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A GH Secretagogue Prevents Ischemic-Induced Mortality Independently of The GH Pathway in Dogs with Chronic Dilated Cardiomyopathy

By

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Running Title: GH Secretagogue GHRP-6 in Cardiomyopathic Dogs

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List of non-standard abbreviations:
coronary artery occlusion (CAO)
GH releasing peptide-6 (GHRP-6)
insulin-like growth factor-I (IGF-1)

Key Words: cardiomyopathy, heart failure, contractile function, hemodynamics, ischemia, ventricular arrhythmias, ventricular function

ABSTRACT

To determine the functional role of growth hormone (GH) secretagogue in myocardium with ischemia, left ventricular (LV) pressure gauge, wall thickness crystals, coronary occluder, pacers and catheters were implanted in 26 dogs. Beginning 1 week after ventricular pacing (240 b/min) was initiated, dogs were treated (s.c.) with GH releasing peptide-6 (GHRP-6, n=8, 0.2 mg/kg/d), GH (n=7, 0.06 mg/kg/d), or vehicle (n=11). Two weeks of pacing was associated with similar decreases in LV pressure, LV dP/dt, systolic wall thickening (WT) and an increase in left atrial pressure in all groups. Coronary artery occlusion (CAO) resulted in a similar loss of WT in ischemic regions, which did not recover during reperfusion period in all groups. WT in non-ischemic regions, however, was enhanced in the GHRP-6 group compared to the GH and vehicle groups, e.g., 53±8% increase of WT ($p<0.05$) in the GHRP-6 group after 1 hr. of reperfusion than that in the GH (+14±12%) or vehicle (+14±6%) groups. There were no differences in myocardial blood flow, hemodynamics or arrhythmic beats among all groups during CAO and reperfusion periods. Strikingly, no dogs in the GHRP-6 group died during CAO while the survival rates for GH and vehicle groups were 57% and 55%, respectively. Our data demonstrate, for the first time, that chronic therapy with a GH secretagogue prevents sudden death in dogs with dilated cardiomyopathy subjected to acute ischemia. This appears to be related to an enhanced non-ischemic compensatory mechanism mediated by the GH secretagogue receptors rather than via the GH/IGF-1 pathway.

Growth hormone (GH) secretagogues compose a heterogeneous group of synthetic peptides and non-peptides that induce potent GH secretion. Recent studies suggest that GH secretagogue receptors exist not only in the human central nervous system but also in peripheral tissues, mainly in the myocardium (Bodart et al., 1999; Papotti et al., 2000). It also has been recently shown that treatment with hexarelin, a synthetic GH releasing hexapeptide, improved ventricular function and protected cardiomyocyte death in acutely anesthetized rodents and isolated perfused rodent hearts subjected to myocardial ischemic or hypoxia injury (Colonna et al., 1997; Rossoni et al., 1998; Locatelli et al., 1999; Tivesten et al., 2000; Filigheddu et al., 2001), as well as in healthy volunteers and GH-deficient patients (Bisi et al., 1999a; 1999b). It is unclear, however, whether the myocardial effects of hexarelin are different from those of exogenous GH, as some of these studies also reported that the effects of hexarelin on cardiac function were similar to those of GH (Locatelli et al., 1999; Tivesten et al., 2000).

The major goal of the present investigation was to determine whether modulation of GH secretagogue receptors in the myocardium would elicit salutary effects in an intact animal model of chronic heart disease. To achieve this goal, experiments were conducted using both conscious dogs with rapid ventricular pacing-induced chronic dilated cardiomyopathy that were subjected to a prolonged period of coronary artery occlusion. The uniqueness of this model is that it not only mimics the process of human chronic heart disease, but that it also allows LV dysfunction induced permanent myocardial injury to be studied. To differentiate the potential effects of GH secretagogues acting directly on the myocardium from the effects of GH secretagogues acting via the GH/insulin-like growth factor-I (IGF-1)

pathway, both GH-releasing peptide-6 (GHRP-6), a synthetic peptidyl GH secretagogue, and GH were studied. The dose of GH selected was based on our preliminary data and on results of other studies (Prahalada et al., 1998) in which IGF-1 levels were similar to those induced by the dose of GHRP-6 used in this study. All measurements were made by using chronically implanted instrumentation to directly and continuously measure LV regional myocardial function and systemic hemodynamics.

METHODS

The animals used in this study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, the NIH (1996) and the Merck Research Laboratories Institutional Animal Care and Use Committee. Twenty-six adult dogs, i.e, seven young mongrel (≈1 years old) and nineteen elderly (9-11 years old) beagles, weighing 9-17 kg, were anesthetized with Pentothal (12-15 mg/kg, i.v.). Following tracheal intubation and ventilation, anesthesia was maintained with isoflurane (1.5-2.0 vol% in oxygen). A left thoracotomy was performed at the fifth intercostal space. Tygon catheters (Norton Plastics, Akron, OH) were implanted in the descending aorta and left atrium to measure their respective pressures and to administer radiolabeled microspheres. The left circumflex coronary artery was isolated, and a flow probe (Transonic Inc., Ithaca, NY) and a hydraulic occluder were implanted to measure coronary blood flow and to temporally occlude the coronary artery, respectively. A solid-state miniature pressure gauge (Konigsberg, Pasadena, CA) was implanted in the left ventricular (LV) cavity through the apex to measure LV pressure and the rate of change of LV pressure (LV dP/dt). Two pairs of piezoelectric ultrasonic dimension crystals were implanted transmurally across the LV anterior and posterior regions to measure their respective wall thicknesses. Proper alignment of the crystals was achieved during surgical implantation by positioning the crystals so as to obtain a signal with the greatest amplitude and shortest transit time. A pacing lead (Medtronic Inc., Minneapolis, MN) was attached to the right ventricular free wall, and stainless steel pacing leads were attached to the left atrial appendage. Catheters and electrical leads were externalized between the scapulae, and the chest was closed in layers.

Hemodynamic recordings were made using a data tape recorder and a multiple-channel oscillograph (Gould, Cleveland, OH). Aortic and left atrial pressures were measured using strain gauge manometers (Argon, Athens, TX), that had been calibrated using a mercury manometer connected to the fluid-filled catheters. The solid-state LV pressure gauge was cross-calibrated with aortic and left atrial pressure measurements. LV dP/dt was obtained by electronically differentiating the LV pressure signal. A triangular wave signal was substituted for the pressure signals to directly calibrate the differentiator (Triton Inc., San Diego, CA). Anterior and posterior LV wall thicknesses were measured using an ultrasonic transit-time dimension gauge (Triton Inc., San Diego, CA). LV end-diastolic dimension for both regions (EDD) was measured at the time that coincided with beginning of the upstroke of the LV dP/dt signal. LV end-systolic dimension (ESD) was measured at minimum LV dP/dt. Systolic wall thickening was calculated as EDD-ESD. A cardiotachometer triggered by the LV pressure pulse provided instantaneous and continuous records of heart rate.

Regional myocardial blood flow was measured by using the radioactive microsphere technique. Microspheres (15±1 μm) labeled with Nb^{95}, Ce^{141}, Sn^{113}, Ru^{103} or Sc^{46} (New Life Science Products, Boston, MA) were suspended by placing them in an ultrasonic water bath for 30 min. Each injection of microsphere suspension, which contained approximately 1 million microspheres, were administered through the left atrial catheter and flushed with saline. An arterial blood reference sample was withdrawn at a rate of 7.75 ml/min for 120 sec. Regional tissue samples were collected at the end of the study and radioactivity was measured using a gamma counter (Packard BioScience, Meriden, CT) with appropriately selected energy windows. After correcting the radioactive counts for background and

crossover, regional blood flow was calculated and expressed as milliliters per minute per gram of tissue.

The experiments were initiated 10-14 days after surgery. One week before surgery and during the postoperative recovery period, the dogs were trained to lie quietly in the right lateral position. After obtaining hemodynamic recordings for baseline measurements of LV systolic pressure, LV dP/dt, mean arterial pressure, left atrial pressure, LV wall thickness and systolic wall thickening in the anterior and posterior regions, and heart rate while the dogs were conscious, rapid (240 beats/min) right ventricular pacing was initiated and continued for 4 weeks using a programmable external cardiac pacemaker (Model EV4543, Pace Medical, Waltham, MA). The dogs were treated subcutaneously with either GHRP-6 at a dose of 0.2 mg/kg (n=11), porcine GH at a dose of 0.06 mg/kg (n=7) or vehicle (n=8) once daily for 3 weeks beginning on the 7th day of ventricular pacing. There were 4 and 3 young dogs in the GHRP-6- and vehicle-treated groups, respectively. The dose of GH selected for the present study was determined by a preliminary study. In that study, we found that administration of GHRP-6 at a dose of 0.2 mg/kg/day for 2 weeks increased plasma IGF-1 by 94±18 ng/ml from a baseline level of 103±15 ng/ml. Administrations of GH increased plasma IGF-1 by 78, 90 and 183 ng/ml at doses of 0.03, 0.06 and 0.10 mg/kg/day, respectively. After the 1st week of treatment (i.e., 2 weeks after rapid ventricular pacing was initiated), the pacing was temporarily stopped and the dogs were administered morphine sulfate (0.3 mg/kg, S.C.). While the dogs were conscious and hemodynamic status continuously monitored, the left circumflex coronary artery was occluded for 90-min duration for the young dogs and 60-min duration for the elderly dogs

by inflating the implanted hydraulic occluder. Our feasibility data showed that elderly dogs were more vulnerable to myocardial ischemia compared to young dogs. Based upon our initial results, the myocardial infarct size developed after 60 minutes of coronary artery occlusion and 4 days of reperfusion in the elderly dogs, was similar to that induced by 90 minutes of occlusion and 4 days of reperfusion in young dogs. Immediately before coronary artery reperfusion, a 1.5-mL bolus injection of lidocaine (2%) was administered via the left atrial catheter. Microspheres were given before coronary artery occlusion, 3-5 min after coronary artery occlusion, and approximately 5 min after coronary artery reperfusion. Hemodynamic recordings were made continuously for up to 3 hr after coronary artery reperfusion and then rapid ventricular pacing was resumed. Four days later, the dogs were euthanized with an overdose of pentobarbital sodium and their hearts were excised and placed on a dual perfusion apparatus as described previously (Shen et al., 1996). Briefly, the ascending aorta was cannulated (distal to the sinus of Valsalva) and perfused retrogradely with Monastral® blue dye (0.2% solution, Sigma Chemical Co., St. Louis, MO). The left circumflex coronary artery was cannulated at the site of occlusion and perfused with saline. The driving pressure for the perfusion apparatus was maintained at approximately 120 mmHg for both cannulas. After perfusion, the hearts were fixed in 5% formalin for 3 days and then sectioned at the atrioventricular junction. The LV was sliced into six to nine rings and both sides of the individual rings were photographed using a digital camera. The previously occluded vascular bed, i.e., the area at risk, was identified. The surface area of each ring was traced using computer-assisted planimetry to measure the

area at risk and infarct size. After pathological analysis, the individual rings were sectioned to measure regional myocardial blood flow.

Since both baseline hemodynamics and hemodynamic responses to all treatments were similar for young vs. elderly dogs, data for both groups of animals were combined and presented in all tables and figures. Data obtained before and after rapid ventricular pacing and in response to coronary artery occlusion and reperfusion were compared using the Student's t-test for paired data with a Bonferroni correction. Comparisons among the GHRP-6-, GH- and vehicle-treated groups were conducted using the unpaired Student's t-test. Survival rates for the groups were compared using Fisher's exact test. All values are expressed as the mean ± S.E. Statistical significance was accepted at the $p<0.05$ level.

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RESULTS

Hemodynamics Before and After Rapid Ventricular Pacing

The values for LV systolic pressure, LV dP/dt, mean arterial pressure, mean left atrial pressure, systolic wall thickening in the non-ischemic and potentially ischemic regions, and heart rate in conscious dogs before and after 2 weeks of rapid ventricular pacing and 1 week of treatment with vehicle, GHRP-6, or GH are shown in Table 1. There were no differences among the three treatment groups in any of these indices before initiation of rapid ventricular pacing. After two weeks of pacing and one week of treatment, LV systolic pressure, LV dP/dt, and mean arterial pressure had decreased ($p<0.05$), while mean left atrial pressure had increased ($p<0.05$) similarly in all three groups. In addition, systolic wall thickening in the non-ischemic and potentially ischemic regions was reduced in the three groups. However, not all of the reductions in regional function were significantly different from the control values, and there were no differences among the three groups.

Effects of Prolonged CAO and CAR

There were no differences in LV systolic pressure, LV dP/dt, mean arterial pressure, heart rate, or systolic wall thickening in the non-ischemic and ischemic zones among the groups treated with vehicle, GHRP-6 and GH before CAO. The changes in LV systolic pressure, LV dP/dt, mean arterial pressure, wall thickening in the non-ischemic and ischemic zone, and heart rate during CAO and CAR are shown in Fig 1. During CAO, rather than systolic wall thickening in the ischemic zone, there was either no wall thickening or wall thinning in all three groups, and systolic wall thickening did not recover during the 3-h

monitoring period following CAR (Fig 1). However, systolic wall thickening in the non-ischemic zone during CAO was significantly ($p<0.05$) increased by 37±8% in the group treated with GHRP-6. Whereas, systolic wall thickening was increased by only 14±8 and 7±14% from the baseline levels in the groups treated with vehicle and GH, respectively. Mean left atrial pressure was increased ($p<0.05$) similarly in the groups treated with vehicle (+15±4 mmHg), GHRP-6 (+15±3 mmHg), or GH (+15±4 mmHg) during CAO. Following CAR, the increase in systolic wall thickening in the non-ischemic zone was significantly ($p<0.05$) greater in the group treated with the GHRP-6 compared to the vehicle- and GH-treated groups. For example, systolic wall thickening in the non-ischemic zone was increased ($p<0.05$) by 53±8% 1 h after CAR in the GHRP-6-treated group, which was significantly ($p<0.05$) greater than in the vehicle- (+14±6%) and GH-treated (+14±12%) groups.

The effects of CAO and CAR on regional myocardial blood flow in the ischemic zone are shown in Table 2. Blood flow in the endo-, mid and epi-myocardial layers before CAO was similar among the three groups. Both early (i.e., 5 min) and late (i.e., just before CAR) during prolonged CAO, blood flow had decreased more in the endo-myocardium than in the epi-myocardium. However, there were no differences among the three groups studied, suggesting that the functional collateral blood flow was developed similarly. After CAR, blood flow in all of the layers was increased slightly more than the baseline level, suggesting that a reactive hyperemic response had occurred. The changes in blood flow in the endo-, mid and epi-myocardium did not differ among the three groups at any of the

time points. The pathology data for the infarct size are summarized in Fig 2. There were no differences among the vehicle-, GHRP-6-, and GH-treated groups with respect to LV, area at risk, or infarct weights. Thus, the infarct size, expressed as a percentage of the area at risk, was similar among the groups treated with vehicle (36±5%), GHRP-6 (42±3%), or GH (44±5%).

There were no differences in the number of ventricular arrhythmic beats among the vehicle-, GHRP-6-, and GH- treated groups either before or during control, 5, 15, 30 or 60 min after CAO as shown in Fig 3. Two dogs in the vehicle-treated group developed ventricular tachycardia, one 7 min and one 40 min after CAO. Two dogs in the GHRP-6-treated group developed ventricular tachycardia, one 3 min and one 16 min after CAO. One dog in the GH-treated group developed ventricular tachycardia 18 min after CAO.

The survival rates for the vehicle-, GHRP-6-, and GH-treated groups during prolonged CAO are shown in Fig 4. The survival rate was 55% (i.e., 6 of 11 dogs) and 57% (i.e., 4 of 7 dogs) for the vehicle-treated and GH-treated groups, respectively. Interestingly, none of the dogs (total n=8) in the GHRP-6-treated group died during CAO. In the subgroup of elderly animals, the survival rate was 43% and 57% in the groups treated with vehicle and GH, respectively.

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DISCUSSION

We have demonstrated that chronic treatment with GHRP-6, a peptidyl GH secretagogue, prevented sudden death in dogs with moderately dilated cardiomyopathy subjected to acute myocardial ischemia. To our knowledge, this is the first time that this effect of a GH secretagogue has been reported. We also found that GHRP-6 considerably enhanced regional myocardial function in the non-ischemic zone when myocardial contraction was depressed in the ischemic zone following both brief and prolonged coronary artery occlusion. This compensatory phenomenon may explain how GHRP-6 prevented acute cardiac failure-induced death during coronary artery occlusion.

It has been postulated that GH secretagogues target cardiac receptors, which potentially mediate their actions independent of GH release. To determine whether the observed effects of GHRP-6 were related to GH/insulin-like growth factor-I (IGF-1) release rather than a specific GH secretagogue pathway, an additional group of dogs treated with GH alone was included. The dose of GH that was selected was based on our preliminary data and on results of other studies (Prahalada et al., 1998) in which IGF-1 levels were similar to those induced by the dose of GHRP-6 used in this study. Interestingly, both mortality and regional myocardial function in the non-ischemic zone in the GH-treated group were similar to those in the vehicle-treated group, but different from those in the GHRP-6-treated group, suggesting that the observed salutary effects of GHRP-6 were most likely mediated by GH secretagogue receptors rather than by the GH/IGF-1 pathway.

Recently, a gastric-derived peptide, ghrelin, has been proposed as the natural ligand of the GH secretagogues receptors (Cassoni et al., 2001; Katugampola et al., 2001). It also

has been shown that administration of ghrelin enhanced cardiac function both in rats with heart failure (Nagaya et al., 2001 a) and in healthy volunteers' (Nagaya et al., 2001 b), further supporting the potential GH secretagogues to affect myocardial function.

In our study, none of the dogs in the GHRP-6-treated group died during prolonged coronary artery occlusion. In contrast, the mortality rates for the vehicle- and GH-treated groups were comparable, i.e., about 50%. Although the precise mechanism that is responsible for this unexpected finding is unclear, it is apparently unrelated to ventricular arrhythmia/tachycardia leading to ventricular fibrillation-induced sudden death, as the frequency of ventricular arrhythmia and tachycardia in each group were similar during sustained coronary artery occlusion. In addition, there were no differences in global hemodynamics between these three groups at baseline or during coronary artery occlusion or reperfusion, which excludes the possibility that hemodynamic changes were responsible for the enhanced regional myocardial function induced by the GHRP-6. Also, we did not observe any differences among the three groups in the area at risk, infarct size, or hemodynamics, including systolic wall thickening in the ischemic zone, regional myocardial blood flow and collateral blood flow within the entire risk area in any layers of the myocardium early or late during the prolonged coronary artery occlusion.

It is conceivable that despite a significant loss of myocardial contractile function in the ischemic zone, the GHRP-6-induced increase in contractile function in the non-ischemic zone facilitated overall cardiac function, thereby effectively preventing acute cardiac failure leading to death. Importantly, unlike other inotropic responses, the enhanced regional myocardial contractile function induced by GHRP-6 was not accompanied by a substantial

increase in myocardial blood flow. The change in myocardial blood flow in any layer of myocardium in the non-ischemic zone was minimal in the GHRP-6-treated group early and near the end of the period of prolonged coronary artery occlusion while systolic wall thickening was increased by approximately 40%.

In conclusion, chronic therapy with GHRP-6, a GH secretagogue, prevents sudden death in dogs with moderately dilated cardiomyopathy subjected to acute myocardial ischemia. This effect appears to be related to an enhanced non-ischemic compensatory mechanism and mediated via specific GH secretagogue receptors rather than via the GH/IGF-1 pathway.

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Figure Legend

Fig 1. Effects of GHRP-6, GH and vehicle on LV systolic pressure, LV dP/dt, mean arterial pressure, systolic wall thickening in the non-ischemic and ischemic zones, and heart rate in conscious dogs during coronary artery occlusion (CAO) and 3 hours of reperfusion. Values are % changes from control (C) baseline levels. During CAO and reperfusion, systolic wall thickening in the ischemic zone was reduced similarly in the GHRP-6-, GH- and vehicle-treated groups. In contrast, systolic wall thickening in the non-ischemic zone was increased significantly ($p<0.05$) in the GHRP-6-treated group as compared with the GH and vehicle-treated groups. There were no differences in any of the other indices among the three groups.

Fig 2. Left ventricular (LV), area at risk (AAR) and infarct (IF) weights for dogs treated with GHRP-6, GH and vehicle. IF is normalized as % of AAR. Coronary artery occlusion did not result in any statistical difference in the AAR, IF or IF/AAR among the three groups.

Fig 3. Effects of coronary artery occlusion (CAO) on the occurrence of ventricular arrhythmic beats in conscious dogs treated with GHRP-6, GH or vehicle. Values are the average number of arrhythmic beats within each of monitored time period that occurred during CAO with no difference among the three groups.

Fig 4. Effects of GHRP-6, GH and vehicle on survival rate during coronary artery occlusion. Clearly, both the GH- and vehicle-treated groups had similar mortality rates. In contrast, no animals in the GHRP-treated group died.

Table 1. LV function and systemic hemodynamics before (control) and after 2 weeks of rapid ventricular pacing and 1 week of treatment in conscious dogs.

Table 2. Regional myocardial blood flow during coronary artery occlusion and reperfusion.

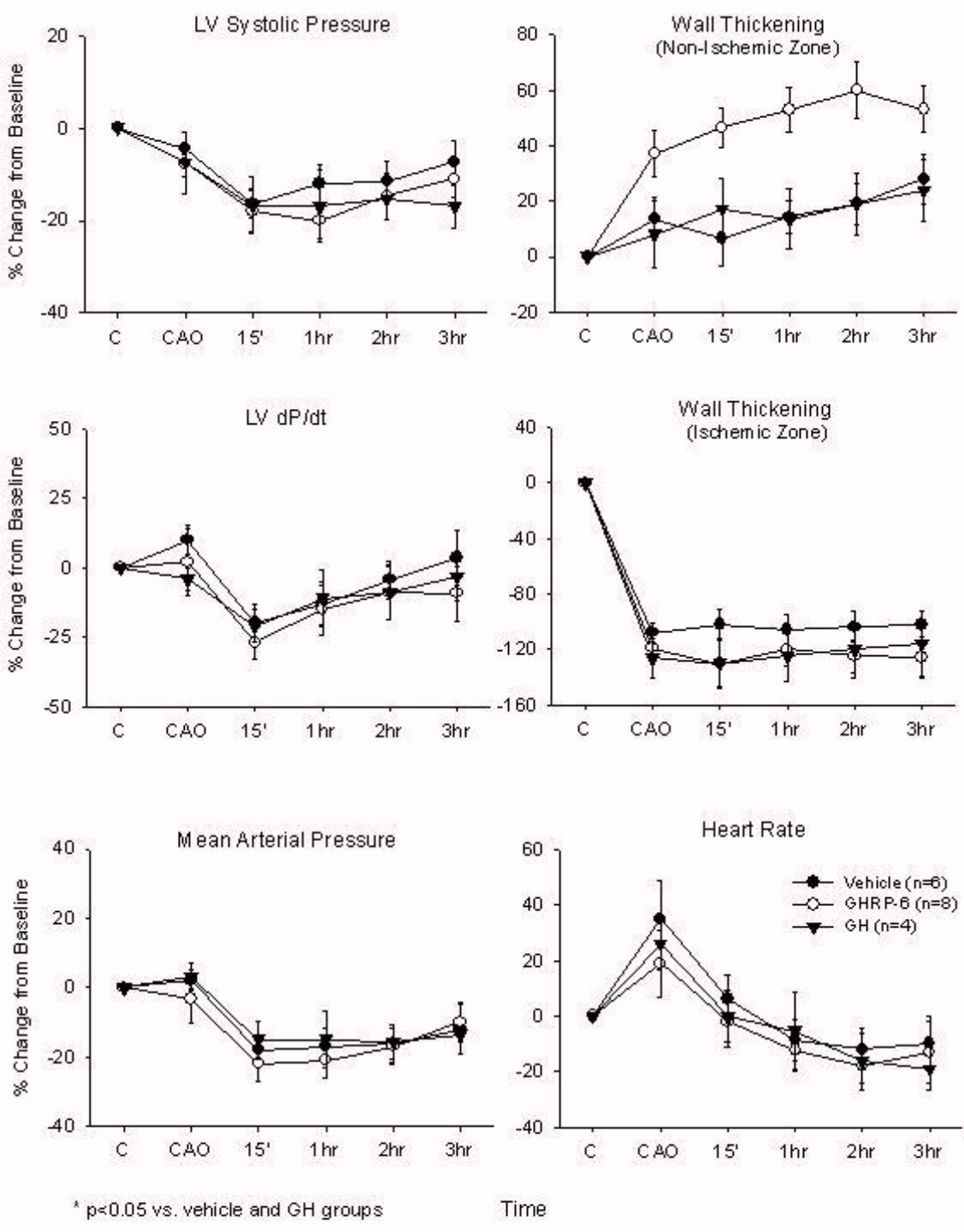
LV Systolic Pressure
Wall Thickening
(Non-Ischemic Zone)
LV dP/dt
Wall Thickening
(Ischemic Zone)
Mean Arterial Pressure
Heart Rate
% Change from Baseline
C
CAO
15'
1hr
2hr
3hr
Vehicle (n=6)
GHRP-6 (n=8)
GH (n=4)
* p<0.05 vs. vehicle and GH groups
Time

Figure 1.

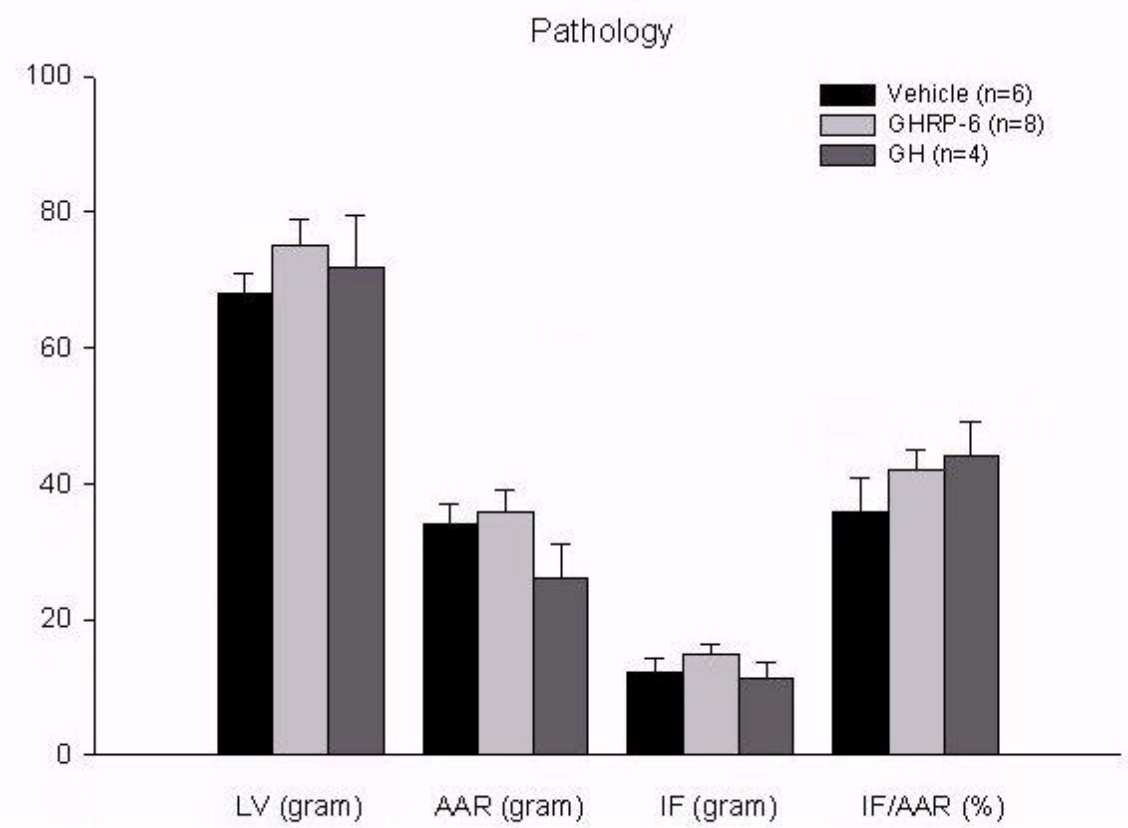
Pathology
Vehicle (n=6)
GHRP-6 (n=8)
GH (n=4)
100
80
60
40
20
0
LV (gram)
AAR (gram)
IF (gram)
IF/AAR (%)

Figure 2.

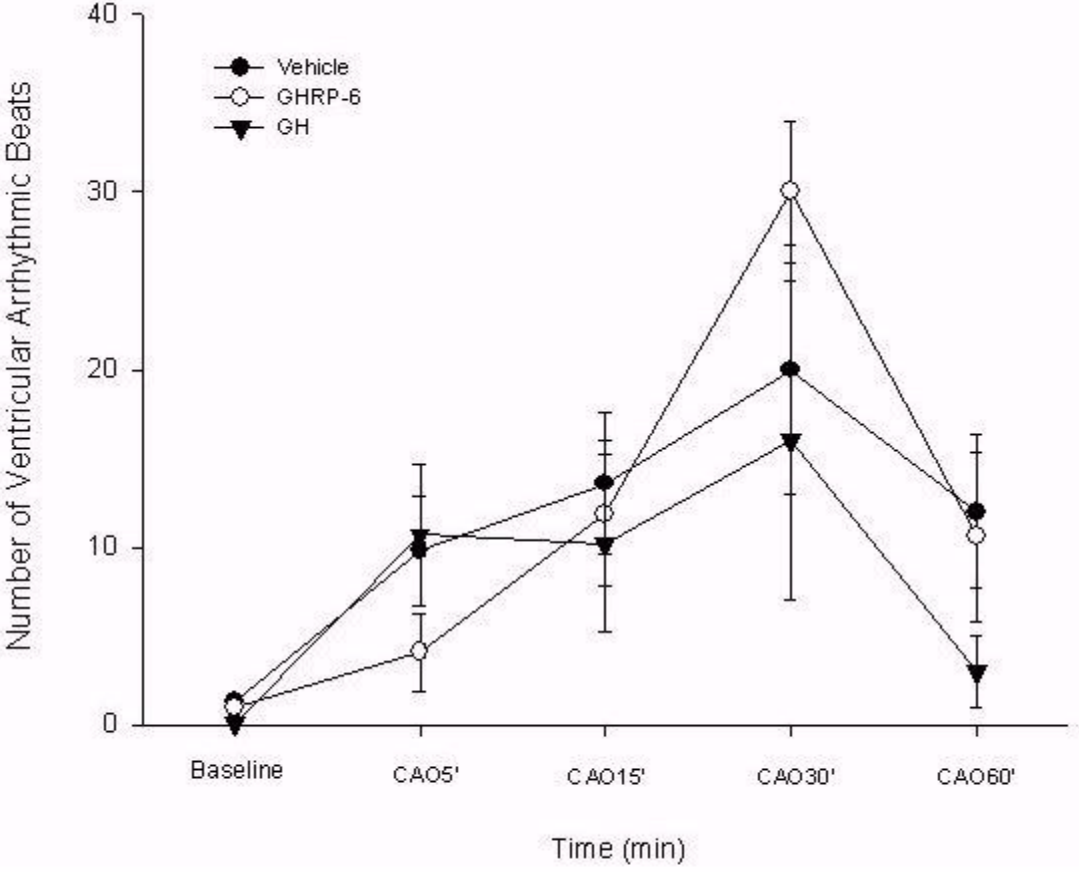
40
30
20
10
0
Vehicle
GHRP-6
GH
Number of Ventricular Arrhythmic Beats
Baseline
CAO5'
CAO15'
CAO30'
CAO60'
Time (min)

Figure 3.

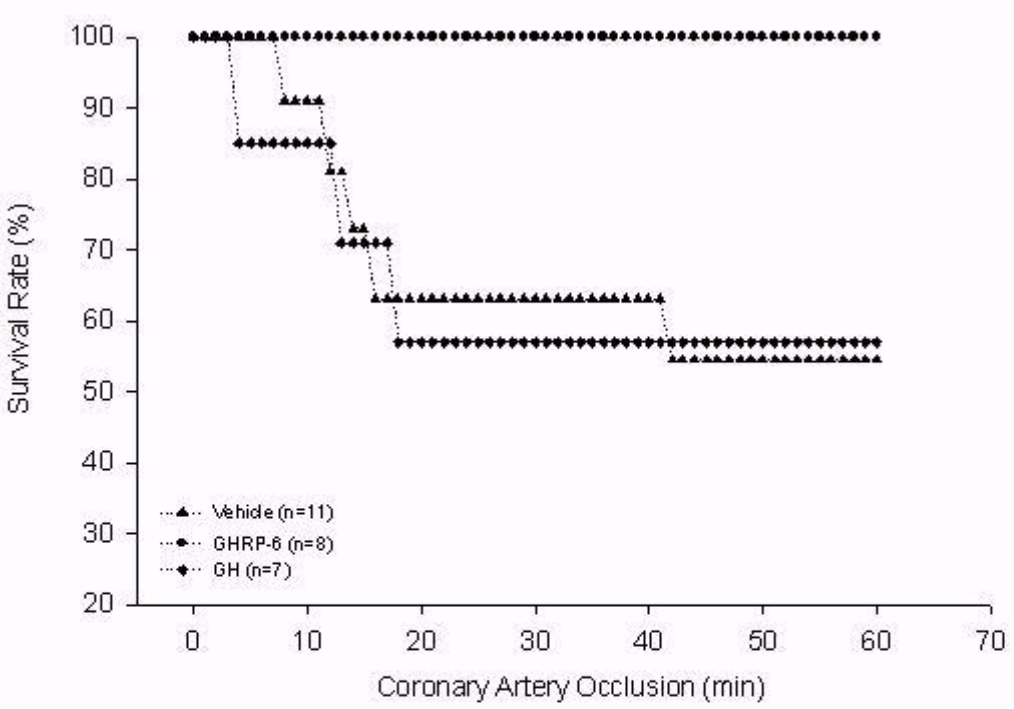
100
90
80
70
60
50
40
30
20
Survival Rate (%)
Vehicle (n=11)
GHRP-6 (n=8)
GH (n=7)
0
10
20
30
40
50
60
70
Coronary Artery Occlusion (min)

Figure 4.

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Table 1. LV function and systemic hemodynamics before (control) and after 2 weeks of rapid ventricular pacing and 1 week of treatment in conscious dogs.

	Control	Pacing & Treatment	Change
LV Systolic Pressure (mmHg)			
Vehicle (n=11)	129±5	104±3	-25±4*
GHRP-6 (n=8)	136±5	112±3	-24±4*
GH (n=7)	136±5	113±6	-24±5*
LV dP/dt (mmHg/sec)			
Vehicle (n=10)	3525±265	1750±107	-1775±178*
GHRP-6 (n=8)	3688±210	2000±97	-1688±219*
GH (n=7)	3571±369	2036±209	-1536±361*
Mean Arterial Pressure (mmHg)			
Vehicle (n=11)	111±5	88±3	-23±3*
GHRP-6 (n=8)	114±5	92±4	-22±4*
GH (n=7)	115±4	94±4	-20±4*
Mean Left Atrial Pressure (mmHg)			
Vehicle (n=11)	5.8±0.8	12.1±1.6	+6.2±1.2*
GHRP-6 (n=8)	5.6±0.8	12.8±1.9	+7.2±1.9*
GH (n=7)	4.4±0.5	10.6±0.8	+6.2±0.8*
Non-Ischemic Wall Thickening (mm)			
Vehicle (n=11)	2.71±0.51	2.24±0.27	-0.47±0.35
GHRP-6 (n=8)	2.88±0.24	2.46±0.15	-0.41±0.17
GH (n=6)	2.88±0.53	2.37±0.29	-0.51±0.45
Ischemic Wall Thickening (mm)			
Vehicle (n=10)	2.87±0.32	2.06±0.36	-0.81±0.25*
GHRP-6 (n=7)	3.13±0.36	2.30±0.29	-0.83±0.27*
GH (n=6)	2.59±0.10	2.04±0.41	-0.55±0.35
Heart Rate (b/min)			
Vehicle (n=11)	121±7	117±8	-5±10
GHRP-6 (n=8)	117±10	129±10	+12±5
GH (n=7)	113±8	114±6	0±6

* $p<0.01$ vs. control

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Table 2. Regional myocardial blood flow during coronary artery occlusion and reperfusion.

	Control	CAO-1	CAO-2	CAR
Non-Ischemic Region				
Endomyocardial (ml/min/g)				
Vehicle (n=6)	0.73±0.05	0.77±0.08	0.98±0.10	0.98±0.14
GHRP-6 (n=8)	1.06±0.11	0.89±0.07	1.10±0.12	1.05±0.13
GH (n=4)	0.90±0.10	0.91±0.04	1.18±0.09	1.04±0.09
Midmyocardial (ml/min/g)				
Vehicle (n=6)	0.59±0.04	0.65±0.07	0.85±0.08	0.84±0.13
GHRP-6 (n=8)	0.91±0.11	0.78±0.07	0.96±0.09	0.97±0.12
GH (n=4)	0.78±0.10	0.79±0.07	1.09±0.09	0.97±0.03
Epimyocardial (ml/min/g)				
Vehicle (n=6)	0.51±0.02	0.60±0.07	0.78±0.10	0.72±0.12
GHRP-6 (n=8)	0.69±0.07	0.65±0.04	0.80±0.05	0.79±0.08
GH (n=4)	0.61±0.04	0.68±0.05	0.94±0.08	0.75±0.03
Ischemic Region				
Endomyocardial (ml/min/g)				
Vehicle (n=6)	0.67±0.04	0.04±0.01*	0.08±0.01*	1.76±0.15*
GHRP-6 (n=8)	0.97±0.11	0.05±0.01*	0.05±0.01*	1.18±0.18
GH (n=4)	0.80±0.06	0.06±0.01*	0.06±0.01*	1.96±0.46
Midmyocardial (ml/min/g)				
Vehicle (n=6)	0.63±0.03	0.07±0.02*	0.17±0.04*	1.94±0.24*
GHRP-6 (n=8)	0.84±0.10	0.08±0.01*	0.11±0.01*	1.56±0.17*
GH (n=4)	0.72±0.04	0.06±0.01*	0.10±0.01*	1.53±0.24
Epimyocardial (ml/min/g)				
Vehicle (n=6)	0.50±0.01	0.12±0.04*	0.27±0.08*	1.01±0.15*
GHRP-6 (n=8)	0.65±0.05	0.12±0.02*	0.20±0.03*	1.34±0.21*
GH (n=4)	0.56±0.07	0.11±0.02*	0.17±0.05*	1.06±0.40

CAO-1: 3-5 min after coronary artery occlusion; CAO-2: before coronary artery reperfusion; CAR: 5-8 min after coronary artery reperfusion; * $p<0.05$, vs control

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