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METHOD OF LOCALIZING AND QUANTIFYING REGIONAL ENERGY METABOLISM IN A WARM-BLOODED LIVING BEING AND COMPOSITION THEREFOR

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(57) Claim

1. A method of localizing and quantifying regional energy metabolism, in particular regional ischemia, in a warm-blooded living being, comprising administering to said being a diagnostically effective quantity of a radiolabelled L-homocysteine or a radiolabelled functional derivative thereof.

3. A pharmaceutical composition when used in the method of claim 1, comprising in addition to a pharmaceutically acceptable carrier and, if desired, at least one pharmaceutical acceptable adjuvant, as the active substance a radiolabelled L-homocysteine or a radiolabelled functional derivative thereof, in a diagnostically effective quantity.

4. A composition as claimed in claim 3, wherein the active substance is L-homocysteine or its functional derivative, labelled with a radioisotope selected from the group consisting of carbon-11, selenium-73, fluor-18, bromine-76, bromine-77 and iodine-123.

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Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD OF LOCALIZING AND QUANTIFYING REGIONAL ENERGY METABOLISM IN A WARM-BLOODED LIVING BEING AND COMPOSITION THEREFOR

(57) Abstract

The invention relates to a method of localizing and quantifying regional energy metabolism, in particular regional ischemia, in a warm-blooded living being, comprising administering to said being a diagnostically effective quantity of a radiolabelled L-homocysteine or a radiolabelled functional derivative thereof. The invention further relates to a pharmaceutical composition to be used for performing the above method.

Method of localizing and quantifying regional energy metabolism in a warm-blooded living being and composition therefor.

The invention relates to a method of localizing and quantifying regional energy metabolism, in particular regional ischemia, in a warm-blooded living being. The invention further relates to a pharmaceutical composition to be used for said method.

The diagnosis and localisation of ischemia in various organs in present clinical practice relies mainly on angiographic methods and the study of tissue perfusion using thallium scintigraphy. While angiography permits the localisation of diseased arteries, it does not allow to draw conclusions as to the functional consequence and extent of the ischemic process. Furthermore, it is often difficult to relate changes in tissue perfusion to abnormalities observed with angiographic techniques. In order to gain information into the dynamics of tissue metabolism during ischemia use was made of various positron emitting tracer substances. Previous studies have largely relied upon only a few tracers, for example N-13 ammonia for blood flow, C-11 palmitate for fatty acid metabolism and F-18 2-fluoro-2-desoxyglucose (FDG) for glucose utilisation. None of these tracers, however, permitted a direct insight into the dynamics of the energy metabolism, mainly because the metabolism of substances applied differs greatly from organ to organ and is dependent on the dietary state of the organism.

Thallium scintigraphy is only a measure of tissue perfusion but not suitable for assessing the dynamics of energy metabolism. Therefore this method has a serious restriction in evaluating and quantifying ischemia. Radiolabelled fatty acids have the drawback, that their kinetic behaviour is seriously influenced by other

substrates which play a role in the metabolism of the organs to be investigated.

It is the object of the invention to enable the localization and quantification of regional energy metabolism, in particular of regional ischemia, without invasive methods and avoiding the above disadvantages. It has been found that this object can be achieved according to the present invention by administering to a warm-blooded living being a diagnostically effective quantity of a radiolabelled L-homocysteine or a radiolabelled functional derivative thereof.

The quantity of radioactive material effective for diagnosing depends on various factors such as the diagnostic method, e.g. planar scintigraphy or emission tomography, the radiolabel used and the organ to be examined. In case the method of the invention is used to localize and quantify regional ischemia, such ischemia does not only encompass cardiac ischemia but also includes ischemia of other organs like the brains and the gastro-intestinal tract. It will be obvious from the above, that the quantity of radioactive material which is effective for diagnosing purposes may vary within broad ranges. Generally the radioactive material is administered to the living being in a quantity of 1 to 1000 MBq per 70 kg of body weight. The radiolabel may be chosen from radionuclides selected from the group consisting of positron emitting nuclides and gamma radiation emitting nuclides. Functional derivatives of L-homocysteine are to be understood to include derivatives of L-homocysteine wherein the S-H function is intact, or, in case the radiolabel is selenium-73, wherein the ^{73}Se-H function is intact.

A preferred radiolabelled compound to be used for the

method of the invention is L-homocysteine or its functional derivative, labelled with a radioisotope selected from the group consisting of carbon-11, selenium-73, fluor-18, bromine-76, bromine-77 and iodine-123. Typical positron emitting nuclides like carbon-11, selenium-73 and fluor-18 enable the in vivo application of the labelled compounds by using the so-called "positron-emission tomography" (PET) technique. By using this technique a computer tomogram can be obtained of the organ to be investigated, e.g. the heart or the brains, enabling the localization and quantification of regional energy metabolism. In the PET technique very short living radioisotopes are used which emit positrons, for example carbon-11 and fluor-18 with half-lives of 20 and 110 minutes respectively. Gamma radiation emitting isotopes like bromine-76, bromine-77 and iodine-123 can be used for the labelling of compounds to be detected by conventional scanning techniques or in the so-called "single photon emission computer tomography" (SPECT) technique. By using conventional scanning techniques the emitted gamma radiation can be detected by suitable apparatuses, e.g. a gamma camera, to produce images of the organ to be investigated. The more advanced SPECT technique is also based upon the detection of gamma radiation by sensible detectors.

The invention further relates to a pharmaceutical composition to be used for the method defined before, comprising in addition to a pharmaceutically acceptable carrier and, if desired, at least one pharmaceutical acceptable adjuvant, as the active substance a radio-labelled L-homocysteine or a radiolabelled functional derivative thereof, in a diagnostically effective quantity. If desired, said composition may be brought into a form

more suitable for intravenous or subcutaneous administration, for example by the addition of a pharmaceutically acceptable liquid vehicle, preferably a physiological saline solution. It will be evident, that the composition should be sterile for intravenous or subcutaneous administration. If desired, one or more adjuvants may be present in the composition, for example suitable stabilizers like ascorbic acid, gentisic acid or salts of these acids, and/or fillers like glucose, lactose, mannitol etc. Dependent on the investigation to be performed and the results desired by performing these experiments, the composition may be administered to the living being, preferably a human being, at once, as a so-called bolus injection, or gradually by a continuous infusion.

The radiolabelled compounds can be prepared by methods which are known per se for related compounds by using readily available radiolabelled synthons like [C-11]CO_2, [C-11]CH_3I, [C-11]HCN, [C-11]CO, [F-18]F_2, [F-18]CH_3CO_2F and [I-123]NaI. The use of [C-11]CO_2 for the preparation of 1-[C-11]-homocysteine thiolactone, a precursor for carbon-11 labelled homocysteine, is described in the examples. The position of carbon-11 as the radiolabel in the homocysteine molecule is not relevant and can be chosen according to the ease of synthesizing the [C-11]homocysteine. Selenium-73 can be introduced as a radioactive label into the homocystein molecule by substituting the mercapto group by a ^{73}Se-H group. Radioactive halogen can be substituted for one of the hydrogen atoms at choice in the homocystein molecule. The same holds, mutatis mutandis, for introducing a radioactive label in a functional derivative of homocysteine.

The invention will now be described in greater detail

with reference to the ensuing specific examples.

EXAMPLE I

Preparation of (C11)-homocysteine thiolactone (11)

The preparation is carried out according to the reactionscheme attached. The precursor, viz. S-(tetrahydropyranyl-(2))-3-thiopropyl isonitrile (8) is prepared starting with 3-chloropropylamine (1) via the intermediates 2 and 3. The oily formamide (2) is purified by distillation; intermediate (3) is purified by column chromatography. The alternative synthetic way (1 → 4 → 5) gives reasonably good yields with column chromatography of 5 only without purification of 4. The formamide of 3-mercaptopropylamine (6) is known, e.g. from U.S. patent 3,278,526. This mercapto compound is converted into compound 7 with an equimolar quantity of 3,4-dihydro-2H-pyran in diethylether at room temperature; TSOH as a catalyst. Formamide 7 is dehydrated in the presence of excess diisopropylamine in dimethoxyethane as a solvent by slowly adding $POCl_3$ while cooling. The overall chemical yield of the precursor (8) synthesis is approx. 10%.

The labelling procedure is based on the carboxylation of the corresponding α-lithioisocyanide (9) with [C-11]CO_2 and subsequent hydrolysation and lactonisation. The labelling procedure is carried out in THF as a solvent. An equimolar quantity of n-butyllithium in hexane is added to a solution of the isonitrile precursor (8) in THF. The labelling is performed by flushing this solution while cooling with [C-11]CO_2 in helium, followed by flushing with CO_2. Subsequent deprotection, hydrolysation and lactonisation of 10 with aqueous acetic acid and 6M hydrochloric acid successively yields the desired thiolactone (11). The

thiolactone (11) obtained is purified by reverse phase HPLC with 0.1 M sodium dihydrogenphosphate as an eluent. The radiochemical yield obtained is approx. 15%.

EXAMPLE II

Use of (C-11) homocysteine

Animal experiments were carried out in anaesthetized, thoracotomized dogs. (C-11)Homocysteine thiolactone (370 MBq) was given i.v. at a dose of 20 mg/kg either as bolus or over 3-10 min. The thiolactone administered is converted within the test animals to (C-11) homocysteine, the active species. Thereafter a side branch of the left anterior descending coronary artery was occluded. Imaging was performed with a positron emission tomograph (Scanditronix® PC-4096) and data were collected for 60 min. after the start of homocysteine infusion. Tracer concentration in plasma decreased with a $T_{1/2}$ of 40 min. Tomograms revealed that accumulation of tracer in the heart was strictly confirmed to the ischemic tissue.

The claims defining the invention are as follows:

1. A method of localizing and quantifying regional energy metabolism, in particular regional ischemia, in a warm-blooded living being, comprising administering to said being a diagnostically effective quantity of a radiolabelled L-homocysteine or a radiolabelled functional derivative thereof.

2. A method as claimed in claim 1, wherein L-homocysteine or its functional derivative, labelled with a radioisotope selected from the group consisting of carbon-11, selenium-73, fluor-18, bromine-76, bromine-77 and iodine-123, is administered to the being.

3. A pharmaceutical composition when used in the method of claim 1, comprising in addition to a pharmaceutically acceptable carrier and, if desired, at least one pharmaceutical acceptable adjuvant, as the active substance a radiolabelled L-homocysteine or a radiolabelled functional derivative thereof, in a diagnostically effective quantity.

4. A composition as claimed in claim 3, wherein the active substance is L-homocysteine or its functional derivative, labelled with a radioisotope selected from the group consisting of carbon-11, selenium-73, fluor-18, bromine-76, bromine-77 and iodine-123.

5. A method according to claim 1 or 2 substantially as hereinbefore described.

6. A pharmaceutical composition according to claim 3 or 4 substantially as hereinbefore described.

DATED this 3 December 1991

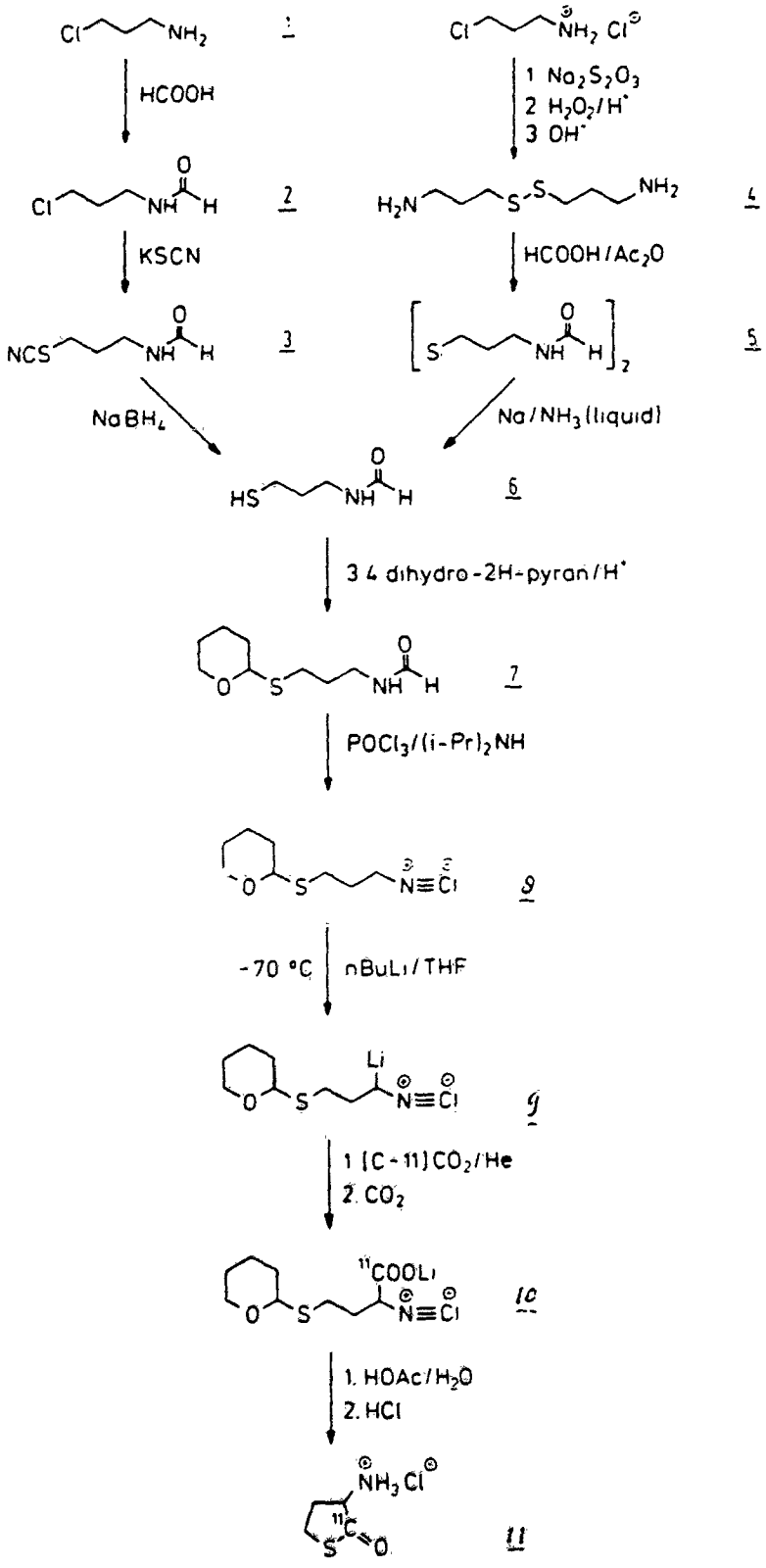
CARTER SMITH & BEADLE

Fellows Institute of Patent Attorneys of Australia

Patent Attorneys for the Applicant:

MALLINCKRODT, INC.

Reaction Scheme



INTERNATIONAL SEARCH REPORT

International Application No PCT/NL 89/00030

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 61 K 49/02

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No [13]
X	Biological Abstracts/RRM, volume 35, 1988, Quest Accession no. 62786, K. Hamacher et al.: "Carbon-11 D L homocysteine thiolactone a potential tracer for myocardial ischemia using pet", see title and terms & Journal of Nuclear Medicine (US) 1988. vol. 29, no. 5, suppl. p. 755 --	3-6
Y,P	Chemical Abstracts, volume 109, 1988, (Columbus, Ohio, US), A. Deussen et al.: "Formation of S-adenosylhomocysteine in the heart I: An index of free intracellular adenosine", see page 484, abstract 71107v & Circ. Res. 1988, 83(1), 240-9 --	3-6
Y,P	Biological Abstracts/RRM, volume 36, 1989, Quest Accession no. 129507, K. Hamacher et al.: "Synthesis of carbon-11 labelled D L homocysteine thiolactone", ./.	3-6

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
11th August 1989	19 09 89
International Searching Authority: EUROPEAN PATENT OFFICE	Signature of Authorized Officer: T.K. WILLIS

International Application No. PCT/NL 89/00030

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	see title and terms & Journal of Labelled Compounds & Radiopharmaceuticals(England), 1989. vol. 26, no. 0, p. 240-242 --	
A	Biological Abstracts/RRM, volume 33, 1987, Quest Accession no. 16368, M. Borst et al.: "Assessment of the free intracellular adenosine concentration in isolated guinea- pig hearts", see title and terms & Pfluegers Archiv European Journal of Physiology(West Germany) 1987. vol. 408, no. suppl. 1, p. R11 --	3-6
A	Biological Abstracts, volume 82, no. 10, 1986, (Philadelphia, P.A., US), K.J. Henrichs et al.: "Intra- cellular trapping of adenosine during myocardial ischemia by L-homocysteine", see page AB-136, abstract 89761, & Basic Res Cardiol 81(3): 267- 275, 1986	3-6

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 1&2 because they relate to subject matter not required to be searched by this Authority, namely:

Methods for treatment of the human animal body by surgery or therapy, as well as diagnostic methods (See Article 17(2)(a)(i) and Rule 39.1 (iv) of the Patent Cooperation Treaty)

2. [ ] Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.