THERAPEUTIC METHODS USING ADIPOSE TISSUE-DERIVED CELL SUSPENSIONS COMPRISING ADIPOCYTES

Related Applications

The present application claims benefit from Australian provisional patent application No. 2008904326 entitled "Therapeutic Methods" filed 22 August 2008 and Australian patent application No. 2009201915 entitled "Therapeutic Methods" filed 14 May 2009, the entire contents of each of which are incorporated herein by reference.

Technical Field

The present invention relates to the use of an adipose-derived cell suspension for the preparation of a pharmaceutical composition for the treatment of an inflammatory disorder and/or the alleviation of pain which is associated with an inflammatory disorder, and to methods of treating an inflammatory disorder and/or alleviating pain associated with an inflammatory disorder using such pharmaceutical compositions.

Background of the Invention

Adipose tissue contains a cell population of large, lipid-filled adipocytes and a population of non-adipocyte cells, which comprises cells associated with various connective fibres and cells associated with capillaries and larger blood vessels. The non-adipocyte cell population also comprises a variety of infiltrating immune cells and cells and cell processes associated with the nervous system. The non-adipocyte cell population is also thought to comprise a population of adipose-derived adult stem cells and consequently there has been interest in using adipose tissue as a source of isolated adult stem cells for various therapeutic applications.

In general, methods for obtaining adipose tissue-derived presumptive adult stem cells involves depleting adipocytes from adipose-derived non-adipocyte cells, which requires digesting adipose tissue with enzymes such as collagenase, and then separating the liberated cells by centrifuging the digested sample. During centrifugation, the adipose-derived non-adipocyte cells separate from the adipocytes to form a pellet, whereas the lipid containing adipocytes float. The fraction containing the non-adipocyte cells is then used as a source of tissue stem cells.

Summary of the Invention

In a first aspect there is provided the use of:

(i) an adipose tissue-derived cell suspension which comprises adipocytes and adipose-derived non-adipocyte cells; or

(ii) a cell-free extract which is prepared from an adipose tissue-derived cell suspension, wherein the adipose tissue-derived cell suspension comprises adipocytes and adipose-derived non-adipocyte cells;

for the preparation of a pharmaceutical composition for use in the treatment of an inflammatory disorder, or the treatment of a cartilage or bone disorder and/or the alleviation of pain associated with an inflammatory disorder in a subject.

Also disclosed is the use of an adipose tissue-derived cell suspension which comprises adipocytes for the preparation of a pharmaceutical

composition for use in the treatment of an inflammatory disorder or the alleviation of pain associated with an inflammatory disorder in a subject.

Also disclosed is~~In a second aspect there is provided~~ the use of an adipose tissue-derived cell suspension which comprises adipocytes for the preparation of a pharmaceutical composition for use in the treatment of a cartilage or bone disorder in a subject.

Also disclosed is~~In a third aspect there is provided~~ a method of treating an inflammatory disorder or a cartilage or bone disorder, or alleviating pain associated with an inflammatory disorder in a subject, comprising administering to the subject a pharmaceutical composition which comprises:

(i) an adipose tissue-derived cell suspension which comprises adipocytes; or

(ii) a cell-free extract which is prepared from an adipose tissue-derived cell suspension, wherein the adipose tissue-derived cell suspension comprises adipocytes,

together with a pharmaceutically-acceptable carrier or diluent.

In certain ~~embodiments~~examples the adipose tissue-derived cell suspension is freshly isolated.

In certain ~~embodiments~~examples, the pharmaceutical composition is a cell free extract.

In certain ~~embodiments~~examples the adipose tissue-derived cell suspension is autologous to the subject. In other ~~embodiments~~examples, the adipose tissue-derived cell suspension is allogeneic to the subject.

In certain ~~embodiments~~examples the subject is a human. In certain ~~embodiments~~examples the pharmaceutical composition is a veterinary composition and the subject is a non-human mammal, such as a canine or feline subject.

In certain ~~embodiments~~examples, the inflammatory disorder is selected from a joint-related inflammatory disorder, corneal inflammation, skin inflammation or inflammation associated with wounding. In certain ~~embodiments~~examples the inflammatory disorder is a skin or corneal ulcer.

In certain ~~embodiments~~examples the cartilage or bone disorder is a cartilage or bone fracture, for instance a non-union bone fracture, or osteoporosis.

Also disclosed is~~In another aspect there is provided~~ a pharmaceutical composition with comprises:

(i) an adipose tissue-derived cell suspension which comprises adipocytes; or

(ii) a cell-free extract which is prepared from an adipose tissue-derived cell suspension, wherein the adipose tissue-derived cell suspension comprises adipocytes,

together with a pharmaceutically-acceptable carrier or diluent.

In another aspect there is provided a method of generating an adipose tissue-derived cell suspension which comprises adipocytes and adipose-derived non-adipocyte cells from a sample of adipose tissue from a mammal, the method comprising:

dissociating the sample of adipose tissue to form a suspension of cells which comprises adipocytes and adipose-derived non-adipocyte cells;

centrifuging the suspension of cells to form a cell pellet, a floating cell layer which comprises adipocytes and an intermediate layer which is depleted of cells relative to the cell pellet and the floating cell layer; and

removing the intermediate layer and mixing the cell pellet and floating cell layer to form an adipose tissue derived cell suspension which comprises adipocytes and adipose - derived non-adipocyte cells.

Also disclosed is a method of generating an adipose tissue-derived cell suspension which comprises adipocytes from a sample of adipose tissue from a mammal, the method comprising:

dissociating the sample of adipose tissue to form a suspension of cells which comprises adipocytes;

centrifuging the suspension of cells to form a cell pellet, a floating cell layer which comprises adipocytes and an intermediate layer which is depleted of cells relative to the cell pellet and the floating cell layer; and

removing the intermediate layer and mixing the cell pellet and floating cell layer to form an adipose tissue derived cell suspension which comprises adipocytes.

In certain ~~embodiments~~examples of this method, the adipose tissue-derived cell suspension which comprises adipocytes is generated from the sample of adipose tissue within 6 hours of the sample of adipose tissue being obtained from the mammal.

Abbreviations

DMEM	Dulbecco's Modified Eagles Medium
RPMI	Roswell Park Memorial Institute Medium

Detailed Description of the Preferred Embodiments

Described herein are compositions comprising adipose tissue-derived cell suspensions which retain at least a substantial portion of the adipocyte fraction, and use of these compositions for treating inflammatory disorders, cartilage or bone disorders or for alleviating pain associated with inflammatory disorders. Without wishing to be bound by any proposed mechanism of action, it is proposed that adipocytes and/or interactions between adipocytes and adipose-derived non-adipocyte cells may contribute to therapeutic activity of an adipose-tissue derived cell suspension for treating inflammatory disorders, cartilage or bone disorders or for alleviating pain associated with inflammatory disorders.

Accordingly, in a first aspect there is provided the use of an adipose tissue-derived cell suspension which comprises adipocytes for the preparation of a pharmaceutical composition for use in the treatment of an inflammatory disorder or the alleviation of pain associated with an inflammatory disorder in a subject.

9. The use according to any one of claims 1 to 7, wherein the inflammatory disorder is arthritis or an autoimmune disease.

10. The use according to claim 9, wherein the arthritis is selected from the group consisting of acute arthritis, osteoarthritis, and rheumatoid arthritis.

11. The use according to any one of claims 1 to 10, wherein the adipose tissue-derived cell suspension is from subcutaneous adipose tissue.

~~12. A pharmaceutical composition which comprises:~~

~~(i) an adipose tissue-derived cell suspension which comprises adipocytes and adipose-derived non-adipocyte cells; or~~

~~(ii) a cell-free extract which is prepared from an adipose tissue-derived cell suspension, wherein the adipose tissue-derived cell suspension comprises adipocytes and adipose-derived non-adipocyte cells, and wherein the cell-free extract comprises cell-derived molecules which are released from the cells following cell death or the break-up of the adipose tissue cells and/or the secretions of cells of the adipose tissue-derived cell suspension;~~

~~together with a pharmaceutically-acceptable carrier or diluent.~~

12~~13~~. A method of generating an adipose tissue-derived cell suspension which comprises adipocytes and adipose-derived non-adipocyte cells from a sample of adipose tissue from a mammal, the method comprising:

dissociating the sample of adipose tissue to form a suspension of cells which comprises adipocytes and adipose-derived non-adipocyte cells;

centrifuging the suspension of cells to form a cell pellet, a floating cell layer which comprises adipocytes and an intermediate layer which is depleted of cells relative to the cell pellet and the floating cell layer; and

removing the intermediate layer and mixing the cell pellet and floating cell layer to form an adipose tissue derived cell suspension which comprises adipocytes and adipose - derived non-adipocyte cells.

13~~14~~. The method according to claim 12~~13~~, wherein the adipose tissue-derived cell suspension which comprises adipocytes and adipose-derived non-adipocyte cells is generated from the sample of adipose tissue within 6 hours of the sample of adipose tissue being obtained from the mammal.

14~~15~~. The method according to claim 12~~13~~ or 13~~14~~, wherein the adipose tissue-derived cell suspension is from subcutaneous adipose tissue.