Rotigaptide (ZP123) Prevents Spontaneous Ventricular Arrhythmias and Reduces Infarct Size During Myocardial Ischemia/Reperfusion Injury in Open-Chest Dogs

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ABSTRACT

The antiarrhythmic and cardioprotective effect of increasing gap junction intercellular communication during ischemia/reperfusion injury has not been studied. The antiarrhythmic peptide rotigaptide (previously ZP123), which maintains gap junction intercellular communication, was tested in dogs subjected to a 60-min coronary artery occlusion and 4 h of reperfusion. Rotigaptide was administered i.v. 10 min before reperfusion as a bolus + i.v. infusion at doses of 1 ng/kg bolus + 10 ng/kg/h infusion (n = 6), 10 ng/kg bolus + 100 ng/kg/h infusion (n = 5), 100 ng/kg bolus + 1000 ng/kg/h infusion (n = 8), 1000 ng/kg bolus + 10 μg/kg/h infusion (n = 6), and vehicle control (n = 5). Premature ventricular complexes (PVCs) were quantified during reperfusion. A series of four or more consecutive PVCs were defined as ventricular tachycardia (VT). The total incidence of VT was reduced significantly with the two highest doses of rotigaptide (20.3 ± 10.9 and 4.3 ± 4.1 events; p < 0.05) compared with controls (48.7 ± 6.0). Total PVCs were reduced significantly from 25.1 ± 4.2% in control animals to 11.0 ± 4.4 and 1.7 ± 1.3% after the two highest doses of rotigaptide. Infarct size, expressed as a percentage of the left ventricle, was reduced significantly from 13.2 ± 1.9 in controls to 7.1 ± 1.0 (p < 0.05) at the highest dose of rotigaptide. Ultrastructural evaluation revealed no differences in myocardial injury in the infarct area, area at risk, border zone, or normal zone in vehicle and rotigaptide-treated animals. However, rotigaptide did increase the presence of gap junctions in the area at risk (p = 0.022, Fisher’s exact test). Rotigaptide had no effect on heart rate, blood pressure, heart rate-corrected QT interval, or left ventricular end-diastolic pressure. In conclusion, these results demonstrate that rotigaptide is a potent antiarrhythmic compound with cardioprotective effects and desirable safety.

Myocardial gap junctions are transmembrane channels that transmit electrical impulses via direct transfer of cytosolic ions, metabolites, and small intracellular messengers between neighboring cells. During electrical excitation and depolarization of cardiomyocytes, gap junctions play a significant role in action potential propagation and are thus a major determinant of conduction velocity (Lin et al., 2003). Acute alterations in electrical coupling during myocardial ischemia and reperfusion are associated with conduction slowing and heterogeneities of repolarization that are important substrates for reentrant arrhythmias (De Groot and Coronel, 2004). Ischemia/reperfusion-induced gap junction uncoupling is determined by several factors including acidosis (Kleber, 1992), calcium overload (Dekker et al., 1996), and accumulation of lipid metabolites (Wu et al., 1993). On the basis of these facts, therapeutic agents aimed to prevent uncoupling or reestablish gap junction intercellular communication may be effective antiarrhythmic agents.

The effect of modulating gap junction intercellular communication (GJIC) on myocardial infarct size after ischemia/reperfusion injury is somewhat controversial. Several studies have demonstrated that reduced GJIC in the presence of the gap junction uncoupler heptanol (0.5–6 mM) may reduce cell-to-cell propagation of hypercontracture and cell death (Garcia-Dorado et al., 1997; Saltman et al., 2002). However,
heptanol has also been shown to relax arteries, activate large conductance calcium-activated potassium channels, and inhibit nifedipine-sensitive calcium currents (Matchkov et al., 2004). Thus, it is difficult to determine whether the cardioprotective effects of heptanol observed at millimolar concentrations are due to gap junction uncoupling or to some other mechanism. Furthermore, heptanol failed to induce cardioprotection in a rabbit model of ischemia/reperfusion injury (Gysenbergh et al., 2001). Reduced GJIC has also been correlated with cardioprotection in studies from connexin43 heterozygous null mice, which develop significantly smaller infarcts compared with wild-type mice (Kanno et al., 2003). In contrast to the evidence that reduced coupling may be cardioprotective, several studies have demonstrated a cardioprotective role for GJIC preservation via dilution of “death signals” or passage of “survival factors” in ischemia/reperfusion injury (Blanc et al., 1998; Yasui et al., 2000; Li et al., 2002).

However, investigation of the cardioprotective effect of increased GJIC has been limited because of an absence of stable compounds that specifically increase GJIC. The novel gap junction modifier rotigaptide represents a unique opportunity to resolve this issue.

Gap junction-modifying peptides were first described in the early 1980s (Aonuma et al., 1980) and later shown to increase gap junction intercellular communication in the absence of changes in membrane conductance or basal current (Muller et al., 1997). The clinical development of the original antiarrhythmic peptide (AAP) (Aonuma et al., 1980) and later synthetic derivatives (HP-5 and AAP10) (Kohama et al., 1987; Dhein et al., 1994) has been limited by their instability and very short half-life. Rotigaptide is a rotation-inversion of AAP10 that incorporates the unnatural D-configuration of the amino acids to provide much improved proteolytic stability. Rotigaptide increases gap junction intercellular communication in paired guinea pig cardiomyocytes measured using dual-cell patch-clamp electrophysiology (Xing et al., 2003). Rotigaptide-mediated increases in GJIC occur without alteration of basal or membrane current, suggesting a direct effect of the compound on gap junctions. Rotigaptide has a low affinity for human ether-a-go-go-related gene and exhibits no binding to a large panel of receptors including numerous ion channels (Haugan et al., 2005). Rotigaptide also prevents metabolic stress-induced conduction velocity slowing in rat atrial strips (Haugan et al., 2005) and improves ventricular conduction velocity in whole guinea pig hearts without affecting cellular repolarization (Eloff et al., 2003). In ischemic canine hearts, rotigaptide reduces the incidence of inducible reentrant ventricular tachycardia, demonstrating the importance of gap junction uncoupling as an arrhythmogenic substrate (Xing et al., 2003). In this study, we investigated the antiarrhythmic and cardioprotective effects of rotigaptide in the dog to elucidate the role of gap junctional intercellular communication on ischemia/reperfusion-induced arrhythmias and myocardial infarction. We present evidence of increased gap junctions in the area at risk after treatment with rotigaptide. We also present cardiovascular safety pharmacology data and a pharmacokinetic profile in dogs administered rotigaptide.

### Materials and Methods

#### Materials

Unless otherwise stated, all chemicals were obtained from Sigma Chemical (St. Louis, MO). Rotigaptide (proposed international nonproprietary name for the drug formerly known as ZP123) was manufactured by Bachem AG (Bubendorf, Switzerland) for Wyeth Research. The chemical structure of rotigaptide (Ac-d-Tyr-d-Pro-d-Hyp-Gly-β-Ala-Gly-NH₂) is presented in Fig. 1.

#### Methods

**Surgical Preparation.** Purpose-bred beagle dogs, weighing 8 to 10 kg, were anesthetized with sodium pentobarbital (30 mg/kg i.v.). The animals were intubated and ventilated with room air using a Harvard respirator (Harvard Apparatus, Inc., Holliston, MA); adjusted to deliver a tidal volume of 30 ml/kg at a frequency of 12 breaths/min. Blood pressure was recorded from the right femoral artery using a Millar Mikro-tip catheter (Millar Instruments, Inc., Houston, TX) interfaced with a Grass model 7 polygraph recorder (Grass Instruments Division, Astro-Med Inc., West Warwick, RI). A standard limb lead II electrocardiogram was recorded continuously to monitor heart rate and rhythm. The heart was exposed through a left thoracotomy at the sixth intercostal space and suspended in a pericardial cradle. The left circumflex (LCX) coronary artery was exposed proximal to the first obtuse marginal branch and instrumented with a Transonic ultrasonic flow probe (model 1.5RB; Transonic Systems Inc., Ithaca, NY) for continuous monitoring of phasic coronary artery blood flow. A ligature stenosis was placed around the LCX coronary artery such that the hyperemic response to a brief 10-s occlusion was reduced by 30%. A Silastic umbilical tape was placed around the LCX coronary artery and through a polyethylene sleeve to create a snare occluder. All hemodynamic parameters were recorded throughout the experiment on a Grass Instruments multichannel recorder interfaced with a PC computer running PO-NEHMAH data acquisition software. Complete LCX coronary artery occlusion was initiated by tightening the snare occluder around the vessel. All dogs were subjected to 60 min of LCX coronary artery occlusion and reperfusion. Restoration of blood flow was performed by slowly releasing the Silastic ligature. Blood flow was restored to preischemic levels over a 10-min period. The ligature stenosis reduces hyperemia during reperfusion. Infarct size was assessed after 4 h of reperfusion.

Rotigaptide or vehicle (saline) was administered 10 min before reperfusion in a randomized blinded fashion. A bolus injection (5-ml volume over 5 min) of drug was followed by a continuous infusion for the duration of the 4-h reperfusion period (10 ml total volume, 42 µl/min; Harvard pump). Four doses of rotigaptide were tested including 1 ng/kg bolus + 10 ng/kg/h infusion (n = 6), 10 ng/kg bolus + 100 ng/kg/h infusion (n = 5), 100 ng/kg bolus + 1000 ng/kg/h infusion (n = 8), 1000 ng/kg bolus + 10 µg/kg/h infusion (n = 6), and vehicle control (n = 5).

![Fig. 1. Chemical structure of rotigaptide.](image-url)
Administration of Radiolabeled Microspheres for Determination of Regional Myocardial Blood Flow. Regional myocardial blood flow (RMBF) was determined using radiolabeled microspheres ($^{103}$Ru, 100 μCi, 15 μm in diameter; New England Nuclear, Boston, MA) by the reference withdrawal method (Black et al., 1998). During the 60-min ischemic period each dog received an injection of microspheres 45 min after occlusion of the LCX coronary artery.

Determination of Myocardial Infarct Size and Area at Risk. At completion of the study period, hearts were excised immediately after the electrical induction of ventricular fibrillation. Histochemical determinations of the anatomic area at risk and zone of infarction were accomplished with a dual perfusion technique. The aorta was perfused in a retrograde fashion with 0.25% Evans blue dye, and the LCX coronary artery was perfused with 1.5% triphenyltetrazolium chloride in 20 mM potassium phosphate buffer (pH 7.4, 37°C). The heart was cut into 1-cm thick transverse sections and fixed in 10% phosphate-buffered formalin. Both surfaces of each ventricular section were traced onto clear plastic overlays and digitized using a flatbed scanner. The PC-Draft software program (Innovative Data Design, Concord, CA) was used to calculate the area of the infarct zone and the area at risk from the digitized heart sections.

Determination of RMBF. Myocardial tissue samples weighing 0.1 to 0.5 g (wet weight) were dissected from the subepicardial, midmyocardial, and subendocardial sections of the heart in the non-ischemic and ischemic zones, which included the posterior papillary muscle. Four transverse sections from each heart were used so that blood flow to each region represents the average of four samples for each experiment. The level of radiolabeled microsphere incorporation into each myocardial tissue sample was measured in a Cobra Quantum Series gamma counter (Packard Instrument Co., Meriden, CT). The mean RMBF from the inner two-thirds of the myocardium was used to determine whether an excess of collateral blood supply (>0.18 ml/min/g of tissue) was present during LCX coronary artery occlusion. RMBF >0.18 ml/min/g of tissue in the inner two-thirds of the ventricular wall or refractory ventricular fibrillation requiring more than three attempts at cardioversion using low-energy pulses (10 J) were exclusion criteria.

Arrhythmia Analysis. Arrhythmias induced by ischemia/reperfusion injury were analyzed according to the Lambeth Conventions (Walker et al., 1988). Premature ventricular complexes, defined as discrete and identifiable premature QRS complexes (premature in relation to the previous ventricular sinus beat), were counted for 2-min periods every 5 min during the first 60 min of reperfusion. A run of four or more consecutive premature ventricular contractions (PVCs) was defined as ventricular tachycardia (VT). Ventricular fibrillation was defined as a signal from which individual QRS deflections could not be distinguished from one another and at which heart rate was no longer measurable.

Electron Microscopy and Ultrastructural Analysis. Small pieces of canine heart from vehicle-treated controls (n = 3) or rotigaptide-treated dogs (100 ng/kg bolus + 1000 ng/kg/h infusion, n = 3) were fixed in modified McDowell-Trump’s fixative and processed for transmission electron microscopic examination as described previously (Keith, 1986). Samples were taken from the infarct area, infarct border zone, area at risk, and normal myocardium. Each sample was postfixed with 1% osmium tetroxide, routinely dehydrated in a graded series of ethanol, immersed in propylene oxide, and embedded in Araldite resin. Thin sections were cut on a Leica EM UC6 microtome using a diamond knife. Sections on grids were poststained with uranyl acetate and lead citrate and viewed on a Zeiss 10C transmission electron microscope operating at 80 kV. Micrographs were recorded on film and printed on photographic paper; 306 electron photomicrographs were produced and randomly coded before evaluation by two investigators (J.C.K. and R.G.S.). The degree of myocardial injury was scored based on four parameters described previously (Trump et al., 1976; Keith, 1986). The swelling or dilation of the tubules and sarcoplasmic reticulum were each scored, ranging from 0 for normal to 2 for severe dilation. The structural integrity of the mitochondria was assessed for swelling and for disruption of the cristae using a scale ranging from 0 for normal to 3 for massive swelling and complete disruption of the cristae. The structure of the myofibrils was graded, ranging from 0 for normal relaxed myofibrils through 1 for hypercontraction to 2 for disruption of myofibrils. Each photomicrograph was examined for the presence of intercellular gap junction profiles clearly associated with zona adherens junctions as described previously (Petrich et al., 2002). If intercellular profiles of gap junctions were seen, they were called present. If zona adherens junctions were present but no gap junction profiles were seen, they were called absent. If no zona adherens junctions and intercellular regions were present in the photomicrograph, the specimen was called nonevaluable for gap junctions. A representative photomicrograph containing gap junctions is seen in Fig. 2. After the analysis was completed, the codes were revealed, and the results of myocardial injury score and presence of gap junctions were tabulated by myocardial region and treatment group.

Pharmacokinetics and Determination of Rotigaptide Concentrations in Dog Plasma. Rotigaptide was formulated in saline with no buffer or pH adjustment and administered via a single intravenous bolus (20 μg/kg) via a cephalic vein in a separate group of eight dogs. Blood (~3 ml) was collected from the jugular vein in tripotassium EDTA at 5, 15, and 30 min and 1, 1.5, 2, 4, 6, 8, and 12 h after dosing. Rotigaptide concentration was determined in plasma samples by a liquid chromatography-tandem mass spectrometry procedure (Kjolbye et al., 2003) with a lower limit of quantitation of 1 ng/ml. The pharmacokinetic parameters for individual animals were determined using i.v. bolus administration, noncompartmental analysis module (model 201) of the PK software package WinNonlin, version 3.2 (Pharsight, Mountain View, CA).

Cardiovascular Safety Profile of Rotigaptide. To further understand the cardiovascular safety of rotigaptide, eight additional dogs (four male and four female) were administered suprapharmacological doses (1, 3, and 10 mg/kg) by single intravenous bolus injection according to a Latin square crossover dosing paradigm. Effects on heart rate, systolic and diastolic blood pressure, and the lead II electrocardiogram (QT, heart rate-corrected QT interval (QTc), PR, and QRS interval) were evaluated in all animals using radiotelemetry units implanted 14 days before dosing. Before dosing, data were collected for 30-s periods every 5 min for 24 h. After dosing, telemetry data were collected for 30 s periods every minute for 2 h followed by measurements every 5 min up to 24-h postdose.

Fig. 2. Representative electron photomicrograph of canine myocardium displaying gap junction profiles. The slender arrows delimit the gap junction profile in the intercellular space, whereas the larger arrows identify the zona adherens junctions. Original magnification, 8800×.
QTc was calculated using the regression relationship estimated from the predose data. The relationship between postdose QT interval and heart rate was examined by fitting a mixed model analysis of variance to the postdose data for the vehicle control and 10 mg/kg dosages.

**Statistical Analysis**

Incidences of spontaneous ventricular arrhythmias (PVC and VT) were compared using a repeated measures ANOVA. The total numbers of PVCs or VT during the first 60 min of reperfusion were compared using a one-way ANOVA followed by Dunnett’s post hoc test. Infarct size and RMBF comparison’s between vehicle control and rotigaptide-treated dogs were performed using a one-way ANOVA followed by Dunnett’s post hoc test. In the safety pharmacology studies, repeated measures ANOVA was used to compare mean response after administration of rotigaptide to mean response after vehicle administration. The ANOVA model used for heart rate data analysis included a random effect to account for animal to animal differences. For heart rate-corrected QT comparisons, a regression model was fit to the combined predose data for vehicle control and compound dosages. The presence of gap junctions in the ultrastructural analysis was evaluated for treatment effect by Fisher’s exact test. For all statistical analysis, \( P < 0.05 \) was considered significant.

**Results**

**Exclusions and Hemodynamic Data.** A total of 45 dogs were used in the ischemia/reperfusion injury study; 36 dogs successfully completed the study. Four dogs were excluded because RMBF was greater than 0.18 ml/min/g of tissue, and five dogs were omitted because of intractable ventricular fibrillation during ischemia or reperfusion. The incidence of ventricular fibrillation was not specific to any one group of animals.

**Effect of Rotigaptide on Spontaneous Ventricular Arrhythmias During Ischemia/Reperfusion Injury.** Spontaneous ventricular arrhythmias were induced by ischemia/reperfusion injury. These arrhythmias had varying origins and were concentrated to the first 60 min of reperfusion after the ischemic episode. During treatment with the two highest dose levels of rotigaptide (100 ng/kg bolus + 1000 ng/kg/h infusion or 1000 ng/kg bolus + 10 µg/kg/h infusion), PVC incidence over time was significantly reduced compared with saline-treated controls in the same period of reperfusion (repeated measures ANOVA, \( P < 0.05 \)) (Fig. 3a). At the highest dose, statistically significant reductions in PVCs were observed at the 25-, 30-, 35-, 40-, and 45-min time points after reperfusion (\( p < 0.05 \)). The total number of PVCs recorded during the first 60 min of reperfusion was reduced by 56% in the 100 ng/kg bolus + 1000 ng/kg/h infusion dose group and by 93% in the 100 ng/kg bolus + 10 µg/kg/h infusion group compared with saline-treated controls (Fig. 3b).

As shown in Fig. 4a, runs of VT were reduced significantly at the 25-, 30-, 35-, and 40-min time points of reperfusion (\( p < 0.05 \)) after treatment with the highest dose of rotigaptide. The trend in VT incidence over time was significantly different from that in control animals at the two highest doses of rotigaptide (repeated measures ANOVA, \( P < 0.05 \)). The total incidence of VT in the first 60 min of reperfusion was reduced significantly during treatment with the two highest doses of rotigaptide (\( p < 0.05 \)) (Fig. 4b). These data suggest that gap junction uncoupling is one of the primary substrates for arrhythmia formation in the ischemic/reperfused dog heart and maintenance of GJIC by rotigaptide is an effective antiarrhythmic strategy.

**Effect of Rotigaptide on Infarct Size After 4 h of Reperfusion.** The effects of rotigaptide on infarct size after 60 min of ligature occlusion and 4 h of reperfusion are illustrated in Fig. 5. The area of the left ventricle at risk, infarct size as a percentage of the area at risk, and infarct size as a percentage of left ventricle are shown. The area at risk was similar in all five groups, indicating that an anatomically similar region of the left ventricle was at risk of ischemia and reperfusion injury. Infarct size, expressed as a percentage of the area at risk or as a percentage of the left ventricle, was significantly reduced after treatment with the highest dose of rotigaptide (\( p < 0.05 \)).

The RMBF of control and rotigaptide-treated dogs is shown in Fig. 6. The data expressed represent the regional blood flow in the inner two-thirds of the myocardium, reflecting myocardial blood flow in the subendocardial and mid myocardial layers of the left ventricular wall. In each of the treatment groups, RMBF in the region of the left ventricle supplied by the left circumflex coronary artery (infarct zone) was reduced significantly compared with the region of the left ventricle supplied by the left anterior descending coronary...
artery (normal zone). The data presented in Fig. 6 indicate that each group of animals was subjected to an equivalent degree of ischemia during the 60-min ligature occlusion.

**Ultrastructural Analysis of Infarcted Hearts.** Two investigators without knowledge of the treatment group evaluated 306 transmission electron photomicrographs. Seventeen micrographs could not be evaluated for the parameters of injury or the presence of gap junctions because of advanced necrosis. Myocardial injury scores were determined for 289 evaluable micrographs, and the results are seen in Table 1. The degree of damage was comparable among the vehicle and the rotigaptide-treated animals. Intercellular gap junction profiles were scored as present or absent in the 146 micrographs that contained intercellular regions adjacent to zona adherens junctions, and the results are displayed by myocardial region in Table 2. In the infarct, border zone, and non-ischemic myocardial regions, the frequency of gap junctions was similar among treatment groups. In the area at risk region, rotigaptide administration appeared to increase the presence of gap junctions ($p = 0.022$, Fisher’s exact test).

**Pharmacokinetics and Estimated Plasma Concentrations of Rotigaptide.** Because of the injection of radiolabeled microspheres in the infarct study plasma concentrations of rotigaptide were not measured. However, a detailed pharmacokinetic analysis was done in the dog to estimate steady-state plasma concentrations of rotigaptide in the ischemia/reperfusion study. The pharmacokinetic profile of rotigaptide following a single bolus intravenous dose of 20 µg/kg showed a linear pharmacokinetic behavior. The mean $C_{\text{max}}$ and exposure (AUC) values were 97.3 ng/ml and 88.8 ng/h/ml, respectively. The mean clearance and volume of distribution ($V_{\text{dss}}$) values were 0.226 l/h/kg and 0.237 l/kg, respec-
from baseline in control and rotigaptide-treated animals, and rate. Mean arterial blood pressure was also not different among groups did not reveal differences in heart rate. Study heart rate was unchanged from the respective baseline in control and rotigaptide-treated animals (Table 3). Com-

<table>
<thead>
<tr>
<th>Group</th>
<th>t-Tubules</th>
<th>Sarcoplasmic Reticulum</th>
<th>Mitochondria</th>
<th>Myofibrils</th>
<th>Mean Injury Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.65 ± 1.02</td>
<td>0.73 ± 0.95</td>
<td>1.69 ± 1.38</td>
<td>0.82 ± 1.05</td>
<td>3.89</td>
</tr>
<tr>
<td>Border zone</td>
<td>0.30 ± 0.53</td>
<td>0.33 ± 0.58</td>
<td>1.86 ± 0.43</td>
<td>1.46 ± 0.47</td>
<td>3.95</td>
</tr>
<tr>
<td>Area at risk</td>
<td>0.61 ± 1.06</td>
<td>0.61 ± 1.05</td>
<td>1.05 ± 1.55</td>
<td>0.81 ± 1.05</td>
<td>3.08</td>
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<tr>
<td>Normal</td>
<td>0.11 ± 0.19</td>
<td>0.11 ± 0.19</td>
<td>0.26 ± 0.35</td>
<td>0.18 ± 0.17</td>
<td>0.86</td>
</tr>
<tr>
<td>Rotigaptide</td>
<td>0.39 ± 0.68</td>
<td>0.47 ± 0.81</td>
<td>1.88 ± 0.36</td>
<td>1.45 ± 0.33</td>
<td>4.19</td>
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<tr>
<td>Border zone</td>
<td>0.67 ± 1.01</td>
<td>0.70 ± 1.06</td>
<td>1.05 ± 0.55</td>
<td>1.28 ± 0.33</td>
<td>3.70</td>
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<tr>
<td>Area at risk</td>
<td>0.30 ± 0.28</td>
<td>0.34 ± 0.30</td>
<td>1.25 ± 1.01</td>
<td>0.75 ± 0.43</td>
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<tr>
<td>Normal</td>
<td>0.09 ± 0.09</td>
<td>0.14 ± 0.15</td>
<td>0.63 ± 0.59</td>
<td>0.22 ± 0.21</td>
<td>1.08</td>
</tr>
</tbody>
</table>

Hemodynamic Effects of Rotigaptide. In the present study heart rate was unchanged from the respective baseline in control and rotigaptide-treated animals (Table 3). Comparisons among groups did not reveal differences in heart rate. Mean arterial blood pressure was also not different from baseline in control and rotigaptide-treated animals, and no difference in blood pressure was observed in a comparison among groups.

Discussion

The present study demonstrates a beneficial antiarrhythmic effect of improving gap junction communication with rotigaptide during reperfusion injury. Furthermore, it provides the first evidence that increasing gap junction intercellular communication during ischemia reperfusion injury reduces infarct size. The antiarrhythmic and cardioprotective effects of rotigaptide were observed at concentrations shown to increase gap junctional conductance in paired cardiomyocytes (Xing et al., 2003), improve conduction velocity in acidic guinea pig ventricle (Eloff et al., 2003), and inhibit metabolic stress-induced conduction velocity slowing in rats.

TABLE 1
Myocardial ultrastructural injury scores after ischemia/reperfusion in dogs treated with vehicle or rotigaptide
Injury scores were determined by scoring t-tubules and sarcoplasmic reticulum: 0–normal, 1–mild dilation, 2–severe dilation; mitochondria: 0–normal, 1–mild swelling, 2–moderate swelling, 3–severe swelling and disruption; and myofibril structure: 0–normal, 1–hypercontraction, 2–disruption of myofibrils.

<table>
<thead>
<tr>
<th>Group/Region</th>
<th>Photomicrographs with Gap Junctions</th>
<th>Present/Photomicrographs with Gap Junctions</th>
<th>Deemed Evaluable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle/infarct</td>
<td>2/19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle/border zone</td>
<td>2/17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle/area at risk</td>
<td>1/13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle/nonischemic</td>
<td>15/19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotigaptide/infarct</td>
<td>5/15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotigaptide/border zone</td>
<td>5/16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotigaptide/area at risk</td>
<td>10/22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotigaptide/nonischemic</td>
<td>9/22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significantly differently higher than vehicle/area at risk (p = 0.022, Fisher’s exact test).

TABLE 3
Mean heart rate and mean arterial blood pressure at baseline and for 4 h after administration of rotigaptide in a canine model of ischemia/reperfusion injury

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
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<tr>
<td>Mean heart rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline control</td>
<td>151 ± 9</td>
<td>128 ± 5</td>
<td>144 ± 10</td>
<td>152 ± 6</td>
<td>159 ± 6</td>
</tr>
<tr>
<td>1 ng/kg bolus + 10 ng/kg/h rotigaptide</td>
<td>160 ± 6</td>
<td>151 ± 15</td>
<td>168 ± 9</td>
<td>179 ± 9</td>
<td>178 ± 8</td>
</tr>
<tr>
<td>10 ng/kg bolus + 100 ng/kg/h rotigaptide</td>
<td>178 ± 22</td>
<td>154 ± 9</td>
<td>178 ± 10</td>
<td>172 ± 7</td>
<td>161 ± 7</td>
</tr>
<tr>
<td>100 ng/kg bolus + 1000 ng/kg/h rotigaptide</td>
<td>160 ± 9</td>
<td>156 ± 12</td>
<td>169 ± 12</td>
<td>172 ± 9</td>
<td>170 ± 7</td>
</tr>
<tr>
<td>1000 ng/kg bolus + 10 µg/kg/h rotigaptide</td>
<td>157 ± 10</td>
<td>146 ± 8</td>
<td>157 ± 5</td>
<td>163 ± 6</td>
<td>165 ± 6</td>
</tr>
<tr>
<td>Mean arterial blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline control</td>
<td>99 ± 7</td>
<td>84 ± 9</td>
<td>88 ± 9</td>
<td>87 ± 9</td>
<td>89 ± 9</td>
</tr>
<tr>
<td>1 ng/kg bolus + 10 ng/kg/h rotigaptide</td>
<td>98 ± 6</td>
<td>79 ± 3</td>
<td>87 ± 3</td>
<td>87 ± 3</td>
<td>84 ± 3</td>
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<tr>
<td>10 ng/kg bolus + 100 ng/kg/h rotigaptide</td>
<td>103 ± 6</td>
<td>93 ± 8</td>
<td>93 ± 6</td>
<td>90 ± 5</td>
<td>82 ± 6</td>
</tr>
<tr>
<td>100 ng/kg bolus + 1000 ng/kg/h rotigaptide</td>
<td>90 ± 5</td>
<td>85 ± 7</td>
<td>85 ± 4</td>
<td>88 ± 4</td>
<td>87 ± 4</td>
</tr>
<tr>
<td>1000 ng/kg bolus + 10 µg/kg/h rotigaptide</td>
<td>81 ± 10</td>
<td>79 ± 7</td>
<td>77 ± 9</td>
<td>82 ± 8</td>
<td>78 ± 8</td>
</tr>
</tbody>
</table>
The process of gap junction uncoupling during ischemia/reperfusion is also associated with detrimental cardiac effects. Despite its proven utility, reperfusion disrupts the progression of necrosis and salvages tissues in the best strategy so far to limit infarct size. Reperfusion arrhythmias observed with rotigaptide suggests that the antiarrhythmic effect of rotigaptide may be mediated through modulation of Cx43 phosphorylation during ischemia. Rotigaptide also increases Cx43 expression in cultured cardiomyocytes (M. Stahlhut, J. S. Peterson, J. K. Hennan, and M. T. Ramirez, unpublished observations, 2005). These new studies provide further evidence that rotigaptide has downstream effects on connexins and gap junctions.

The extent of gap junction uncoupling during ischemia is directly related to the duration of ischemia and well correlated with the onset of ischemic rigor and calcium overload (Delmar et al., 1987). Upon reperfusion of the ischemic myocardium, rapid normalization of pH and cytosolic calcium elicits further hypercontracture and the genesis of reperfusion arrhythmias (Yamazaki et al., 1986). These arrhythmias occur most often through reentrant circuits that develop in the injured myocardium because of slowed conduction and conduction block (Yamazaki et al., 1986; Dillon et al., 1988). Confocal microscopy and thin-section electron microscopy have shown that gap junctions are severely disturbed in the infarct and border zone, leading to heterogeneous conduction and increased susceptibility to arrhythmias (Smith et al., 1991; Peters et al., 1995). The dramatic reduction in reperfusion arrhythmias observed with rotigaptide suggests that under these pathological conditions the gap junction may be the predominant factor implicated in arrhythmogenesis and thus agents that improve gap junction intercellular communication may be effective for the prevention of VT postmyocardial infarction. These results are in agreement with previously published data on rotigaptide demonstrating a reduction in reentry VT in ischemic canine hearts subjected to programmed extrastimulation to induce VT and three-dimensional activation mapping to confirm reentry (Xing et al., 2003). Interestingly, rotigaptide does not appear to be efficacious against focal VT in canine hearts subjected to programmed extrastimulation (Xing et al., 2005). Thus, the spontaneous arrhythmias observed in this study probably originate from reentry circuits in the ischemic zone. Rotigaptide had no effect on heart rate or mean arterial blood pressure confirming the previously reported lack of effect of rotigaptide on cardiac contractility or systemic hemodynamics (Kjolbye et al., 2003; Xing et al., 2003; Haugan et al., 2005).

Myocardial reperfusion of the ischemic heart remains the best strategy so far to limit infarct size. Reperfusion interrupts the progression of necrosis and salvages tissues in the border zone of the infarct. Despite its proven utility, reperfusion is also associated with detrimental cardiac effects including extension of myocyte hypercontracture and a deleterious inflammatory response against host tissue, mediated by free radicals, neutrophils, and complement activation. The process of gap junction uncoupling during ischemia/reperfusion injury is well documented, but the role of gap junctions as a determinant of final infarct size is controversial (Garcia-Dorado et al., 1997; Blanc et al., 1998; Yasui et al., 2000; Gysembergh et al., 2001; Li et al., 2002; Saltman et al., 2002; Kanno et al., 2003; Matchov et al., 2004). It has been suggested that gap junction intercellular communication may act to dilute potentially lethal signals during reperfusion injury thereby limiting the spread of cell death. In rat neonatal cardiomyocytes, preservation of cell-to-cell coupling is associated with a reduction in apoptosis (Yasui et al., 2000). Low cell density in culture or inhibition of gap junction formation through Cx43 antisense treatment leads to increased cell death (Yasui et al., 2000). It has been suggested that one or more humoral factors may transfer through gap junctions acting as “survival signals” to reduce cell death. Gap junctions also permit transfer of antioxidants from nonischemic cells to ischemic cells, which protect the cell against further oxidative stress and prevent cardiomyocytes from induction of apoptosis (Inserte et al., 2000). Further studies are required to specifically identify those apoptosis-affecting humoral factors that are transferred through gap junctions to prevent spread of cell death.

The observed preservation of gap junctions in the rotigaptide-treated hearts is a possible indication of improved conduction in the ischemic heart. However, we did not assess functional coupling or conduction velocity in this model so it remains to be determined whether the gap junctions are functional. Previous rotigaptide studies have demonstrated increased conduction velocity (Eloff et al., 2003; Haugan et al., 2005) and reduced dispersion of the action potential (Kjolbye et al., 2003) in intact cardiac muscle. Whether these effects result from improved gap junction preservation or decreased degradation of gap junctions requires further study. Several studies have demonstrated reduced conduction velocity after ischemia-induced gap junction uncoupling or after administration of gap junction uncouplers (for review, see Rohr, 2004), further suggesting that preservation of gap junctions should result in sustained or increased conduction velocity. Aside from cardiac tissue the potential benefit of increasing gap junction communication in bone osteoblast membrane has been linked to improved bone density in rat osteoporosis (Jorgensen et al., 2005).

The effect of improving gap junction coupling or reducing uncoupling in the setting of ischemia/reperfusion injury has not been studied extensively in vivo because of a lack of compounds that selectively increase gap junction coupling. The reduction in infarct size with rotigaptide demonstrated in this study provides the first evidence that improved gap junction communication during ischemia/reperfusion may protect the myocardium. These data are supported by previous studies demonstrating an important role for gap junctional intercellular communication during ischemia/reperfusion may protect the myocardium. Other mechanisms of cardioprotection including altered intracellular calcium dynamics, ATP preservation or improved perfusion of the infarct and border zone due to increased gap junction communication may be responsible for the decrease in infarct size observed with rotigaptide. Further studies investigating these cardioprotective pathways are required to clearly identify the mechanism of rotigaptide-induced cardioprotection.

Despite our finding of a protective effect for rotigaptide, several investigators have studied the effect of inhibiting gap
junction communication during reperfusion injury. Four gap junction uncouplers with different mechanisms of action (heptanol, 18-glycyrrhetinic acid, halothane, and palmitoleic acid) have demonstrated a protective effect against myocardial necrosis when administered during reperfusion. These agents limit cell-to-cell hypercontracture and reduce overall infarct size in rat, rabbit, and dog models of ischemia/reperfusion injury (Schlack et al., 1994; Garcia-Dorado et al., 1997). The mechanism of action of the most commonly used uncoupler, the organic solvent heptanol, remains unknown. It reportedly reduces the open probability of gap junction channels by eliciting a conformational change at the interface between gap junction proteins and membrane lipids (Takens-Kwak et al., 1992; Christ et al., 1999). However, heptanol has also been shown to relax arteries, activate large conductance calcium-activated potassium channels, and inhibit nifedipine-sensitive calcium current (Matchov et al., 2004). The nonselective actions of this agent were clearly apparent in the mouse heart, where 20% of hearts treated with 1 mM heptanol exhibited spontaneous arrest (Li et al., 2002). Rotigaptide is a selective gap junction modifier with no observed cardiovascular side effects at doses well above efficacy. Thus, it is possible that the role of gap junctions in the spread of infarction has been misled by poor uncouplers and a lack of good gap junction coupled proteins.

In conclusion, our data demonstrate that increased gap junction intercellular communication in the presence of rotigaptide prevents spontaneous reperfusion-induced ventricular arrhythmias and protects the heart against the spread of infarction. Rotigaptide represents a novel approach to acute prevention of VT/ventricular fibrillation after myocardial infarction.

References


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