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(54) Title: NUCLEAR MAGNETIC RESONANCE IMAGING AGENT

(57) Abrégé/Abstract:
A nuclear magnetic resonance imaging agent comprises a complex compound composed of (a) dialdehyde-saccharide having a molecular weight of from 500 to 10,000, at least one of constituent monosaccharides of which is oxidation-cleaved, (b) at least one complexing agent that is chemically coupled to an aldehyde group of the dialdehyde-saccharide and (c) a paramagnetic metal ion that is chemically coupled to the complexing agent. The agent has improved performance, included a more desirable retention time in blood.

ABSTRACT

A nuclear magnetic resonance imaging agent comprises a complex compound composed of (a) dialdehyde-saccharide having a molecular weight of from 500 to 10,000, at least one of constituent monosaccharides of which is oxidation-cleaved, (b) at least one complexing agent that is chemically coupled to an aldehyde group of the dialdehyde-saccharide and (c) a paramagnetic metal ion that is chemically coupled to the complexing agent. The agent has improved performance, included a more desirable retention time in blood.

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NUCLEAR MAGNETIC RESONANCE IMAGING AGENT

The present invention relates to a nuclear magnetic resonance imaging (hereinafter sometimes abbreviated as MRI) agent, in particular, to a nuclear magnetic resonance diagnostic agent containing a paramagnetic metal species.

Diethylenetriaminepentaacetic acid-gadolinium (DTPA-Gd) is the only pharmaceutical to be used as a MRI agent whose effectiveness as a diagnostic agent in the brain or spinal regions has been almost established. Since, however, it is rapidly excreted in the urine after administration, its half-life period in blood is extremely short, such as about 14 minutes [Hiroki Yoshikawa et al., Gazoshindan, 6, 959-969 (1986)]. It is therefore difficult to diagnose several parts of the body (blood vessel distribution, blood stream distribution, distribution volume, permeation and the like in a lesion) with a single injection. Further, since it is nonspecifically distributed from the interior of a blood vessel to the interstice of tissue cells, sometimes no clear contrast can be obtained due to an undistinguishable difference in the concentration between normal tissue and a lesion.

Furthermore, since the imaging time in a nuclear magnetic resonance diagnosis method depends upon the magnetic field in the MRI spectrometer to be used, it requires a longer imaging time, for example, when using a widely popularized low magnetic field type of MRI spectrometer. Then, the conditions of a lesion cannot be appreciated precisely by using DTPA-Gd which disappears from the blood within a short period of time. Thus, diagnosis with DTPA-Gd has a natural limitation depending upon the diagnosing site or the particular type of a diagnosing apparatus.

To solve these problems, there has been an increased demand for an MRI agent that can localize in a blood vessel for a constant period of time from immediately after administration, stay therein for a relatively longer period of time, and have a medium or long half-life period in blood. As

a result, as prototype imaging agents, paramagnetic metal complex compounds using as their carriers polymer materials, such as HSA [Ogan, M.D. et al., Invest. Radiol., 22, 665-671 (1987)], dextran [Brasch, R.C. et al., Radiology, 175, 483-488 (1990)], polylysine (JP-A 64-54028) and the like have been studied and developed. However, since all these carriers are polymer compounds having a molecular weight of tens of thousands or more, the retention time in blood is unnecessarily long, as from ten and several hours to several days, and there are problems in residence of this length in the body, antigenicity and the like.

The main object of the present invention is to provide a MRI agent containing a paramagnetic metal ion and having appropriate localization in a blood vessel and retention in blood. In other words, the technical problem to be solved by the present invention is to improve the retention of DTPA-Gd in blood, among the various behaviors of DTPA-Gd in the body. Accordingly, an imaging agent of the present invention requires that (1) it stays in blood and does not permeate out of a blood vessel, (2) it is excreted mainly and relatively rapidly into the urine, (3) it scarcely accumulates in the body, and (4) it has non-antigenicity and low toxicity.

In accordance with one aspect of the present invention there is provided a nuclear magnetic resonance imaging agent which comprises a complex compound composed of (a) a dialdehyde-saccharide having a molecular weight of from 500 to 10,000, at least one of constituent monosaccharides of which is oxidation-cleaved, (b) at least one complexing agent that is chemically coupled to an aldehyde group of the dialdehyde-saccharide and (c) a paramagnetic metal ion that is chemically coupled to the complexing agent.

The aforementioned complex compound may have a retention time in blood from 0.5 to 5 hours as its half-life period in

blood. The concentration of the paramagnetic metal ion in this complex compound may be from $1x10^{-5}$ to 1x10 mol/liter.

These as well as other objects and advantages of the present invention will become apparent to those skilled in the art from the following description with reference to the accompanying drawings, in which:

Figure 1 is a MRI showing a transverse view of a chest region including the heart of a rat sacrificed immediately after administration of a (dialdehyde-starch)-p-aminobenzyl-DTPA-Gd (DAS-DTEN-Gd) solution;

Figure 2 is a MRI showing a transverse view of a chest region including the heart of a rat sacrificed 30 minutes after administration of a DAS-DTEN-Gd solution;

Figure 3 is a MRI showing a transverse view of a chest region including the heart of a rat sacrificed 30 minutes after administration of DTPA-Gd (MAGNEVIST).

According to the present invention, there is provided a nuclear magnetic resonance imaging agent that comprises a complex compound composed of (a) dialdehyde-saccharide having a molecular weight of from 500 to 10,000, at least one of constituent monosaccharides of which is oxidation-cleaved, (b) at least one complexing agent that is chemically coupled to an aldehyde group of the dialdehyde-saccharide and (c) a paramagnetic metal ion that is chemically coupled to the complexing agent.

It has been found that a complex compound obtained by chemically coupling a paramagnetic metal ion through a complexing agent that is chemically coupled to a certain dialdehyde-saccharide is suitable for a nuclear magnetic resonance diagnostic agent that satisfies the above demand. Further, it has been found that such a diagnostic agent improves the retention of DTPA-Gd in blood and has a clinically effective half-life period in blood.

For example, in the case of (dialdehyde-starch)-DTEN-In-111 and (dialdehyde-amylose)-DTEN-In-111 composed of dialdehyde-starch (average molecular weight: 7,000, hereinafter abbreviated as DAS) and dialdehyde-amylose (average molecular weight: 2,900, hereinafter abbreviated as DAA) as the dialdehyde-saccharide, p-aminobenzyl-DTPA [Martin, W.B. et al, Inorg. Chem., 25, 2772-2781 (1986)] (hereinafter abbreviated as DTEN) as the complexing agent, radioactive In-111 as the metal species (the use of a radioactive metal species in place of a paramagnetic metal ion results from the handling restriction and is a conventional experimental procedure in this art field), the half-life periods of the blood of rats are calculated as 2 hours and 45 minutes, respectively, based on the radioactivity distribution ratio in blood with time after intravenous injection. This supports the point that these compounds show the effective retention in blood that is clinically required. Further, the excretion of these compounds into the urine at 24 hours after administration are calculated as 78%/dose and 87%/dose, respectively, based on the above radioactivity distribution

experiment. In view of this, it is clear that these compounds have good excretion properties. Furthermore, from this experiment, it has been confirmed that these compounds have no problems in specific distribution and residence in the body.

The present invention is based on the above findings and, as described above, the gist thereof is a MRI agent that comprises a complex compound composed of (a) dialdehyde-saccharide having a molecular weight of from 500 to 10,000, at least one of constituent monosaccharides of which is oxidation-cleaved, (b) at least one complexing agent that is chemically coupled to an aldehyde group of the dialdehyde-saccharide and (c) a paramagnetic metal ion that is chemically coupled to the complexing agent.

As described above, in the conventional prototype paramagnetic metal complex imaging agents using polymer materials, such as HSA, dextran, polylysine and the like having the molecular weights of tens of thousands or more, the retention in blood is considerably improved. However, their disappearance half-life periods in blood are unnecessarily long, and their residence in the body causes trouble. These factors result in such clinical disadvantages that administration cannot be repeated. Further, in view of safety, the chemical toxicity due to the compound per se and, in some cases, the metal toxicity due to the paramagnetic metal ion released from the complexing agent during residence in the body for a long period of time are not negligible. Thus, various drawbacks are recognized in the use of the polymer materials composed of polymerization of repetition units.

On the other hand, since an oxidation-cleaved dialdehyde-saccharide having a molecular weight of from 500 to 10,000, is used as a parent skeleton, the present invention has been successful in providing a clinically useful imaging agent that has no such drawbacks and solves the above-described problems.

The dialdehyde-saccharide to be used as the above component (a) in the complex compound of the imaging agent of the present invention has a molecular weight of from 500 to 10,000, preferably, not more than 3,000, and is preferably an oxide of oligosaccharide which is tri- to deca-saccharide. Examples of the dialdehyde-saccharide includes maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose, isomaltotriose, isomaltotetraose, isomaltopentaose, isomaltohexaose, isomaltoheptaose, cellotriose, cellotetraose, cellopentaose, cellohexaose, laminaritriose, laminaritetraose, laminaripentaose, laminarihexaose, laminariheptaose, cyclodextrin, amylose (average molecular weight: 2,900), dextran (average molecular weight: 2,000 to 8,000), starch (average molecular weight: 7,000) and the like. Preferably, there can be used a dialdehyde-saccharide obtained by oxidation of the constituent monosaccharide, D-glucose. The oxidation-cleavage can be carried out according to a known method, for example, using sodium periodate.

As the complexing agents of the above component (b), there can be used a linear or cyclic polyaminopolycarboxylic acid having an active amino group as a crosslinking chain and a bifunctional structure capable of trapping a metal ion to form a complex, preferably, a bifunctional complexing agent having an active amino group and a DTPA (diethylenetriamine-pentaacetic acid) skeleton or a DOTA (1,4,7,10-tetraazacyclo-dodecane-1,4,7,10-tetraacetic acid) skeleton. Examples of the complexing agent include 1-(p-aminobenzyl)diethylenetriamine-pentaacetic acid [Martin, W.B. et al., Inorg. Chem., 25, 2772-2781 (1986)], 2-(p-aminobenzyl)-1,4,7,10-tetraazacyclo-dodecane-1,4,7,10-tetraacetic acid (US Patent No. 4,678,667), 2-aminobutyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra-acetic acid [Parker, D. et al., Pure & Appl. Chem., 61, 1637-1641 (1989)] (hereinafter abbreviated as "AB-DOTA") and the like.

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The oxidation-cleaved dialdehyde-saccharide is coupled with the complexing agent according to a known method. For example, the dialdehyde-saccharide is reacted with the complexing agent in an alkaline solution to obtain a compound wherein both of them are coupled through -CH=N-. If necessary, the compound can be reduced to convert -CH=N- into $-CH_2-NH-$.

The paramagnetic metal ion as the component (c) can be selected from lanthanide elements having an atomic number of from 57 to 70, and is preferably Gd or Dy. The paramagnetic metal can be the lanthanide element per se or a compound containing such an element, for example, a chloride or oxide. The complexing can be carried out according to a conventional method.

The complex compound thus obtained has a structure in which, at least one, preferably, two or more complexing agents are chemically coupled to the dialdehyde-saccharide having a molecular weight of from 500 to 10,000, at least one, preferably, two or more of the constituent monosaccharides of which are oxidation-cleaved, and the paramagnetic metal ion is coupled to the complexing agent part.

The above-described complex compound can be optionally admixed with one or more pharmaceutically acceptable additives by a conventional method to prepare an imaging agent in various suitable forms. Preferably, the complex compound is dissolved in an aqueous physiologically acceptable solvent to prepare an imaging agent in the form of a solution.

For using a complex compound of the present invention as a MRI agent, it is administered in a dose from 0.0001 to 10 mmol/kg, preferably, from 0.005 to 0.5 mmol/kg as the dose of the paramagnetic metal ion. It is usually administered intravenously. In some cases, it can be administered orally or intra-arterially.

Retention in blood of the complex compound of the present invention is from 0.5 to 5 hours as a half-life period in blood. Therefore, the compound can be appropriately selected and used according to the particular desired retention in

blood and the particular kind of MRI spectrometer. For example, in the case of a low magnetic field MRI spectrometer, it is preferred to use an imaging agent having a relatively long retention in blood, so as to promote collection efficiency for proton relaxation of the imaging agent. When the complex compound of the present invention contains Gd as the paramagnetic metal ion, since the effect on shortening of the relaxation time per Gd ion is predominantly stronger than that of DTPA-Gd, it can be used more advantageously than DTPA-Gd. Further, in diagnosis with a low magnetic field MRI spectrometer having a lower collection efficiency for the proton relaxation effect, the detection efficiency is increased in another sense and thereby the imaging time can be shortened. Furthermore, when it is desired to obtain the same contrast effect as that in DTPA-Gd with the same magnetic field, a complex compound of the present invention can be used in a less amount than that of DTPA-Gd, and therefore it is also advantageous in view of safety. Contrary to this, in the case of the same dose, a complex compound of the present invention can provide much more information of a living body than the imaging agent, and thereby its clinical usefulness is improved. Accordingly, the present invention can provide an imaging agent having appropriate retention in blood and an effectively enhanced effect, which matches the magnetic field of a MRI spectrometer or other imaging conditions.

Since a complex compound of the present invention has appropriate retention in blood and localization in a blood vessel, a blood vessel distribution image (vascularity) can be evaluated. Therefore, the imaging agent of the present invention is also expected to be useful as a transvenous imaging agent for MR angiography which has been remarkably advanced.

Further, since a complex compound of the present invention is hydrophilic, it can be used, as is, to prepare a concentrated solution. In the case of DTPA-Gd, the addition of a certain solubilizer is required for the preparation of a solution having the desired concentration. Accordingly, when

a solution containing the same concentration of Gd as that of DTPA-Gd is prepared, in some cases a complex compound of the present invention does not require any solubilizer. Further, since a complex compound of the present invention is polynuclear, when the same concentration of Gd solution is prepared, the total number of moles becomes small, resulting in a decrease in the osmotic pressure. Thus, a complex compound of the present invention is also advantageous from the pharmaceutical viewpoint.

As described hereinabove, an imaging agent of the present invention comprises a complex compound composed of the specific dialdehyde-saccharide, the complexing agent which is chemically coupled to an aldehyde group of the dialdehyde-saccharide and the paramagnetic metal ion which is chemically coupled to the complexing agent. Thus, by using this novel and specific complex compound, a clinically effective retention time in blood and an enhanced contrast effect in the magnetic field as employed in an MRI can be realized.

The following Examples, Tests and Reference Examples further illustrate the present invention in detail, but are not to be construed to limit the scope thereof.

Example 1

Synthesis of DAS-DTEN

Starch (average molecular weight: 7,000) was oxidation-cleaved with periodic acid according to the conventional method to obtain DAS.

DAS (0.5 g, 0.07 mmol) was dissolved in 0.1M phosphate buffer (pH 7.0, 50 ml), followed by addition of DTEN (0.8 g, 1.6 mmol). Triethylamine (1.66 g, 16.4 mmol) was added thereto and the pH was adjusted to about 12. The mixture was reacted by stirring at room temperature for 24 hours. To the reaction mixture was added sodium borohydride (0.121 g, 3.2 mmol), and the mixture was further reacted by stirring at room temperature for another 24 hours. The pH was adjusted to 2 or below, with the addition of

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7N hydrochloric acid, and then the mixture was neutralized with the addition of a 10N aqueous solution of sodium hydroxide to obtain crude DAS-DTEN.

A part of the reaction mixture (50 μl) was taken out and admixed with 0.1M citrate buffer (pH 5.9, 100 μl) and indium chloride (In-111) solution (50 μl). The ratio of DAS-DTEN-In-111 and DTEN-In-111 was determined by thin layer chromatography and it was confirmed that 6.4 molecules of DTEN were coupled per one molecule of DAS.

The above reaction mixture was purified by gel filtration chromatography (Sephadex™ G-75) to obtain DAS-DTEN (0.57 g).

Proton-NMR spectrum (solvent/D_2O, 270 MHz): 2.10-3.33 (10H, m, CH_2), 3.37-4.11 (m, CH and CH_2), 4.30 (1H, m, N-CH), 6.80 (2H, d, benzene ring), 7.08 (2H, d, benzene ring).

IR absorption spectrum (KBr tablet method): 780 cm^{-1} (CH in benzene ring), 1100 cm^{-1} (OH), 1410 cm^{-1} (CH_2), 1615 cm^{-1} (COOH)

Example 2

Synthesis of DAA-DTEN

Amylose (average molecular weight: 2,900) was oxidation-cleaved by periodic acid according to the conventional method to obtain DAA.

DAA (0.5, 0.17 mmol) was dissolved in 0.1M phosphate buffer (pH 7.0, 50 ml), followed by the addition of DTEN (0.678 g, 1.4 mmol). The pH was adjusted to about 12 with the addition of triethylamine (1.4 g, 13.8 mmol). The mixture was treated according to the same manner as that described in Example 1 to obtain crude DAA-DTEN.

A part of the reaction mixture (50 μl) was taken out and admixed with 0.1M citrate buffer (pH 5.9, 100 μl) and indium chloride (In-111) solution (50 μl). The ratio of DAA-DTEN-In-111 and DTEN-In-111 was determined by thin layer chromatography and it was confirmed that 2.1 molecules of DTEN were coupled per one molecule of DAA.

The above reaction mixture was purified by gel filtration chromatography (Sephadex™ G-75) to obtain DAA-DTEN (0.32 g).

Proton-NMR spectrum (solvent/D_2O, 270 MHz): 2.45-3.40 (10H, m, CH_2), 3.45-4.52 (m, CH and CH_2), 4.36 (1H, m, N-CH), 6.86 (2H, d, benzene ring), 7.13 (2H, d, benzene ring)

Example 3

Synthesis of dialdehyde-maltopentaose (DAMP)-DTEN

Maltopentaose (molecular weight: 828) was oxidation-cleaved by periodic acid according to the conventional method to obtain DAMP.

DAMP (0.127 g, 0.15 mmol) was dissolved in 0.1M phosphate buffer (pH 7.0, 5 ml), followed by the addition of DTEN (0.296 g, 0.6 mmol). The pH was adjusted to about 12 with the addition of triethylamine (0.6 g, 6.0 mmol) and the mixture was treated according to the same manner as that described in Example 1 to obtain crude DAMP-DTEN.

A part of the reaction mixture (50 μ1) was taken out and admixed with 0.1M citrate buffer (pH 5.9, 100 μ1) and indium chloride (In-111) solution (50 μ1). The ratio of DAMP-DTEN-In-111 and DTEN-In-111 was determined by thin layer chromatography and it was confirmed that 1.2 molecules of DTEN were coupled per one molecule of DAMP.

The above reaction mixture was purified by gel filtration chromatography (Sephadex™ G-75) to obtain DAMP-DTEN (0.047 g).

Proton-NMR spectrum (solvent/D_2O, 270 MHz): 2.24-3.40 (10H, m, CH_2), 3.40-4.13 (m, CH, CH_2 and NH), 4.28 (1H, bs, N-CH), 6.78 (d, benzene ring), 7.05 (dd, benzene ring)

IR absorption spectrum (KBr tablet method): 810 cm^{-1} (CH in benzene ring), 1080 cm^{-1} (OH), 1400 cm^{-1} (CH_2), 1630 cm^{-1} (COOH)

Example 4

Synthesis of DAMP-(AB-DOTA)

DAMP-(AB-DOTA) is obtained according to the same manner as that described in Example 3, except that AB-DOTA is used instead of DTEN.

Example 5

Synthesis of DAS-DTEN-Gd

DAS-DTEN (0.7 g, 0.07 mmol) was dissolved in distilled water (3 ml). Gadolinium chloride hexahydrate (0.024 g, 0.066 mmol) was added thereto and the mixture was reacted by stirring at room temperature to obtain DAS-DTEN-Gd.

Gd concentration (ICP emission spectral analysis): 19 mM

Example 6

Synthesis of Gd complex

According to the same manner as that described in Example 5, except that DAA-DTEN, DAMP-DTEN or DAMP-(AB-DOTA) was used instead of DAS-DTEN, the corresponding Gd complex was obtained.

Example 7

Synthesis of DAS-DTEN-Dy

DAS-DTEN (0.2 g, 0.02 mmol) was dissolved in distilled water (3 ml). Dysprocium chloride hexahydrate (0.007 g, 0.018 mmol) was added thereto and the mixture was reacted by stirring at room temperature to obtain DAS-DTEN-Dy.

Dy concentration (ICP emission spectral analysis): 6.5 mM

Example 8

Synthesis of Dy complex

According to the same manner as that described in Example 7, except that DAA-DTEN, DAMP-DTEN or DAMP-(AB-DOTA) was used instead of DAS-DTEN, the corresponding Dy complex was obtained.

Test 1

Relaxivity of DAS-DTEN-Gd (in vitro test)

An appropriate amount of DAS-DTEN-Gd was dissolved in distilled water. The relation to water proton exposed to this compound was determined as a proton relaxation time (T_1 and T_2, msec.) at room temperature (24 to 26°C) using NMR (6.35T, manufactured by Nihondenshi K.K., Japan). Respective relaxation times are shown in Table 1.

Table 1

Relaxation time of DAS-DTEN-Gd

Concentration (mM)	T_1 (msec.)	T_2 (msec.)
2.3	55	29
0	3275	2208

DAS-DTEN-Gd (2.3 mM) shortened the T_1 and T_2 values of water about 60 times and about 76 times, respectively. Relaxivity on the T_1 and T_2 (each R_1 or R_2 $(mM \cdot S)^{-1}$) were calculated based on the values in Table 1. The results are shown in Table 2.

Table 2

Relaxivity of DAS-DTEN-Gd

Compound	R_1 $(mM \cdot S)^{-1}$	R_2 $(mM \cdot S)^{-1}$
DAS-DTEN-Gd	7.9	15.1
DTPA-Gd	3.9	4.8

DAS-DTEN-Gd has good *in vitro* relaxation effect, which is significantly higher than that of DTPA-Gd (also shown in Table 2) measured in the same manner. This clearly shows the effectiveness of a complex compound of the present invention.

Test 2

Relaxation time in blood of mouse after intravenous administration of DAS-DTEN-Gd (*ex vivo* test)

A DAS-DTEN-Gd solution (Gd concentration: 19 mM) was administered to a thiopental-anesthetized ICR female mouse (body weight: 54 g) through the tail vein (the dose of Gd administered: 0.025 mmol/kg). Blood was taken from the aorta descendence at 15 minutes after administration and the relaxation time (T_1, msec.) of blood at room temperature (24-26°C) was determined with 6.35T NMR (manufactured by Nihondenshi K.K., Japan).

Further, as a control, blood was taken from the aorta descendence of a thiopental-anesthetized ICR female mouse (body weight: 55 g) and, in the same manner, the relaxation time was determined. The results are shown in Table 3.

Table 3

Relaxation time of DAS-DTEN-Gd in blood

	T_1 in blood (msec.)
Mouse given DAS-DTEN-Gd	1292
Control mouse	1769

T_1 relaxation time of DAS-DTEN-Gd in blood is about 1.4 times effective in comparison with that of the control mouse and it has thus been found that the relaxation time of blood is effectively shortened.

Test 3

Contrast enhancement of the heart in a rat immediately after intravenous administration of DAS-DTEN-Gd (in vivo test)

A DAS-DTEN-Gd solution (Gd concentration: 19.0 mM) was administered to a thiopental-anesthetized Sprague-Dawley female rat (body weight: 198 g, 9-weeks old) through a cannula fixed at the femoral vein (the dose of Gd administered: 0.087 mmol/kg). After about 30 seconds, the animal was sacrificed by administration of a pentobarbital solution (1 ml) through the above cannula and fixed in the dorsal position in the magnetic field of a MRI spectrometer. MRI measurement (transverse sectional view) of the chest region including the heart was carried out.

As a control, a Sprague-Dawley female rat (body weight: 188 g, 9-weeks old) was sacrificed by administration of a pentobarbital solution (1 ml) through a cannula fixed at the femoral vein and was subjected to the same MRI measurement (transverse sectional view).

The apparatus was SIGNA™ (manufactured by GE, U.S.A.) with magnetic field intensity of 1.5T and, as an imaging coil, a 26 cm ϕbird-cage type head QD coil was used. Imaging was carried out according to spin echo technique of T_1 weighted (TR/TE, 600/30 msec.) under the conditions of 10 mm in slice thickness, a resolution of 256 x 128.

The heart and its vascular system of the rat to which DAS-DTEN-Gd was administered were imaged at high signal intensity, which demonstrated that the effective contrast enhancement was also obtained in vivo. The signal intensity from the heart imaged with DAS-DTEN-Gd was about 4.7 times higher than that of the same part of the control rat.

Test 4

Contrast enhancement of the heart in a rat at 30 minutes after intravenous administration of DAS-DTEN-Gd (in vivo test)

A DAS-DTEN-Gd solution (Gd concentration: 19.0 mM) was administered to a thiopental-anesthetized Sprague-Dawley female rat (body weight: 186 g, 9-weeks old) through a cannula fixed at the femoral vein (the dose of Gd administered: 0.087 mmol/kg). The animal was sacrificed by administration of a pentobarbital solution (1 ml) through the above cannula 30 minutes after administration and fixed in the dorsal position in the magnetic field of a MRI spectrometer. MRI measurement (transverse sectional view) of the chest region including the heart was carried out.

As a control, DTPA-Gd (MAGNEVIST™) was administered to a Sprague-Dawley female rat (body weight: 234 g, 9-weeks old) (0.1 mmol/kg) through a cannula fixed at the femoral vein and MRI measurement (transverse sectional view) of the chest region including the heart was carried out in the same manner.

The apparatus was SIGNA™ (manufactured by GE, U.S.A.) with the magnetic field intensity of 1.5T and, as an imaging coil, a 26 cm ϕbird-cage type head QD coil was used. Imaging was carried out according to spin echo technique of T_1 weighted (TR/TE, 600/30 msec.) under the conditions of 10 mm in slice thickness, a resolution of 256 x 128.

The signal intensity from the rat to which DAS-DTEN-Gd was administered was found to be about 1.4 times higher than that of the control rat when comparing the signal intensity from the same part of the heart. The superiority in retention in blood of DAS-DTEN-Gd over that of DTPA-Gd together with the dose of Gd demonstrated the advantages of the present invention.

Reference Example 1

Radioactivity distribution in rat after intravenous administration of DAS-DTEN-In-111 (in vivo test)

DAS-DTEN (10 mg) was dissolved in distilled water (0.5 ml) and 0.1M citrate buffer (pH 5.9, 1 ml) was added thereto. The mixture was admixed with an indium chloride (In-111) solution (0.5 ml, 59 MBeq) to obtain DAS-DTEN-In-111. The radiochemical purity was 100%.

Sprague-Dawley female rats (three rats/group) (body weight: 110 to 130 g) were anesthetized with thiopental and DAS-DTEN-In-111 (50 μl/rat) was administered through the tail vein. The animals were sacrificed by dehematization at 0.25, 1, 3, 6 and 24 hours after administration. The main organs were removed and the radioactivity of each organ was measured. The radioactivity distribution ratio in blood and urine at each measurement time are shown in Table 4.

Table 4

Radioactivity distribution ratio of DAS-DTEN-In-111 in blood and urine

Time (hr)	Blood (%/dose)	Urine (%/dose)
0.25	4.02±0.92	48.26±4.42
1.0	2.28±1.18	63.74±2.29
3.0	1.15±0.14	72.09±2.54
6.0	0.94±0.02	74.67±1.98
24.0	0.19±0.10	78.33±2.16

As seen from the results in Table 4, the half-life period of DAS-DTEN-In-111 in blood was about 2 hours and was found to be a clinically effective retention in blood. Since excretion into the urine was good, there was no problem of residence in the body.

Reference Example 2

Radioactivity distribution in rats after intravenous administration of DAA-DTEN-In-111 (in vivo test)

DAA-DTEN (10 mg) was dissolved in distilled water (0.5 ml), followed by addition of 0.1M citrate buffer

(pH 5.9) (1 ml). The mixture was admixed with an indium chloride (In-111) solution (0.5 ml, 473 MBeq) to obtain DAA-DTEN-In-111. The radiochemical purity was 100%.

Sprague-Dawley female rats (three rats/group) (body weight: 150 to 190 g) were anesthetized with thiopental and DAA-DTEN-In-111 (25 μ1/rat) was administered through the tail vein. The animals were sacrificed by dehematization at 0.25, 1, 3, 6 and 24 hours after administration. The main organs were removed and the radioactivity of each organ was measured. The radioactivity distribution ratio in blood and urine at each measurement time are shown in Table 5.

Table 5

Radioactivity distribution ratio of DAA-DTEN-In-111 in blood and urine

Time (hr)	Blood (%/dose)	Urine (%/dose)
0.25	3.77±0.29	48.90±3.74
1.0	1.11±0.51	72.92±2.10
3.0	0.32±0.05	81.76±1.84
6.0	0.19±0.06	84.56±1.14
24.0	0.08±0.02	86.81±1.87

As seen from the results in Table 5, the half-life period of DAA-DTEN-In-111 in blood was about 45 minutes and was found to be a clinically effective retention in blood. Since excretion into the urine was good, there was no problem of residence in the body.

CLAIMS:

1. A nuclear magnetic resonance imaging agent which comprises a complex compound composed of (a) dialdehyde-saccharide having a molecular weight of from 500 to 10,000, at least one of constituent monosaccharides of which is oxidation-cleaved, (b) at least one complexing agent that is chemically coupled to an aldehyde group of the dialdehyde-saccharide and (c) a paramagnetic metal ion that is chemically coupled to the complexing agent.

2. The imaging agent according to claim 1, wherein the retention time of the complex compound in blood is from 0.5 to 5 hours as its half-life period in blood.

3. The imaging agent according to claim 2, wherein the constituent monosaccharide in the complex compound is D-glucose.

4. The imaging agent according to claim 2, wherein the number of repetition units of the constituent monosaccharide in the complex compound is from 3 to 10.

5. The imaging agent according to claim 2, wherein the complexing agent in the complex compound is a derivative of diethylenetriaminepentaacetic acid or 1,4,7,10-tetraazacyclo-dodecane-1,4,7,10-tetraacetic acid.

6. The imaging agent according to claim 2, wherein the paramagnetic metal ion in the complex compound is a lanthanide element having an atomic number of from 57 to 70.

7. The imaging agent according to claim 6, wherein the paramagnetic metal ion is Gd or Dy.

8. The imaging agent according to claim 2, wherein the concentration of the paramagnetic metal ion in the complex compound is from 1×10^{-5} to 1 x 10 mol/liter.

Application number / numéro de demande: # 2073482

Figures: figures #1-2-et 3

Unscannable items
received with this application
(Request original documents in File Prep. Section on the 10th floor)

Documents reçu avec cette demande ne pouvant être balayés
(Commander les documents originaux dans la section de préparation des dossiers au 10ème étage)