angioplasty. Reperfusion is necessary for the salvage of myocardial cells and cardiac function in general. However, reperfusion initiates a cascade of events that leads to "reperfusion injury". This also occurs following recovery from cardioplegic arrest of the heart during bypass surgery. Reperfusion injury is characterized by arrhythmias, endothelial dysfunction leading to the no-reflow phenomenon and myocardial stunning (reversible loss of myocardial contractility).

Reperfusion injury culminates in apoptotic death of cardiac cells that were viable immediately before myocardial reperfusion. The involvement of a highly-regulated form of cell death during myocardial ischemia/reperfusion may lead to novel therapeutic interventions in the reperfusion phase. However, the apoptosis signalling pathways that are involved during myocardial ischemia/reperfusion have not yet been fully delineated *in vivo*.

Finding new treatments for inhibiting apoptosis (*i.e.*, "programmed cell death"), and in particular for treating myocardial infarction and reperfusion injury, thus constitutes a real challenge to protect cardiac function and to save lives.

Summary of the Invention

The ~~invention~~disclosure is based on the finding that it is possible to decrease apoptosis of cardiac cells after myocardial infarction by inhibiting the Fas signalling pathway. The Fas Receptor trimerizes upon binding to the FasL (Fas Ligand) and induces apoptosis through a cytoplasmic domain called DD (Death Domain) that interacts with signalling adaptors such as FAF-1 (Fas-Associated Factor-1), FADD (Fas-Associated Death Domain), DAXX (Death-Domain Associated protein), FAP-1, FLASH (FLICE-associated huge) and RIP (Receptor-Interacting Protein). DAXX and FADD independently bind to Fas, and activate distinct apoptotic pathways. DAXX can enhance Fas-mediated apoptosis by activating the JNK kinase cascade, culminating in phosphorylation and activation of transcription factors such as c-Jun. In contrast, FADD triggers, through a cascade of signalling caspases, the release of mitochondrial pro-apoptotic factors like CytoC (Cytochrome-C) and SMAC (Second Mitochondria-derived Activator of Caspases) also called Diablo.

The inventors have shown that inhibiting the interaction of the Fas Receptor with DAXX (SEQ ID NO: 1) or with FADD (SEQ ID NO: 8) leads to a strong decrease in the apoptosis of cardiac cells after myocardial infarction. Furthermore, the present inventors

have unexpectedly found that small fragments of DAXX and of FADD retain the anti-apoptotic ability of the full proteins DAXX and FADD, respectively.

Accordingly, the present ~~invention~~disclosure relates to a peptide consisting of:

- a fragment of 16, 17, 18, 19, 20, 21, 22, 23 or 24 consecutive amino acid residues of the DAXX protein of SEQ ID NO: 1, wherein said fragment comprises the amino acid sequence set forth in SEQ ID NO: 5, or

- a fragment of 9, 10, 11, 12, 13, 14, 15, 16 or 17 consecutive amino acid residues of the FADD protein of SEQ ID NO: 8, wherein said fragment comprises the amino acid sequence set forth in SEQ ID NO: 12,

wherein said peptide is capable of inhibiting cell apoptosis.

In one aspect, there is provided a peptide consisting of:

a fragment of 16, 17, 18, 19, 20, 21, 22, 23 or 24 consecutive amino acid residues of the DAXX protein of SEQ ID NO:1, wherein said fragment comprises the amino acid sequence set forth in SEQ ID NO: 5, and

wherein said peptide is capable of inhibiting cell apoptosis.

In certain embodiments, an anti-apoptotic peptide ~~according to the invention~~as disclosed herein is a DAXX protein fragment consisting of the amino acid sequence set forth in SEQ ID NO: 5. In other embodiments, an anti-apoptotic peptide is a DAXX fragment consisting of the amino acid sequence set forth in any one of SEQ ID NOs: 21-44.

In other embodiments, an anti-apoptotic peptide ~~according to the invention~~as disclosed herein is a FADD protein fragment consisting of the amino acid sequence set forth in SEQ ID NO: 12 or SEQ ID NO: 9. In other embodiments, an anti-apoptotic peptide is a FADD fragment consisting of the amino acid sequence set forth in any one of SEQ ID NOs: 45-57.

In another aspect, the present invention relates to a peptidomimetic of an anti-apoptotic peptide ~~according to the invention~~as defined herein.

In yet another aspect, the present invention provides a conjugate comprising an anti-apoptotic peptide or a peptidomimetic ~~according to the invention~~as defined herein linked to a Cell Penetrating Peptide. The Cell Penetrating Peptide may be Tat, RXR, Bpep or Pip2b.

In certain embodiments, the Cell Penetrating Peptide is linked to the peptide or to the peptidomimetic through a linker.

In certain embodiments, the Cell Penetrating Peptide is selected from the group consisting of Tat, RXR, Bpep and Pip2b.

In particular, in certain preferred embodiments, the conjugate consists of the amino acid sequence set forth in SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60 or SEQ ID NO: 61.

In yet another aspect, the present invention relates to a pharmaceutical composition comprising at least one pharmaceutically acceptable carrier or excipient and an effective amount of at least one peptide, or at least one peptidomimetic or at least one conjugate ~~according to the invention~~as defined herein.

In certain embodiments, a pharmaceutical composition ~~according to the invention~~as defined herein further comprises at least one additional biologically active agent. In particular, the biologically active agent may be selected from the group consisting of cyclosporine A, BH4, and combinations thereof.

In still another ~~aspect~~embodiment, the present ~~invention~~disclosure provides the peptides, peptidomimetics, conjugates and pharmaceutical compositions according to the invention for use in a method of treatment of the human or animal body, in particular for use in a method for inhibiting cell apoptosis in the human or animal body.

In certain embodiments, the disclosed peptides, peptidomimetics, conjugates and pharmaceutical compositions ~~according to the invention~~ are used in a method for the treatment of acute myocardial infarction (AMI), cerebral infarction, organ transplantations, cardiac interventions (extra-corporally circulation and temporary vessel occlusion), or acute circulation perturbations (state of shock), in the human or animal body.

In other embodiments, the disclosed peptides, peptidomimetics, conjugates and pharmaceutical compositions ~~according to the invention~~ are used in a method for treatment of ischemia, in particular cardiac ischemia, kidney ischemia, ischemic colitis, mesenteric ischemia, brain ischemia, limb ischemia or skin ischemia, in the human or animal body.

In yet other embodiments, the disclosed peptides, peptidomimetics, conjugates and pharmaceutical compositions ~~according to the invention~~ are used in a method for treatment of reperfusion injury in the human or animal body.

In a related ~~aspect~~embodiment, the present ~~invention~~disclosure also provides a method for treating a disease or condition associated with apoptosis in a subject, comprising a step of: administering to said subject an effective amount of at least one peptide, or at least one peptidomimetic, or at least one conjugate or at least one pharmaceutical composition according to the invention.

In certain embodiments, the method further comprise a step of administering to said subject at least one additional biologically active agent selected from the group consisting of cyclosporine A, BH4, and combinations thereof.

In yet another aspect, there is provided the use of a peptide as defined herein, or a peptidomimetic as defined herein, or a conjugate as defined herein, for the manufacture of a medicine for the treatment of a disease or condition associated with apoptosis.

As mentioned above, in these methods of treatment, the disease or condition associated with apoptosis may be selected from the group consisting of acute myocardial infarction (AMI), cerebral infarction, organ transplantations, cardiac interventions, acute circulation perturbations, reperfusion injury, and ischemia.

These and other objects, advantages and features of the present invention will become apparent to those of ordinary skill in the art having read the following detailed description of the preferred embodiments.

Brief Description of the Drawing

Figure 1: Determination of the DAXX epitope by SPOT synthesis. (A) The amino acid sequence of DAXX was dissected in overlapping peptide arrays (pepscan; 15 mer peptides with a shift of 3 amino acids) and analyzed in enzyme-linked blot. The black rectangle shows the four brightest spots with the corresponding epitope sequence. Incubation conditions: His-tagged intracellular region of Fas receptor [10 µg/ml]; antibodies: anti-His-(mouse) (Sigma H1029; 1:6,000) / anti-mouse-HRP (Calbiochem 401207; 1:2,000), exposure time: 1 minute. **(B)** Alignment of the human DAXX sequence comprising the 16-mer KKSRKEKKQTGSGPLG (=DAXXp of SEQ ID NO: 5) with the DAXX sequences of other species (mouse, rat, dog (CANFA) and green monkey (CHLAE)).

Figure 2: Determination of the optimal length of the DAXX epitope (=DAXXp) **by SPOT synthesis. (A)** The DAXXp-211 and DAXXp-209 peptides (of SEQ ID NO: 2 and SEQ ID NO: 3, respectively) were successively shortened by one amino acid residue at the N-terminus, at the C-terminus and both at the N- and the C-termini, and analyzed using enzyme-linked blot. The spots indicated by an arrow exhibited the highest signal intensities (BLU). Incubation conditions: His-tagged intracellular region of Fas receptor

[10 μg/ml]; antibodies: anti-His-(mouse) (Sigma H1029; 1:6,000) / anti-mouse-HRP (Calbiochem 401207; 1:2,000), exposure time: 1 minute. **(B)** Alignment of both peptide sequences DAXXp-211 and DAXXp-209; corresponding to the brightest spots, to determine the optimal DAXX peptide sequence.

Claims

1. A peptide consisting of:
   a fragment of 16, 17, 18, 19, 20, 21, 22, 23 or 24 consecutive amino acid residues of the DAXX protein of SEQ ID NO:1, wherein said fragment comprises the amino acid sequence set forth in SEQ ID NO: 5, and
   wherein said peptide is capable of inhibiting cell apoptosis.

2. The peptide according to claim 1, wherein said peptide is a DAXX protein fragment and consists of the amino acid sequence set forth in SEQ ID NO: 5 or in any one of SEQ ID NOs: 17-44.

3. A peptidomimetic of a peptide according to claim 1or claim 2.

4. A conjugate comprising a peptide according to claim 1 or claim 2 or a peptidomimetic according to claim 3, wherein said peptide or peptidomimetic is linked to a Cell Penetrating Peptide.

5. The conjugate according to claim 4, wherein said peptide or said peptidomimetic is linked to the Cell Penetrating Peptide through a linker.

6. The conjugate according to claim 4 or claim 5, wherein said Cell Penetrating Peptide is selected from the group consisting of Tat, RXR, Bpep and Pip2b.

~~7. The conjugate according to claim 6, wherein Tat has the sequence set forth in SEQ ID NO:13, RXR has the sequence set forth in SEQ ID NO: 12, Bpep has the sequence set forth in SEQ ID NO: 14 and Pip2b has the sequence set forth in SEQ ID NO: 23.~~

~~8~~7. The conjugate according to claim ~~7~~6, wherein said conjugate consists of the amino acid sequence set forth in SEQ ID NO: 58.

~~9~~8. A pharmaceutical composition comprising an effective amount of at least one peptide according to claim 1 or claim 2, or of at least one peptidomimetic according to claim 3, or of at least one conjugate according to any one of claims ~~4-8~~4-7, and at least one pharmaceutical acceptable carrier or excipient.

~~10~~9. The pharmaceutical composition of claim ~~9~~8 further comprising at least one additional biologically active agent.

~~11~~10. The pharmaceutical composition according to claim ~~10~~9, wherein said at least one additional biologically active agent is selected from the group consisting of cyclosporine A, BH4, and combinations thereof.

~~12~~11. The peptide according to claim 1 or claim 2, or a peptidomimetic according to claim 3, or a conjugate according to any one of claims ~~4-8~~4-7 or a pharmaceutical composition according to any one of claims ~~9-11~~8-10, for use in a method of treatment of the human or animal body.

~~13~~12. The peptide according to claim 1 or claim 2, or a peptidomimetic according to claim 3, or a conjugate according to any one of claims ~~4-8~~4-7 or a pharmaceutical composition according to any one of claims ~~9-11~~8-10, for use in a method for inhibiting cell apoptosis in the human or animal body.

~~14~~13. The peptide according to claim 1 or claim 2, or a peptidomimetic according to claim 3, or a conjugate according to any one of claims ~~4-8~~4-7 or a pharmaceutical composition according to any one of claims ~~9-11~~8-10, for use in a method for the treatment of acute myocardial infarction (AMI), cerebral infarction, organ transplantations, cardiac interventions (extra-corporally circulation and temporary vessel occlusion), or acute circulation perturbations (state of shock), in the human or animal body.

~~15~~14. The peptide according to claim 1 or claim 2, or a peptidomimetic according to claim 3, or a conjugate according to any one of claims ~~4-8~~4-7 or a pharmaceutical composition according to any one of claims ~~9-11~~8-10, for use in a method for treatment of ischemia, in particular cardiac ischemia, kidney ischemia, ischemic colitis, mesenteric ischemia, brain ischemia, limb ischemia or skin ischemia, in the human or animal body.

~~16~~15. The peptide according to claims 1or claim 2, or a peptidomimetic according to claim 3, or a conjugate according to any one of claims ~~4-8~~4-7 or a pharmaceutical composition according to any one of claims ~~9-11~~8-10, for use in a method for treatment of reperfusion injury in the human or animal body.

~~17~~16. The peptide, the derivative, the conjugate or the pharmaceutical composition for the use according to any one of claims ~~12-16~~11-15, wherein cyclosporine A and/or BH4 is also to be administered to said human or animal body.

~~18~~17. Use of a peptide according to claim 1 or claim 2, or a peptidomimetic according to claim 3, or a conjugate according to any one of claims ~~4-8~~4-7, for the manufacture of a medicine for the treatment of a disease or condition associated with apoptosis.

~~19~~18. Use according to claim ~~18~~17, wherein the medicine further comprises at least one additional biologically active agent selected from the group consisting of cyclosporine A, BH4, and combinations thereof.

~~20~~19. Use according to claim ~~18~~17 or claim ~~19~~18, wherein said disease or condition associated with apoptosis is selected from the group consisting of acute myocardial infarction (AMI), cerebral infarction, organ transplantations, cardiac interventions, acute circulation perturbations, reperfusion injury, and ischemia.