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— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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(54) Title: METHODS OF PREVENTING OR TREATING DISEASE OF THE CENTRAL NERVOUS SYSTEM USING RLIP76

(57) Abstract: Provided herein are methods for treating a disease or condition of the central nervous system. The methods include administering to the subject having or at risk of having the disease or condition a composition comprising RLIP76. Also provided herein are methods of inhibiting oxidative stress in a subject. The methods include selecting a subject having a disease or condition of the central nervous system and administering to the subject an effective amount of a composition comprising RLIP76.

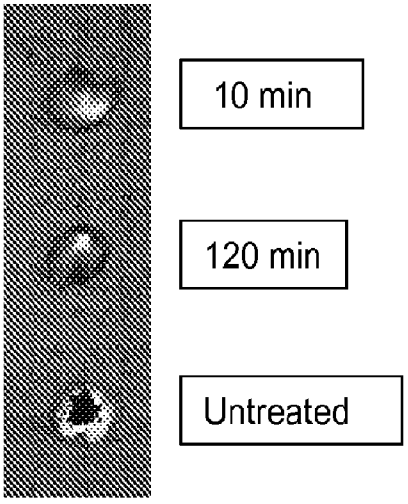

FIG. 1

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METHODS OF PREVENTING OR TREATING DISEASE OF THE CENTRAL NERVOUS SYSTEM USING RLIP76

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application No. 61/651,925, filed May 25, 2012, which is incorporated by reference herein in its entirety.

BACKGROUND

As the most complex system, the nervous system serves as the body control center and communications electrical-chemical wiring network. As a key homeostatic regulatory and coordinating system, it detects, interprets, and responds to changes in internal and external conditions. The nervous system integrates countless bits of information and generates appropriate reactions by sending electrochemical impulses through nerves to effector organs such as muscles and glands. The brain and spinal cord are the central nervous system (CNS); the connecting nerve processes to effectors and receptors serve as the peripheral nervous system (PNS). Special sense receptors provide for taste, smell, sight, hearing, and balance. Nerves carry all messages exchanged between the CNS and the rest of the body.

There are many central nervous system diseases, including infections of the central nervous system such as encephalitis and poliomyelitis, neurodegenerative diseases such as Alzheimer's disease and amyotrophic lateral sclerosis, autoimmune and inflammatory diseases such as multiple sclerosis or acute disseminated encephalomyelitis, and genetic disorders such as Krabbe's disease, Huntington's disease, or adrenoleukodystrophy. Last, cancers of the central nervous system can cause severe illness and, when malignant, can have very high mortality rates.

Traumatic brain injury (TBI), also known as intracranial injury, occurs when an external force traumatically injures the brain. TBI can be classified based on severity, mechanism (closed or penetrating head injury), or other features (e.g., occurring in a specific location or over a widespread area). Head injury usually refers to TBI, but is a broader category because it can involve damage to structures other than the brain, such as the scalp and skull.

TBI is a major cause of death and disability worldwide, especially in children and young adults. Causes include falls, vehicle accidents, and violence. Brain trauma can be caused by a direct impact or by acceleration alone. In addition to the damage caused at the moment of injury, brain trauma causes secondary injury, a variety of events that take place in the minutes and days following the injury. These processes, which include alterations in cerebral blood flow and the pressure within the skull, contribute substantially to the damage from the initial injury.

Several, related mechanisms have been proposed to underlie the secondary injury resulting from traumatic brain injury (TBI), including differentiated pathways leading to neuronal cell death (apoptosis) in the brain. Free radical-induced oxidative damage reactions and membrane lipid peroxidation (LP) resulting in the formation of neurotoxic aldehydes 4-HNE, are one of the most validated secondary injury mechanisms in preclinical TBI models.

Alzheimer's disease (AD) and Parkinson's disease (PD) are the most progressive neurodegenerative diseases affecting millions of people in the world. Neurodegenerative diseases and acute injuries may all share a common pathway of oxidative stress leading to neuronal cell death.

Therefore, a reasonable approach for developing new pharmaceutical compounds for treating neurodegenerative and acute brain injury conditions may be designing compounds that inhibit cellular oxidative stress. The brain is a center of high metabolic activity, consuming 20% of the body's oxygen, but does not possess an extremely high capacity for anti-oxidant activity. Reactive oxygen species (ROS), such as oxygen radical superoxide (O2) or hydrogen peroxide (H2O2), are produced during normal metabolic processes and perform several useful functions under normal conditions. Cells are provided with several mechanisms to control levels of these oxidative agents, for instance, superoxide dismutase (SOD), glutathione or vitamin E. In normal physiological conditions, a balance between ROS and these anti-oxidative mechanisms exists. An excessive production of ROS and/or a loss of efficiency of the anti-oxidative defenses can lead to pathological conditions in cells and provoke tissue damage and cell death. This event can occur dramatically in neurons, because of their high rate of metabolic activity, and seems to be involved in a series of degenerative processes, diseases and syndromes, for example, Alzheimer's disease, Parkinson's

disease, amyotrophic lateral sclerosis (ALS) and schizophrenia. Also other diseases or pathological conditions have been related to oxidative stress, such as Huntington's disease, brain injury (such as traumatic brain injury (TBI) and ischemic stroke), diabetes, multiple sclerosis, epilepsy, age-related macular degeneration (AMD), atherosclerosis, and heart failure.

SUMMARY

Provided herein are methods for treating a disease or condition of the central nervous system. The methods include administering to the subject having or at risk of having the disease or condition a composition comprising RLIP76.

Also provided herein are methods of inhibiting oxidative stress in a subject. The methods include selecting a subject having a disease or condition of the central nervous system and administering to the subject an effective amount of a composition comprising RLIP76.

The details of one or more embodiments are set forth in the accompanying drawings and the description below. Other features, objects, and advantages will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

Figure 1 shows pictures of mouse brains from animals treated with RLIP76 proteoliposome (top and middle) as compared to a brain from an untreated control animal (bottom).. RLIP76 proteoliposome was administered intravenously and distribution to the mouse brain was detecting using proteoliposome tagged with Dylight 650. The treated brains show that systemically administered RLIP76 enters the brain.

Figure 2 is a graph showing the effect of RLIP76 on brain infarction volume after temporal middle cerebral artery occlusion (MCAO) in rats.

Figure 3 are pictures showing a comparison of the infarct areas in brain sections after administration of test articles. Infarct size is larger and involving the striatum and fronto-parietal cortex in the vehicle group (V1-V6). The infarction is less involved in the cortex and sub-cortex structures with treatment of RLIP76 (R1-R6). Each section represents an individual brain sample region from each study group.

Figure 4 is a graph showing assessment neurological scores following MCAO in rats. RLIP76 improves neurological deficits after cerebral ischemia. The *p* value of Day 1-3 was 0.476, 0.303, and 0.374 respectively.

DETAILED DESCRIPTION

The present disclosure is based on the original discovery relating to RLIP76's protective abilities and extends to the use of RLIP76 to prevent or treat subjects having or at risk of having neurodegenerative diseases or conditions of the central nervous system (CNS). This is based on the principle of reducing oxidative stress or the results thereof, including reducing reactive oxygen species (ROS) and/or 4-HNE, in a subject. While not wanting to be bound by a particular theory, it is also believed that RLIP76 is effective in reducing oxidative damage, affecting the NOS pathway or intracellular inflammatory response, for example, in the subject. For example, RLIP76 reduces reactive oxygen species (ROS) and/or 4-HNE in the subject.

Thus, provided herein is a method of inhibiting oxidative stress in a subject. The method includes selecting a subject having a disease or condition of the central nervous system or a subject with one or more indications of oxidative stress and administering to the subject an effective amount of a composition comprising RLIP76. Also provided is a method of treating a disease or condition of the central nervous system. The method includes selecting a subject having a disease or condition of the central nervous system and administering to the subject an effective amount of a composition comprising RLIP76. Optionally, the central nervous system disease or condition is associated with oxidative stress. Optionally, the disease or condition is traumatic brain injury, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), ischemia, or stroke.

RLIP76 (also known as RALBP1 or RIP1) is a ubiquitous protein found in Drosophila to humans that serves multiple roles in cellular physiology. When membrane-associated, the protein functions as a multi-specific efflux pump for a variety of compounds, including amphiphilic small molecules such as Vinca alkaloids and anthracylines, which are common anticancer drugs. However, RLIP76 transport also involves movement from the cell of endogenous glutathione electrophile conjugates (GS E) formed from reactive oxygen species (ROS). ROS are produced by a variety of insults such as radiation and a plethora of organic chemicals, and are toxic

to the cell on many levels. As their name implies, ROS are highly reactive and bind to almost anything in their path, including proteins, lipids and nucleic acids, modifying each of these as they are contacted. The damage done by ROS to lipids (lipid peroxidation) is particularly pernicious since the resulting peroxidation products are themselves toxic. These include proapoptotic reactive alkenals, such as 4 hydroxynonenal (4-HNE), which are long lived and can accumulate in the cell, ultimately leading to further damage and death. As such, RLIP76 is an important component of stress response in cultured cells and provides protection from stressors including heat, oxidant chemicals, chemotherapeutic agents, UV irradiation and X-irradiation. RLIP76 as used throughout optionally comprises one or more ATP binding domains. Optionally, the RLIP76 comprises amino acids 1-367 of SEQ ID NO:1 and/or amino acids 410-655 of SEQ ID NO:1.

The normal cell has defense mechanisms designed to bind up (conjugate) ROS-associated toxins, for example, glutathione. Glutathione binds electrophilic compounds to sequester the reactive electrons. However, the resulting conjugates (GS-E) are harmful or fatal to the cell if allowed to accumulate, and so must be removed by the cell. Although not wishing to be bound to any particular theory, it appears that the active efflux of GS-E derived from these toxic intermediates is the principal mechanism by which RLIP76 confers resistance to oxidant and radiant stressors.

The protective effect of RLIP76 goes beyond its protection of potentially toxic chemical substituents and their by-products. For example, electrophilic products of lipid peroxidase (LPO) caused by reactive oxygen species generated during radiation may partly account for cell killings by radiation. As detailed herein, RLIP76-mediated transport of GSH conjugates of these electrophiles provides protection from radiation. Such protection may be readily transferred to a larger scale to protect mammals against damaging radiation, including ionizing, electromagnetic, thermal, and laser radiation, wherein either long- or short-range electrons are involved.

Therefore, RLIP76 mediates transport of endogenously generated chemicals, metabolic products, their by-products and exogenously administered drugs or radiation, and their by-products. RLIP76 mediates the transport of most chemicals and by-products that also involve GS-E (e.g., conjugate of 4-HNE). For example, RLIP76-

enriched cells are resistant to toxicity in the form of chemical toxicity (organic or inorganic) or from damage (e.g., from stress, oxidation, alkylation, radiation). The function of RLIP76 via an ATP-dependent efflux of xenobiotics (e.g., GS-E and exogenous and endogenous electrophiles) is disclosed herein. Xenobiotics, radiation, their metabolites, mitochondrial electron transport and metal ions generate ROS that can cause membrane lipid peroxidation and 4-hydroxynonenal (the toxic end product of lipid peroxidation), which can cause DNA damage leading to mutagenesis, carcinogenesis and apoptosis as well as modulate the stress mediated signaling pathways. RLIP76 mediates the ATP-dependent efflux of a wide variety of metabolic, stress, and pharmaceutical by-products, such as amphiphilic drugs, GSH-conjugates (GS-E) of both xeno and endo-biotics, GS-HNE and leukotrienes, from eukaryotic cells. The transport of GS-E is important for maintaining functionality of GSTs and glutathione reductase (GR), because these enzymes are inhibited by GS-E. RLIP76 regulates the intracellular concentrations of 4-HNE by a coordinated mechanism with cellular GSTs.

The RLIP76 protein may be divided into four regions out of which two central domains carry a Rac-1/CDC42 GAP activity and a Ral binding domain. Representative nucleotide sequences encoding human RLIP76 (GenBank Accession Number NM--006788) and mouse RLIP76 (NM--009067), and amino acid sequences of human RLIP76 (GenBank Accession Number NP--006779) and mouse RLIP76 (GenBank Accession Number NP--033093), have been described. The human RLIP76 amino acid sequence includes sites for N-glycosylation (amino acids 341-344), cAMP (amino acids 113-116), cGMP-dependent protein kinase phosphorylation (amino acids 650 653), tyrosine kinase phosphorylation (amino acids 308-315), N-myristolation (amino acids 21-26, 40-45, and 191-196), leucine zipper pattern (amino acids 547-578) and several protein kinase C phosphorylation, casein kinase II phosphorylation, trypsin and chymotrypsin cut sites. The presence of such motifs in the primary structure of RLIP76, and its facile proteolytic degradation, shows RLIP76 to be involved in several intra- and extracellular processes (e.g., protein processing, intracellular signaling, protein degradation, recognition, tagging, etc.) and that proteolytic processing of RLIP76 is required for the multiple functions. The peptide fragments of RLIP76 individually or in association with other fragments may catalyze

these various functions. For example, N terminal and C-terminal fragments of RLIP76, fragments that are individually incapable of mediating ATP-dependent transport, can catalyze the transport of electrically charged drugs (e.g., DOX, colchicines) when reconstituted together in proteoliposomes.

Optionally, RLIP76 comprises a sequence of 655 amino acids as set forth in GenBank Accession Number NP-006779). Optionally, RLIP76 comprises a sequence as disclosed in US 2005/0123594, US 2006/0182749, US 2008/0279919, US 2010/0124566, or WO 2009/100446A1, the contents of which are incorporated by reference in their entireties. Thus, RLIP76 is used throughout as an example. In each case, an active fragment or variant of RLIP76 can be used similarly.Unlike the ABC transporters, no transmembrane alpha-helices are evident in the RLIP76 sequence. The association of RLIP76 with membranes has, however, been demonstrated by immunohistochemical studies using specific antibodies (Awasthi, et al., Proceedings of the American Association for Cancer Research, 43:Abst. 4717, 2002; herein incorporated by reference). The extraction of RLIP76 from cell lysates requires detergent, suggesting membrane association, a feature important for transport. These findings show a greater diversity in this transporter, in terms of structural elements defining ATP binding and mode of membrane insertion, than is currently accepted. In addition, the distinction between transporters for anions as opposed to neutral or cationic substrates is blunted because RLIP76 catalyzes the transport of both, and, in contrast to MRP 1, does so without co-transporting GSH.

RLIP76 expressed in cultured cells or in *E. coli* undergoes facile proteolysis during purification. The most prominent peptides, N-RLIP761-367 and C-RLIP76 410-655, arising from the N and C termini of RLIP76, respectively, appear as 49 kDa and 38 kDa bands in SDS-gels. Both these peptides display constitutive ATPase activity that may be stimulated in the presence of the anionic or cationic ligands transported by RLIP76. Both peptides bind ATP, as shown by photoaffinity labeling that increased in the presence of vanadate, indicating the trapping of a reaction intermediate in the ATP binding site. Neither of the two fragments catalyze transport when reconstituted alone in proteoliposomes. However, when reconstituted together, ATP dependent transport of charged chemicals (e.g., DNP-SG, DOX) is observed with kinetic parameters similar to those for RLIP76. The ATP binding sites in N-

RLIP761-367 and C RLIP76 410-655 were identified to be amino acids 69-74 and amino acids 418-425, respectively. Mutations of K74 and K425 in the N and C-terminal peptides, respectively, abrogate the ATPase activity, ATP binding capacity, and transport function. The sequence of these ATP binding sites is not identical to the consensus sequence for the P-loop (Walker motif).

In addition to the human RLIP76 nucleic acid sequence described above, a number of single nucleotide polymorphisms (SNPs) have been described in the art within the human RLIP76 gene, three of which (an A to G mutation at nucleotide 660 of the coding sequence, a G to A mutation at nucleotide 838 of the coding sequence, and a C to T mutation at nucleotide 2065 of the coding sequence) fall within the RLIP76 coding sequence. These nucleotide changes result in changing the amino acid sequence from lysine to glutamate at amino acid position 149, from arginine to glutamine at amino acid position 208, and from alanine to valine at amino acid position 617, respectively. These SNPs, along with SNPs that occur in the introns of the human RLIP76 gene, and well as SNPs that occur in the 5' and 3' untranslated regions of the human RLIP76 gene, are described in the Single Nucleotide Polymorphism (SNP) database on the National Center for Biotechnology Information web site. The term RLIP76 includes these variants.

In the methods throughout, one or more fragments of RLIP76 amino acid sequence or mutants of the RLIP76 can be used. Optionally, the methods involve RLIP76 comprising SEQ ID NO:1, fragments thereof, or modified variants (e.g., conservatively modified variants that include one or more conservative amino acid substitutions) thereof. Optionally, modified RLIP76 refers to an amino acid sequence that has about 99% identity with the human RLIP76 amino acid sequence as shown in GenBank Accession Number NP-006779 or SEQ ID NO:1, about 98% identity or homology, about 95% identity or homology, about 90% identity or homology, about 85% identity or homology, or about 80% identity or homology to the human RLIP76 amino acid sequence as shown in GenBank Accession Number NP-006779 or SEQ ID NO:1. The percentage of sequence identity or homology may reflect certain additions, deletions, substitutions, silent or conservative mutations to the sequences.

As used herein, the terms peptide, polypeptide, or protein are used broadly to mean two or more amino acids linked by a peptide bond. Protein, peptide, and

polypeptide are also used herein interchangeably to refer to amino acid sequences. It should be recognized that the term polypeptide is not used herein to suggest a particular size or number of amino acids comprising the molecule and that a peptide of the invention can contain up to several amino acid residues or more.

It is understood that the nucleic acids that can encode those peptide, polypeptide, or protein sequences, variants and fragments thereof are also disclosed. This would include all degenerate sequences related to a specific polypeptide sequence, i.e. all nucleic acids having a sequence that encodes one particular polypeptide sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the disclosed variants and derivatives of the polypeptide sequences. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every sequence is in fact disclosed and described herein through the disclosed polypeptide sequence.

As with all peptides, polypeptides, and proteins, including fragments thereof, it is understood that additional modifications in the amino acid sequence of the provided polypeptide can occur that do not alter the nature or function of the peptides, polypeptides, or proteins. Such modifications include, for example, conservative amino acids substitutions and are discussed in greater detail below.

Thus, the provided agents comprising polypeptides or nucleic acids can be further modified and varied so long as the desired function is maintained. It is understood that one way to define any known modifications and derivatives or those that might arise, of the disclosed nucleic acid sequences and proteins herein is through defining the modifications and derivatives in terms of identity to specific known sequences. Specifically disclosed are polypeptides which have at least 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83 , 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 percent identity to the polypeptides provided herein. Those of skill in the art readily understand how to determine the identity of two polypeptides. For example, the identity can be calculated after aligning the two sequences so that the identity is at its highest level.

Another way of calculating identity can be performed by published algorithms. Optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman, Adv. Appl. Math 2:482 (1981), by

the identity alignment algorithm of Needleman and Wunsch, J. Mol Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

The same types of identity can be obtained for nucleic acids by, for example, the algorithms disclosed in Zuker, Science 244:48-52 (1989); Jaeger et al., Proc. Natl. Acad. Sci. USA 86:7706-7710 (1989); Jaeger et al., Methods Enzymol. 183:281-306 (1989), which are herein incorporated by reference for at least material related to nucleic acid alignment. It is understood that any of the methods typically can be used and that in certain instances the results of these various methods may differ, but the skilled artisan understands if identity is found with at least one of these methods, the sequences would be said to have the stated identity and to be disclosed herein.

Protein modifications include amino acid sequence modifications. Modifications in amino acid sequence may arise naturally as allelic variations (e.g., due to genetic polymorphism), may arise due to environmental influence (e.g., exposure to ultraviolet light), or may be produced by human intervention (e.g., by mutagenesis of cloned DNA sequences), such as induced point, deletion, insertion, and substitution mutants. These modifications can result in changes in the amino acid sequence, provide silent mutations, modify a restriction site, or provide other specific mutations. Amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional, or deletional modifications. Insertions include amino and/or terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions preferably are made in adjacent pairs, i.e., a deletion of 2 residues or insertion of 2 residues. Substitutions,

deletions, insertions or any combination thereof may be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional modifications are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Table 1 and are referred to as conservative substitutions.

Table 1. Amino Acid Substitutions

Amino Acid	Substitutions (others are known in the art)
Ala	Ser, Gly, Cys
Arg	Lys, Gln, Met, Ile
Asn	Gln, His, Glu, Asp
Asp	Glu, Asn, Gln
Cys	Ser, Met, Thr
Gln	Asn, Lys, Glu, Asp
Glu	Asp, Asn, Gln
Gly	Pro, Ala
His	Asn, Gln
Ile	Leu, Val, Met
Leu	Ile, Val, Met
Lys	Arg, Gln, Met, Ile
Met	Leu, Ile, Val
Phe	Met, Leu, Tyr, Trp, His
Ser	Thr, Met, Cys
Thr	Ser, Met, Val
Trp	Tyr, Phe
Tyr	Trp, Phe, His
Val	Ile, Leu, Met

Modifications, including the specific amino acid substitutions, are made by known methods. By way of example, modifications are made by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the modification, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example, M13 primer mutagenesis and PCR mutagenesis.

Provided herein are compositions comprising RLIP76 or fragments thereof. The provided compositions are suitable for formulation and administration *in vitro* or *in vivo*. Optionally, the compositions comprise RLIP76 and a pharmaceutically

acceptable carrier. Suitable carriers and their formulations are described in Remington: The Science and Practice of Pharmacy, 21st Edition, David B. Troy, ed., Lippicott Williams & Wilkins (2005). By pharmaceutically acceptable carrier is meant a material that is not biologically or otherwise undesirable, i.e., the material is administered to a subject without causing undesirable biological effects or interacting in a deleterious manner with the other components of the pharmaceutical composition in which it is contained. If administered to a subject, the carrier is optionally selected to minimize degradation of the active ingredient and to minimize adverse side effects in the subject. Optionally, the provided compositions are formulated for oral administration. Optionally, the compositions include one or more buffers, one or more mucoadhesive polymers, one or more permeation enhancers and combinations thereof.

The compositions can be administered in a number of ways as selected by one skilled in the art and depending on whether local or systemic treatment is desired, on the target area to be treated, and other variables. The compositions are administered via any of several routes of administration, including topically, orally, parenterally, intravenously, intra-articularly, intraperitoneally, intramuscularly, intraventricularly, subcutaneously, intracavity, transdermally, intrahepatically, intracranially, pulmonary, nebulization/inhalation, or by installation via bronchoscopy. Optionally, the provided compositions are administered orally.

Optionally, the RLIP76 is formulated as a liposome composition or proteoliposome. Liposomes are vesicles consisting of amphipathic lipids arranged in one or more concentric bilayers. When lipids are placed in aqueous medium, the hydrophilic interaction of the lipid head groups with water results in the formation of multilamellar and unilamellar systems or vesicles which resemble biological membranes in the form of a spherical shell. Liposomes may be small (0.025-0.05 .mu.m) to large (0.05-10 .mu.m) multilamellar vesicles. Lipids used to prepare the liposomes can include, but are not limited to, phospholipids, sphingolipids, glycosphingolipids, saturated glycerides, steroids (e.g., cholesterol) and synthetic phospholipids. Liposomes are typically prepared by melting the lipid together in aqueous solvent with an emulsifier like POE. The agent is then added and the liposomes are generated through mixing or sonication. The agent is usually entrapped

in the vesicle structure. These basic liposomes are sometimes referred to as conventional liposomes. Several other types of liposomal preparations exist, including (1) sterically stabilized liposomes, which are surface coated with an inert hydrophilic polymer, such as polyethylene glycol; (2) targeted liposomes, to which are attached targeting ligands, such as antibodies or fragments thereof, lectins, oligosaccharides or peptides (e.g., choleratoxin B (CTB) is used to target liposomes to the gastrointestinal epithelium); and (3) reactive or polymorphic liposomes, which change their phase and structure in response to a particular interaction (this group includes liposomes sensitive to ions (pH, cations), heat and light, among other stimuli). Lipids and liposomes include, but are not limited to, neutral (e.g., dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, and distearolyphosphatidyl choline) negative (e.g., dimyristoylphosphatidyl glycerol DMPG) and cationic (e.g., dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA) liposomes.

Optionally, the compositions provided herein include proteoliposomes. As used herein, a proteoliposome is generally a protein and lectin or glyco- or phospholipid combination that forms a spherical micellular-like or vesicular structure. The structures may form spontaneously or by chemical or mechanical manipulation, or combinations thereof. Proteoliposomes take advantage of the amphipathic nature of the lipid (or lectin) that causes them to form bilayers when in solution resulting in at least one of several shapes, including: (a) spherical micelle with the tails inward, or (b) bimolecular sheets that are bilayers with hydrophobic tails sandwiched between hydrophilic head groups. In general, proteoliposomes may reseal themselves when torn or broken. Proteoliposomes may contain only one lectin or lipid or a variety and combination of each. Examples of phospholipids include phosphatidylcholine, sphingomyelin, phosphatidylserine, inositol phospholipids, and phosphatidylethanolamine. When used, proteoliposomes may be charged or electrically neutral and are generally used at physiological pH. They may also be structures mixed with detergent (e.g., detergent/lipid/protein, detergent/lectin/protein). Methods for preparing proteoliposomes of defined lipid-protein or lectin-protein ratios and size are well-known to one of ordinary skill in the art of molecular biology and protein/lipid biochemistry. The proteoliposomes of the disclosure can be made by

any method known in the art, including methods disclosed and described in U.S. Publication No. 2005/0123594, the disclosure of which is incorporated herein in its entirety by reference. Optionally, the liposomes comprising RLIP76 or proteoliposomes are made using microfluidics, for example, by known techniques such as those described in, e.g., Pradhan et al., Anticancer Research 28:943-8 (2008) and Jahn et al., Langmuir, 23(11):6289-93 (2007), which are incorporated by reference herein in their entireties.

Optionally, the compositions disclosed herein comprise a pharmaceutically acceptable carrier. As used herein, pharmaceutically acceptable carrier includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such pharmaceutically acceptable carriers with pharmaceutical active agents is well known in the art. Except insofar as any conventional media or agent is incompatible with the active agent, its use in the compositions disclosed herein is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

Optionally, the compositions comprise one or more buffers. Most commonly used buffers are salts of weak acids such as carbonates, citrates, gluconates, phosphate and tartrates. Buffers include, but are not limited to, citric acid, sodium phosphate, sodium acetate, dipotassium hydrogen phosphate, phosphoric acid, and L-methionine.

A protein can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include, but are not limited to, the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

As used herein, pharmaceutically-acceptable salts refer to compounds disclosed herein wherein the parent compound is modified by making acid or base salts thereof. Examples of pharmaceutically-acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. Thus, the term

acid addition salt refers to the corresponding salt derivative of a parent compound that has been prepared by the addition of an acid. The pharmaceutically-acceptable salts include, but are not limited to, the conventional salts or the quaternary ammonium salts of the parent compound formed, for example, from inorganic or organic acids. For example, such conventional salts include, but are not limited to, those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, palmoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, and the like. Certain acidic or basic compounds may exist as zwitterions. All forms of the active agents, including free acid, free base, and zwitterions, are contemplated to be within the scope of the present disclosure.

RLIP76 compositions can be complexed with polyethylene glycol (PEG), metal ions, or incorporated into polymeric compounds such as polylactic acid, polyglycolic acid, hydrogels, dextran, etc., or incorporated into liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts or spheroblasts. Such compositions will influence the physical state, solubility, stability, rate of in vivo release, and/or rate of in vivo clearance, and are thus chosen according to the intended application.

In addition, RLIP76, or one or more active fragments thereof, can be bound, for example, by covalent, non-covalent, ionic, or hydrophobic bonds, with any number of different delivery vehicles, including, but not limited to, liposomes, proteoliposomes, vesicles, nanoparticles, noisosomes, carrier proteins, gold particles, chitin, polymers, organic "cages," viruses, and bacteria. In addition, preferential uptake of any of the above RLIP76 compositions by one or more specific organs, tissues, or cell types can be accomplished by the inclusion of one or more specific targeting moieties with RLIP76 or any of the delivery vehicles listed above. Such targeting moieties include, but are not limited to, antibodies, or fragments thereof, peptides, lipids, chemicals, charged particles, receptors, proteins, viral promoters, transcription factors, DNA promoters, and nucleic acids that have a particular two- or three-dimensional structure.

The disclosed compounds can be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or they can be enclosed in hard or soft shell gelatin capsules, or they can be incorporated directly with the food of the diet. For oral therapeutic administration, the active compounds can be incorporated with excipients and used in the form of tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active agent. The percentage of the compositions and preparations may, of course, be varied. The amount of active agents in such therapeutically useful compositions is such that a suitable dosage will be obtained.

Compositions and formulations for oral administration include, but are not limited to, powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, and tablets. Such compositions may also include thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders. Optionally, the compositions formulated for oral administration include one or more buffers, one or more mucoadhesive polymers, one or more permeation enhancers and combinations thereof.

Tablets, troches, pills, capsules and the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the composition is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the composition. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup of elixir may contain sucrose as a sweetening agent methyl and propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor. Of course, any material used in preparing any composition should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active agents may be incorporated into sustained-release preparation and formulations.

The active agents may be administered parenterally or intraperitoneally. Solutions of the active agents as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 mL of isotonic NaCI solution and either added to 1000 mL of hypodermoclysis fluid or injected at the proposed site of infusion. Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be suitably fluid. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or

sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use of compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active agents in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active agents into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The disclosed compositions can be formulated to be administered by use of a skin patch or other transdermal delivery system. Transdermal administration can be accomplished by any of a number of systems known in the art. Examples of systems that may be adapted for use with the compositions described herein include those systems of transdermal administration described in U.S. Pat. No. 4,816,252; U.S. Pat. No. 5,122,382; U.S. Pat. No. 5,198,223; U.S. Pat. No. 5,023,084; U.S. Pat. No. 4,906,169; U.S. Pat. No. 5,145,682; U.S. Pat. No. 4,624,665; U.S. Pat. No. 4,687,481; U.S. Pat. No. 4,834,978; and U.S. Pat. No. 4,810,499, each of which is incorporated herein by reference.

The provided methods may include an adhesive matrix or drug reservoir system and may include a skin permeation enhancement agent such as ethanol, polyethylene glycol 200 dilaurate, isopropyl myristate, glycerol trioleate, linolenic acid saturated ethanol, glycerol monooleate, glycerol monolaurate, n-decyl alcohol, capric acid, and certain saturated and unsaturated fatty acids, and their esters, alcohols, monoglycerides, acetate, diethanolamides and N,Ndimethylamides (see, for examples, U.S. Pat. No. 4,906,169).

The provided compositions can be administered one or more times daily, weekly or monthly. Optionally, the composition is administered twice daily. Optionally, the composition is administered for one or more days or weeks prior to or

after the onset of one or more symptoms of oxidative stress or CNS disease, injury or condition. Optionally, the composition is administered daily for one week prior to and for four weeks the onset of symptoms. In the case of traumatic brain injury or stroke, for example, the composition is optionally administered daily for at least one week prior to the injury and for at least four weeks after the injury. However, the treatment periods before and after the lesion or onset of symptoms can vary.

As used herein, and unless otherwise indicated, the terms treat, treating, and treatment contemplate an action that occurs while a patient is suffering from a disease or condition, that reduces the severity of one or more symptoms or effects of the disease or condition, or a related disease or condition. Thus in the disclosed method, treatment can refer to a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% reduction in the severity of an established disease or condition or symptom of the disease or condition. For example, a method for treating a disease is considered to be a treatment if there is a 10% reduction in one or more symptoms of the disease in a subject as compared to a control. Thus the reduction can be a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or any percent reduction in between 10% and 100% as compared to native or control levels. It is understood that treatment does not necessarily refer to a cure or complete ablation of the disease, condition, or symptoms of the disease or condition.

As used herein, and unless otherwise indicated, the terms manage, managing, and management encompass preventing, delaying, or reducing the severity of a recurrence of a disease or condition in a patient who has already suffered from the disease or condition. The terms encompass modulating the threshold, development, and/or duration of the disease or condition, or changing the way that a patient responds to the disease or condition.

As used herein, the terms prevent, preventing, and prevention of a disease or condition refers to an action, for example, administration of a therapeutic agent, that occurs before or at about the same time a subject begins to show one or more symptoms of the CNS disease or condition, which inhibits or delays onset or exacerbation of one or more symptoms of the disease or condition. As used herein, references to decreasing, reducing, or inhibiting include a change of 10%, 20%, 30%,

40%, 50%, 60%, 70%, 80%, 90% or greater as compared to a control level. Such terms can include but do not necessarily include complete elimination.

As used herein, and unless otherwise specified, a therapeutically effective amount of a compound is an amount sufficient to provide any therapeutic benefit in the treatment or management of a disease or condition, or to delay or minimize one or more symptoms associated with a disease or condition. A therapeutically effective amount of a compound means an amount of the compound, alone or in combination with one or more other therapy and/or therapeutic agent, which provides any therapeutic benefit in the treatment or management of a disease or condition, or related diseases or conditions. The term therapeutically effective amount can encompass an amount that cures a disease or condition, improves or reduces a disease or condition, reduces or avoids symptoms or causes of a disease or condition, improves overall therapy, or enhances the therapeutic efficacy of another therapeutic agent.

Toxicity and therapeutic efficacy of the described compounds and compositions can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, expressed as the ratio LD50/ED50. Compounds that exhibit large therapeutic indices are preferred. Compounds that exhibit toxic side effects may be used; however, care should usually be taken to design delivery systems that target such compounds preferentially to the site of affected tissue (for example, central nervous system), in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

Data obtained from cell culture assays and animal studies can be used in formulating a range of dosages for use in humans. In certain aspects of the present disclosure, the dosages of such compounds lie within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending on the dosage form employed and the route of administration utilized. For any compound used in the disclosed methods, the therapeutically effective dose can be estimated initially from cell culture assays. A

dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (i.e., the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Plasma levels may be measured, for example, by high performance liquid chromatography.

When therapeutic treatment is contemplated, the appropriate dosage may also be determined using animal studies to determine the maximal tolerable dose, or MTD, of a bioactive agent per kilogram weight of the test subject. In general, at least one animal species tested is mammalian. Those skilled in the art regularly extrapolate doses for efficacy and avoiding toxicity to other species, including human. Before human studies of efficacy are undertaken, Phase I clinical studies help establish safe doses. Additionally, the bioactive agent may be complexed with a variety of well-established compounds or structures that, for instance, enhance the stability of the bioactive agent, or otherwise enhance its pharmacological properties (e.g., increase *in vivo* half-life, reduce toxicity, etc.).

The provided compositions can contain from 0.1 microgram/kg body weight to 1000 mg/kg body weight of the RLIP76. Optionally, the composition contains 100 to 1500 micrograms of RLIP76. Optionally, the composition contains 1 mg of RLIP76. Optionally, the effective dose of the composition or dosage unit can be in the range of about 14 mg/kg to about 0.01 mg/kg, about 14 mg/kg to about 0.025 mg/kg, about 14 mg/kg to about 0.05 mg/kg, about 14 mg/kg to about 0.1 mg/kg, about 14 mg/kg to about 0.25 mg/kg, about 14 mg/kg to about 0.5 mg/kg, about 14 mg/kg to about 1 mg/kg, about 14 mg/kg to about 2.5 mg/kg, about 14 mg/kg to about 5 mg/kg, about 5 mg/kg to about 0.01 mg/kg, about 2.5 mg/kg to about 0.01 mg/kg, about 1 mg/kg to about 0.01 mg/kg, about 0.5 mg/kg to about 0.01 mg/kg, about 0.25 mg/kg to about 0.01 mg/kg, about 0.1 mg/kg to about 0.01 mg/kg, about 0.05 mg/kg to about 0.01 mg/kg, about 0.025 mg/kg to about 0.01 mg/kg, about 5 mg/kg to about 0.025 mg/kg, about 2.5 mg/kg to about 0.05 mg/kg, about 1 mg/kg to about 0.1 mg/kg, about 0.5 mg/kg to about 0.25 mg/kg, or about 3 mg/kg to about 0.1 mg/kg, or so. Thus, the effective dose of the composition or dosage unit can be about 0.01 mg/kg, about 0.025 mg/kg, about 0.05 mg/kg, about 0.075 mg/kg, about 0.1 mg/kg,

about 0.25 mg/kg, about 0.5 mg/kg, about 0.75 mg/kg, about 1 mg/kg, about 2.5 mg/kg, about 3 mg/kg, about 5 mg/kg, about 7.5 mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg, about 13 mg/kg, about 14 mg/kg, or so.

The methods and agents as described herein are useful for both prophylactic and therapeutic treatment. For prophylactic use, a therapeutically effective amount of the agents described herein are administered to a subject prior to onset (e.g., before obvious signs of disease) or during early onset (e.g., upon initial signs and symptoms of disease). Prophylactic administration can occur for several days to years prior to the manifestation of symptoms of disease. Prophylactic administration can be used, for example, in the preventative treatment of subjects diagnosed with a genetic predisposition to disease. Therapeutic treatment involves administering to a subject a therapeutically effective amount of the agents described herein after diagnosis or development of disease.

As used throughout, subject can be a vertebrate, more specifically a mammal (e.g., a human, horse, cat, dog, cow, pig, sheep, goat, mouse, rabbit, rat, and guinea pig), birds, reptiles, amphibians, fish, and any other animal. The term does not denote a particular age or sex. Thus, adult and newborn subjects, whether male or female, are intended to be covered. As used herein, patient or subject may be used interchangeably and can refer to a subject with a disease or condition (e.g., autoimmune disease, viral infection, or cancer). The term patient or subject includes human and veterinary subjects.

Optionally, the subject has or is at risk of having a disease or condition of the central nervous system (CNS). Optionally, the disease or condition of the central nervous system is associated with oxidative stress. Diseases or conditions of the central nervous system include, but are not limited to, Traumatic Brain Injury (TBI), Alzheimer's Disease (AD), Parkinson's Disease (PD), Huntington's Disease, macular degeneration, age-related macular degeneration, Amyotrophic Lateral Sclerosis (ALS), Multiple Sclerosis (MS), brain injury, ischemia, reperfusion injury, stroke, schizophrenia, epilepsy, glioma (or brain cancer), post-chemotherapy cognitive dysfunction ("chemo brain"), Friedreich's ataxia and AIDS dementia. By way of example, age-related macular degeneration (AMD) is a disease or the CNS and is associated with high levels of ROS due to the high metabolic activity in the macula.

With age, the balance between production of ROS and local antioxidant levels is shifted, and damage ensues. Optionally, the disease or condition is Alzheimer's Disease (AD), traumatic brain injury (TBI), ischemia, stroke, or amyotrophic lateral sclerosis (ALS).

Since clinically effective TBI therapy is not currently available, pharmacological agents like RLIP76, a combined treatment with mechanistically complementary antioxidants that simultaneously scavenge LP-initiating free radicals and remove neurotoxic LP byproducts, may be an effective approach to interrupting post-traumatic injury. By inhibiting free radical-induced LP and its neurotoxic effects, RLIP76 may act as a neuroprotectant to reduce neuroinflammation and increase neurorepair, which have clinically relevant therapeutic potential in TBI.

One of skill in the art can determine whether a subject has or is at risk of developing a central nervous system disease or condition or a central nervous system disease or condition associated with oxidative stress. For example, various clinical symptoms, signs, and assays are associated with various diseases and conditions. These are well known to those of skill in the art. Furthermore, genetic testing or family history can be used to determine if one is at risk of developing such a disease or condition. Finally, occupations or activities can place subjects at risk of developing a central nervous system disease or condition or a central nervous system disease or condition associated with oxidative stress. Examples of such occupations or activities include military occupations, sports (such as boxing and football), and the like.

Kits for treating a disease or condition of the CNS are also provided. A typical kit comprises one or more dosage units of a composition comprising RLIP76, or a pharmaceutically acceptable salt, prodrug, solvate, hydrate, stereoisomer, liposome, or proteoliposome thereof. Kits can further comprise devices that are used to administer the active ingredients. Examples of such devices include, but are not limited to, syringes, drip bags, patches, and inhalers. Optionally, the kits include instructions for use.

The disclosed kits can further comprise pharmaceutically acceptable vehicles that can be used to administer one or more disclosed compositions. For example, if a disclosed composition is provided in a solid form that is to be reconstituted for

parenteral administration, the kit can comprise a sealed container of a suitable vehicle in which the disclosed composition can be dissolved to form a particulate-free sterile solution that is suitable for parenteral administration. Examples of pharmaceutically acceptable vehicles include, but are not limited to, water; aqueous vehicles such as, but not limited to, sodium chloride injection, Ringer's injection, dextrose injection, dextrose and sodium chloride injection, and lactated Ringer's injection; water miscible vehicles such as, but not limited to, ethyl alcohol, polyethylene glycol, and polypropylene glycol; and non-aqueous vehicles such as, but not limited to, corn oil, cottonseed oil, peanut oil, sesame oil, ethyl oleate, isopropyl myristate, and benzyl benzoate.

Disclosed are materials, compositions, and components that can be used for, can be used in conjunction with, can be used in preparation for, or are products of the disclosed methods and compositions. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutations of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a composition is disclosed and discussed and a number of modifications that can be made to a number of molecules including the composition are discussed, each and every combination and permutation of the composition, and the modifications that are possible are specifically contemplated unless specifically indicated to the contrary. Likewise, any subset or combination of these is also specifically contemplated and disclosed. This concept applies to all aspects of this disclosure including, but not limited to, steps in methods using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed, it is understood that each of these additional steps can be performed with any specific method steps or combination of method steps of the disclosed methods, and that each such combination or subset of combinations is specifically contemplated and should be considered disclosed.

Publications cited herein and the material for which they are cited are hereby specifically incorporated by reference in their entireties.

A number of embodiments have been described. Nevertheless, it will be understood that various modifications may be made. Accordingly, other embodiments are within the scope of the claims below.

Examples

Example 1. Investigation of Neuroprotective Properties of RLIP76 in a Model of Acute Ischemia in Rats.

The neuroprotective effect of RLIP76 on neurological deficits and infarct volumes induced by a temporal focal cerebral ischemia in a rat model of stroke was evaluated. Middle cerebral artery occlusion (MCAO) results in a rapid onset of focal neurologic deficit resulting from brain infarction in the territory supplied by the middle cerebral artery (MCA). In man, MCAO is the most common cause of cerebral ischemia stroke. The objective of this study was to investigate the therapeutic activity of RLIP76 in brain ischemic injury and associated neurological deficits induced by a temporal MCAO in rats.

Materials and Methods

RLIP76, 0.7 mg/mL or vehicle alone were used in solution form (10 mM Tris, 2 mM MgCl2, 1 mM EDTA, 100 mM NaCl, 1 % dextrose, 0.5% glutathione, and 0.025% Tween 80).

Male Sprague-Dawley rats (250 to 300 g) were obtained from Shanghai Laboratory Animal Co. LTD (SLAC). The rats were selected for inclusion based upon acceptable clinical condition and body weight. Animals were randomly assigned to 2 groups (12/group for vehicle and RLIP76 treatment groups). The rats were kept one week acclimation in the animal facility prior to any procedures.

Upon receipt and throughout the study, the animals were housed in groups (n = 4-5/cage) in regular bedding (wood dust) cages. Animals were provided food and water ad libitum. Room humidity (50 – 70%) and temperature (22 – 25 °C) were maintained consistently. In addition, the study room was maintained on a 12-hour light/dark cycle.

RLIP76 was administered (IV) three times, immediately after removing the occluder, at 24 and 48 hours after MCAO surgery.

Table 2. Study Design

Group	Treatment	N	Route of Administration	Dosing Level	Dosing Volume	Schedule
1	Vehicle	12	IV	-	1.43 mL	Once a day for 3 days
2	RLIP76	12	IV	1 mg/mouse	1.43 mL	Once a day for 3 days

Clinical signs and abnormal behaviors were checked at least twice daily and carefully recorded during the experiment. All animals were examined daily for mortality and signs of ill health.

The animals were fasted overnight but allowed free access to water prior to the MCAO surgery. Under anesthesia, the right common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) were exposed through a midline incision of the neck. A commercial monofilament (silicon-coated) was used as an occluder and inserted via the ECA. The occluder was advanced into the ICA 18 ± 0.5 mm beyond the carotid bifurcation. Mild resistance indicated that the occluder was properly lodged in the anterior cerebral artery and blocked blood flow to the middle cerebral artery (MCA). After 1 hour, reperfusion was allowed by withdrawing the monofilament approximately 10 mm. Body temperature was kept around 36.5°C with a heating pad during the surgery process. Clinical signs (a standardized neurobehavioral battery) were tested at 60 minutes after occlusion by the observer blinded to the treatment group to confirm neurological deficit (rats without left upper extremity paresis was excluded from further study). The neurological deficits were evaluated again at 24, 48, and 72 hours after MCAO. Three days after MCAO, animals were euthanized and the brains were cut into 6 coronal sections (2 mm in thickness by use of a rat brain matrix). The fresh brain sections were stained with 2% solution of triphenyltetrazolium chloride (TTC) at 37°C and then fixed with 4% paraformaldehyde. Pictures of all sections were taken with a digital camera. These digital pictures were put into a computer. After a blind measurement of infarct area (%) for each section with Image-Pro or Photoshop 7.0 (pixels), the infarct volumes per brain were obtained. The infarct area (%) = (contralateral hemisphere area –

ipsilateral non-infarct area)/contralateral hemisphere area. Tissue swelling was corrected with this formula.

The standardized battery test was performed based on the previous description (Belayev et al. Stroke. 1996; 27:1616-1623). The score is as follows:

0 - no neurological deficit

1 - failure to extend left forepaw fully

2 - inconstant circling to the left

3 - constant circling to the left;

4 - falling to the left;

5 - no spontaneous walking with depressed level of consciousness;

6 - death

All animals were euthanatized with asphyxiation by CO_2 and decapitation.

Student's t-test was used in this study. $p<0.05$ was considered as significant differences between the groups.

Animals were weighed before compound administration. Dosing regimen of test compound and vehicle was selected following Sponsor's guideline (Experiment Design).

All animals showed good general condition before the study. During this experiment, the mortality was 3 of 12 (25%) in the vehicle group and 2 of 12 (16.7%) in the RLIP76-treated group, respectively. The animal death occurred within 24 hr (2 controls and 1 RLIP76 group) or within 48 hr (1 control and 1 RLIP76 group) after MCAO surgery. All other animals remained in a relatively stable health condition until euthanasia.

Animals were monitored carefully until consciousness. All animals were fully recovered after the last step of surgery (the occluder was removed).

The rats treated with RLIP76 showed remarkably less infarct volumes (23.35% ± 3.08) when compared with the vehicle group (38.88% ± 3.02). This difference was statistically significant ($p < 0.01$, t-Test). Most infarction in the vehicle- and RLIP76-treated groups was involved in the striatum and fronto-parietal cortex that were typically supplied by the middle cerebral artery. Notably, the infarct size in the RLIP76 group was smaller with less involvement of the striatum and the

corresponding cortex comparing with that in the Vehicle group. The detailed analysis results of infarct volume are shown in Figure 2 and Table 3.

Table 3. Infarct volume (%)

Groups	Infarct Volume (%, Mean±SEM)
Vehicle	38.88±3.02
RLIP76	23.53±3.08

Typical infarct areas in brain sections for each group are represented in Figure 3.

The ischemic insult caused clinical signs of motor function impairments on the left side. Treatment with RLIP76 tended to reduce the neurological deficit scores although it did not reach statistical significance (p=0.476, 0.303, 0.374 in Day 1-3 respectively). The detailed information on assessment of neurological deficit scores is shown in Figure 4 and Table 4.

Table 4. Assessment of neurological deficits immediately after removing the occluder, 24, 48 and 72 hours after MCAO in rat.

Groups	Neurological scores of clinical signs (Mean±SEM)			
	Day 0	Day 1	Day 2	Day 3
Vehicle	2.83±0.17	2.67±0.59	2.50±0.62	2.17±0.67
RLIP76	2.75±0.25	2.08±0.54	1.58±0.61	1.50±0.62

This study investigated the therapeutic efficacy of RLIP76 on cerebral ischemia in a rat MCAO model. RLIP76 exhibited a significant and marked neuroprotective effect on brain ischemic injury and this was associated with a non-significant improvement in neurological deficits and survival. Rat mortalities after MCAO surgery were 25% in the vehicle group and 16.7% in the RLIP76-treated group. RLIP76 lowered neurological scores of clinical signs. The infarct volumes were significantly reduced in the RLIP76-treated group (23±3%), when compared

with the vehicle (39±3%; $p<0.01$, t-Test). This study suggests that IV administration of RLIP76 improves neurological deficits and protects against ischemic in a 1-hour MCAO model in rats.

Example 2. Investigation of the Efficacy of RLIP76-PL in a Traumatic Brain Injury (TBI) Mouse Model.

To investigate the effect of RLIP76 proteoliposome in TBI, a controlled closed skull injury model in the C57BL/6 mouse is used (Watts et al., J Neurotrauma. 30(1):55-66 (2013)).

Efficacy of RLIP76 proteoliposome (RLIP76-PL) is measured via reduced edema formation, reduced levels of oxidative metabolites and lipid peroxidation, and/or improved neuronal recovery in a controlled closed skull injury TBI mouse model. Efficacy of RLIP76-PL is measured via reduced cell swelling and reactive gliosis.

Table 5: Study Design

Group #	Description	Administration Route for Treatment	Treatment Dose of RLIP76-PL	Number of Mice (n)
Group 0	Sham injury, RLIP76-PL treatment	IV	100 µg/dose	6
Group 1	Sham injury, vehicle treatment	IV	0	6
Group 2	TBI injury, vehicle treatment	IV	0	6
Group 3	TBI injury, RLIP76-PL treatment	IV	100 µg/dose	6

Study animals receive intravenous (IV) injection of vehicle or RLIP76-PL treatment as described by the schedule in Table 6 below.

Table 6. Dosing & Harvest Schedule

Group #	Dosing Schedule Post-TBI	Day 3 Harvest
0	3 doses per animal: +30 min, +4 hours, +8 hours	n = 6
1		n = 6
2		n = 6
3		n = 6

Brains are harvested at 3 days (n=6 from each group) post TBI. To do so, at specified survival times, three mice from each group are anesthetized under isoflurane, sacrificed, and prepared accordingly for the study of edema formation-measure brain water contents, Nissl staining-identify neuronal changes and morphology of the brain, GFAP staining- examine astrocytes, TUNEL staining-apoptosis, SOD and GPx- oxidative stress, and 4-HNE and MDA- lipid peroxidation.

RLIP76 is expected to reduce edema formation, reduce levels of oxidative metabolites and/or improve neuronal recovery in TBI.

SEQ ID NO:1 Amino Acid Sequence of RLIP76

mtecflpptsspsehrrvehgsgltrtpsseeisptkfpglyrtgepspphdilheppdvvsddekdhgkkkg
kfkkkekrtegyaafqedssgdeaespskmkrskgihvfkkpsfskkkekdfkikekpkeekhkeekhkeekhke
kkskdltaadvvkqwkekkkkkkpiqepevpqidvpnlkpifgipladavertmmydgirlpavfrecidyvekyg
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llkelpecnylliswlivhmdhviakeletkmniqnisivlsptvqisnrvlyvffthvqelfgnvvlkqvmkplrwsn
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akeqpkagkepakpspsrdrketsi

WHAT IS CLAIMED IS:

1. A method of inhibiting oxidative stress in a subject comprising:
   (a) selecting a subject having a disease or condition of the central nervous system; and
   (b) administering to the subject an effective amount of a composition comprising RLIP76.
2. A method of treating a disease or condition of the central nervous system comprising:
   (a) selecting a subject having a disease or condition of the central nervous system; and
   (b) administering to the subject an effective amount of a composition comprising RLIP76.
3. The method of claim 1 or 2, wherein the central nervous system disease or condition is associated with oxidative stress.
4. The method of claim 1 or 2, wherein the disease or condition is traumatic brain injury.
5. The method of claim 1 or 2, wherein the disease or condition is Alzheimer's disease.
6. The method of claim 1 or 2, wherein the disease or condition is ALS.
7. The method of claim 1 or 2, wherein the disease or condition is ischemia.
8. The method of claim 1 or 2, wherein the disease or condition is stroke.
9. The method of any one of claims 1-8, wherein the composition further comprises a liposome.
10. The method of claim 9, wherein the liposome is selected from the group consisting of lectin, glycolipid, phospholipid and combinations thereof.
11. The method of any one of claims 1-10, wherein the composition is administered orally.
12. The method of any one of claims 1-10, wherein the composition is administered intravenously.
13. The method of any one of claims 1-12, wherein the composition is administered to the subject in one or more doses.

14. The method of any one of claims 1-13, wherein the composition comprises 0.1 micrograms to 1000 mg of RLIP76.
15. The method of claim 14, wherein the composition comprises 100 to 1500 micrograms of RLIP76.
16. The method of claim 14, wherein the composition comprises 1 mg of RLIP76.
17. The method of any one of claims 1-16, wherein the composition is administered one or more times daily.
18. The method of claim 17, wherein the composition is administered once daily.
19. The method of any one of claims 1-18, wherein the RLIP76 comprises one or more ATP binding domains.
20. The method of any one of claims 1-18, wherein the RLIP76 comprises amino acids 1-367 of SEQ ID NO:1.
21. The method of any one of claims 1-18, wherein the RLIP76 comprises amino acids 410-655 of SEQ ID NO:1.

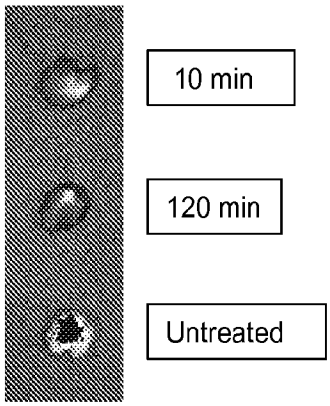
10 min
120 min
Untreated

FIG. 1

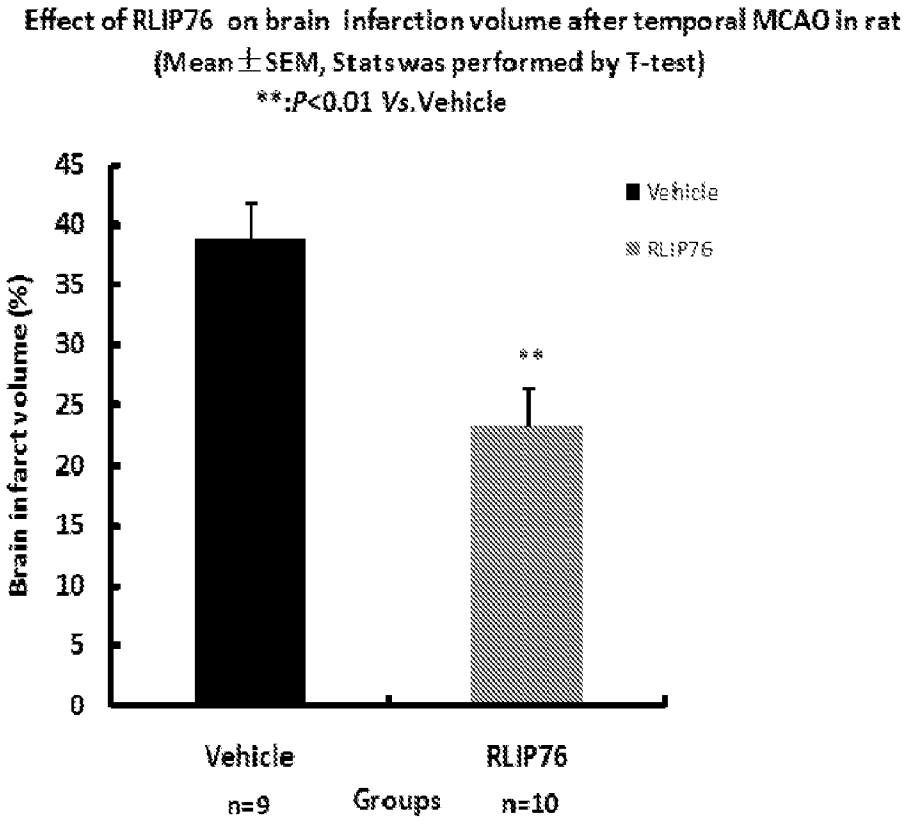
Effect of RLIP76 on brain infarction volume after temporal MCAO in rat
(Mean±SEM, Stats was performed by T-test)
**:P<0.01 Vs.Vehicle
Brain infarct volume (%)
45
40
35
30
25
20
15
10
5
0
Vehicle
RLIP76
**
Vehicle
n=9
Groups
RLIP76
n=10

FIG. 2

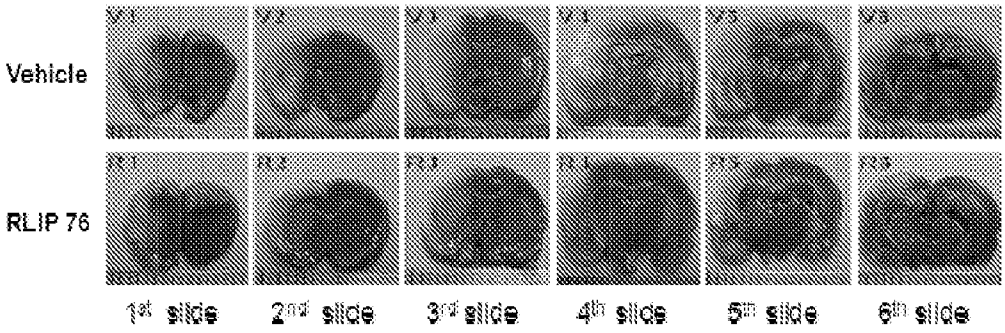
Vehicle
RLIP 76
1st slide
2nd slide
3rd slide
4th slide
5th slide
6th slide

FIG. 3

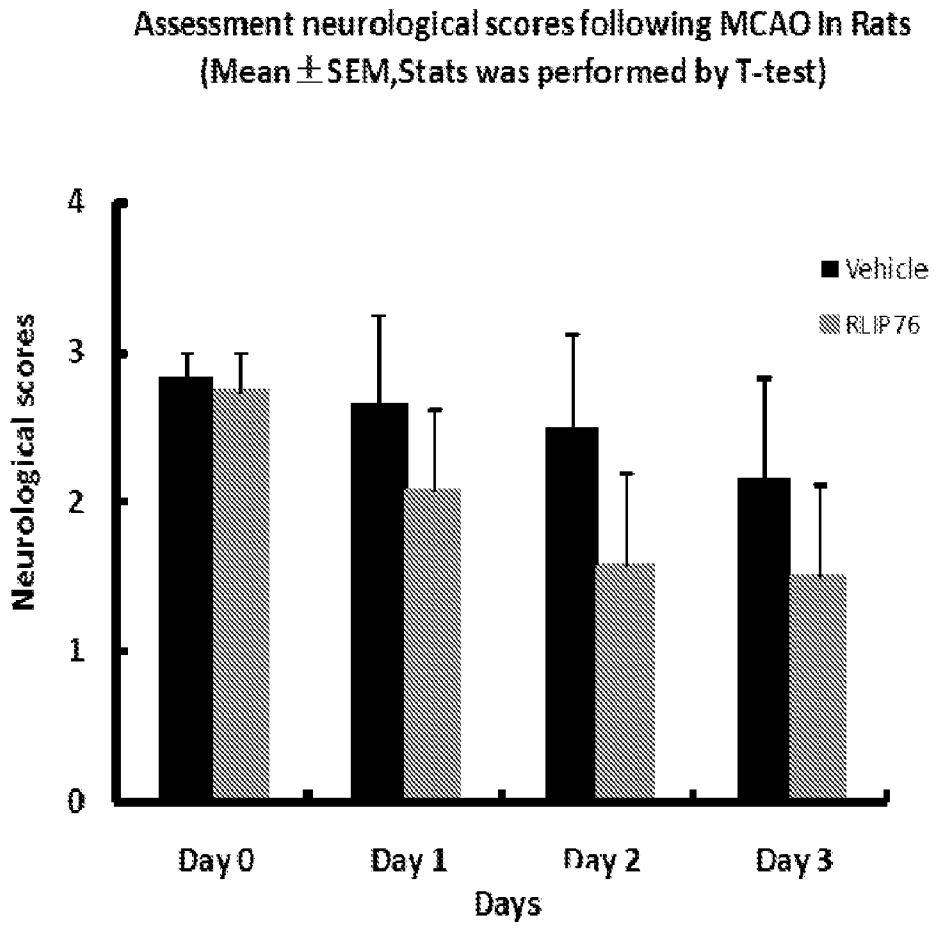
Assessment neurological scores following MCAO In Rats
(Mean ± SEM,Stats was performed by T-test)
Neurological scores
0
1
2
3
4
Vehicle
RLIP 76
Day 0
Day 1
Day 2
Day 3
Days

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/042756

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/17 A61P25/00 A61P25/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE, MEDLINE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/100446 A1 (TERAPIO LLC [US]; CUNNINGHAM C CASEY [US]) 13 August 2009 (2009-08-13) page 1, line 26 - line 35 page 6, line 22 - page 7, line 3 page 12, line 15 - page 13, line 30	1-21
X	US 2010/124566 A1 (AWASTHI SANJAY [US] ET AL) 20 May 2010 (2010-05-20) cited in the application paragraphs [0009], [0013], [0014], [0109]	1-21
X	US 2011/020432 A1 (CUNNINGHAM C CASEY [US]) 27 January 2011 (2011-01-27) paragraphs [0004], [0008], [0043], [0100]	1-21

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 July 2013	29/07/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Pilling, Stephen

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/042756

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		EP 2456455 A1	30-05-2012
		JP 2013500264 A	07-01-2013
		KR 20120052985 A	24-05-2012
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		WO 2011011713 A1	27-01-2011