DESCRIPTION

NOVEL METHOD FOR TREATING CARDIAC INFARCTION USING HMGB1 FRAGMENT

Technical Field

The present invention relates to novel pharmaceutical compositions for treating myocardial infarction comprising an HMGB1 fragment peptide, and uses thereof.

Background Art

Acute myocardial infarction which results in the necrosis of cardiac muscle due to coronary artery occlusion is a disease with poor prognosis and is the main underlying condition for heart diseases which are the second cause of death in Japan. Existing therapies, acute phase catheterization and coronary artery bypass surgery, alleviate myocardial damage and contribute to the reduction of mortality rate; however, there are problems such as myocardial damage that occurs when the blocked coronary blood flow restarts (ischemia-reperfusion injury). Thus, development of further therapies is desired.

Recently, in basic experiments using animal models, bone marrow mesenchymal stem cell transplantation has been reported to suppress the development of myocardial infarction *via* regeneration of cardiac muscle by direct differentiation of the transplanted cells into constituent cardiac muscle cells, and also *via* suppression of left ventricular remodeling caused by the paracrine effect of the produced cytokines. Currently, clinical trials of autologous cell transplantations *via* various administration routes are being carried out. However, many issues remain to be resolved as problems concerning autologous cell transplantation, such as physical burden imposed at cell collection, cost and manpower involved in cell culturing, and the time required until cell transplantation. Meanwhile, it has been recently confirmed that a mechanism exists in which damaged tissue releases factors that recruit bone marrow pluripotent stem cells into blood to induce tissue regeneration at the damaged site. Tissue regeneration that uses the mechanism of inducing regeneration of damaged tissue *in vivo* by administering bone marrow stem cell-inducing factors is a non-conventional novel concept in regenerative therapy, and is believed to be advantageous compared to cell therapy that uses stem cells in that manpower is not required, stable supply is possible, and administration at an early stage of damage is possible.

In previous studies, the present inventors have identified the HMGB1 protein as a novel factor that mobilizes bone marrow pluripotent stem cells. HMGB1 is the major component of non-histone nuclear protein and is extracellularly released from dendritic cells, macrophages, or

necrotic cells that accumulate in damaged sites, and is confirmed to be associated with various diseases.

Prior Art Documents

[Patent Documents]

[Patent Document 1] WO2008/053892
[Patent Document 2] WO2007/015546
[Patent Document 3] WO2009/133939
[Patent Document 4] WO2009/133940
[Patent Document 5] WO2009/133940
[Patent Document 6] WO2004/004763
[Patent Document 7] WO2002/074337

[Non-patent Documents]

[Non-patent Document 1] Bustin *et al.*, Mol Cell Biol, 19: 5237-5246, 1999
[Non-patent Document 2] Hori *et al.*, J. Biol. Chem., 270, 25752-25761, 1995
[Non-patent Document 3] Wang *et al.*, Science, 285: 248-251, 1999
[Non-patent Document 4] Muller *et al.*, EMBO J, 20: 4337-4340, 2001
[Non-patent Document 5] Wang *et al.*, Science, 285: 248-251, 1999
[Non-patent Document 6] Germani *et al.*, J Leukoc Biol., 81(1): 41-5, 2007
[Non-patent Document 7] Palumbo *et al.*, J. Cell Biol., 164: 441-449, 2004
[Non-patent Document 8] Merenmies *et al.*, J. Biol. Chem., 266: 16722-16729, 1991
[Non-patent Document 9] Wu Y *et al.*, Stem cells, 25: 2648-2659, 2007
[Non-patent Document 10] Tamai *et al.*, Proc Natl Acad Sci U S A., 108(16): 6609-6614, 2011
[Non-patent Document 11] Yang *et al.*, J Leukoc Biol., 81(1): 59-66, 2007

Summary of the Invention

[Problems to be Solved by the Invention]

Recently, in diseases such as cerebral infarction, it has been confirmed that a mechanism exists in which damaged tissue induces tissue regeneration at the damaged site by releasing factors that mobilize bone marrow pluripotent stem cells into blood, and that the mechanism contributes to the prevention of further expansion of the damage. In the present invention, the present inventors aimed to develop novel therapeutic agents for myocardial infarction using fragment peptides derived from an HMGB1 protein, which was identified as a new bone marrow pluripotent stem cell mobilizing factor.

[Means for Solving the Problems]

The present inventors prepared a fragment peptide (1-44) of an HMGB1 protein which has a cell migration activity, and revealed in the myocardial infarction model for the first time that systemic administration of the fragment peptide enhances the accumulation of PDGFRα-positive cells in infarction sites and vicinities thereof. Further, long-standing improvement of the heart function was demonstrated in the myocardial infarction model animals administered with the fragment peptide (1-44). The above suggests that the fragment peptide (1-44) induces tissue regeneration by making use of the mechanism of inducing damaged tissue regeneration *in vivo*, and suppresses the development of acute myocardial infarction. Thus, it is shown that the fragment peptide of the present invention can be a useful therapeutic agent for poor-prognosis human acute myocardial infarction.

The present invention discloses novel pharmaceutical compositions for treating myocardial infarction comprising an HMGB1 fragment peptide, and uses thereof.

Specifically, the present inventors produced a peptide consisting of amino acids at positions 1 to 44 of an HMGB1 protein (SEQ ID NO: 3) by peptide synthesis. To model mice that enable evaluation of the therapeutic effect on myocardial infarction, each of the produced HMGB1 fragments was administered, and the therapeutic effects of the fragments on myocardial infarction were confirmed.

Based on these findings, the present application provides the following invention:

[1] a pharmaceutical composition for use in the treatment of myocardial infarction, comprising an HMGB1 fragment peptide;

[2] the pharmaceutical composition of [1], wherein the HMGB1 fragment peptide is a peptide comprising the amino acid sequence of SEQ ID NO: 3;

[3] the pharmaceutical composition of [1], wherein the HMGB1 fragment peptide is a peptide consisting of the amino acid sequence of SEQ ID NO: 3;

[4] a method for treating myocardial infarction, comprising the step of administering an HMGB1 fragment peptide;

[5] the method of [4], wherein the HMGB1 fragment peptide is a peptide comprising the amino acid sequence of SEQ ID NO: 3;

[6] the method of [4], wherein the HMGB1 fragment peptide is a peptide consisting of the amino acid sequence of SEQ ID NO: 3;

[7] an HMGB1 fragment peptide for use in the treatment of myocardial infarction;

[8] the HMGB1 fragment peptide of [7], wherein the HMGB1 fragment peptide is a peptide comprising the amino acid sequence of SEQ ID NO: 3;

[9] the HMGB1 fragment peptide of [7], wherein the HMGB1 fragment peptide is a peptide consisting of the amino acid sequence of SEQ ID NO: 3;

[10] use for the production of a medicament for use in the treatment of myocardial infarction, comprising an HMGB1 fragment peptide;

[11] the use of [10], wherein the HMGB1 fragment peptide is a peptide comprising the amino acid sequence of SEQ ID NO: 3; and

[12] the use of [10], wherein the HMGB1 fragment peptide is a peptide consisting of the amino acid sequence of SEQ ID NO: 3.

Brief Description of the Drawings

Fig. 1A is a graph indicating the number of PDGFRα-positive bone marrow mesenchymal stem cells in infarction sites (INFARCT), border sites (BORDER), and non-infarction sites (REMOTE) seven days after creating myocardial infarction. PDGFRα-positive bone marrow mesenchymal stem cells were significantly recruited (1-WAY ANOVA, *P < 0.05 vs. 1-44) in all areas of the infarction, border, and non-infarction sites in the fragment peptide (1-44)-administered group (1-44; N = 4) compared to the negative control group (CONT; N = 4).

Fig. 1B is a photograph showing immunofluorescence staining in the border site of a fragment peptide (1-44)-administered mouse. Cells positive for green fluorescence are GFP-positive bone marrow-derived cells and cells positive for red fluorescence indicate PDGFRα-expressing cells. Cells positive for both GFP and PDGFRα are indicated with arrows.

Fig. 1C is a graph showing the mRNA level of inflammatory cytokine TNFα in infarction sites (INFARCT), border sites (BORDER), and non-infarction sites (REMOTE) 7 and 56 days after creating myocardial infarction. The inflammatory cytokine (TNFα, IL-1β) levels in non-infarction sites were significantly lower (1-WAY ANOVA, #P < 0.05 1-44 vs. CONT) in the fragment peptide (1-44)-administered group (1-44; N = 4) compared to the negative control group (CONT: N = 4).

Fig. 1D is a graph showing the mRNA level of inflammatory cytokine IL-1β in infarction sites (INFARCT), border sites (BORDER), and non-infarction sites (REMOTE) 7 and 56 days after creating myocardial infarction. The inflammatory cytokine (TNFα, IL-1β) levels in non-infarction sites were significantly lower (1-WAY ANOVA, #P < 0.05 1-44 vs. CONT) in the fragment peptide (1-44)-administered group (1-44; N = 4) compared to the negative control group (CONT: N = 4).

Fig. 1E is a graph showing the left ventricular ejection fraction (EF), left ventricular end-diastolic diameter (DD), and left ventricular end-systolic diameter (DS) of the negative control group and the fragment peptide (1-44)-administered group 56 days after creating myocardial infarction.

Modes for Carrying Out the Invention

The present invention provides pharmaceutical compositions for use in the treatment of myocardial infarction, which comprises an HMGB1 fragment peptide having a cell migration-stimulating activity. Herein, pharmaceutical compositions for use in the treatment of myocardial infarction of the present invention are also expressed as medicaments, pharmaceutical agents, or medicinal compositions.

In the present invention, a cell migration-stimulating activity refers to the activity of stimulating cell migration. Herein, a cell migration-stimulating activity is also expressed as a cell migration-inducing activity or a cell-attracting activity.

The pharmaceutical compositions of the present invention can be administered/added to any sites. That is, the compositions can exert their effects no matter at which site they are administered, such as a site of myocardial infarction in need of regeneration, a site other than the infarction site, or blood. For example, when the compositions are administered/added, cells are recruited to the site of administration/addition or its nearby sites, thereby inducing or promoting regeneration of the lesion. Also, for example, when the compositions are administered/added to an infarction site or to the vicinity thereof, cells are recruited to the infarction site, thereby inducing or promoting regeneration of the infarction site. Further, for example, when the compositions are administered/added to a tissue other than a tissue in need of regeneration, bone marrow cells are mobilized from bone marrow to the site in need of regeneration through peripheral circulation, thereby inducing or promoting regeneration. Here, "peripheral circulation" is also called "blood circulation" or "peripheral circulation bloodstream".

Administration to a tissue other than a tissue in need of regeneration refers to administration to a site that is not a site in need of regeneration (a site other than a site in need of regeneration). Accordingly, "a tissue other than a tissue in need of regeneration" can also be referred to as:

a site other than a tissue in need of regeneration; a site other than a site in need of regeneration; a site distant from a tissue in need of regeneration; a site distant from a site in need of regeneration; a site distal to a site in need of regeneration; a tissue distal to a tissue in need of regeneration; a distal site; or a distal tissue.

Thus, compositions of the present invention are effectively used to regenerate tissues to which it is difficult to directly administer pharmaceutical agents from outside of the body. Examples of the tissue other than a tissue in need of regeneration include blood tissues, muscle tissues, subcutaneous tissues, intradermal tissues, abdominal cavity, and such.

In the present invention, cells that are stimulated to migrate or cells mobilized from bone marrow to peripheral blood include undifferentiated cells and cells in various stages of differentiation, but are not limited thereto. In the present invention, cells that are stimulated to migrate or cells mobilized from bone marrow to peripheral blood include stem cells, non-stem

cells, and such, but are not limited thereto. Stem cells include circulatory stem cells and non-circulatory stem cells. Non-circulatory stem cells are, for example, stem cells residing in a tissue. Circulatory stem cells are, for example, circulatory stem cells in blood.

Further, cells stimulated to migrate or cells mobilized from bone marrow to peripheral blood include bone marrow-derived cells and hematopoietic stem cells, but are not limited thereto. In the present specification, "hematopoietic stem cells" are stem cells that can differentiate into blood cells such as red blood cells, platelets, mast cells, and dendritic cells, as well as white blood cells including neutrophils, eosinophils, basophils, lymphocytes, monocytes, macrophages, and such. Their markers are known to be CD34-positive and CD133-positive in human, and CD34-negative, c-Kit-positive, Sca-1-positive, and lineage marker-negative in mouse. Hematopoietic stem cells are difficult to be cultured alone when cultured in culture dishes, and they need to be co-cultured with stromal cells.

In the present specification, "bone marrow cells" means cells present inside bone marrow while "bone marrow-derived cells" means "bone marrow cells" mobilized from bone marrow to outside of bone marrow. "Bone marrow cells" include cells containing tissue progenitor cell populations present inside bone marrow. Further, "bone marrow-derived cells" may be cells containing mesoangioblasts or cells free of mesoangioblasts.

Tissue progenitor cells are defined as undifferentiated cells having a unidirectional potency to differentiate into cells of a specific tissue other than the blood system, and include undifferentiated cells having the potency to differentiate into mesenchymal tissues, epithelial tissues, nerve tissues, parenchymatous organs, and vascular endothelium as mentioned above.

"Bone marrow cells" and "bone marrow-derived cells" are hematopoietic stem cells and differentiated cells derived therefrom such as leukocytes, erythrocytes, platelets, osteoblasts, and fibrocytes, or are stem cells represented by cells which have been hitherto called bone marrow mesenchymal stem cells, bone marrow stromal pluripotent stem cells, or bone marrow pluripotent stem cells. As used herein, "bone marrow stem cells" refer to stem cells present inside bone marrow, while "bone marrow-derived stem cells" refer to "bone marrow stem cells" mobilized from bone marrow to outside of bone marrow. In the present invention, cells stimulated to migrate or cells mobilized from bone marrow to peripheral blood include "bone marrow-derived stem cells", but are not limited thereto. "Bone marrow cells" and "bone marrow-derived cells" can be isolated by bone marrow collection (bone marrow cell collection) or peripheral blood collection. Hematopoietic stem cells are nonadherent, while some of the "bone marrow cells" and "bone marrow-derived cells" are obtained as adherent cells by means of a cell culture of a monocyte fraction of blood obtained by the bone marrow collection (bone marrow cell collection) or peripheral blood collection.

Moreover, "bone marrow cells" and "bone marrow-derived cells" include mesenchymal

stem cells, and have a potential to differentiate into, preferably, osteoblasts (which can be identified by observing calcification after inducing differentiation), chondrocytes (which can be identified by alcian blue positive staining, safranin O positive staining, or the like), adipocytes (which can be identified by Sudan III positive staining), and other mesenchymal cells such as fibroblasts, smooth muscle cells, stromal cells, and tendon cells; and further nerve cells, epithelial cells (for example, epidermal keratinocytes and intestinal epithelial cells express cytokeratin family), and vascular endothelial cells. The cells to be differentiated into are not limited to the above cells, and the potential to differentiate into cells of parenchymatous organs such as liver, kidney, and pancreas is also included.

Herein, "bone marrow mesenchymal stem cells", "bone marrow stromal pluripotent cells" or "bone marrow pluripotent stem cells" refer to cells existing in the bone marrow, which are directly collected from the bone marrow or indirectly collected from other tissues (blood, skin, fat, and other tissues), and can be cultured and proliferated as adherent cells on a culture dish (made of plastic or glass). These cells are characterized in having a potential to differentiate into mesenchymal tissues such as bone, cartilage, and fat (mesenchymal stem cells), or into skeletal muscle, heart muscle, nervous tissues, and epithelial tissues (pluripotent stem cells), and can be obtained by collection of bone marrow cells.

On the other hand, "bone marrow-derived bone marrow mesenchymal stem cells", "bone marrow-derived bone marrow stromal pluripotent cells", or "bone marrow-derived bone marrow pluripotent stem cells" mobilized from bone marrow to outside of the bone marrow are cells that can be obtained by collection from peripheral blood, mesenchymal tissues such as fat, epithelial tissues such as skin, or nervous tissues such as brain.

In addition, these cells are also characterized in having a potential to differentiate into, for example, epithelial tissues such as keratinocytes that constitute skin, or nervous tissues that constitute brain, when administered to a lesion area of the living body immediately after collection or after once being adhered onto a culture dish.

Bone marrow mesenchymal stem cells, bone marrow stromal pluripotent stem cells, bone marrow pluripotent stem cells, or these cells recruited from bone marrow to outside of the bone marrow preferably have a potency to differentiate into: osteoblasts (which can be identified by observing calcification after inducing differentiation), chondrocytes (which can be identified by alcian blue positive staining, safranin O positive staining, or the like), adipocytes (which can be identified by Sudan III positive staining or the like), and other mesenchymal cells such as fibroblasts, smooth muscle cells, skeletal muscle cells, stromal cells, and tendon cells; nerve cells, pigment cells, epidermal cells, hair follicle cells (which express cytokeratin family, hair keratin family, or the like), epithelial cells (for example, epidermal keratinocytes and intestinal epithelial cells express cytokeratin family or the like), and endothelial cells; and further into cells of

parenchymatous organs such as liver, kidney, and pancreas. However, differentiated cells are not limited to the above cells.

Human bone marrow cells and human bone marrow-derived cells can be exemplified by, but are not limited to, cells which can be directly obtained by collecting bone marrow (bone marrow cells), peripheral blood, or fat, or obtained as adherent cells through culturing of an isolated monocyte fraction. Markers for human bone marrow cells and human bone marrow-derived cells include, for example, all or some of the following but are not limited thereto: PDGFRα-positive, Lin-negative, CD45-negative, CD44-positive, CD90-positive, and CD29-positive, Flk-1-negative, CD105-positive, CD73-positive, CD90-positive, CD71-positive, Stro-1-positive, CD106-positive, CD166-positive, and CD31-negative.

Moreover, mouse bone marrow cells and mouse bone marrow-derived cells can be exemplified by, but are not limited to, cells which can be directly obtained by collecting bone marrow (bone marrow cells), peripheral blood, or fat, or obtained as adherent cells through culturing of an isolated monocyte fraction. Markers for mouse bone marrow cells and mouse bone marrow-derived cells include, for example, all or some of the following but are not limited thereto: CD44-positive, PDGFRα-positive, PDGFRβ-positive, CD45-negative, Lin-negative, Sca-1-positive, c-kit-negative, CD90-positive, CD29-positive, and Flk-1-negative.

In the present invention, cells stimulated to migrate include PDGFRα-positive cells, but are not limited thereto. In addition, PDGFRα-positive cells stimulated to migrate are not particularly limited, but preferably are bone marrow-derived PDGFRα-positive cells. Further, markers other than PDGFRα can be exemplified by all or some of CD29-positive, CD44-positive, CD90-positive, CD271-positive, CD11b-negative, and Flk-1-negative, but are not limited thereto. PDGFRα-positive cells include, but are not limited to, for example, PDGFRα-positive bone marrow-derived cells, PDGFRα-positive bone marrow-derived mesenchymal stem cells, tissue cells residing in PDGFRα-positive tissues (for example, fibroblasts and such), PDGFRα-positive bone marrow-derived cells obtained as adherent cells by means of cell culture of a monocyte fraction of blood obtained by bone marrow collection (bone marrow cell collection) or peripheral blood collection.

The HMGB1 protein in the present invention includes, but is not limited to, for example, a protein comprising the amino acid sequence of SEQ ID NO: 1 as a human-derived HMGB1 protein, and DNA encoding said protein includes, but is not limited to, for example, a DNA comprising the nucleotide sequence of SEQ ID NO: 2.

In the present invention, "an HMGB1 fragment peptide having a cell migration-stimulating activity" refers to a peptide consisting of a portion of an HMGB1 protein and having a cell migration-stimulating activity. Fragment peptides consisting of a portion of an HMGB1 protein of the present invention are not particularly limited as long as they have cell migration-

stimulating activity, but are preferably HMGB1 fragment peptides comprising at least the amino acid sequence of positions 1 to 44 of the HMGB1 protein (SEQ ID NO: 3), which are fragment peptides having a cell migration-stimulating activity.

In the present invention, the HMGB1 fragment peptides having a cell migration-stimulating activity can be exemplified by the fragments below, without being limited thereto.

In the present invention, the HMGB1 fragment peptides having a cell migration-stimulating activity include HMGB1 fragment peptides that comprise the amino acid sequence of SEQ ID NO: 3 and have a cell migration-stimulating activity. Such fragment peptides include, without being limited thereto, for example, as an upper limit any arbitrary HMGB1-derived fragment peptide that comprise at least the HMGB1 fragment peptide (1-44) but do not contain the full-length HMGB1 protein.

In the present invention, HMGB1 fragment peptides having a cell migration-stimulating activity include the HMGB1 fragment peptide (1-44).

Methods for administering a composition of the present invention include oral administration and parenteral administration. Specifically, parenteral administration includes, but is not limited to, injection, transnasal administration, transpulmonary administration, transdermal administration, and such. As examples of injection, intravenous injection, intramuscular injection, intraperitoneal injection, subcutaneous injection, and such can be used to administer a composition of the present invention systemically or locally (for example, under the skin, in the skin, on the surface of skin, eyeball or palpebral conjunctiva, nasal cavity mucosa, intraoral mucosa and mucosa of the gastrointestinal tract, vaginal mucosa/intrauterine mucosa, damage site or such).

Methods of administering a composition of the present invention include, but are not limited to, for example, intravascular administration (intra-arterial administration, intravenous administration, or such), blood administration, intramuscular administration, subcutaneous administration, intradermal administration, intraperitoneal administration.

There is no limitation on the site of administration, and for example, it may be a tissue site in need of regeneration or its nearby region, a site different from the tissue in need of regeneration, or a site distant to and different from the tissue in need of regeneration. The site is, for example, in the blood (in arteries, in veins, or such), muscle, under the skin, in the skin, in the abdominal cavity, or such, without being limited thereto.

The method of administration may be appropriately selected according to the age and the symptoms of the patient. When a peptide of the present invention is administered, the dose per time of the peptide can be selected within a range of 0.0000001 mg to 1000 mg per kg body weight of a patient. Alternatively, the dose can be selected within a range of 0.00001 mg to 100000 mg per body of patient, for example. When administering cells secreting a peptide of the

present invention or gene therapy vectors inserted with DNA encoding the peptide, they may be administered such that the amount of the peptide is within the above range. However, the dose of pharmaceutical compositions of the present invention is not limited thereto.

HMGB1 fragment peptides of the present invention can be obtained as recombinants by incorporating a DNA encoding the peptide into an appropriate expression system, or can be synthesized artificially. Pharmaceutical compositions of the present invention can be formulated according to the usual methods (for example, Remington's Pharmaceutical Science, latest edition, Mark Publishing Company, Easton, U.S.A), and may contain pharmaceutically acceptable carriers and additives together. Examples include surfactants, excipients, colorants, perfumes, preservatives, stabilizers, buffers, suspending agents, isotonizing agents, binders, disintegrants, lubricants, flow promoters, and flavoring agents, although they are not limited thereto and other common carriers may be appropriately used. Specific examples include light anhydrous silicic acid, lactose, crystalline cellulose, mannitol, starch, carmellose calcium, carmellose sodium, hydroxypropylcellulose, hydroxypropylmethylcellulose, polyvinylacetaldiethylamino acetate, polyvinylpyrrolidone, gelatin, medium-chain fatty acid triglyceride, polyoxyethylene hydrogenated castor oil 60, white sugar, carboxymethyl cellulose, corn starch, and inorganic salts.

All prior art documents cited herein are incorporated herein by reference.

Herein below, the present invention will be further illustrated with reference to Examples, but it is not to be construed as being limited thereto.

[Example 1]

(Objective)

The therapeutic effect by systemic administration of the HMGB1 protein fragment peptide (1-44) is comprehensively evaluated using an acute stage myocardial infarction mouse model by means of cardiac physiology, histopathology, and molecular biology.

(Materials and Methods)

Bone marrow cells were collected from the thigh bone/lower leg bone of three-week-old mice (C57BL6-CAG-GFP transgenic mice), and the bone marrow cell suspension was administered (300 ml/mouse) to the tail vein of six-week-old mice (C57BL6) after irradiation (10GY) to produce bone marrow chimeric mice. Sixty days after bone marrow transplantation, left fourth interspace thoracotomy was performed under general anesthesia in the supine position, and the proximal left anterior descending coronary artery was ligated to produce an extensive myocardial infarction model (left anterior descending coronary artery/circumflex occlusion model). In making the HMGB1 protein low molecular, a fragment peptide (1-44) of the HMGB1 protein was developed in consideration of retaining the activity of recruiting bone

marrow mesenchymal stem cells. At 6, 24, 48, 72, and 96 hours after infarction creation, the HMGB1 protein fragment peptide (1-44) (5 μg/300 μl), or 300 μl of PBS (negative control group) was administered five times to the tail vain. Mice were euthanized by administration of excess ketamine and xylazine, and their hearts were collected. After the tissues were embedded in OCT compound, cryosections of 5 μm thickness were prepared. Immunohistological studies were performed as follows by using an anti-mouse PDGFRα antibody. The PDGFRα Rabbit Anti-PDGF Receptor Alpha Antibody (Abcam, AB61219) was used as the primary antibody. The Alexa 555 Goat Anti-rabbit Antibody (Molecular Probes) was used as the secondary antibody, and 4,6-diamino-2-phenylinodole (DAPI, Molecular Probes) was used for nuclear staining. Confocal laser scanning microscope (FV300, Olympus) was used for photographing. Regarding the number of bone marrow-derived mesenchymal stem cells accumulated in the tissue, the number of cells positive for both GFP and PDGFRα present in all areas of randomly-selected 5 sections were determined and the mean value was calculated. Further, RNA was extracted from myocardial tissue using the RNeasy Kit (Qiagen) and cDNA was obtained by reverse transcription reaction using the Omniscript Reverse Transcriptase (Qiagen). Real-time PCR was performed with the Gene Amp(R) PCR System 9700 (Life Technologies Japan) using the above-mentioned cDNA as a template, and the levels of TNFα and IL-1β mRNA were determined as a ratio to the GAPDH mRNA level. Improvement in the cardiac function was evaluated by measuring the left ventricular end-diastolic diameter (LVDd: Dd), left ventricular end-systolic diameter (LVDs: Ds), and left ventricular ejection fraction (LVEF: EF) using the Vivid 7 Echocardiography System and 12-MHZ Transducer (General Electric).

By systemic administration of the HMGB1 protein fragment peptide (1-44), PDGFRα-positive bone marrow-derived mesenchymal cells were recruited significantly in all areas of non-infarction sites, boundary sites, and infarction sites on day 7 post infarction creation (non-infarction site: partial fragment of the HMGBI protein (1-44 peptide), 52 ± 11#*; negative control, 32 ± 9 cells/mm^2, N = 4 each, #P < 0.05 vs. negative control) (Fig. 1A). Further, in the HMGB1 protein fragment peptide (1-44) group, the levels of inflammatory cytokines (TNFα, IL-1β) in the non-infarction sites were significantly low (TNFα: HMGB1 protein fragment peptide (1-44), 4 ± 2#*; negative control, 11 ± 3 GAPDH; N = 4 each, #P < 0.05 vs. negative control), (IL-1β: HMGBI protein partial fragment (1-44 peptide), 2 ± 1#*; negative control, 6 ± 1 GAPDH; N = 4 each, #P < 0.05 vs. negative control) (Figs. 1C, 1D). Ultrasonic examination of the heart on Day 56 revealed that left ventricular systolic dysfunction after myocardial infarction was significantly suppressed in the HMGB1 protein fragment peptide (1-44) group (EF: HMGB1 protein fragment peptide (1-44) (N = 8), 26 ± 4#*; negative control (N = 16), 20 ± 4%, #P < 0.05 vs. negative control). Further, left ventricular enlargement was significantly suppressed in the HMGB1 protein fragment peptide (1-44) group (DD: HMGB1 protein fragment peptide (1-44)

(N = 8), 5.8 ± 0.2#*, negative control (N = 16), 6.3 ± 0.2 mm, #P < 0.05 VS. negative control) (Fig. 1E).

In the present investigation, it was demonstrated that five-day-administration of the HMGB1 protein fragment peptide (1-44) recruits PDGFRα-positive bone marrow mesenchymal stem cells to the infarction site and its neighboring region in the acute stage (Day 7) after infarction creation (Figs. 1A, 1B). In particular, it was demonstrated that administration of the fragment peptide (1-44) enhances the recruitment of PDGFRα-positive mesenchymal stem cells to the myocardial tissue of the non-infarction site. Bone marrow mesenchymal stem cells are known to have an inflammation-suppressing effect and a tissue-regenerating effect. In the present Example, they were also found to suppress inflammatory reactions (Figs. 1C, 1D). Further, administration of the HMGB1 fragment peptide (1-44) significantly suppressed the aggravation of the left ventricular systolic function, left ventricular enlargement, and left ventricular remodeling. Significant long-standing improvement of the myocardial function was also observed (Fig. 1E).

Industrial Applicability

The present invention provides novel uses of HMGB1 fragment peptides that retain the activity of recruiting PDGFRα-positive cells for treating myocardial infarction. The HMGB1 fragment peptides of the present invention include fragment peptides with a molecular weight of about 20 percent of the full-length HMGB1 protein consisting of 215 amino acids. Such fragment peptides can be produced by chemical synthesis using peptide synthesizers, and are thus expected to improve purity, provide stable production, and reduce costs in the situation of producing peptides as pharmaceuticals.

Moreover, full-length HMGB1 is known to have an activity to bind to lipopolysaccharides (LPS) which are one type of endotoxin. Contamination of even a trace amount of LPS in pharmaceuticals causes fever and such, and often results in serious side effects. Thus, LPS contamination in pharmaceuticals is strictly regulated. As HMGB1 has affinity for LPS, it is difficult to completely eliminate LPS from pharmaceuticals contaminated therewith. However, making HMGB1 into peptides reduces its affinity to LPS, and thus it is expected to reduce LPS contamination in pharmaceuticals. Accordingly, by using an HMGB1 fragment comprising the portion for recruiting PDGFRα-positive cells as specified in the present invention, it is possible to develop safer pharmaceutical compositions for treating myocardial infarction.

Direct administration of the HMGB1 fragment peptide of the present invention to the myocardial infarction site in need of regeneration, or to the vicinity thereof, may induce or enhance regeneration of the infarction site or the tissue damaged by the infarction. Further, regeneration of myocardial infarction may be induced or enhanced by administering the HMGB1

fragment peptide of the present invention to a site different from the site in need of regeneration by means such as intravenous administration. As such, the present invention enables treatment of myocardial infarction with intravenous administration, which is widely performed in general practice; and thus, therapeutic agents can be safely and easily administered at arbitrary concentrations for an arbitrary number of times. This fact is one of the extremely superior aspects of the present invention in comparison to conventional therapeutic methods.

In current practice of regenerative medicine or cell therapy, scarce bone marrow pluripotent stem cells derived from patients are cultured *ex vivo* and used for therapies after proliferation; however, since the culturing process carries the risk of cell deterioration (canceration or contamination of bacteria, viruses, and such), sufficient safety control is required. In contrast, therapeutic agents based on the present invention do not include the step of taking cells outside the body or the step of including manual operation, and thus, they are believed to be relatively safe. This fact is also one of the superior aspects of the present invention in comparison to conventional therapeutic methods.

CLAIMS

1. A pharmaceutical composition for use in the treatment of myocardial infarction, comprising an HMGB1 fragment peptide.

2. The pharmaceutical composition of claim 1, wherein the HMGB1 fragment peptide is a peptide comprising the amino acid sequence of SEQ ID NO: 3.

3. The pharmaceutical composition of claim 1, wherein the HMGB1 fragment peptide is a peptide consisting of the amino acid sequence of SEQ ID NO: 3.

SEQUENCE LISTING

<110> Genomix Co., Ltd.
Osaka University

<120> Novel method for treating cardiac infarction using HMGB1 fragment

<130> G6-A1201P

<150> JP 2012-235785
<151> 2012-10-25

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Arg Pro Pro Ser Ala Phe Phe Leu Phe Cys Ser Glu Tyr Arg Pro Lys
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35 40