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A Novel Adenylyl Cyclase Type 5 Inhibitor That Reduces Myocardial Infarct Size Even When Administered After Coronary Artery Reperfusion

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Abstract

We developed a novel adenylyl cyclase type 5 (AC5) inhibitor, C90, that reduces myocardial infarct size even when administered after coronary reperfusion. This is key, since it is not practical to administer a drug to a patient with myocardial infarction before revascularization, and is one reason why so many prior drugs, which reduced infarct in experimental animals, failed in clinical trials. C90 is the most potent AC5 inhibitor, as exhibited by its IC50 value for AC5 inhibition, which was 5 times lower than the next most potent AC5 inhibitor. C90 reduced cAMP in response to forskolin in wild type mice by 42%, but no longer reduced cAMP in response to forskolin in mice with disruption of AC5, indicating that the mechanism of C90 was specific for AC5 inhibition. Compared with vehicle treatment, C90 reduced infarct size by 64% at a dose of 0.6mg/kg. Thus, C90 is a novel, selective and potent AC5 inhibitor that reduces infarct size, when delivered after coronary artery reperfusion, rendering it potentially clinically useful. It also reduces beta-adrenergic receptor signaling, which will provide additional benefit to patients with coronary artery disease or heart failure.

Keywords

Myocardial Ischemia; Myocardial Infarction; Adenylyl Cyclase; Coronary Occlusion; Coronary Reperfusion

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DISCLOSURE:

JZ; DL; MO; CB; SY: None

DEV; SFV: are owners of Vasade Biosciences, Inc., which developed this AC5 inhibitor.

INTRODUCTION

Myocardial infarction remains one of the leading causes of death and disability. There has been considerable interest in discovering mechanisms to reduce infarct size in experimental animals over the past half century [1]. Many pharmacological interventions, proven effective in reducing infarct size in animal models have failed, when tested clinically [2–7], including the most potent cardioprotective agent, adenosine [2]. Our hypothesis is that one reason for success in animal models and have failed in patients, since most compounds that reduce infarct size in animals are given prior to coronary artery occlusion (CAO) or at least prior to coronary artery reperfusion (CAR). One exception is Vidarabine, an antiviral compound that has properties of inhibition of adenylyl cyclase type 5 (AC5), which reduces infarct size, even when administered after CAR [8]. Unfortunately, the toxicity of that compound precludes it from ever being considered clinically to treat myocardial infarction. Accordingly, we developed a new more specific AC5 inhibitor, C90, which is less toxic and more soluble, and can be structured for future oral use. The goal of this study was to determine its efficacy in reducing infarct size in mice when delivered after CAR and to highlight its specificity for inhibiting AC5.

MATERIALS AND METHODS

All protocols concerning animal use were approved by the Institutional Animal Care and Use Committee at the Rutgers, New Jersey Medical School. All of the investigations conformed to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health.

Ischemia and Reperfusion Model

3–5 month old male C57BL/6 or mixed background SVJ129/C57BL/6 mice. As described previously [8], after pentobarbital anesthesia the left anterior descending (LAD) coronary artery was occluded for 30 min followed by CAR. After 24 hours the mice were re-anesthetized with tribromoethanol, 12.5 mg/ml, and the area at risk was measured using Alcian Blue and the infarct size was measured using triphenyl tetrazolium chloride (TTC) staining. A protocol of 60 min CAO and 4 hours CAR was used to assess infarct size in 9–11 month old Watanabe rabbits purchased from Kobe University [9].

In Vitro Biochemistry—Membranes were prepared from AC5 WT and AC5 KO hearts as described previously [10]. AC activity and cAMP were also measured as described previously [10].

Data Analysis and Statistics

The comparison between two groups was performed using the Student’s t-test. For comparison among multiple groups, we used one-way ANOVA followed by Bonferroni post-hoc analysis.

RESULTS

A Novel AC5 Inhibitor, C90, Reduced Infarct Size in Mice Even When Administered After Reperfusion

The area at risk was similar in all groups (Figure 1A). Compared with vehicle treatment C90 reduced infarct size, $p<0.05$, by 64% at a dose of 0.6mg/kg, delivered at 5 min after CAR. Even when C90 was administered at 10 min after CAR, infarct size was reduced by 50% (Figure 1B).

C90 Reduces Infarct Size in a Model of Coronary Atherosclerosis, the Watanabe Rabbit

In the Watanabe rabbit, a model of chronic atherosclerosis, C90 at a dose of 0.6 mg/kg reduced infarct size by 50% (Figures 1C and 1D), when administered at 5 min after CAR.

C90 Inhibits Forskolin-Induced Increase in cAMP Production in the Heart

Forskolin induced increases in AC activity and cAMP were reduced to a greater extent with C90, than with the earlier version of an AC5 inhibitor, PMC6, in H9c2 cells (Figure 2B). C90 reduced forskolin induced AC activity and cAMP in WT mice by 42±9%, compared with vehicle, $p<0.05$. However, in AC5 KO C90 no longer reduced cAMP in response to forskolin, indicating that the mechanism of reduction by C90 involved AC5 inhibition and not just inhibition of any AC isoform (Figure 2A).

The Novel AC5 Inhibitor, C90, has Enhanced Efficacy and Selectivity as an AC5 inhibitor

C90 exhibited enhanced AC5 inhibition efficacy and selectivity, as reflected by 5 times lower IC_{50} values (0.03 μM) for type 5 AC, than PMC6 (0.15 μM), and 37 times lower than Vidarabine (1.11 μM) (Figure 2C). C90 was at least 5 times less potent for inhibiting AC2 and AC6 isoforms.

DISCUSSION

There are 11 isoforms of AC, the key enzyme mediating beta adrenergic signaling, with type 5 being one of the major isoforms in the heart. Since increased AC activity leading to increased sympathetic tone exerts an adverse influence on the heart in the presence of heart failure and myocardial infarction [11], it follows that inhibiting AC in general, and AC5 in particular, would be salutary for heart failure and myocardial infarction therapy. Indeed, disruption of AC5 exerts a protective action in heart disease [8, 11]. However, since disrupting a gene is not generally feasible in patients, developing a small molecule AC5 inhibitor that reduces myocardial infarction and can be delivered i.v. or orally after coronary revascularization would be extremely useful clinically.

Myocardial infarction is a leading cause of death and disability, but prior drugs aimed at reducing infarction have failed [2–7], potentially because they are only effective when administered prior to CAO. This study presents the first data on the development of a novel AC5 inhibitor, termed C90, that reduces myocardial infarction even when administered after CAR, a critical feature for patients coming to the hospital with impending infarction, since the occluded artery must be opened before a drug is administered. C90, the AC5 inhibitor

used in this investigation, is a novel carbocyclic nucleoside analog possessing a metal chelating functional group. It acts through simultaneous binding at the AC5 P-site and through chelation to one of two magnesium atoms in the enzyme active site. Currently, C90 is the most potent and selective AC5 inhibitors identified. As a carbocyclic nucleoside analog, C90 is resistant to hydrolytic digestion in the gastrointestinal tract thus making this compound a viable candidate for development as an orally administered therapeutic agent. Furthermore, there is little toxicity as even a dose of 180 mg/kg was well tolerated in rodents.

In summary, this investigation describes a novel AC5 inhibitor, C90, that reduces infarct size (Figure 1), even when administered after coronary artery reperfusion, a key point of contrast with many other prior therapeutic modalities designed to be cardioprotective. AC5 inhibition has several mechanistic features that result in cardioprotection, e.g., protection against oxidative stress [11, 12], which mediates reperfusion injury, exacerbating the myocardial infarction process. In addition, its ability to reduce beta adrenergic receptor signaling, will be salutary for patients with myocardial infarction and heart failure, where reducing beta adrenergic stimulation is used for patients with heart failure.

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HIGHLIGHTS

- C90 is a novel, selective and potent AC5 inhibitor.
- C90 delivered after coronary reperfusion reduces infarction, important clinically.
- C90 reduces beta receptor signaling, an additional benefit to cardiac patients.

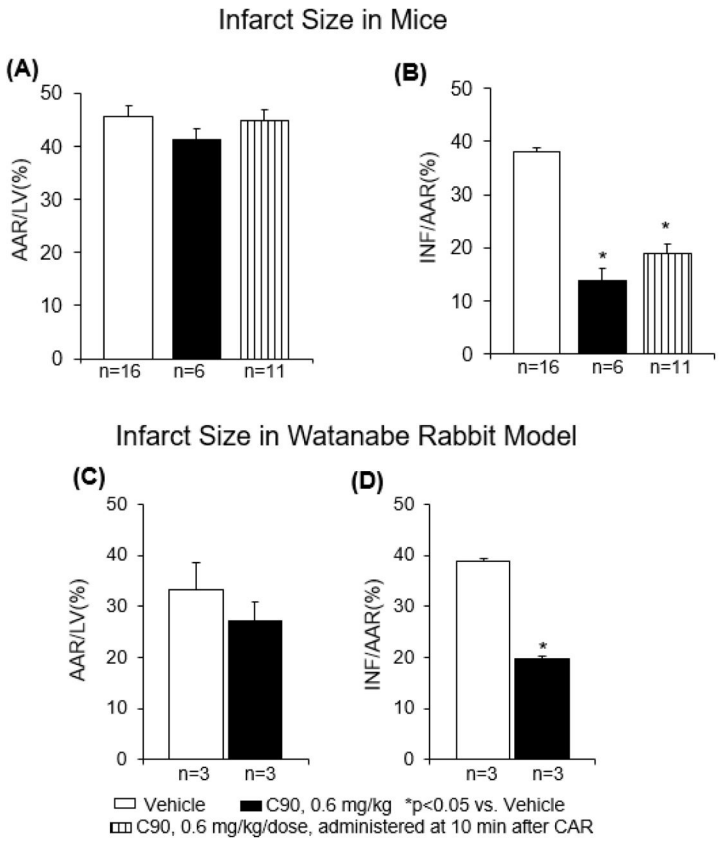
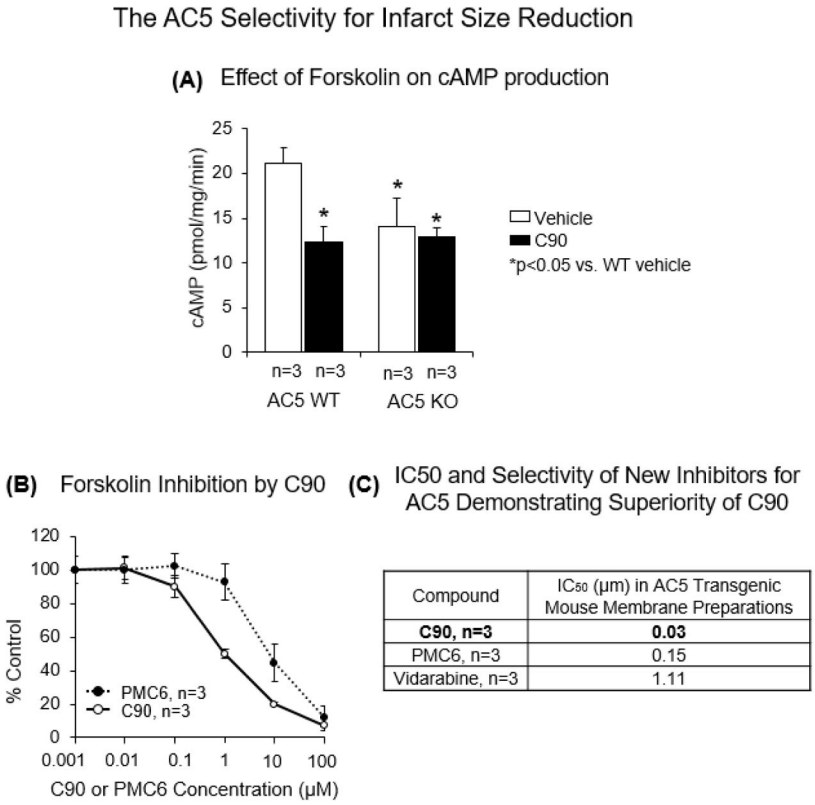


Figure 1. C90 protects against myocardial ischemia when delivered after CAR

C90 demonstrated marked cardiac protection, even when delivered after CAR. The area at risk was similar in vehicle vs treated groups (A). (B) C90 reduced infarct size, measured as a fraction of the area at risk, with a 64% reduction at a dose of 0.6 mg/kg (solid bar), when administered 5 minutes after CAR, and with a 50% reduction, when administered 10 minutes after CAR (striped bar). The numbers in each group are shown at the bottom of each bar. (D) C90 also reduced infarct size when administered after CAR in the atherosclerotic Watanabe rabbit. *p<0.05 vs. vehicle.



Compound	IC_{50} (μm) in AC5 Transgenic Mouse Membrane Preparations
C90, n=3	**0.03**
PMC6, n=3	0.15
Vidarabine, n=3	1.11

Figure 2.

(A) C90 inhibited forskolin induced cAMP in WT, but not in AC5 KO, indicating the AC5 specificity for C90 and that the mechanism of reduction of cAMP by C90 involved AC5 inhibition and not inhibition of any other AC isoform. (B) C90 inhibition of forskolin induced cAMP was greater than by a traditional AC5 inhibitor (PMC6). (C) Using membrane preparations from the hearts of AC5 transgenic mice C90 induced more potent AC5 inhibition, compared with other compounds with AC5 inhibitory properties, as shown by the lowest IC50 (μM) values for AC5 inhibition with C90, shown in bold. *p<0.05 vs. WT vehicle.