(54) Title: USE OF CERAMIDES FOR THE TREATMENT OF CYSTIC FIBROSIS

(57) Abstract:
The invention relates to the use of ceramides and/or substances which contain ceramides as a building block. Said ceramides and substances are used for the treatment of cystic fibrosis. The ceramides are especially C2 and/or C6 ceramides. The invention also relates to the use of the above-mentioned substances for the treatment of diseases which are linked to a disturbed regulation of transport processes at membranes.
ABSTRACT

USE OF CERAMIDES FOR THE TREATMENT OF CYSTIC FIBROSIS

The invention relates to the use of ceramides and/or the use of substances containing ceramides as the building block for the treatment of cystic fibrosis. The ceramides are in particular C2 and/or C6 ceramides. The invention also covers the use of the aforementioned substances for the treatment of diseases, which are linked with a disturbed regulation of transport processes at membranes.
USE OF CERAMIDES FOR THE TREATMENT OF CYSTIC FIBROSIS

[001] The invention relates to the use of ceramides, derivatives of ceramides and/or precursors of ceramides. The invention more particularly relates to the use of these substances in conjunction with the treatment of cystic fibrosis.

[002] Cystic fibrosis is the most frequently encountered hereditary metabolic disease with several hundred thousand deaths annually throughout the world making it the most frequently encountered lethal genetic disease. The frequency of occurrence of this disease regionally differs and in Europe one out of 2,000 newborn babies are affected.

[003] Cystic fibrosis is a recessive autosomal metabolic disorder, which is linked with a generalized malfunction of exocrine glands. As a result of an increased electrolyte content of the secretion of sweat glands liquid and electrolyte losses occur. In addition, the viscosity of the secretions is increased, so that serious complications arise in the area of the respiratory tracts and digestive tract with secondary cystogenesis. The disease gives rise to a considerable reduction of life expectancy.

[004] One of the pathophysiological mechanisms in cystic fibrosis (CF) is a disturbed regulation of mainly epithelial Cl−, Na+ and K+ channels. Chloride channels are in particular affected, transporting Cl− ions out of the cell, namely outwardly rectifying chloride channels or ORCC.

[005] The lack of activatability of these chloride channels as a result of the disease and disturbed regulation of Na+ and water-resorbing transport processes leads to a reduced epithelial secretion of liquids and bicarbonate. This leads to viscous secretions in the lungs, pancreas and intestine of affected patients leading to an obstruction of respiratory tracts, pancreatic ducts and the intestine. This is favourable to pulmonary infections and leads to malabsorption.

[006] Cystic fibrosis is caused by numerous different mutations of the CFTR gene, which for chloride channels and/or regulators of chloride channels codes Na+ and K+ channels. The mutations lead to a disturbed transepithelial transport with reduced Cl− and HCO3− secretion and increased Na+ resorption.

[007] The problem of the invention is to make available substances and processes positively influencing the aforementioned processes disturbed in the case of CF patients. For example it is intended to activate liquid secretion in the epithelia of CF patients and in this way at least improve
pathogenesis.

[008] This problem is solved by the use of ceramides and/or substances with a ceramide building block according to claim 1 and the dependent claims 2 to 4. The problem is also solved by the use of biological precursor molecules according to claim 5 and activators according to claim 6. Pharmaceutical compositions containing at least one of these active substances are claimed in claims 7 to 12. Claims 13 to 17 relate in general terms to the use of said substances in the treatment of diseases, which are linked with disturbed transport processes. By reference the wording of all of the claims is made into part of the content of the present description.

[009] The chloride channels in cells of healthy people, but not in those of CF patients, can be activated by cAMP (cyclic adenosine monophosphate) [Rich et al, Nature 347: 358-363, 1990]. According to the prior art it was to be assumed that it would not be possible to activate the chloride channels in the cells of CF patients. However, this defect gives rise to the lack of liquid secretion of the cells, which is an important cause of the symptoms of cystic fibrosis. It has recently been possible to show that the outwardly rectifying chloride channels (ORCC channels) in cells from healthy patients can be activated by ceramides [Szabo et al, Acad. Sci. USA, 95(11): 6169-6174, 1998].

[010] Surprising results forming the basis for the present invention show that also the chloride channels in lymphocytes of CF patients can be activated by ceramides, although the regular activatability by cAMP (cyclic adenosine monophosphate) is disturbed. The channels activated by ceramides bring about a liquid secretion of the cells from CF patients, so that through the use of ceramides the symptoms of cystic fibrosis are improved.

[011] As is known ceramides are endogenic substances, which in particular occur in the brain substance and myelin of the central nervous system. They are lipophilic amides. Ceramides occur in the organism as choline phosphate esters or as glycosides. Ceramides form the building blocks of choline phosphate esters, also known as sphingomyelins, or glycosides occurring as so-called cerebrosides, gangliosides and sulphatides. The two groups of substances with ceramides as building blocks are called sphingolipids. From the chemical standpoint they are lipids, which in place of glycerin (in the case of fats and oils) contain as the alcohol component sphingosine (4-sphingogenin), which does not freely occur in nature.

[012] The invention relates to the use of ceramides and/or other of the aforementioned substances for the treatment of cystic fibrosis. Through the administration of an effective quantity of such substances it is preferably possible to stimulate liquid secretion in the epithelia of patients.
Particular reference is made to the pulmonary, pancreatic and intestinal epithelia of CF patients. The invention also covers the treatment of cystic fibrosis by the administration of ceramides and/or the other aforementioned substances, as well as the use of all these substances for the production of medicaments, particularly those for the treatment of cystic fibrosis.

[013] The ceramides or substances with a ceramide building block used according to the invention are, in a preferred embodiment, longer chain ceramides, such as e.g. the C12 ceramide or derivatives thereof, which as frequently naturally occurring substances can be directly isolated from cell material. The ceramides/substances used can also be obtained by enzymatic treatment of corresponding precursors isolatable from cell material. In a further embodiment of the invention use is made of ceramides/substances, which are obtained with the aid of molecular biological methods which are known to the expert. In a particularly preferred embodiment use is made according to the invention of synthetically produced ceramides/substances, more particularly the C2 or C6 ceramides/substances with said ceramides as the building block.

[014] According to the invention it is also possible to use derivatives of ceramides, which are chemically or biologically modified in their ceramide building blocks. With respect to cystic fibrosis, these derivatives evolve a similar and preferably better action than ceramides and/or have similar or better pharmaceutical characteristics. These can in particular be e.g. slower decomposition rates of the active substance in the body or better absorption rates in the cells.

[015] In a preferred embodiment, the substances used according to the invention are biological precursor molecules of ceramides or substances with a ceramide building block which, e.g. through an enzymatic cleaving by sphingomyelinases, are transformed in the cell into active ceramides.

[016] According to a further preferred embodiment of the invention, the substances used according to the invention act metabolically leading to the formation of ceramides. In particular, use is made of activators of sphingomyelinases, which bring about the cleavage of precursor molecules to active ceramides.

[017] A further possible point of attack for activators are cell components, preferably enzymes, which follow the ceramides in the signal chain. In particular, consideration can be given to kinases, which are activated by ceramides.

[018] Apart from the direct administration of the aforementioned active substances, the invention also covers the introduction of nucleic acids,
which code for these substances.

[019] The invention also covers pharmaceutical compositions, which contain at least one ceramide and/or the other said substances and preferably additionally a pharmaceutically acceptable carrier. As to whether use is made of a pharmaceutical carrier and optionally which carrier, is dependent on the medicament administration form.

[020] The administration of all said substances or the pharmaceutical compositions according to the invention can take place systemically. In a preferred embodiment the pharmaceutical composition is administered via the digestive tract. Particular preference is given to administration by inhalation, the active substance being rapidly and directly introduced into the lung. This administration form is particularly appropriate in the treatment of cystic fibrosis, because with this disease there are serious complications with respect to the respiratory organs.

[021] Also in the case of other diseases and illnesses linked with a disturbed regulation of molecular transport processes, preferably at membranes, a treatment with ceramides and/or the other indicated substances can lead to positive results. Therefore the invention covers the use of all these substances in the treatment of such diseases. Preferably the transport processes in the invention take place at ion channels, particularly on outwardly rectifying (from a cell or cell organelle) ion channels, preferably chloride channels.

[022] The described and further features of the invention can be gathered from the following description of preferred embodiments in conjunction with the subclaims and example. The individual features can be implemented singly or in combination with one another. In the drawings show:

Fig. 1 Influencing of the chloride channels in normal lymphocytes (A, B) and in CF lymphocytes (C, D) by cAMP.

Fig. 2 Influencing of chloride channels in CF lymphocytes by ceramide.

Fig. 3 Influencing of chloride channels in normal lymphocytes (A, B) and CF lymphocytes (C, D) by tyrosine kinase Lck56.

Example

[023] The surprising stimulating action of C2/C6 ceramide on chloride channels could be proved in the following experiments. In these experiments the known patch-clamp technique was used. The latter makes it possible to detect the conductivity on biological membranes. Reference is made in this
connection to the document of Szabo et al, Acad. Sci. USA, 95(11): 6169-6174, 1998, whose content in this connection is made into part of the content of the present description.

[024] As is known a micropipette is used to exert suction action on a membrane and by underpressure sealing occurs on the edge of the micropipette. The membrane fixed in this way as part of an intact cell or as an isolated membrane portion is immersed in a suitable electrolyte bath and the conductivity between the latter and the electrolyte solution within the pipette is measured (patch-clamp electrode). By adding different agents to the electrolyte solutions the opening state of ion channels of the membrane can be influenced. Such an influencing can be detected by a sudden conductivity change.

[025] In the present case T-lymphocytes of normal persons and patients suffering from cystic fibrosis (CF) were isolated, introduced into a perfusion chamber and over them was allowed to flow an electrolyte solution (145 mM NaCl, 5 mM KCl, 1 mM MgCl2, 2 mM CaCl2, 10 mM glucose, 10 mM hepes/NaOH, pH 7.4). Under the inverse microscope the patch-clamp electrodes (filled with 160 mM Cs-glutamate, 2 mM MgCl2, 0.1 mM CaCl2, 1.1 mM EGTA, 4 mM ATP, 10 mM hepes/NaOH, pH 7.2) was brought to the cell membrane and by the sucking in of the cell membrane a link was produced with the intracellular space (whole cell patch clamp) or the conductivity of the sucked membrane patch was measured (cell attached patch clamp). Thus, a continuous registration of the cell membrane conductivity was possible. Following a control period the cells were stimulated either in extracellular manner with cAMP (200 μM), in extracellular with C2 ceramide (50 μM) or in intracellular manner with tyrosine kinase Lck56 (10 U/ml) and the conductivity was continuously measured.

[026] The electrodes were connected by means of suitable preamplifiers to a patch-clamp amplifier (EPC-9) with the aid of which the potential between the patch-clamp electrode and a reference electrode was varied in the bath from -100 mV to +100 mV. The flows over the cell membrane between the patch-clamp electrode and the reference electrode were recorded with the aid of the patch-clamp amplifier. The choice of solutions permitted an exclusive analysis of flows through chloride channels.

[027] The curve traces shown in the individual parts of figs. 1 and 3 reflect the conductivity change (whole cell) over a period of several minutes following the administration of the substances in question. The data shown in fig. 2 represent the conductivity of administration of ceramide (cell attached) and 7 minutes following ceramide administration. The administration of cAMP and Lck56 took place in the pipette, so that from the curves of figs. 1 and 3, which were recorded at different times, it is not
possible to directly derive the kinetics of activation of chloride channels. This time dependent can be attributed to the diffusion of the supplied substances within the pipette and is consequently dependent on the method chosen. What is mainly decisive for the interpretation is the end point of the conductivity change, i.e. the top curve in each case.

[028] Ceramide was applied outside the pipette. Therefore diffusion played no part in the test, whose results are represented in fig. 2. In the representation chosen only the control value (without ceramide) and the modified conductivity after 7 minutes incubation with ceramide are plotted.

[029] As shown in fig. 1, the administration of cAMP, as expected, led to an activation of the Cl flow in normal lymphocytes (figs. 1 A, B), but not in CF lymphocytes (fig. 1 C, D). Thus, in fig. 1 A, C, D the calibration curves determined with time intervals are in each case superimposed, but not in fig. 1 B. As opposed to this and surprisingly ceramide in CF lymphocytes (fig. 2) led to an activation of chloride channels (CF curve before and curve after administration). In addition, the addition of tyrosine kinase Lck\textsuperscript{10}, which is known to be activatable by ceramides, led to an activation of chloride channels in normal lymphocytes (fig. 3 A, B) and in CF lymphocytes (fig. 3 C, D). The curves are superimposed in figs. 3 A and C, but not in figs. 3 B and D, which is in opposition to the results with cAMP.

[030] These results clearly show that the defect in CF cells, i.e. the disturbed activatability of chloride channels, can be positively influenced by the use of ceramides according to the invention.
1. Use of at least one ceramide and/or at least one substances containing a ceramide as the building block for the preparation of a pharmaceutical composition for the treatment of cystic fibrosis.

2. Use according to claim 1, characterized in that the ceramide is a C2 or a C6 ceramide or the substance contains such a ceramide as the building block.

3. Use according to claim 1 or 2, characterized in that the ceramide and/or the substance with the ceramide component is isolated from biological cell material, particularly from molecular biologically modified cell material.

4. Use according to claim 1 or 2, characterized in that the ceramide and/or the substance with the ceramide building block is synthetically produced.

5. Use of at least one biological precursor of a ceramide or a substance containing a ceramide as the building block for the preparation of a pharmaceutical composition for the treatment of cystic fibrosis.

6. Use of at least one activator, in particular at least one biological activator, which has an activating action on a ceramide, on a substance containing a ceramide as the building block, or on a biological precursor of said ceramide or said substance, for the preparation of a pharmaceutical composition for the treatment of cystic fibrosis.

7. Pharmaceutical composition comprising an effective quantity of at least one ceramide and preferably a pharmaceutical carrier.

8. Pharmaceutical composition according to claim 7, characterized in that the ceramide is a C2 or a C6 ceramide.

9. Pharmaceutical composition according to claim 7 or 8, characterized in that the ceramide is isolated from biological cell material, particularly from molecular biologically modified cell material.

10. Pharmaceutical composition according to claim 7 or 8, characterized in that the ceramide is synthetically produced.

11. Pharmaceutical composition comprising an effective quantity of at least one biological precursor of a ceramide or a substance containing a ceramide as the building block and preferably at least one pharmaceutical carrier.

12. Pharmaceutical composition comprising an effective quantity of at least one activator, preferably at least one biological activator having an
activating action on a ceramide, on a substance containing a ceramide as the building block or on a biological precursor of said ceramide or said substance and preferably at least one pharmaceutical carrier.

13. Use of at least one ceramide and/or at least one substance containing a ceramide as the building block for the preparation of a pharmaceutical composition for the treatment of diseases linked with a disturbed regulation of transport processes at membranes.

14. Use of at least one biological precursor of a ceramide or a substance containing a ceramide as the building block for the preparation of a pharmaceutical composition for the treatment of diseases linked with a disturbed regulation of transport processes at membranes.

15. Use of at least one activator, particularly at least one biological activator having an activating action on a ceramide, on a substance containing a ceramide as the building block, or on a biological precursor of said ceramide or said substance for the preparation of a pharmaceutical composition for the treatment of diseases linked with a disturbed regulation of transport processes at membranes.

16. Use according to one of the claims 13 to 15, characterized in that the transport processes relate to ion channels, particularly outwardly rectifying chloride channels.

17. Use according to one of the claims 13 to 16, characterized in that the ceramide is a ceramide having a feature according to one of the claims 2 to 4.
FIG. 2

CF-T-Lymphocyte
C2 Ceramide

after ceramide application
before ceramide application