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(54) Title: METHODS AND PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF MYOCARDIAL INFARCTION

(57) Abstract: The present invention relates to methods and pharmaceutical compositions for the treatment of myocardial infarction and myocardial infarction induced cardiovascular dysfunction. In particular, the present invention relates to a method of treating myocardial infarction in a subject in need thereof comprising administering to the subject a therapeutically effective amount of selenoprotein T or a nucleic acid molecule encoding for selenoprotein T.

METHODS AND PHARMACEUTICAL COMPOSITIONS FOR THE TREATMENT OF MYOCARDIAL INFARCTION

FIELD OF THE INVENTION:

The present invention relates to methods and pharmaceutical compositions for the treatment of myocardial infarction and myocardial infarction induced cardiovascular dysfunction.

BACKGROUND OF THE INVENTION:

Cardiovascular diseases are the leading cause of death in industrialized countries. Indeed, in France, despite major advances in drug treatment, more than 150 000 cardiovascular deaths per year are observed, mainly due to ischemic heart disease such as myocardial infarction and heart failure. This 'relative' ineffectiveness of existing treatments (angiotensin converting enzyme inhibitors, beta-blockers, aldosterone antagonists and If current inhibitors) on overall mortality despite marked improvement of systemic and cardiac hemodynamics, prevention of cardiac remodeling and correction of endothelial dysfunction, suggests that other factors, among which enhanced oxidative stress, continue or start to exert deleterious effects. Oxidative stress, due to an excess of reactive oxygen species, is now recognized as a key player in the progression of heart failure due to its cellular toxicity. In pathological conditions such as myocardial infarction and heart failure, excessive reactive oxygen species production causes cell damage in terms of both structural and metabolism such as oxidation of DNA and proteins, lipid peroxidation and disruption of calcium homeostasis. All these changes contribute to cardiac dysfunction and left ventricular hypertrophy. Moreover, experimental data clearly demonstrated that inhibitors of pro-oxidant enzymes such as apocynin and allopurinol, respectively targeting NADPH- oxidase and xanthine-oxidase, improves myocardial function in animal models of heart failure. It must be stressed that a decrease antioxidant defenses and/or systems, which is also observed in heart failure and even in the case of normal reactive oxygen species production, results in excessive reactive oxygen species levels. In most tissues, antioxidant defenses, such as glutathione peroxidase, superoxide dismutase and catalase but also non-enzymatic antioxidant molecules such as vitamins C, E, A and glutathione, are decreased in overt heart failure, and this decrease in antioxidant defenses is accompanied by an increase in markers of oxidative stress and is correlated with the severity of heart failure. However, although the administration of

anti-oxidants / ROS chelators, i.e. vitamins C / E or mitoQ, slows the progression of heart failure in animal models, but these treatments of exogenous origin are ineffective in humans. In this context, boosting endogenous anti-oxidant defenses might be effective.

Selenoproteins (Sel) are mediators of the biological effects of the micronutrient selenium. Up to today, 25 selenoproteins are identified and are essential in a broad spectrum of biological processes, ranging from embryonic development and cellular redox status regulation to intracellular calcium handling. Indeed, in-utero death during embryonic development is observed after non-selective abrogation of all selenoproteins expression, but also after selective abrogation of the selenoproteins glutathione peroxidase-4, thioredoxine reductase-1 or thioredoxine reductase-22, while the selenoproteins SelW, SelH, gluthatione peroxidase are implicated in the regulation of cellular redox status by neutralizing reactive oxygen species. Among Sels with up to day unknown biological effects, there is selenoprotein T (SelT) that was a recently identified member of a Sel subfamily possessing a thioredoxin-like motif in its structure and is mainly localized in the Golgi apparatus as well as in the endoplasmic reticulum. Although abundant SelT protein expression is observed during embryonic development, SelT expression declines in adulthood, suggesting that in physiological conditions its biological role is restricted to embryonic development. However, in pathological conditions, in particular experimental cerebral ischemia and hepatic regeneration, SelT protein becomes re-expressed and in the case of cerebral ischemia associated with a reduction in ischemic lesions via its anti-oxidant properties related with SelT's thioredoxin-like motif. However no studies has yet addressed the involvement of SelT in the deleterious myocardial and vascular effects of heart failure or the possible underlying cellular mechanisms(e.g. altered oxidative status or cellular Ca2+ handling). Moreover it is unknown whether increasing cardiac SelT, by limiting the immediate, short- and/or long- term consequences of myocardial infarction, could be a therapeutic option.

SUMMARY OF THE INVENTION:

The present invention relates to methods and pharmaceutical compositions for the treatment of myocardial infarction and myocardial infarction induced cardiovascular dysfunction. In particular, the present invention is defined by the claims.

DETAILED DESCRIPTION OF THE INVENTION:

The present invention relates to a method of treating myocardial infarction in a subject in need thereof comprising administering to the subject a therapeutically effective amount of selenoprotein T or a nucleic acid molecule encoding for selenoprotein T.

As used herein, the term "treatment" or "treat" refer to both prophylactic or preventive treatment as well as curative or disease modifying treatment, including treatment of subjects at risk of contracting the disease or suspected to have contracted the disease as well as subjects who are ill or have been diagnosed as suffering from a disease or medical condition, and includes suppression of clinical relapse. The treatment may be administered to a subject having a medical disorder or who ultimately may acquire the disorder, in order to prevent, cure, delay the onset of, reduce the severity of, or ameliorate one or more symptoms of a disorder or recurring disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment. By "therapeutic regimen" is meant the pattern of treatment of an illness, e.g., the pattern of dosing used during therapy. A therapeutic regimen may include an induction regimen and a maintenance regimen. The phrase "induction regimen" or "induction period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the initial treatment of a disease. The general goal of an induction regimen is to provide a high level of drug to a subject during the initial period of a treatment regimen. An induction regimen may employ (in part or in whole) a "loading regimen", which may include administering a greater dose of the drug than a physician would employ during a maintenance regimen, administering a drug more frequently than a physician would administer the drug during a maintenance regimen, or both. The phrase "maintenance regimen" or "maintenance period" refers to a therapeutic regimen (or the portion of a therapeutic regimen) that is used for the maintenance of a subject during treatment of an illness, e.g., to keep the subject in remission for long periods of time (months or years). A maintenance regimen may employ continuous therapy (e.g., administering a drug at a regular intervals, e.g., weekly, monthly, yearly, etc.) or intermittent therapy (e.g., interrupted treatment, intermittent treatment, treatment at relapse, or treatment upon achievement of a particular predetermined criteria [e.g., disease manifestation, etc.]).

In particular, the method of the present invention is suitable for the treatment of left ventricular (LV) dysfunction after myocardial infarction. More particularly, the method of the present invention is suitable for restoring cardiac output and LV fractional shortening associated with improvement of LV end-diastolic/end-systolic pressures and LV tissue

perfusion. Typically, the improved LV function is an improvement in New York Heart Association Class, incorporated by reference herein. In addition, or separately, the improved LV function is preferably an improvement in hemodynamics which include reductions in LV diastolic pressures, reductions in pulmonary artery pressures, increases in cardiac output and declines in heart rate. In addition, or separately, the improved LV function is preferably a greater than 5% increase in LV ejection fraction.

In some embodiments, the Selenoprotein T or the nucleic acid encoding thereof is administered to a subject having one or more signs or symptoms of acute myocardial infarction injury. In some embodiments, the subject has one or more signs or symptoms of myocardial infarction, such as chest pain described as a pressure sensation, fullness, or squeezing in the mid portion of the thorax; radiation of chest pain into the jaw or teeth, shoulder, arm, and/or back; dyspnea or shortness of breath; epigastric discomfort with or without nausea and vomiting; and diaphoresis or sweating.

As used herein, the term “Selenoprotein T” or “SelT” has its general meaning in the art and refers to the SELT gene (Gene ID: 51714). This gene encodes a selenoprotein, which contains a selenocysteine (Sec) residue at its active site. The selenocysteine is encoded by the UGA codon that normally signals translation termination. The 3' UTR of selenoprotein genes have a common stem-loop structure, the sec insertion sequence (SECIS), that is necessary for the recognition of UGA as a Sec codon rather than as a stop signal. An exemplary human amino acid sequence is SEQ ID NO:1 and an exemplary human nucleic acid sequence is SEQ ID NO:2. According to the present invention, the term "selenoprotein T" here means any polypeptide having at least 90% of identity with SEQ ID NO:1.

SEQ ID NO:1 (NCBI Reference Sequence: NP_057359.2)

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  1 mrllllllva asamvrseas anlggvpskr lkmqyatgpl lkfqicvsug yrrvfeeymr
 61 visqrypdir iegenylpqp iyrhiasfls vfklvligli ivgkdpfaff gmqapsiwqw
121 gqenkvyacm mvfflsnmie nqcmstgafe itlndvpvws klesghlpsm qqlvqildne
181 mklnvhmdsi phhrs
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SEQ ID NO:2 (NCBI Reference Sequence: NM_016275.3)

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   1 tgcgcagtgg ggagcagctc gctcctgggc tttgggctgg ctgcagtctg tctgagggcg
  61 gccgaagtgg ctggctcatt taagatgagg cttctgctgc ttctcctagt ggcggcgtct
 121 gcgatggtcc ggagcgaggc ctcggccaat ctgggcggcg tgcccagcaa gagattaaag
 181 atgcagtacg ccacggggcc gctgctcaag ttccagattt gtgtttcctg aggttatagg
 241 cgggtgtttg aggagtacat gcgggttatt agccagcggt acccagacat ccgcattgaa
 301 ggagagaatt acctccctca accaatatat agacacatag catctttcct gtcagtcttc
 361 aaactagtat taataggctt aataattgtt ggcaaggatc cttttgcttt ctttggcatg
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 421 caagctccta gcatctggca gtggggccaa gaaaataagg tttatgcatg tatgatggtt
 481 ttcttcttga gcaacatgat tgagaaccag tgtatgtcaa caggtgcatt tgagataact
 541 ttaaatgatg tacctgtgtg gtctaagctg gaatctggtc accttccatc catgcaacaa
 601 cttgttcaaa ttcttgacaa tgaaatgaag ctcaatgtgc atatggattc aatcccacac
 661 catcgatcat agcaccacct atcagcactg aaaactcttt tgcattaagg gatcattgca
 721 agagcagcgt gactgacatt atgaaggcct gtactgaaga cagcaagctg ttagtacaga
 781 ccagatgctt tcttggcagg ctcgttgtac ctcttggaaa acctcaatgc aagatagtgt
 841 ttcagtgctg gcatattttg gaattctgca cattcatgga gtgcaataat actgtatagc
 901 tttccccacc tcccacaaaa tcacccagtt aatgtgtgtg tgtgtttttt ttttaaggta
 961 aacattacta cttgtaactt tttttcttag tcatatttga aaaagtagaa aattgagtta
1021 caatttgatt ttttttccaa agatgtctgt taaatctgtt gtgcttttat atgaatattt
1081 gttttttata gtttaaaatt gatcctttgg gaatccagtt gaagttccca aatactttat
1141 aagagtttat cagacatctc taatttggcc atgtccagtt tatacagttt acaaaatata
1201 gcagatgcaa gattatgggg gaaatcctat attcagagta ctctataaat ttttgtgtat
1261 gtgtgtatgt gcgtgtgatt accagagaac tactaaaaaa accaactgct ttttaaatcc
1321 tattgtgtag ttaaagtgtc atgccttgac caatctaatg aattgattaa ttaactgggc
1381 ctttatactt aactaaataa aaaactaagc agatatgagt taaatttaaa agtttcaatt
1441 tattgctcag tgtacctgtt aacattatat ttaacaattg cttaaatttt tgtttttgat
1501 ttatggataa tttcttaaga gtacacactt tagatacaca aataatcgtt catttaccat
1561 ctttaggatc attgaaactc atctcactaa agaaagttca cttgaacctc tttatagcat
1621 tgatactagg tgaacagaaa ttacctgact aataatttgt ctaacatcat atatcagaat
1681 tttattgtat atgatgaaca aaacttaaaa ttttttaaat ttaattttta aatactgttt
1741 cagagttcta aaaaggcagt tttttaaaaa acttaagttg ataaaaactg taagaataat
1801 ttagcagaaa tagaaccaga atgtagaaga gtagtcatgt aacagcagta ataacatact
1861 tcagcttcca tataggaata gaagtggtag agccaaaagt gatttaggaa aagttataag
1921 gtacaggttg agtatccctt ttccaaaaat gcttgggaca agaagtattt cagatttcat
1981 aatttttttc aaagtttgga atatttgcat tatacttacc agttgggcat cccaaatctg
2041 aaatctgaaa tgttccatga gcatttcctt tgagtgtcat gttggcactc aaaaaggttc
2101 aacattgagt ccacttaaca cttaggtgtt agaagaccta actttctgta acaattaacc
2161 ttatactttg tttgtcatcg aatatttgtt gaatgcatgt caggtaatgg tcttgattgt
2221 gatagcttca aggtggaaca tactgtaatc tccagatgct aggaagttag tctaataatt
2281 cactgcagaa aattgattaa gtggctgtcc ttttaattaa gagtgtggag tcataaactt
2341 aagttcttca tatagtgaca agagtcctta gagattgtta ttcaagttcc ttagaaattg
2401 ttatttaggt ataatatcat cttgtctttg actagagctt gaaaccttgt tatctgattg
2461 tgtaccactc caaattccct gccttctgca agttgaatgt cttgctgaat gtgtctaggg
2521 gttcatcttc agtaatcgac attccactag tgccatagtt aacttcatga catgtagaca
2581 ttcaaaactt gagccttgga tgttcctgtg gacctgacag ttaaaaatat aaagaaccta
2641 ggattcaatt ccaactttct ctgtttgcct tgggttgaat aacttatctt ttggagaata
2701 gctttaagtg gcttagacac tgataaaatt cagctgtgtt gttgacgctc atctcttttg
2761 tcttacgctt agccatattt aaatcttgaa tttaatagag tctagtgaaa aaaatgagtg
2821 ggaagaatga atataaaagt aataatataa ggaaaaaggg aaagtaaact atttagaatg
2881 tagttttgtt atattcccag catttcaata tttattagtt acttgtaaat tactgtggct
2941 gtgtagttta taaatgtctg tgcactatat taattagaag accatagaac atgccagcag
3001 gttggctaat gctatggggg ttttttaccac agttgccatt gtggaagaaa ttatttggta
3061 cattaataaa aaaagttggt aaaacatggt tttatacctc agtgtataag atgtgcaaga
3121 caaatatgct tatttccttt tctagaatat aagtgatatt atttgcttat gacactaaca
3181 ctattaatga caggagtcaa tcagccttta cagctatcaa aatataatga gatcccaatg
3241 atgattcttt tttactttga atgttaatta gtttgggact ttgattggct ggcaaacatt
3301 ttatcattgt cagaatttaa tttagatttc aaaaatagct tacaggattt taaacatggt
3361 gtggtattct aaagcctttt ttttaaaaaa agagatcttt ttgagagaaa caaatgagga
3421 ttgtaaagtt tggggactta cctctgtagc attgtgaaaa taaactttga ttaagctgat
3481 ttgaaaggaa aaaaaaaa
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According to the invention a first amino acid sequence having at least 90% of identity with a second amino acid sequence means that the first sequence has 90; 91; 92; 93; 94; 95; 96; 97; 98; 99 or 100% of identity with the second amino acid sequence. Amino acid sequence identity is typically determined using a suitable sequence alignment algorithm and default parameters, such as BLAST P (Karlin and Altschul, 1990).

According to the invention, the selenoprotein T may be produced by conventional automated synthesis methods or by recombinant expression. General principles for designing and making proteins are well known to those of skill in the art. The selenoprotein T of the invention may be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. The selenoprotein T of the invention may also be synthesized by solid-phase technology employing an exemplary peptide synthesizer such as a Model 433A from Applied Biosystems Inc. The purity of any given protein generated through automated peptide synthesis or through recombinant methods may be determined using reverse phase HPLC analysis. Chemical authenticity of each peptide may be established by any method well known to those of skill in the art. As an alternative to automated peptide synthesis, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes a protein of choice is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression as described herein below. Recombinant methods are especially preferred for producing longer polypeptides. A variety of expression vector/host systems may be utilized to contain and express the peptide or protein coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transfected with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (e.g., Ti or pBR322 plasmid); or animal cell systems. Those of skill in the art are aware of various techniques for optimizing mammalian expression of proteins. Mammalian cells that are useful in recombinant protein productions include but are not limited to VERO cells, HeLa cells, Chinese hamster ovary (CHO) cell lines, COS cells (such as COS-7), W138, BHK, HepG2, 3T3, RIN, MDCK, A549, PC12, K562 and 293 cells. Exemplary protocols for the recombinant expression of the peptide substrates or fusion polypeptides in bacteria, yeast and other invertebrates are known to those of skill in the art and a briefly described herein below. Mammalian host systems for the expression of recombinant proteins also are well known to those of skill in the art. Host cell strains may be chosen for a particular ability to process the expressed protein or produce certain post-translation modifications that will be useful in providing protein activity. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-

translational processing, which cleaves a "prepro" form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, 293, WI38, and the like have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein.

Typically the nucleic acid molecule as above described are delivered in vivo alone or in association with a vector. In its broadest sense, a "vector" is any vehicle capable of facilitating the transfer of the nucleic acid to the cells and in particular cardiomyocytes. In particular, the vector transports the nucleic acid to cells with reduced degradation relative to the extent of degradation that would result in the absence of the vector. In general, the vectors useful in the invention include, but are not limited to, plasmids, phagemids, viruses, other vehicles derived from viral or bacterial sources that have been manipulated by the insertion or incorporation of the nucleic acid sequences. Viral vectors are a preferred type of vector and include, but are not limited to nucleic acid sequences from the following viruses: retrovirus, such as moloney murine leukemia virus, harvey murine sarcoma virus, murine mammary tumor virus, and rous sarcoma virus; adenovirus, adeno-associated virus; SV40-type viruses; polyoma viruses; Epstein-Barr viruses; papilloma viruses; herpes virus; vaccinia virus; polio virus; and RNA virus such as a retrovirus. One can readily employ other vectors not named but known to the art. Typically viral vectors are based on non-cytopathic eukaryotic viruses in which non-essential genes have been replaced with the gene of interest. Non-cytopathic viruses include retroviruses (e.g., lentivirus), the life cycle of which involves reverse transcription of genomic viral RNA into DNA with subsequent proviral integration into host cellular DNA. Retroviruses have been approved for human gene therapy trials. Most useful are those retroviruses that are replication-deficient (i.e., capable of directing synthesis of the desired proteins, but incapable of manufacturing an infectious particle). Such genetically altered retroviral expression vectors have general utility for the high-efficiency transduction of genes in vivo. Standard protocols for producing replication-deficient retroviruses (including the steps of incorporation of exogenous genetic material into a plasmid, transfection of a packaging cell lined with plasmid, production of recombinant retroviruses by the packaging cell line, collection of viral particles from tissue culture media, and infection of the target cells with viral particles) are provided in Kriegler, 1990 and in Murry, 1991. Preferred viruses are the adenoviruses and adeno-associated (AAV) viruses, which are double-stranded DNA viruses that have already been approved for human use in gene therapy.

Actually 12 different AAV serotypes (AAV1 to 12) are known, each with different tissue tropisms (Wu, Z Mol Ther 2006; 14:316-27). The adeno-associated virus type 1 to 12 can be engineered to be replication deficient and is capable of infecting a wide range of cell types and species (Wu, Z Mol Ther 2006; 14:316-27). It further has advantages such as, heat and lipid solvent stability; high transduction frequencies in cells of diverse lineages, including hemopoietic cells; and lack of superinfection inhibition thus allowing multiple series of transductions. Reportedly, the adeno-associated virus can integrate into human cellular DNA in a site-specific manner, thereby minimizing the possibility of insertional mutagenesis and variability of inserted gene expression characteristic of retroviral infection. In addition, wild-type adeno-associated virus infections have been followed in tissue culture for greater than 100 passages in the absence of selective pressure, implying that the adeno-associated virus genomic integration is a relatively stable event. The adeno-associated virus can also function in an extrachromosomal fashion. In some embodiments, the nucleic acid as above described is packaged in a cardiotropic adeno-associated viral vector, such AAV9. Other vectors include plasmid vectors. Plasmid vectors have been extensively described in the art and are well known to those of skill in the art. See e.g. Sambrook et al., 1989. In the last few years, plasmid vectors have been used as DNA vaccines for delivering antigen-encoding genes to cells in vivo. They are particularly advantageous for this because they do not have the same safety concerns as with many of the viral vectors. These plasmids, however, having a promoter compatible with the host cell, can express a peptide from a gene operatively encoded within the plasmid. Some commonly used plasmids include pBR322, pUC18, pUCl9, pRC/CMV, SV40, and pBlueScript. Other plasmids are well known to those of ordinary skill in the art. Additionally, plasmids may be custom designed using restriction enzymes and ligation reactions to remove and add specific fragments of DNA. Plasmids may be delivered by a variety of parenteral, mucosal and topical routes. For example, the DNA plasmid can be injected by intramuscular, intradermal, subcutaneous, or other routes. It may also be administered by intranasal sprays or drops, rectal suppository and orally. It may also be administered into the epidermis or a mucosal surface using a gene-gun. The plasmids may be given in an aqueous solution, dried onto gold particles or in association with another DNA delivery system including but not limited to liposomes, dendrimers, cochleate and microencapsulation. Typically, the nucleic acid sequence is under the control of a heterologous regulatory region, e.g., a heterologous promoter that will drive the expression of the nucleic acid. In some embodiments, the nucleic acid sequence is under the control of a

heterologous promoter that will drive the expression of the nucleic acid specifically in cardiomyocyte (e.g. NFAT promoter).

Typically, the agent (i.e. the selenoprotein T or the nucleic acid molecule encoding thereof) is administered to the subject in a therapeutically effective amount. By a "therapeutically effective amount" is meant a sufficient amount of the agent of the present invention for reaching a therapeutic effect (e.g. the treatment of left ventricular (LV) dysfunction). It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific polypeptide employed; and like factors well known in the medical arts. For example, it is well known within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. However, the daily dosage of the products may be varied over a wide range from 0.01 to 1,000 mg per adult per day. Typically, the compositions contain 0.01, 0.05, 0.1, 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100, 250 and 500 mg of the active ingredient for the symptomatic adjustment of the dosage to the subject to be treated. A medicament typically contains from about 0.01 mg to about 500 mg of the active ingredient, typically from 1 mg to about 100 mg of the active ingredient. An effective amount of the drug is ordinarily supplied at a dosage level from 0.0002 mg/kg to about 20 mg/kg of body weight per day, especially from about 0.001 mg/kg to 7 mg/kg of body weight per day.

Typically, the agent is combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form pharmaceutical compositions. "Pharmaceutically" or "pharmaceutically acceptable" refer to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Typically,

the pharmaceutical compositions contain vehicles, which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. 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. Sterile injectable solutions are prepared by incorporating the agent 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 ingredients 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.

In some embodiments, the Selenoprotein T or the nucleic acid encoding thereof is administered simultaneously or sequentially (i.e. before or after) with a revascularization procedure performed on the subject. In some embodiments, the subject is administered with the Selenoprotein T or the nucleic acid encoding thereof before, during, and after a revascularization procedure. In some embodiments, the subject is administered with the Selenoprotein T or the nucleic acid encoding thereof as a bolus dose immediately prior to the revascularization procedure. In some embodiments, the subject is administered with the Selenoprotein T or the nucleic acid encoding thereof continuously during and after the revascularization procedure. In some embodiments, the subject is administered with the Selenoprotein T or the nucleic acid encoding thereof for a time period selected from the group consisting of at least 3 hours after a revascularization procedure; at least 5 hours after a revascularization procedure; at least 8 hours after a revascularization procedure; at least 12 hours after a revascularization procedure; at least 24 hours after a revascularization procedure.

In some embodiments, the subject is administered with the Selenoprotein T or the nucleic acid encoding thereof in a time period selected from the group consisting of starting at least 8 hours before a revascularization procedure; starting at least 4 hours before a revascularization procedure; starting at least 2 hours before a revascularization procedure; starting at least 1 hour before a revascularization procedure; starting at least 30 minutes before a revascularization procedure. In some embodiments, the revascularization procedure is selected from the group consisting of percutaneous coronary intervention; balloon angioplasty; insertion of a bypass graft; insertion of a stent; directional coronary atherectomy; treatment with a one or more thrombolytic agent(s); and removal of an occlusion.

In some embodiments, the Selenoprotein T or the nucleic acid encoding thereof is administered in combination with an additional active agent. In some embodiments, the additional active agent is a cardiovascular agent selected from the group consisting of hyaluronidase, a corticosteroid, recombinant superoxide dismutase, prostacyclin, fluosol, magnesium, poloxamer 188, trimetazidine, eniporidine, cariporidine, a nitrate, anti-P selectin, an anti-CD18 antibody, adenosine, and glucose-insulin-potassium. In some embodiments, the cardiovascular agent is selected from the group consisting of an anti-arrhthymia agent, a vasodilator, an anti-anginal agent, a corticosteroid, a cardioglycoside, a diuretic, a sedative, an angiotensin converting enzyme (ACE) inhibitor, an angiotensin II antagonist, a thrombolytic agent, a calcium channel blocker, a throboxane receptor antagonist, a radical scavenger, an anti-platelet drug, a β- adrenaline receptor blocking drug, oreceptor blocking drug, a sympathetic nerve inhibitor, a digitalis formulation, an inotrope, and an antihyperlipidemic drug. In some embodiments, the active agent is an inotrope. Positive inotropic agents increase myocardial contractility, and are used to support cardiac function in conditions such as decompensated congestive heart failure, cardiogenic shock, septic shock, myocardial infarction, cardiomyopathy, etc. Examples of positive inotropic agents include, but are not limited to, Berberine, Bipyridine derivatives, Inamrinone, Milrinone, Calcium, Calcium sensitizers, Levosimendan, Cardiac glycosides, Digoxin, Catecholamines, Dopamine, Dobutamine, Dopexamine, Epinephrine (adrenaline), Isoprenaline (isoproterenol), Norepinephrine (noradrenaline), Eicosanoids, Prostaglandins, Phosphodiesterase inhibitors, Enoximone, Milrinone, Theophylline, and Glucagon. Negative inotropic agents decrease myocardial contractility, and are used to decrease cardiac workload in conditions such as angina. While negative inotropism may precipitate or exacerbate heart failure, certain beta blockers (e.g. carvedilol, bisoprolol and metoprolol) have been shown to reduce morbidity

and mortality in congestive heart failure. Examples of negative inotropic agents include, but are not limited to, Beta blockers, Calcium channel blockers, Diltiazem, Verapamil, Clevidipine, Quinidine, Procainamide, disopyramide, and Flecainide. In some embodiments, the cardiovascular agent is cyclosporine. As used herein, the term "cyclosporine" refers to cyclosporine A, cyclosporine G, and functional derivatives or analogues thereof, e.g., NIM81 1. Cyclosporine A refers to the natural Tolypocladium inflation cyclic non-ribosomal peptide. Cyclosporine G differs from cyclosporine A in the amino acid 2 position, where an L-norvaline replaces the a-aniinobutyric acid. (See generally, Wenger, R. M. 1986. Synthesis of Ciclosporin and analogues: structural and conformational requirements for immunosuppressive activity. Progress in Allergy, 38:46-64).

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURES:

Figure 1. Left ventricular (LV) end-systolic pressure, LV end-systolic pressure-volume relation, LV end-diastolic pressure and LV end-diastolic pressure-volume relation determined 7 days after surgery in sham-operated (white bars), placebo-treated ischemia-reperfusion (I/R; black-bars) and selenoprotein T-treated I/R animals (hatched bars). *: $p<0.05$ vs. sham operated; †: $p<0.05$ vs. placebo-treated I/R.

Figure 2. Left ventricular (LV) diastolic, LV systolic, LV fractional shortening and cardiac output determined 7 days after surgery in sham-operated (white bars), placebo-treated ischemia-reperfusion (I/R; black-bars) and selenoprotein T-treated I/R animals (hatched bars). *: $p<0.05$ vs. sham operated; †: $p<0.05$ vs. placebo-treated I/R.

Figure 3. Left ventricular tissue perfusion, in the septum and in the ischemia-reperfusion zone, determined 7 days after surgery in sham-operated (white bars), placebo-treated I/R (black-bars) and selenoprotein T-treated I/R animals (hatched bars). *: $p<0.05$ vs. sham operated; †: $p<0.05$ vs. placebo-treated I/R.

Figure 4. Coronary endothelium-dependent and independent relaxation determined 7 days after surgery in sham-operated (open circles), placebo-treated ischemia-reperfusion (I/R; filled circles) and selenoprotein T-treated I/R animals (triangles). *: $p<0.05$ vs. sham operated.

Figure 5. Left ventricular remodelling 13 weeks after coronary artey ligation (CAL) and 12 weeks after intramuscular administration of rAAV8-SelT. *: $p<0.05$ vs. historical sham values; †: $p<0.05$ vs. coronary artery ligation.

Figure 6. Stoke volume and cardiac output 13 weeks after coronary artey ligation (CAL) and 12 weeks after intramuscular administration of rAAV8-SelT. *: $p<0.05$ vs. historical sham values; †: $p<0.05$ vs. coronary artery ligation.

Figure 7. Myocardial tissue perfusion, measured by MRI, 13 weeks after coronary artey ligation (CAL) and 12 weeks after intramuscular administration of rAAV8-SelT. *: $p<0.05$ vs. sham values; †: $p<0.05$ vs. coronary artery ligation.

EXAMPLES:

EXAMPLE 1: Selenoprotein T attenuates the development of LV dysfunction after myocardial infarction in rats.

Introduction. Selenoproteins are mediators of the essential micronutrient selenium, but their biological effects are not yet fully understood. Selenoprotein T (SelT), a recently discovered selenoprotein, is abundant during embryonic development but declines during adulthood. Preliminary data obtained in our laboratory showed that myocardial infarction increases SelT protein expression in mouse heart as revealed by Western blotting analysis, suggesting that SelT may play a yet unrevealed role in the cardiovascular system. Thus, we sought to investigate SelT's role in myocardial infarction. **Methods and results.** Male Wistar rats were subjected to either cardiac ischaemia (45 min) followed by reperfusion (I/R) or sham surgery. Five days before I/R SelT (15 μg/kg/day, IP) or saline infusion was started by osmotic minipump. Eight days after myocardial infarction, left ventricular (LV) function was assessed by echocardiography, MRI and LV hemodynamics (pressure-volume loops). I/R resulted in a significant increase of LV systolic and diastolic diameters, in a decrease of cardiac output and fractional shortening. This was associated with a decrease of LV end-

systolic pressure and an increase of LV end-diastolic pressure. SelT restored cardiac output and LV fractional shortening associated with improvement of LV end-diastolic/end-systolic pressures and LV tissue perfusion. Furthermore, SelT did not modify LV infarct size. **Conclusion.** SelT, administered before I/R, preserves LV function and perfusion in a rat model of myocardial infarction and might be a new therapeutic target in the treatment of myocardial infarction.

EXEMPLE 2: Cardio-protective properties of rAAV-SeltT in a rat model of heart failure induced by coronary ligation.

Coronary artery ligation (CAL) induced major left ventricular remodeling, i.e. dilatation, associated with cardiac dysfunction, illustrated by the marked increases in left ventricular diastolic and systolic diameters (Figure 5), as well as the reductions in fractional shortening, stroke volume and cardiac output. (Figure 5 and 6).

After 12 weeks genetic therapy via rAAV8-SelT administration improved left ventricular function. This is illustrated by the significant reduction of left ventricular diastolic and systolic diameters (Figure 5) and the increases fractional shortening, stroke volume and cardiac output. (Figure 5 and 6).

The impairment of left ventricular dysfunction observed 13 weeks after coronary artery ligation (CAL) was associated with a reduction of myocardial tissue perfusion. (Figure 7).

After 12 weeks genetic therapy via rAAV8-SelT administration significantly increased myocardial perfusion. (Figure 7).

In conclusion, intramuscular rAAV-SeltT administrated 1 weeks after coronary artery ligation exerts cardio-protective properties in a rat model of heart failure induced by coronary ligation.

REFERENCES:

Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

CLAIMS:

1. A method of treating myocardial infarction in a subject in need thereof comprising administering to the subject a therapeutically effective amount of selenoprotein T or a nucleic acid molecule encoding for selenoprotein T.

2. The method of claim 1 which is suitable for the treatment of left ventricular (LV) dysfunction after myocardial infarction.

3. The method of claim 1 which is suitable for restoring cardiac output and LV fractional shortening associated with improvement of LV end-diastolic/end-systolic pressures and LV tissue perfusion.

4. The method of claim 1 wherein the subject has one or more signs or symptoms of acute myocardial infarction injury.

5. The method of claim 1 wherein the subject has one or more signs or symptoms of myocardial infarction, such as chest pain described as a pressure sensation, fullness, or squeezing in the mid portion of the thorax; radiation of chest pain into the jaw or teeth, shoulder, arm, and/or back; dyspnea or shortness of breath; epigastric discomfort with or without nausea and vomiting; and diaphoresis or sweating.

6. The method of claim 1 wherein the selenoprotein T is polypeptide having at least 90% of identity with SEQ ID NO:1

7. The method of claim 1 wherein the nucleic acid molecule is delivered in vivo alone or in association with a vector.

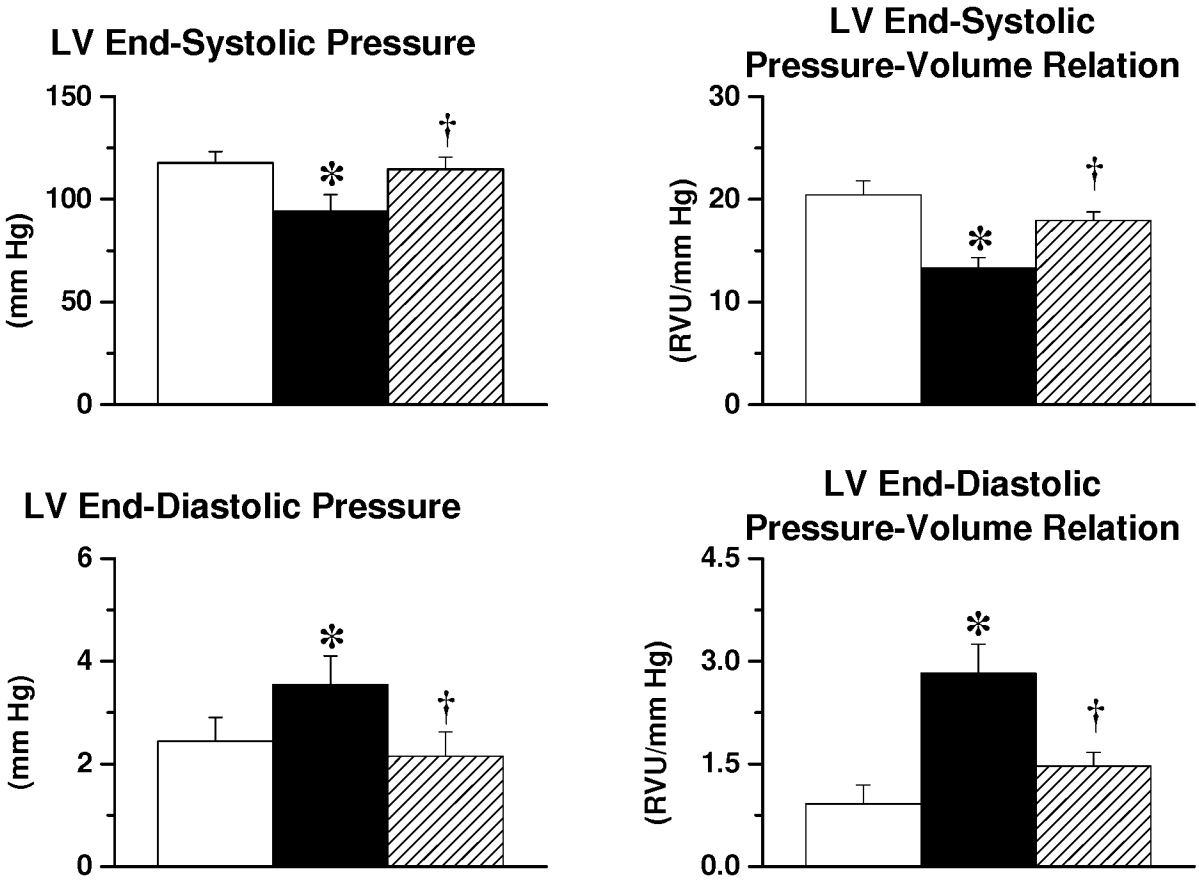
8. The method of claim 1 wherein the Selenoprotein T or the nucleic acid encoding thereof is administered simultaneously or sequentially with a revascularization procedure performed on the subject.

9. The method of claim 1 wherein the subject is administered with the Selenoprotein T or the nucleic acid encoding thereof as a bolus dose immediately prior to the revascularization procedure.

10. The method of claim 1 wherein the subject is administered with the Selenoprotein T or the nucleic acid encoding thereof for a time period selected from the group consisting

of at least 3 hours after a revascularization procedure; at least 5 hours after a revascularization procedure; at least 8 hours after a revascularization procedure; at least 12 hours after a revascularization procedure; at least 24 hours after a revascularization procedure.

11. The method of claim 8 wherein the revascularization procedure is selected from the group consisting of percutaneous coronary intervention; balloon angioplasty; insertion of a bypass graft; insertion of a stent; directional coronary atherectomy; treatment with a one or more thrombolytic agent(s); and removal of an occlusion.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150
100
50
0
*
†
LV End-Systolic Pressure-Volume Relation
(RVU/mm Hg)
30
20
10
0
*
†
LV End-Diastolic Pressure
(mm Hg)
6
4
2
0
*
†
LV End-Diastolic Pressure-Volume Relation
(RVU/mm Hg)
4.5
3.0
1.5
0.0
*
†

Figure 1

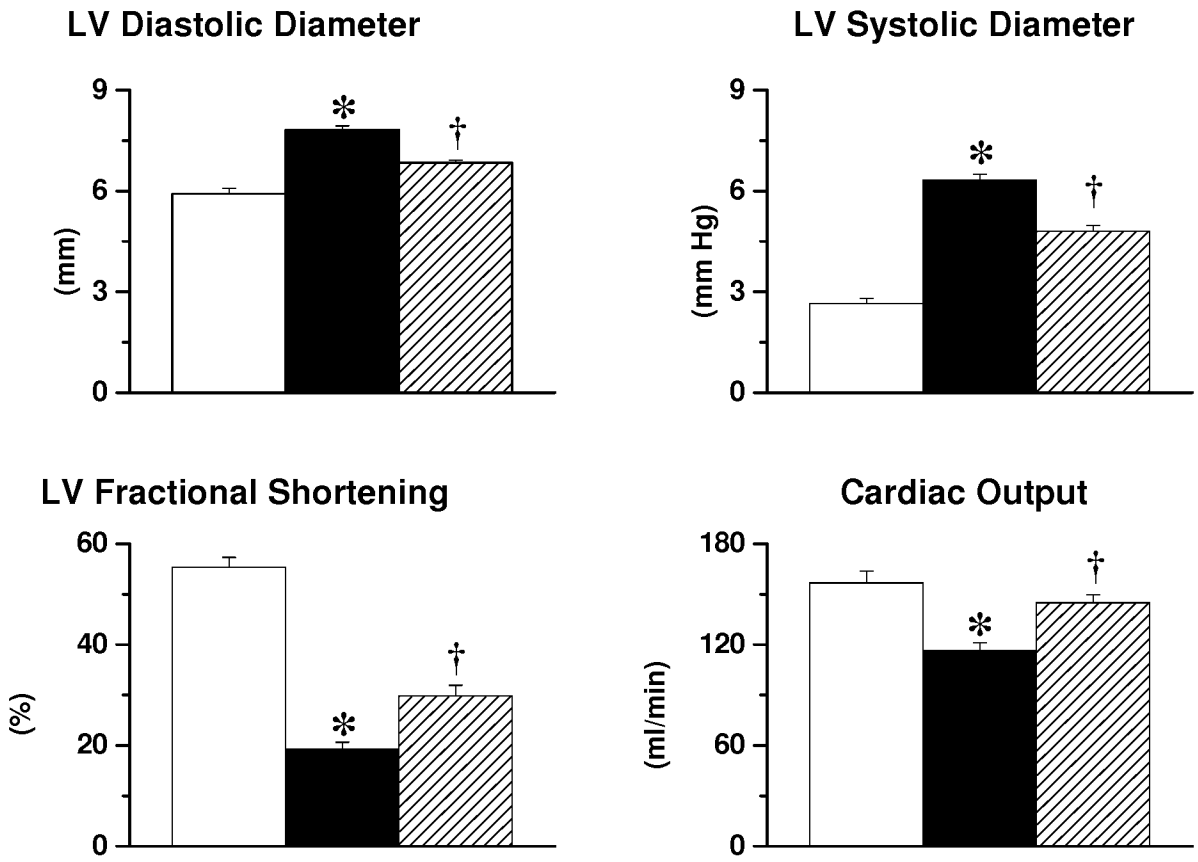
LV Diastolic Diameter
(mm)
9
6
3
0
*
†
LV Systolic Diameter
(mm Hg)
9
6
3
0
*
†
LV Fractional Shortening
(%)
60
40
20
0
*
†
Cardiac Output
(ml/min)
180
120
60
0
*
†

Figure 2

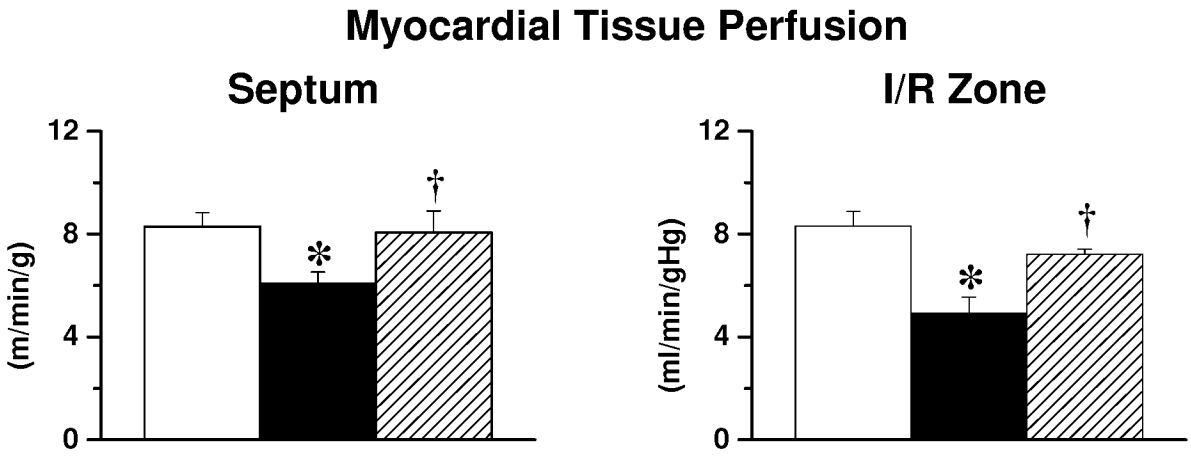

Figure 3

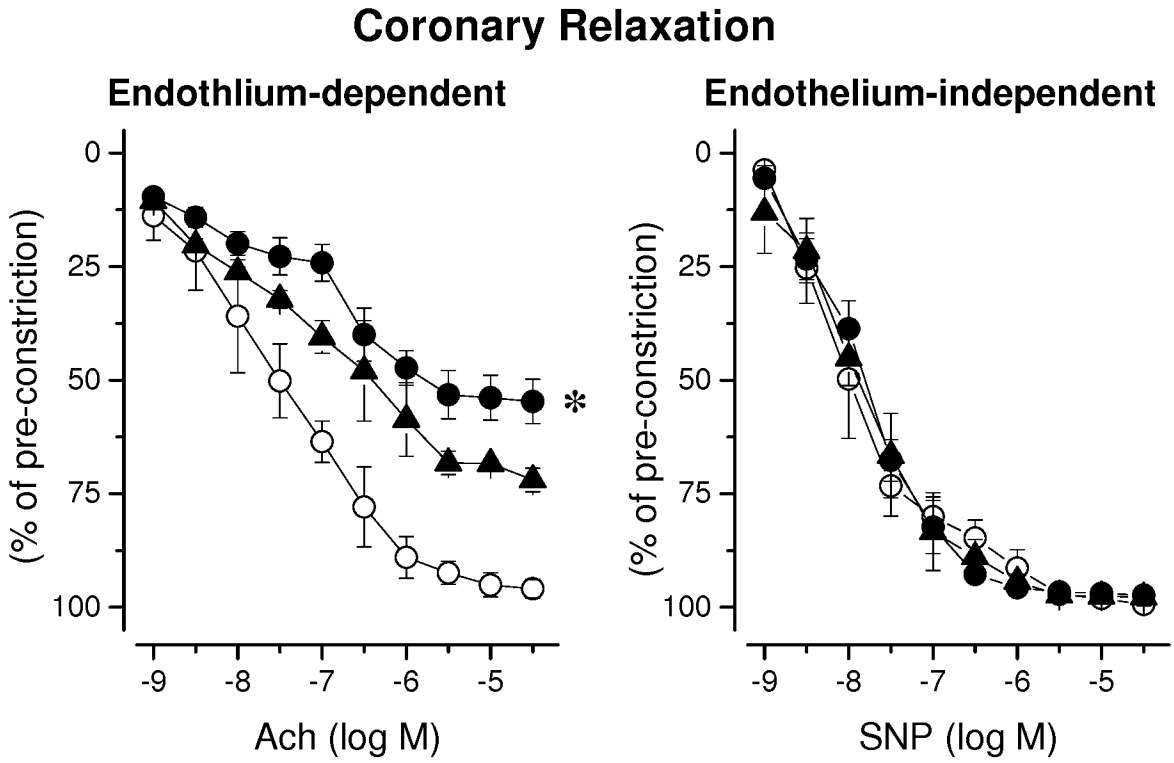

Figure 4

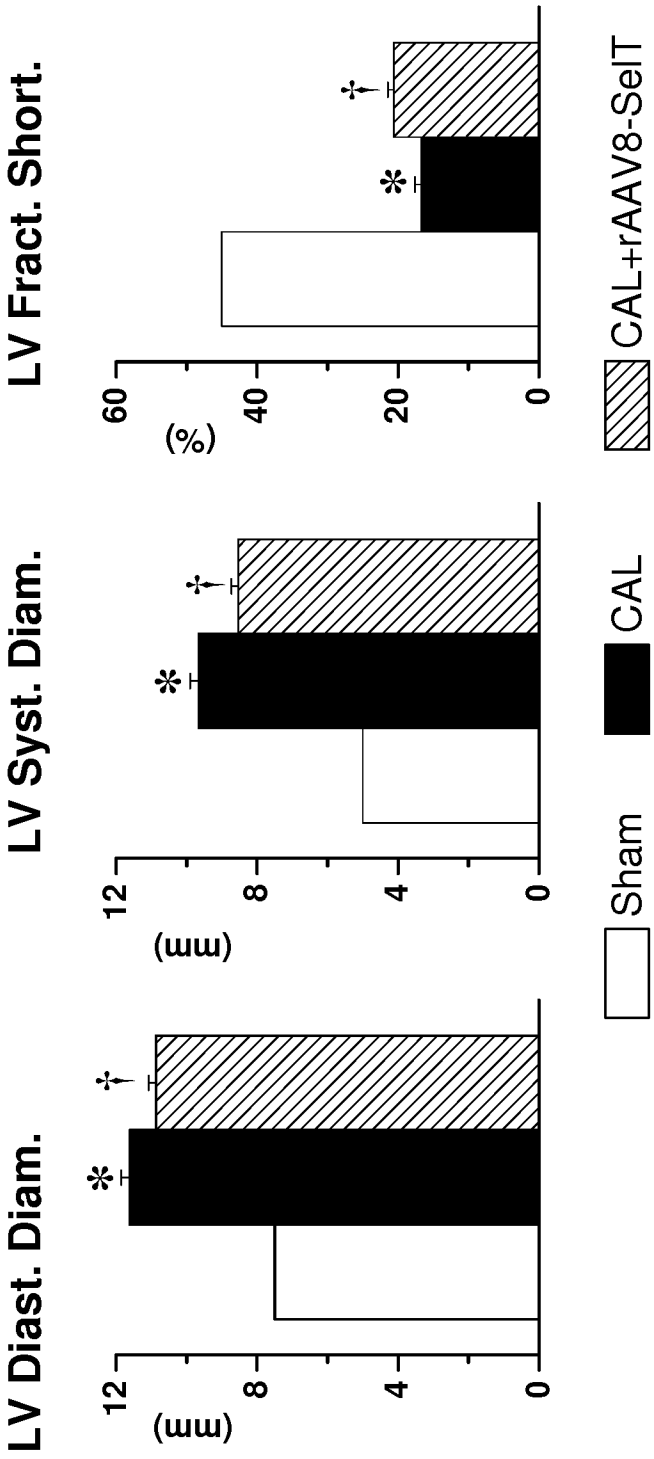
LV Fract. Short.
60
40
20
0
(%)
LV Syst. Diam.
12
8
4
0
(mm)
LV Diast. Diam.
12
8
4
0
(mm)
Sham
CAL
CAL+rAAV8-SelT

Figure 5

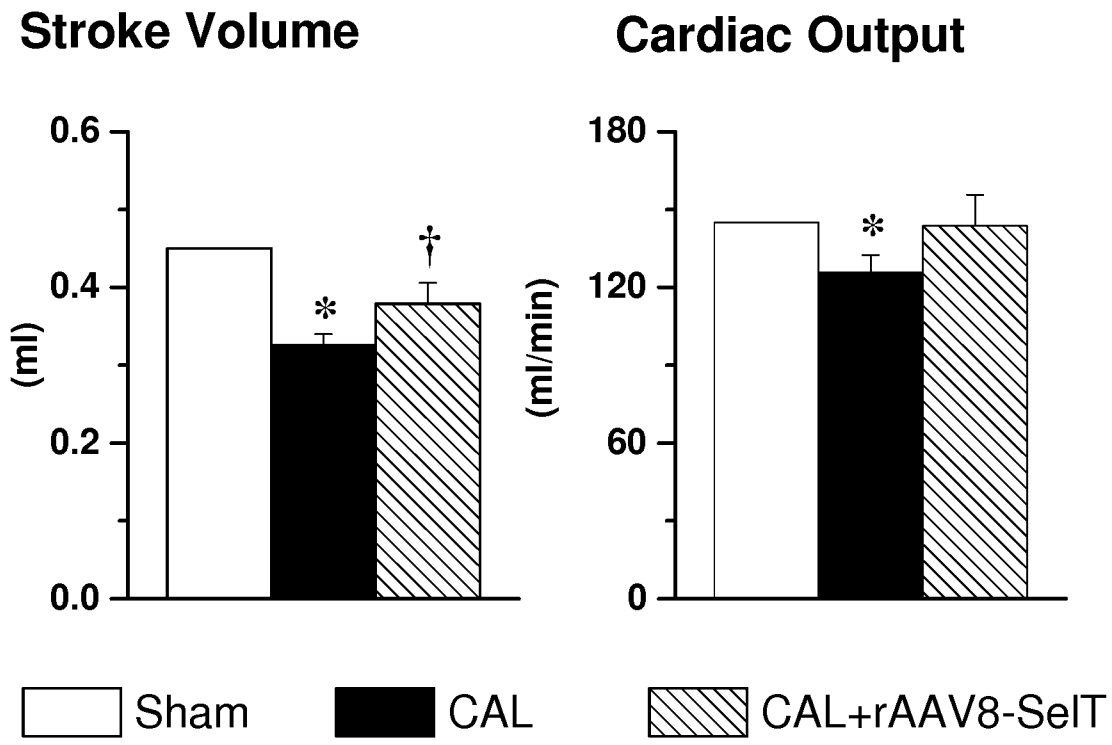

Figure 6

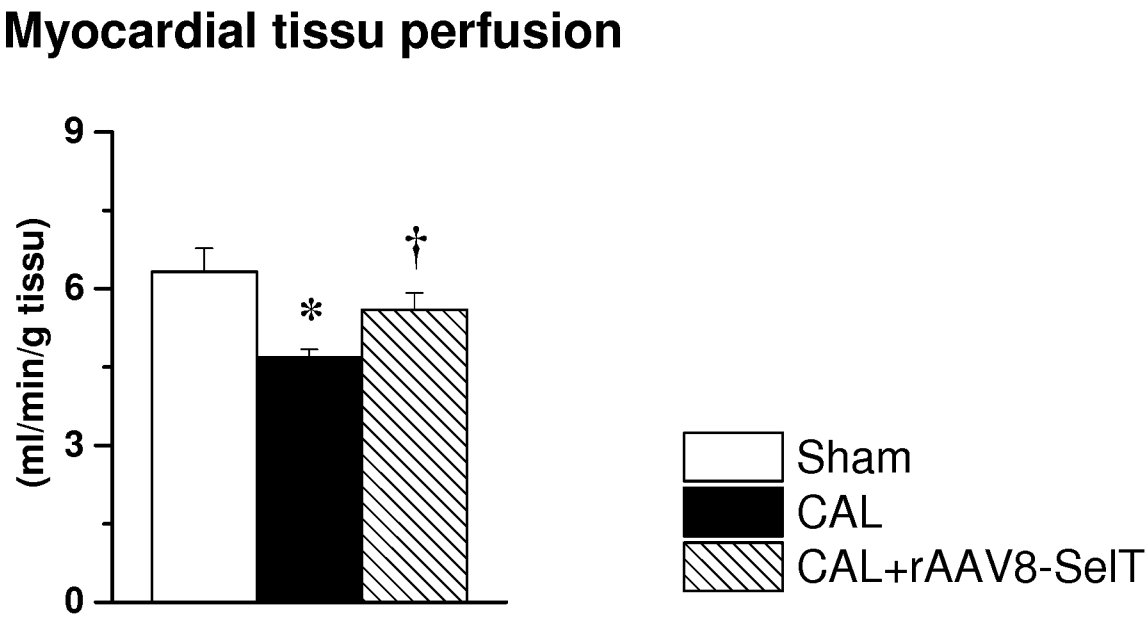

Figure 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2016/056552

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/056552

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/17 A61P9/10
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

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, EMBASE, BIOSIS, COMPENDEX, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ROSE A H ET AL: "Selenoproteins and cardiovascular stress", THROMBOSIS AND HAEMOSTASIS 2015 SCHATTAUER GMBH DEU, vol. 113, no. 3, 30 October 2014 (2014-10-30), pages 494-504, XP002743616, ISSN: 0340-6245 the whole document	1-11

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[X] 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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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
23 May 2016	15/06/2016

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	Bochelen, Damien

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/056552

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KASAIKINA MARINA V ET AL: "Understanding selenoprotein function and regulation through the use of rodent models", BIOCHIMICA ET BIOPHYSICA ACTA. MOLECULAR CELL RESEARCH, vol. 1823, no. 9, 18 February 2012 (2012-02-18), pages 1633-1642, XP028932667, ISSN: 0167-4889, DOI: 10.1016/J.BBAMCR.2012.02.018 the whole document -----	1-11
A	SHRIMALI ET AL: "Selenoprotein expression is essential in endothelial cell development and cardiac muscle function", NEUROMUSCULAR DISORDERS, PERGAMON PRESS, GB, vol. 17, no. 2, 20 February 2007 (2007-02-20), pages 135-142, XP005895422, ISSN: 0960-8966, DOI: 10.1016/J.NMD.2006.10.006 the whole document -----	1-11

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