Methods of treating myocardial event related conditions with Thymosin beta 4
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

Inflammation or damage associated with myocardial events is usualy prevented by administration of an angiogenesis-inducing, anti-inflammatory peptide such as Thymosin β4, an isofrom of Thymosin β4 or oxidized Thymosin β4.
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BACKGROUND OF THE INVENTION

CROSS-REFERENCE TO RELATED APPLICATION

The present application claims the benefit of U.S. Provisional Application Serial
No. 60/315,547, filed August 29, 2001.

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 LKKTE or a conservative variant thereof having myocardial event-inhibiting activity.

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 LKKTE 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 KLKKTE and LKKTE. 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 4.5 kDa ubiquitously 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. PCT/US99/17282,
incorporated herein by reference, 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.
PCT/GB99/00833 (WO 99/49883), incorporated herein by reference, 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, Tβ4*, 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-thromosins 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 Tβ4*, 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 Tβ4*, 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 sequestration, 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 gelatin, vitamin D binding protein (DBP), profilin, coflin, aisevertin, propomycin, falcinil, depacin, Dnasil, vilin, fragmin, severin, capping protein, β-actin and acumenitin, for example. As such methods include those practiced in a subject, the
invention further provides pharmaceutical compositions comprising gelsolin, vitamin D binding protein (DBP), prolin, coflin, depactin, Drasal, viil, fragnlin, scellen, capping protein, S-actinin and acumenlin as set forth herein. Thus, the invention includes the use of an angiogenesis-inducing, anti-inflammatory polypeptide comprising the amino acid sequence LKDTET (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.

TB4 has been localized to a number of tissue and cell types and thus, agents which stimulate the production of TB4 can be added to or comprise a composition to effect TB4 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), thyromosin α1 (TGF1) 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. TB4 compositions of the invention may reduce the effects 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 TB4 or a TB4 isoform. Such agents include angiogenic agents, growth factor, agents that direct differentiation of cells. For example, and not by way of limitation, TB4 or a TB4 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, prothromosin α and thyromosin 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 TBI4 may be utilized.

In another embodiment, the invention provides a method for utilizing compounds that modulate TBI4 activity. Compounds that affect TBI4 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 TBI4 and an antibody to TBI4 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 TBI4 markedly enhances the transformation of endocardial cells to mesenchymal cells in the collagen gel assay. Furthermore, an antibody to TBI4 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.
CLAIMS

1. A method of treatment for promoting healing or preventing damage to coronary tissue, comprising administering to a subject in need of such treatment an effective amount of a polypeptide comprising amino acid sequence LKKTET, or a conservative variant thereof.

2. The method of claim 1 wherein said damage results from an injurious myocardial event.

3. The method of claim 1 wherein said polypeptide comprises amino acid sequence KLKKTET or LKKTETQ, Thymosin β4 (Tβ4), an N-terminal variant of Tβ4, a C-terminal variant of Tβ4, an isoform of Tβ4, oxidized Tβ4 or Tβ4 sulfoxide.

4. The method of claim 1 wherein said composition is administered systemically.

5. The method of claim 1 wherein said composition is administered directly to said coronary tissue.

6. The method of claim 1 wherein said polypeptide is recombinant or synthetic.

7. The method of claim 1 wherein said polypeptide is an antibody.

8. The method of claim 7 wherein said antibody is polyclonal or monoclonal.

9. A method of treatment for promoting healing or preventing damage to coronary tissue, comprising administering to a subject in need of such treatment an effective amount of a composition comprising an agent that stimulates production of an angiogenesis-inducing, anti-inflammatory polypeptide comprising amino acid sequence LKKTET, or a conservative variant thereof having angiogenesis-inducing, anti-inflammatory activity.

10. The method of claim 9 wherein said polypeptide is Thymosin β4.

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11. The method of claim 9 wherein said agent is an antagonist of Thymosin B4

12. A composition when used to promote healing or prevent damage to coronary tissue, comprising an effective amount of a composition including an angiogenesis-inducing, anti-inflammatory polypeptide comprising amino acid sequence LKKTET or a conservative variant thereof having angiogenesis-inducing, anti-inflammatory activity.

13. The composition when used according to claim 12 wherein said composition comprises an N- or C-terminal variant of LKKTET.

14. The composition when used according to claim 12 wherein said composition comprises KLKKTET or LKKTETQ.

15. The composition when used according to claim 12 wherein said polypeptide comprises TB4, an isoform of TB4, oxidised TB4 sulfoxide.

16. Use of an angiogenesis anti-inflammatory polypeptide comprising amino acid sequence LKKTET or a conservative variant thereof having angiogenesis-inducing, anti-inflammatory activity for preparing a medicament for promoting healing or preventing damage to coronary tissue.

17. Use according to claim 16 wherein said polypeptide or variant thereof comprises an N- or C-terminal variant of LKKTET.

18. Use according to claim 16 wherein said polypeptide or variant thereof comprises KLKKTET or LKKTETQ.

19. Use according to claim 16 wherein said polypeptide or variant thereof comprises TB4, an isoform of TB4, oxidised TB4 or TB4 sulfoxide.