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MAGNETIC RESONANCE SPECTROSCOPY

Effects of a pharmacologically-induced shift of hemoglobin-oxygen dissociation on myocardial energetics during ischemia in patients with coronary artery disease

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Background. Conventional strategies to treat myocardial ischemia include interventions that reduce oxygen demand and/or increase myocardial blood flow. Animal experiments suggest that right-shifting the hemoglobin-oxygen dissociation curve may also attenuate the metabolic consequences of myocardial ischemia. We evaluated whether exercise-induced myocardial ischemia can be alleviated in subjects with coronary artery disease (CAD) by enhancing oxygen release with an allosteric modifier of hemoglobin's affinity for oxygen (RSR13). *Methods and Results.* Seven subjects with CAD underwent a randomized, double-blind, cross-over study of the metabolic consequences of RSR13 administration on myocardial ischemia. Myocardial high-energy phosphates were quantified with ^{31}P nuclear magnetic resonance (NMR) spectroscopy before, during, and after isometric handgrip-exercise. Subjects underwent NMR studies at baseline and on two separate occasions following the infusion of RSR13 (100 mg/kg) or placebo. RSR13 infusion significantly increased mean p50 by 8.1 ± 2.7 mmHg at the end of the infusion, and it was still elevated by 4.9 ± 3.3 mmHg after the completion of the treadmill tests while placebo had no effect. The myocardial creatine-phosphate (PCr) to adenosine-triphosphate (ATP) ratio decreased during handgrip-exercise in the baseline studies (from 1.39 ± 0.23 before exercise to 0.95 ± 0.21 during handgrip-exercise, p = 0.0001) and in the placebo studies (from 1.29 ± 0.16 to 0.98 ± 0.37, p = 0.06) but not during administration of RSR13 (from 1.28 ± 0.18 to 1.02 ± 0.24, p = 0.12). However, the mean values of cardiac PCr/ATP during handgrip-exercise did not differ significantly among the three measurements (baseline, placebo, RSR13). *Conclusions.* A single infusion of RSR13 to subjects with CAD increased mean p50 by 4.9–8.1 mmHg but did not significantly alter myocardial PCr/ATP during exercise. This is the largest right-shift in hemoglobin-oxygen binding affinity achieved in CAD subjects, and it did not provide clear evidence of protection from cardiac ischemia.

Key Words: Coronary artery disease; Myocardial ischemia; Magnetic resonance spectroscopy; Myocardial energetics; Hemoglobin-oxygen dissociation; Cardiac metabolism

1. Introduction

Myocardial ischemia occurs when there is an imbalance between myocardial oxygen supply and demand. Conventional strategies to treat or alleviate the tissue hypoxia resulting from myocardial ischemia focus on interventions that reduce oxygen demand (e.g. beta-blockers) or augment blood supply (e.g. vasodilator medications or coronary revascularization). A conceptually novel approach to attenuate the oxygen supply deficit in ischemic myocardium is through interventions that diminish the affinity of hemoglobin for oxygen, resulting in enhanced oxygen unloading in hypoxic tissues (1–7).

Decreasing the binding affinity of hemoglobin for oxygen shifts the sigmoidal hemoglobin-oxygen dissociation curves to the right so that less oxygen is bound to hemoglobin at any given oxygen tension. This is typically quantified as an increase in the oxygen tension at which hemoglobin is 50% saturated (p50). A novel synthetic organic molecule was found to be a potent allosteric modifier of the hemoglobin-oxygen binding affinity (8, 9). This compound is RSR13, 2-[4-[{(3,5-disubstituted anilino)carbonyl}methyl]phenoxyl]-2-methyl-propionic acid. It is a small molecule that stabilizes deoxyhemoglobin, thus reducing hemoglobin's binding affinity for oxygen. Several phase I studies demonstrated the safety

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and tolerability of RSR13 administration in human subjects (10–12).

Spatially localized phosphorus-31 nuclear magnetic resonance (^{31}P NMR) spectroscopy is the only noninvasive method to directly quantify in vivo the cardiac high energy phosphates, adenosine triphosphate (ATP), and creatine phosphate (PCr). During myocardial ischemia, PCr falls rapidly, and if ischemia is sufficiently prolonged, the ATP content decreases (13–18). Image-guided, spatially-localized ^{31}P NMR can detect 30%–50% reductions in the cardiac PCr/ATP ratio during continuous isometric handgrip exercise in subjects with coronary artery disease (CAD) (14, 15, 19). The reductions in PCr/ATP are thought to be a specific marker for the metabolic evidence of ischemia in these patients because these changes are not observed in normal individuals or in patients with CAD following successful revascularization (14).

In a canine model of low-flow ischemia, we showed that the administration of RSR13 (100 mg/kg) increased mean p50 by 18 mmHg, attenuated the decline in cardiac high energy phosphates and intracellular pH detected by ^{31}P NMR, and improved regional myocardial function (20). These effects occurred without an increase in regional blood flow or a reduction in hemodynamic determinants of myocardial oxygen demand and suggest that a substantial right-shift of the hemoglobin-oxygen dissociation curve can limit the metabolic and functional consequences of myocardial ischemia. Based on these observations, we sought to 1) determine the extent to which a single parenteral 100 mg/kg infusion of RSR13 alters the p50 in subjects with CAD; and to 2) test whether a right-shift in the hemoglobin-oxygen dissociation curve induced by RSR13 would improve exercise treadmill times and/or attenuate the handgrip-exercise induced decline in myocardial PCr/ATP in subjects with CAD.

2. Subjects and methods

This was a randomized, double-blind, placebo-controlled, crossover study of a single dose of 100 mg/kg RSR13 or placebo (0.45% normal saline) in subjects with CAD. This dose of RSR13 was selected because pilot studies established its safety in oncologic patients (10–12) and 100 mg/kg was the dose that was used in our previous canine study (20).

2.1. *Study population*

All subjects gave informed consent for the study, which was approved by the Johns Hopkins Joint Committee on Clinical Investigation. Subjects were eligible for this study if they had chronic symptomatic atherosclerotic CAD, with a $\geq$ 70% stenosis of either the left anterior descending (LAD) coronary artery system or the left main coronary artery that was not amenable to further revascularization. Subjects were excluded if they had a myocardial infarction within 30 days prior to screening, history of congestive heart failure, uncontrolled hypertension, hypoxemia, significant lung, renal or liver disease, anemia, or a contraindication to magnetic resonance imaging.

2.2. *Protocol description*

The study protocol is illustrated in Fig. 1. Twelve subjects met the initial eligibility criteria and underwent a baseline ^{31}P NMR spectroscopy isometric handgrip exercise study, followed by a baseline exercise treadmill test. Five subjects did not complete the study. One of those subjects developed pruritis and a rash during study drug infusion, which resulted in discontinuation of the infusion and withdrawal from the study. The remaining seven subjects (6 men and 1 woman) completed the entire study protocol. These subjects returned several days after the baseline study for administration of the study medication and a repeat of the ^{31}P NMR spectroscopy and the exercise treadmill test. They were randomized in a double-blinded fashion to receive an infusion of either RSR13 (100 mg/kg) or matching placebo (0.45% normal saline) administered through a peripheral vein over 90 minutes. At a dose volume of 10 ml/kg, the average total volume of infusate was 909 ± 122 ml (range 686 to 1100 ml). The infusion of the study medication was completed approximately 30 minutes before the ^{31}P NMR spectroscopy isometric handgrip exercise and within two hours of the exercise treadmill stress test. The half-life of RSR13 is approximately 6 hours. Of note, 4 L/min of oxygen were continuously administered through a nasal cannula on all days that involved stress testing. Subjects returned the next day for a safety evaluation. Several days after the initial administration of the study medication, subjects were crossed-over to an infusion of the second study medication, either placebo or RSR13, and then underwent a repeat handgrip exercise study with ^{31}P NMR spectroscopy, and an exercise treadmill stress test. They returned for safety evaluations the next day and again a week later.

2.3. *^{31}P NMR spectroscopy*

Subjects were placed in a clinical 1.5 T *SIGNA* magnetic resonance scanner (General Electric, Milwaukee, WI) equipped with broad-band spectroscopy (14). The subjects lay prone but rotated slightly to the left with the thorax anterior to the heart centered over a set of commercial coplanar ^{31}P NMR surface coils. Conventional proton (^{1}H) NMR images were acquired with the body coil to confirm the location of the coil relative to the heart and to identify the anatomy corresponding to each localized ^{31}P spectrum. In each exam, three sets of ^{31}P NMR spectra were collected: one before the isometric handgrip exercise, a second beginning one to two minutes after initiation of handgrip exercise and continuing until the end of exercise, and a third during

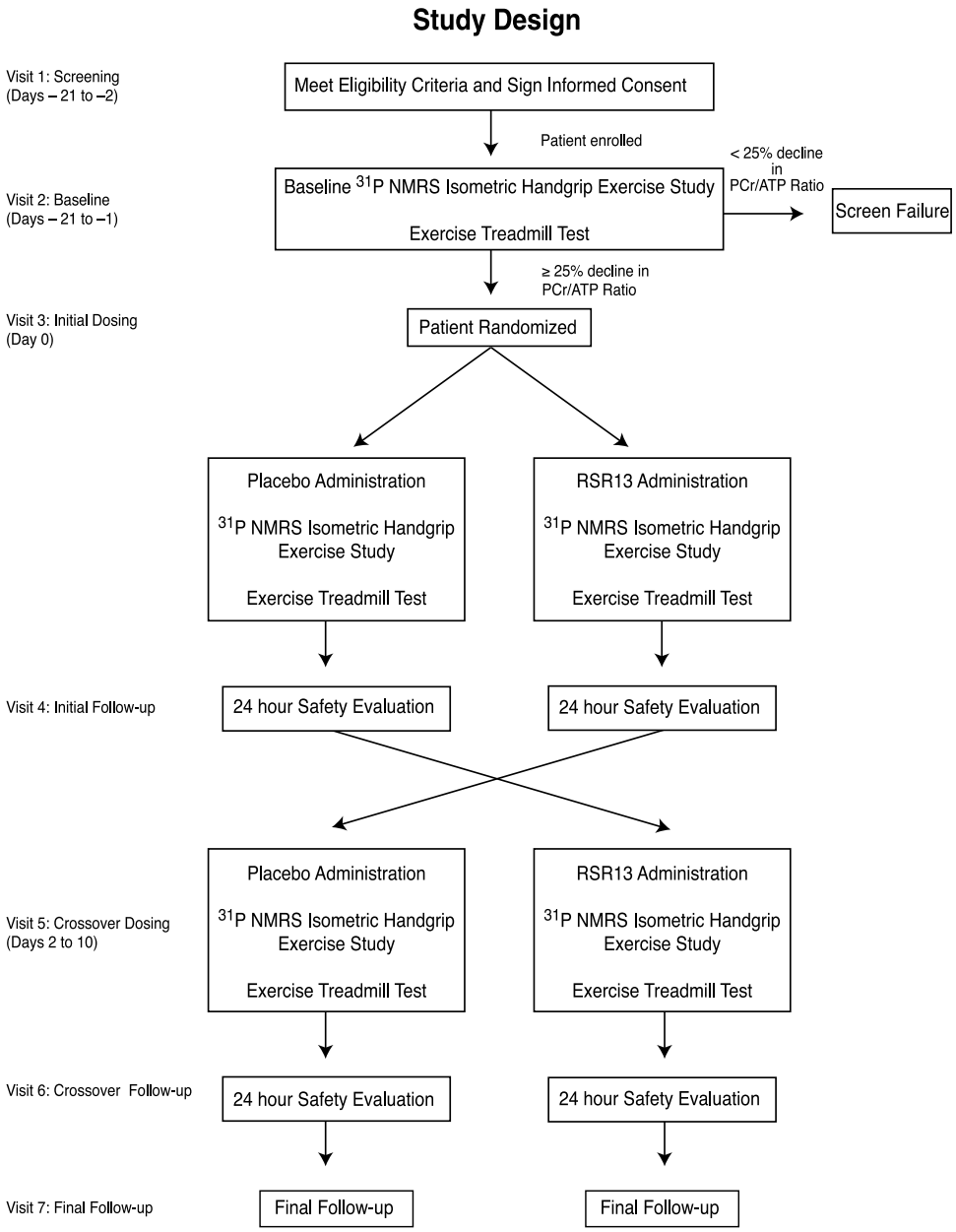

Figure 1. Experimental study design. The left column shows the number of visits and the approximate time intervals between each visit, referenced to the day of the first study medication infusion (day 0). NMRS denotes nuclear magnetic resonance spectroscopy, PCr phosphocreatine, and ATP adenosine triphosphate.

recovery beginning one minute after completion of the exercise. The isometric hand-grip exercise was performed on a custom modified dynamometer as previously described (14), with each subject maintaining 30% of their maximal strength. ^{31}P NMR spectra were obtained from slices parallel to the surface coils using cardiac-gated (once per cycle) 32 step 1-D phase-encoding gradient sequence with a 1 millisecond acquisition delay and 14–20 acquisitions per step (five to ten minute acquisition time). All investigators were blinded to the study medication, including the one (PAB) who processed the free induction decays and performed computerized peak fitting for each peak to determine the integrated peak areas of PCr and ATP. The results were corrected for partial saturation effects and blood ATP using previously described methods (14, 21, 22). Myocardial pH was not determined as inorganic phosphate and can not be routinely identified in all subjects at 1.5 T.

2.4. *Exercise treadmill tests*

Standard symptom-limited exercise treadmill tests were performed (23) using a modified Bruce protocol. The exercise

stress tests were stopped upon the development of moderate angina, defined as the severity of pain that would ordinarily cause the subject to stop his or her exercise during normal daily activity.

2.5. *Pharmacodynamic and pharmacokinetic evaluations*

Shifts in the hemoglobin oxygen dissociation curve (p50) were determined by multipoint tonometry on venous blood samples collected prior to the initiation, at the end of, and 30 minutes after completion of the infusion. Plasma and RBC RSR13 concentrations were assessed by high-performance liquid chromatography.

2.6. *Statistical analysis*

Differences in the means among the baseline, placebo, and RSR13 groups were compared with analysis of variance. The changes in PCr/ATP within a group were analyzed with paired *t*-tests. The absolute and relative changes in the PCr/ATP ratio before and during exercise, and the absolute and relative changes in the PCr/ATP ratio during and after exercise, were compared among the 3 groups (baseline, placebo and RSR13) with a two-way repeated measures analysis of variance. Data are presented as mean ± standard deviation. Statistical significance was inferred for $p < 0.05$.

The initial study size estimates were based on the previously observed effect of RSR13 in canine models of ischemia and on the variability in ^{31}P NMR spectroscopy measures in patients with CAD. The study was terminated by the sponsor after an interim analysis on seven of the planned ten subjects indicated no significant difference in the primary endpoint, the stress cardiac PCr/ATP, between the two groups. The smaller than anticipated observed differences between placebo and RSR13 during exercise require a much larger population size to obtain the usual level of significance.

Table 1. Clinical characteristics of the 7 subjects who completed the protocol

	Range	Mean (SD)
Age (years)	50–78	59.4 (10.2)
Height (cm)	162–185	173 (8.8)
Weight (kg)	67–113	92 (13.7)
BMI (kg/m^2)	21–39	31.1 (6.7)
EF (%)	35–52	43.9 (6.6)
Treadmill time (sec)	223–701	480 (181)
Cholesterol (mg/dL)	112–211	168 (37)
Triglycerides (mg/dL)	72–404	170 (110)
Fasting glucose (mg/dL)	67–189	112 (40)
BUN (mg/dL)	9–23	14.7 (4.6)
Creatinine (mg/dL)	0.7–1.3	0.9 (0.2)
WBC (× 1000)	4.6–9.1	6.5 (1.6)
Hematocrit (%)	36.4–44.7	40.4 (3.3)

Table 2. Clinical history of the 7 subjects who completed the protocol

	Number of subjects
Hypertension	6
Diabetes mellitus	2
Smoking (past or present)	4
Triple vessel CAD	6
Myocardial infarction	4
Angioplasty	3
Coronary artery bypass	3
Medications	
ASA	7
Beta-blockers	5
ACE-inhibitors	6
Statins	4

Based on these findings, p50 data and emerging animal data suggesting a larger shift in p50 may be required for anti-ischemic benefit, and the current inability to achieve greater p50 shifts in patients with cardiac disease, enrollment was terminated.

3. Results

The clinical characteristics and clinical history of the study cohort (6 men and 1 woman) that completed the entire protocol are summarized in Tables 1 and 2. Activity was limited by symptoms of exertional angina (or anginal equivalent) in six subjects and by claudication in one.

3.1. *Pharmacodynamic and pharmacokinetic effects of RSR13*

Mean p50 was 26.7 ± 2.7 mmHg before infusion and was not significantly altered by placebo (27.4 ± 2.1 mmHg at

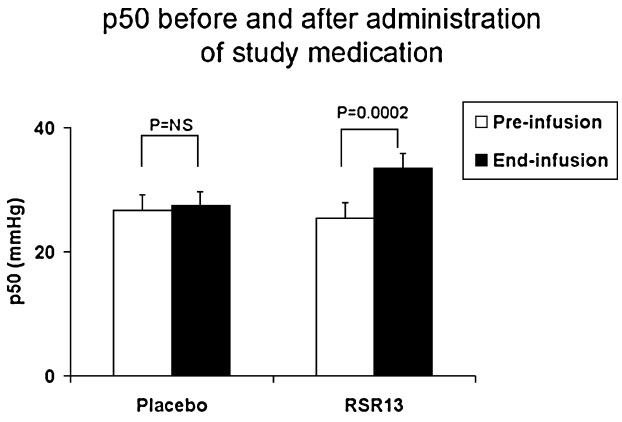

Figure 2. The mean (± SD) p50 (the oxygen tension at which hemoglobin is 50% saturated) before administration of the study medication (placebo or RSR13) and at the end of the infusion.

end-infusion, p = NS, Fig. 2). In contrast, RSR13 significantly increased mean p50 by 8.1 ± 2.7, from 25.4 ± 2.3 mmHg before the infusion to 33.5 ± 2.5 mmHg at the end of the infusion (relative increase of 32%, p = 0.0002). Mean p50 was 31.6 ± 2.4 mmHg approximately 41 ± 16 minutes (range 24–58 minutes) after completion of the RSR13 infusion, representing a net increase of 6.2 ± 2.5 from baseline (relative increase of 24%, p = 0.01); mean p50 was 30.0 ± 3.3 mmHg fifteen minutes after completion of the treadmill test, representing a net increase of 4.9 ± 3.3 from baseline (relative increase of 19%, p = 0.05). The concentrations of RSR13 were 441.4 ± 79.4 μg/mL in red blood cells, and 544 ± 58.6 μg/mL in the plasma in the 5 subjects in whom they were measured at the completion of the infusion. These were respectively 338.3 ± 39.9 μg/mL and 402.7 ± 58.2 μg/mL in the 3 subjects in whom they were measured 15 minutes after the completion of the treadmill stress test.

3.2. *^{31}P NMR spectroscopy*

Each subject underwent three NMR spectroscopy stress tests: A baseline study, a study during infusion of placebo, and a study during infusion of RSR13 (100 mg/kg over 90 minutes). One subject developed moderate angina during the handgrip test on the baseline study that required termination of this stress test. This subject did not develop angina on the subsequent 2 tests, which involved the administration of study medications. Two other subjects developed angina during the handgrip test, one during administration of placebo, and one during all three tests.

There were no significant differences in mean heart rate at peak isometric handgrip exercise among the 3 studies (73.7 ± 11.4 bpm for baseline studies, 75.6 ± 8.6 bpm for placebo infusion studies, and 77.3 ± 11.9 bpm for RSR13 infusion studies, p = NS). Similarly, neither the mean systolic blood pressure at peak exercise (180 ± 38 mmHg, 183 ± 28 mmHg, and 180 ± 25 mmHg, p = NS) nor the mean double product (maximal heart rate × maximal systolic blood pressure) (13,214 ± 2,041, 13,956 ± 3,164, 13,934 ± 2,930, p = NS) differed among the 3 sessions.

In each session the myocardial PCr/ATP ratio was assessed at rest, during isometric handgrip exercise, and during recovery (Fig. 3). In the baseline screening studies of the initial 12 subjects, the mean myocardial PCr/ATP was

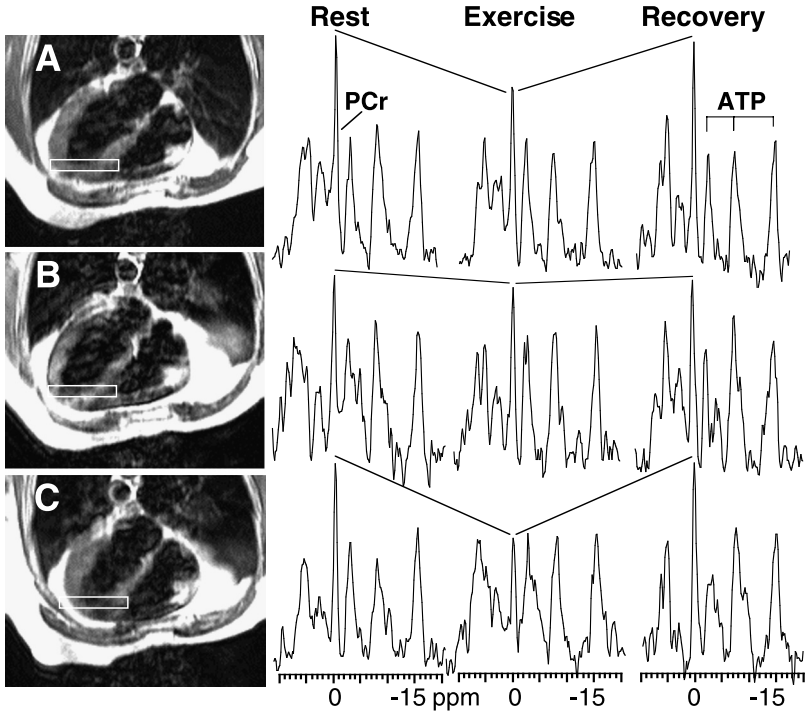

Figure 3. Representative cardiac-gated spin-echo images (left), and anterior myocardial ^{31}P spectra acquired at rest, during hand-grip exercise and during recovery (right) from a patient with myocardial ischemia meeting the study inclusion criteria. Data at top (A) were acquired at initial screening. The PCr/ATP at rest was 1.2, and during exercise was 0.9, representing a 26% decrease, with PCr dropping due to ischemia (connecting lines). Data at center (B) were acquired during the RSR13 infusion studies. The PCr/ATP at rest and exercise was unchanged at 1.1, exhibiting no decrease (lines). Data at bottom (C) were acquired during the placebo infusion studies. The PCr/ATP at rest was 1.15, decreasing to 0.7 during exercise, a 40% decrease. The approximate locations of the spectra are indicated by boxes in the images on the left. Magnetic resonance spectra data are extracted from a 32-spectra data set at 1cm resolution acquired in about 12 min at rest, and about 6 min during exercise and recovery with reduced averaging. PCr/ATP ratios were blood and partial-saturation corrected, and averaged from two adjacent myocardial slices. The signal-to-noise ratio (SNR) in rest and recovery spectra ranges from 7–17 (mean 14 ± 4) for PCr, and 5–11 (mean 9 ± 2) for the β–ATP resonances.

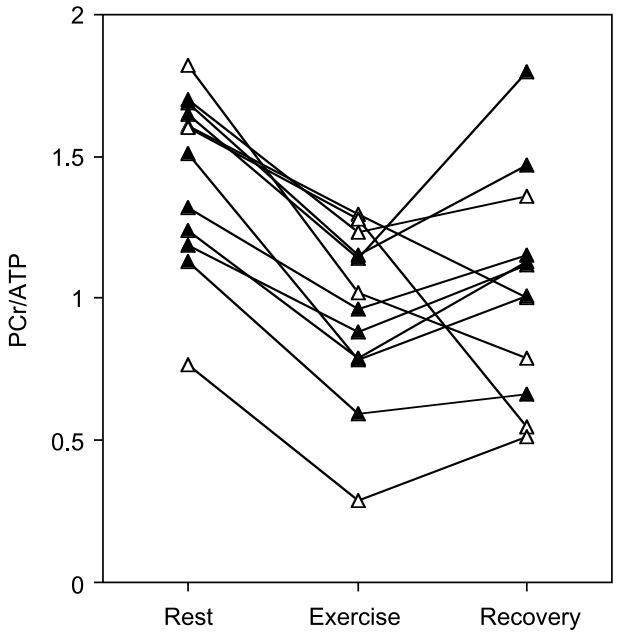

Figure 4. Baseline myocardial PCr/ATP response for the 12 subjects meeting initial eligibility criteria. Mean myocardial PCr/ATP decreased significantly during exercise ($p < 10^{-4}$), and recovered slightly during recovery. The filled triangles represent the 7 subjects who completed the entire protocol.

1.39 ± 0.32, which decreased significantly to 0.94 ± 0.30 during handgrip exercise ($p < 10^{-4}$) and recovered slightly to 1.04 ± 0.39 (p = NS vs. stress) on the scan taken during the immediate post-exercise period (Fig. 4).

In the seven subjects who qualified for serial testing and completed the entire protocol, the cardiac PCr/ATP ratio at rest did not differ among the three sessions (1.39 ± 0.23, 1.29 ± 0.16, 1.28 ± 0.18, p = NS). Similarly, the PCr/ATP ratio did not significantly differ during handgrip exercise among the three sessions. The mean ratio before exercise was 1.39 ± 0.23, and it decreased significantly during handgrip exercise to 0.95 ± 0.21 (p = 0.0001) (Fig. 5A). This represented a 31% reduction from that at rest (p = 0.0001) (Fig. 5B). In the placebo infusion studies, the mean ratio before exercise was 1.29 ± 0.16, and it fell during handgrip exercise to 0.98 ± 0.37 (p = 0.06) (Fig. 5A). This represented a 24% reduction from that at rest (p = 0.06) (Fig. 5B). In the RSR13-infusion studies, the mean ratio before exercise was 1.28 ± 0.18, which fell only to 1.02 ± 0.24 during handgrip exercise (p = 0.12) (Fig. 5A). This represented a 20% decline from that at rest (p = 0.13 = NS) and suggests an attenuation in the decline of the PCr/ATP ratio with RSR13 (Fig. 5B). The change in PCr/ATP from the resting state to peak exercise was not significantly different among the 3 groups (p = NS by two-way analysis of variance). The possibility that a training effect may have influenced these results is unlikely as the change in PCr/ATP from the resting state to peak exercise during administration of placebo was not significantly different between subjects randomized to receive placebo at the first infusion and those randomized to receive placebo at the second infusion.

In the baseline studies, the mean PCr/ATP ratio was 0.95 ± 0.21 at peak exercise and 1.16 ± 0.34 during recovery (p = NS). Similarly, in the placebo group, the ratio was 0.98 ± 0.37 at peak exercise and 1.31 ± 0.32 during recovery (p = NS); in the RSR13 group, the ratio was 1.02 ± 0.24 at peak exercise and 1.35 ± 0.09 during recovery (p = NS). The PCr/ATP ratio did not significantly differ during recovery among the three sessions, and the change in PCr/ATP from peak exercise to recovery did not significantly differ among the 3 groups (p = NS by two-way analysis of variance).

3.3. *Treadmill stress tests*

Six subjects completed all 3 treadmill stress tests. At baseline, the mean time on the treadmill for the cohort was 8 ± 3 minutes (range 3.72 min–11.85 min), the maximal heart rate was 111 ± 17 bpm, the maximal systolic blood pressure was 195 ± 28 mmHg, and the double product was 21,794 ± 5,114 bpm × mmHg. Administration of the study medication (placebo or RSR13) did not significantly alter any of these values. The time to onset of symptoms on the treadmill was shorter in two subjects, longer in two, and unchanged in three with RSR13.

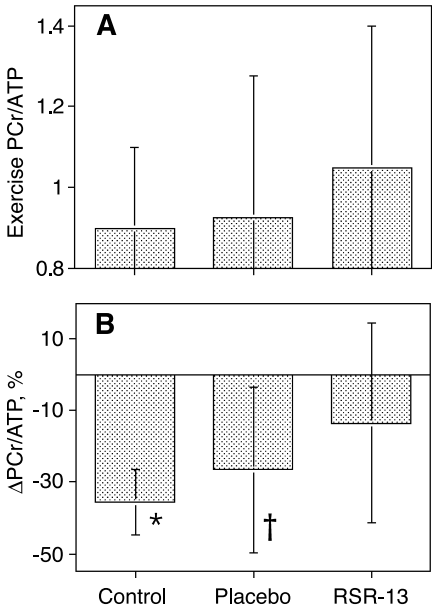

Figure 5. (A) Mean raw myocardial PCr/ATP values recorded during hand-grip exercise for the 7 subjects completing the entire protocol. Bars denotes one standard deviation. (B) Mean percentage reduction in myocardial PCr/ATP at exercise compared to rest, during control, placebo and RSR-13 infusion. Bars denote one standard deviation. * denotes p < 0.0001, † denotes p = 0.06.

3.4. *Adverse events*

Only one patient had a significant reaction to the administration of study medication (RSR13) that necessitated discontinuing the infusion. The subject developed burning, pain, and a rash at the peripheral intravenous catheter site.

Among the seven subjects who completed the study protocol, 4 subjects reported adverse events after administration of placebo and 7 subjects after administration of RSR13. The most frequent symptoms reported after the RSR13 infusion included headaches (n = 3) and discomfort at the peripheral venous infusion site (n = 3). Adverse events rated moderate in severity occurred only after administration of RSR13 and included headaches in one subject, headaches with associated nausea in another, dizziness in one participant, and an episode of transient hypotension, emesis, and transient renal dysfunction in another. The latter subject had the highest serum creatinine at baseline (1.3 mg/dL), which increased to 2.7 mg/dL the next day, decreased to 1.6 mg/dL within a week, and returned to its baseline value four weeks later.

4. Discussion

In this study, a single intravenous infusion of RSR13, a novel allosteric modifier of hemoglobin's oxygen binding affinity, increased mean p50 by 8.1 ± 2.7 mmHg, which was still elevated by 4.9 ± 3.3 mmHg after the completion of the treadmill test, but it did not significantly improve myocardial high-energy phosphates during isometric handgrip exercise or increase treadmill exercise performance in subjects with CAD. The observations that the absolute and relative exercise-induced decline in cardiac PCr/ATP attained borderline significance during placebo infusion but not during RSR13 are consistent with the premise that a right-shift in hemoglobin-oxygen affinity induced by RSR13 may modestly attenuate the metabolic consequences of myocardial ischemia.

4.1. *A right shift in hemoglobin-oxygen binding affinity*

This is the first clinical study of the effects of a significant right shift of hemoglobin-oxygen binding affinity on myocardial hypoxia secondary to CAD. Modest right shifts in hemoglobin-oxygen binding affinity ($\Delta p50 \sim 2$ mmHg) occur in patients with heart failure, myocardial infarction, and shock and may represent a beneficial adaptive response that increases oxygen delivery (1–7). Some anti-anginal medications such as nitroglycerin and propranolol also modestly right shift the hemoglobin-oxygen dissociation curve ($\sim$2 mmHg), prompting some investigators to suggest that the shift may contribute to their anti-anginal effects (24–26). Some agents, including fibrate derivatives, increase p50 by about 2–6 mmHg and reduce ischemic manifestations in animal models (8, 27). However, in each of those studies, the agents also increased coronary blood flow, reduced heart rate or blood pressure, and possibly enhanced glycolysis. Thus the myocardial anti-ischemic effects of these agents could not be attributed unambiguously to the right-shift in hemoglobin-oxygen binding affinity.

RSR13 is useful for studying the effects of a right-shift in hemoglobin-oxygen binding affinity on myocardial ischemia because RSR13 increases p50 more profoundly than earlier agents and does so without significantly altering coronary blood flow or myocardial oxygen demand. In a canine model of myocardial ischemia induced by lowering coronary flow, we previously showed that an infusion of 100 mg/kg of RSR13 over 15 minutes prior to the onset of ischemia increased the p50 by 54% (absolute increase of 17.9 ± 5.4 mmHg), and did not affect heart rate, blood pressure, or microsphere measures of myocardial blood flow (20). In that study, RSR13 did not prevent, but did significantly attenuate, the declines in myocardial PCr/ATP and intracellular pH during ischemia. Furthermore, when RSR13 was administered after the onset of ischemia, it improved the PCr/ATP ratio, prevented the progressive decline in intracellular pH, and improved regional shortening and mechanical work (20). A right shift in hemoglobin-oxygen affinity by RSR13 was also shown to provide some myocardial protection in other canine models of ischemia, including coronary occlusion (28) and hypothermic cardiopulmonary bypass (29).

4.2. *Reconciling preclinical and clinical studies of RSR13 in myocardial ischemia*

There are several factors that may contribute to the differences between our earlier experimental and the present clinical results. There are potentially important species differences such as the availability of collaterals as well as the impact of physiologic allosteric modifiers of hemoglobin (e.g. pCO_2, pH, DPG) (30, 31). In addition the types of ischemia (reduced coronary perfusion versus isometric handgrip) and magnetic resonance localization techniques differed between the two studies. However, two factors merit expanded discussion. The first is that the shift in p50 with RSR13 in all prior canine studies demonstrating myocardial protection was greater than the shift in p50 achieved here in patients. The mean increase in p50 was 17.9 mmHg in the open-chest, low coronary blood flow canine studies of myocardial high-energy phosphates (20), which is about 2.5 times that observed in this clinical study at the completion of the infusion (8 mmHg) (Fig. 6), despite the same total dose of RSR13 (100 mg/kg). The much smaller shift with the same dose in people is likely explained by the longer infusion times (90 vs. 15 minutes) necessitated by the relatively large volume of infusate and injection through a peripheral vein. A recent canine study that evaluated two doses of RSR13 demonstrated that a large right shift is needed for myocardial protection (28). Specifically, in open-chest dogs exposed to

repetitive episodes of LAD ischemia, only high dose RSR13, which increased mean p50 by 13 mmHg (39% over baseline), but not low dose of RSR13, which increased mean p50 by 8 mmHg (26% over baseline), was effective in improving the recovery of stunned myocardium (28).

A second factor is the ability to quantify metabolic changes due to ischemia in identical regions over time. Both our canine experiments (20) and this clinical trial used ^{31}P NMR spectroscopy but the experimental settings were different in ways that may have affected our ability to detect more subtle effects of a synthetic modifier of hemoglobin-oxygen binding affinity. In canine studies, a small surface coil was visually placed directly over the controlled ischemic region and high-quality spectra were obtained with a high-field magnet (4.7 T) in a single study without repositioning each animal. In our present patient study, external surface coils were used with spatial-localization techniques in a 1.5 T scanner but the regions manifesting exercise-induced ischemia were variable in size and location among subjects and impossible to identify prior to the test. In addition, the study design, requiring three

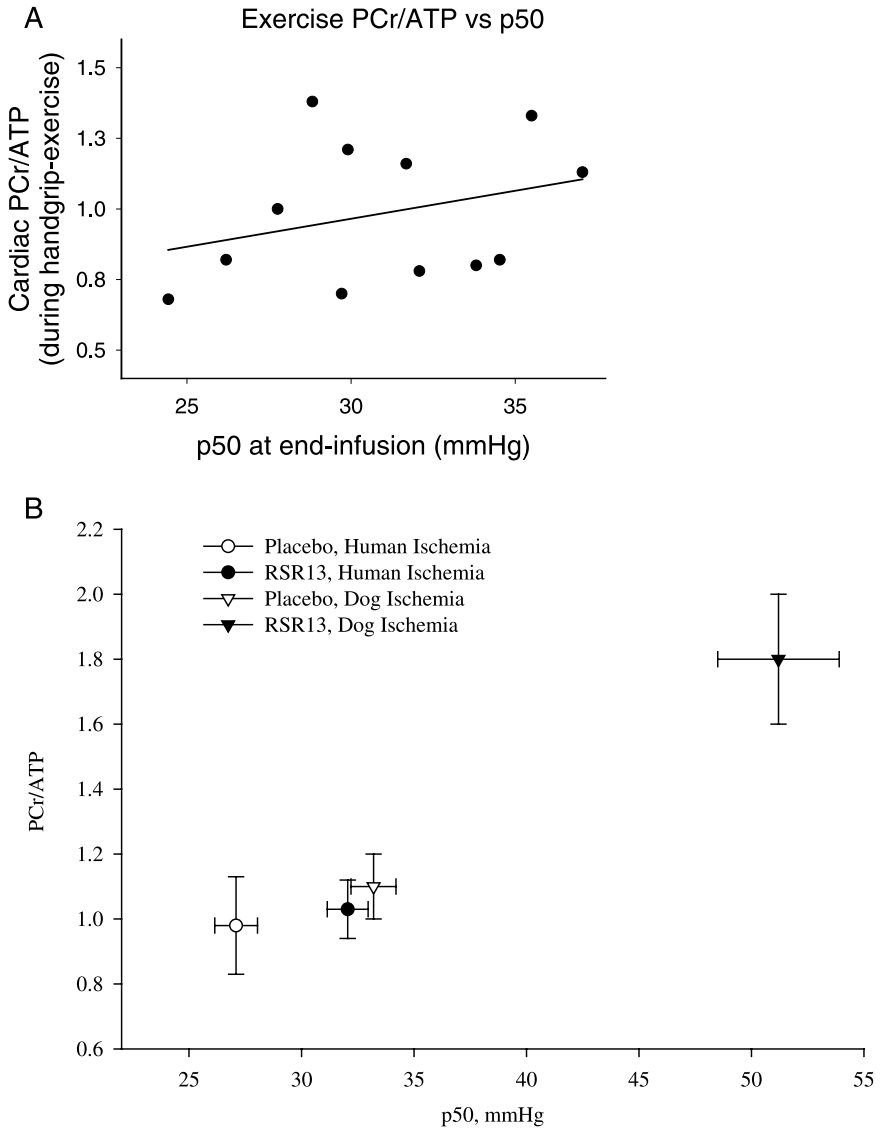

Figure 6. Relationship between cardiac PCr/ATP during ischemia and p50. (A) Myocardial PCr/ATP values recorded during isometric hand-grip exercise vs. p50 measured at end-infusion, in the placebo and RSR13 groups. The equation of the fitted linear regression line is: $y = 0.02x + 0.37$. The correlation ($r = 0.3$) does not achieve statistical significance ($p = 0.3$) likely because of the narrow range of p50 values. (B) In a low coronary blood flow model of canine coronary ischemia, the administration of RSR13 [dark triangle from Ref. (20)] resulted in a significant increase in p50 compared to dogs administered placebo (open triangles), and this was associated with a markedly higher PCr/ATP. In humans, the administration of RSR13 (dark circle from the current data) during ischemia resulted in a much more modest increase in p50 compared to those given placebo (open circle), and was associated with a more modest improvement in PCr/ATP.

separate studies in a given patient on different days (baseline, placebo, RSR13), necessitated studying the same region at three different times and attempting to induce the same degree of ischemia each time. We minimized this variability by choosing patients with large ischemic regions and by repositioning patients based on anatomical images acquired during the baseline studies.

Although we used the same RSR13 dose (100 mg/kg) shown to be effective in dogs and did observe the largest shift in p50 to date in people with CAD (8 mmHg), this did not increase p50 to the range where benefits to myocardial high-energy phosphate metabolism were seen in the ^{31}P NMR canine studies (13–19 mmHg Fig. 6). Future investigations aimed at achieving a greater increase in p50 than that achieved in this study are needed to determine whether such a strategy can attenuate the functional and metabolic consequences of ischemia. A greater right shift in hemoglobin-oxygen binding capacity could be accomplished by increasing the rate at which RSR13 is administered, as done in oncology patients with less tenuous volume status and indwelling central catheters (11, 32) or by utilizing a more potent modifier of hemoglobin-oxygen binding affinity.

4.3. *^{31}P NMR stress-testing for myocardial ischemia*

A stress-induced decline in myocardial PCr/ATP during isometric handgrip exercise is a specific marker of myocellular ischemia in patients with CAD (14, 15, 19) and resolves after successful revascularization (14). In women with chest pain but no critical coronary disease, a significant exercise-induced decline in cardiac PCr/ATP occurs in a minority of subjects (19) and predicts worse cardiovascular outcomes in those subjects (33). We believe our study is the first report to use ^{31}P NMR stress-testing to assess the efficacy of a new anti-anginal agent in humans by comparing the exercise-induced decline in PCr/ATP between RSR13 and placebo infusions. Future studies that utilize ^{31}P NMR methodology should note the variability in the magnitude of the decline among ^{31}P NMR stress tests observed here when calculating sample sizes. Also, the uncertainty in the PCr/ATP due to the ^{31}P NMR spectroscopy signal-to-noise ratio is about 13% based on the mean fractional errors for PCr and ATP given in Fig. 3. Although this means that drug-induced changes in PCr/ATP of less than about 13% may not be reliably detected in individual data sets, a drug effect comparable to this could certainly introduce sufficient bias to reveal a statistically significant effect if enough studies are performed.

5. Conclusion

A single parenteral infusion of 100 mg/kg RSR13 in patients with CAD and anterior wall ischemia increased mean p50 by 4.9–8.1 mmHg, the largest increase in p50 yet evaluated for anti-ischemic properties in people. This, however, did not significantly alter treadmill exercise times or myocardial high-energy phosphates during exercise. The exercise-induced decline in cardiac PCr/ATP was modestly attenuated by RSR13 but the stress myocardial PCr/ATP was not significantly altered. Because recent studies in animal models suggest that right-shifts of greater magnitude in p50 are required to provide myocardial protection during ischemia, future clinical work should focus on the effects of strategies that provide a greater shift in hemoglobin-oxygen binding affinity as they become available.

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