THE PHARMACOLOGICAL USE OF CERTAIN CYSTINE DERIVATIVES

Field of the Invention

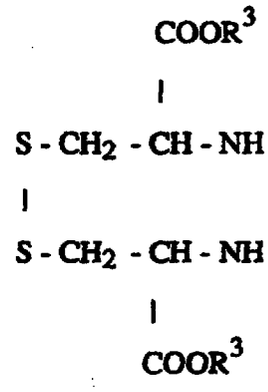
The present invention relates to a new medical use of a certain cystine derivatives.

In particular the invention relates to the use of such cystine derivatives for the preparation of medicaments with effect against restenosis.

Background of the Invention

Such cystine derivatives as to which the present invention has found a new pharmacological use are known from WO 91/185 94 and EP 463 514 to have immunomodulating activity. Nothing is reported or generally known concerning the pharmacological and/or therapeutic properties of these compounds with respect to effects or restenosis.

In connection with the present invention compounds of the general formula I are employed:



(I)

wherein R is hydrogen, methyl, ethyl, n-propyl or a moiety $-COR^1$, wherein R^1 is a straight or branched akyl group having 1 - 12 carbon atoms, R^2 is a straight or branched alkyl group having 1 - 12 carbon atoms and R^3 is a moiety which provides an ester hydrolysable in body fluids to release the active compound in free acid form, or a physiologically acceptable salt and/or a stereochemical isomer thereof.

In particular compounds of the formula I wherein R is hydrogen or a moiety -CO-R^1 wherein R^1 is methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, isopropyl, 1-methylpropyl, 2-methylpropyl, tert. butyl, 3-methylbutyl or 2-metylbutyl, R^2 is methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, isopropyl, 1-methylpropyl, 2-methylpropyl, tert. butyl, 3-methylbutyl or 2-methylbutyl and R^3 is hydrogen, methyl, ethyl, propyl, isopropyl, butyl or isobutyl, or a physiologically acceptable salt and/or a stereochemical isomer thereof, are preferred.

The compounds of formula I are used in racemic form as well as stereoisomers (enantiomers, diastereomers). Of particular interest are the compounds having the L configuration, particularly interesting is N,N'-diacetyl-L-cystine.

The invention also involves the compound of formula I in the form of its physiologically acceptable salts, such as the salts of sodium, potassium, ammonium, calcium or magnesium. Also included are salts of the unesterified compounds with pharmaceutically acceptable organic bases, such as lysine, ethylenediamine, N,N'-dibenzylethylenediamine, adamantanamine, N-benzyl-2-phenylethylamine and piperazine.

Lysine and arginine can be used in its D- or L- forms. Most preferred is the L-form.

The most preferred compound is di-L-lysinium-N,N'-diacetyl-L-cystinate (compound A).

The compounds of the general formula I may be prepared by any of the processes disclosed in EP 463 514. Salts of such compounds with organic bases are prepared according to any conventional method or any method disclosed in WO 93/11104.

Prior art

Rapid development of the tunica intima occurs following mechanical injury to arteries. The most common such complication is the so called restenosis that occurs after percutaneous transluminal coronary angioplasty (PTCA), i.e. the removal of arteriosclerotic lesions causing stenosis in coronary vessels wih a balloon catheter, but similar complications can occur also as a result of other procedures and interventions.

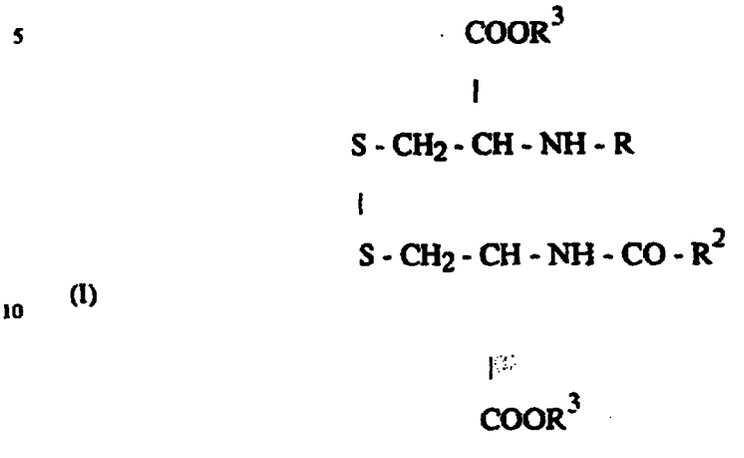
After PTCA, restenosis develops in all patients. In approximately 1/3 of the patients, this consequence is so severe that within 3 to 6 months the developed restenosis requires renewed PTCA or coronary by-pass surgery. The need for chemotherapy that can substantially reduce the incidence of severe restenosis is thus obvious.

Acute thrombosis is a distinct risk during angioplastic manoeuvres, and heparin is therefore given during the surgical treatment. For the same reason aspirin is usually given for a period of time after the treatment. However,this medication does not reduce the development of restenosis in the months following the PTCA. Thus, there is today no generally accepted treatment available which reduces sufficiently the incidence of restenosis. However, recently published clinical trials have shown that the antiplatelet agent trapedil (Maresta et al, Circulation 1994;90:2710-15) and the hypolipidemic (and antioxidant) probucol (Setsuda et al, Clin Ther 1993;15:174-82) can at least partly prevent the development of restenosis.

The current possibilities to prevent restenosis are still inadequate, and can also be associated with unwanted side effects, such as increased bleeding risk following aspirin and trapedil treatment.

Disclosure of the Invention

It has unexpectedly been found that compounds of the general formula



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wherein R is hydrogen, methyl, ethyl, n-propyl or a moiety $-COR^1$, wherein R^1 is straight or branched alkyl group having 1 - 12 carbon atoms, R^2 is a straight or branched alkyl group having 1 - 12 carbon atoms and R^3 is a moiety which provides an ester hydrolysable in body fluids to release the active compound in free acid form, or a physiologically acceptable salt and/or a stereochemical isomer thereof are potent in an experimental animal model for assessing restenosis.

In particular the compounds of formula I wherein R is hydrogen or a moiety $-CO-R^1$ wherein R^1 is methyl, ethyl, *n*-propyl, n-butyl, *n*-pentyl, *n*-hexyl, *n*-heptyl, *n*-octyl, *n*-nonyl, n-decyl, n-undecyl, isopropyl, 1-methylpropyl, 2-methylpropyl, tert. butyl, 3-methylbutyl or 2-methylbutyl, R^2 is methyl, ethyl, *n*-propyl, n-butyl, *n*-petnyl, *n*-hexyl, *n*-heptyl, n-octyl, *n*-nonyl, n-decyl, n-undecyl, isopropyl, 1-methylpropyl, 2-methylpropyl, tert. butyl, 3-methylbutyl or 2-methylbutyl and R^3 is hydrogen, methyl, ethyl, propyl, isopropyl, butyl or isobutyl, or a physiologically acceptable salt and/or a stereochemical isomer thereof are of interest.

Therefore, the compounds of the general formula I, their stereoisomers (enantiomers, diastereomers) and salts thereof may be used for the prophylaxis or treatment of restenosis. Accordingly, the invention includes the use of these compounds for the preparation of a medicament for prophylaxis or treatment of restenosis. The invention also includes a method for the prophylaxis or treatment of restenosis using compounds of formula I.

Effects of compounds in experimental models of restenosis.

The most commonly used animal model of restenosis is rat carotid arteries or aortas that have been dilated with balloon catheters. For this purpose, rats were anaesthetized, and the carotid bifurcation was exposed surgically. A balloon catheter (a 2F Fogarty catheter) was introduced from the bifurcation to the aorta, inflated and withdrawn. This procedure leads to the development of a "neointima" with a microscopic appearance similar to that of restenosis in humans.

The active compounds were administered as a solution in the drinking water in doses corresponding to 0.003-30 μmoles/kg/day for 2-4 weeks.
The extent of the cross-section area of neointima and the media was evaluated microscopically 2 to 4 weeks after denudation. At termination of the studies, the rats were therefore perfusion-fixed and microscopic specimens from the carotid arteries were produced, and the cross-section areas were obtained through morphometric procedures using point-counting and/or cutting out and weighing photos of the neointima and media.

Initial studies were performed in Sprague-Dawley rats. Neointima formation was dependent on a sufficient response in the media layer, and proportional to the media area when this exceeded a certain threshold value. The results are therefore expressed as the neointima-to-media ratio. Doses from 0.03 to 3.0 μmoles/kg/day could reduce the neointima/media ratio by more than 20 %.

Pharmaceutical formulations

The described active substances for human use can be prepared in different dosage forms e.g. tablets, coated tablets, gelatin capsules, oral solutions, solutions for injection, and aerosols and dry powder for inhalation.

For the preparation of tablets, coated tablets and gelatin capsules the active substances can be combined with pharmaceutic ally acceptable carriers or diluents, e.g. lactose, starch, dicalcium phosphate, microcrystalline cellulose, polyvinylpyrrolidone, gelatin, cellulose derivatives, colloidal silicone dioxide, talc and stearic acid or its salts.

For the preparation of oral solutions suitable excipients are water or solutions of saccharose, glucose, sorbitol, fructose or zylitol.

Solutions for parenteral administration may be prepared as a solution of a compound of the invention in a pharmaceutically acceptable solvent, preferably in a concentration from 0.1% to 10% by weight. These solutions may also contain stabilizing ingredients and/or buffering ingredients and are conveniently dispensed into unit doses in the form of ampoules or vials. Solutions for parenteral administration may also be prepared as a dry preparation to be reconstituted with a suitable solvent extemporaneously before use.

The substance can be inhaled from a pressurized metered dose inhaler, from a dry powder inhaler, e.g. a Turbuhaler® or from a dry powder inhaler utilizing gelatine, plastic or other capsules. Non-toxic and chemically inert substances e.g. lactose, trehalose, mannitol or glucose can be added to the powdered substance for inhalation therapy.

Pressurized aerosols are intended for oral or nasal inhalation. The aerosol system is generally designed in such a way that each delivered dose contains 10-1000 µg, preferably 20-250 µg of the active compound. The most active compounds are administered in the lower part of the dose range. For inhalation therapy micronized compound are used and

there consists of particles substantially smaller than 5 μm, which are suspended in a propellent mixture with the assistance of a dispersant, such as sorbitan trioleate, oleic acid, lecithin or sodium salt of dioctylsulphosuccinic acid.

The dosage forms can contain in addition to mentioned excipients preservatives, stabilizers, viscosity regulating agents, emulsifiers, sweetening agents, colouring agents, flavouring agents, tonicity regulating agents, buffers or antioxidants. They can also contain other therapeutically valuable substances.

The compounds of the formula I will normally be administered orally, rectally, by injection or inhalation, in the form of pharmaceutical preparations comprising the active ingredient either as the compound per se or as a pharmaceutically acceptable non-toxic, acid addition salt. The dosage form may be a solid, semisolid or liquid preparation. Usually the active substance will constitute between 0.1 and 99 % by weight of the preparation, more usually between 0.5 and 20 % by weight for preparations for injection and between 0.2 and 50 % by weight for preparations for oral administration.

Effective amounts of the compounds of the formula I for use in the prophylaxis and treatment of restenosis are in the range 0.5 - 500 mg, preferably 1 - 100 mg for a daily dose.

The following examples illustrate medicaments for prophylaxis or treatment of restenosis.

Example 1

Tablet containing 10 mg of compound A per tablet:

Compound A	10 mg
Lactose	100 mg
Potato starch	50 mg
Polyvinylpyrrolidone	5 mg
Microcrystalline cellulose	15 mg
Magnesium sterate	1 mg

Example 2

Direct compression tablet containing 5 mg of compound A per tablet:

Compound A	5 mg
Lactose, anhydrous	150 mg
Microcrystalline cellulose	50 mg
Colloidal silicon dioxide	1 mg
Magnesium sterate	2 mg

If desired, the obtained tablets can be film coated with e.g. hydroxypropyl methylcellulose, hydroxypropyl cellulose or dimethylaminoethyl methacrylate methacrylic acid ester copolymer.

Example 3

Solution for injection containing compound A 1 mg/ml

Compound A	1.0 mg
Sodium chloride	8.8 mg
Water for injection to 1 ml	

Example 4

Oral solution containing Compound A 1 mg/ml

Compound A		1.0 mg
Sorbitol		150 mg
Glycering		100 mg
Disodium edetate		0.5 mg
Preservative		q.s.
Flavour		q.s.
Water, purified	to	1 ml

Example 5

Powder aerosol giving 1 mg compound A per dose

The micronized compound A can be filled into a powder inhaler device e.g. Turbuhaler® giving 1 mg/dose.

Example 6. Presurised aerosol for inhalation

The aerosol system is arranged so that each metered dose contains 0.1-1.0 mg.

Compound A, micronized	1.0 % w/w
Sorbitan trioleate	0.7 % w/w
Trichloromonofluoromethane	24.4 % w/w
Dichlorotetrafluoroethane	24.4 % w/w
Dichlorodifluoromethane	49.5 % w/w

Example 7. Powder aerosol for inhalation of pure substance

Pure substance prepared for inhalation from Turbuhaler.

Each single dose contains 0.1 - 1.0 mg.

Compound A, processed	0.1 - 1.0 mg

Example 8. Powder aerosol for inhalation

Each single dose contains 0.1 - 1.0 mg in a capsule.

Compound A, micronized	0.1 - 1.0 mg
Lactose	50 mg

Example 9. Solution for nebulising

The solution contains 1.0 - 10.0 mg/mL and 1 - 3 mL may be administered in a single dose.

Compound A	1.0 - 10.0 mg
Water for injection	to 1.0 mL

Example 10. Tablets

Each tablet contains:

Compound A	0.1 - 100 mg
Maize starch	50 mg
Lactose	150 mg
Polyvidone	7 mg
Microcrystalline cellulose	20 mg
Magnesium stearate	2 mg

Example 11. Oral solution

A single dose of 10 mL contains 10 - 100 mg.

Compound A	1 - 10 mg
Sorbitol 70 %	150 mg
Glycerol	100 mg
Sodium benzoate	1 mg
Flavour	q.s.
Water purified to	1.0 mL

Example 12. Tablet for controlled release

1 tablet:

Compound A	1 - 100 mg
Paraffin Special	145 mg
Lactose Powder	50 mg
Colloidal Silicon Dioxide	5 mg
Ethylcellulose 10 cps	13 mg
Ethanol 99,5 vol%	85 mg
Magnesium Stearate	2.5 mg

Example 13. Granulate for controlled release

1 g of granulate:

Compound A		1 - 100 mg
Ethylcellulose Dispersion		10 mg
Acetyltributylcitrate		0.5 mg
Eudragit L 100-55		55 mg
Triethylcitrate		5 mg
Talc		30 mg
Water newly distilled		350 mg
Pellets, neutral	to	1000 mg

Example 14. Solution for injection

1 mL in a single dose contains 1.0 - 10.0 mg

Compound A			1.0 - 10.0 mg
Sodium chloride			8.9 - 7.7 mg
Water for injection		to	1.0 mL

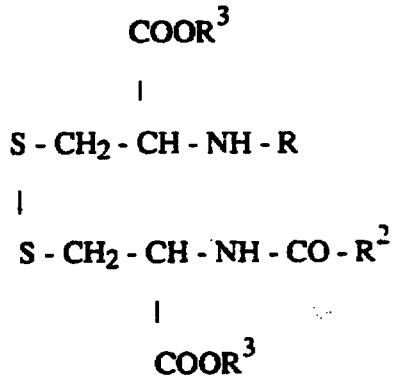
Example 15. Cream for topical application

1 g of cream contains:

Compound A			0.1 - 1 mg
White soft paraffin			75 mg
Liquid paraffin			10 mg
Cetostearyl alcohol			75 mg
Cetomacrogol 1000			20 mg
Metagin			0.8 mg
Propagin			0.2 mg
Water, purified		to	1.0 g

CLAIMS

1. The use of a compound of the general formula



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2. The use of a compound according to claim 1 where in formula I R is hydrogen or a moiety - CO-R^1 wherein R^1 is methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, isopropyl, l-methylpropyl, 2-methylpropyl, tert. butyl, 3-methylbutyl or 2-methylbutyl, R^2 is methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, iso-propyl, l-methylpropyl, 2-methylpropyl, tert. butyl, 3-methylbutyl or 2-methylbutyl and R^3 is hydrogen, methyl, ethyl, propyl, isopropyl, butyl or 2-methylpropyl, or a physiologically acceptable salt and/or a stereochemical isomer thereof for the preparation of a medicament for the prophylaxis and treatment of restenosis.



3. The use according to claim 1 or 2 of the L isomer of N,N'-diacetylcystine or a physiologically acceptable salt thereof for the preparation of a medicament for the prophylaxis or treatment of restenosis.

4. The use according to claim 3 of di-L-lysinium-N,N'-diacetyl-L-cystinate.

5. A pharmaceutical preparation for use in the treatment of restenosis wherein the active ingredient is a compound as defined in claim 1.

6. A pharmaceutical preparation according to claim 5 in dosage unit form.

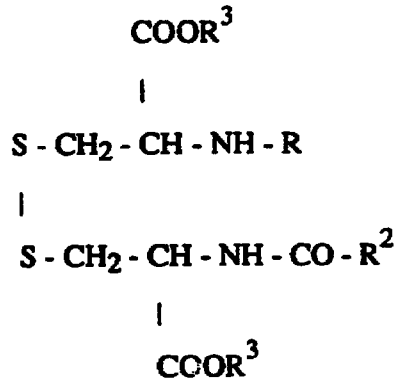
7. A pharmaceutical preparation according to claim 5 or claim 6 comprising the active ingredient in association with a pharmaceutically acceptable carrier.

8. A pharmaceutical preparation according to any of claims 5 to 7 comprising as active ingredient N,N'-diacetyl-L-cystine.

9. A pharmaceutical preparation according to any of claims 5 to 8 comprising as active ingredient di-L-lysinium-N,N'-diacetyl-L-cystinate.

10. A method for the prophylaxis or treatment of restenosis in mammals including man, characterized by the administration to a host in need of such prophylaxis or treatment of an effective amount of a compound of the formula I





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11. A method according to claim 10 where in formula I R is hydrogen or a moiety -CO-R^1 wherein R^1 is methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, isopropyl, l-methylpropyl, 2-methylpropyl, tert. butyl, 3-methylbutyl or 2-methylbutyl, R^2 is methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, isopropyl, l-methylpropyl, 2-methylpropyl, tert. butyl, 3-methylbutyl or 2-methylbutyl and R^3 is hydrogen, methyl, ethyl, propyl, iso-propyl, butyl or 2-methylpropyl, or a physiologically acceptable salt and/or a stereochemical isomer thereof.

12. A method according to any of claim 10 or 11 wherein the L isomer of N,N'-diacetylcystine or a physiologically acceptable salt thereof is administered.

13. A method according to claim 12 wherein di-L-lysinium-N,N'-diacetyl-L-cystinate is administered.