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(54) Title: COMPOSITIONS AND METHODS FOR THE TREATMENT OF FIBROSIS AND FIBROTIC DISEASES

(57) Abstract: The present invention provides pharmaceutical compositions comprising at least one relaxin polypeptide and at least one ACE inhibitor for use in the treatment of fibrosis and fibrotic diseases and in reducing or preventing the progression of pre-existing fibrosis. The invention also provides methods of treatment using such compositions.



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Compositions and methods for the treatment of fibrosis and fibrotic diseases

Field of the Invention

[001] The present invention relates generally to the treatment of fibrosis and fibrotic diseases and disorders using combination therapy comprising a relaxin polypeptide together with an angiotensin-converting enzyme (ACE) inhibitor.

Background of the Invention

[002] Fibrosis, the abnormal and excessive accumulation of fibrous connective tissue, may result from a number of causes, including physical injury or trauma, inflammation or infection. Fibrosis can effect a range of tissues, most commonly the heart, lungs, kidneys, liver, stomach, skin and joints. If undetected, or untreated or inadequately treated the abnormal accumulation of fibrous tissue can cause organ failure and/or develop into debilitating and life threatening fibrotic diseases. Current therapies for fibrosis and fibrotic diseases are limited and are commonly hampered by a lack of efficacy and significant side effects. There remains a need for the development of improved treatment options.

[003] Relaxin is a member of a protein hormone superfamily which also includes insulin, insulin-like growth factors I and II (IGF-I and IGF-II), and the insulin-like hormones INSL3, 4, 5 and 6. The relaxin superfamily members have a wide range of biological activities that are well described in the art. Relaxin is a heterodimeric peptide hormone composed, in its mature form, of an A chain and a B chain linked via disulphide bridges. Human relaxins in their mature form are stabilised by three disulphide bonds, two inter-chain disulphide bonds between the A chain and B chain and one intra-chain disulphide bond between cysteine residues in the A chain.

[004] Relaxin has been conserved through vertebrate evolution and has been characterised in a large and diverse range of vertebrate species. In particular the cysteine residues in the B and A chains responsible for the intra- and inter-chain disulphide bonds are highly conserved. Whilst in most species only two forms of relaxin have been identified (relaxin and relaxin-3), in humans three distinct forms of relaxin have been identified (relaxin and relaxin-3), in humans three distinct forms of relaxin have been described and the genes and polypeptides characterised. These have

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been designated H1, H2 and H3. Homologues of H1 and H2 relaxin have been identified in other higher primates including chimpanzees, gorillas and orangutans. Differing expression patterns for H1, H2 and H3 relaxin may suggest some differences in biological roles, however all three forms display similar biological activities, as determined for example by their ability to stimulate cAMP activity in cells expressing relaxin receptors, and accordingly share many biological functions in common.

[005] The biological functions of relaxins include an ability to inhibit myometrial contractions, to stimulate remodelling of connective tissue and to induce softening of the tissues of the birth canal. Additionally, relaxins increase growth and differentiation of the mammary gland and nipple and induce the breakdown of collagen, one of the main components of connective tissue. Relaxins can cause a widening of blood vessels (vasodilatation) in the kidney, mesocaecum, lung and peripheral vasculature, which leads to increased blood flow or perfusion rates in these tissues. Relaxins can also stimulate an increase in heart rate and coronary blood flow, and increase both glomerular filtration rate and renal plasma flow.

[006] The biological actions of relaxins are mediated through G protein coupled receptors (reviewed in Bathgate *et al.*, 2006, *Pharmacol Rev* 58:7-31). To date, H1, H2 and H3 relaxins have been shown to primarily recognise and bind four receptors, RXFP1 (LGR7), RXFP2 (LGR8), RXFP3 (GPCR135) and RXFP4 (GPCR142). Interestingly, receptors RXFP1 and RXFP2 are structurally distinct from receptors RXFP3 and RXFP4, yet despite the differences there is significant cross-reactivity between different relaxin molecules and different receptors. RXFP1 is the most widely expressed of these receptors and binds each of H1, H2 and H3 with high affinity. H1 and H2 relaxin also bind RXFP2. The endogenous receptor in the brain for H3 relaxin is RXFP3, however H3 relaxin has also been shown to bind and activate both RXFP1 and RXFP4.

[007] Relaxin has been shown to have anti-fibrotic activity. For example, H2 relaxin has been demonstrated to inhibit fibrosis in several experimental models of fibrosis (see, for example, Samuel *et al.*, 2011, *Lab Invest* 91:675-690; and Hewitson *et al.*, 2010, *Endocrinology* 151:4938-4948), and H3 relaxin has been shown to inhibit and reverse fibrosis in a model of fibrotic cardiomyopathy (Hossain *et al.*, 2011, *Biochemistry* 50:1368-1375). The anti-fibrotic effects of both H2 and H3 relaxin are mediated via their interaction with the RXFP1 receptor. However further work has been required to

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better understand the anti-fibrotic activity and mechanism of action of relaxin, and to exploit this to develop more effective treatments for fibrosis and fibrotic diseases.

Summary of the Invention

[008] The present invention is predicated on the inventors' finding that a combination of an ACE inhibitor and relaxin provides improved anti-fibrotic efficacy when compared to either ACE inhibitor or relaxin treatment alone in two different mouse models of human fibrotic disease.

[009] Accordingly, a first aspect of the invention provides a pharmaceutical composition for use in the treatment of fibrosis or a fibrotic disease, the composition comprising at least one relaxin polypeptide and at least one ACE inhibitor.

[010] The fibrosis or fibrotic disorder may affect any tissue or organ including, for example, kidney, heart, lung, liver, skin, bone or bone marrow. In exemplary embodiments the fibrosis may be renal or cardiac fibrosis. The fibrosis may result from tissue or organ damage or injury, such as cardiac injury. In exemplary embodiments the fibrotic disorder may be tubulointerstitial kidney disease, myocardial infarction or other disorders associated with cardiac damage.

[011] Typically the relaxin polypeptide comprises an A chain and a B chain. Typically the A chain comprises an amino acid sequence as set forth in SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5 or a variant or derivative thereof. Typically the B chain comprises an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6 or a variant or derivative thereof. The relaxin polypeptide may be a relaxin-1, relaxin-2 or relaxin-3 polypeptide. In a particular embodiment, the relaxin is a relaxin-2 polypeptide. Where the subject is human, typically the relaxin-2 is H2 relaxin. The H2 relaxin may comprise an A chain comprising the sequence set forth in SEQ ID NO:1 and a B chain comprising the sequence set forth in SEQ ID NO:2.

[012] The relaxin polypeptide may be administered to the subject in the form of a polynucleotide encoding the polypeptide such that the polynucleotide is expressed *in vivo* producing the polypeptide.

[013] The ACE inhibitor may be a synthetic or naturally derived. The synthetic ACE inhibitor may be, for example, a dicarboxylate-containing agent. In particular embodiments the ACE inhibitor may be selected from enalapril and perindopril.

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[014] According to a second aspect the invention provides a pharmaceutical composition for use in inhibiting fibrosis, the composition comprising at least one relaxin polypeptide and at least one ACE inhibitor.

[015] According to a third aspect the invention provides a pharmaceutical composition for use in reducing or preventing progression of existing fibrosis, the composition comprising at least one relaxin polypeptide and at least one ACE inhibitor.

[016] According to a fourth aspect the invention provides a method for treating fibrosis or a fibrotic disorder in a subject in need thereof, the method comprising administering to the subject an effective amount of at least one relaxin polypeptide and an effective amount of at least one ACE inhibitor.

[017] The relaxin polypeptide and the ACE inhibitor may be formulated together in a single composition or may be administered separately. Where administered separately, administration may be simultaneous or sequential.

[018] According to a fifth aspect the invention provides a method for inhibiting fibrosis in a subject in need thereof, the method comprising administering to the subject an effective amount of at least one relaxin polypeptide and an effective amount of at least one ACE inhibitor.

[019] According to a sixth aspect the invention provides a method for reducing or preventing progression of existing fibrosis in a subject in need thereof, the method comprising administering to the subject an effective amount of at least one relaxin polypeptide and an effective amount of at least one ACE inhibitor.

[020] According to a seventh aspect the invention provides the use of at least one relaxin polypeptide and at least one ACE inhibitor for the manufacture of a medicament for the treatment of fibrosis or a fibrotic disorder.

[021] According to an eighth aspect the invention provides the use of at least one relaxin polypeptide and at least one ACE inhibitor for the manufacture of a medicament for the inhibition of fibrosis.

[022] According to a ninth aspect the invention provides the use of at least one relaxin polypeptide and at least one ACE inhibitor for the manufacture of a medicament for reducing or preventing progression of existing fibrosis.

Brief Description of the Drawings

[023] The present invention is described, by way of non-limiting example only, with reference to the accompanying drawings.

[024] **Figure 1.** Effects of ACE inhibitors and H2 relaxin on renal collagen concentration in UUO model of tubulointerstitial kidney disease (A and B) and ISO model of myocardial infarction and cardiac damage (C). Numbers in parentheses indicate numbers of animals. *** $p < 0.01$ vs D0; # $p < 0.05$; ## $p < 0.01$ vs D5 alone; § $p < 0.05$ vs D5+ 200mg/L enalapril alone; ++ $p < 0.01$ vs D0 (control) group.

[025] **Figure 2.** MMP-2 activity in renal myofibroblasts from rats 3 days post UUO, in the presence and absence of H2 relaxin (RLX) and various concentrations of Irbesartan (AT1 antagonist) as measured by gelatin zymography (A) and densitometric analysis of the resulting bands. (B). Data are presented as the relative mean $\pm$ SEM OD MMP from $n=4$ separate experiments; ** $p < 0.01$ vs untreated group; # $p < 0.05$, ## $p < 0.01$ vs H2 relaxin alone group.

[026] **Figure 3.** MMP-9 activity in renal myofibroblasts from rats 3 days post UUO, in the presence and absence of H2 relaxin (RLX) and various concentrations of Irbesartan (AT1 antagonist) as measured by densitometric analysis of bands resulting from gelatin zymography. Data are presented as the relative mean $\pm$ SEM OD MMP from $n=4$ separate experiments; ** $p < 0.01$ vs untreated group; # $p < 0.05$, ## $p < 0.01$ vs H2 relaxin alone group.

[027] **Figure 4.** Levels of smad2 phosphorylation in renal myofibroblasts from rats 3 days post UUO in the presence and absence of H2 relaxin (RLX) and various concentrations of Irbesartan (AT1 antagonist) as measured by densitometric analysis of bands resulting from Western blotting. Data are presented as the relative mean $\pm$ SEM OD MMP from $n=3$ separate experiments; ** $p < 0.01$ vs untreated group; # $p < 0.05$, ## $p < 0.01$ vs H2 relaxin alone group.

[028] **Figure 5.** Staining of TGF- β 1 (A and B) and staining of phosphorylated smad2 (C and D) in left ventricular tissue in mice from the ISO model of myocardial infarction. H2 relaxin used at 0.5mg/kg/d and enalapril used at 200mg/L. Numbers in parentheses indicate numbers of animals. *** $p < 0.001$ vs untreated group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs ISO alone group. § $p < 0.05$, §§ $p < 0.01$ vs ISO + enalapril group.

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[029] **Figure 6.** Relative collagen concentration (A), collagen staining (B), TGF- β 1 staining (C) and phosphorylated smad2 staining (D) in response to enalapril and H2 relaxin administered after the establishment of fibrosis in ISO model of myocardial infarction. Numbers in parentheses indicate numbers of animals. *** $p < 0.001$ vs untreated group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs ISO alone group. (P) refers to fibrosis prevention studies (i.e. H2 relaxin and enalapril administration between days 1 and 17 in the ISO model). (R) refers to fibrosis reversal studies (i.e. H2 relaxin and enalapril administration between days 10 and 17 in the ISO model).

[030] Amino acid sequences of native human relaxin-2 (H2) A and B chains are set forth in **SEQ ID NOs: 1 and 2**, respectively. Amino acid sequences of native human relaxin-1 (H1) A and B chains are set forth in **SEQ ID NOs: 3 and 4**, respectively. Amino acid sequences of native human relaxin-3 (H3) A and B chains are set forth in **SEQ ID NOs: 5 and 6**, respectively.

Detailed Description of the Invention

[031] The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[032] In the context of this specification, the term "about," is understood to refer to a range of numbers that a person of skill in the art would consider equivalent to the recited value in the context of achieving the same function or result.

[033] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[034] The term "fibrosis" as used herein refers to any pathological condition resulting from an accumulation of excess fibrous tissue through either overproduction or insufficient degradation of extracellular matrix.. The term "fibrotic disease" as used herein means any disease, condition or disorder that is associated with fibrosis. The disease, condition or disorder may be characterised by, caused by, or otherwise associated with fibrosis, either directly or indirectly.

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[035] The term "polypeptide" means a polymer made up of amino acids linked together by peptide bonds. The term "peptide" may also be used to refer to such a polymer although in some instances a peptide may be shorter (i.e. composed of fewer amino acid residues) than a polypeptide. Nevertheless, the terms "polypeptide" and "peptide" may be used interchangeably herein.

[036] The term "relaxin polypeptide" as used herein means a polypeptide, whether corresponding to a naturally occurring relaxin molecule or a modified form thereof which displays biological activity typically associated with relaxin. The level of such relaxin biological activity displayed by a relaxin polypeptide of the invention may be equivalent to that of a naturally occurring or native relaxin, or may be enhanced or reduced when compared with the activity of a naturally occurring or native relaxin. The "biological activity" typically comprises the ability to bind the relaxin receptor RXFP1. In the context of the present disclosure, the term relaxin polypeptide refers to polypeptides that in the mature form either comprise a biologically active B chain capable of binding the RXFP1 receptor or heterodimeric polypeptides comprising an A chain and a B chain. Also encompassed by the term "relaxin polypeptide" are precursor relaxin polypeptides also comprising a C chain. In the context of relaxin polypeptides the terms "naturally occurring" and "native" refer to relaxin polypeptides as encoded by and produced from the genome of an organism. For example in humans three distinct forms of relaxin have been identified to date, H1, H2 and H3. Each of these forms is considered herein as a different "naturally occurring" or "native" relaxin.

[037] As used herein the term "derived" in the context of relaxin A and B chains in relaxin polypeptides means that the A and B chain sequences correspond to, originate from, or otherwise share significant sequence homology with naturally occurring A and B chain sequences. Those skilled in the art will also understand that by being "derived" from a naturally occurring or native relaxin sequence, the sequence in a relaxin polypeptide need not be physically constructed or generated from the naturally occurring or native sequence, but may be chemically synthesised such that the sequence is "derived" from the naturally occurring or native sequence in that it shares sequence homology and function with the naturally occurring or native sequence.

[038] The term "modified" as used herein in the context of a relaxin polypeptide means a polypeptide that differs from a naturally occurring or native relaxin polypeptide

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at one or more amino acid positions in one or more peptide chains of such naturally occurring or native polypeptide.

[039] The term "conservative amino acid substitution" as used herein refers to a substitution or replacement of one amino acid for another amino acid with similar properties within a polypeptide chain (primary sequence of a protein). For example, the substitution of the charged amino acid glutamic acid (Glu) for the similarly charged amino acid aspartic acid (Asp) would be a conservative amino acid substitution. The nature of other conservative amino acid substitutions are well known to those skilled in the art.

[040] The term "polynucleotide" as used herein refers to a single- or double- stranded polymer of deoxyribonucleotide, ribonucleotide bases or known analogues of natural nucleotides, or mixtures thereof. The term includes reference to the specified sequence as well as to the sequence complimentary thereto, unless otherwise indicated. The terms "polynucleotide" and "nucleic acid" are used interchangeably herein.

[041] As used herein the terms "treating", "treatment", "preventing", "prevention" and variations thereof refer to any and all uses which remedy a disease, disorder or condition or symptoms, prevent the establishment of a condition or disease, or otherwise prevent, hinder, retard, or reverse the progression of a condition or disease or other undesirable symptoms in any way whatsoever. Thus, terms "treating" and the like are to be considered in their broadest context. For example, treatment does not necessarily imply that a patient is treated until total recovery. In the context of some conditions, methods of the present invention involve "treating" the disease, disorder or condition in terms of reducing or eliminating the occurrence of a highly undesirable and irreversible outcome of the progression of the condition but may not of itself prevent the initial occurrence of the disease, disorder or condition. Accordingly, treatment includes amelioration of the symptoms of a particular disease, disorder or condition or preventing or otherwise reducing the risk of developing a particular disease, disorder or condition.

[042] The terms "inhibits" and "inhibiting" and variations thereof as used herein as they relate to fibrosis and fibrotic diseases do not necessarily mean complete inhibition. Rather, inhibition may be to an extent, and/or for a time, sufficient to produce the desired effect. Thus inhibition of fibrosis for example may be partial or complete

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attenuation of one or more biological causes or effects of fibrosis, and inhibition may be temporally and/or spatially limited. By temporally and/or spatially limited is meant that the inhibition may be limited to particular physiological conditions or circumstances and/or to particular regions of the body.

[043] As used herein the term "effective amount" includes within its meaning a non-toxic but sufficient amount of an agent or compound to provide the desired effect. The exact amount required will vary from subject to subject depending on factors such as the species being treated, the age and general condition of the subject, the tissue or organ in which the fibrosis occurs, the severity or extent of the fibrosis or fibrotic disease being treated, the particular agent being administered and the mode of administration and so forth. Thus, it is not possible to specify an exact "effective amount". However, for any given case, an appropriate "effective amount may be determined by one of ordinary skill in the art using only routine experimentation.

[044] The term "subject" as used herein typically refers to mammals including humans, primates, livestock animals (eg. sheep, pigs, cattle, horses, donkeys), laboratory test animals (eg. mice, rabbits, rats, guinea pigs), companion animals (eg. dogs, cats) and captive wild animals (eg. foxes, kangaroos, deer). Preferably, the mammal is human or a laboratory test animal. Even more preferably, the mammal is a human.

[045] Provided herein are compositions and methods for the treatment of fibrosis and fibrotic disorders and for the inhibition of fibrosis. The compositions and methods involve combination therapy comprising the administration of at least one relaxin polypeptide and at least one ACE inhibitor.

[046] In accordance with such combination therapies the relaxin polypeptide and the ACE inhibitor as described herein are coadministered to facilitate the desired therapeutic outcome. By "coadministered" is meant simultaneous administration in the same formulation or in different formulations via the same or different routes or sequential administration by the same or different routes. By "sequential" administration is meant a time difference of from seconds, minutes, hours, days, weeks, months or years between the administration of the different agents. The agents may be administered in any order.

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[047] The relaxin polypeptide to be administered may be any suitable relaxin polypeptide, either in mature form or precursor form, which is capable of binding and inducing a biological effect via the RXFP1 relaxin receptor. Typically the relaxin polypeptide comprises an A chain and a B chain. The A chain may comprise an amino acid sequence as set forth in SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5. The B chain may comprise an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6. The relaxin polypeptide may be a relaxin-1, relaxin-2 or relaxin-3 polypeptide. In a particular embodiment, the relaxin is a relaxin-2 polypeptide. Where the subject is human, typically the relaxin-2 is H2 relaxin.

[048] The present disclosure also contemplates variants and fragments of relaxin polypeptides disclosed herein.

[049] The term "variant" as used herein refers to substantially similar sequences. Generally, polypeptide sequence variants possess qualitative biological activity in common. Further, these polypeptide sequence variants may share at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity. Also included within the meaning of the term "variant" are homologues of polypeptides of the disclosure. A homologue is typically a polypeptide from a different species but sharing substantially the same biological function or activity as the corresponding polypeptide disclosed herein.

[050] Further, the term "variant" also includes analogues of the polypeptides of the present disclosure, wherein the term "analogue" means a polypeptide which is a derivative of a polypeptide of the disclosure, which derivative comprises addition, deletion, substitution of one or more amino acids, such that the polypeptide retains substantially the same function.

[051] The term "fragment" refers to a polypeptide molecule that is a constituent of a polypeptide of the disclosure or variant thereof. Typically the fragment possesses qualitative biological activity in common with the polypeptide of which it is a constituent. The peptide fragment may be between about 5 to about 26 amino acids in length, between about 5 to about 25 amino acids in length, between about 5 to about 20 amino acids in length, or between about 5 to about 15 amino acids in length. Alternatively, the peptide fragment may be between about 5 to about 10 amino acids in length.

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[052] Relaxin polypeptides of the disclosure can also be modified, for instance, by glycosylation, amidation, carboxylation, or phosphorylation, or by the creation of acid addition salts, amides, esters, in particular C-terminal esters, and N-acyl derivatives of the polypeptides. The polypeptides can also be further modified to create polypeptide derivatives by forming covalent or noncovalent complexes with other moieties. Covalently-bound complexes can be prepared by cross-linking the chemical moieties to functional groups on the side chains of amino acids comprising the peptides, or at the N-or C terminus. For example, as polypeptide sequence minimisation is often accompanied by increased susceptibility to enzymatic attack and degradation with a corresponding decrease in plasma half life and *in vivo* activity, a modified polypeptide of the present disclosure may be generated with a polyethylene moiety conjugated at one or more locations (PEGylation) to increase *in vivo* half life of the polypeptide. Those skilled in the art will appreciate that a number of other well known approaches exist to extend the *in vivo* half life of polypeptides, such as for example the addition of albumin affinity tags, and the present disclosure is not limited by reference to the exemplary means specifically discussed herein.

[053] In accordance with the present disclosure relaxin polypeptides may be produced using standard techniques of recombinant DNA and molecular biology that are well known to those skilled in the art. Guidance may be obtained, for example, from standard texts such as Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, New York, 1989 and Ausubel *et al.*, *Current Protocols in Molecular Biology*, Greene Publ. Assoc. and Wiley-Intersciences, 1992. Methods described in Morton *et al.*, 2000 (*Immunol Cell Biol* 78:603-607), Ryan *et al.*, 1995 (*J Biol Chem* 270:22037-22043) and Johnson *et al.*, 2005 (*J Biol Chem* 280:4037-4047) are examples of suitable purification methods for relaxin polypeptides, although the skilled addressee will appreciate that the present invention is not limited by the method of purification or production used and any other method may be used to produce relaxin for use in accordance with the methods and compositions of the present disclosure. Relaxin peptide fragments may be produced by digestion of a polypeptide with one or more proteinases such as endoLys-C, endoArg-C, endoGlu-C and staphylococcus V8-protease. The digested peptide fragments can be purified by, for example, high performance liquid chromatographic (HPLC) techniques.

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[054] The purification of modified relaxin polypeptides of the present disclosure may be scaled-up for large-scale production purposes. For this purpose a range of techniques well known to those skilled in the art are available.

[055] Relaxin polypeptides of the present disclosure, as well as fragments and variants thereof, may also be synthesised by standard methods of liquid or solid phase chemistry well known to those of ordinary skill in the art. For example such molecules may be synthesised following the solid phase chemistry procedures of Steward and Young (Steward, J. M. & Young, J. D., Solid Phase Peptide Synthesis. (2nd Edn.) Pierce Chemical Co., Illinois, USA (1984). In general, such a synthesis method comprises the sequential addition of one or more amino acids or suitably protected amino acids to a growing peptide chain. Typically, either the amino or carboxyl group of the first amino acid is protected by a suitable protecting group. The protected amino acid is then either attached to an inert solid support or utilised in solution by adding the next amino acid in the sequence having the complimentary (amino or carboxyl) group suitably protected and under conditions suitable for forming the amide linkage. The protecting group is then removed from this newly added amino acid residue and the next (protected) amino acid is added, and so forth. After all the desired amino acids have been linked, any remaining protecting groups, and if necessary any solid support, is removed sequentially or concurrently to produce the final polypeptide.

[056] Embodiments of the invention contemplate the administration of a polynucleotide encoding a relaxin polypeptide as disclosed or contemplated herein. In such situations the polynucleotide is typically operably linked to a promoter such that the appropriate polypeptide sequence is produced following administration of the polynucleotide to the subject. In particular embodiments, the polynucleotide may be cloned into a vector. The vector may be a plasmid vector, a viral vector, or any other suitable vehicle adapted for the insertion of foreign sequences, their introduction into eukaryotic cells and the expression of the introduced sequences. Typically the vector is a eukaryotic expression vector and may include expression control and processing sequences such as a promoter, an enhancer, ribosome binding sites, polyadenylation signals and transcription termination sequences. The polynucleotide to be administered may comprise naked DNA or may be in the form of a composition, together with one or more pharmaceutically acceptable carriers.

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[057] Those skilled in the art will appreciate that any ACE inhibitor may be used in combination with a relaxin polypeptide in accordance with embodiments of the invention. By way of example, the ACE inhibitor may be a dicarboxylate-containing ACE inhibitor, a sulfhydryl-containing ACE inhibitor or a phosphonate-containing ACE inhibitor. Examples of suitable ACE inhibitors that may be employed include, but are not limited to, captopril, zofenopril, enalapril, ramipril, quinapril, perindopril, lisinopril, benazepril, imidapril, trandolapril, alacepril, cilazapril, delapril, moexipril, rentiapril, spirapril, temocapril and fosinopril. In addition, several naturally derived molecules such as the lactotripeptides Val-Pro-Pro and Ile-Pro-Pro, for example as produced by *Lactobacillus helveticus*, have also been shown to act as ACE inhibitors, and may also be employed in accordance with present embodiments.

[058] In addition to comprising combination therapy using relaxin polypeptides and ACE inhibitors, those skilled in the art will recognise that the compositions and methods of the present invention may further comprise the administration of one or more additional therapeutic agents. Such additional agents are typically anti-fibrotic agents or other agents useful in the treatment of one or more symptoms of a fibrotic disease. Such additional agents may be formulated into the same composition for administration with a relaxin polypeptide and/or an ACE inhibitor or may be administered separately. Where formulated into a separate composition administration may be simultaneous or sequential and may be by the same or different routes.

[059] The compositions and methods disclosed herein find application in the treatment and inhibition of fibrosis and the treatment of fibrotic disorders. Fibrosis affects a wide range of organs and tissues including the kidneys, heart, lungs, liver, epidermis, endodermis, muscle, tendon, cartilage, pancreas, uterus, nervous system, testis, ovary, adrenal gland, cardiovascular system such as arteries and veins, the gastrointestinal tract including the stomach, small intestine and colon, and the biliary tract. In particular embodiments the fibrosis may be cardiac fibrosis, renal fibrosis, hepatic fibrosis, pulmonary fibrosis or myelofibrosis.

[060] Treating or inhibiting fibrosis in accordance with embodiments of the present invention may involve decreasing the level of fibrosis relative to an untreated control, as measured by any standard method known to those skilled in the art. A reduction in fibrosis may also be measured by a reduction in any symptom associated with fibrosis or a fibrotic disease.

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[061] Fibrotic diseases amenable to treatment using the compositions and methods disclosed herein include those caused by or associated with, for example, trauma, acute or chronic tissue or organ damage or injury, surgery, wound healing, inflammation, infection or toxin exposure. Examples of cardiac fibrosis and cardiac-related fibrotic diseases that may be treated in accordance with the present invention include, but are not limited to myocardial infarction, ischemia, fibrotic cardiomyopathy, heart failure, atherosclerosis, cardiac hypertension, cardiac reperfusion injury and type 1 diabetes. Examples of renal fibrosis and renal-related fibrotic diseases that may be treated in accordance with the present invention include, but are not limited to renal hypertension, tubulointerstitial kidney disease, chronic papillary necrosis, crescentic glomerulonephritis, and renal insufficiency due to loss of renal mass. Examples of pulmonary fibrosis and pulmonary-related fibrotic diseases that may be treated in accordance with the present invention include, but are not limited to hypoxic pulmonary hypertension, idiopathic pulmonary fibrosis, pulmonary reperfusion injury, interstitial pulmonary fibrosis and allergic airways diseases. Examples of hepatic fibrosis and liver-related fibrotic diseases that may be treated in accordance with the present invention include, but are not limited to cirrhosis. Other fibrotic diseases that may be treated in accordance with the present invention include, but are not limited to scleroderma, systemic sclerosis, dermal fibrosis, familial cutaneous collagenoma, acute pancreatitis, diabetic retinopathy, and proliferative vitreoretinopathy.

[062] Compositions in accordance with embodiments of the invention may be administered by standard routes. In general, the compositions may be administered by the parenteral (e.g., intravenous, intraspinal, intracerebroventricular, intranasal, subcutaneous or intramuscular), oral or topical route. Administration may be systemic, regional or local. The particular route of administration to be used in any given circumstance will depend on a number of factors, including the nature of the fibrosis or fibrotic disease to be treated, the severity and extent of the condition, the required amount or dosage of the agents or composition to be delivered and the potential side-effects of the agents or composition.

[063] In general, suitable compositions may be prepared according to methods which are known to those of ordinary skill in the art and may include a pharmaceutically acceptable diluent, adjuvant and/or excipient. The diluents, adjuvants and excipients

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must be "acceptable" in terms of being compatible with the other ingredients of the composition, and not deleterious to the recipient thereof.

[064] Examples of pharmaceutically acceptable carriers or diluents are demineralised or distilled water; saline solution; vegetable based oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oil, arachis oil or coconut oil; silicone oils, including polysiloxanes, such as methyl polysiloxane, phenyl polysiloxane and methylphenyl polysiloxane; volatile silicones; mineral oils such as liquid paraffin, soft paraffin or squalane; cellulose derivatives such as methyl cellulose, ethyl cellulose, carboxymethylcellulose, sodium carboxymethylcellulose or hydroxypropylmethylcellulose; lower alkanols, for example ethanol or iso-propanol; lower aralkanols; lower polyalkylene glycols or lower alkylene glycols, for example polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, 1,3-butylene glycol or glycerin; fatty acid esters such as isopropyl palmitate, isopropyl myristate or ethyl oleate; polyvinylpyrrolidone; agar; carrageenan; gum tragacanth or gum acacia, and petroleum jelly. Typically, the carrier or carriers will form from 10% to 99.9% by weight of the compositions.

[065] Compositions may be in a form suitable for administration by injection, in the form of a formulation suitable for oral ingestion (such as capsules, tablets, caplets, elixirs, for example), in the form of an ointment, cream or lotion suitable for topical administration, in a form suitable for delivery as an eye drop, in an aerosol form suitable for administration by inhalation, such as by intranasal inhalation or oral inhalation, in a form suitable for parenteral administration, e.g., intravenous, intraspinal, intracerebroventricular, intranasal, subcutaneous or intramuscular.

[066] For administration as an injectable solution or suspension, non-toxic parenterally acceptable diluents or carriers can include, Ringer's solution, isotonic saline, phosphate buffered saline, ethanol and 1,2 propylene glycol. In some embodiments cerebrospinal fluid (CSF) may be used as a carrier.

[067] Some examples of suitable carriers, diluents, excipients and adjuvants for oral use include peanut oil, liquid paraffin, sodium carboxymethylcellulose, methylcellulose, sodium alginate, gum acacia, gum tragacanth, dextrose, sucrose, sorbitol, mannitol, gelatine and lecithin. In addition these oral formulations may contain suitable flavouring

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and colourings agents. When used in capsule form the capsules may be coated with compounds such as glyceryl monostearate or glyceryl distearate which delay disintegration.

[068] Adjuvants typically include emollients, emulsifiers, thickening agents, preservatives, bactericides and buffering agents.

[069] Methods for preparing parenterally administrable compositions are apparent to those skilled in the art, and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa., hereby incorporated by reference herein.

[070] The composition may incorporate any suitable surfactant such as an anionic, cationic or non-ionic surfactant such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as siliceous silicas, and other ingredients such as lanolin, may also be included.

[071] The compositions may also be administered in the form of liposomes. Liposomes are generally derived from phospholipids or other lipid substances, and are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolisable lipid capable of forming liposomes can be used. The compositions in liposome form may contain stabilisers, preservatives, excipients and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art, and in relation to this specific reference is made to: Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 *et seq.*, the contents of which is incorporated herein by reference.

[072] For the purposes of the present disclosure agents may be administered to subjects as compositions either therapeutically or preventively. In a therapeutic application, compositions are administered to a patient already suffering from a disease, disorder or condition, in an amount sufficient to cure or at least partially arrest the disease, disorder or condition and its complications. The composition should provide a quantity of the agent sufficient to effectively treat the patient.

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[073] The therapeutically effective amount 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 molecule or agent employed; the composition employed; the age, body weight, general health, sex and diet of the subject; the time of administration; the route of administration; the rate of sequestration of the agent; the duration of the treatment; drugs used in combination or coincidental with the treatment, together with other related factors well known in medicine. One skilled in the art would be able, by routine experimentation, to determine an effective, non-toxic amount of agent which would be required to treat applicable diseases.

[074] Generally, an effective amount is expected to be in the range of about 0.0001mg to about 1000mg per kg body weight per 24 hours; typically, about 0.001mg to about 750mg per kg body weight per 24 hours; about 0.01mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 250mg per kg body weight per 24 hours; about 1.0mg to about 250mg per kg body weight per 24 hours. More typically, an effective dose range is expected to be in the range about 1.0mg to about 200mg per kg body weight per 24 hours; about 1.0mg to about 100mg per kg body weight per 24 hours; about 1.0mg to about 50mg per kg body weight per 24 hours; about 1.0mg to about 25mg per kg body weight per 24 hours; about 5.0mg to about 50mg per kg body weight per 24 hours; about 5.0mg to about 20mg per kg body weight per 24 hours; about 5.0mg to about 15mg per kg body weight per 24 hours.

[075] Alternatively, an effective amount may be up to about 500mg/m². Generally, an effective dosage is expected to be in the range of about 25 to about 500mg/m², preferably about 25 to about 350mg/m², more preferably about 25 to about 300mg/m², still more preferably about 25 to about 250mg/m², even more preferably about 50 to about 250mg/m², and still even more preferably about 75 to about 150mg/m².

[076] It will also be apparent to one of ordinary skill in the art that the optimal quantity and spacing of individual dosages will be determined by the nature and extent of the disease being treated, the form, route and site of administration, and the nature of the particular individual being treated. Also, such optimum conditions can be determined by conventional techniques. It will also be apparent to one of ordinary skill in the art that the optimal course of treatment, such as, the number of doses of the composition given

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per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

[077] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

[078] The present invention will now be described with reference to the following specific examples, which should not be construed as in any way limiting the scope of the invention.

Examples

Example 1 – Anti-fibrotic efficacy of relaxin and clinically used ACE inhibitors

[079] To first determine the dose at which clinically used ACE inhibitors specifically inhibit fibrosis progression, 6-8 week old male mice were subjected to the unilateral ureteric obstruction (UUO)-induced model of tubulointerstitial kidney disease. This model mimics several features of human disease. Affected mice i) experience fibrosis progression in a relatively short time frame; ii) experience fibrosis progression independently of gender; and iii) experience fibrosis progression independently of blood pressure changes. This latter point is particularly important as it avoids the complications of the blood pressure changes that can be induced by ACE inhibitors.

[080] To establish the model, following an abdominal incision, the left ureter of animals (n=3-6 per group) was ligated with 5.0 surgical silk, while the contralateral ureter was left intact. The incision was sutured and mice were allowed to recover following temgesic administration (Burprenorphine; for pain relief). UUO results in inflammation and fibrogenesis of the obstructed kidney by day 3 post-UUO and established fibrosis in this obstructed kidney by day 5. Day 5 post-injury was specifically chosen as it was a time-point that has been previously studied to demonstrate the anti-fibrotic efficacy of ACE inhibitors. In each treatment group, animals were administered with the ACE inhibitor enalapril (100mg/L or 200mg/L) or

perindopril (also known as coversyl; 200mg/L) in their drinking water, from 1-2 days prior to UUO surgery until day 5 post-surgery. Animals with intact un-ligated ureters (n=7) were used as controls. The animals were housed in a controlled environment with free access to rodent lab chow and water during the experimental period.

[081] At 5-days post-surgery, all animals were killed for collection of their kidneys. Kidney tissues were cut into four transverse sections (each containing cortex and medulla) for western blotting, hydroxyproline analysis, and fixation in two different fixatives for histochemistry. To ensure standardization, and to enable inter-group comparisons, each assay used the same portion from each animal. Hydroxyproline analysis was used to determine total collagen content and collagen concentration (% collagen content/dry weight tissue) from similar kidney portions from each of the groups studied.

[082] As shown in Figure 1A, both enalapril and perindopril were able to significantly prevent the UUO-induced increase in collagen concentration (a measure of fibrosis) by 25-30% when administered at a concentration of 200mg/L, but did not have any effects on collagen concentration at 100mg/L.

[083] The effect of 200mg/L enalapril was then compared to 0.5mg/kg/day H2 relaxin, being a dose at which H2 relaxin has consistently been able to inhibit fibrosis in several experimental models of fibrosis (Samuel *et al.*, 2011, *Lab Invest* 91:675-690; Hewitson *et al.*, 2010, *Endocrinology* 151:4938-4948; and Samuel *et al.*, 2004, *Endocrinology* 145:4125-4133). The combined effects of enalapril and H2 relaxin were also investigated to determine if H2 relaxin could enhance the anti-fibrotic actions of an ACE inhibitor and thus have potential as a suitable adjunct therapy. These effects were assessed in both the UUO model (described above) and in an isoproterenol (ISO)-induced model of myocardial infarction and cardiac damage. The ISO model mimics several features of human disease with affected mice experiencing fibrosis progression independently of gender and blood pressure changes. In the ISO model, 8 week old mice were given subcutaneous injections of ISO for 5 consecutive days (which causes the heart to beat faster, leading to injury and myocardial infarction) and then left for a further 12 days for fibrosis to progress.

[084] A sub-group of mice (n=5-6 per model) were administered 200mg/L enalapril in their drinking water, from 2 days prior to surgery until day 5 post-UUO (over 7 days) or

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immediately before the first ISO injection until 12-days after the 5th ISO injection (over 17 days). A separate sub-group of mice (n=5-6 per model) were subcutaneously implanted with osmotic mini-pumps (with an infusion rate of 0.5 μ l/hour; Alzet, Cupertino, CA) containing recombinant H2 relaxin, from 3 days prior to surgery until day 5 post-UUO (over 8 days) or immediately before the first ISO injection until 12 days after the 5th ISO injection (over 17 days). Recombinant H2 relaxin is bioactive in mice, and this rate of infusion produces circulating relaxin levels of ~20-40ng/ml. A third sub-group of mice (n=5-6 per model) were administered both enalapril and H2 relaxin (as described above). The animals were housed in a controlled environment with free access to rodent lab chow and water during the experimental period.

[085] At 5 days post-UUO or 12 days after the 5th ISO injection, all animals were killed for collection of their kidneys and heart, respectively. The left ventricle was separated from the right ventricle and atria; and then left ventricle and kidney tissues were cut into four transverse sections for western blotting, hydroxyproline analysis, and fixation in two different fixatives for histochemistry. To ensure standardization, and to enable inter-group comparisons, each assay used the same portion from each animal. Total collagen content in each tissue and collagen concentration was extrapolated from hydroxyproline analysis.

[086] As shown in Figures 1B and 1C, 200mg/L enalapril modestly but significantly inhibited collagen concentration (fibrosis progression) in both models studied, by 25-30%. On the other hand, 0.5mg/kg/d H2 relaxin inhibited fibrosis by approximately 40% in the UUO model (Figure 1B) and approximately 45% in the ISO model (Figure 1C). Combination therapy inhibited fibrosis progression by 50% in the UUO model (Figure 1B) and 60% in the ISO model (Figure 1C), which in both cases was significantly greater than the effects of ACE inhibitors alone.

Example 2 – Mechanistic aspects of H2 relaxin anti-fibrotic activity

[087] H2 relaxin is known to mediate its anti-fibrotic actions via interference of TGF- β 1 signal transduction. Specifically, H2 relaxin activation of its receptor, RXFP1 (in TGF- β 1-stimulated human renal fibroblasts and renal myofibroblasts isolated from the obstructed kidneys of rats, 3 days post-UUO), inhibits Smad2 phosphorylation (where Smad2 is a regulatory protein that promotes TGF- β 1 activity). It has also recently been shown in rat renal myofibroblasts that H2 relaxin promotes neuronal nitric oxide (NO)

synthase (nNOS) expression and signals through an nNOS (NOS D)-NO-cyclic guanosine monophosphate (cGMP)-dependent pathway to abrogate Smad2 phosphorylation, as a means of interfering with TGF- β 1-signal transduction of renal myofibroblast differentiation and collagen production (fibrosis) (Mookerjee *et al.*, 2009, *FASEB J* 23:1219-1229).

[088] As H2 relaxin has also been shown to separately interfere with TGF- β 1 and angiotensin II-stimulation of cardiac fibroblast proliferation, differentiation and collagen production, relaxin signal transduction studies were extended to determine if H2 relaxin interferes with angiotensin II stimulation of TGF- β 1, as angiotensin II has been shown to promote TGF- β 1 activity and fibrosis progression via the AT1 receptor. Alternatively, angiotensin II conversion to angiotensin 1-7 can act via the AT2 receptor to block TGF- β 1 activity and subsequent fibrosis, via a NO-cGMP-dependent mechanism. Hence, H2 relaxin (acting through its receptor, RXFP1) may either block the angiotensin II-induced promotion of TGF- β 1 activity via the AT1 receptor or promote the inhibition of TGF- β 1 activity via the AT2 receptor and NO signal transduction.

[089] To determine if H2 relaxin was able to abrogate the actions of angiotensin II on TGF- β 1 activity via the AT1 receptor, renal myofibroblasts isolated from the obstructed kidneys of rats, 3 days post-UUO were treated with H2 relaxin (100ng/ml; 16.8nM) in the absence or presence of increasing concentrations (0.1, 1, 10 μ M) of the AT1 receptor antagonist, Irbesartan over 72 hours in culture. Untreated cells were used as controls. After 72 hours in culture, media from untreated and treated cells were assessed for MMP-2 and MMP-9 activity (known to be targets of relaxin activity; by gelatin zymography and densitometry of the resulting MMP bands), while the cell layer was extracted for protein and assessed by Western blotting for changes in Smad2 phosphorylation (as determined by densitometry of the resulting phosphorylated Smad2 bands).

[090] As shown in Figures 2 and 3, the H2 relaxin-induced promotion of MMP-2 and MMP-9 (respectively) were dose-dependently blocked by Irbesartan. Irbesartan was able to completely inhibit the H2 relaxin-induced upregulation of MMP-2 at 10 μ M, and MMP-9 at 1 μ M and 10 μ M, to levels measured in untreated control cultures. All concentrations of AT1 receptor antagonist evaluated (0.1-10 μ M) did not affect MMP activity in the absence of H2 relaxin treatment. Similarly, Irbesartan completely

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inhibited the H2 relaxin-induced upregulation of MMP-13 at concentrations of 0.1 μ M, 1 μ M and 10 μ M (data not shown), but did not have any effect on the basal levels of MMP-13 (in the absence of H2 relaxin).

[091] As shown in Figure 4, the H2 relaxin-induced inhibition of smad2 phosphorylation was completely blocked by Irbesartan, at concentrations of 0.1 μ M, 1 μ M and 10 μ M, to levels measured in untreated control cultures. Importantly, the same concentrations of AT1 receptor antagonist did not affect smad2 phosphorylation in the absence of H2 relaxin treatment. Similar results were obtained for TGF- β 1 and α -SMA (data not shown).

[092] The combined findings from Figures 2 to 4 suggest that there is an interaction between H2 relaxin and the angiotensin II-AT1 receptor axis, and that this interaction inhibits TGF- β 1 signal transduction, and promotes MMP activity.

[093] The inventors then investigated the effect of an AT2 receptor antagonist (PD12331) on the ability of H2 relaxin to promote latent and active MMP-2 levels. Similar to the results obtained for the AT1 receptor antagonist Irbesartan, at PD12331 concentrations of 0.1 μ M, 1 μ M and 10 μ M, H2 relaxin-induced upregulation of MMP-2 was completely inhibited (data not shown), but no effect on MMP-2 levels was observed in the absence of H2 relaxin.

Example 3 – Immunohistochemical staining and morphometric analysis of TGF- β 1 and Smad2 phosphorylation

[094] The inventors also used immunohistochemical staining and morphometric analysis to investigate TGF- β 1 expression and Smad2 phosphorylation (the phosphorylation of Smad2 being required to promote TGF- β 1 signal transduction and pro-fibrotic action) from the ISO model of myocardial injury (described above). Serial sections left ventricular tissue obtained from mice in the ISO model of myocardial infarction were immunohistochemically assessed using a rabbit polyclonal antibody to TGF- β 1 (1:200, Santa Cruz Biotechnology, CA, USA) and rabbit monoclonal antibody to phosphorylated-Smad2 (Ser465/467) (pSmad2; 1:500, Cell Signalling Technology Inc., MA, USA). Slides from all treatment groups were dewaxed and incubated in 3% hydrogen peroxide for 10 minutes to block the endogenous peroxidase activity. Citrate

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antigen retrieval (TGF- β 1) was performed by heating the slides in pre-warmed citrate buffer (pH 6.0) in a microwave for 5 minutes and cooled in a water bath at room temperature for 25 minutes. No antigen retrieval was performed for pSmad2. Sections were circled with a Dako pen (Dako Cytomation, CA, USA), blocked with antibody diluents (Dako Cytomation, CA, USA) for 10 minutes to prevent non-specific binding and incubated overnight with the specific primary antibody detailed above. Following overnight incubation, the slides were washed in three x 7 minute washes of phosphate buffer saline (PBS) before being incubated with the species specific secondary antibody (anti-rabbit for 30 minutes, Dako Cytomation and anti-mouse for 60 minutes, Dako Cytomation). The secondary antibody was removed with washes of PBS before sections were incubated with one drop of 3,3-diaminobenzidine (DAB) (Dako Cytomation, CA, USA) in 1ml of substrate buffer solution (pH 7.5) for 5 minutes. Slides were rinsed in water and counter stained in haematoxylin, dehydrated and mounted. Morphometric analysis was then performed on the stained sections for the picrosirius red-staining of interstitial collagen.

[095] It was found that H2 relaxin (0.5mg/kg/d) had a significantly enhanced ability to inhibit TGF- β 1 staining/expression and Smad2 phosphorylation ($p < 0.01$) compared to enalapril (200mg/L) (Figure 5). Furthermore, compared to enalapril alone, the combination of H2 relaxin and enalapril (0.5mg/kg/d H2 relaxin + 200mg/L enalapril) also significantly more effective at inhibiting TGF- β 1 staining (double that of enalapril alone; $p < 0.05$) and Smad2 phosphorylation (triple that of enalapril alone; $p < 0.01$) (Figure 5). This is consistent with combination therapy of H2 relaxin and enalapril inhibiting ISO- and UVO-induced collagen concentration and staining by double that of enalapril alone, further confirming that relaxin is able to enhance the anti-fibrotic efficacy of ACE inhibitors.

Example 4 – Reduction of established fibrosis

[096] The inventors also investigated if combination therapy (0.5mg/kg/d H2 relaxin + 200mg/L enalapril) could reduce established fibrosis, representing the clinically relevant situation, when administered over a one week (7 day) period beginning 10 days after the onset of ISO-induced injury (as described above). Experiments were carried out as described above in Example 1. As shown in Figure 6, combination therapy was able to significantly prevent further progression of established fibrosis (total collagen

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concentration and interstitial collagen staining) by 30-35% of that measured in the ISO alone group; which was also associated with its ability to reduce TGF- β 1 staining and signal transduction (at the level of Smad2 phosphorylation).

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Claims

1. A pharmaceutical composition for use in the treatment of fibrosis or a fibrotic disease, the composition comprising at least one relaxin polypeptide and at least one ACE inhibitor.
2. A composition according to claim 1 wherein the fibrosis is renal or cardiac fibrosis.
3. A composition according to claim 1 or 2 wherein the fibrosis results from tissue or organ damage or injury, such as cardiac injury.
4. A composition according to claim 3 wherein the fibrosis results from cardiac injury.
5. A composition according to claim 1 wherein the fibrotic disorder is interstitial kidney disease, myocardial infarction or other disorder associated with cardiac damage.
6. A composition according to any one of claims 1 to 5 wherein the treatment comprises reducing or preventing progression of existing fibrosis.
7. A composition according to any one of claims 1 to 6 wherein the relaxin polypeptide comprises an A chain and a B chain.
8. A composition according to any one of claims 1 to 7 wherein the relaxin polypeptide comprises an A chain comprising an amino acid sequence as set forth in SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5 or a variant or derivative thereof.
9. A composition according to any one of claims 1 to 7 wherein the relaxin polypeptide comprises an B chain comprising an amino acid sequence as set forth in SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6 or a variant or derivative thereof.
10. A composition according to any one of claims 1 to 9 wherein the relaxin polypeptide is a relaxin-2 polypeptide.
11. A composition according to claim 10 wherein the relaxin-2 polypeptide comprises an A chain comprising the sequence set forth in SEQ ID NO:1 and a B chain comprising the sequence set forth in SEQ ID NO:2.
12. A composition according to any one of claims 1 to 11 wherein the relaxin polypeptide is administered to the subject in the form of a polynucleotide encoding the polypeptide such that the polynucleotide is expressed *in vivo* producing the polypeptide.

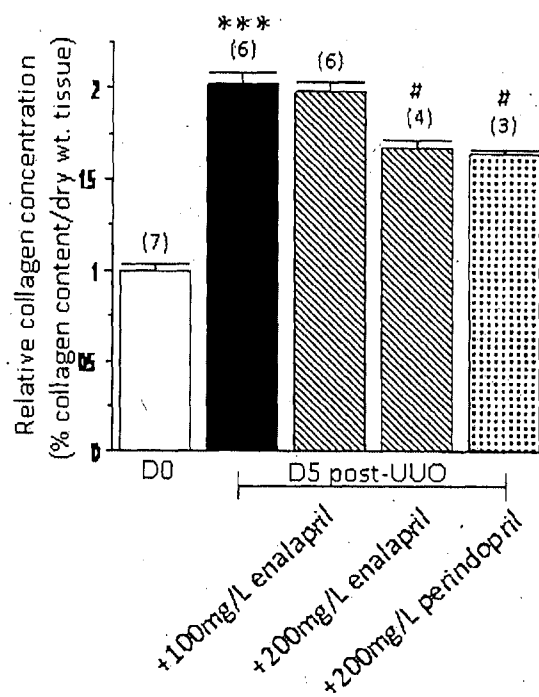
- 26 -

13. A composition according to any one of claims 1 to 12 wherein the ACE inhibitor comprises a dicarboxylate-containing agent.
14. A composition according to any one of claims 1 to 13 wherein the ACE inhibitor is selected from enalapril and perindopril.
15. A pharmaceutical composition for use in inhibiting fibrosis, the composition comprising at least one relaxin polypeptide and at least one ACE inhibitor.
16. A pharmaceutical composition for use in reducing or preventing progression of existing fibrosis, the composition comprising at least one relaxin polypeptide and at least one ACE inhibitor.
17. A method for treating fibrosis or a fibrotic disorder in a subject in need thereof, the method comprising administering to the subject an effective amount of at least one relaxin polypeptide and an effective amount of at least one ACE inhibitor.
18. A method according to claim 17 wherein the relaxin polypeptide and the ACE inhibitor are formulated together in a single composition.
19. A method according to claim 17 wherein the relaxin polypeptide and the ACE inhibitor are administered separately.
20. A method for inhibiting fibrosis in a subject in need thereof, the method comprising administering to the subject an effective amount of at least one relaxin polypeptide and an effective amount of at least one ACE inhibitor.
21. A method for reducing or preventing progression of existing fibrosis in a subject in need thereof, the method comprising administering to the subject an effective amount of at least one relaxin polypeptide and an effective amount of at least one ACE inhibitor.
22. Use of at least one relaxin polypeptide and at least one ACE inhibitor for the manufacture of a medicament for the treatment of fibrosis or a fibrotic disorder.
23. Use of at least one relaxin polypeptide and at least one ACE inhibitor for the manufacture of a medicament for the inhibition of fibrosis.
24. Use of at least one relaxin polypeptide and at least one ACE inhibitor for the manufacture of a medicament for reducing or preventing progression of existing fibrosis.

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FIGURE 1

A



B

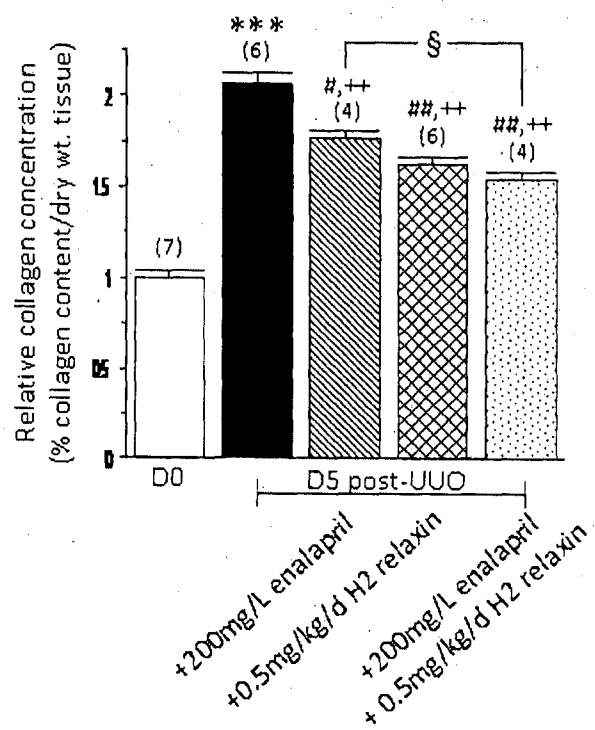


FIGURE 1

C

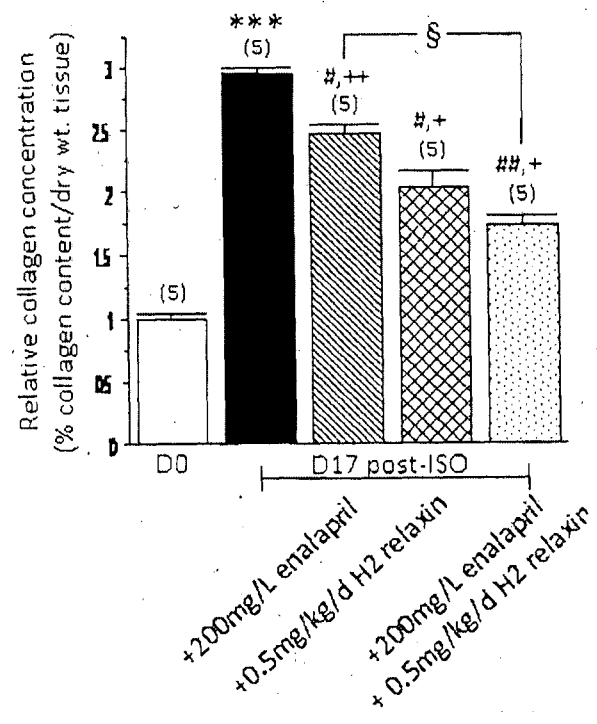
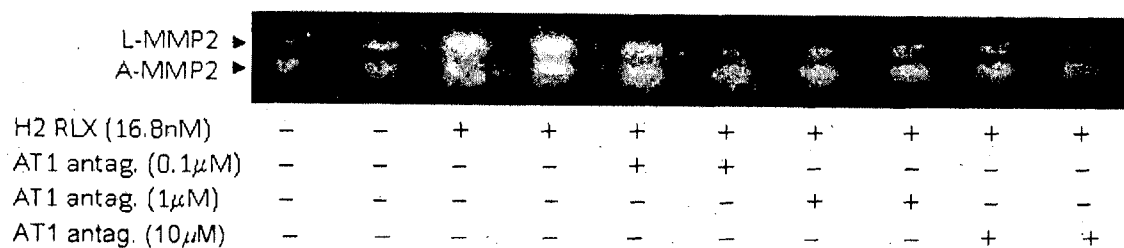


FIGURE 2

A



B

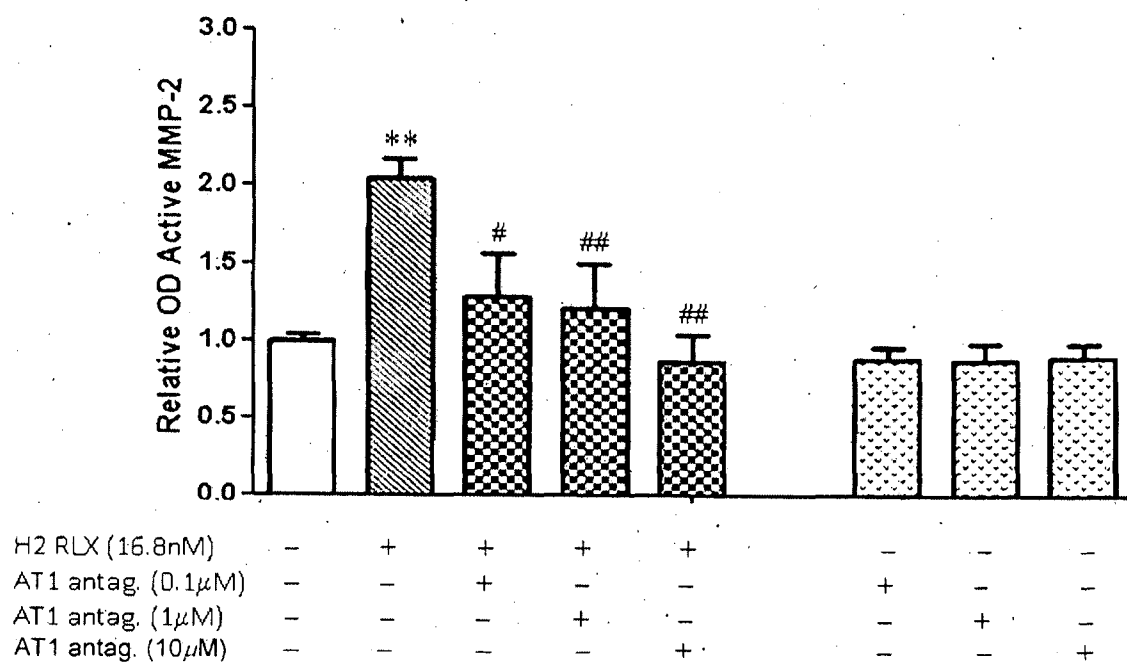


FIGURE 3

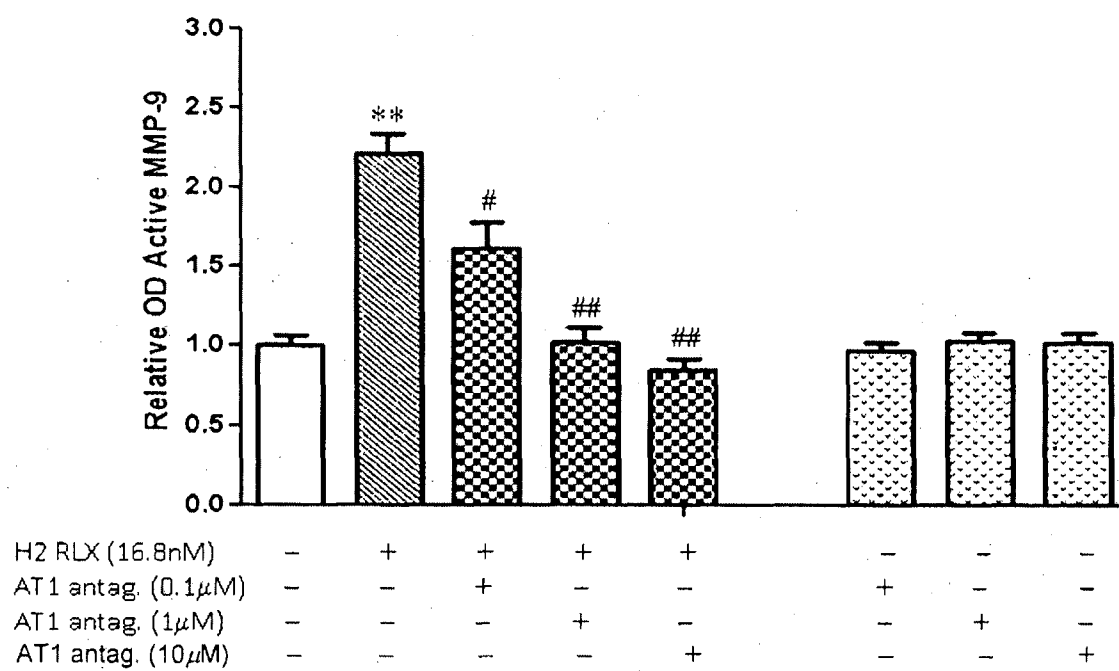


FIGURE 4

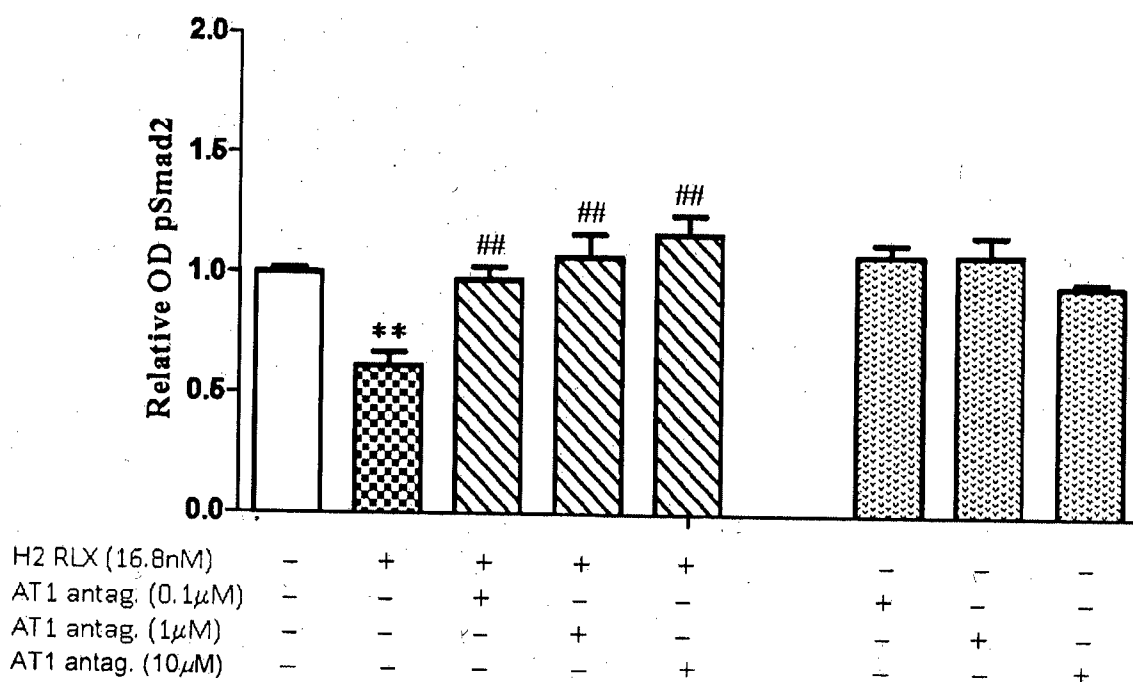
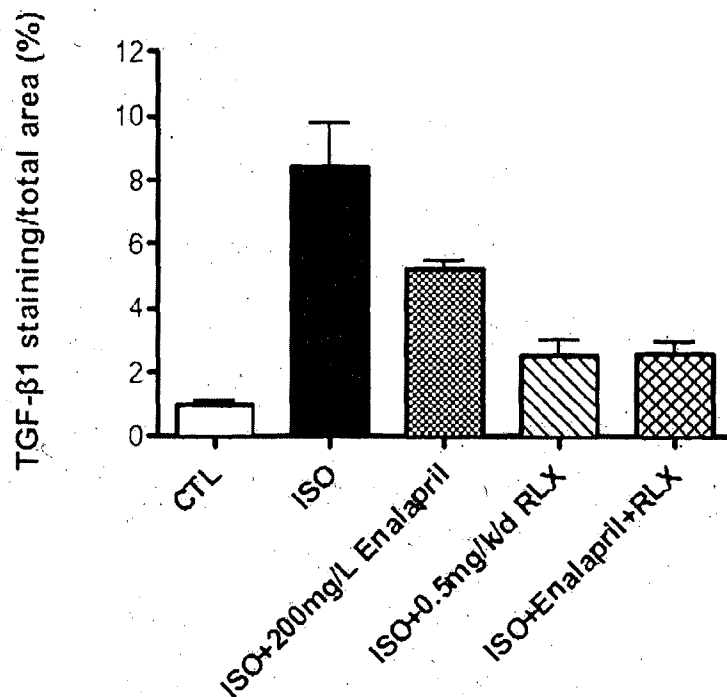
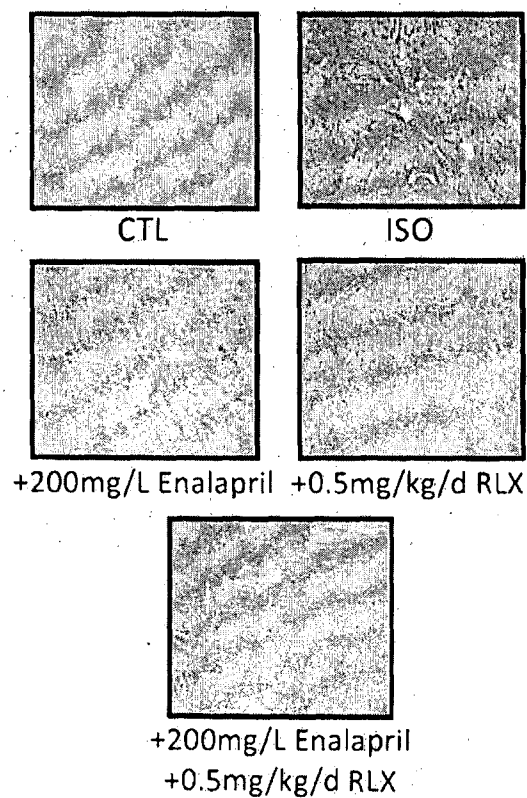


FIGURE 5

A



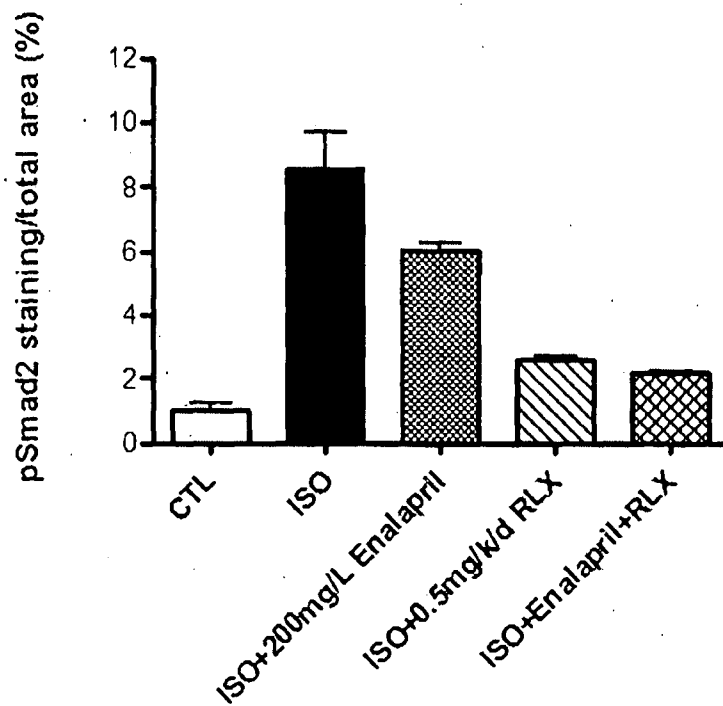
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FIGURE 5 (cont'd)

C



D

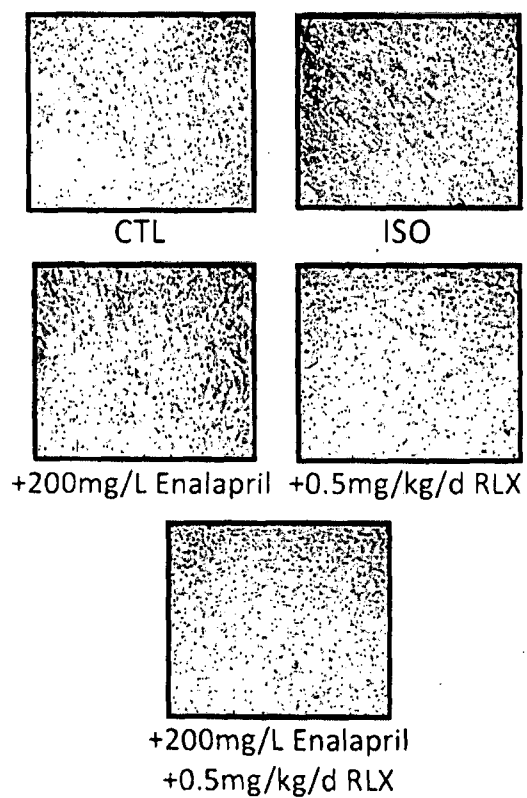
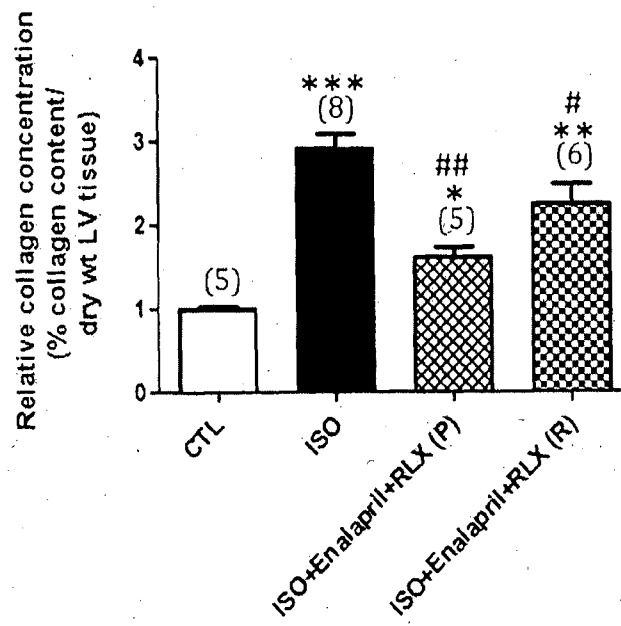
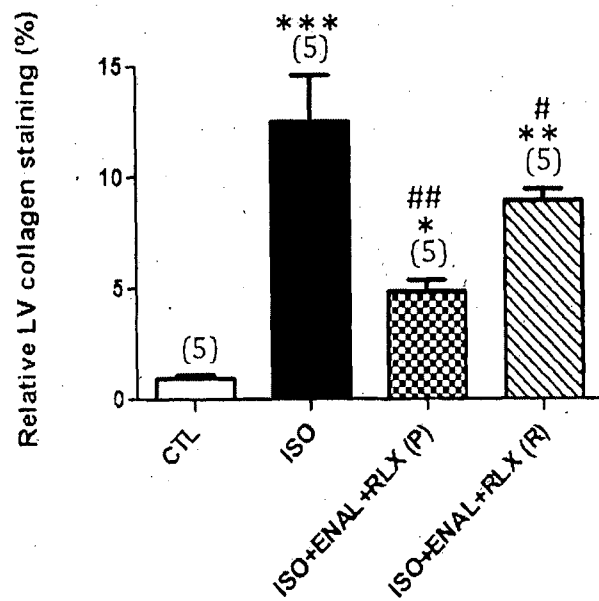


FIGURE 6

A



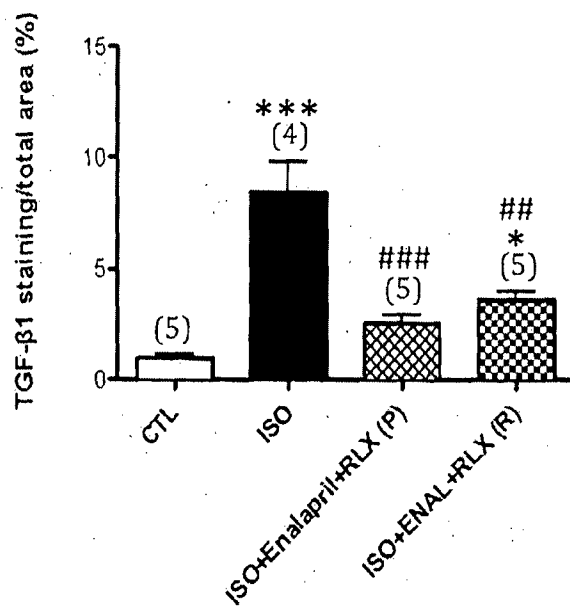
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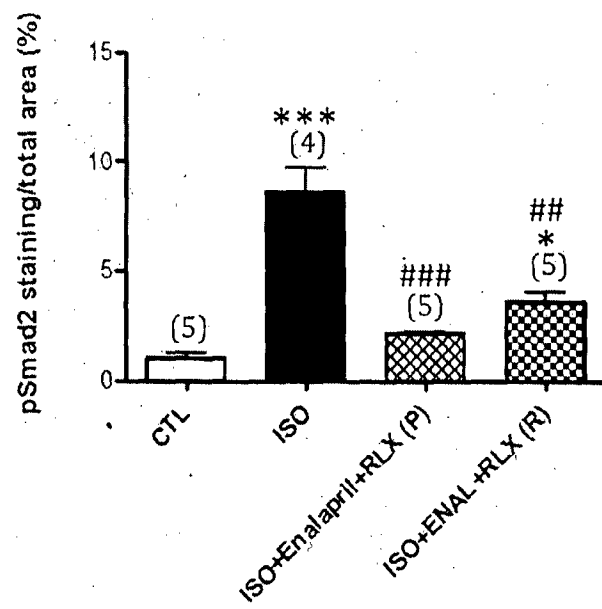
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FIGURE 6 (cont'd)

C



D



INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2012/001313

A. CLASSIFICATION OF SUBJECT MATTER

A61K 38/22 (2006.01) A61K 38/30 (2006.01) A61K 31/401 (2006.01) A61K 31/405 (2006.01) A61K 31/19 (2006.01)
A61P 9/10 (2006.01) A61P 9/00 (2006.01)

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)

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)

WPI, EPODOC, MEDLINE

Relaxin, RLN, Insulin like peptide, INSL, ACE Inhibitor, angiotensin converting enzyme inhibitor, enalapril, perindopril, captopril, fibrosis, ischemia, cardiac fibrosis, renal fibrosis, tubulointerstitial disease, and like terms.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



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	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"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
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
26 November 2012

Date of mailing of the international search report
26 November 2012

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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 filed or furnished:
 - a. (means)
☐ on paper
☒ in electronic form
 - b. (time)
☐ in the international application as filed
☒ together with the international application in electronic form
☐ subsequently to this Authority for the purposes of search
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 in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:
This international application has filed a sequence listing, however the sequence was not searched for the purpose of this search and opinion.

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. PCT/AU2012/001313
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 2459983 B (CORTHERA, INC.) 28 April 2010 [0001], [0010], [00124], [00114]-[00115], [00124]-[00128], [0058], [0033], Figure 1A, [0018]	1-24
Y	[00124], [00114]-[00115], [00124]-[00128], [0058], [0033], Figure 1a, [0018]	1-24
X	US 2011/0243942 A1 (WANG) 06 October 2011 Abstract, [0018], [0064], [0113], [0114], [0117], [0120]	1-24
Y	Abstract, [0018], [0064], [0113], [0114], [0117], [0120]	1-24
X	WO 2010/105081 A1 (MEDTRONIC, INC.) 16 September 2010 Abstract, Page 7 lines 6-10, Example 1 at page 19, page 14 lines 1-14, page 4 lines 26-30, page 7 line 9, page 6 lines 25-34, adjoining paragraphs at pages 8-9, page 9 lines 3-9, page 8 lines 17-22	1-24
Y	Abstract, Page 7 lines 6-10, Example 1 at page 19, page 14 lines 1-14, page 4 lines 26-30, page 7 line 9, page 6 lines 25-34, adjoining paragraphs at pages 8-9, page 9 lines 3-9, page 8 lines 17-22	1-24
X	US 2008/0045609 A1 (DeVORE) 21 February 2008 Abstract, [0006], [0057], [0059], [0116], [0155]-[0164]	1-24
Y	Abstract, [0006], [0057], [0059], [0116], [0155]-[0164]	1-24
Y	US 2006/0264367 A1 (SAMUEL et al.) 23 November 2006 Abstract, [0010], [0043], [0049], [0060]-[0062], [0051], [0080]	1-24
A	CHAN et al. 'The Relaxin Peptide Family - Structure, Function and Clinical Applications,' Protein & Peptide Letters, (March 2011) Vol 18 Issue 3 pp 220-229 Whole Document, In particular Figures 2-3 on page 222	1-24
A	WO 2009/055854 A1 (HOWARD FLOREY INSTITUTE OF EXPERIMENTAL PHYSIOLOGY AND MEDICINE) 07 May 2009 Whole document	1-24
Form PCT/ISA/210 (fifth sheet) (July 2009)		

INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/AU2012/001313	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
GB 2459983 B	28 Apr 2010	None	
US 2011/0243942 A1	06 Oct 2011	None	
WO 2010/105081 A1	16 Sep 2010	None	
US 2008/0045609 A1	21 Feb 2008	None	
US 2006/0264367 A1	23 Nov 2006	None	
WO 2009/055854 A1	07 May 2009	None	
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2009)			