CMX-2043 Mechanisms of Action In Vitro

Alan S. Lader, PhD, Alexander Baguisi, MS, Ralph Casale, MS, Steven A. Kates, PhD, and Reinier Beeuwkes III, PhD

Abstract: α-Lipoic acid has been shown to provide cytoprotection in some tissues through antioxidant and antiapoptotic mechanisms. We have enhanced these properties by synthetic modification, resulting in a new chemical entity, CMX-2043, with proven efficacy in an animal model of cardiac ischemia-reperfusion injury. The present studies compare cytoprotective cellular pathways of R-α-lipoic acid and CMX-2043. Biochemical and cellular assays were used to compare antioxidant potency, tyrosine kinase activation, and protein kinase B (Akt) phosphorylation. CMX-2043 was more effective than lipoic acid in antioxidant effect, activation of insulin receptor kinase, soluble tyrosine kinase, and Akt phosphorylation. Activation of insulin-like growth factor 1 receptor was similar for both. CMX-2043 stimulation of Akt phosphorylation was abolished by the phosphatidylinositide 3-kinase inhibitor LY294002. Consistent with Akt activation, CMX-2043 reduced carbachol-induced calcium overload. The S-stereoisomer of CMX-2043 was less active in the biochemical assays than the R-isomer. These results are consistent with cytoprotection through activation of Akt and antioxidant action. CMX-2043 may thus provide a pharmacological approach to cytoprotection consistent with established anti-apoptotic mechanisms.

Key Words: ischemia-reperfusion injury, cytoprotection, Akt activation, CMX-2043, lipoic acid

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INTRODUCTION

Coronary revascularization procedures have transformed the clinical approach to the treatment of patients with advanced coronary artery disease. However, these procedures are associated with the risk of injury due to operative interruption of blood flow and microembolization by dislodged plaque. The term "ischemia-reperfusion injury" (IRI) reflects the understanding that reestablishment of normal blood flow may also result in damage through accumulation of reactive oxygen species and triggering of energy-requiring apoptotic mechanisms. Its occurrence is not limited to the heart but is recognized as clinically significant in both the kidney and liver. Protective strategies have been put forward to prevent or diminish cardiac IRI, including administration of antioxidants,[1–4] and insulin treatment with and without glucose[5] without a clear demonstration of benefit. These and other pharmacological approaches have been ably reviewed by Hausenloy and Yellon.[6]

We have synthesized and evaluated novel R-α-lipoic acid (LA) derivatives to identify a compound that could potentially be clinically useful. LA is as a natural cofactor found in mammalian tissue.[7] Its administration is known to protect cells from oxidative damage[8–10] and is reported to offer protection against IRI in the liver,[11–14] kidney,[15–18] heart,[19–23] and brain.[24] In addition to its antioxidant action, exogenous LA activates the intracellular domain of the insulin receptor kinase (IRK).[25,26] By this and other potential pathways, LA activates protein kinase B (Akt) and thus could inhibit many downstream apoptotic processes.[11,26,27]

CMX-2043 (α-*N*-[(R)-1, 2-dithiolane-3-pentanoyl]-L-glutamyl-L-alanine) is a novel compound composed of R-α-LA covalently linked to the dipeptide L-glutamyl-L-alanine (EA). This molecule was selected for further study based on demonstration of efficacy in a rat model of cardiac IRI.[28,29] The study reported here sought to define the cellular and molecular pathways responsible for this efficacy and to compare the actions of CMX-2043 with those of the parent molecule.

METHODS

Oxygen Radical Absorbance Capacity Assay

Peroxyl radicals are produced by cells during reperfusion and can be detected by fluorescein oxidation-induced fluorescence that decays over time. The rate of decay is diminished in the presence of an oxygen radical scavenger. The change in the rate of decay between control and test samples is used to measure the peroxyl radical scavenging potency of a test compound.[30,31] These studies were done to examine whether or not the addition of the peptide had an effect on the antioxidant capacity of the dithiolane part of the LA molecule.

Each compound tested was diluted to a concentration ranging from 250 mmol/L to 15 mmol/L in 10 mmol/L phosphate buffer (pH = 7.4) containing 10 nmol/L fluorescein. The buffer and compound were incubated at 37°C for 10 minutes. Fluorescein fluorescence was measured using a plate reader (Molecular Devices Flexstation II, Ex = 485, Em = 520). Baseline fluorescence measurements were recorded for 15 minutes before the addition of the antioxidant trigger, 2,2′-azobis-2-methyl-propanimidamide hydrochloride. Fluorescence decay was recorded for 90 minutes. Decay

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Reprints: Reinier Beeuwkes III, PhD, Ischemix, LLC, 63 Great Road, Maynard, MA 01754 (e-mail: rbeeuwkes@ischemix.com).

without compound was subtracted from the decay with compound. The slope of the decay versus concentration difference is reported as the absorbance capacity.

Tyrosine Kinase Activation Assay

Many pathways for cell survival are activated through tyrosine kinases. We studied purified intracellular domains of several human tyrosine kinases for enhancement of activity by CMX-2043.[32] These experiments were performed in collaboration with Caliper Life Sciences (now part of PerkinElmer), Hanover, MD.

Activity of human IRK, insulin-like growth factor 1 receptor kinase (IGF1R) and soluble tyrosine kinase (Src) were measured by mobility shift assay using a Caliper LabChip 3000 and a 12-sipper LabChip to detect both phosphorylated and nonphosphorylated substrate. This assay used a microfluidic chip to measure the conversion of fluorescent peptide substrate to a phosphorylated product.[32] The reaction mixture from a microtiter plate well was transferred through a capillary sipper onto the chip and the nonphosphorylated substrate and phosphorylated product were separated by electrophoresis and detected via laser-induced fluorescence. The signature of the fluorescence signal over time was used to assess kinase activity. The catalytic subunits of these tyrosine kinases had endogenous levels of activity in the presence of adenosine triphosphate (ATP) and substrate, thus the results are presented as percent of control.

IRK, IGF1R, and Src tyrosine kinases have baseline levels of activity in the presence of ATP and substrate. Enzyme, substrate, and ATP concentrations were optimized for each assay. For the IRK assay, the final concentrations of the enzyme, peptide, and ATP were 40 nmol/L, 1.5 μmol/L, and 810 μmol/L, respectively. For the IGF1R1 kinase assay, the final concentrations of the enzyme, peptide, and ATP were 20 nmol/L, 1.5 μmol/L, and 1220 μmol/L, respectively. For the Src assay, the final concentrations of the enzyme, peptide, and ATP were 2.5 nmol/L, 1.5 μmol/L, and 17 μmol/L, respectively.

Cytoblot Assay for Akt Activation

The ability of CMX-2043 and LA to increase phosphorylated Akt was evaluated using an in-cell western blot or cytoblot assay.[33–35] These experiments were performed in collaboration with the University of Wisconsin, Small Molecule Screening Facility, Madison, WI. Human non–small-cell lung cancer cells (A549; ATCC, Manassas, VA) were used for this assay because they could be manipulated to increase or decrease the level of phosphorylated Akt.

A549 cells were plated at 70% confluence in microtiter plates. After overnight incubation to allow cell attachment, the medium was changed to 0.1% fetal bovine serum (FBS) and incubation continued for another 24 hours. Cells were treated with test compounds for 45 minutes (standard in the Wisconsin laboratory), then fixed and permeabilized for the Akt assay. Total Akt and phosphorylated Akt at each of the 2 phosphorylation sites (threonine 308 and serine 473) were determined at 7 test concentrations, each performed in quadruplicate. The phospho-Akt data were normalized to total Akt and background subtracted for analysis.[35]

Experiments were also done in the presence of the phosphatidylinositide 3-kinase (PI3K) inhibitor, LY294002. These experiments tested if the phosphorylation of Akt was a result of upstream activation of the tyrosine kinases. After overnight serum starvation to reduce basal Akt phosphorylation, cells were stimulated with vehicle test agent. Cells were treated for 45 minutes or 3 hours and studied for phosphorylation at serine 473 or threonine 308, respectively.

Immunocytochemistry Assay for Phosphorylated Akt

To extend the data obtained in the A549 human lung cells, the effect of CMX-2043 on Akt phosphorylation was assayed in rat polyclonal cardiac myocyte (H9c2; ATCC) cells by immunocytochemistry followed by visualization using microscopy.[36]

H9c2 cells were plated at 70% confluence and incubated overnight to allow cell attachment. The cells were then incubated in low serum medium (0.5% FBS) for an additional 48 hours. Cells were treated for 3 hours with vehicle, CMX-2043 (50 μmol/L), or cotreatment with LY294002 (25 μmol/L); then fixed with 3.7% formaldehyde in phosphate buffered saline and permeabilized with 0.5% Triton X-100 in phosphate buffered saline. After permeabilization, the cells were treated with antibody specific for Akt phosphorylated at threonine 308, followed by treatment with a fluorescent-labeled secondary antibody to quantify the amount of bound primary antibody.

Cytosolic Calcium Overload Assay

Akt phosphorylation can result in activation of mechanisms that inhibit apoptosis by promoting uptake of cytosolic calcium into intracellular calcium stores.[37] Cytosolic calcium levels increase in Chinese hamster ovary (CHO) cells (CHO-M1-WT3; ATCC) when stimulated with carbachol. This free calcium was measured with a fluorescent dye using a Flexstation II (Molecular Devices Flexstation Application Note #3). The CHO cells from the cell line CHO-M1-WT3 were seeded onto 96-well plates in Hams F12 medium supplemented with 10% FBS and 5 μg/mL G418 to maintain expression of the M1 muscarinic receptor. After 24 hours, cells were incubated for 30 minutes with Fluo-4 NW or Calcium-3 in Hank's balanced salt solution along with 2.5 mmol/L water-soluble probenecid and the test compound. Carbachol was added automatically by the Flexstation. Dye fluorescence was measured before and after the addition of carbachol. Data were normalized to the peak carbachol response in a control sample.

Thapsigargin treatment of Jurkat cells (human T lymphocytes; ATCC) will also result in an increase in cytosolic calcium. Jurkat cells were seeded onto 96-well plates in RPMI-1640 medium supplemented with 10% FBS. After 24 hours cells were incubated Fluo-4 NW or Calcium-3 in Hank's balanced salt solution and the test compound for 30 minutes. Dye fluorescence was measured before and after the addition of thapsigargin. Cytosolic calcium was measured 5 minutes following the addition of thapsigargin. Data were normalized to the peak thapsigargin response in a control sample.

Statistical Analysis

Data are presented as mean and standard error. The significance of the difference between treatment groups was determined by Student's *t* test.

RESULTS

Oxygen Radical Absorbance Capacity Assay

Peroxyl radicals are produced by cells during tissue reperfusion, and LA is a known antioxidant protective against oxidative damage.[9] The peroxyl radical absorbance capacity values are shown in Table 1. These data show that CMX-2043 had greater peroxyl radical absorbance capacity than R-α-LA and that the dipeptide adduct alone had no significant effect. These results clearly indicated that the lipoyl moiety was necessary for the ability of CMX-2043 to scavenge peroxyl radicals in the oxygen radical absorbance capacity assay. Furthermore, there is little difference between the peroxyl radical scavenging capability of the R- and S-isomers of the novel compound, reflecting the lack of stereospecificity in its antioxidant action.

Tyrosine Kinase Activation Assays

The effect of CMX-2043 and LA at 300 μM on a broad panel of tyrosine kinases was evaluated. IRK was activated by both molecules, although CMX-2043 was 1.5-fold more potent. There was no difference for IGF1R. Src was 4-fold more activated by CMX-2043 than by LA. Spleen tyrosine kinase (Syk) and tunica interna endothelial cell kinase (Tie2) were weakly inhibited by CMX-2043 but not by LA. Abl was activated by CMX-2043 but not by LA. Neither compound had an effect on the activity of FGFR1, KDR, ErbB2, EphB2, Flt1, LTK, Mer, BMX, BTK, Met, Ret, and PDGFRα.

The stereochemistry of the activation of IRK, IGF1R, and src were further evaluated. CMX-2043 incorporating the R-isomer of lipoic acid (denoted as (R)-CMX-2043) activated all the 3. The S-isomer was inactive. The racemic (R/S)-CMX-2043 marginally activated only IGF1R and src (Table 2).

CMX-2043 was further compared with LA and with EA, the peptide modifier. CMX-2043 stimulated IRK activity in a dose-dependent manner. CMX-2043 was more potent than LA with an EC_{50} of 35 μmol/L versus 93 μmol/L for LA. EA alone was inactive (Fig. 1).

TABLE 1. Oxygen Radical Absorbance

Compound	Relative Absorbance (% of CMX-2043)
CMX-2043	100 ± 5
R-α-LA	61 ± 4*
EA	8 ± 5†
(R/S)-CMX-2043	102 ± 10
(S)-CMX-2043	89 ± 4

Absorbance capacity was measured (oxygen radical absorbance capacity assay) then normalized to CMX-2043 (R-isomer) for comparison. The absorbance capacities of the 3 optical isomers were not statistically different.
*$P < 0.05$ when compared with CMX-2043.
†$P < 0.001$ when compared with CMX-2043.

TABLE 2. CMX-2043 Stereoisomer and Kinase Activation

Compound	IRK	IGF1R	src
(R)-CMX-2043	78 ± 15	42 ± 1	70 ± 1
(R/S)-CMX-2043	14 ± 1	27 ± 3	43 ± 1
(S)-CMX-2043	4 ± 12	−4 ± 10	19 ± 1

Experiments were conducted with 100 μmol/L concentrations of each compound. Activity is measured as percentage activity above control. The standard baseline range of activity in these assays ranges between −20% to +20%, and compounds showing activity within this range are considered to be inactive. Compounds exhibiting 21%–49% activity are considered marginally active. Values are mean ± the standard error of mean of 3 experiments.

Cytoblot Assay for Akt Activation

Akt is activated via phosphorylation resulting from activation of signal transduction pathways triggered by plasma membrane receptors. Phosphorylated Akt is readily detected with specific antibodies in situ in fixed and permeabilized cells. The effect of CMX-2043 and LA on Akt phosphorylation relative to total Akt was assessed in A549 (human lung adenocarcinoma) cells using a cytoblot assay. A 3-fold and 2-fold increase in phosphorylated Akt at serine 473 was observed following 45 minutes of incubation[35] with CMX-2043 and LA, respectively (Fig. 2A). Both CMX-2043 and LA increased the amount of phosphorylated Akt relative to total Akt in a dose-dependent manner. CMX-2043 was more effective than LA at lower doses.

Akt phosporylation by CMX-2043 was completely inhibited by cotreatment with 5 μmol/L LY294002 indicating dependence on the PI3K pathway (Fig. 2B).

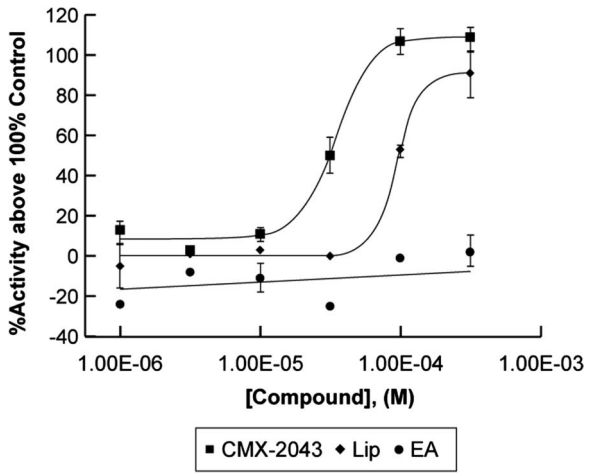

FIGURE 1. Human insulin receptor tyrosine kinase activation. CMX-2043 and R-α-LA (Lip) were compared in a kinase activation assay together with the peptide modifier (EA). Both CMX-2043 and LA activated the intracellular kinase domain of the insulin receptor in a dose-dependent manner, but EA did not. The EC50 s for CMX-2043 and LA were 35 and 93 μmol/L, respectively. Data are mean ± the standard error of mean (n = 3 for each point).

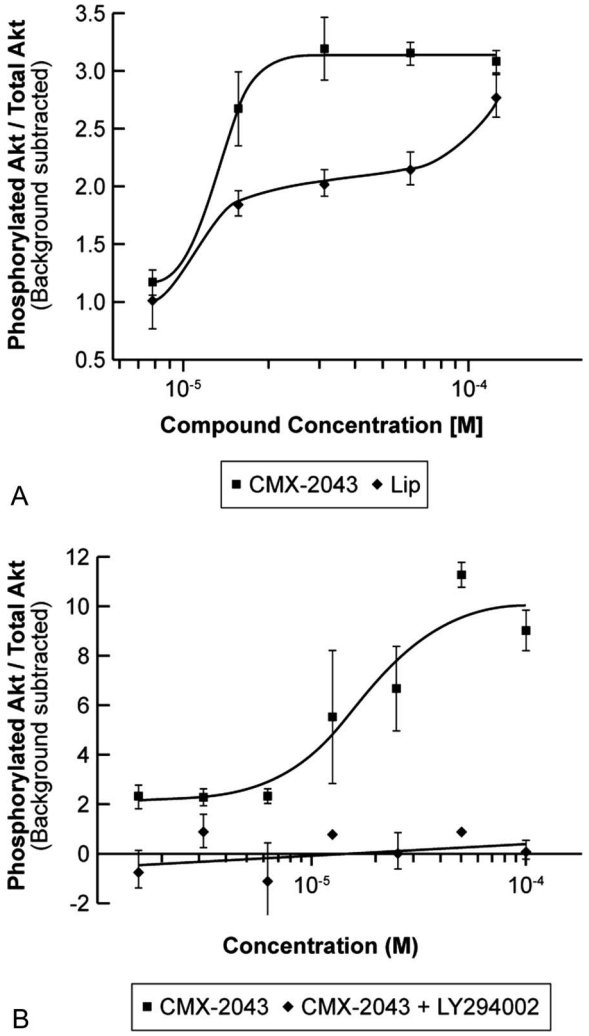

FIGURE 2. A, Akt phosphorylation assay in A549 cells. Akt phosphorylation at Ser473 in response to CMX-2043 or R-α-LA treatment was examined in a cytoblot assay using serum-starved A549 cells, a human lung adenocarcinoma cell line. Both compounds increased the amount of phosphorylated Akt in a dose-dependent manner. However, CMX-2043 was more potent than R-α-LA. Data are mean ± the standard error of mean (SEM) (n = 4) of background-subtracted phospho-Akt to total Akt ratio. B, Akt phosphorylation in the presence of LY294002. The effect of CMX-2043 and the PI3K inhibitor, LY294002, on Akt phosphorylation was assessed in A549 human adenocarcinoma cells. The complete inhibition of Akt phosphorylation shows that CMX-2043 acts through the PI3-kinase pathway. Data are mean ± SEM (n = 3) of the background-subtracted phospho-Akt to total Akt ratio.

Immunocytochemistry Assay for Phosphorylated Akt

The effect of CMX-2043 on Akt phosphorylation was also assessed in H9c2 (rat cardiac myocyte) cells using an immunocytochemistry assay as described. Cells treated with vehicle alone showed little fluorescence. Fluorescence intensity was much brighter in cells treated for 3 hours with CMX-2043 (Fig. 3). Some cells in this polyclonal cell line showed a very high activation. These cells have not been identified. Cotreatment of cells with LY294002 for 30 minutes before the addition of CMX-2043 diminished the fluorescence intensity due to Akt phosphorylation (data not shown).

Cytosolic Calcium Overload Assay

The ability of CMX-2043, LA, and the dipeptide alone (EA) to prevent calcium overload in CHO-M1-WT3 cells was determined. The peak rise in cytosolic calcium of CHO cells expressing muscarinic M1 receptor was measured in response to carbachol stimulation. CMX-2043 diminished the rise in cytosolic calcium in a concentration-dependent manner, whereas LA and EA had no significant effect (Fig. 4). CMX-2043 was without effect with short incubation times (ie, when CMX-2043 was administered immediately before carbachol stimulation).

To confirm the results of the CHO cell experiments, the ability of CMX-2043 to prevent calcium overload in thapsigargin-treated Jurkat cells was also assessed. CMX-2043 prevented the thapsigargin-induced cytosolic calcium increase in a dose-dependent manner (data not shown).

DISCUSSION

It is well established that some clinical conditions and procedures can induce IRI with serious pathological consequences. Many protective strategies have been studied. Proposed therapies include administration of antioxidants,[1–4] insulin with and without glucose,[5] atrial natriuretic peptide,[38] protein kinase C delta inhibitors,[39] glucagon-like peptide,[40,41] nicorandil,[42,43] statins,[44] G-protein-coupled receptor activators such as adenosine,[45] and erythropoietin.[46,47] In general, these therapies focus on single receptors or mechanisms. None has achieved clinical acceptance. Recently, Hausenloy and Yellon[6] proposed that in contrast to single pathways, a multifactorial approach based on protein kinase pathways may be the best way to prevent myocardial IRI.

Lipoic acid, a natural fatty acid, stimulates both antioxidant and antiapoptotic mechanisms.[7–24] Studies from our laboratory have demonstrated that LA modified by the covalent addition of the dipeptide glutamyl-alanine (CMX-2043) is effective in reducing cardiac IRI in an established rat model.[28,29] The studies reported here have identified several biochemical and cellular mechanisms associated with this cytoprotection. These mechanisms confirm both antioxidant activity and the activation of multiple cell survival pathways through Akt. Figure 5 summarizes the results in the context of these mechanisms.

The antioxidant properties of LA are well known and the dithiolane region of the molecule is critical for this property.[7] In CMX-2043, the peptide addition to LA is to the carboxylic acid and the dithiolane remains intact. Therefore, we expected the modification to have little or no effect on oxygen radical scavenging ability. In fact, the modification unexpectedly resulted in an improved ability of the molecule to scavenge reactive oxygen. The S-enantiomer appeared to have slightly lower oxygen radical scavenging ability than the R-enantiomer, although this difference was not statistically

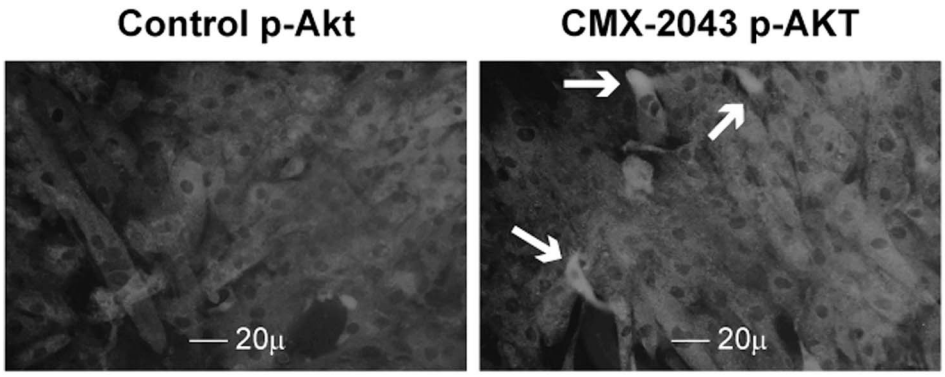

FIGURE 3. Immunocytochemistry of H9c2 cells for phosphorylated Akt. The effect of CMX-2043 on phosphorylated Akt was assessed in the rat cardiac myocyte cell line H9c2 by immunocytochemistry. CMX-2043 increased the immunofluorescence indicating an increase in phosphorylation of Akt. Some cells in this polyclonal cell line showed very high activation (arrows). These cells have not been identified.

significant. Scavenging ability is not directly related to oxidative metabolic and ATP production. These important issues remain to be evaluated in further studies.

In addition to addressing free radicals, IRI cytoprotection might ideally activate a broad range of cell survival pathways. Hausenloy and Yellon[48] have developed the concept of the reperfusion injury salvage kinase pathway. This includes Akt activation triggered by receptor tyrosine kinases such as the insulin receptor and IGF1 receptor. Our results confirm the functional interaction between lipoic acid and IRK which has been reported previously.[25,26] CMX-2043 was more effective in activation of IRK compared with LA. Further studies demonstrated that the R-enantiomer of CMX-2043 was clearly superior to the S-enantiomer. Diesel et al[25] have proposed that lipoic acid binds to the cytosolic domain of the insulin receptor through the dithiolane portion of the LA molecule. Since CMX-2043 has the same dithiolane group, it is not surprising that the molecule activates IRK. It is, however, surprising that the peptide addition to the acidic domain of LA enhanced activation, suggesting a more complex interaction worthy of further study.

It has not been reported if other tyrosine kinases could be activated by LA or similar structures. Because the structure of IGF1 receptor kinase is similar to IRK,[49] we tested the ability of CMX-2043 and LA to activate this other kinase. Although there was weak activation by both compounds, CMX-2043 was more active than LA. The cytosolic tyrosine kinase Src is also known to be cytoprotective.[50] CMX-2043 activated this kinase, but LA did not. We interpret these findings as further evidence that the peptide adduct plays a role in the molecular interaction with such kinases.

The ability of both CMX-2043 and LA to activate or inhibit other receptors and tyrosine kinases was further investigated. Although there was some very weak inhibition of Syk and Tie2 by CMX-2043, neither molecule had significant effect in this broad panel. This finding is important from a drug safety perspective because it suggests a low probability of drug-related adverse reactions.[51]

The tyrosine kinases activated by CMX-2043 are upstream of Akt phosphorylation.[52–54] Although Akt phosphorylation after treatment with LA has been reported previously,[11,26,27] our studies with A549 cells and H9c2 cells showed that CMX-2043 was more potent in Akt phosphorylation than LA. This activity was dependent on PI3K, as demonstrated by LY294002 inhibition of the effect of CMX-2043. This result demonstrated that

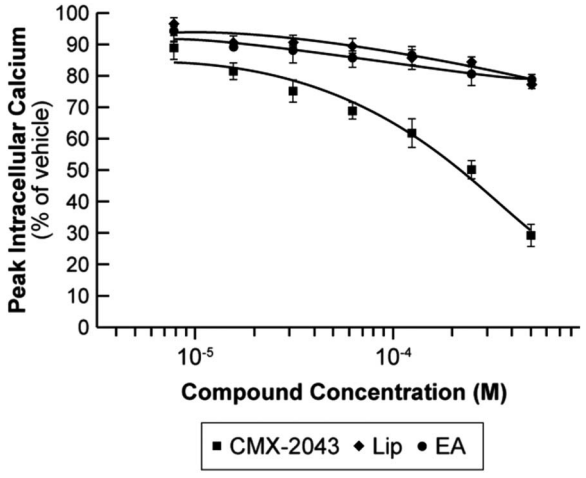

FIGURE 4. Calcium flux assay. CHO-M1 cells. The peak rise in cytosolic calcium of CHO-M1 cells expressing the muscarinic M1 receptor was measured in response to carbachol stimulation. CMX-2043 diminished the carbachol-stimulated increase in cytosolic calcium in a dose-dependent manner. R-α-LA (Lip) and the peptide modifier (EA) had no significant effect. Each point is the mean ± the standard error of mean of 6 experiments.

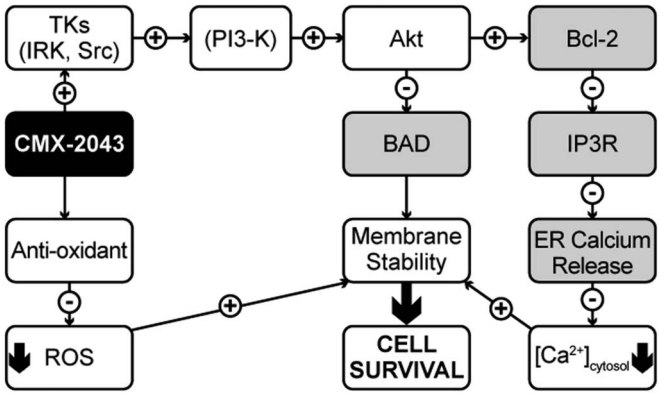

FIGURE 5. Pathways illustrating the potential biological effects of CMX-2043. Phosphorylation of the molecules/pathways downstream of Akt leads to cell survival.[42]

Akt-phosphorylation was not direct but followed a well-established signal transduction pathway.

The reperfusion injury salvage kinase pathway described by Hausenloy and Yellon[48] included activation through IRK of both Akt and extracellular signal-regulated kinase. In our studies, we did not detect extracellular signal-regulated kinase activation by CMX-2043 (data not shown) suggesting some unexpected downstream specificity of IRK. In vivo gene transfer of a constitutively activated Akt (myristoylated Akt) alone has been shown to reduce infarct size and improve cardiac function in both a mouse and rat model of cardiac IRI.[55,56] Furthermore, Akt activation by way of the hepatocyte growth factor receptor has recently been demonstrated to confer cardiac cytoprotection in the setting of IRI.[57]

The broad downstream effects of Akt phosphorylation extend to regulation of the mitochondrial permeability transition pore (mPTP). This is a nonselective ion channel that, when open, uncouples oxidative phosphorylation.[58] The pore is closed during ischemia but opens during reperfusion in response to oxidative stress, calcium overload, and ATP depletion.[59] LA supplementation in old rats decreased oxidative damage and improved mitochondrial function and increased metabolic rate.[60] Approaches to keep the mPTP closed during cardiac reperfusion are the subject of research.[58,61] Akt activation is reported to keep the pore closed.[59] Therefore, it is reasonable to assume that CMX-2043 cytoprotection through Akt activation will inhibit mPTP opening.

We also demonstrated an inhibitory effect of CMX-2043 on the increase in intracellular calcium caused by carbachol stimulation, whereas LA had little effect. Carbachol increases calcium release from the endoplasmic reticulum (ER) into the cytosol. The effect of CMX-2043 could have resulted from either a decrease in the calcium release or an increase in the rate of reuptake of calcium by the ER. Because CMX-2043 remained effective in the presence of thapsigargin, which reduces calcium reuptake, we conclude that the drug action resulted from a reduction in the release of calcium from the ER. This conclusion is consistent with CMX-2043 activation of Akt and the known activity of Akt to inhibit inositol 1,4,5-triphosphate receptors and, hence, ER calcium release.[62,63] Given that cytosolic calcium has been shown to play a role via mPTP in progression of apoptosis,[64] its inhibition by CMX-2043 provides a further cytoprotective pathway.

The whole-cell experiments provided further insight into the biology of CMX-2043 action. We were not able to show an effect of CMX-2043 on calcium or Akt activation with short incubation times (ie, adding the drug within a few minutes before the readout). The cytoblot experiments for Akt activation, and the carbachol stimulation calcium experiments required 30–45 minutes of incubation with the drug. In these experiments, the greater potency of CMX-2043 was presumably not due to relative membrane permeability since the calculated LogP of CMX-2043 is −0.9 versus 2.25 for LA indicating much lower membrane stability. This indicated that CMX-2043 was not interacting with extracellular receptors but presumably entered the cytosol to interact with the intracellular domains. This is consistent with the findings of the tyrosine kinase experiments, which were performed with purified human recombinant intracellular domains or soluble cytosolic tyrosine kinases, and with the report by Diesel.[25]

CONCLUSION

CMX-2043 has been proven effective in protecting cardiac cells from IRI.[28,29] The present studies show that this effectiveness is associated not only with antioxidant activity but also with well-established downstream pathways dependent on Akt phosphorylation. This new molecule is similar to the parent LA but has important differences in potency and specificity. The multimodal mechanism of action combined with low toxicity[51] suggests use in prevention of IRI in the clinical setting.

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