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(54) **Title**: EAR DROP COMPOSITIONS AND PROCESS FOR PRODUCING THE EAR DROP COMPOSITIONS

(57) **Abstract**: The subject of the invention is ear drop compositions, which contain adenosine or a mixture of adenosine and lecithin - as active agent. The invention is relating to the process for producing ear drop compositions applying adenosine or a mixture of adenosine and lecithin, which are applicable for treatment of inflammation of the middle ear.

Ear drop compositions and process for producing the ear drop compositions

The invention relates to an ear drop compositions and a process for producing the ear drop compositions primarily for treating inflammation of the middle ear.

Hundreds of thousands of people, primarily in childhood, get sick each year with serous inflammation of the middle ear (*otitis media*) resistant to antibiotics in the whole world.

In the first decade of their life 93% of children get over at least once while 74% of them get over inflammation of the middle ear three or more times.

The treatment of inflammations of the middle ear requires also surgical intervention in numerous cases.

In the greater group of cases of inflammation of the middle ear an inflammatory process runs its course in the hollow system of the middle ear while the eardrum is closed, and the process can be acute or chronic.

There is a close relationship between the acute, mostly purulent inflammation of middle ear and the chronic serous one.

It is a widely accepted opinion, that they do not constitute two separate disease entities, but they represent two states of one disease and the chronic serous inflammation of the middle ear is a not healed state of an acute inflammation, which ran its course previously.

The early recognition of the serous inflammation and the treatment of the inflammation of the middle ear are very important from the viewpoint to avoid the later complications, particularly the later hardness of hearing or deafness.

The characteristics of the serous inflammation of the middle ear are the abundant thick discharge, which is frequently pus, and the perforation of the eardrum in a great number of cases.

The later hardness of hearing and deafness can be traced back to a serous inflammation of the middle ear in the childhood.

The patent description HUP 198125 describes a compositions containing an amino acid including a sulfhydryl group, advantageously acetyl-cysteine, an

antibiotic of amino-glycoside type and steroid inflammation inhibitor compounds for the treatment of the inflammation of the middle ear.

According to the patent description HUP 196558 cold squeezed juice of elderflower, houseleek, melilot, dill and leaves of mullein is mixed with a mixture of oily yarrow extract, bee-glue extract, juices of elderflower, mallow and houseleek.

The oily compositions gained in this way is used as ear-drop.

We set the aim to develop a compositions of ear-drop, which is applicable particularly for the treatment of serous inflammation of the middle ear of childhood, resistant to antibiotic during several weeks or months and the development of serious complication can be prevented or inhibited using it.

We established by means of our explorations that the concentration of several inflammatory cytoquinines increases in the ear secretion of children suffering inflammation of the middle ear (Rezes Sz. et al.: American Otologycal Society American Neurotology Society and European Academy of Otology and Neurology (2007), volume 28, issue 5, pp. 663-667).

It is known, that the adenosine compounds being present in the organism, among others the adenosine triphosphate (ATP) and the adenosine diphosphate (ADP) fulfill important biochemical role in the energy household of the organism.

Adenosine, being intermediate product of the above mentioned compounds, is a purine-nucleoside, which is present in the organism, occurs as four adenosine receptor subtypes: A1, A2A, A2B and A3.

The molecular mass of adenosine is 267.241 g/mol.

In case of inflammatory processes the concentration of adenosine increases in the cells from the normal value of 300 nM and it can reach even 600 to 1200 nM.

The increased adenosine concentration in the organism cannot, however, combat or heal the serous inflammation of the middle ear in most cases.

The adenosine derivatives, as known inflammation inhibiting active agents, are used for treatment of wounds on the feet occurring in case of diabetes (USP 6020321).

The published European patent application EP 0297630 recommends one or more nucleosides among others adenosine or inosine for the treatment of myasthenia.

Adenosine is widely used in form of intravenous injection or in infusion in case of supraventricular tachycardia.

The injection in solution under brand name Adenocor is a known compositions, which is administered intravenously or through a venous cannula for quick restoration of sinal rhythm of the heart.

The compositions in form injection containing adenosine have, however, a number of side effects this is the reason why only certain adenosine derivatives are recently used in cardiologic treatments.

It is another disadvantage, that the adenosine containing compositions have extraordinarily short half-period i. e. they decompose quickly.

The published international patent application WO 2007/112986 describes the usage of a mixture of adenosine and inosine as cardiologic compositions for infusion.

While we experimented seeking a compositions adequate for the treatment of inflammation of the middle ear, we found an astonishing result that a significant inflammation inhibiting effect can be reached using an adenosine containing solution introduced into the middle ear through a tube provided with a ventilator even in such cases where antibiotics proved to be useless.

There is no compositions applicable for treating the diseases of inflammation of the ear described in the specialized literature, which contains adenosine.

While experimenting, we reached an unexpected result that the phagocyte cell activation inhibiting, essentially inflammation inhibiting effect of adenosine can be multiplied in such a way that vegetable lecithin, advantageously soy lecithin is applied additionally to the adenosine.

This unexpected effect may be probably due to the fact that lecithin and adenosine are linked together by secondary bonds.

The adenosine is released in long acting way from the great molecule bound together by secondary bonds and so it can exert a longer inflammation inhibiting effect.

The invention relates to compositions of ear drop containing adenosine or a mixture of adenosine and vegetable lecithin - as active agent and known, therapeutically acceptable ingredients.

The compositions of ear drop according to the invention contain advantageously 0.2 to 4.0 mass parts of adenosine, or 0.2 to 4.0 mass parts of adenosine and 0.01 to 1.0 mass parts of vegetable lecithin, particularly soy lecithin, in given case 0.001 to 1.0 mass parts of a therapeutically acceptable ingredient and purified water and/or physiological salt solution in the quantity necessary to the 100 mass parts.

The compositions contain advantageously a stabilizer for example benzoate salts, methyl-p-hydroxy-benzoate, propyl-p-hydroxy-benzoate and other ingredient for example propylene-glycol.

The invention relates as well to the process for producing ear drop compositions applying adenosine or a mixture of adenosine and lecithin which are applicable for treatment of inflammation of the middle ear.

According to the process the inventive compositions are produced in such a way that 0.2 to 4.0 mass parts of adenosine is mixed with purified water or physiological salt solution, in given case 0.01 to 1.0 mass parts of vegetable lecithin is added to the mixture which previously was solved in water, then in given case 0.001 to 1.0 mass parts of a therapeutically applicable ingredient, advantageously a stabilizer is added to it, then purified water and/or physiological salt solution of quantity necessary to the 100 mass parts is admixed, then the mixture is homogenized then packaged.

The compositions and the process are presented by means of the following examples.

Example 1

1.34 mass parts ($0.5x10^{-2}$ moles) of adenosine is mixed with physiological sodium chloride solution.

The mixture is homogenized and packaged.

Example 2

0.1 mass parts of soy lecithin is admixed to 1.34 mass parts ($0.5x10^{-2}$ moles) of adenosine in form of aqueous solution. Physiological NaCl solution is added to the mixture in the quantity necessary to the 100 mass parts.

The mixture is homogenized and packaged.

Example 3

One proceeds in all according to the Example 2 but the quantity of adenosine is 2.67 mass parts ($1x10^{-2}$ moles) and the quantity of soy lecithin is 0.02 mass parts.

Example 4

One proceeds in all as in the Example 2 but in addition 0.001 mass parts of methyl-p-hydroxy-benzoate is added to the mixture.

Example 5

Pharmacological example

Three types of ear drop containing adenosine as active agent were examined and compared applying individually and in combination with varying concentration (0.5 to $5x10^{-2}$ moles) of adenosine and identical concentration (1 mg/ml) of soy lecithin found optimal in view of pharmaceutical technology *in vitro*. The inhibiting effects were measured in suspensions of human neutrophylic granulocytes (inflammatory phagocytes) activated with forbol myristate acetate (FMA), as well as in non-activated ones (10^6 cells/ml) in Hank's type culture solution in presence of 10^{-7} moles of luminol for chemical luminescence originating from free radicals containing oxygen produced in the cells, using Berthold-type luminometer.

The unit of measure of the chemical luminescence is " KLE", the numbers in the table indicate the KLE values.

The result of the examinations are the following:

1. the quantity of adenosine is 0,5x 10^{-2} mole (Ado1)
2. the quantity of adenosine is 1,0x 10^{-2} mole (Ado2)
3. the quantity of adenosine is 5,0x 10^{-2} mole (Ado3)
4. soy lecithin solved in water (1mg/ml) (solution 4)
5. 0,5x 10^{-2} mole of adenosine + soy-lecithin (Ado5)
6. 1,0x 10^{-2} mole of adenosine + soy-lecithin (Ado6)
7. 5,0x 10^{-2} mole of adenosine + soy-lecithin (Ado7)

These solutions exerted their effects in the reaction mixture being diluted in proportion of 1:6.

The results of the examination are as follows.

Table 1

The influence of the solution of 0.5 x 10^{-2} mole of adenosine according to Example 2 (Ado1) and 1 mg/ml of soy lecithin (solution 4) to the chemical luminescence (KLU) of human neutrophylic granulocytes induced with FMA during its individual application and in combination (Ado5).

Cells	Cells + Ado1	Cells + FMA	Cells + Ado1 + FMA
589	382	1919	1646
Cells + lecithin	Cells + lecithin + Ado5	Cells + lecithin + FMA	Cells + Ado5 + FMA
876	672	935	656

Table 2

The influence of the solution of 1 x 10^{-2} mole of adenosine according to the Example 2 (Ado1) and 1 mg/ml of soy lecithin (solution 4) to the chemical luminescence (KLU) of human neutrophylic granulocytes induced with FMA during its individual application and in combination (Ado6).

Cells	Cells + Ado1	Cells + FMA	Cells + Ado1 + FMA
589	131	1919	1374
Cells + lecithin	Cells + lecithin + Ado6	Cells + lecithin + FMA	Cells + Ado6 + FMA
876	415	935	163

Table 3

The influence of the solution of 5 x 10^{-2} moles of adenosine according to the Example 2 (Ado3) and 1 mg/ml of soy lecithin to the chemical luminescence (KLU) of human neutrophylic granulocytes induced with FMA during its individual application and in combination (Ado7).

Cells	Cells + Ado3	Cells + FMA	Cells + Ado3 + FMA
589	105	1919	976
Cells + lecithin	Cells + lecithin + Ado7	Cells + lecithin + FMA	Cells + Ado7 + FMA
876	356	935	284

<u>The conclusions, which can be derived from the examinations:</u>

1. The adenosine hindered chemical luminescence of non-stimulated human neutrophylic cells, as well as those stimulated with FMA in increasing degree depending on its concentration.

2. The soy lecithin produces a small increase of chemical luminescence in the inert cells and it results in hindering of luminescence in the cells stimulated with FMA.

A possible explanation of this phenomenon is that the lecithin emulsion enters the cells and produces a slight activation of cells.

The activating effect of FMA becomes effective in smaller extent at the same time in the activated cells.

3. The lecithin of 1mg/ml applied in the same solutions in addition to the adenosine in increasing concentrations unexpectedly increased "made potentiation" the chemical luminescence inhibiting influence of adenosine mainly in the cells activated with FMA and at the concentration of 1.0×10^{-2} moles of adenosine in the sample Ado7.

This phenomenon can be explained in such a way that the optimal physic-chemical connection between the molecules of adenosine and lecithin can come into being at this concentration of adenosine and in the course of that adenosine-lecithin bound by secondary bonds enters the cells.

In the cytoplasm the adenosine gets free from the lecithin coating and it can create its immediate hindering effect to the cell activation processes.

Claims

1. Compositions of ear drop characterized by that it contains adenosine – or a mixture of adenosine and lecithin as active agent.

2. The compositions of ear drop according to the Claim 1 characterized by that it contains 0.2 to 4.0 mass parts of adenosine or 0.2 to 4 mass parts of adenosine and 0.01 to 1.0 mass parts of vegetable lecithin, advantageously 0.001 to 1 mass parts of a therapeutically applicable ingredient, and purified water and/or physiological salt solution in a quantity necessary to 100 mass parts.

3. The compositions of ear drop according to any of the Claims 1 or 2 characterized by that it contains soy lecithin as vegetable lecithin.

4. The compositions of ear drop according to any of the Claims 1 to 3 characterized by that the ingredient advantageously a stabilizer for example benzoate salts, methyl-p-hydroxy-benzoate or propyl-p-hydroxy-benzoate.

5. The compositions of ear drop according to any of the Claims 1 to 3 characterized by that it contains propylene glycol as ingredient.

6. Process for producing ear drop compositions applying adenosine or a mixture of adenosine and lecithin, which are applicable for treatment of inflammation of the middle ear characterized by that 0.2 to 4.0 mass parts of adenosine is mixed with purified water or physiological salt solution, in given case 0.01 to 1.0 mass parts of vegetable lecithin is added to the mixture, which previously was solved in water, then in given case a therapeutically applicable ingredient, advantageously a stabilizer is admixed to the mixture in a mass proportion of 0.001 to 1.0 mass parts, then purified water and/or physiological salt solution is added to it in a quantity necessary to 100 mass parts, then the mixture is homogenized and packaged.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/HU2012/000053

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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