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(54) Titre : PROCEDE DE PREVENTION OU DE TRAITEMENT DE L'INFLAMMATION, DES DOMMAGES ET D'AUTRES CHANGEMENTS QUI SE PRODUISENT AVANT, PENDANT OU IMMEDIATEMENT APRES UN EVENEMENT MYOCARDIQUE PARLA THYMOSINE BETA 4, DES ANALOGUES, DES ISOFORMES ET AUTRES DERIVES

(54) Title: METHODS OF HEALING OR PREVENTING INFLAMMATION, DAMAGE AND OTHER CHANGES THAT OCCUR PRIOR TO, DURING OR IMMEDIATELY AFTER A MYOCARDIAL EVENT WITH THYMOSIN BETA 4, ANALOGUES, ISOFORMS AND OTHER DERIVATIVES

(57) Abrégé/Abstract:
Inflammation or damage associated with myocardial events is treated or prevented by administration of an angiogenesis-inducing, anti-inflammatory peptide such as Thymosin β4, an isoform of Thymosin β4 or oxidized Thymosin β4.

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(54) Title: METHODS OF HEALING OR PREVENTING INFLAMMATION, DAMAGE AND OTHER CHANGES THAT OCCUR PRIOR TO, DURING OR IMMEDIATELY AFTER A MYOCARDIAL EVENT WITH THYMOSIN BETA 4, ANALOGUES, ISOFORMS AND OTHER DERIVATIVES

(57) Abstract: Inflammation or damage associated with myocardial events is treated or prevented by administration of an angiogenesis-inducing, anti-inflammatory peptide such as Thymosin β4, an isoform of Thymosin β4 or oxidized Thymosin β4.

METHODS OF HEALING OR PREVENTING INFLAMMATION, DAMAGE AND OTHER CHANGES THAT OCCUR PRIOR TO, DURING OR IMMEDIATELY AFTER A MYOCARDIAL EVENT WITH THYMOSIN BETA 4, ANALOGUES, ISOFORMS AND OTHER DERIVATIVES

1. FIELD OF THE INVENTION

The present invention relates to the field of healing or preventing inflammation, damage and other changes that occur in the heart, heart valves and septa just prior to, during or immediately after a myocardial event (e.g., myocardial infarction).

2. DESCRIPTION OF THE BACKGROUND ART

There are many causes of myocardial and coronary vessel and tissue injuries, including but not limited to myocardial ischemia, clotting, vessel occlusion, infection, developmental defects or abnormalities and other such myocardial events. Myocardial infarction results from blood vessel disease in the heart. It occurs when the blood supply to part of the heart is reduced or stopped (caused by blockage of a coronary artery). The reduced blood supply causes injuries to the heart muscle cells and may even kill heart muscle cells. The reduction in blood supply to the heart is often caused by narrowing of the epicardial blood vessels due to plaque. These plaques may rupture causing hemorrhage, thrombus formation, fibrin and platelet accumulation and constriction of the blood vessels.

There remains a need in the art for improved methods and compositions for healing or preventing inflammation, damage and other changes that occur prior to, during or immediately after a myocardial event.

SUMMARY OF THE INVENTION

In accordance with the present invention, a method of treatment for promoting healing or prevention of damage associated with myocardial events involves administration to a subject or patient in need of such treatment an effective amount of a

composition comprising an angiogenesis-inducing and anti-inflammatory polypeptide comprising amino acid sequence LKKTET or a conservative variant thereof having myocardial event-inhibiting activity.

According to one aspect of the present invention, there is provided the use of a polypeptide in the manufacture of a pharmaceutical composition for promoting healing or preventing damage to a coronary tissue of a subject by enhancing or inducing cardiac cell differentiation in said coronary tissue, wherein said polypeptide comprises at least one of the following amino acid sequence LKKTET, a conservative variant of amino acid sequence LKKTET and amino acid sequence LKKTETQ, and wherein the polypeptide is selected from the group consisting of Thymosin β4 (Tβ4), oxidized Tβ4, Tβ4 sulfoxide, $T\beta4^{ala}$, Thymosin β9 (Tβ9), Thymosin β10 (Tβ10), Thymosin β11 (Tβ11), Thymosin β12 (Tβ12), Thymosin β13 (Tβ13), Thymosin β14 (Tβ14), Thymosin β15 (Tβ15), gelsolin, vitamin D binding protein (DBP), profilin, cofilin, adsevertin, propomyosin, fincilin, depactin, Dnase 1, vilin, fragmin, severin, capping protein, β-actinin and acumentin.

According to another aspect of the present invention, there is provided the use of a polypeptide in the manufacture of a pharmaceutical composition for restoring functionality of a damaged myocardial tissue or vessels due to infection of a coronary tissue, wherein said polypeptide comprises at least one of the following amino acid sequence LKKTET, a conservative variant of amino acid sequence LKKTET and amino acid sequence LKKTETQ, and wherein the polypeptide is selected from the group consisting of Thymosin β4 (Tβ4), oxidized Tβ4, Tβ4 sulfoxide, $T\beta4^{ala}$, Thymosin β9 (Tβ9), Thymosin β10 (Tβ10), Thymosin β11 (Tβ11), Thymosin β12 (Tβ12), Thymosin β13 (Tβ13), Thymosin β14 (Tβ14), Thymosin β15 (Tβ15), gelsolin, vitamin D binding protein (DBP), profilin, cofilin, adsevertin, propomyosin, fincilin, depactin, Dnase 1, vilin, fragmin, severin, capping protein, β-actinin and acumentin.

According to still another aspect of the present invention, there is provided the use of a polypeptide in the manufacture of a pharmaceutical composition for treating heart valve defect or damage, wherein said polypeptide comprises at least one of the following amino acid sequence LKKTET, a conservative variant of amino acid sequence LKKTET and amino acid sequence LKKTETQ, and wherein the polypeptide is selected from the group consisting of Thymosin β4 (Tβ4), oxidized Tβ4, Tβ4 sulfoxide, $T\beta4^{ala}$, Thymosin β9 (Tβ9), Thymosin

β10 (Tβ10), Thymosin β11 (Tβ11), Thymosin β12 (Tβ12), Thymosin β13 (Tβ13), Thymosin β14 (Tβ14), Thymosin β15 (Tβ15), gelsolin, vitamin D binding protein (DBP), profilin, cofilin, adsevertin, propomyosin, fincilin, depactin, Dnase 1, vilin, fragmin, severin, capping protein, β-actinin and acumentin.

According to yet another aspect of the present invention, there is provided the use of a polypeptide in the manufacture of a pharmaceutical composition for promoting healing or promoting prevention of damage to coronary tissue due to coronary vessel occlusion, wherein said polypeptide comprises at least one of the following amino acid sequence LKKTET, a conservative variant of amino acid sequence LKKTET and amino acid sequence LKKTETQ, and wherein the polypeptide is selected from the group consisting of Thymosin β4 (Tβ4), oxidized Tβ4, Tβ4 sulfoxide, $T\beta4^{ala}$, Thymosin β9 (Tβ9), Thymosin β10 (Tβ10), Thymosin β11 (Tβ11), Thymosin β12 (Tβ12), Thymosin β13 (Tβ13), Thymosin β14 (Tβ14), Thymosin β15 (Tβ15), gelsolin, vitamin D binding protein (DBP), profilin, cofilin, adsevertin, propomyosin, fincilin, depactin, Dnase 1, vilin, fragmin, severin, capping protein, β-actinin and acumentin.

According to a further aspect of the present invention, there is provided the use of a polypeptide for promoting healing or preventing damage to a coronary tissue of a subject by enhancing or inducing cardiac cell differentiation in said coronary tissue, wherein said polypeptide comprises at least one of the following amino acid sequence LKKTET, a conservative variant of amino acid sequence LKKTET and amino acid sequence LKKTETQ, and wherein the polypeptide is selected from the group consisting of Thymosin β4 (Tβ4), oxidized Tβ4, Tβ4 sulfoxide, $T\beta4^{ala}$, Thymosin β9 (Tβ9), Thymosin β10 (Tβ10), Thymosin β11 (Tβ11), Thymosin β12 (Tβ12), Thymosin β13 (Tβ13), Thymosin β14 (Tβ14), Thymosin β15 (Tβ15), gelsolin, vitamin D binding protein (DBP), profilin, cofilin, adsevertin, propomyosin, fincilin, depactin, Dnase 1, vilin, fragmin, severin, capping protein, β-actinin and acumentin.

According to yet a further aspect of the present invention, there is provided the use of a polypeptide for restoring functionality of damaged a myocardial tissue or vessels due to infection of a coronary tissue, wherein said polypeptide comprises at least one of the following amino acid sequence LKKTET, a conservative variant of amino acid sequence LKKTET and amino acid sequence LKKTETQ, and wherein the polypeptide is selected from the group consisting of Thymosin β4 (Tβ4), oxidized Tβ4, Tβ4 sulfoxide, $T\beta4^{ala}$, Thymosin β9 (Tβ9), Thymosin β10 (Tβ10), Thymosin β11 (Tβ11), Thymosin β12 (Tβ12), Thymosin

β13 (Tβ13), Thymosin β14 (Tβ14), Thymosin β15 (Tβ15), gelsolin, vitamin D binding protein (DBP), profilin, cofilin, adsevertin, propomyosin, fincilin, depactin, Dnase 1, vilin, fragmin, severin, capping protein, β-actinin and acumentin.

According to still a further aspect of the present invention, there is provided the use of a polypeptide for treating heart valve defect or damage, wherein said polypeptide comprises at least one of the following amino acid sequence LKKTET, a conservative variant of amino acid sequence LKKTET and amino acid sequence LKKTETQ, and wherein the polypeptide is selected from the group consisting of Thymosin β4 (Tβ4), oxidized Tβ4, Tβ4 sulfoxide, $T\beta4^{ala}$, Thymosin β9 (Tβ9), Thymosin β10 (Tβ10), Thymosin β11 (Tβ11), Thymosin β12 (Tβ12), Thymosin β13 (Tβ13), Thymosin β14 (Tβ14), Thymosin β15 (Tβ15), gelsolin, vitamin D binding protein (DBP), profilin, cofilin, adsevertin, propomyosin, fincilin, depactin, Dnase 1, vilin, fragmin, severin, capping protein, β-actinin and acumentin.

According to another aspect of the present invention, there is provided Use of a polypeptide for promoting healing or promoting prevention of damage to coronary tissue due to coronary vessel occlusion, wherein said polypeptide comprises at least one of the following amino acid sequence LKKTET, a conservative variant of amino acid sequence LKKTET and amino acid sequence LKKTETQ, and wherein the polypeptide is selected from the group consisting of Thymosin β4 (Tβ4), oxidized Tβ4, Tβ4 sulfoxide, $T\beta4^{ala}$, Thymosin β9 (Tβ9), Thymosin β10 (Tβ10), Thymosin β11 (Tβ11), Thymosin β12 (Tβ12), Thymosin β13 (Tβ13), Thymosin β14 (Tβ14), Thymosin β15 (Tβ15), gelsolin, vitamin D binding protein (DBP), profilin, cofilin, adsevertin, propomyosin, fincilin, depactin, Dnase 1, vilin, fragmin, severin, capping protein, β-actinin and acumentin.

DETAILED DESCRIPTION OF THE INVENTION

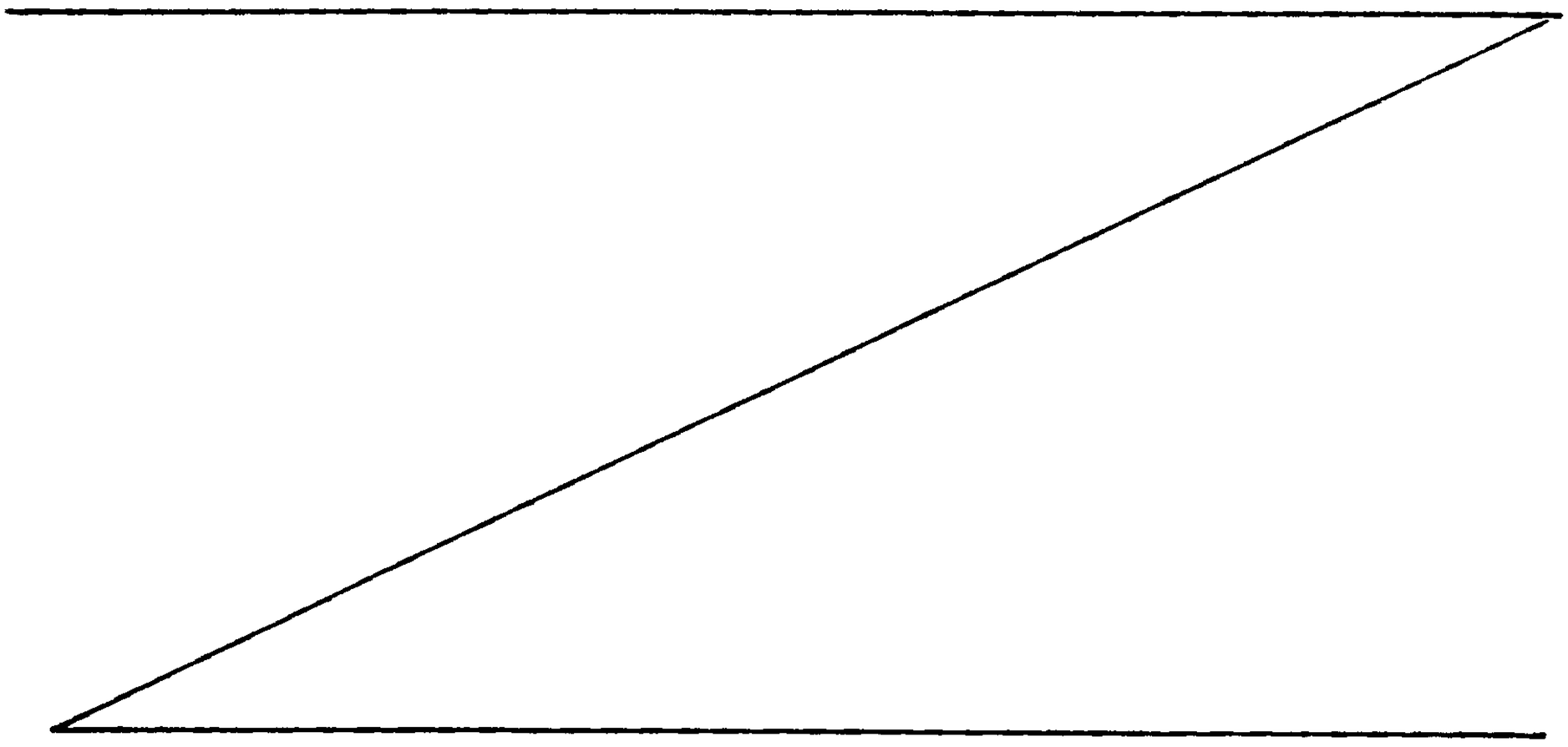
The present invention is based on a discovery that actin-sequestering peptides such as thymosin β4 (Tβ4) and other actin-sequestering peptides or peptide fragments containing amino acid sequence LKKTET or conservative variants thereof, promote healing or prevention of damage and other changes associated with myocardial events. Included are N– or C-terminal variants such as KLKKTET and LKKTETQ. Tβ4 has been suggested as being a factor in angiogenesis in rodent models. However, there heretofore has been no known indication that such properties may be useful in treating myocardial and coronary vessel events such as myocardial infarction, vessel occlusion or heart valve defects and damage. Without being bound to any particular theory, these

peptides may have the capacity to promote repair, healing and prevention by having the ability to induce terminal deoxynucleotidyl transferase (a non-template directed DNA polymerase), to decrease and modulate the levels of one or more inflammatory cytokines or chemokines, and to act as a chemotactic and/or angiogenic factor for endothelial cells and thus heal and prevent degenerative changes in patients afflicted with myocardial events.

The present invention provides factors and compositions that can enhance or down regulate mesenchymal epithelial cell differentiation and restore the functionality of damaged myocardium tissue and vessels due to the effects of ischemia, infection, aging, and other insult or injury.

Thymosin β4 was initially identified as a protein that is up-regulated during endothelial cell migration and differentiation *in vitro*. Thymosin β4 was originally isolated from the thymus and is a 43 amino acid, 4.9 kDa ubiquitous polypeptide identified in a variety of tissues. Several roles have been ascribed to this protein including a role in a endothelial cell differentiation and migration, T cell differentiation, actin sequestration and vascularization.

In accordance with one embodiment, the invention is a method of treatment for promoting healing and prevention of damage and inflammation associated with myocardial events comprising administering to a subject in need of such treatment an effective amount of a composition comprising an angiogenesis-inducing, anti-



inflammatory peptide comprising amino acid sequence LKKTET, or a conservative variant thereof having angiogenesis-inducing, anti-inflammatory activity, preferably Thymosin β4, an isoform of Thymosin β4, oxidized Thymosin β4, Thymosin β4 sulfoxide, or an antagonist of Thymosin β4.

Compositions which may be used in accordance with the present invention include Thymosin β4 (Tβ4), Tβ4 isoforms, oxidized Tβ4, Thymosin β4 sulfoxide, polypeptides or any other actin sequestering or bundling proteins having actin binding domains, or peptide fragments comprising or consisting essentially of the amino acid sequence LKKTET or conservative variants thereof, having angiogenesis-inducing, anti-inflammatory activity. International Application Serial No. WO/2000/006190 discloses isoforms of Tβ4 which may be useful in accordance with the present invention as well as amino acid sequence LKKTET and conservative variants thereof having angiogenesis-inducing, anti-inflammatory activity, which may be utilized with the present invention. International Application Serial No. WO 99/49883 discloses oxidized Thymosin β4 which may be utilized in accordance with the present invention. Although the present invention is described primarily hereinafter with respect to Tβ4 and Tβ4 isoforms, it is to be understood that the following description is intended to be equally applicable to amino acid sequence LKKTET, LKKTETQ, peptides and fragments comprising or consisting essentially of LKKTET or LKKTETQ, conservative variants thereof having angiogenesis-inducing, anti-inflammatory activity, as well as oxidized Thymosin β4.

In one embodiment, the invention provides a method for healing and preventing inflammation and damage in a subject by contacting the damaged site with an effective amount of an angiogenesis-inducing, anti-inflammatory composition which contains Tβ4 or a Tβ4 isoform. The contacting may be direct or systemically. Examples of contacting the damaged site include contacting the site with a composition comprising Tβ4 alone, or in combo with at least one agent that enhances Tβ4 penetration, or delays or slows release of Tβ4 peptides into the area to be treated. Administration may include, for example, intravenous, intraperitoneal, intramuscular or subcutaneous injections, or inhalation, transdermal or oral administration of a composition containing Tβ4 or a Tβ4 isoform, etc. A subject may be a mammal, preferably human.

Tβ4, or its analogues, isoforms or derivatives, may be administered in any suitable myocardial event damage-inhibiting or -reducing amount. For example, Tβ4

may be administered in dosages within the range of about 0.1-50 micrograms of Tβ4, more preferably in amounts within the range of about 1-25 micrograms.

A composition in accordance with the present invention can be administered daily, every other day, etc., with a single administration or multiple administrations per day of administration, such as applications 2, 3, 4 or more times per day of administration.

Tβ4 isoforms have been identified and have about 70%, or about 75%, or about 80% or more homology to the known amino acid sequence of Tβ4. Such isoforms include, for example, $T\beta4^{ala}$, Tβ9, Tβ10, Tβ11, Tβ12, Tβ13, Tβ14 and Tβ15. Similar to Tβ4, the Tβ10 and Tβ15 isoforms have been shown to sequester actin. Tβ4, Tβ10 and Tβ15, as well as these other isoforms share an amino acid sequence, LKKTET, that appears to be involved in mediating actin sequestration or binding. Although not wishing to be bound to any particular theory, the activity of Tβ4 isoforms may be due, in part, to the ability to regulate the polymerization of actin. β-thymosins appear to depolymerize F-actin by sequestering free G-actin. Tβ4's ability to modulate actin polymerization may therefore be due to all, or in part, its ability to bind to or sequester actin via the LKKTET sequence. Thus, as with Tβ4, other proteins which bind or sequester actin, or modulate actin polymerization, including Tβ4 isoforms having the amino acid sequence LKKTET, are likely to be effective, alone or in a combination with Tβ4, as set forth herein.

Thus, it is specifically contemplated that known Tβ4 isoforms, such as $T\beta4^{ala}$, Tβ9, Tβ10, Tβ11, Tβ12, Tβ13, Tβ14 and Tβ15, as well as Tβ4 isoforms not yet identified, will be useful in the methods of the invention. As such Tβ4 isoforms are useful in the methods of the invention, including the methods practiced in a subject. The invention therefore further provides pharmaceutical compositions comprising Tβ4, as well as Tβ4 isoforms $T\beta4^{ala}$, Tβ9, Tβ10, Tβ11, Tβ12, Tβ13, Tβ14 and Tβ15, and a pharmaceutically acceptable carrier.

In addition, other proteins having actin sequestering or binding capability, or that can mobilize actin or modulate actin polymerization, as demonstrated in an appropriate sequestering, binding, mobilization or polymerization assay, or identified by the presence of an amino acid sequence that mediates actin binding, such as LKKTET, for example, can similarly be employed in the methods of the invention. Such proteins include gelsolin, vitamin D binding protein (DBP), profilin, cofilin, adsevertin, propomyosin, fincilin, depactin, DnaseI, vilin, fragmin, severin, capping protein, β-actinin and acumentin, for example. As such methods include those practiced in a subject, the

invention further provides pharmaceutical compositions comprising gelsolin, vitamin D binding protein (DBP), profilin, cofilin, depactin, DnaseI, vilin, fragmin, severin, capping protein, β-actinin and acumentin as set forth herein. Thus, the invention includes the use of an angiogenesis-inducing, anti-inflammatory polypeptide comprising the amino acid sequence LKKTET (which may be within its primary amino acid sequence) and conservative variants thereof.

As used herein, the term "conservative variant" or grammatical variations thereof denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the replacement of a hydrophobic residue such as isoleucine, valine, leucine or methionine for another, the replacement of a polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like.

Tβ4 has been localized to a number of tissue and cell types and thus, agents which stimulate the production of Tβ4 can be added to or comprise a composition to effect Tβ4 production from a tissue and/or a cell. Such agents include members of the family of growth factors, such as insulin-like growth factor (IGF-1), platelet derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor beta (TGF-β), basic fibroblast growth factor (bFGF), thymosin α1 (Tα1) and vascular endothelial growth factor (VEGF). More preferably, the agent is transforming growth factor beta (TGF-β) or other members of the TGF-β superfamily. Tβ4 compositions of the invention may reduce the affects of myocardial events by effectuating growth of the connective tissue through extracellular matrix deposition, cellular migration and vascularization.

In accordance with one embodiment, subjects are treated with an agent that stimulates production in the subject of an angiogenesis-inducing, anti-inflammatory peptide as defined above.

Additionally, agents that assist or stimulate healing of damage caused by a myocardial event may be added to a composition along with Tβ4 or a Tβ4 isoform. Such agents include angiogenic agents, growth factors, agents that direct differentiation of cells. For example, and not by way of limitation, Tβ4 or a Tβ4 isoform alone or in combination can be added in combination with any one or more of the following agents: VEGF, KGF, FGF, PDGF, TGFβ, IGF-1, IGF-2, IL-1, prothymosin α and thymosin α1 in an effective amount.

The invention also includes a pharmaceutical composition comprising a therapeutically effective amount of Tβ4 or a Tβ4 isoform in a pharmaceutically acceptable carrier. Such carriers include those listed above with reference to parenteral administration.

The actual dosage, formulation or composition that heals or prevents inflammation, damage and degeneration associated with myocardial events may depend on many factors, including the size and health of a subject. However, persons of ordinary skill in the art can use teachings describing the methods and techniques for determining clinical dosages as disclosed in PCT/US99/17282, *supra*, and the references cited therein, to determine the appropriate dosage to use.

Suitable formulations include Tβ4 or a Tβ4 isoform at a concentration within the range of about 0.001 - 10% by weight, more preferably within the range of about 0.01 - 0.1% by weight, most preferably about 0.05% by weight.

The therapeutic approaches described herein involve various routes of administration or delivery of reagents or compositions comprising the Tβ4 or other compounds of the invention, including any conventional administration techniques to a subject. The methods and compositions using or containing Tβ4 or other compounds of the invention may be formulated into pharmaceutical compositions by admixture with pharmaceutically acceptable non-toxic excipients or carriers.

The invention includes use of antibodies which interact with Tβ4 peptide or functional fragments thereof. Antibodies which consists essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art as disclosed in PCT/US99/17282, *supra*. The term antibody as used in this invention is meant to include monoclonal and polyclonal antibodies.

In yet another embodiment, the invention provides a method of treating a subject by administering an effective amount of an agent which modulates Tβ4 gene expression. The term "modulate" refers to inhibition or suppression of Tβ4 expression when Tβ4 is over expressed, and induction of expression when Tβ4 is under expressed. The term "effective amount" means that amount of Tβ4 agent which is effective in modulating Tβ4 gene expression resulting in effective treatment. An agent which modulates Tβ4 or Tβ4 isoform gene expression may be a polynucleotide for example. The polynucleotide may be an antisense, a triplex agent, or a ribozyme. For example,

an antisense directed to the structural gene region or to the promoter region of Tβ4 may be utilized.

In another embodiment, the invention provides a method for utilizing compounds that modulate Tβ4 activity. Compounds that affect Tβ4 activity (*e.g.*, antagonists and agonists) include peptides, peptidomimetics, polypeptides, chemical compounds, minerals such as zincs, and biological agents.

While not be bound to any particular theory, the present invention may promote healing or prevention of inflammation or damage associated with myocardial events by inducing terminal deoxynucleotidyl transferase (a non-template directed DNA polymerase), to decrease the levels of one or more inflammatory cytokines, or chemokines, and to act as a chemotactic factor for endothelial cells, and thereby promoting healing or preventing degenerative changes in cardiac vessels and tissue brought about by myocardial event or other degenerative or environmental factors.

The invention is further illustrated by the following example, which is not to be construed as limiting.

Example

Synthetic Tβ4 and an antibody to Tβ4 was provided by RegeneRx Biopharmaceuticals, Inc. (3 Bethesda Metro Center, Suite 700,, Bethesda, MD 20814) and were tested in a collagen gel assay to determine their effects on the Transformation of cardiac endothelial cells to mesenchymal cells. It is well established that development of heart valves and other cardiac tissue are formed by epithelial-mesenchymal transformation and that defects in this process can cause serious cardiovascular malformation and injury during development and throughout life. At physiological concentrations Tβ4 markedly enhances the transformation of endocardial cells to mesenchymal cells in the collagen gel assay. Furthermore, an antibody to Tβ4 inhibited and blocked this transformation. Transformation of atrioventricular endocardium into invasive mesenchyme is critical in the formation and maintenance of normal cardiac tissue and in the formation of heart valves.

SEQUENCE LISTING

<110> REGENERX BIOPHARMACEUTICALS, INC.

<120> METHODS OF HEALING OR PREVENTING INFLAMMATION, DAMAGE AND OTHER CHANGES THAT OCCUR PRIOR TO, DURING OR IMMEDIATELY AFTER A MYOCARDIAL EVENT WITH THYMOSIN BETA 4, ANALOGUES, ISOFORMS AND OTHER DERIVATIVES

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CLAIMS

1. Use of a thymosin β4 (Tβ4) and a pharmaceutically acceptable carrier in the manufacture of a pharmaceutical composition for enhancing myocardial tissue cell differentiation in a cardiac tissue of a subject in need thereof, wherein:

   – the cardiac tissue comprises cardiac endothelial cells;

   – the cardiac endothelial cells are transformed to mesenchymal cells;

   – the pharmaceutical composition comprises between 0.1 to 50 μg of the Tβ4; and

   – the pharmaceutical composition comprises between 0.001 to 10% (w/w) of the Tβ4.

2. Use of a thymosin β4 (Tβ4) for enhancing myocardial tissue cell differentiation in a cardiac tissue of subject in need thereof, wherein:

   – the cardiac tissue comprises cardiac endothelial cells;

   – the cardiac endothelial cells are transformed to mesenchymal cells; and

   – the Tβ4 is for administration at a dose between 0.1 to 50 μg.

3. The use of claim 1 or 2, wherein the subject has damage to the cardiac tissue or vessels.

4. The use of claim 3, wherein the cardiac tissue is a myocardium tissue.

5. The use of claim 3 or 4, wherein said damage results from an injurious myocardial event or vessel occlusion.

6. The use of claim 3, wherein the subject has had a myocardial infarction.

7. The use of any one of claims 1 to 6, wherein the subject is a mammal.

8. The use of claim 7, wherein the mammal is a human.

9. The use of any one of claims 1 to 8, wherein the pharmaceutical composition or is Tβ4 for direct administration to the cardiac tissue.

10. The use according to any one of claims 1 to 8, wherein the pharmaceutical composition or Tβ4 is for systemic administration.