Journal of Cardiovascular Pharmacology and Therapeutics
2016, Vol. 21(6) 563-569

DOI: 10.1177/1074248416640118
cpt.sagepub.com

CMX-2043 Efficacy in a Rat Model of Cardiac Ischemia–Reperfusion Injury

Alexander Baguisi, MS[1], Ralph A. Casale, MS[1], Steven A. Kates, PhD[1], Alan S. Lader, PhD[1], Kenneth Stewart, BS[1], and Reinier Beeuwkes III, PhD[1]

Abstract

α-Lipoic acid (LA) has been shown to offer protection against ischemia–reperfusion injury (IRI) in multiple organ systems. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine (CMX-2043), a novel analogue of LA, was studied as part of a preclinical development program intended to identify safe and efficacious drug candidates for prevention or reduction in myocardial IRI. This study was designed to evaluate the efficacy of CMX-2043 in an animal model of myocardial IRI and to establish effective dosing conditions. CMX-2043 or placebo was administered at different doses, routes, and times in male Sprague-Dawley rats subjected to 30-minute left coronary artery ligation. Fluorescent microsphere injection defined the area at risk (AR). Animals were euthanized 24 hours after reperfusion, and the hearts were excised, sectioned, and stained with triphenyltetrazolium. Cytoprotective effectiveness was determined by comparing the unstained myocardial infarction zone (MI) to the ischemic AR. The reduction in the MI–AR ratio was used as the primary measure of drug efficacy relative to placebo injections. Treatment with CMX-2043 reduced myocardial IRI as measured by the MI–AR ratio and the incidence of arrhythmia. The compound was effective when administered by injection, both before and during the ischemic injury and at reperfusion. The most efficacious dose was that administered 15 minutes prior to the ischemic event and resulted in a 36% ($P < .001$) reduction in MI–AR ratio compared to vehicle control.

Keywords

ischemia–reperfusion injury, cytoprotection, arrhythmia, CMX-2043, lipoic acid

Introduction

Myocardial ischemia–reperfusion injury (IRI) occurs when the blood flow to the heart is interrupted and subsequently reestablished. It is now well accepted that both the ischemic event and the reperfusion, that is, the restoration of the flow of oxygen and nutrients, contribute to cell damage and the overall injury.[1] Ischemia–reperfusion injury is a complicating factor in interventional procedures such as percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG). In the United States alone, there are estimated to be 950,000 PCI and nearly 400,000 cardiac revascularization procedures each year.[2] Although many pharmacological and mechanical interventions have been evaluated to mitigate IRI, none has reached clinical acceptance. There remains a need for a safe, efficacious, and clinically practical approach to this problem.[3]

The experiments described in this report are part of a drug discovery program focused on the synthesis and evaluation of proprietary molecules based on R-α-lipoic acid (LA). α-Lipoic acid is an endogenous molecule present in both eukaryotic and prokaryotic cells including human tissue.[4] In addition to its antioxidant properties, LA has been shown to upregulate the cytoprotective kinase protein kinase B (Akt) through phosphatidylinositide 3-kinase (PI3K).[5-7] Protection has been demonstrated in wide variety of animal and organ model systems.[8-17] The single stereo isomer R-α-LA has an attractive safety profile and is available in Germany in oral and parenteral formulations for treatment of diabetic neuropathy.[18,19]

N-[(R)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine (CMX-2043) is R-α-LA condensed with the amino terminus of the dipeptide glutamyl-alanine. We report here preclinical studies to establish optimal dose, dose timing, route of administration, and duration of protective action on infarct size in a modified version of a widely used rat model of myocardial IRI.[20-25] Additionally, we address the effects of CMX-2043 on the incidence of ventricular arrhythmias, cardiac arrest, and death associated with ischemic injury in this model.

Methods

Rat Model of IRI Injury

These studies employed a standard rat model of myocardial injury as reported previously.[20-21,26] Because pharmacologic

[1] Ischemix, LLC, Maynard, MA, USA

Manuscript submitted: December 28, 2015; **accepted:** January 28, 2016.

Corresponding Author:
Reinier Beeuwkes III, Ischemix, LLC, 63 Great Road, Maynard, MA 01754, USA.
Email: rbeeuwkes@ischemix.com

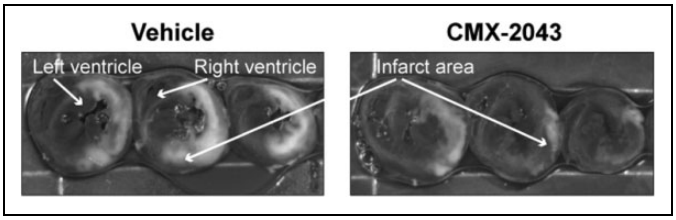

Figure 1. Examples of treated and untreated rat myocardium. A bright field view of 2-mm thick cardiac tissue slices stained with a 1% 2,3,5-triphenyl-2*H*-tetrazolium chloride (TTC) solution as obtained from vehicle and *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine (CMX-2043)-treated rats. Hearts exposed to 30 minutes of ischemia followed by reperfusion were excised after 24 hours and stained to delineate the live (stained) and infarcted (white) tissue. Horizontal white lines are 1 cm apart.

efficacy in IRI is very much dependent on the specifics of the technique, details of this approach are repeated here. These studies were approved by the Ischemix Institutional Animal Care and Use Committee and conformed to the *Guide for the Care and Use of Laboratory Animals*.

Male Sprague Dawley rats (Charles River) weighing between 300 and 325 g were anesthetized with 3% to 4% isoflurane through an endotracheal tube. During surgery animals were ventilated with 1.5% to 2.0% isoflurane. Body temperature was monitored and maintained.

The heart was exposed by anterior thoracotomy, and the left coronary artery was ligated approximately 4 mm from the aorta. To define the ischemic area at risk (AR), fluorescent microspheres were injected into the left ventricle 15 minutes after the ligation. The ligature was removed after 30 minutes of ischemia. After chest closure, the temperature was controlled until recovery of consciousness.

Anesthesia was reinduced with intraperitoneal ketamine after 24 hours and the chest reopened. To sacrifice the heart in diastole, 15% potassium chloride solution was injected into the left ventricle. The heart was removed and washed to remove residual blood. Sagittal slices of 2 mm were stained by immersion in 1% 2,3,5-triphenyl-2*H*-tetrazolium chloride (TTC) in isotonic saline.[27] Fluorescence microscopy was used to define the AR by absence of microsphere, and the infarct was defined by absence of TTC staining (Figure 1).

Formulation of CMX-2043

For injection, CMX-2043 was formulated as an isotonic solution at pH 6.8 to 7.4 and filtered to remove any particles larger than 0.2 μm.[26] Stock solutions were confirmed for concentration and purity by reverse-phase high pressure liquid chromatography (HPLC). Normal saline was used for dilution of stocks to achieve a constant 1 mL/kg injection volume for each dose concentration. Doses were aliquoted into single vials per injection, and aliquots were grouped and letter coded to blind the studies, excepting experiments with varied injection timing. Vehicle control solutions consisted of filtered and aliquoted 0.9% saline.

Route of Administration

Intracardiac injection. Intracardiac doses were administered into the left ventricular cavity following pericardotomy as a bolus using a 1 mL tuberculin syringe and 30-gauge needle.

Intravenous injection. Intravenous doses were administered as a bolus into the left femoral vein following a cut down using a 1 mL tuberculin syringe and 30-gauge needle. The incision was closed using surgical wound clips.

Oral. Food and water were withheld from the rats (N = 8 per study arm) for 24 hours prior to the passive administration of CMX-2043 at doses of 50, 100, and 200 mg/kg. CMX-2043 was offered in 5 mL of normal saline solution containing 2% vanilla extract as flavoring. Test animals drank this dose within 30 minutes. Myocardial IRI was induced 30 to 60 minutes after oral dosing. Ad libitum food and water were provided after the animals were returned to the colony.

Pharmacokinetics of CMX-2043

Toxicokinetic analysis in Sprague Dawley rats was performed by Bioanalytical Systems, (West Lafayette, Indiana). CMX-2043 was administered at 30 mg/kg (6/sex) by single intravenous dose. The pharmacokinetic (PK) analysis was performed using WinNonlin version 5.1 (Pharsight Corp; Cary, North Carolina). Area under the plasma concentration curve extrapolated to infinity, terminal phase half-life ($t_{½}$), and total body clearance were determined.

Observance of Arrhythmia and Mortality

A high incidence of nonresolving arrhythmia was achieved in the model by moving the ligation site closer to the base of the aorta (approximately 2-4 mm from the base). Intravenous vehicle control or CMX-2043 treatment was administered 15 minutes prior to the ischemic episode. During the 30 minutes of ischemia, the incidence of ventricular arrhythmia and the duration of arrhythmic episodes were observed.

Statistical Analysis

Data are presented as mean and standard error. One-way analysis of variance and post hoc analysis using Bonferroni were used for infarct size. The chi-square test was used for testing intergroup differences in incidence of ischemia-induced arrhythmias and mortality rate (actual vs expected).

Results

Model (MI–AR Ratio of Vehicle Control)

The animal model of IRI proved consistent and practical for the evaluation of the protective effects of CMX-2043. Preliminary vehicle control experiments using saline injections routinely

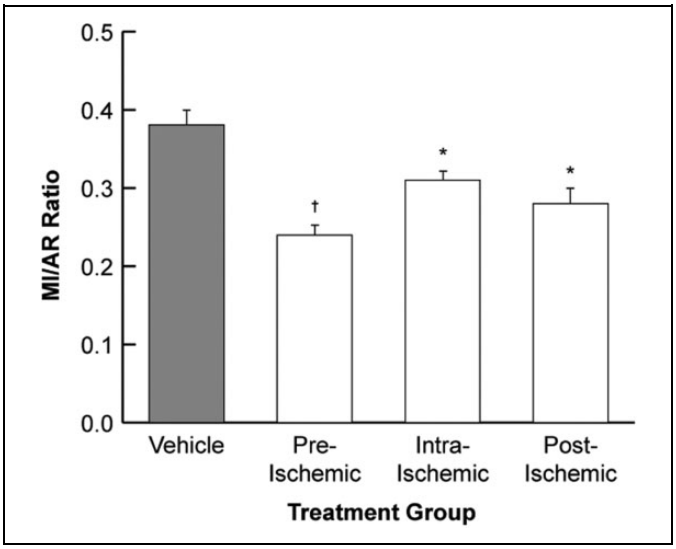

Figure 2. Therapeutic Timing. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine (CMX-2043) was most effective in reducing ischemia–reperfusion injury when administered 15 minutes prior to the ischemic event, reducing the ratio of myocardial infarct to area at risk (MI–AR) by 36% compared to vehicle. The drug was also effective when provided during the occlusion and at 1 minute after establishing reperfusion, reducing MI-AR by 18% and 26% compared to control, respectively. Values are presented as means ± standard error of the mean (SEM) from N = 12-15/group. *$P < .01$, †$P < .001$, compared to vehicle; 1-way analysis of variance and post hoc analysis using Bonferroni.

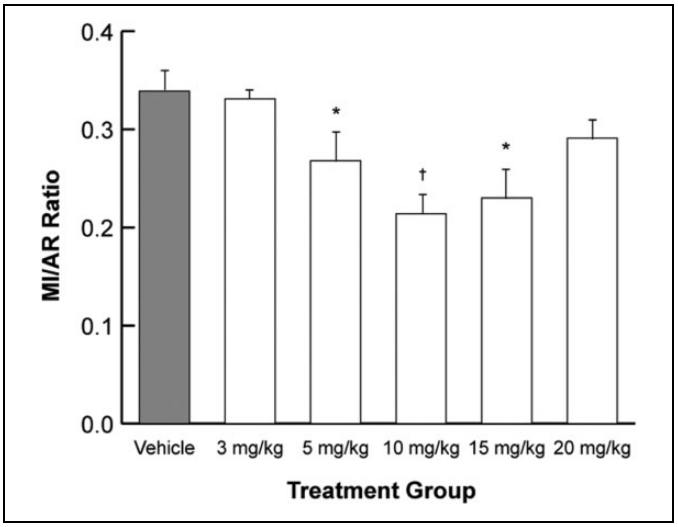

Figure 3. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine (CMX-2043) dose response. CMX-2043 was administered via the femoral vein 15 minutes prior to coronary artery ligation. CMX-2043 show a U-shaped dose–response curve with an efficacious dose range between 5 and 20 mg/kg. At 10 mg/kg the myocardial infarct to area at risk (MI–AR) ratio was reduced by 37% compared to vehicle. Values are presented as means ± standard error of the mean (SEM) from N = 10-12/group. *$P < .01$, †$P < .001$, compared to vehicle; 1-way analysis of variance and post hoc analysis using Bonferroni.

showed a mean AR of 156 mm^2 (± 36 mm^2) and myocardial infarct (MI–AR) ratios between 0.3 and 0.4 (data not shown).

Initial Dose Timing

Timing of CMX-2043 administration was initially investigated with intraventricular dosing. Drug was administered 15 minutes prior to the arterial ligation, 15 minutes into the 30-minute occlusion, or 1 minute after the removal of the ligation and establishment of reperfusion. CMX-2043 was provided to groups of at least 12 animals per study arm. CMX-2043 at 1 mg/kg reduced myocardial injury whether administered before, during, or after the ischemic insult when compared to a corresponding 0.9% saline solution control provided 15 minutes prior to the arterial ligation. CMX-2043 administration prior to the ischemic event was most effective, reducing the MI–AR by 36% compared to saline control ($P < .001$; Figure 2).

Intracardiac and Intravenous Dosing

CMX-2043 was initially evaluated by intracardiac administration. As the program advanced, intravenous administration was used. When dosed at 15 minutes prior to the occlusion injury, the efficacy observed with a 10 mg/kg intravenous dose was not significantly different from that observed with a 1 mg/kg dose delivered intracardiac. The observed average MI–AR ratios for the vehicle control, intracardiac, and intravenous dosing were 0.34, 0.24 ($P < .01$ vs control), and 0.21 ($P < .001$ vs control), respectively.

Intravenous Dose Response

Dose–response studies of CMX-2043 administered intravenously 15 minutes prior to the arterial ligation were conducted; each included a control 0.9% saline injection at 1 mL/kg and at least 9 animals per group. Efficacious doses for CMX-2043 were found in the range of 5 to 20 mg/kg and with a U-shaped dose–response curve. None of the doses evaluated had a detrimental effect. The 10 mg/kg dose provided the greatest reduction in the MI–AR ratio compared to the vehicle (37%, $P < .001$ vs control; Figure 3).

Oral Dosing

Oral dosing of CMX-2043 was evaluated in groups of at least 10 animals. CMX-2043 was passively administered to rats dehydrated for 24 hours at doses of 50, 100, and 200 mg/kg. Ischemic injury was induced 30 to 60 minutes after oral dosing. CMX-2043 was increasingly effective with dose up to 200 mg/kg. At 200 mg/kg, CMX-2043 reduced MI–AR ratio by 38% ($P < .01$ vs control; Figure 4).

Duration of Effect

A 10 mg/kg intravenous injection of CMX-2043 was provided at 15 minutes, 3, 6, or 24 hours prior to the occlusion and compared to a 0.9% saline control. The optimal time of

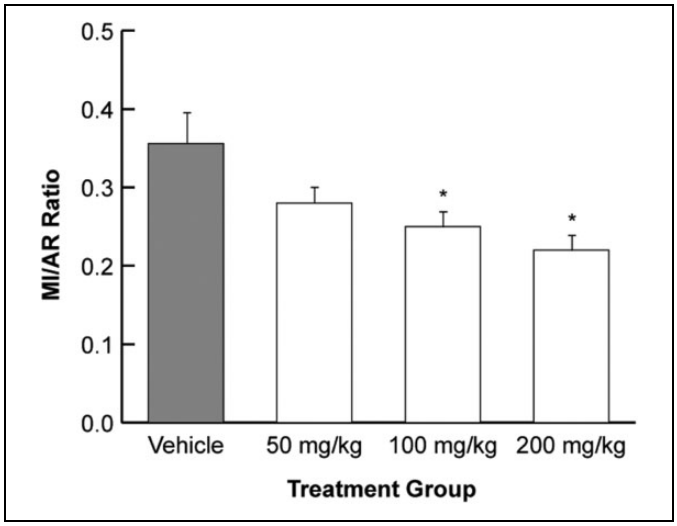

Figure 4. Oral efficacy. *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine (CMX-2043) was administered orally 30 minutes prior to coronary arterial ligation. CMX-2043 showed increasing efficacy with dose from 50 up to 200 mg/kg. Myocardial infarct to area at risk (MI–AR) ratio was reduced by 38% with the 200 mg/kg dose and was also protective at 100 mg/kg reducing MI-AR ratio by 30% compared to vehicle. Values are presented as means ± standard error of the mean (SEM) from N = 10-13/group. **P* < .01, compared to vehicle; 1-way analysis of variance and post hoc analysis using Bonferroni.

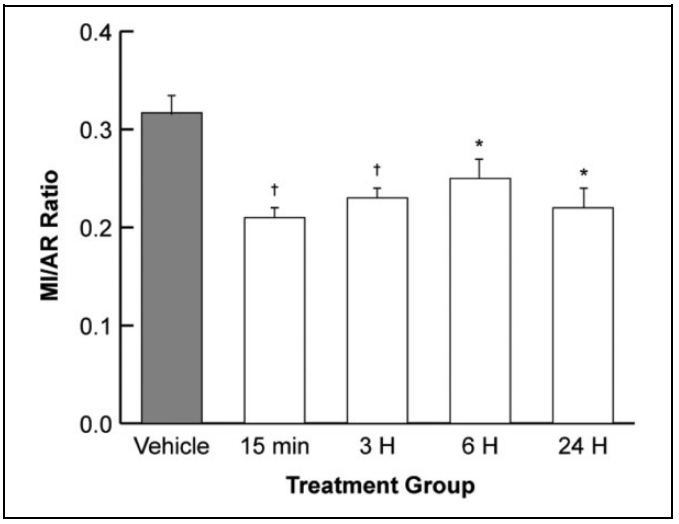

Figure 5. Duration of therapeutic effect. The duration of effectiveness of a single intravenous bolus injection of *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine (CMX-2043) at 10 mg/kg was evaluated by delaying the induction of ischemia–reperfusion injury (IRI) by up to 24 hours. Efficacy when compared to vehicle injection at 15 minutes prior to IRI was observed for even up to 24 hours post administration. Optimal efficacy was observed with the dosing at 15 minutes prior to the ischemic event. Values are presented as means ± standard error of the mean (SEM) from N = 7-8/group. **P* < .01, †*P* < .001, compared to vehicle; 1-way analysis of variance and post hoc analysis using Bonferroni.

administration was within 3 hours prior to the occlusion. A diminished but still significant protective effect was observed at 24 hours (Figure 5).

Pharmacokinetics

The PK analysis was performed at an intravenous dose of 30 mg/kg. Drug concentration decayed in a biexponential manner, and the $t_{½}$ was 0.16 hour (9.6 minutes) in males and 0.14 hour (8.4 minutes) for females, indicating >98% clearance of the unbound parent molecule from the blood stream in about 1 hour.

Incidence of Arrhythmia and Mortality

CMX-2043 was compared to vehicle control in animals subjected to a greater region of myocardial ischemia. Table 1 presents the incidence of ventricular arrhythmias (tachycardia, bradycardia, and fibrillation). Arrhythmia was observed in 42% (10 of 24) of the vehicle control-treated animals. Mortality in this group was 38% (9 of 24). The population of the control group was increased to obtain at least 14 surviving animals. Treatment with CMX-2043 at 10 mg/kg intravenously reduced the incidence of ventricular arrhythmias, $\chi^2(1, N = 40) = 3.89$, $P < .05$, and resulting death to 13%, $\chi^2(1, N = 40) = 3.07$, $P = .08$.

Table 1. Incidence of Arrhythmia and Mortality.[a]

Treatment	Saline vehicle	CMX-2043
Arrhythmia (%)	10/24 (42%)	2/16 (13%)
Mortality (%)	9/24 (38%)	2/16 (13%)

Abbreviations: CMX-2043, *N*-[(R)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine; IRI, ischemia–reperfusion injury.
[a]The incidence of arrhythmia was increased over the standard IRI model by ligating closer to the base of the aorta to increase the area of ischemic injury. CMX-2043 (10 mg/kg intravenous) administered 15 minutes prior to the ischemic episode reduced the incidence of ventricular arrhythmias, χ^2 (1, N = 40) = 3.89, $P < .05$, and death, χ^2 (1, N = 40) = 3.07, $P = .08$, compared to vehicle treatment.

Discussion

The studies reported here support development of CMX-2043 as a protective agent against ischemia and IRI. CMX-2043 is the condensation product of the dipeptide glutamyl-alanine with R-α-LA. α-Lipoic acid is a naturally occurring fatty acid that has demonstrated efficacy in prior studies of IRI and has been shown to trigger multiple cytoprotective mechanisms and antiapoptotic pathways.[28] The dithiolane ring (cyclic disulfide) structure provides antioxidant properties in both its readily reversible reduced form and its oxidized form, and the fatty acid structure enables solubility in both aqueous and lipophilic environments.[29,30] Studies have shown a protective effect of LA in an isolated perfused heart model[8] and reduced mortality in rats given a bilateral carotid artery occlusion.[9] α-Lipoic acid alone, and in combination with apocynin, enhanced protection in a rat model of ischemic stroke.[10-12] α-Lipoic acid has demonstrated protective utility in spinal IRI where it appeared

to aid in the maintenance of endogenous antioxidant homeostasis.[13] Testicular[14] and ocular[15] models of IRI have also demonstrated treatment benefit. Renal studies have shown protective effects of both LA[16] and LA derivatives.[31]

We have evaluated a number of LA condensation products with natural and unnatural amino acids and dipeptides.[26] Of this group, CMX-2043 demonstrated an attractive pharmacologic and toxicologic profile[32] together with multimodal cytoprotective properties. Mechanistically, CMX-2043 acts through the insulin receptor/PI3K pathway to increase the phosphorylation of Akt.[33] The PI3K/Akt pathway attenuates multiple processes that activate the apoptotic program, inhibit necrosis, modulate metabolic function, promote energy generation, and regulate inflammatory responses.[34] In in vitro studies, the drug has been observed to activate or potentiate endogenous cellular survival pathways and downregulate inflammatory responses. CMX-2043 also exhibits greater potency and antioxidant activity than its parent compound in vitro[33] and in vivo.[26]

We evaluated CMX-2043 in a modified version of a widely used rat model of myocardial IRI.[20,25] The model was selected for its analogy to the IRI observed in patients following coronary occlusion and cardiac surgery procedures, such as PCI and CABG. Variations in this model have been used for the evaluation of many protective approaches, including mechanical remote conditioning,[22] mitochondrial targeted peptides,[23] *N*-acetylcysteine,[24] angiotensin-converting enzyme inhibitors,[20] and statin[25] Bolli et al in 2004, while applauding the use of live animal models rather than in vitro approaches to the discovery of therapeutic interventions, lamented that comparisons between published animal studies were nearly impossible.[35] Differences such as ligation time and placement, the use of potentially protective anesthetics, and recovery time all influence the extent of injury and the therapeutic challenge.

With these concerns in mind, we first established a model that was consistent, sufficiently injurious, and reflective of clinical experience. Ligations near the origin of the coronary artery resulted in large areas of ischemia, about 40% to 50% of the left ventricle. Higher ligations compromised survival. Lower ligations resulted in smaller infarcts and better appearing drug efficacy. In these small infarcts, small variations in optical measurements had greater effect on calculated ratios and hence data consistency. To compensate for small differences in occlusion placement, we normalized the dead tissue (myocardial infarction zone) to the ischemic area. The AR was measured with the postocclusion injection of fluorescent microspheres.[36] The isoflurane anesthetic has itself been shown to have cytoprotective properties.[37] We confirmed this in our model, accepted it as clinically relevant, and so were careful to control our experimental procedures with vehicle injections. Where practical, such as in dose–response studies, experimental arms were blinded to the operator conducting the surgical procedure and to the independent evaluator measuring and calculating the MI–AR ratio.

The initial studies to evaluate the efficacy of CMX-2043 used direct intracardiac injection to reduce the effect of first pass dilution. Early studies also used a molecule based on racemic (R/S)-α-LA. Efficacy studies with the enantiomeric pure molecules showed CMX-2043 superior to both *N*-[(S)-1,2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine and the mixed stereoisomer.[26] Optimal stereochemistry of the dipeptide moiety was not as rigorously evaluated.

CMX-2043 for injection was readily formulated as a pH-balanced isotonic saline solution at up to 30 mg/mL. The concentration of the formulated drug product was adjusted so as to inject the drug and corresponding vehicle control consistently at a volume of 1 mL/kg. The tendency of LA to form polymer via intermolecular disulfide linkages has been described in the literature[38] but is infrequently discussed in usage studies. Adherence to the established formulation procedure was critical to avoid overconcentration, which could lead to polymer formation. The addition of the chemical entity to a premixed basic diluent provided pure test product. Each stock formulation was evaluated by HPLC to ensure concentration and purity.

Our interest in evaluating timing of the therapeutic intervention was based on recognition of anticipated clinical use. While it is ideal to administer a protective agent prior to an event, patients often present themselves after the onset of ischemia. We found that CMX-2043 was protective when administered prior to, during, and after ischemia. It was optimally protective against IRI when administered just prior to the ischemic insult.

Once effective dose and timing were identified using the intracoronary injection route, we established the comparable intravenous dose. The 10-fold higher dose for intravenous versus intracardiac suggests that first pass dilution and distribution of the molecule to other tissues diminish drug availability to the myocardium. The rat intravenous dose extrapolates to a clinically practical corresponding human dose. In a subsequent clinical study, we have demonstrated a protective effect of CMX-2043 on biomarkers of IRI in patients undergoing elective PCI.

Dose range studies in the rat IRI model showed the protective effect was absent below 1 mg/kg and was diminished above 20 mg/kg. This effect of increased dose did not appear to be related to toxicity, as toxicology studies identified a no observed adverse event level in rats of 30 mg/kg.[32] The U-shaped dose–response curve is consistent with prior observations on LA. In studies of cell proliferation, LA was stimulatory at 1 μmol/L but antiproliferative at 100 μmol/L.[39] This type of response is not an unusual outcome with cellular reactions to stress response.[40] It is also consistent with earlier observations with antioxidant compounds.[41] The breadth of the effective dose range is anticipated to provide an ample clinical margin.

We also explored the protective effect of the drug when orally administered. The addition of a drop of vanilla extract to an oral dosing solution insured that the animals readily ingested the drug product. Oral dosing provided cardioprotection in an optimal dose range of 100 to 200 mg/kg, a 10- to 20-fold increase over the optimal intravenous dose. Evaluation of the oral bioavailability of CMX-2043 (data not shown) was consistent with this high dose requirement.

To determine whether reducing MI–AR ratio reflected improved electrical stability, we induced a high incidence of arrhythmia by increasing the AR in the model. Most unresolved arrhythmias occurred in the control animals. These started with tachycardia 3–5 minutes into the ischemic episode, followed by fibrillation a few minutes later, ultimately ending in death. Administration of CMX-2043 prior to the ischemic insult markedly reduced the incidence of arrhythmia and death.

The PK study showed rapid plasma clearance of the drug. A 30 mg/kg dose, nearest to but in excess of the optimal intravenous dose of 10 mg/kg, exhibited a half-life in rats of about 10 minutes. It was therefore of great interest that the protective effect of the intravenous dose persisted for as long as 24 hours. This indicates activity in a compartment, presumably intracellular, not reflected in the plasma concentration. This characteristic is consistent with strategies such as mechanical remote conditioning, which has been observed to exhibit both early and late stage organ protection.[42] The results support further investigation of CMX-2043 in clinical settings.

Authors' Note

No other persons besides the authors have made substantial contributions to this manuscript.

Author Contribution

Baguisi, A contributed to conception and design and acquisition and analysis; critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy. Casale, R contributed to conception and analysis; drafted the manuscript; gave final approval; and agreed to be accountable for all aspects of work ensuring integrity and accuracy. Kates, S contributed to interpretation, drafted the manuscript, gave final approval, and agreed to be accountable for all aspects of work ensuring integrity and accuracy. Lader, A contributed to conception and interpretation, critically revised the manuscript, gave final approval, and agreed to be accountable for all aspects of work ensuring integrity and accuracy. Stewart, K contributed to acquisition, gave final approval, and agreed to be accountable for all aspects of work ensuring integrity and accuracy. Beeuekws, R contributed to conception and design and interpretation, critically revised the manuscript, gave final approval, and agreed to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article. All authors are current or past employees or officers of Ischemix LLC which provided funding for this study.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was funded by the manufacturer of CMX-2043, Ischemix LLC.

References

1. Hausenloy DJ, Yellon DM. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. *J Clin Invest*. 2013; 123(1):92-100.
2. Mozaffarian D, Benjamin BJ, Go AS, et al. Heart disease and stroke statistics-2015 update: a report from the American Heart Association. *Circulation*. 2015;131(4):e29-e322.
3. Sharma V, Bell RM, Yellon DM. Targeting reperfusion injury in acute myocardial infarction: a review of reperfusion injury pharmacotherapy. *Expert Opin Pharmacother*. 2012;13(8):1153-1175.
4. Packer L. Alpha-lipoic acid: a metabolic antioxidant which regulates NF-kappa B signal transduction and protects against oxidative injury. *Drug Metab Rev*. 1998;30(2):245-275.
5. Zhang L, Xing GQ, Barker JL, et al. Alpha-lipoic acid protects rat cortical neurons against cell death induced by amyloid and hydrogen peroxide through the Akt signalling pathway. *Neurosci Lett*. 2001;312(3):125-128.
6. Muller C, Dunschede F, Koch E, Vollmar AM, Kiemer AK. Alpha-lipoic acid preconditioning reduces ischemia-reperfusion injury of the rat liver via the PI3-kinase/Akt pathway. *Am J Physiol Gastrointest Liver Physiol*. 2003;285(4):G769-G778.
7. Deng C, Sun Z, Tong G, et al. Alpha-lipoic acid reduces infarct size and preserves cardiac function in rat myocardial ischemia/reperfusion injury through activation of PI3K/Akt/Nrf2 pathway. *PloS One*. 2013;8(3):e58371.
8. Schonheit K, Gille L, Nohl H. Effect of alpha-lipoic acid and dihydrolipoic acid on ischemia/reperfusion injury of the heart and heart mitochondria. *Biochim Biophys Acta*. 1995;1271(2-3): 335-342.
9. Panigrahi M, Sadguna Y, Shivakumar BR, et al. Alpha-lipoic acid protects against reperfusion injury following cerebral ischemia in rats. *Brain Res*. 1996;717(1-2):184-188.
10. Connell BJ, Saleh M, Khan BV, Saleh TM. Lipoic acid protects against reperfusion injury in the early stages of cerebral ischemia. *Brain Res*. 2011;1375:128-136.
11. Connell BJ, Saleh MC, Khan BV, Rajagopal D, Saleh TM. Upei-100, a conjugate of lipoic acid and apocynin, mediates neuroprotection in a rat model of ischemia/reperfusion. *Am J Physiol Regul Integr Comp Physiol*. 2012;302(7):R886-R895.
12. Richard MJ, Connell BJ, Khan BV, Saleh TM. Cellular mechanisms by which lipoic acid confers protection during the early stages of cerebral ischemia: a possible role for calcium. *Neurosci Res*. 2011;69(4):299-307.
13. Shaafi S, Afrooz MR, Hajipour B, Dadadshi A, Hosseinian MM, Khodadadi A. Anti-oxidative effect of lipoic acid in spinal cord ischemia/reperfusion. *Med Princ Pract*. 2011;20(1):19-22.
14. Ozbal S, Ergur BU, Erbil G, Tekmen I, Bagriyanik A, Cavdar Z. The effects of alpha-lipoic acid against testicular ischemia-reperfusion injury in rats. *ScientificWorldJournal*. 2012;2012:489248.
15. Chidlow G, Schmidt KG, Wood JP, Melena J, Osborne NN. Alpha-lipoic acid protects the retina against ischemia-reperfusion. *Neuropharmacology*. 2002;43(6):1015-1025.
16. Sehirli O, Sener E, Cetinel S, Yuksel M, Gedik N, Sener G. Alpha-lipoic acid protects against renal ischaemia-reperfusion injury in rats. *Clin Exp Pharmacol Physiol*. 2008;35(3):249-255.

17. Wolz P, Krieglstein J. Neuroprotective effects of alpha-lipoic acid and its enantiomers demonstrated in rodent models of focal cerebral ischemia. *Neuropharmacology*. 1996;35(3):369-375.
18. Ziegler D, Nowak H, Kempler P, Vargha P, Low PA. Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a meta-analysis. *Diabet Med*. 2004;21(2): 114-121.
19. Ziegler D, Ametov A, Barinov A, et al. Oral treatment with alpha-lipoic acid improves symptomatic diabetic polyneuropathy: the SYDNEY 2 trial. *Diabetes Care*. 2006;29(11):2365-2370.
20. Kobara M, Tatsumi T, Kambayashi D, et al. Effects of ACE inhibition on myocardial apoptosis in an ischemia-reperfusion rat heart model. *J Cardiovasc Pharmacol*. 2003;41(6):880-889.
21. Matsui T, Tao J, del Monte F, et al. Akt activation preserves cardiac function and prevents injury after transient cardiac ischemia in vivo. *Circulation*. 2001;104(3):330-335.
22. Wei M, Xin P, Li S, et al. Repeated remote ischemic postconditioning protects against adverse left ventricular remodeling and improves survival in a rat model of myocardial infarction. *Circ Res*. 2011;108:1220-1225.
23. Cho J, Won K, Wu D, et al. Potent mitochondria-targeted peptides reduce myocardial infarction in rats. *Coron Artery Dis*. 2007; 18(3):215-220.
24. Calvillo L, Masson S, Salio M, et al. In vivo cardioprotection by N-acetylcysteine and isosorbide 5-mononitrate in a rat model of ischemia-reperfusion. *Cardiovasc Drugs Ther*. 2003;17(3): 199-208.
25. Wayman NS, Ellis BL, Thiemermann C. Simvastatin reduces infarct size in a model of acute myocardial ischemia and reperfusion in the rat. *Med Sci Monit*. 2003;9(5):BR155-BR159.
26. Kates SA, Casale RA, Baguisi A, Beeuwkes III R. Lipoic acid analogs with enhanced pharmacological activity. *Bioorg Med Chem*. 2014;22(1):505-512.
27. Fishbein MC, Meerbaum S, Rit J, et al. Early phase acute myocardial infarct size quantification: validation of the triphenyl tetrazolium chloride tissue enzyme staining technique. *Am Heart J*. 1981;101(5):593-600.
28. Shay KP, Moreau RF, Smith EJ, Smith AR, Hagen TM. Alpha-lipoic acid as a dietary supplement: molecular mechanisms and therapeutic potential. *Biochim Biophys Acta*. 2009;1790(10): 1149-1160.
29. Packer L, Witt EH, Tritschler HJ. Alpha-lipoic acid as a biological antioxidant. *Free Radic Biol Med*. 1995;19(2):227-250.
30. Ghibu S, Richard C, Vergely C, Zeller M, Cottin Y, Rochette L. Antioxidant properties of an endogenous thiol: alpha-lipoic acid, useful in the prevention of cardiovascular diseases. *J Cardiovasc Pharmacol*. 2009;54(5):391-398.
31. Koga H, Hagiwara S, Kusaka J, et al. New alpha-lipoic acid derivative, DHL-His-Zn, ameliorates renal ischemia-reperfusion injury in rats. *J Surg Res*. 2012;174(2):352-358.
32. Kates SA, Lader AS, Casale R, Beeuwkes III R. Pre-clinical and clinical safety studies of CMX-2043: a cytoprotective lipoic acid analogue for ischaemia-reperfusion injury. *Basic Clin Pharmacol Toxicol*. 2014;115(5):456-464.
33. Lader AS, Baguisi A, Casale R, Kates SA, Beeuwkes III R. CMX-2043 mechanisms of action in vitro. *J Cardiovasc Pharmacol*. 2016;68(3):241-247.
34. Zhang WJ, Wei H, Hagen T, Frei B. Alpha-lipoic acid attenuates LPS-induced inflammatory responses by activating the phosphoinositide 3-kinase/Akt signaling pathway. *Proc Natl Acad Sci U S A*. 2007;104(10):4077-4082.
35. Bolli R, Becker L, Gross G, Mentzer R Jr, Balshaw D, Lathrop DA. Myocardial protection at a crossroads: the need for translation into clinical therapy. *Circ Res*. 2004;95(2):125-134.
36. Hale SL, Vivaldi MT, Kloner RA. Fluorescent microspheres: a new tool for visualization of ischemic myocardium in rats. *Am J Physiol*. 1986;251(4 pt 2):H863-H868.
37. Newberg LA, Michenfelder JD. Cerebral protection by isoflurane during hypoxemia or ischemia. *Anesthesiology*. 1983;59(1):29-35.
38. Thomas RC, Reed LJ. Disulfide polymers of dl-α-lipoic acid. *J Am Chem Soc*. 1956;78(23):6148-6149.
39. Bilska A, Wlodek L. Lipoic acid - the drug of the future? *Pharmacol Rep*. 2005;57(5):570-577.
40. Calabrese V, Cornelius C, Dinkova-Kostova AT, et al. Cellular stress responses, hormetic phytochemicals and vitagenes in aging and longevity. *Biochim Biophys Acta*. 2012;1822(5):753-783.
41. Forman HJ, Davies KJ, Ursini F. How do nutritional antioxidants really work: Nucleophilic tone and para-hormesis versus free radical scavenging in vivo. *Free Radic Biol Med*. 2014;66:24-35.
42. Guo Y, Wu WJ, Qiu Y, Tang XL, Yang Z, Bolli R. Demonstration of an early and a late phase of ischemic preconditioning in mice. *Am J Physiol*. 1998;275(4 pt 2):H1375-H1387.