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- (71) Applicant (for all designated States except US):
MOLYPHARMA, S.A. [ES/ES]; Dr. Severo Ochoa,
37, E-28100 Alcobendas (ES).
- (72) Inventor; and
- (75) Inventor/Applicant (for US only): **ORTIZ ARMUA, Pedro** [ES/ES]; Sta. Maria Micaela, 4, E-28008 Madrid (ES).
- (74) Agents: **PONTI SALES, Adelaida** et al.; C. Consell de Cent, 322, E-08007 Barcelona (ES).
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(54) Title: A RADIOPHARMACEUTICAL FOR RADIONUCLIDE TUMOR THERAPY

(57) Abstract: The present invention is related to a radiopharmaceutical comprising a 5-thio-D-Glucose molecule labeled with a ¹⁸⁸Re isotope for use in radionuclide tumor therapy; to a composition for radionuclide therapy comprising the radiopharmaceutical; and a method of preparing the radiopharmaceutical; and to a method of preparing the radiopharmaceutical, comprising the step of combining 5-thio-D-Glucose, stannous ions, and a solution of ¹⁸⁸Re isotope in a pharmaceutically acceptable solvent. This radiopharmaceutical is generally applicable to the majority of the different types of tumors related to the different types of cancer, and it is efficiently cleared from the body after the therapeutic use.

TITLE: A radiopharmaceutical for radionuclide tumor therapy

FIELD OF THE INVENTION

The present invention relates to a radiopharmaceutical for radionuclide
5 tumor therapy; a composition for radionuclide therapy comprising the
radiopharmaceutical; and a method of preparing the radiopharmaceutical.

BACKGROUND OF THE INVENTION:

Within the therapy of tumors in an organism the concept of carrying a
10 cytotoxic agent, such as a radionuclide (radioactive isotope), direct to an aberrant cell
remains an attractive alternative to conventional forms of radiation treatment. In
theory, the intimate contact between a radioactive conjugate and a cell enables the
absorbed radiation dose to be concentrated at the site of abnormality with minimal
injury to normal tissues.

15 The art describes radiopharmaceuticals for tumor therapy comprising an
antibody labeled with a radioactive isotope. The objective of such a strategy is
generally to identify an antibody which specifically recognizes special tumor cells with
the aim to direct the cytotoxic radioactive isotope to these tumor cells. See e.g. the
review articles (Chatal J-F et al., Chapter 84 "Therapeutic use of radiolabeled
20 antibodies"; and Duncan A., Chapter 78, "Principles of Radionuclide therapy"; both in
the book "Nuclear Medicine, Clinical Diagnostic and Treatment, Editor: Murray
I.P.C., Ell Peter, Hong Kong 1998") for further details.

The article "Ozker K, et al., Biodistribution of ⁹⁹Tcm-labelled 5-thio-D-
glucose, *Nucl Med Commun* 1999 Nov;20(11):1055-8" describes the use of a 5-thio-D-
25 glucose labeled with a ⁹⁹Tcm isotope for diagnostic purpose. Diagnostic purpose
relates to the identification of where the tumor is situated within the body, i.e. it is not
a therapeutic treatment in the sense of reducing the size of a tumor within the body.

SUMMARY OF THE INVENTION

30 The problem to be solved by the present invention is to provide a
radiopharmaceutical suitable for radionuclide tumor therapy and which should be

generally applicable to the majority of the different types of tumors related to the different types of cancer, such as lung cancer, testicle cancer, uterus cancer, pancreatic cancer, etc.

Further, it should have a good clearance effect in the sense that both the
5 radioactive isotope and the vector molecule labeled with the isotope, of the radiopharmaceutical, are efficiently cleared from the body after the therapeutic use.

The solution is a novel complex comprising a 5-thio-D-Glucose molecule labeled with a ^{188}Re isotope.

Accordingly, in a first aspect the invention relates to a radiopharmaceutical
10 comprising a 5-thio-D-Glucose molecule labeled with a ^{188}Re isotope for use in medical therapy;

and in a second aspect the invention relates to use of a radiopharmaceutical comprising a 5-thio-D-Glucose molecule labeled with a ^{188}Re isotope for the manufacture of a medicament for radionuclide tumor therapy.

15 As demonstrated in a working example herein (*vide infra*) such a radiopharmaceutical is capable of directing the cytotoxic ^{188}Re isotope to tumor cells in a preferentially manner, i.e. the tumor tissue takes up the radiopharmaceutical at a must higher frequency that other normal tissue within the organism. The working example is performed in a mice. Mice is known to be a very good model for a human.
20 Accordingly, these data demonstrate that the radiopharmaceutical as described herein is very suitable for radionuclide tumor therapy.

An advantage of such a radiopharmaceutical is that it is more general applicable that radiopharmaceuticals which use antibodies as a vector molecule to direct the radioactive isotope to the tumor cell. This is due to that the antibody only
25 recognizes the specific tumor cells which they are constructed to bind. On the contrary, the 5-thio-D-Glucose used as a vector molecule in a radiopharmaceutical as described herein is more general useable since the tumor uptake of the radiopharmaceutical is related to the general D-Glucose transport pathway within the body. A theory behind the preferential uptake of the radiopharmaceutical is that tumor
30 tissue is a region of low oxygen tension and decreased availability of oxygen causes an increased extraction of glucose via an anaerobic pathway. However, independently of

the exact mechanism behind it, the final result is that a radiopharmaceutical as described herein is preferentially taken of by a tumor cell independently of which specific cancer disease the tumor relates to.

A further advantage of a radiopharmaceutical as described herein is that it has a good clearance effect in the sense that both the radioactive ^{188}Re isotope and the 5-thio-D-Glucose vector molecule are efficiently cleared from the body after the therapeutic use. The normal catabolic pathway of the organism degrades the D-Glucose and the remaining ^{188}Re isotope is rejected from the organism due to it is recognized as a foreign non wanted element. This rejection generally passes through the normal urinary pathway. To have a good clearance is important due to that if the radionuclide is not efficiently removed from the body it may cause destruction of normal tissue in the body. See the article (Li et. al. "A distribution and elimination study of ^{188}Re in pig", J. Nuc. Medicine Vol 40(5): 88p, May 1999) for further description of the clearance issue.

However, even though the radiopharmaceutical has the above described good clearance effect it still maintains an adequate biological half-life within the tumor tissue. The biological half-life denotes the time the molecule are maintained in the tissue to be treated.

Consequently, a radiopharmaceutical as described herein exhibits a perfect balance between an adequate biological half-life within the tumor tissue and a good clearance effect.

In a third aspect the invention relates to a composition for radionuclide therapy comprising a 5-thio-D-Glucose molecule labeled with a ^{188}Re isotope and a pharmaceutically acceptable solvent, and

in a fourth aspect the invention relates to a method of preparing a radiopharmaceutical comprising a 5-thio-D-Glucose molecule labeled with a ^{188}Re isotope comprising the step of combining 5-thio-D-Glucose, stannous ions, and a solution of ^{188}Re isotope in a pharmaceutically acceptable solvent.

DEFINITIONS:

Prior to a discussion of the detailed embodiments of the invention is provided a definition of specific terms related to the main aspects of the invention.

The term "tumor therapy" should herein be understood in its general sense, 5 i.e. that the objective is to significantly reduce the size the tumors within the organism. Preferably the tumors should be completely eliminated.

Embodiment(s) of the present invention is described below, by way of example(s) only

10 DETAILED DESCRIPTION OF THE INVENTION:

Use of a radiopharmaceutical comprising a 5-thio-D-Glucose molecule labeled with a ^{188}Re isotope for the manufacture of a medicament for radionuclide tumor therapy:

The radiopharmaceutical of the second aspect of the invention is preferably used for tumor therapy, wherein tumors are disseminated at different places within the 15 organism. Due to that the radiopharmaceutical directs itself to tumors cell in general, it is perfectly suitable to treat tumors situated at different places in the organism in a one time treatment, i.e. the radiopharmaceutical is delivered to the organism, it finds it way to the different tumors, and performs the cytotoxic killing of these.

Preferably, the tumors to be treated are relatively small tumors of less than 8 20 mm in diameter, preferably less than 5 mm in diameter. The ^{188}Re isotope has a β -radiation of 2 MeV which makes it highly suitable to treat such tumors.

However, the radiopharmaceutical, as described herein, may also be used to treat more bulky tumors, such as tumors of more than 1 cm in diameter.

Preferably, the amount of radiopharmaceutical delivered to the organism is 25 from 25 MBq to 750 MBq per kilogram body weight of the organism, more preferably from 50 MBq to 400 MBq per kilogram body weight of the organism, even more preferably from 75 MBq to 250 MBq per kilogram body weight of the organism, and most preferably from 90 MBq to 150 MBq per kilogram body weight of the organism.

A working example herein (*vide infra*) demonstrates that a 30 radiopharmaceutical, as described herein, is capable of directing the cytotoxic ^{188}Re isotope to tumor cells in a preferentially manner, i.e. the tumor tissue takes up the

radiopharmaceutical at a must higher frequency that other normal tissue within the organism. The working example is performed in a mice and since mice is known to be a very good model for a human it is reasonably expected that a radiopharmaceutical, as described herein, can be effectively employed with any mammalian species, including
5 humans. The preferred organism to be treated is a human.

The Radiopharmaceutical may be delivered to the organism by a number of conventional routes, such as by an intraperitoneal route, an intrathecal route, or an intravesical route. See (Chatal J-F et al., Chapter 84 "Therapeutic use of radiolabeled antibodies"; in the book "Nuclear Medicine, Clinical Diagnostic and Treatment,
10 Editor: Murray I.P.C., Ell Peter, Hong Kong 1998") for further details in relation to the routes of delivery.

Preferably the radiopharmaceutical is delivered by an intravenously route.

A composition for radionuclide therapy comprising a 5-thio-D-Glucose molecule
15 labeled with a ^{188}Re isotope and a pharmaceutically acceptable solvent.

A preferred composition of the third aspect of the invention is wherein at least 90% of the 5-thio-D-Glucose present in the composition is labeled with a ^{188}Re isotope.

An working example herein (*vide infra*) describes preparation of such a
20 composition wherein 98% of the 5-thio-D-Glucose present in the composition is labeled with a ^{188}Re isotope.

An preferred way of preparing a radiopharmaceutical as described herein is by the use of stannous ions during the process. See below for further details.

Consequently, an embodiment of the invention relates to the composition of
25 the third aspect of the invention, which further comprises stannous ions,
and more preferably, wherein the stannous ions is supplied as stannous tin, stannous chloride dihydrate, and/or stannous flouride.

A pharmaceutically acceptable solvent may e.g. be normal saline. However it is anticipated that any pharmaceutically acceptable carrier in which the complex is
30 soluble may be used as a suitable solvent.

A method of preparing a radiopharmaceutical comprising a 5-thio-D-Glucose molecule labeled with a ^{188}Re isotope comprising the step of combining 5-thio-D-Glucose, stannous ions, and a solution of ^{188}Re isotope in a pharmaceutically acceptable solvent.

A working example herein (*vide infra*) describes in detail the preparation
5 with high labeling efficiency of a ^{188}Re labeled 5-thio-D-glucose (^{188}Re -5TG) complex using stannous ion reduction. The ^{188}Re -5TG complex thus prepared was found to exhibit high stability. Stability is measured by the percentage of 5TG that is labeled with ^{188}Re after 24 hours from the time that the complex was prepared. Preferably, at least 90% of the 5TG is labeled with ^{188}Re after 24 hours. More preferably, at least
10 95% or even as much as 98% of the ^{188}Re is labeled with Tc-99m after 24 hours.

The complex of the present invention is preferably prepared by dissolving 5-thio-D-glucose in a suitable solvent such as normal saline (0.85 M NaCl) with low dissolved oxygen, adding a stannous ion source such as stannous chloride or stannous tin, for example, and lyophilizing the mixture to form a solid that can be conveniently
15 stored and readily reconstituted when needed. Preferably, around 20 mg of 5-thio-D-glucose and around 74 μg stannous ion are present. Optionally, an antioxidant such as gentisic acid can be included in the mixture to be lyophilized.

Stannous tin and stannous chloride are shown in the examples to be suitable for the preparation of ^{188}Re -5TG. It is expected that other stannous ion sources
20 will be equally suitable for the practice of this invention.

At the time of use, the lyophilized solid is preferably reconstituted with between 185 and 555 MBq [^{188}Re] in isotonic saline per milligram 5-thio-D-glucose to form the desired 5-thiogluco-
25 se and ^{188}Re complex. It is anticipated that any pharmaceutically acceptable carrier in which the complex is soluble may be used in the present invention. One of skill in the art would recognize that one could prepare the ^{188}Re -labeled 5TG by combining a solution of 5-TG and stannous ions together with a solution of ^{188}Re without first lyophilizing the 5-TG and stannous ion solution.

In the working example below, a preparation of ^{188}Re -labeled 5TG was prepared by reconstituting lyophilized 5-TG and stannous ions with a ^{188}Re solution
30 and incubating the mixture at room temperature for 30 minutes prior to chromatography analysis. It is reasonably expected that ^{188}Re -labeled 5TG could be

prepared using shorter or longer periods of incubation. Preferably, to insure high efficiency of labeling, the incubation should be at least 5 minutes long, and more preferably the incubation should be at least 10 minutes long.

5 EXAMPLES:

Example 1:

Preparation of ^{188}Re -5TG

^{188}Re -TG was prepared using 74 μg stannous tin, 20 mg 5-Thio-D-glucose (Aldrich Chemical Company) and 50-150 mCi (1.85-5.55 GBq) ^{188}Re (in the form of
10 pertechnetate and derived from an ^{188}Re generator (Aldrich National Laboratory, Tennessee, USA)) in a final volume of 2-4 mL. After a 30 minute incubation at 20 room temperature, the solution was chromatographed using Whatman 3MM paper strips developed in methylethyl ketone (MEK) and Gelman instant thin-layer silica gel (ITLC-SG) strips developed in saline.

15 Labeling efficiency of ^{188}Re -TG was 98.5+0.8%, stable for over 24 hours. Any unreacted ^{188}Re impurity present would appear at the solvent front on the chromatograms developed in MEK; insoluble ^{188}Re species would appear at the origin of saline chromatogram.

The labeling reaction can be performed using the same stannous reduction
20 method and a formulation having suitable ratios of 10 mg 5-thio-D-glucose and stannous ions, such as a composition comprising about 10 mg 5-thio-D-glucose and between about 0.01 mg and 2 mg stannous tin as stannous chloride dihydrate or stannous fluoride.

25 Example 2:

Pharmacokinetic studies of ^{188}Re -5TG in mice:

Tumor localization experiments were performed in 5 nude mice. 3 of the mice were C57BL/6 strain male mice bearing MC26 colon carcinoma and 2 were female nude mice bearing breast cancer.

0.25 mCi (9.25 MBq) of $^{188}\text{Re-TG}$ was injected into the tail vein of the mice. Each mice weighted around 29 g. Consequently the dose was around 318 MBq per kilogram body weight of the mice.

Animals were sacrificed by cervical dislocation at 1, 2 and 3 hours after 5 injections. Tissue specimens were removed from each mouse, weighed, and counted in a scintillation counter.

The data obtained clearly demonstrated that the $^{188}\text{Re-TG}$ radiopharmaceutical was accumulated in the tumors of the mice. No significant background was identified within the normal tissue of the mice.

CLAIMS

1. A radiopharmaceutical comprising a 5-thio-D-Glucose molecule labeled with a ^{188}Re isotope for use in medical therapy.
- 5
2. Use of a radiopharmaceutical comprising a 5-thio-D-Glucose molecule labeled with a ^{188}Re isotope for the manufacture of a medicament for radionuclide tumor therapy.
- 10
3. The use of claim 2, wherein the tumors are disseminated at different places within the organism.
4. The use of claim 2 or 3, wherein the tumor is a relatively small tumor of less than 8 mm in diameter, preferably less than 5 mm in diameter.
- 15
5. The use of any of claims 2 to 4, wherein the amount of radiopharmaceutical delivered to the organism is from 25 MBq to 750 MBq per kilogram body weight of the organism, more preferably from 50 MBq to 400 MBq per kilogram body weight of the organism, even more preferably from 75 MBq to 250 MBq per kilogram body
- 20 weight of the organism, and most preferably from 90 MBq to 150 MBq per kilogram body weight of the organism.
6. The use of any of claims 2 to 5, wherein the organism is a human.
- 25
7. The use of any of claims 2 to 6, wherein the radiopharmaceutical is delivered intravenously.
8. A composition for radionuclide therapy comprising a 5-thio-D-Glucose molecule labeled with a ^{188}Re isotope and a pharmaceutically acceptable solvent.

9. The composition of claim 8, wherein at least 90% of the 5-thio-D-Glucose present in the composition is labeled with a ^{188}Re isotope.

10. The composition of any of claim 8 to 9, which further comprises stannous ions.

11. The composition of claim 10, wherein the stannous ions is supplied as stannous tin, stannous chloride dihydrate, and/or stannous flouride.

10 12. A method of preparing a radiopharmaceutical comprising a 5-thio-D-Glucose molecule labeled with a ^{188}Re isotope comprising the step of combining 5-thio-D-Glucose, stannous ions, and a solution of ^{188}Re isotope in a pharmaceutically acceptable solvent.