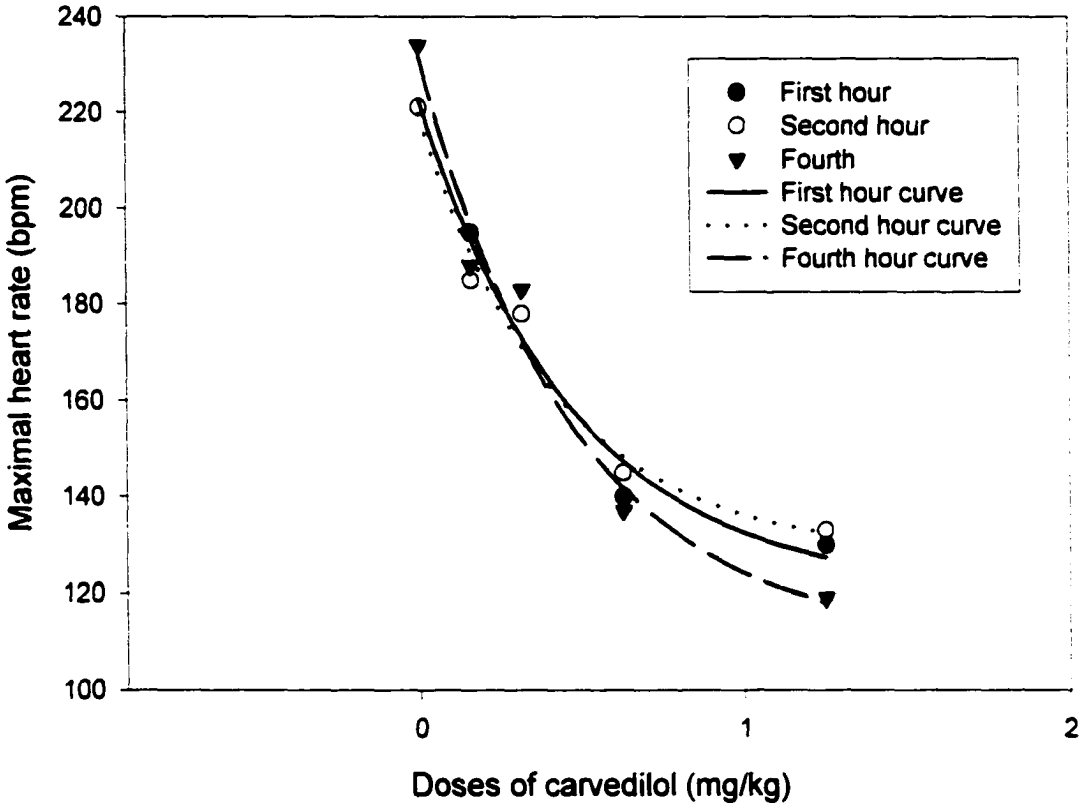

Figure 3.8 Dose-response curves between doses of carvedilol and heart rates. Graphs are plotted between doses of carvedilol and heart rates after isoproterenol infusion at a dose of 0.512 μg/kg/min. Heart rates were recorded at 1, 2 and 4 hours after the last doses of carvedilol (0.15625, 0.3125, 0.625, and 1.25 mg/kg, bid, for 3 days).

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Carvedilol was measured in plasma samples collected after the oral dose at 1.25 mg/kg in three dogs in experiment II at 0, 1, 4, 8, 16, 24 hours just before starting isoproterenol infusion. Peaks plasma concentrations were reached between 2 and 4 hours after the last dosing with the mean of maximal plasma concentrations were 181.5 ng/ml (SD = 30 ng/ml). The elimination half-life was surprisingly short at about 67 minutes. However, the frequency of plasma sampling (only three points) may limit the time-resolution of this calculation. Studies in humans have shown that doubling the dose of carvedilol produces a greater larger AUC without any effect on the elimination half-life (Neugebauer, 1987; Louis, 1987). This implies that the rate of metabolism between two doses (25 mg and 50 mg orally) may not be different. Therefore, the higher dose of carvedilol should produce greater and longer effects.

Recently, a study in humans conducted by Stoschitzky et al. has been shown that the R(+) enantiomer increases heart rate both during exercise and recovery. On the contrary, the S(-) enantiomer depressed heart rate at both activities, and the combination of both enantiomers decreases heart rate and systolic blood pressure only during exercise (Stoschitzky, 2001a). These may be due to the contribution of S(-) enantiomer on β-adrenergic blockade, and both enantiomers on α_1-adrenergic blockade. Moreover, the higher dose of carvedilol may increase heart rate rather than decrease the hear rate (Stoschizky, 2001b). The increase in heart rate may be a consequence of the baroreceptor reflex in response to vasodilatation and systemic arterial hypotension. The metabolism between both stereoisomers is also different. Oldham and Clarke show that the metabolic rate of the S(-) enantiomer is higher than the R(+) enantiomer, although both are

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metabolized in liver microsomes by the cytochrome P450 enzymes (Oldham, 1997). Therefore, the mixture of equal stereoisomers may cause higher plasma concentration of the R(+)-enantiomer, which plays a role in the balance between cardiac contractility β-adrenergic blockade) and vasodilating properties (α_1-adrenergic blockade). A lower activity of P450 enzymes in humans may play a role on the rate and pathways of metabolism, especially phase I (Oldham, 1997). P450 inhibitors e.g. quinidine (CYP2D6), ketoconazole (CYP3A), sulfaphenazole (CYP2C9), and furafylline (CYP1A2) may interfere carvedilol metabolism (Oldham, 1997).

Therefore, the ratio of both stereoisomers in the commercial drug and administration dosage may affect the duration of α_1-adrenergic and β-adrenergic blockades. The combination of carvedilol with some P450 inhibitors may change the rate and pathways of carvedilol metabolism. Species, race, and pathways of metabolism may affect both active metabolites and the actions of carvedilol on living organisms.

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CHAPTER 4

POTENTIAL FOR CARVEDILOL TO MODIFY DOXORUBICIN CARDIOTOXICITY

ABSTRACT

Doxorubicin-induced cardiomyopathy is known to limit the use of this antineoplastic drug in patients with cancer. Although the origin of the cardiomyopathy is not known for certain, production of free radicals of oxygen are thought to be important. There are many free radical scavenging drugs which have demonstrated the ability of modifying doxorubicin cardiotoxicity, but only one has been approved for use in humans, dexrazoxane. However, there is no drug which alleviates the cardiac dysfunction together with prevention injury from free radicals. Carvedilol is a beta blocker which has been approved for use in the treatment of patients with dilated cardiomyopathy and heart failure. It also has free radical scavenging activity which may play a protective role. The purpose of this study was to determine if carvedilol has a protective effect in a canine model of doxorubicin-induced cardiomyopathy. Fourteen healthy beagle hounds were given intracoronary injections of doxorubicin at a dose of 0.75 mg/kg once a week for 4 or 5 weeks. They were divided into those receiving nothing in addition (Group 1) and

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those receiving orally 2.5 mg/kg of carvedilol daily (Group 2). Animals in Group 2 had lower PEP/ET ratio's, shorter tau's, higher systemic blood pressures and V_{max}, and had shorter QTc intervals compared to animals in Group1. Heart rate variability of animals in Group 1 was also decreased at the ninth week compared to the baseline, but there was no change in animals in Group 2. This represents a cardioprotective action of carvedilol.

INTRODUCTION

The dose of doxorubicin used to treat cancer is limited by production of doxorubicin-induced cardiomyopathy characterized by reduced contractility, arrhythmia, and altered conductivity. It is thought that these adverse effects are mediated through the production of reactive oxygen species generated by the semiquinone moiety of the molecule doxorubicin. There is a report of doxorubicin-induced cardiotoxicosis in which 18% of dogs receiving doxorubicin showed clinical cardiac abnormalities, and cumulative doses which produced cardiomyopathy in dogs were lower than in humans (Mauldin, 1992). Research has been conducted to develop less cardiotoxic anthracyclines (e.g. idarubicin, epirubucin), or to develop compounds (e.g. dexrazoxane, probucol) that might either prevent or salvage from cardiotoxicity (Horenstein, 2000; Iliskovic, 1999). Alternatively, afterload reducers (e.g. captopril and prazosin) have been proposed to blunt doxorubicin-induced cardiotoxicity (Khaper, 1997). Recently, a case report which showed a beneficial effect of carvedilol in a human patient who received doxorubicin for treatment of breast cancer (Fazio, 1998). She developed heart failure which was not responsive to digoxin, furosemide and enalapril, but did respond to carvedilol. Carvedilol-- a non-specific β-adrenergic blocker, α_1-adrenergic blocker and scavenger of

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free radicals of oxygen-- possesses, in the same molecule, properties that might limit or reverse cardiotoxicity from higher doses of doxorubicin. The objective of the present study was to evaluate the potential value of carvedilol for permitting the use of greater doses of doxorubicin. Information from this study may be relevant to the more effective treatment of both humans and dogs with the most commonly-used antineoplastic, whose therapeutic potential has not been achieved because of cardiotoxicity.

MATERIALS AND METHODS

Animals use:

Experiments were performed in 14 healthy mature beagle hounds of either sex. All dogs were given 0.75 mg/kg of doxorubicin, weekly for 4 weeks (except two dogs which were given for 5 weeks) into the coronary circulation. Animals were divided into two groups, and each group had a dog which was given doxorubicin for 5 weeks. Animals in the first group (n=7) received nothing else. Animals in the second group (n=7) received doxorubicin for 4 weeks as described (except a dog that received 5 injections of doxorubicin) plus carvedilol given orally at a dose of 2.5 mg/ kg of body weight daily starting just before the first dose of doxorubicin and ending three days before euthanasia (approximately 9 weeks).

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Echocardiographic study:

A day before surgery, animals were narcotized with 0.05 mg/kg of butorphanol given intramuscularly, and 15 minutes later animals were positioned in right lateral recumbency. ECG leads were connected, and echocardiograms were obtained using a 5 mHz probe and an Aloka echocardiograph (model SSD-1400). M-mode echocardiography were performed from a 2-D guided, right parasternal short-axis view (for the left ventricle) and from the right parasternal long-axis view (for the left atrium and aorta). These echocardiograms were analyzed for the following dimensions: left atrial, aortic, left ventricle, and for left ventricular wall thickness. The measurements were performed weekly for four weeks and during the ninth week before animals were sacrificed.

Surgical preparation:

Animals were fasted 12 hours before starting surgery, but were given free access to water. They were pre-medicated with butorphanol (0.1 mg/kg) and acepromazine (0.1 mg/kg) intramuscularly. Anesthesia was induced with 5% isofurane, animals were intubated, and surgical anesthesia was maintained with 2% isoflurane until completion of cardiac catheterization. ECG leads were connected to monitor heart rate and heart rhythms. Aseptic technique was used to prepare the inguinal area for catheterization. The skin was opened by a surgical blade, and the femoral artery was accessed by a 5F introducer sheath. A catheter-tip pressure transducer (Millar catheter, 5F) was inserted into the aorta and into the left ventricle to measure pressures. After that, the Millar catheter was removed, and a left Judkins coronary catheter (Cook Cardiology, 5F) was

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introduced to the left main coronary artery. The position at the left coronary artery was confirmed by injection of diluted iohexol (2 ml of 240 mgI/ml, diluted by isotonic saline to 5 ml.) and visualization of opacification of the coronary circulation. Next, doxorubicin (1.5 mg/ml, diluted with isotonic normal saline to 10 ml.) was injected into the left main coronary artery over 1 minute. Finally, the Judkins catheter and the introducer sheath were removed. Bleeding was stopped by manual compression for at least 30 min. The entire procedure was performed weekly and was repeated for 3 times in all but two dogs (one dog in each group) in which they were repeated for 4 times. Dogs were rested for 4 weeks. Finally, physiological measurements were made during the 9^{th} week, before they were euthanatized with pentobarbital sodium.

Data analysis:

Four to five consecutive cardiac cycles of M-mode echocardiograms were analyzed for the following:

a. Left ventricular internal dimension at end-diastole ($LVID_d$) in cm is the distance between the left ventricular face of the interventricular septum and the endocardium of the left ventricular posterior wall (free wall) at the papillary muscle just below the mitral valve measured at the beginning of the QRS complex.

b. Left ventricular internal dimension at end-systole ($LVID_s$) in cm is the distance between the left ventricular face of the interventricular septum and the left ventricular posterior wall (free wall) at the papillary muscle level measured at the end of T wave.

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c. Fraction (FS) shortening (%) = $[(LVID_d - LVID_s) / LVID_d] * 100$

e. Pre-ejection period or PEP (sec) is the time from the beginning of QRS complex until the aortic valve begins to open.

f. Ejection time (ET) is time in seconds from the opening of the aortic valve until it closes.

g. Interventricular septal thickness at end-diastole (IVS_d) was measured at the papillary muscle level just before the beginning of QRS complex.

h. Interventricular septal thickness at end-systole (IVS_s) was measured at the papillary muscle level just before the end of T wave.

i. Aortic root dimension (AOD) in cm. was the diameter of the aortic root downstream from the Valsalva sinus measured at the beginning of QRS complex (end-diastole).

j. Left atrial dimension (LAD) in cm. was the diameter of the left atrium measured at the end of T wave.

Electrocardiograms and left ventricular pressure recordings were analyzed by formulas which were described in the Chapter 2.

For statistical analysis, a two-way ANOVA with repeated measures design was used to compare all mean values. The specific means in the same group but different periods were compared by the one-way ANOVA with repeated measures design. If indicated by a significant F-statistic, specific means were compared by the Student-Newman-Keuls multiple comparisons. The specific means between two groups at the same period were compared by the unpaired t-test.

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Heart rate Variability study:

Animals were trained to stand in a sling for 30 minutes at least 2 weeks before the experiment started. Electrocardiographic leads I and III were recorded for 5 minutes with the Biopac system (sampling rate of 1000/second) during a control period and at the ninth week of administration of compounds. RR interval tachograms were generated from electrocardiograms of all beats over five minutes, they were resampled at 5 Hz, and were subjected to Fast Fourier Transform (FFT) and Power spectral density (PSD). Time and frequency domains of HRV were calculated following the guideline of the Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology (Malik, 1996).

The time Domain method:

a) SDNN (ms) is the standard deviation of RR intervals
b) SDSD (ms) is the standard deviation of differences between adjacent RR intervals
c) RMSSD (ms) is the square root of the mean of the sum of the squares of differences between adjacent RR intervals.
d) NN50 count is the number of pairs of adjacent RR intervals which are different more than 50 ms.
e) pNN50 (%) is the percentage of NN50 in all RR intervals

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The frequency domain method:

a) The power spectral density (PSD) were calculated by the non-parametric method (Fast Fourier Transform) using the following formula (Kay, 1981);

$$PSD = \frac{1}{N\Delta t} \left| \Delta t \sum_{n=0}^{N-1} X_n \exp(-j2\pi fn\Delta t) \right|^2$$

b) Total Power (ms^2) is the variance of RR intervals (Frequency $\leq$ 0.6 Hz)

b) VLF (ms^2) is the power in the very low frequency range (Frequency $\leq$ 0.04 Hz)

c) LF (ms^2) is the power in the low frequency range (0.04-0.15 Hz)

d) HF (ms^2) is the power in the high frequency rage (0.15-0.6 Hz)

e) Normalization

i. LF_{norm} = LF/(Total Power-VLF)*100

ii. HF_{norm} = HF/(Total Power-VLF)*100

For statistical analysis, the Wilcoxon Signed Rank Test was used to make comparisons between the baseline and the ninth week. Specific means between two groups at the same period were compared by an unpaired t-test. To claim significance, a $P < 0.05$ was required.

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RESULTS

Echocardiography:

Although there were no differences in heart rate during the pretest period (baseline), dogs in Group 1 (just doxorubicin) had significantly higher heart rates than dogs in Group 2 (doxorubicin plus carvedilol) ($p<0.05$) at the 1, 3 and 9 weeks recordings. There were trends for this difference at weeks 2 and 4, but the difference did not achieve statistical significance (Figure 4.1). At the recording 3 days after stopping carvedilol, heart rate in Group 2 tended to increase toward that maintained by dogs in Group 1. Although fractional shortening did not differ between groups during baseline, it was significantly lower in Group 2 at recording times of 2, 3, and 4 weeks, insignificantly lower during week 1, and at 9 weeks when fractional shortening for Group 1 decreased precipitously, there was no difference between Groups 1 and 2 (Figure 4.2). Interestingly, factional shortening of dogs in Group 2 improved after stopping carvedilol for three days although the difference did not reach statistical significance. PEP/ET ratios of dogs in Group 1 increased significantly at the ninth week compared to dogs in Group 2 (Figure 4.3). This occurred because PEP lengthened and ET shortened. Left ventricular internal dimensions at end-diastole and end systole in dogs of Group 2 significantly increased starting from the third week and the second week respectively compared to dogs in Group1. However, dogs in both groups had significant dilation of their left ventricles at the ninth week compared to the baseline (Figures 4.4 and 4.5). Once carvedilol was stopped, left ventricular chamber sizes of dogs in Group 2 tended to decrease. At end-systole, the interventricular septum of dogs in Group 2 was significantly thinner than Group 1 from the second week. The left ventricular free wall of dogs in Group 2 was

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thinner than the baseline starting from the second week, but the left ventricular free wall of dogs in Group 1 tended to lower than dogs in Group 2 at the ninth week (Figures 4.6, 4.7, 4.8, and 4.9). Again, the left ventricular free wall thickness of dogs in Group 2 increased after cessation of carvedilol ($p<0.07$). Aortic root dimensions at end-diastole of dogs in Group 1 decreased significantly at the ninth week compared to the baseline (Figure 4.10). Left atrial dimension at end-systole of dogs in Group 2 increased significantly at the ninth week compared to the baseline (Figure 4.11), but the left atrial enlargement returns to normal after cessation of carvedilol.

Electrocardiography:

RR intervals of dogs in Group 2 significantly increased at the ninth week compared to the baseline under general anesthesia (Fig 4.12). PQ intervals of dogs in both groups were significantly increased at the ninth week compared to the baseline (Figure 4.13). QRS durations were significantly longer in Group1 at the ninth week compared to the baseline (Figure 4.14). QT intervals of dogs in Group 1 significantly increased at the ninth week and QT intervals of dogs in Group 2 significantly increased at the fourth week and the ninth week (Figure 4.15). However, QTc prolongations were observed only in Group 1 at the fourth week and the ninth week (Figure 4.16).

Three out of seven dogs in Group1 developed cardiac arrhythmias. Two dogs in Group1 had ventricular arrhythmias and another one had supraventricular arrhythmia. Only one dog in Group 2 developed supraventricular arrhythmia.

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Left ventricular and aortic pressures:

End diastolic pressures of dogs in both groups tended to increase at the fourth week, but declined at the ninth week (Figure 4.17). Left ventricular peak pressures of dogs in Group 2 were significantly higher than dogs in Group 1 at the baseline (first dose), and increases of peak pressures in both groups continued to the fourth week (Figure 4.18). dP/dt_{max} of dogs in Group 2 was higher than for dogs in Group 1 at the baseline (first dose of carvedilol), and increased in both groups at the fourth week (Figure 4.19). However, V_{max} of dogs in Group 2 at the fourth week and the ninth week was higher than the baseline, and was also higher than dogs in Group 1 at the ninth week (Figure 4.20). dP/dt_{min} of dogs in Group 2 tended to increase (i.e. became more negative), but this did not reach statistical significance (Figure 4.21). Tau for dogs in Group 2 was significantly decreased at the fourth week and the ninth week, but tau for dogs in Group 1 tended to increase at the ninth week compared to the fourth week. A significant difference for tau between the two groups was reached at the ninth week (Figure 4.22).

Aortic systolic, diastolic, and mean pressures for dogs in Group 2 is higher than for dogs in Group1 at the baseline (first dose), and these blood pressures of dogs in both groups were dropped at the ninth week (Figure4.23-4.26). Blood pressure for dogs in Group 1 increased at the fourth week compared to the baseline, but pulse pressures at the fourth week and the ninth weeks were increased only in dogs in Group 2.

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Heart rate variability:

RR intervals of dogs in Group 1 were significantly decreased at the ninth week compared to the baseline, and RR intervals of dogs in Group 2 tended to increase but that increase did not achieve statistical significance (Table 4.1). However, a significant difference ($p=0.005$) in RR intervals between groups was found at the ninth week. SDNN and RMSSD were decreased in Group 1 at the ninth week compared to the baseline. There was a trend for RMSSD ($p=0.08$) for a difference between groups at the ninth week. SDSD and pNN50 of dogs in Group 1 tended to decrease at the ninth week but the difference did not reach statistical significance ($P=0.078$). Again, the difference in pNN50 between groups at the ninth week did not reach ($P=0.09$) significance. There were no changes in any of the parameters in the time domain in animals of Group 2.

For the frequency domains, total power of HRV was significantly decreased in dogs of Group 1 at the ninth week compared to the baseline (Table 4.2), but there was no change in total power for dogs in Group 2. HF (high frequency) component HRV for dogs in Group 1 was also decreased significantly at the ninth week compared to the baseline. Decreases of VLF and LF in Group 1 were found at the ninth week when compared to dogs in Group 2, and HF between groups tended to decrease, but did not reach statistical significance.

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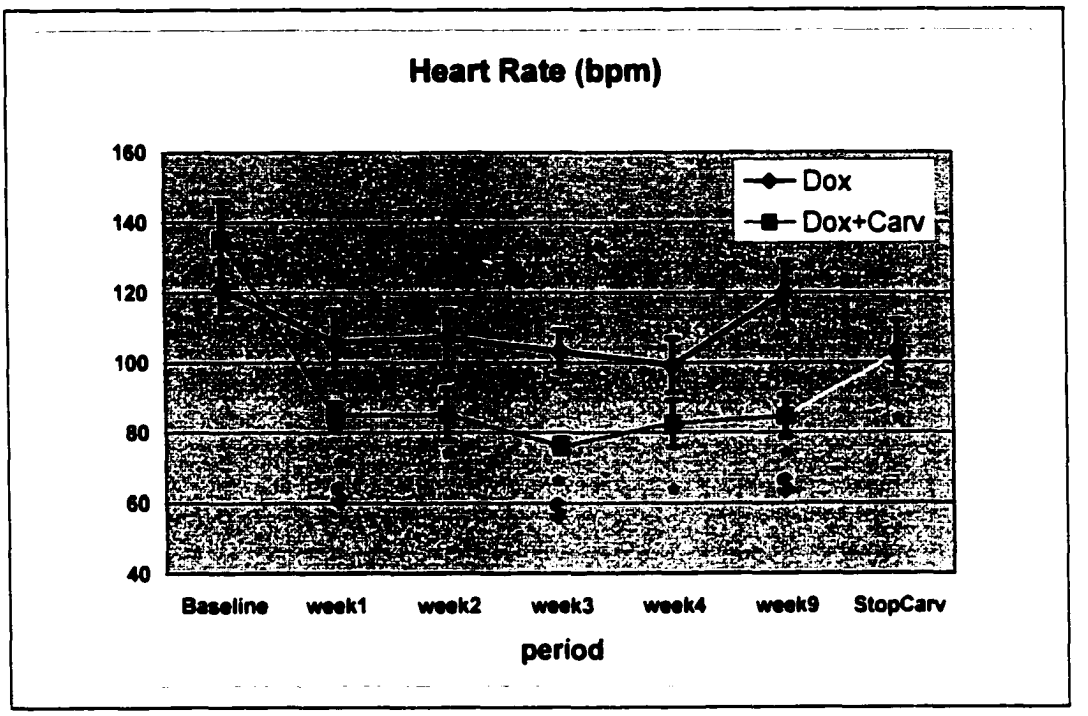

Figure 4.1: The graph of heart rate. Heart rates are compared between beagle hounds receiving doxorubicin (Dox) (n=7), and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.
($) significant differences ($p<0.05$) between group at the same period.

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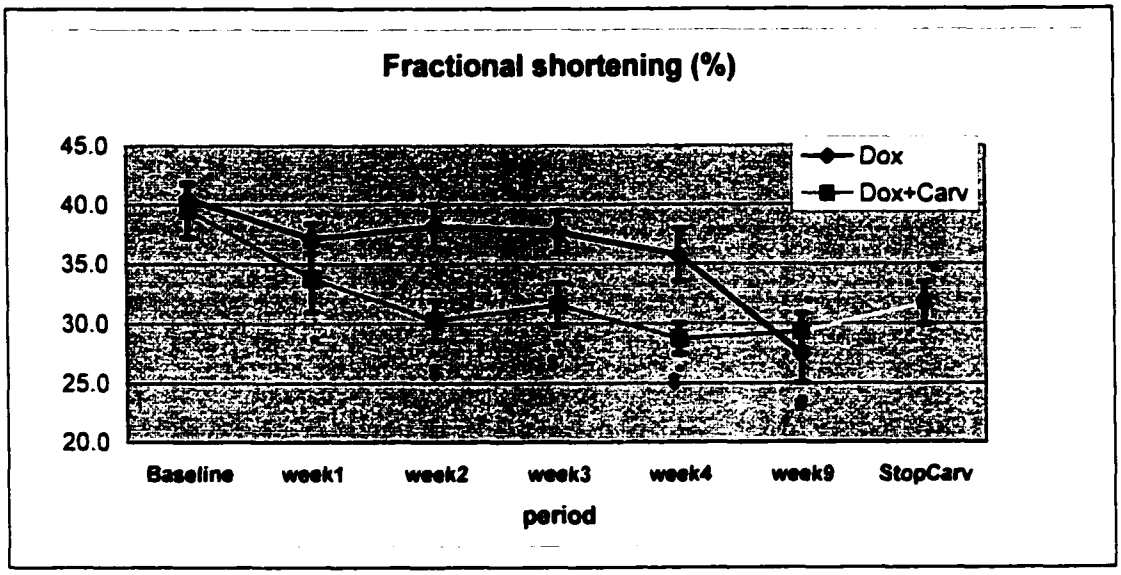

Figure 4.2: The graph of fractional shortening. Fractional shortenings are compared between beagle hounds receiving doxorubicin (Dox) (n=7), and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(#) significant differences ($p<0.05$) from the baseline in the Dox group
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.
($) significant differences ($p<0.05$) between group at the same period.

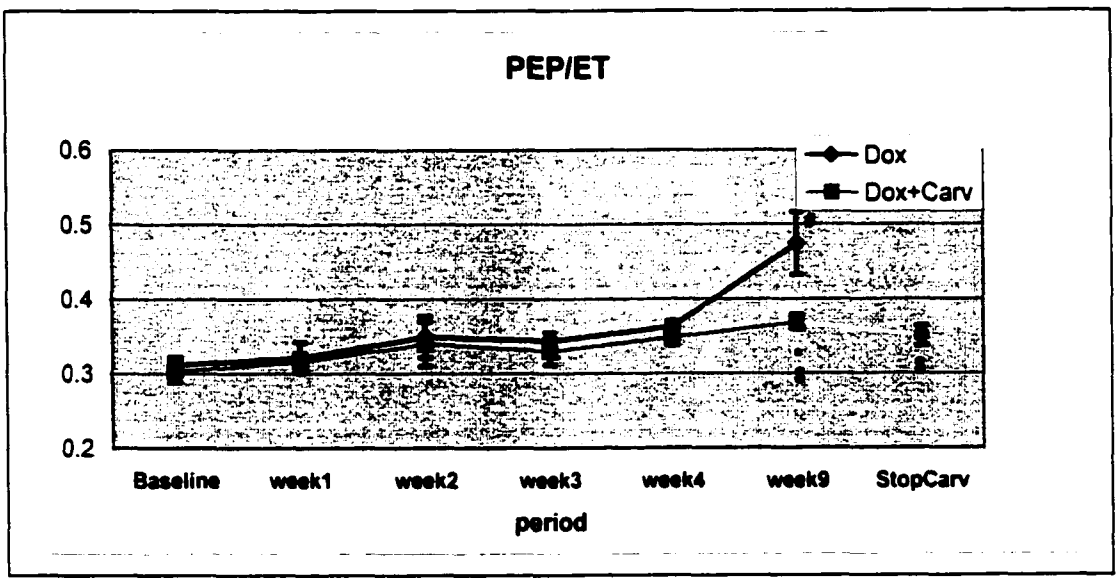

Figure 4.3: The graph of ratio between pre-ejection period (PEP) and ejection time (ET). PEP/ET ratios are compared between beagle hounds receiving doxorubicin (Dox) (n=7), and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(#) significant differences ($p<0.05$) from the baseline in the Dox group
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.
($) significant differences ($p<0.05$) between group at the same period.

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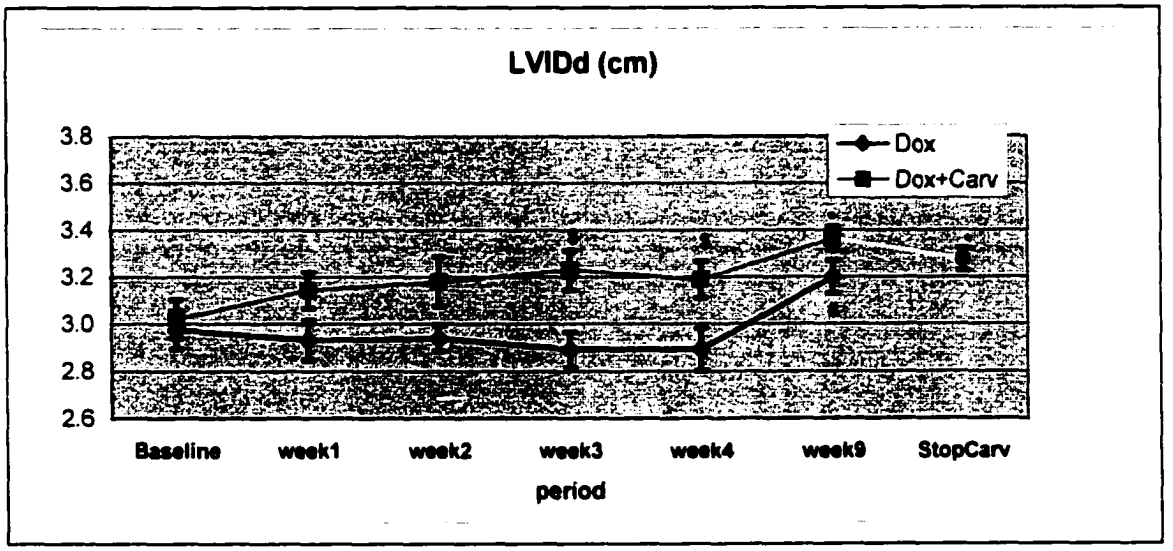

Figure 4.4: The graph of left ventricular internal dimension at end-diastole ($LVID_d$). $LVID_d$s are compared between beagle hounds receiving doxorubicin (Dox) (n=7), and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(#) significant differences ($p<0.05$) from the baseline in the Dox group
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.
($) significant differences ($p<0.05$) between group at the same period.

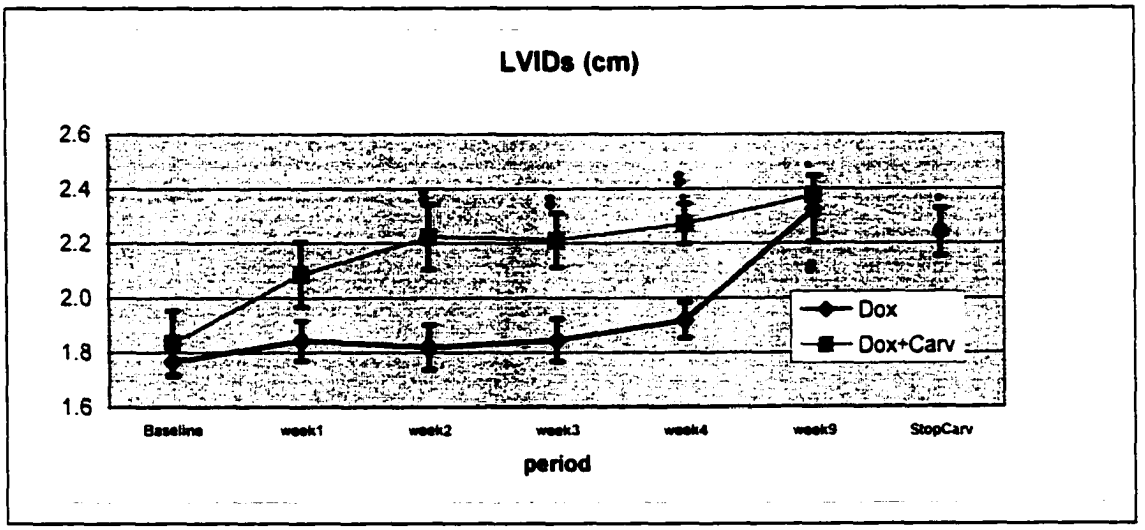

Figure 4.5: The graph of left ventricular internal dimension at end-systole ($LVID_s$). $LVID_s$s are compared between beagle hounds receiving doxorubicin (Dox) (n=7), and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(#) significant differences ($p<0.05$) from the baseline in the Dox group
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.
($) significant differences ($p<0.05$) between group at the same period.

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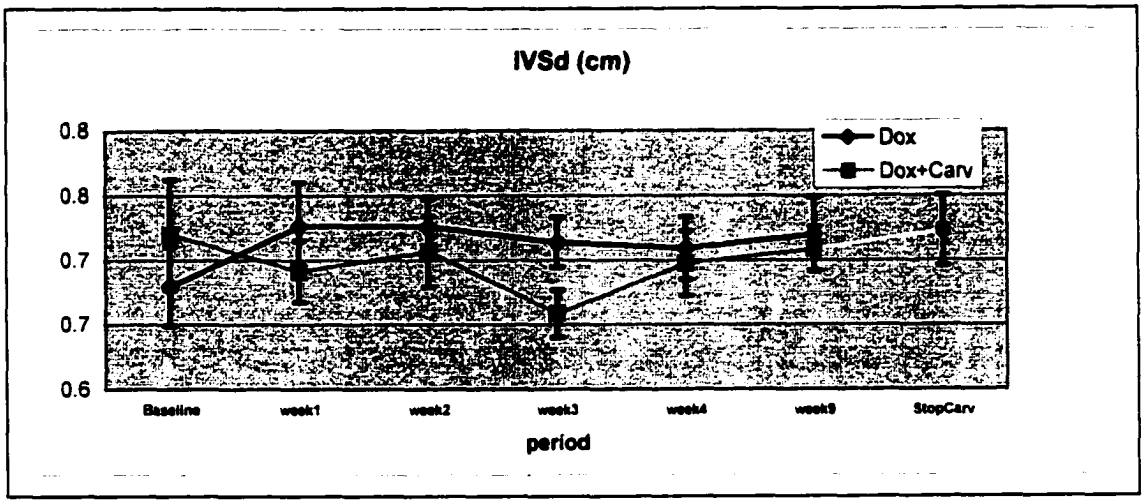

Figure 4.6: The graph of interventricular septal thickness at end-diastole (IVS_d). IVS_ds are compared between beagle hounds receiving doxorubicin (Dox) (n=7), and doxorubicin plus carvedilol (Dox+Carv) (n=7).

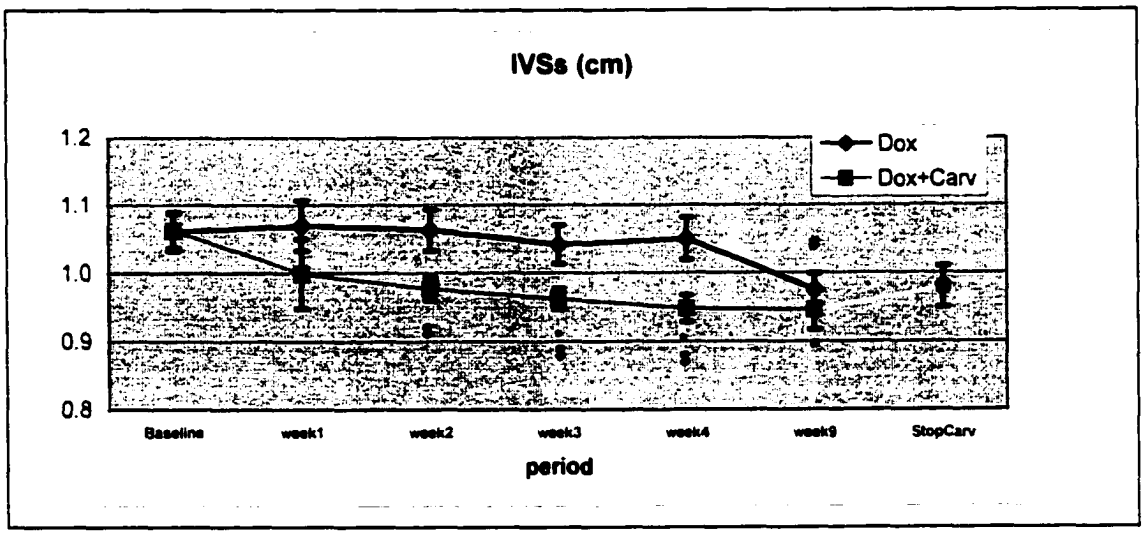

Figure 4.7: The graph of interventricular septal thickness at end-systole (IVS_s). IVS_ss are compared between beagle hounds receiving doxorubicin (Dox) (n=7), and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(#) significant differences ($p<0.05$) from the baseline in the Dox group
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.
($) significant differences ($p<0.05$) between group at the same period.

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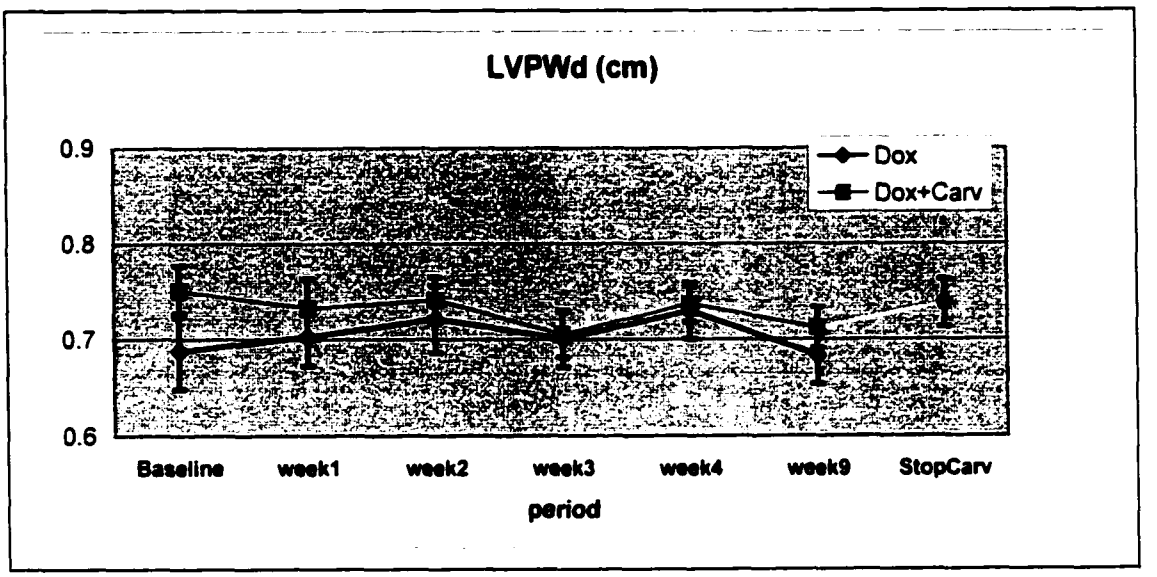

Figure 4.8: The graph of left ventricular posterior wall thickness at end-diastole ($LVPW_d$). $LVPW_d$s are compared between beagle hounds receiving doxorubicin (Dox) (n=7), and doxorubicin plus carvedilol (Dox+Carv) (n=7).

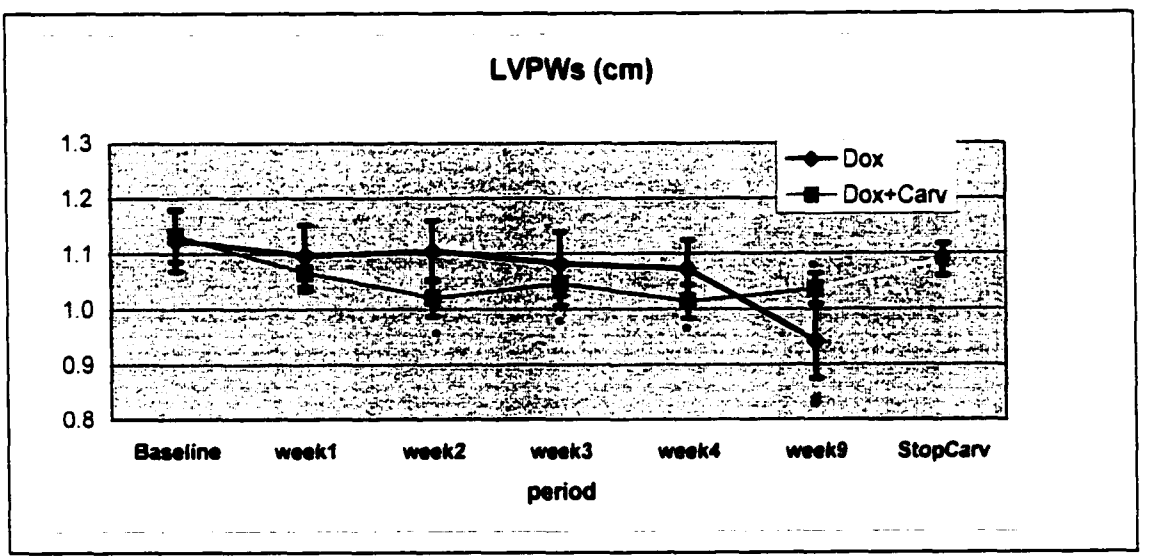

Figure 4.9: The graph of left ventricular posterior wall thickness at end-systole (LVPWs). $LVPW_s$s are compared between beagle hounds receiving doxorubicin (Dox) (n=7), and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(#) significant differences ($p<0.05$) from the baseline in the Dox group
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.
($) significant differences ($p<0.05$) between group at the same period.

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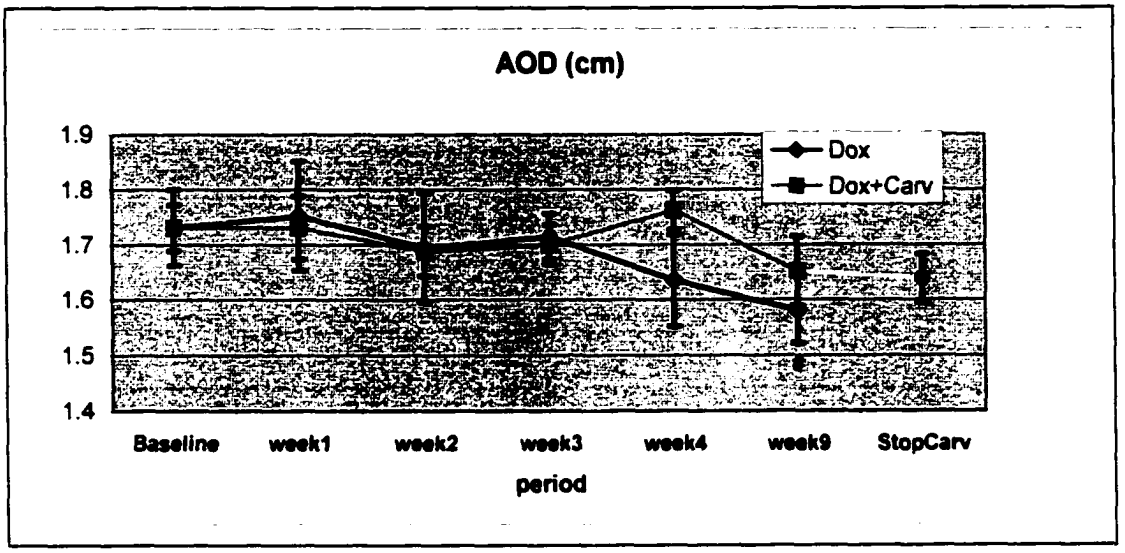

Figure 4.10: The graph of aortic root dimension at end-diastole (AOD). AODs are compared between beagle hounds receiving doxorubicin (Dox) (n=7), and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(#) significant differences ($p<0.05$) from the baseline in the Dox group

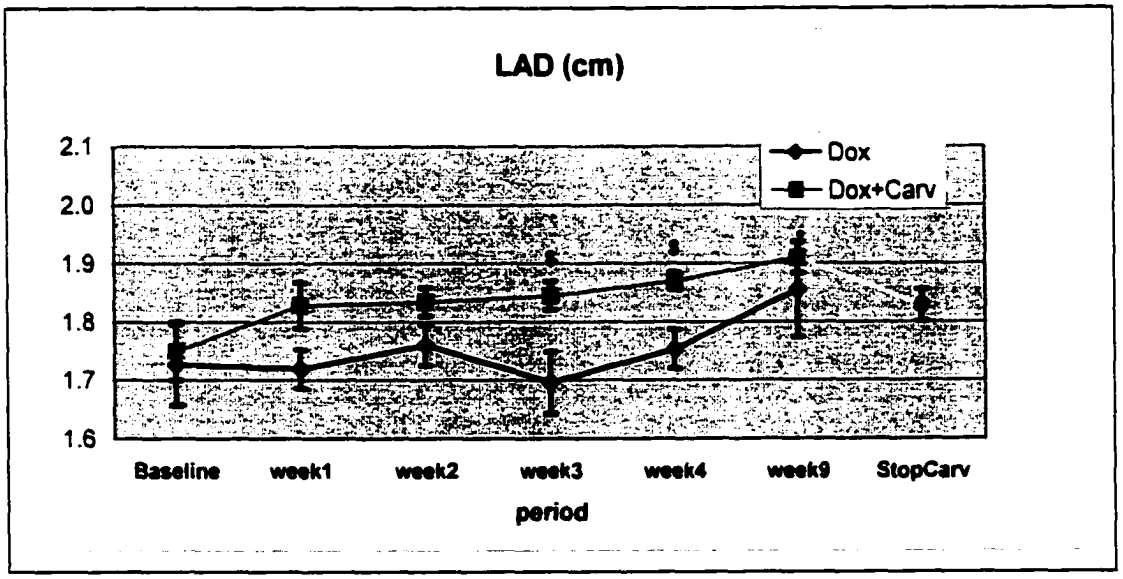

Figure 4.11: The graph of left atrial dimension at end-systole (LAD). LADs are compared between beagle hounds receiving doxorubicin (Dox) (n=7), and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.
($) significant differences ($p<0.05$) between group at the same period.

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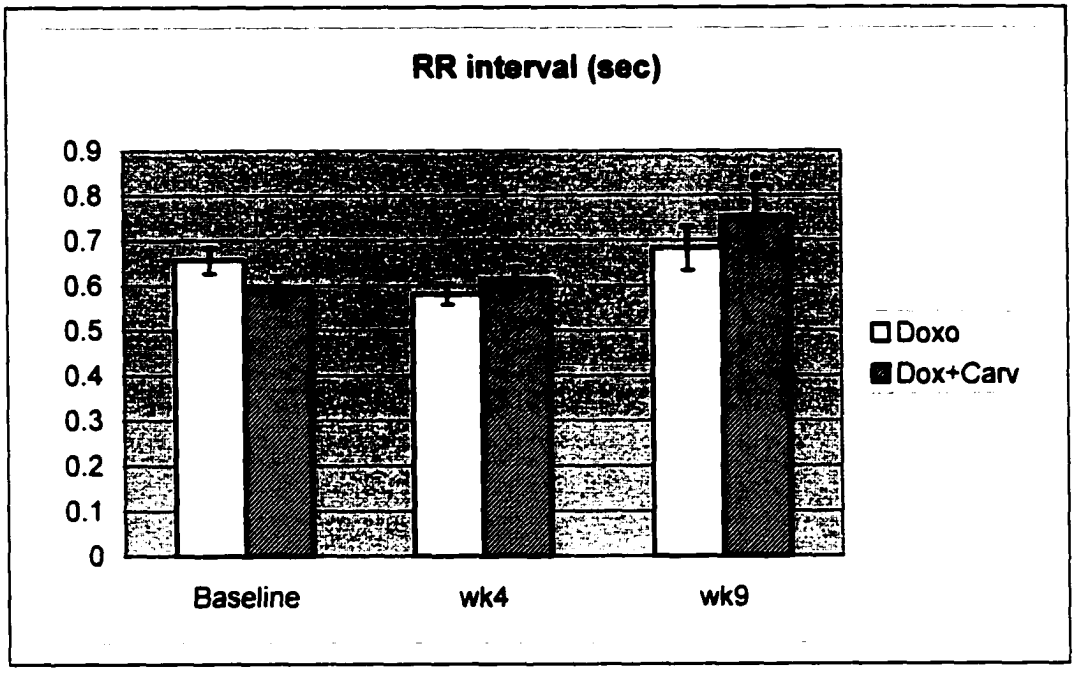

Figure 4.12: The graph of RR intervals. RR intervals are compared between beagle hounds receiving doxorubicin (Dox) and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.

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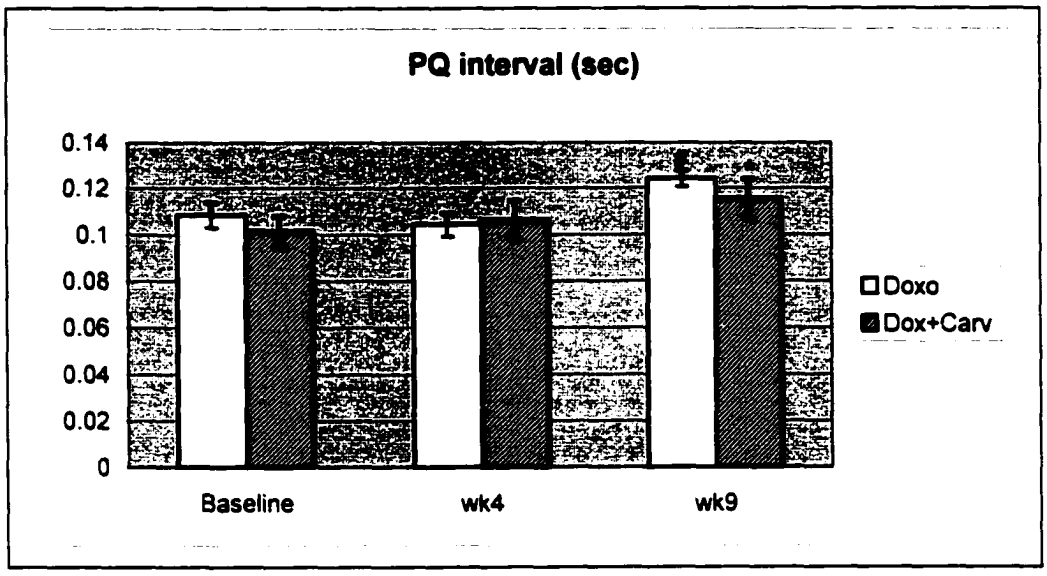

Figure 4.13: The graph of PQ intervals. PQ intervals are compared between beagle hounds receiving doxorubicin (Dox) (n=7) and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(#) significant differences ($p<0.05$) from the baseline in the Dox group.
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.

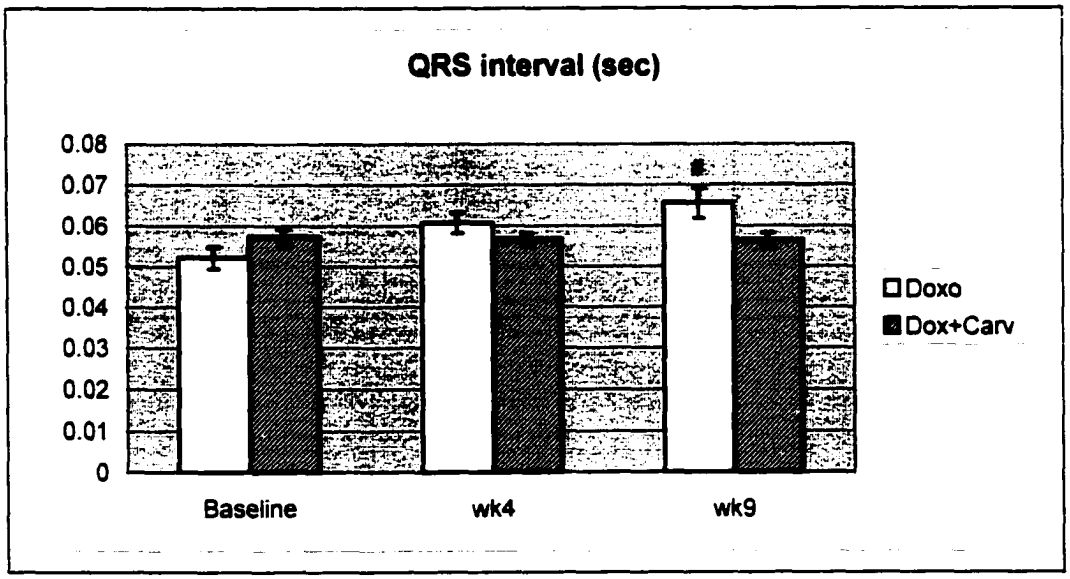

Figure 4.14: The graph of QRS intervals. QRS intervals are compared between beagle hounds receiving intracoronary doxorubicin (Dox) (n=7), and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(#) significant differences ($p<0.05$) from the baseline in the Dox group.

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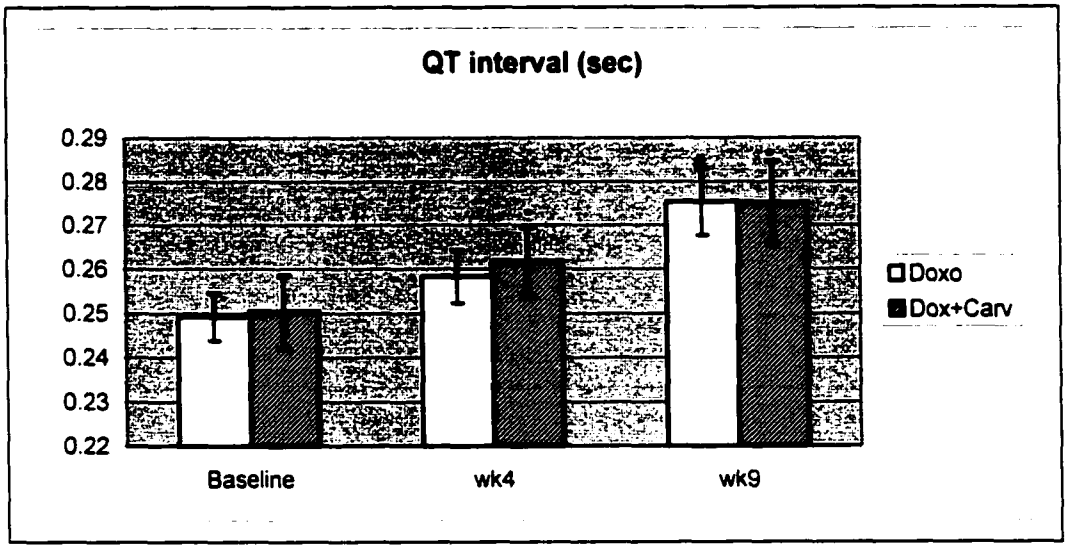

Figure 4.15: The graph of QT intervals. QT intervals are compared between beagle hounds receiving doxorubicin (Dox) (n=7) and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(#) significant differences ($p<0.05$) from the baseline in the Dox group.
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.

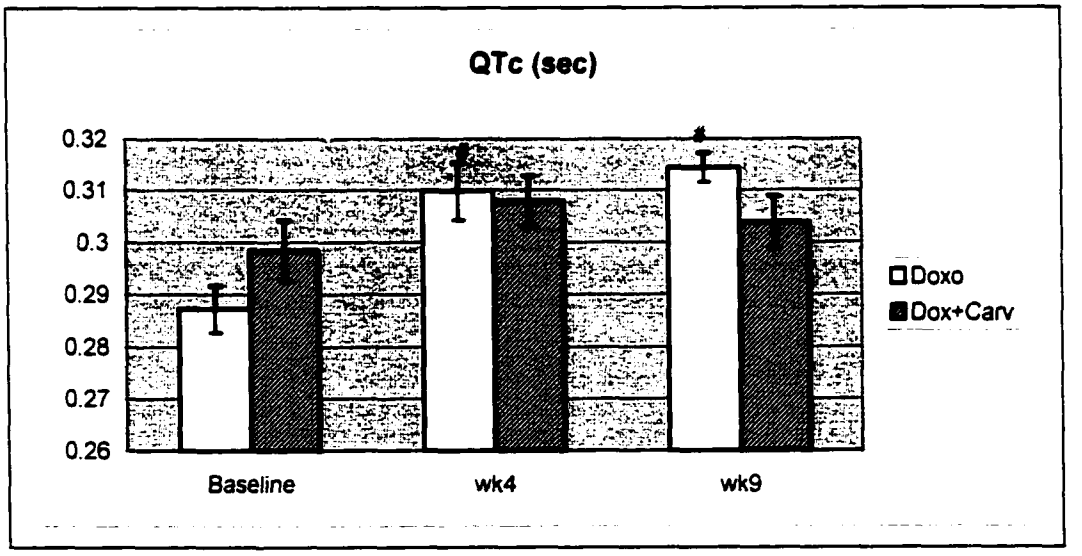

Figure 4.16: The graph of QTc intervals. QTc intervals are compared between beagle hounds receiving doxorubicin (Dox) (n=7) and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(#) significant differences ($p<0.05$) from the baseline in the Dox group.

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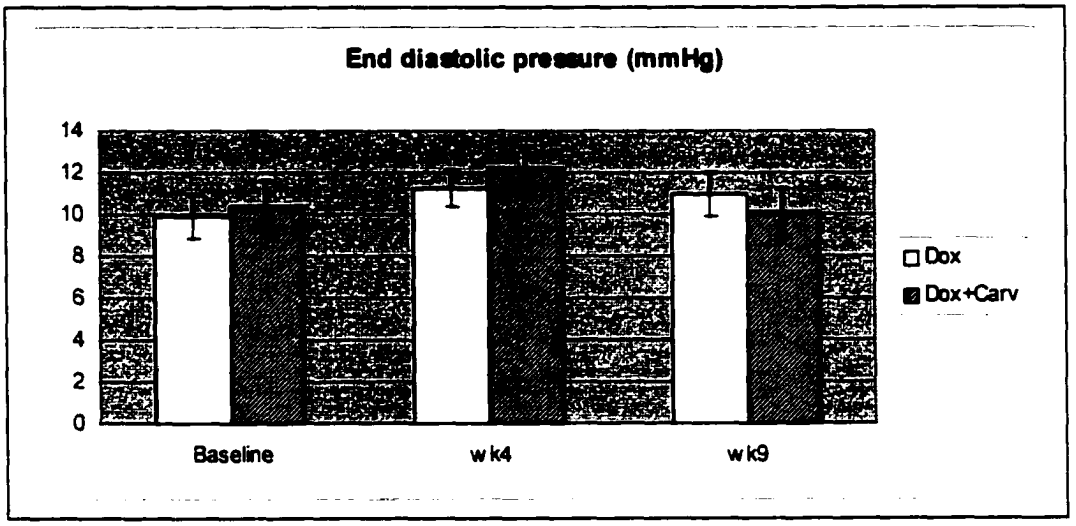

Figure 4.17: The graph of end diastolic pressures. End diastolic pressures are compared between beagle hounds receiving doxorubicin (Dox)(n=7) and doxorubicin plus carvedilol (n=7).

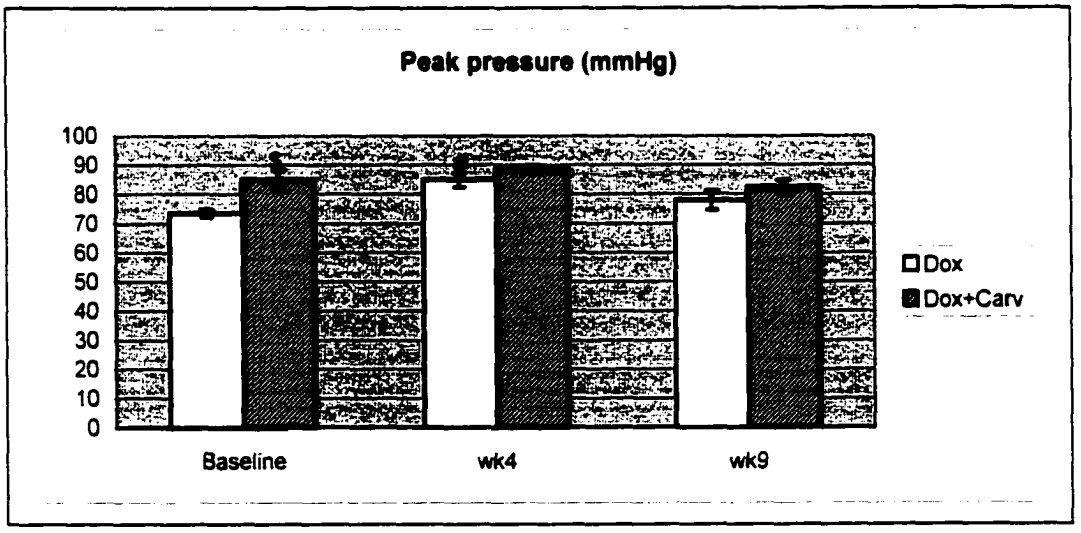

Figure 4.18: The graph of peaks of left ventricular pressures. Peaks of ventricular pressures are compared between beagle hounds receiving doxorubicin (Dox) (n=7) and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(#) significant differences ($p<0.05$) from the baseline in the Dox group.
($) significant differences ($p<0.05$) between groups at the same period.

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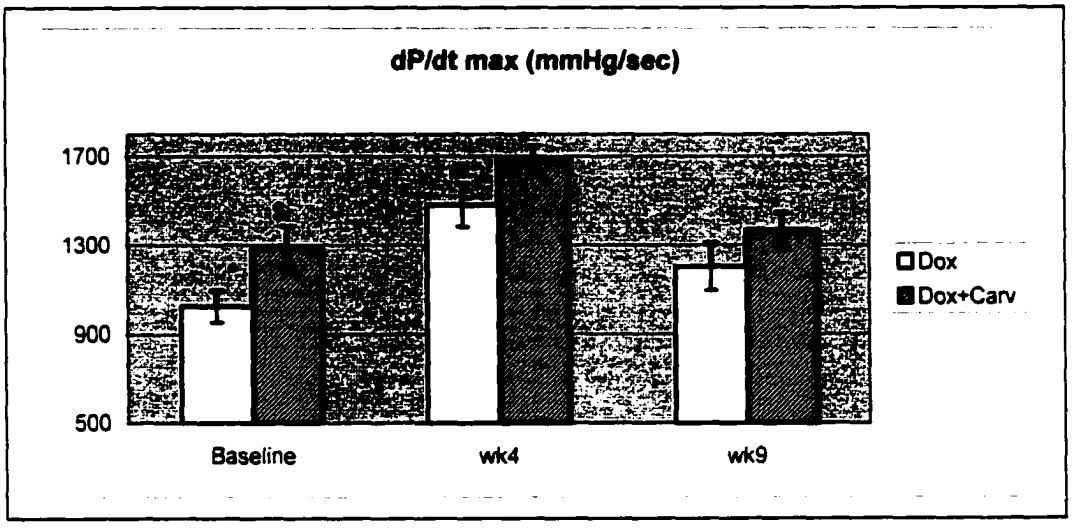

Figure 4.19: The graph of maximal rates of rise (dP/dt_{max}). Maximal rates of rise are compared between beagle hounds receiving doxorubicin (Dox) (n=7), and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(#) significant differences ($p<0.05$) from the baseline in the Dox group.
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.
($) significant differences ($p<0.05$) between groups at the same period.

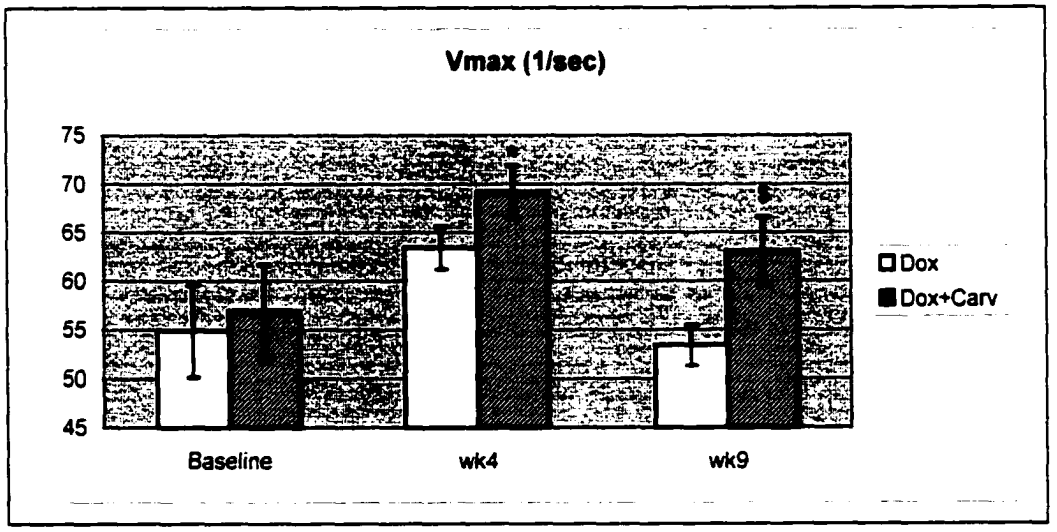

Figure 4.20: The graph of velocities of fiber shortening at zero load (V_{max}). Velocities of fiber shortening at zero load are compared between beagle hounds receiving doxorubicin (Dox) (n=7) and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.
($) significant differences ($p<0.05$) between groups at the same period.

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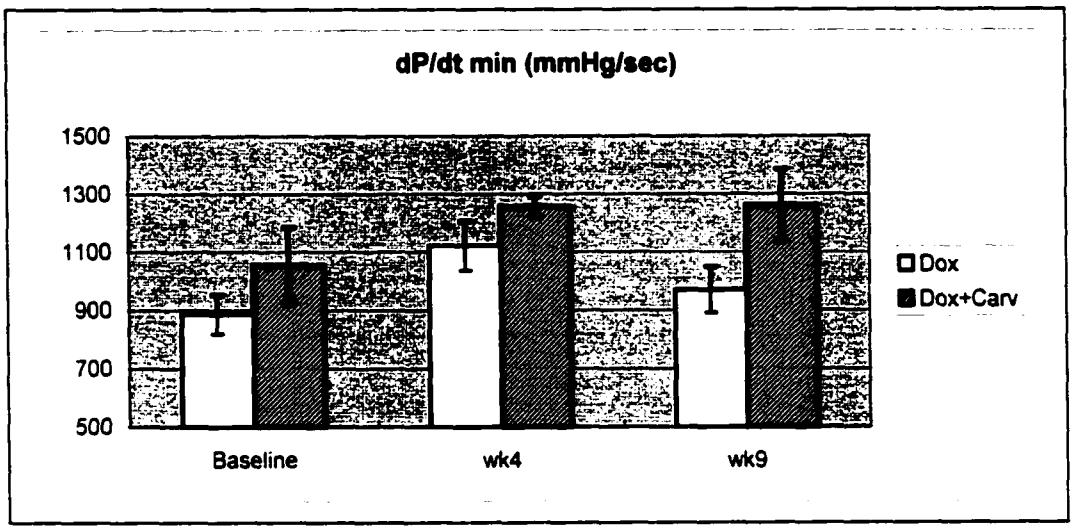

Figure 4.21: The graph of maximal rates of fall (dP/dt_{min}). Maximal rates of fall are compared between beagle hounds receiving doxorubicin (Dox) (n=7), and doxorubicin plus carvedilol (Dox+Carv) (n=7).

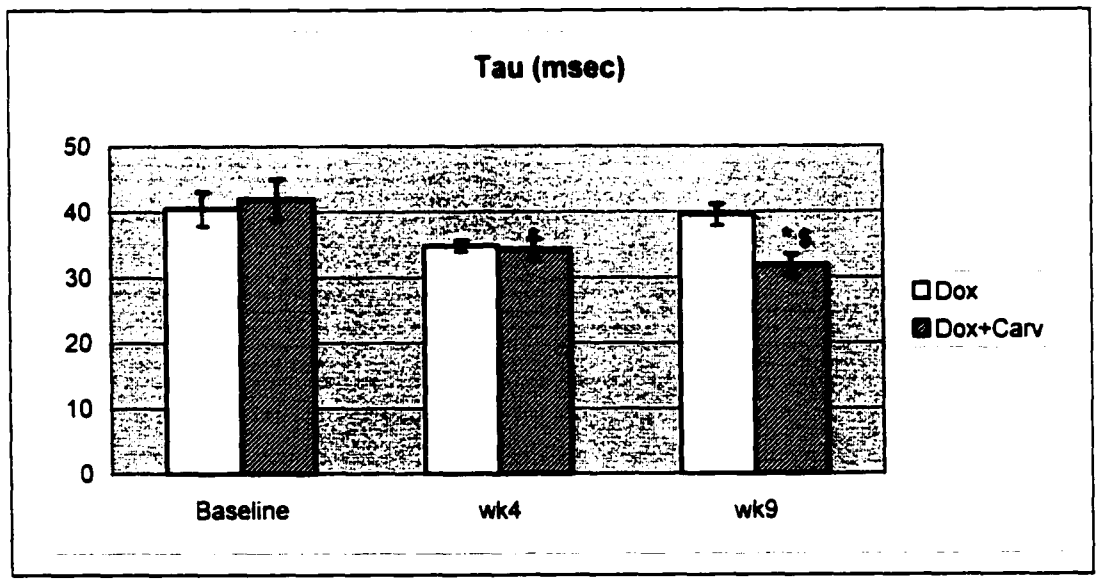

Figure 4.22: The graph of tau's. Tau's are compared between beagle hounds receiving doxorubicin (Dox) (n=7) and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.
($) significant differences ($p<0.05$) between groups at the same period.

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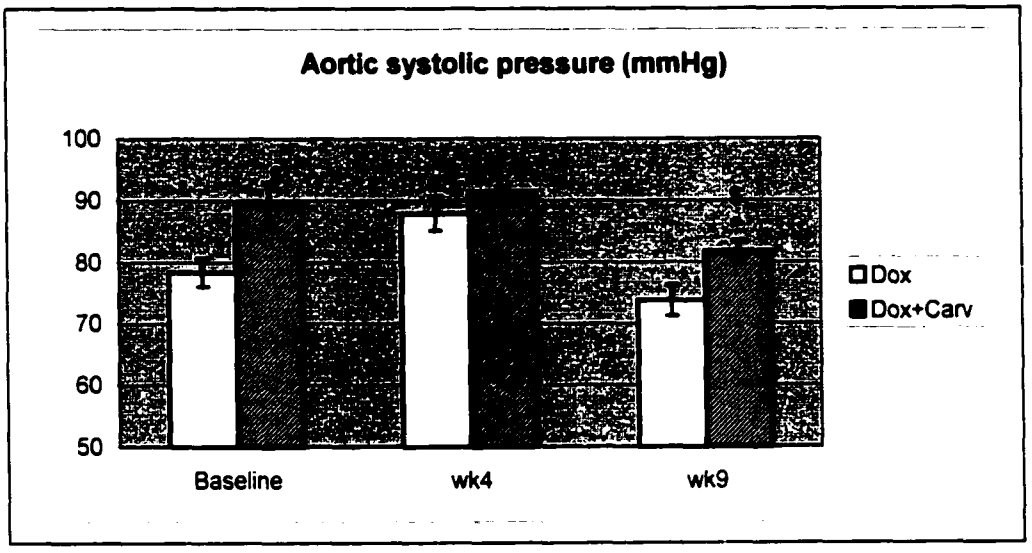

Figure 4.23: The graph of aortic systolic pressures. Aortic systolic pressures are compared between beagle hounds receiving doxorubicin (Dox) (n=7) and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(#) significant differences ($p<0.05$) from the baseline in the Dox group.
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.
($) significant differences ($p<0.05$) between groups at the same period.

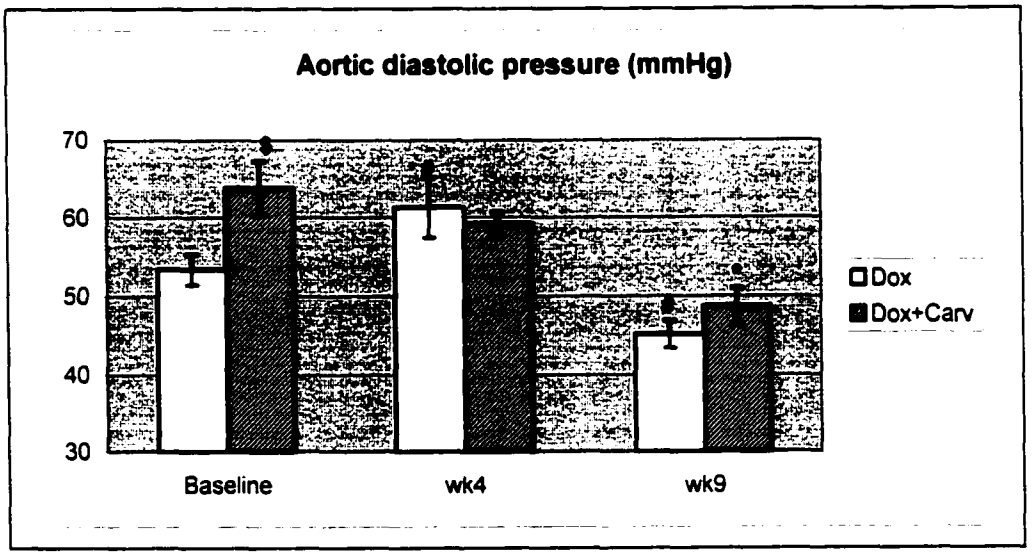

Figure 4.24: The graph of aortic diastolic pressures. Aortic systolic pressures are compared between beagle hounds receiving doxorubicin (Dox) (n=7) and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(#) significant differences ($p<0.05$) from the baseline in the Dox group.
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.
($) significant differences ($p<0.05$) between groups at the same period.

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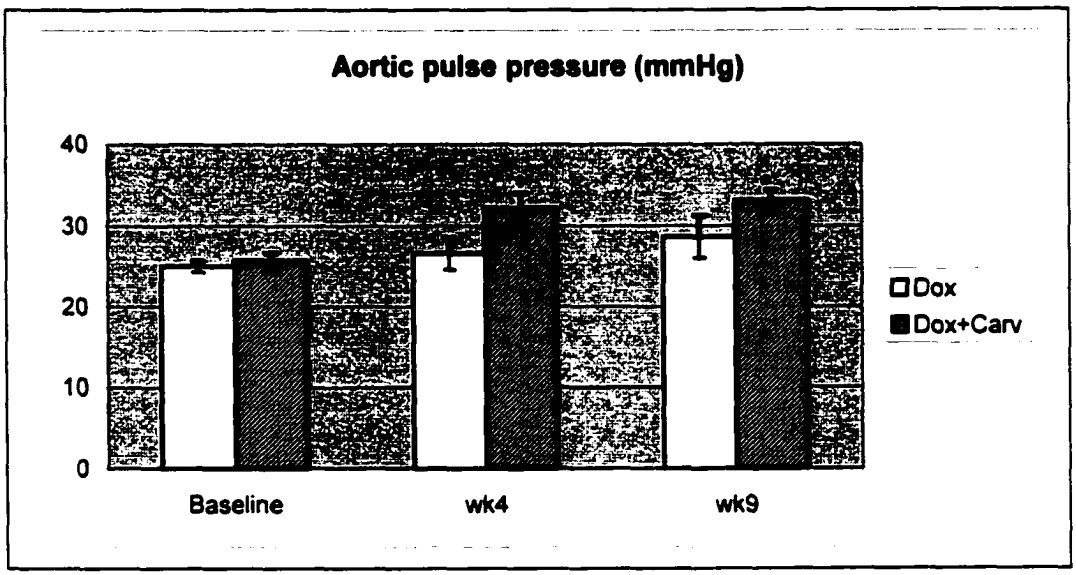

Figure 4.25: The graph of aortic pulse pressures. Aortic pulse pressures are compared between beagle hounds receiving doxorubicin (Dox) (n=7) and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.

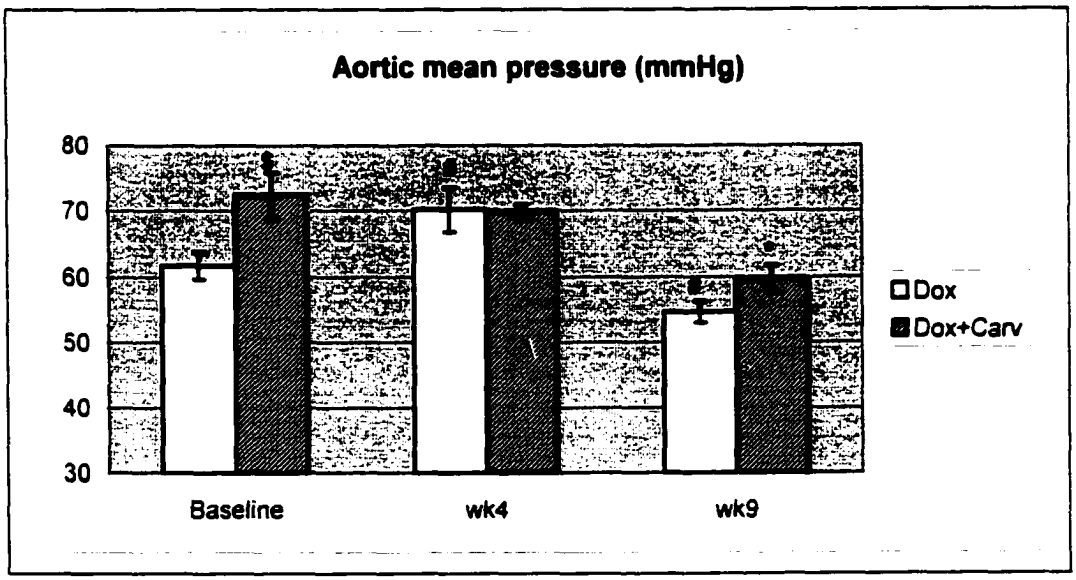

Figure 4.26: The graph of aortic mean pressures. Aortic mean pressures are compared between beagle hounds receiving doxorubicin (Dox) (n=7) and doxorubicin plus carvedilol (Dox+Carv) (n=7).
(#) significant differences ($p<0.05$) from the baseline in the Dox group.
(*) significant differences ($p<0.05$) from the baseline in the Dox+Carv group.
($) significant differences ($p<0.05$) between groups at the same period.

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Parameter	Unit	Group	Baseline Mean ± SE	Week 9th Mean ± SE	P(Period)
RR interval	ms	Dox	708.1 ± 28.9	563.1 ± 42.5	0.047
		Dox+Carv	700.9 ± 35.7	746.2 ± 33.3	0.376
			P = 0.878	*P = 0.005*	
SDNN	ms	Dox	242.1 ± 29.2	139.3 ± 22.3	0.047
		Dox+Carv	220.0 ± 20.7	196.8 ± 37.1	0.578
			P = 0.548	*P = 0.208*	
SDSD	ms	Dox	189.5 ± 23.7	105.3 ± 19.2	0.078
		Dox+Carv	176.6 ± 14.4	173.5 ± 41.2	0.813
			P = 0.650	*P = 0.159*	
RMSSD	ms	Dox	300.1 ± 34.2	147.7 ± 25.7	0.031
		Dox+Carv	283.9 ± 26.4	259.4 ± 52.4	0.688
			P = 0.714	*P = 0.080*	
NN50 count		Dox	334 ± 14	274 ± 47	0.375
		Dox+Carv	346 ± 16	300 ± 14	0.156
			P = 0.591	*P = 0.611*	
pNN50	%	Dox	78.3 ± 3.5	54.6 ± 10.2	0.078
		Dox+Carv	79.8 ± 3.4	74.8 ± 4.1	0.688
			P = 0.770	*P = 0.090*	

Table 4.1. Indices of heart rate variability in time domain. Means between periods (baseline VS the ninth week) are compared by the Wilcoxon Signed Rank Test, and means between groups at the same period are compared by the student t-test.

Dox = animals receiving doxorubicin only (n = 7)

Dox + Carv = animals receiving doxorubicin and carvedilol (n =7)

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Parameter	Unit	Group	Basline Mean SE	Week 9th Mean SE	P(Period)
RR interval	ms	Dox	708.1 ± 28.9	563.1 ± 42.5	0.047
		Dox+Carv	700.9 ± 35.7	746.2 ± 33.3	0.376
			P = 0.878	*P = 0.005*	
Total Power	ms^2	Dox	5764.1 ± 1635.8	1958.4 ± 446.7	0.031
		Dox+Carv	4635.3 ± 1096.5	4373.4 ± 1269.6	0.688
			P = 0.577	*P = 0.098*	
VLF	ms^2	Dox	678.3 ± 275.5	254.0 ± 55.5	0.578
		Dox+Carv	354.8 ± 139.8	422.5 ± 54.8	0.297
			P = 0.316	*P = 0.052*	
LF	ms^2	Dox	485.1 ± 199.3	165.1 ± 31.4	0.109
		Dox+Carv	334.9 ± 79.5	496.2 ± 142.2	0.688
			P = 0.497	*P = 0.042*	
HF	ms^2	Dox	4600.7 ± 1218.9	1539.3 ± 390.1	0.031
		Dox+Carv	3945.5 ± 1084.8	3454.7 ± 1184.1	0.578
			P = 0.695	*P = 0.150*	
LF %	%	Dox	10.3 ± 2.5	19.8 ± 9.1	0.375
		Dox+Carv	10.0 ± 2.0	16.5 ± 3.8	0.156
			P = 0.947	*P = 0.739*	
HF %	%	Dox	89.7 ± 2.5	80.2 ± 9.1	0.375
		Dox+Carv	90.0 ± 2.0	83.5 ± 3.8	0.156
			P = 0.947	*P = 0.739*	
LF/HF		Dox	0.120 ± 0.032	0.497 ± 0.372	0.469
		Dox+Carv	0.115 ± 0.025	0.214 ± 0.060	0.156
			P = 0.914	*P = 0.466*	

Table 4.2. Indices of heart rate variability in frequency domain. Means between periods (baseline VS the ninth week) are compared by the Wilcoxon Signed Rank Test, and means between groups at the same period are compared by the student t-test.

Dox = animals receiving doxorubicin only (n = 7)

Dox + Carv = animals receiving doxorubicin and carvedilol (n =7)

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DISCUSSION

Unlike acute intravenous injection in which carvedilol increases heart rate, chronic oral administration of carvedilol in the present study reduces heart rate significantly in both conscious and anesthetized dogs. The negative chronotropic effect of carvedilol may be explain by its binding to and blocking of β-adrenergic receptors in the SAN. Normally, catecholamines produce hyperpolarization of pacemaker cells, probably mediated through an increase in the sodium-potassium pump (Opie, 1998). This hyperpolarization stimulates the inward current (I_f) leading to an increase slope in Phase 4 depolarization (DiFrancesco, 1991). This change increases the transient (T-type) calcium current and the long-lasting (L-type) calcium current, and initiates depolarization. Moreover, β-adrenergic blockers also inhibit the L-type calcium current. Consequently, the decrease in slope of phase 4 of pacemaker cells results in the slower heart rate (Irisawa, 1993; Opie, 1998).

Fractional shortening of dogs in Group2 was decreased, but the dimensions of the left ventricle were increased during the first four weeks. However, the fractional shortening and left ventricular dimensions of both groups did not differ at the ninth week due to continuous deterioration in Group 1. Interestingly, after stopping carvedilol, dogs in Group 2 tended to have improved fractional shortening and smaller dimensions, with an increase in heart rate of approximately 25 %. Therefore, a slower heart rate may cause an increase in left ventricular dimensions due to lengthening of diastole. Slight prolongation of PEP would be expected with the reduction in heart rate observed in dogs of Group 2. The reduction in heart rate also lengthens ET. Afterload reduction from the

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vasodilatory action of carvedilol should allow blood to leave the heart earlier and should abbreviate PEP. The fact that PEP lengthened indicates that the effect of the reduction in heart rate dominated the reduction in afterload. Stroke volume may actually have increased when shortening fraction decreased (due to the beta blockade) by virtue of the increase in end-diastolic volume. Therefore, left ventricular function may be maintained or even improved in dogs of Group 2. This assumption is supported by results at the fourth and ninth weeks, at which aortic systolic and mean pressures and aortic dimension decreased less in dogs of Group 2 than dogs of Group 1.

The degree of dilated cardiomyopathy induced by doxorubicin depends upon the cumulative dose of the antineoplastic (Oakley, 1993; Bristow, 1998); therefore dilatation of the left ventricular chamber increases with time (Toyoda, 1998). Hearts from animals in the present study were not significant dilated until the ninth week. This implies that dilatation is a slow process requiring significant time to develop (Bristow, 1998), and is consistent with what is known about doxorubicin-induced cardiomyopathy. However, it is important to administer compounds, thought to minimize doxorubicin-induced cardiomyopathy, before or simultaneous with administration of the doxorubicin. Herman and Ferrans reported that administration of a protective agent simultaneously with doxorubicin yielded better protection than when administered two hours after doxorubicin (Herman, 1998).

Bristow et al. have shown that doxorubicin causes release of both histamine and catecholamines and reduction of coronary flow (Bristow, 1980). Histamine and catecholamines cause the reduction of coronary flow; hence, they limit the oxygen supply to the cardiac myocytes at a time when the heart requires more energy due to a higher

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heart rate and more forceful contraction. Increased myocardial oxygen demand and decreased oxygen availability lead to myocardial ischemia and reduction in ATP stores—the source of energy for both contraction and relaxation. In addition this may constitute oxidative-stress (production of free radicals of oxygen) leading to lipid peroxidation of cellular membranes. Moreover, doxorubicin also inhibits carnitine palmitoyl transferase I which is important for fatty acid oxidation (Abdel-aleem, 1997). This may result in the lower energy supply from fatty acid oxidation. Therefore, still more highly reactive oxygen species may be produced in addition to free radicals produced by doxorubicin itself. Doxorubicin also increases oxygen radicals which are produced in the cardiac mitochondria, and required NADH (Doroshow, 1983) for their production. On the other hand, carvedilol protects mitochondrial function via mechanisms which involve the inhibition of NADH dehydrogenase (Oliveira, 2000) and decrease in mitochondrial transmembrane potential (Oliveira, 2001). Therefore, it is possible that carvedilol may protect mitochondria by inhibiting NADH dehydrogenase and minimizing production of highly reactive free radicals of oxygen.

Recently, it has been shown that carvedilol inhibits endothelin-1 (a potent vasoconstrictor and proliferative stimulator) biosynthesis in cultured human coronary artery endothelial cells (Ohlstein, 1998), and reduces plasma endothelin-1 in patients with chronic heart failure (Krum, 1996). This action cannot be found in other beta blockers (e.g. propanolol, celiprolol) nor probucol (an antioxidant) (Ohlstein, 1998). High circulating levels of endothelin have been found in patients who receive doxorubicin and develop heart failure (Yamashita, 1995). Thus the anti-vasoconstrictive and anti-

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proliferative properties of carvedilol increase blood flow and slow the progression of heart failure.

Dogs in Group1 had significant higher PEP/ET ratio than dogs in Group2. This parameter is useful for identification of patients who have impairment of myocardial contractility. When the heart becomes weaker, it requires more time to generate sufficient pressure during isovolumetric contraction—before the aortic valve opens—thus prolonging PEP. Also myocardial weakening shortens ET by preventing development of adequate force to sustain the aortic valves in the opened position. This results in the shorter time for blood to cross the aortic valve, and lowers both stroke volume and cardiac output.

The increases in left ventricular pressure and dP/dt_{max} of dogs in Group1 at the fourth week may reflect a positive inotropic effect of doxorubicin which has been reported by Bottone el al. They showed that doxorubicin might interact directly with muscle cross-bridges as shown by an increase in rigor tension in skinned fibers (Bottone, 1997; Brown, 1989). Moreover, doxorubicin causes the accumulation of calcium in the cytosol of cardiac myocytes (Wang, 1995). The mechanism of calcium accumulation may be due to the increase in calcium leak from the sarcoplasmic reticulum, but not the calcium-induced calcium release. However it appears that the more tension developed, the more cardiotoxicity produced by anthracyclines. This of course is consistent with the notion that there is an increase in demand for oxygen, and decrease in oxygen delivery, and oxygen debt serving as oxidative stress. Besides, myofibrillar degeneration is found in chronic toxicity of anthacyclines (Bristow, 1998; Herman, 1998). These acute and

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chronic effects support the biphasic (i.e. increased and decreased contractility) action of doxorubicin (Bottone, 1997).

The relatively preserved V_{max} and Tau of dogs in Group 2 at the ninth week may be due to the lesser degree of myofibrillar loss and vacuolization observed histopathologically, and the less thinning of the left ventricular free wall observed echocardiography. These may relatively less of a decrement in cardiac contractility in Group 2 compared to Group 1. Moreover, the lower reduction of V_{max} in Group 2 may also be caused by conservation of β-adrenergic receptors despite high circulating levels of catecholamines (Flesch, 2001).

Leicht et al. have demonstrated that carvedilol inhibits proliferation of cardiac fibroblasts induced by catecholamines (Leicht, 2000). Moreover, Grimm et al. have demonstrated that carvedilol decreases formations of collagen types I and III, and fibronectin produced by cardiac fibroblasts (Grimm, 2001). These effects should reduce myocardial stiffness. It has also been reported that plasma vascular endothelial growth factor, which is important for angiogenesis, was increased by carvedilol (de Boer, 2001). The higher ratio of vessels to myofibrils may increase oxygen supply and delivery of nutrients to cardiac myocytes, especially in critical situations in which oxygen demands are not met.

A relationship exists between histopathological changes in pulmonary tissue and the right ventricular cardiomyopathy (Minamide, 1998). Animals which have stenosis of pulmonary vessels show degenerative changes of the right ventricle. Pressure load and mechanical stress on the myocardium have been proposed to produce or to exacerbate degenerative changes in cardiac myocytes by increasing protooncogene expression and

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protein synthesis. This supports the use of afterload and preload reducers—both of which decrease mechanical stress—therefore partially retard or stop the progression of cardiomyopathy.

Prolongation of the PQ interval of dogs in both groups may result from the decrease in heart rate or possibly by a negative dromotropic action of doxorubicin. Increases in QRS durations of dogs in Group1 may be cause by ability of doxorubicin to block sodium channels (Binah, 1983). This slows phase 0 of action potential. Moreover, late potentials occurred at the end of QRS complex in dogs of the present study. Myocardial fibrosis leads to anisotropy which impedes the process of normal ventricular activation and both prolongation of QRS and imposition of high-frequency late potentials. The prolonged QTc of dogs in Group1 may result from impaired conductances over specific potassium or sodium ion channels, or from the inhibition of sodium-calcium exchangers induced by doxorubicin (Binah, 1983). This may lead to development of reentrant ventricular arrhythmias and to sudden cardiac death.

There are reports of doxorubicin-related arrhythmias (Doherty, 1990; Mauldin, 1992). These were observed in 4 (1 with carvedilol and 3 without carvedilol) out of 14 dogs in this study. The number is too low to obtain statistical significance, but the differences indicate a trend. Mauldin et al. also found that dogs with severe myocardial lesions developed arrhythmias at an earlier state (Mauldin, 1992). However, carvedilol appeared to reduce the incidence of cardiac arrhythmias in the present study as was reported in another study in humans (Senior, 1992). Yildirir et al. showed that carvedilol reduces the QT dispersion in patients with heart failure (Yildirir, 2001). Carvedilol has been shown to inhibit the human ether-a-go-go related gene (HERG) potassium channels

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encoding the rapid component of the delayed rectifier potassium channels (Karle, 2001). This should prolong action potential duration, and increase possibility to develop arrhythmias. Another study showed that carvedilol blocks both rapid and slow components of the delayed rectifier potassium current, and the transient outward potassium current (Cheng, 1999a). This paradox may be explained by the fact that carvedilol also blocks the L-type calcium current which may limit the increase in action potential duration from potassium channel blockade, resulting in moderate prolongation of action potential duration with minimal reverse frequency-dependence (Cheng, 1999a). Therefore, carvedilol should protect arrhythmias more than to precipitate arrhythmias. In the present study, at the fourth week, doxorubicin alone and doxorubicin with carvedilol, both lengthened QTc; however, at the ninth week QTc remained prolonged but less so with carvedilol. The difference did not reach statistical significance ($p=0.09$) but supports the conclusion that carvedilol produces moderate prolongation of QTc, and possibly protects the heart from development of vulnerable ventricular arrhythmias.

HRV is considered to be an index which reflects autonomic control (sympatho-vagal balance) of the heart. A decrease in HRV has been shown to correlate with mortality, ventricular arrhythmias, and sudden cardiac death after acute myocardial infarction (Fallen, 1997). The pattern of change is an increase in LF and a reduction of HF indicating high sympathetic and low parasympathetic efferent activity (Lombardi, 1996). Moreover, Lotze et al. demonstrated that the HRV is correlated well with the sympathetic activity in dilated cardiomyopathy (Lotze, 1999). The combination of HRV and other indices (e.g., ejection fraction, late potentials, and arrhythmia frequency) increase the prognostic power (Fallen, 1997).

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The relationships between indices in time domain and frequency domain have been established (Malik, 1996). SDNN in time domain is related to the total power in frequency domain, whereas RMSSD, SDSD, and pNN50 are related to the HF component in frequency domain. In the present study, indices in time and frequency domains are changed in the same direction. However the peak of HF occurs at higher frequencies in dogs than in man because of the relatively elevated respiratory rates in dogs compared to man. Therefore in performing this type of analysis on dogs, the upper limit for the HF component must be increased to 0.6 Hz rather than 0.4 Hz which was recommended in the guidelines for man.

The relationship between respiratory rate and the peak of HF may be explained by the juxtaposition of respiratory and cardiovascular centers in medulla oblongata were communicated occurs via the reticular formation. In addition, both utilize the vagus for their efferent pathways. When respiratory rate and effort are changed, the effect of parasympathetic control to the heart is also changed (Kotel'nikov, 2002).

In the present study, decreases in SDNN in time domain and total power in frequency domain in Group 1 may represent the development and progression of heart failure due to exposure of the dogs to doxorubicin. Both indices have been shown to correlate well (Malik, 1996). Although no dogs in Group 1 showed clinical signs of heart failure (e.g. anorexia, cough, dyspnea, or fluid retention)[1] during rest, the HRV still decreased.

[1] Because no dog in this study appeared to be distressed, this model of injecting doxorubicin directly into the coronary arteries carries little liability for being considered inhumane. Furthermore much less doxorubicin is required to produce a failing heart, therefore the expense is much less than when the compound is given intravenously.

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This demonstrates the great potential to utilize HRV as an index to predict doxorubicin-induced cardiotoxicity in an early state. Normally, the LF component increases due to increased sympathetic activity. The reduction in or absence of LF is thought to be due to down regulation of beta receptors—a consequence of elevated levels of catecholamines that occurs in heart failure. This elevation occurs, in great part, because of norepinephrine spillover (Sanderson, 1998). Moreover, the impairment of baroreceptor function—due in part to loading of the high pressure baroreceptors from the action of aldosterone-- may also produce a decrease in the LF component (Sanderson, 1998). The HF component is controlled by parasympathetic activity which is known to decrease in heart failure—also caused by abnormal high pressure baroreceptor function. Moreover, a reduction of HF is associated with the inducibility of ventricular tachycardia (Fetsch, 1998). The decrease in HF components of HRV observed in dogs in Group 1 may reflect the increased incidence of cardiac arrhythmias in the present study. The decrease in VLF in animals receiving doxorubicin may be caused, in part, by the reduction of total power, or may be due to the change of catecholamine levels or levels of other endocrines. However, the true generator of VLF is still unclear. Interestingly, carvedilol seems to preserve the autonomic control to the heart, or even protect the heart from doxorubicin toxicity.

In conclusion, the present study shows that carvedilol maintains PEP/ET ratio, systemic blood pressures and V_{max}. Moreover, it also decreases tau, minimizes QT prolongation, and maintains heart rate variability which may support the protective effect of carvedilol on doxorubicin-induced cardiotoxicity.

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CHAPTER 5

CONCLUSION

Carvedilol is a novel beta blocker which has vasodilating property due to its α_1-adrenergic blocking and calcium channel blocking activities. The vasodilating property of carvedilol does not result in a reflex tachycardia, and provides an advantage for use in patients with congestive heart failure. Non-specific beta blockers have proven more useful than cardiospecific beta blockers for treating patients with heart failure, yet because of the β_2-adrenergic blockade they might produce vasoconstriction. However the added alpha-1 blockade prevents this vasoconstriction and increase in afterload. Furthermore, the ability of carvedilol to scavenge free radicals of oxygen adds additional protective benefits to the typical beat blocker that does not possess the scavenging potential. Since free radicals of oxygen have been incriminated in the pathogenesis of doxorubicin-induced cardiomyopathy or in ischemic heart failure, the benefits of carvedilol should be clear.

It has been shown that α_1-adrenergic receptors, mainly the α_{1b}-subtype, play a role in deterioration of patients due to negative effects on contractility, metabolism, cellular hypertrophy, and electrical activity (Fedida, 1993). Moreover, the change in α-adrenergic receptor density in heart diseases may also affect the physiological and

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pharmacological responses of adrenergic antagonists. In cardiac ischemia, stimulation of α_1-adrenergic receptors may cause free radical formation, calcium overload, endothelin release, and ventricular arrhythmias (Kern, 1999; Wilber, 1987). Therefore, benefits from α_1-adrenergic blocking activity may be far more important than its ability to decrease afterload (vasodilation property). However, the variation among species of α_1-adrenergic receptor density in the heart may limit extrapolation from one species to another.

Three reports show protective effects of other beta blockers on dilated cardiomyopathy and cardiac ischemia (Glass, 1993; Gilbert, 1993; Liu, 1991). However, doses of beta blockers (e.g., propranolol, pindolol, and metoprolol) which limit free radicals of oxygen are far beyond what are required for β-adrenergic blocking activity (Mak, 1988). The favorable effects from antioxidant activity may be concealed by side effects e.g. negative inotropy, upregulation of β-adrenergic receptor, and bradycardia. However, doses of carvedilol between 0.3-1 mg/kg intravenously, studied in dogs, show significant protective effect after left coronary occlusion (Feuerstein, 1993). Mechanisms of protections include prevention of lipid peroxidation and inhibition of superoxide release from neutrophils (Feuerstein, 1993). At these doses, carvedilol does not produce adverse effects. The dose used in this study should be safe and effective at treating heart failure. Variable portions of the benefit may be attributable to the varying pharmacologic properties of the compound.

Effects of carvedilol on left ventricular function are different from other beta blockers. There is evidence which shows free radical formation in both the failing heart and heart in stable failure (Singal, 1999a; Singal, 1999b). One reason for continued deterioration in heart failure may well be the production of free radicals of oxygen

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unabated by inadequate production of natural scavengers, i.e. the cells cannot produce enough antioxidants to protect themselves from free radicals. Therefore, drugs which possess free radical scavenging may become more important for the treatment of heart failure in order to stop or slow the progression. This third generation beta blocker has advantages of free radical scavenging, conserves energy by decreasing afterload, and preserving mitochondrial functions. This molecule may limit myocardial degeneration resulting from inflammation, and may even limit apoptosis resulting from free radicals of oxygen.

There are some cautions with the use of beta blockers in heart failure. Plasma concentrations may not be able to predictor a response, especially some beta blockers that may have inhomogeneous distribution among tissues. This is particularly applicable to carvedilol, which tends to accumulate in the plasma membranes (Cheng, 1996). Therefore, physiological and pharmacological responses should be monitored together with the plasma concentration. Furthermore not all patients can tolerate beta blockers at the same level. Some patients may show adverse effects even at low doses, but toxicity to beta blockers seems to be dose-related. Therefore, it is essential to titrate, slowly, individual patients to ascertain the possible highest dose which patients can tolerate without any adverse effects.

The route and duration of carvedilol administration also affect its action. Acute intravenous injection causes an increase in heart rate, possibly by activation of the baroreceptor reflex. On the other hand, chronic oral administration of carvedilol reduces heart rate significantly from β-adrenergic blockade. A study shows the degree of α_1-adrenergic blockade after chronic administration of carvedilol is lower than prazosin. On

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the other hand, acute administration of carvedilol has the same effect as prazosin (Giannattasio, 1992). It is possible that during acute intravenous injection may produce temporary higher plasma concentrations at which carvedilol may also block calcium channels, and enhance the α_1-adrenergic blocking activity. Moreover, the bioavailability of both stereoisomers is also different. Reasons for differences in mechanisms of action by different routes of administration are still unclear. Stereoisomers may vary in metabolic pathways, and active metabolites may differ between the isomers and by rate of increase in plasma concentration.

In the future, combination-drug therapy may provide some advantages in patients who suffer from cardiovascular diseases related to free-radical formation, for example, cardiac ischemia, heart failure, and doxorubicin-induced cardiomyopathy. By using the more potent (10-40 fold) antioxidant metabolite of carvedilol, e.g. SB 211475 (or BM-910228) together with carvedilol, a more favorable effect and may be produced with fewer adverse effects (e.g. bradycardia, negative inotropy), especially at high doses.

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