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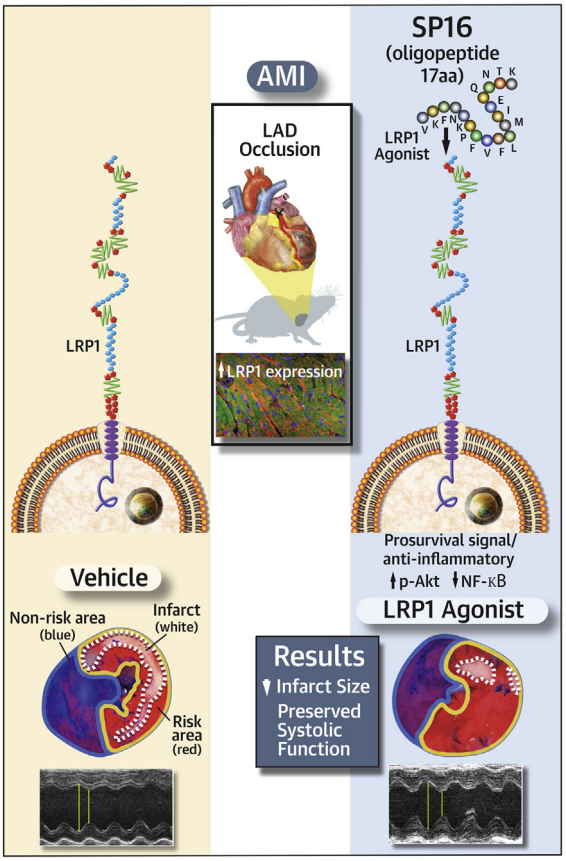
PRECLINICAL RESEARCH

Low-Density Lipoprotein Receptor–Related Protein-1 Is a Therapeutic Target in Acute Myocardial Infarction

Stefano Toldo, PhD,[a,b] Dana Austin, MS,[c] Adolfo G. Mauro, MS,[a,b] Eleonora Mezzaroma, PhD,[b,d] Benjamin W. Van Tassell, PharmD,[b,d] Carlo Marchetti, PhD,[a,b] Salvatore Carbone, MS,[a,b] Soren Mogelsvang, PhD,[c] Cohava Gelber, PhD,[c] Antonio Abbate, MD, PhD[a,b]

VISUAL ABSTRACT



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HIGHLIGHTS

- **LRP1 is a scavenger receptor with regulatory function, that also transduces a cytoprotective and anti-inflammatory signal.**
- **The expression of LRP1 in the heart is increased in a mouse model of acute ischemia and in patients with ischemic heart failure.**
- **The binding of the serine protease inhibitor–enzyme complex to the LRP1 receptor induces a protective signal, that can be leveraged by the use of a small peptide functioning as LRP1 agonist.**
- **The LRP1 agonist recapitulates the cytoprotective effect of serine protease inhibitor–enzyme complex, and reduces the myocardial ischemia-reperfusion injury in the mouse model.**

From the [a]Division of Cardiology, VCU Pauley Heart Center, Virginia Commonwealth University, Richmond, Virginia; [b]Johnson Research Center for Critical Care, Virginia Commonwealth University, Richmond, Virginia; [c]Serpin Pharma, Manassas, Virginia; and the [d]Department of Pharmacotherapy and Outcome Sciences, School of Pharmacy, Virginia Commonwealth University, Richmond, Virginia. The study was completed as part of a collaboration between Virginia Commonwealth University and Serpin Pharma. Ms. Austin and Dr. Gelber are employees of Serpin Pharma. Dr. Abbate is supported by National Heart, Lung, and Blood Institute grants HL121402 and HL136816. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. Drs. Toldo and Austin contributed equally to this work.

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ABBREVIATIONS AND ACRONYMS

A2MG = alpha-2 macroglobulin

AAT = alpha-1 antitrypsin

AMI = acute myocardial infarction

AT_{III} = antithrombin III

HRP = horseradish peroxidase

IL = interleukin

IV = intravenous

LPS = lipopolysaccharide

LRP1 = low-density lipoprotein receptor-related protein-1

LV = left ventricular

LVFS = left ventricular fractional shortening

PBS = phosphate-buffered saline

SEC = serine protease inhibitor–enzyme complex

SERPIN = serine protease inhibitor

TBS = tris-buffered saline

TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labeling

SUMMARY

Low-density lipoprotein receptor–related protein-1 (LRP1) is a ubiquitous membrane receptor functioning as a scavenger and regulatory receptor, inducing anti-inflammatory and prosurvival signals. Based on the known structure–activity of the LRP1 receptor binding site, the authors synthesized a small peptide (SP16). SP16 induced a >50% reduction in infarct size ($p < 0.001$) and preservation of left ventricular systolic function ($p < 0.001$), and treatment with an LRP1 blocking antibody eliminated the protective effects of SP16. In conclusion, LRP1 activation with SP16 given within 30 min of reperfusion during experimental acute myocardial infarction leads to a cardioprotective signal reducing infarct size and preservation of cardiac systolic function. (J Am Coll Cardiol Basic Trans Science 2017;2:561–74)

The inflammatory response as a result of tissue injury is necessary for the clearance of cellular debris (1,2). Low-density lipoprotein receptor-related protein-1 (LRP1) is a ubiquitous membrane receptor functioning as a scavenger and regulatory receptor (3–5). Its expression increases during hypoxia, ischemia, and tissue injury (3–6). LRP1 is a nonselective receptor, binding several plasma proteins (3–6). Also known as an alpha-2-macroglobulin (A2MG) receptor, LRP1 has the ability to bind the complex of A2MG and plasma proteases (7,8). The binding of the protease-inhibitor complex to LRP1 is seen across the entire spectrum of serine protease inhibitors (SERPINs) such as alpha-1 antitrypsin (AAT) and antithrombin III (AT_{III}), and referred to as a SERPIN-enzyme complex (SEC) receptor (3–5) The binding of protease-inhibitor complexes to LRP1 inhibits the inflammatory response (5) and induces a prosurvival signal through phosphorylation of protein kinase Akt (9). In preclinical animal studies, the administration of plasma-derived AAT or AT_{III} led to a significant reduction in myocardial injury in experimental acute myocardial infarction (AMI) (10,11). Therefore, we hypothesized that the synthesis of an LRP1 agonist in the form of a small peptide would provide cardioprotection in AMI.

METHODS

SYNTHESIS OF THE LRP1 AGONIST. SERPINs are a family of proteins characterized by the ability to inhibit plasma serine proteases such as trypsin, elastase, thrombin, and others (12). When SERPINs bind the serine proteases and consequently inactivate them, a conformational change occurs by which a short peptide containing a unique motif (5 to 11 amino acids) is exposed (13). This unique motif is responsible for the binding to LRP1 (3,14). Based on the known structure-activity of the LRP1 receptor binding site, we synthesized a series of short peptides ranging from 11 to 38 amino acids in length. Based on the anti-inflammatory activity in several in vitro assays we chose a 17 amino acid sequence (VKFNKPFVFLMIEQNTK). This peptide was then modified via replacement of a methionine (M) with norleucin (Nle) to improve stability and binding to LRP1 leading to an oligopeptide, named SP16 (Ac-VKFNKPFVFLNleIEQNTK-NH2). We also created a scrambled control peptide, SP34, containing all the amino acids of the SP16 peptide, including the core motif responsible for binding LRP1, in a randomly scrambled sequence order (FPKMVPQFNTELKIFPEVNIK). Peptides were synthesized by CPC Scientific Inc. (Sunnyvale, California) with purity >90% as verified by high-performance liquid chromatography and mass spectrometry. The pharmacokinetic profile of SP16 in rats was evaluated following single administration of 3 different intravenous (IV) doses (5 mg/kg bolus, 25 mg/kg bolus, 25 mg/kg infusion). Each dose was administered to 2 healthy adult rats (1 male and 1 female) for a total of 6 rats, and blood samples were obtained at baseline, 5, 15, 30, 60, 120, and 240 min, and at 8 and 24 h after IV administration. SP16 concentration was measured using high-performance liquid chromatography-mass spectrometry analysis (Shimadzu CBM20A; Applied Biosystems API 5500 LC/MS/MS instrument) and a Waters XSelect CSH C18 2.5 μm (2.1 mm × 50 mm) column. Pharmacokinetic parameters were calculated based on a noncompartmental model (WinNonlin 6.4).

CELL CULTURE EXPERIMENTS. Murine macrophage J774A.1 cells were maintained in Dulbecco's modified minimum essential medium (Dulbecco's modified Eagle medium) (Hyclone, Thermo Fisher Scientific, Waltham, Massachusetts) supplemented with 10% fetal bovine serum (Hyclone) and 1%