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(57) Abrégé/Abstract:
Radioactive high molecular weight metal-binding protein compositions and a method for therapeutic radiation treatment including the treatment of rheumatoid arthritis comprising injection of a radioactive high molecular weight metal-binding protein composition are disclosed.

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(54) Title: RADIOLABELED METAL-BINDING PROTEIN FOR THE TREATMENT OF ARTHRITIS

(57) Abstract

Radioactive high molecular weight metal-binding protein compositions and a method for therapeutic radiation treatment including the treatment of rheumatoid arthritis comprising injection of a radioactive high molecular weight metal-binding protein composition are disclosed.

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RADIOLABELED METAL-BINDING PROTEIN FOR THE TREATMENT OF ARTHRITIS

This invention relates to radioactive high molecular weight metal-binding protein compositions and to a method for the treatment of rheumatoid arthritis by administering such radioactive high molecular weight metal-binding protein compositions.

Rheumatoid arthritis is a prevalent disease characterized by chronic inflammation of the synovial membrane lining the afflicted joint. Current treatment methods for severe cases of rheumatoid arthritis include the removal of the synovial membrane, e.g., synovectomy. Surgical synovectomy has many limitations including the risk of the surgical procedure itself, and the fact that a surgeon often cannot remove all of the membrane. The diseased tissue remaining may eventually regenerate, causing the same symptoms which the surgery was meant to alleviate.

Radiation synovectomy is radiation-induced ablation of diseased synovial membrane tissue accomplished by injecting a radioactive compound into the diseased synovium. Early attempts to perform radiation synovectomy were hampered by an instability of the radioactive compounds utilized and by leakage of

such compounds from the synovium into surrounding healthy tissues. The instability of labile radionuclide-complexes resulted in displacement of the radionuclide from the colloid complex and retention of the radionuclide in soft tissues. Significant leakage of the radioactive compound from the injection site exposed normal tissues to dangerous levels of radiation. Because of these limitations, new radiolabeled compositions were sought which would have minimal leakage.

U.S. Patent No. 4,752,464 describes a composition comprising a radioactive colloid in which a radionuclide is entrapped within an iron hydroxide matrix. Radioactive colloids are useful in radiation ablation procedures, for example, ablation of a synovium in rheumatoid arthritis; however, their use may still result in significant leakage of radioactivity from a site of injection, e.g., a synovium, and into the surrounding normal tissues, exposing normal tissues to an undesirable amount of radiation. To compensate for the leakage, a radioactive metal having a short half-life, such as dysprosium (Dy-165) with a half-life of 2.3 hours has been proposed for use as the labeling radionuclide. Because of its short half-life, the majority of Dy-165 radioactivity decays before significant leakage can occur, thereby minimizing the dose of radiation seen by normal tissues.

The use of radioactive metals having a short half-life severely limits the utility of the therapeutic radiation procedure in two significant ways. First, radioactive compositions prepared with short half-life isotopes lose a significant amount of radioactivity because of decay during shipment to distant locations.

Second, to achieve a therapeutic dose of a composition comprising a radioactive metal having a short half-life, large amounts of radioactive materials must be used. As a result, clinical personnel must handle large amounts of radioactive materials.

Therefore, there remains a need for a therapeutic radioactive composition which upon injection into a synovium, would remain at the site of injection, e.g., within a synovium, for a prolonged period of time. Prolonged retention at the site of injection would allow use of radionuclides having a longer half-life in therapeutic procedures, including radiation synovectomy, without fear of significant leakage from the site of injection and radiation exposure to normal tissues.

It has now been found that when radioactive compositions prepared from radionuclides and high molecular weight metal-binding proteins are injected into a synovium, they are retained at the site of injection for a prolonged period of time, without significant leakage of radioactivity. Radioactive compositions prepared from high molecular weight metal--binding proteins may be prepared with radionuclides having longer half-lives than previously used in radiation ablation procedures, greatly minimizing fear of significant leakage from the site of injection and radiation exposure to normal tissues.

The invention includes a compound which comprises a therapeutic radionuclide bound to a metal--binding protein wherein the radionuclide is a beta--emitter with a half-life from 2 hours to 7 days and the protein has a molecular weight of greater than 50,000.

The invention is also directed to a pharmaceutical formulation which comprises as the active ingredient a therapeutic radionuclide bound to a metal-binding protein associated with one or more pharmaceutically acceptable carriers, wherein the radionuclide is a beta-emitter with a half-life from 2 hours to 7 days and the protein has a molecular weight of greater than 50,000.

In another aspect, the invention is a pharmaceutical formulation which comprises as the active ingredient for use as an agent in treating rheumatoid arthritis a protein having a molecular weight of greater than 50,000, a bifunctional chelator covalently attached to the protein, and a therapeutic radionuclide chelated to the bifunctional chelator, wherein the active ingredient is associated with one or more pharmaceutically acceptable carriers and the radionuclide is a beta-emitter having a half-life of 2 hours to 7 days.

In particular, there is provided a pharmaceutical composition for the treatment of rheumatoid arthritis in a subject in need of such treatment, the composition comprising a metal-binding protein, a therapeutic radionuclide bound to the protein, and a physiologically acceptable carrier, wherein the radionuclide is a beta-emitter with a half-life from 2 hours to 7 days and the protein has a molecular weight of greater than 50,000.

According to another aspect of the present invention, there is provided a pharmaceutical composition

comprising a protein having a molecular weight of greater than 50,000, a bifunctional chelator covalently attached to the protein, a therapeutic radionuclide chelated to the bifunctional chelator, and a physiologically acceptable carrier, wherein the radionuclide is a beta-emitter having a half-life of 2 hours to 7 days, for treating rheumatoid arthritis in an animal in need of such treatment.

According to still another aspect of the present invention, there is provided use of a therapeutically effective amount of a pharmaceutical composition comprising a metal-binding protein, a therapeutic radionuclide bound to the protein, and a physiologically acceptable carrier, wherein the radionuclide is a beta-emitter with a half-life from 2 hours to 7 days and the protein has a molecular weight of greater than 50,000, for the treatment of rheumatoid arthritis in a subject in need of such treatment.

According to yet another aspect of the present invention, there is provided use of an effective amount of a pharmaceutical composition, which comprises a protein having a molecular weight of greater than 50,000, a bifunctional chelator covalently attached to the protein, a therapeutic radionuclide chelated to the bifunctional chelator, and a physiologically acceptable carrier, wherein the radionuclide is a beta-emitter having a half-life of 2 hours to 7 days, for treating rheumatoid arthritis in an animal in need of such treatment.

According to a further aspect of the present invention, there is provided use of a metal-binding protein and a therapeutic radionuclide capable of binding to the protein, wherein the radionuclide is a beta-emitter with a half-life from 2 hours to 7 days and the protein has a molecular weight of greater than 50,000, in the preparation

of a medicament for the treatment of rheumatoid arthritis in a subject in need of such treatment.

According to yet another aspect of the present invention, there is provided use of a protein having a molecular weight of greater than 50,000, a therapeutic radionuclide, and a bifunctional chelator capable of being covalently attached to the protein and chelated to the therapeutic radionuclide, wherein the radionuclide is a beta-emitter having a half-life of 2 hours to 7 days, in the preparation of a medicament for treating rheumatoid arthritis in an animal in need of such treatment.

The compositions of the present invention include high molecular weight metal-binding proteins complexed with therapeutic radionuclides. The preferred metal-binding proteins are those having a high molecular weight as determined in the absence of bound metals, preferably those having a molecular weight of greater than 50,000, more preferably greater than 100,000 and most preferably greater than 250,000.

In the phrase "metal-binding protein", the word "metal" designates a metal cation wherein a portion of the metal is radioactive. The term "metal-binding protein" designates a protein which naturally possesses the ability to bind metal cations; the term "binding"

means an attraction between the protein and metal cation, including covalent or ionic bonding, which can be measured by standard techniques after mixing the protein and metal cation in an aqueous solution, such as separation of proteins containing metal cations from non-metal binding proteins by gel permeation chromatography, dialysis, ion exchange chromatography, electrophoresis, high performance liquid chromatography or thin layer chromatography. The term "metal-binding protein" also designates a protein which has been modified by the conjugation of a metal chelating ligand to said protein. The word "protein" includes proteins containing only amino acids connected by peptide linkages and conjugated proteins containing amino acids plus nucleic acids, carbohydrates or lipids. The metal-binding protein is preferably inert to having the metal separate from the protein when used *in vivo*. Biodegradation of the protein will occur over time; but the metal does not readily leach from the protein during the desired treatment time.

Examples of preferred high molecular weight metal-binding proteins include ferritin, transferrin, hemoglobin, ceruloplasmin, hemocyanin, and other proteins which can inherently bind metal cations, as well as proteins modified by the addition of a bifunctional chelator to impart metal-binding capability to the protein. An example of a preferred metal-binding protein is ferritin, an iron storage protein having an approximate molecular weight of 460,000 in the absence of bound metals. Ferritin contains a central structural core capable of binding as many as 4,500 ferric atoms per molecule.

The preferred high molecular weight metal--binding proteins of the present invention also include high molecular weight proteins, such as immunoglobulins, which have been modified to impart metal-binding capability by the addition of a bifunctional chelator. The addition of a bifunctional chelator renders the protein capable of stably binding metals.

A bifunctional chelator is a chemical compound that has a metal chelating moiety, which is capable of sequestering or chelating metals, and a reactive group by which the compound is covalently coupled to a protein. Bifunctional chelators for use in the present invention are those that contain polyaminocarboxylates or polyaminophosphonates as the metal chelating moiety. Preferred are bifunctional chelators which contain cyclic polyaminocarboxylates or cyclic polyaminophosphonates, such as macrocyclic hetero rings of 12 to 16 total atoms in the ring.

Example of compounds known in the art which can be activated and function as bifunctional chelators according to the present invention include substituted 1,4,7-tris-carboxymethyl-1,4,7,10-tetraazacyclododecane and analogs disclosed in published European Patent Application 292689, 2-(p-nitrobenzyl)-1,4,7,10--tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid disclosed in *J. Am. Chem. Soc.* 11, 6206-6207 (1988); PA--DOTA (α-[2-(4-aminophenyl)ethyl]-1,4,7,10--tetraazacyclododecane-1,4,7,10-tetraacetic acid), PA-DOTMA (α-[2-(4-aminophenyl)ethyl]-1,4,7,10--tetraazacyclododecane-1-acetic-4,7,10-tris(methylacetic acid), BA-DOTA (α-(4-aminophenyl)-1,4,7,10--tetraazacyclododecane-1,4,7,10-tetraacetic acid), OMe-BA-DOTA (α-(5-amino-2-methoxyphenyl)-1,4,7,10-

-tetraazacylododecnae-1,4,7,10-tetraacetic acid) and EA-DO_3A (1-[2-(4-aminophenyl)ethyl]-1,4,7,10--tetraazacyclododecnae-4,7,10-triacetic acid) disclosed in PCT application WO 89/12631, published December 28, 1989; 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''--tetraacetic acid, 1,4,7,10-tetraazacyclotridecane-N,N'N'',N'''-tetraacetic acid, 1,4,8,11--tetraazacyclotetradecane-N,N'N'',N'''-tetraacetic acid and 1,5,9,13-tetraazacyclohexadecane-N,N'N'',N'''--tetraacetic acid disclosed in U.S. Patent 4,678,667; and derivatives of diethylenetriaminepentaacetic acid disclosed in U.S. Patent 4,831,175.

Polyamino phosphonates known in the art which can be activated and function as bifunctional chelators are 1,4,7,10-tetraazacyclododenace-1,4,7,10--tetramethylenephosphonic acid and analogs disclosed in U.S. Patent 4,882,142; and the polyamino phosphonates such as 1,3-propanediamine-N-(carboxypropyl)-N,N',N'--trimethylenephosphonic acid, ethylenediamine--N-(4-aminophenethyl)-N,N',N',-trimethylenephosphonic acid, ethylenediaminetetramethylenephosphonic acid, 1-(carboxy)ethylenediaminetetramethylenephosphonic acid, 1-(4-aminobenzyl)ethylenediaminetetramethylenephosphonic acid, N''-(4-aminophenyl)-dipropylenetriamine--N',N',N''',N'''-tetramethylenephosphonic acid and N''-(4-aminophenethyl)-diethylenetriamine--N'N',N''',N'''-tetramethylenephosphonic acid.

The aminophosphonic acids can be prepared by a number of known synthetic techniques. Of particular importance is the reaction of a compound containing at least one reactive amine hydrogen with a carbonyl compound (aldehyde or ketone) and phosphorous acid or derivative thereof as described in U.S. Patent 3,288,846

and described by Moeritzer and Irani, *J.Org.Chem.*, 31, 1603 (1966).

Methods for making bifunctional chelators are well known in the art. In one method, one or more carboxylic acid groups of a polyamine, polycarboxylic acid chelator are activated by conversion to such activating groups as internal or mixed anhydrides, activated esters (e.g., para-nitrophenyl, N-hydroxysuccinimide, etc.) or with other derivatives known to those skilled in the art. The activated acid group is then reacted with the protein. The metal ion is then added to the protein-chelator complex.

Another method for making a bifunctional chelator is to prepare a chelating ligand with a unique reactive group, such as an isothiocyanate, attached to the chelating moiety at a position that does not substantially diminish the strength with which the chelating ligand binds the metal ion. An article by M. W. Brechbiel et al., *Inorg.Chem.* 25, 2772-2781 (1986) is illustrative of this second procedure. Examples of other protein-binding functional groups of bifunctional chelators include electrophilic or nucleophilic moieties such as bromoacetamide, maleimide, imidoester, thiophthalimide, N-hydroxysuccinimyl ester, pyridyl disulfide, phenyl azide, o-acylisourea, anhydride, diazonium, carboxyl, amino, acyl hydrazide, semicarbazide, and thiosemicarbazide groups.

The modification of proteins by the addition of bifunctional chelators may be accomplished by methods known in the art. Generally, these methods include formation of a covalent linkage with an amino acid residue of the protein and a functional group of the

bifunctional chelator which is capable of binding proteins.

Binding of a therapeutic radioactive metal to a high molecular weight protein or protein-chelator conjugate may be accomplished by exposing the metal binding protein to an aqueous solution of the metal ion, at a pH of about 3 to about 12, preferably about 4 to about 9.

The bifunctional chelator may be reacted with a therapeutic radioactive metal and the complex then attached to a protein. Alternatively, a protein may first be modified by the addition of the bifunctional chelator, and the modified protein conjugate then reacted with a therapeutic radioactive metal.

Radionuclides for use in the present invention are beta-emitters with half-lives in the range of from about 2 hours to about 7 days. Preferred radionuclides are the radionuclides Holmium (Ho-166), Samarium (Sm-153), Rhodium (Rh-105), Lutetium (Lu-177), Indium (In-115m), Dysprosium (Dy-165), Yttrium (Y-90), Lanthanum (La-140), Gadolinium (Gd-159), Ytterbium (Yb-175), Rhenium (Re-186), (Re-188) and Scandium (Sc-47). More preferred are the radionuclides Ho-166, Sm-153, Re-186, Re-188, Rh-105, Lu-177, In-115m, and Dy-165.

The respective radionuclides can be produced by methods known in the art. For example, in a nuclear reactor, a nuclide is bombarded with neutrons to obtain a nuclide with additional neutrons in its nucleus. For example:

Ho-165 + neutron = Ho-166 + gamma

Typically, the desired radionuclide can be prepared by irradiating an appropriate target, such as a metal oxide. Another method of obtaining radionuclides is by bombarding nuclides with particles in a linear accelerator or cyclotron. Yet another way of obtaining radionuclides is to isolate them from fission product mixtures.

The ratio of the amount of radioactive metal to the amount of high molecular weight metal-binding protein to be used in the preparation of the compositions of the present invention will vary according to the specific protein to be radiolabeled, its specificity and metal-binding capacity. For example, the metal-binding protein, ferritin has a molecular weight of approximately 460,000 in the absence of bound metals, and in nature one molecule of protein may bind as many as 4,500 ferric atoms. Such a high binding capacity would permit a molar ratio of up to 1:4,500 ferritin:metal. In contrast, the metal-binding protein, transferrin, having an approximate molecular weight of 77,000 binds only 2 ferric atoms per molecule, permitting a molar ratio of 1:2 transferrin:metal.

In general, the bifunctional chelators used in the present invention are capable of binding (chelating) one atom of metal cation per molecule of chelator, and in general will bind one molecule of protein per molecule of chelator. Thus, the molar ratio of chelator-protein conjugate to metal will generally be at least 1:1, protein conjugate:metal. It is possible for one protein molecule to bind more than one bifunctional chelator molecule, and thus increase the ratio of metal

to protein; however, at least a 1:1 conjugate ratio is preferred.

In the radioactive compositions of the present invention, the metal-binding protein need not be saturated, i.e., fully occupied with radioactive metal. A given mass of protein may be complexed with radioactive metal to produce a radioactive composition having a molar ratio of protein to metal of 1 to less than or equal to the binding capacity of the metal--binding protein.

The pharmaceutical compositions of the present invention contain radioactive metals complexed with high molecular weight metal-binding proteins, in a physiologically-acceptable carrier. Examples of suitable physiologically-acceptable carriers include aqueous carriers such as phosphate-buffered saline (PBS), glycols or saline. The pharmaceutical compositions may be administered to a patient for therapeutic treatments by methods known in the art, e.g., intravenously or by injection. For example, a ferritin-Ho-166 composition may be prepared in saline and injected into a joint for radiation synovectomy.

The formulations of the present invention are in solid or liquid form. These formulation may be in kit form such that the various components are mixed at the appropriate time prior to use. Whether premixed or as a kit, the formulations usually require a pharmaceutically-acceptable carrier.

The quantity of the radioactive composition administered to the patient will depend upon several factors including the specific radionuclide, its

specific activity and emissions, the particular type of therapeutic treatment, e.g., type of injection site, duration of therapy desired, and type of disease being treated, and the amount of radioactivity desired at the site of injection.

A therapeutic dosage of radioactivity is that which is sufficient when administered to a patient, to achieve the therapeutic radiation ablation result, for example, the amount sufficient, when injected into the synovium of a patient, to ablate the synovial membrane. In general, the therapeutic dosage will be that which delivers approximately 5 Gy to 1,500 Gy. A more preferred dosage is that which delivers from about 20 Gy to about 500 Gy to the site of injection. Gy is Greys wherein 1 Gy equals 100 rads.

The invention will be further clarified by a consideration of the following examples, which are intended to be purely exemplary of the present invention.

Example 1 Preparation of Ferritin - Sm-153 by Metal Exchange

A 50 µl quantity of ferritin containing bound iron (100 mg/mL, MW 900,000) was added to a vial containing 150 µl of 0.1 M sodium citrate with 10 µL of Sm-153 solution (3×10^{-4} M in 0.1 N HCl) at a pH of 7. This solution was heated for five minutes at 80°C. After heating, 100 µl of the solution was injected into a 10 cm long SEPHADEX™ G-50 gel filtration column (a polysaccharide dextran sold by Pharmacia) and eluted with water, collecting 10 drop fractions in a fraction collector. The amount of activity in each fraction was determined using an NaI-well scintillation counter.

The majority of the radioactivity eluted in an early fraction corresponding to protein-bound Sm-153. A smaller percentage of the radioactivity eluted more slowly and corresponded to a smaller molecule, e.g., Sm-153-citrate.

A volume of 100 µl of the radiolabeled protein (fraction No. 7) was injected into the synovium of the left knee joint of an anesthetized rabbit. A NaI gamma detector was placed above the injected knee joint and the Sm-153 gamma activity remaining in the synovium was determined in counts per minute over a period of 1.6 hours. This procedure was repeated with injection into in the synovium of the right knee of the same rabbit.

The results indicated almost no leakage of radioactivity from the synovium. After corrections for decay, a curve was obtained by plotting the activity as a function of time. The curve was assumed to be a straight line and calculated slopes of -0.08 and -0.26 counts per 30 seconds using a least squares method were obtained for the left and the right knees, respectively.

Example 2 Radiolabeled Ferritin Prepared by Addition Through a Bifunctional Chelant

Into a vial was placed 1 mL of $SmCl_3$ solution (3×10^{-4} M, in 0.1 N HCl). To this was added 2 µL of tracer Sm-153 (3×10^{-4} M Sm in 0.1 N HCl) solution and 10 µl of α-aminophenyl-1,4,7,10-tetraazacyclo-dodecane-1,4,7,10-tetraacetic acid (14.9 mg/mL). The pH was adjusted to 7.5 with NaOH and the mixture was heated for 30 minutes at 100°C. Free metal was removed from the solution by passing it through an ion exchange resin. The percent metal in the complex of the purified

solution was determined by ion exchange chromatography to be 98 percent. To 150 µl of this resultant metal complex solution was added 2 µL of thiophosgene and 150 µL of $CHCl_3$. The solution was shaken for 30 minutes and then extracted with ether to remove the $CHCl_3$. Ion exchange chromatography was used to determine that the percent Sm in the resulting isothiocyanate complex was 98 percent.

A volume of 50 µl of ferritin containing bound iron (1000 mg/mL, MW 900,000) was placed into a vial and 150 µL of HEPES buffer (N-[2-hydroxyethyl]piperazine-N'--[2-ethane sulfonic acid]) was added (pH of 7.4). The pH was adjusted to 8 with NaOH and 100 µL of the bifunctional isothiocyanato-Sm-153 complex was added. The solution was permitted to stand overnight. Gel permeation chromatography as described for Example 1 was used to isolate the radiolabeled protein fraction.

Injection of the resultant radiolabeled protein composition into the synovium of a knee of a rabbit was performed as described for Example 1. The data was recorded and calculated as described for Example 1 and a slope of -0.03 counts per 30 seconds was obtained, again showing little to no leakage of this formulation from the synovium of the rabbit.

Example A Comparison of Radiolabeled Compositions for Use in Radiation Synovectomy

Ferritin was iodinated using NaI-131 and 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril (TDPG), a mediator for protein iodination sold under the trademark "IODOGEN" by Pierce Chemical Company. A 1.4 mg quantity of TDPG was placed in a polypropylene tube and 1.4 mL of chloroform was added. Aliquots of 50 µL (20 µg) were placed into one-gram glass vials and left unstoppered

until the tubes were dry. The prepared TDPG was stored frozen until used. A volume of 50 µL of ferritin (100 mg/mL, MW 900,000) was placed into a vial and 100 mL of 0.2 M phosphate buffer (pH of 7.2) was added. This mixture was then added to a prepared TDPG tube and 200 µL of NaI-131 (959 µCi) was added. After standing 10 minutes, the iodinated solution was passed through a PD-10 column (G-25 gel permeation) and eluted with 0.01 M sodium phosphate buffer. The protein-bound fraction (3.5 to 4.5 mL) was collected.

A rabbit was injected with the collected protein-bound composition and the data obtained and calculated in the same manner as described for Example 1. The retention slope calculated was -20.7 counts per 30 seconds indicating a significant loss of the iodinated radioactivity from the synovium.

The procedure described above was repeated to iodinate the antibody protein B-72.3 (approximate MW, 150,000) (D. Colcher et al. *Cancer Research*, 48, 4597-5603 1988). The iodinated protein was injected into the synovium of a rabbit and the activity determined and calculated as described for Example 1. A calculated slope of -4.2 counts per 30 seconds was observed. When NaI-131 alone was injected into the synovium of the opposite leg, a calculated slope of -155.1 was observed.

These data indicate that the high molecular weight protein ferritin when radiolabeled with a bifunctional chelate (Example 2) or with natural metal adsorption (Example 1) remained in the synovium of the rabbit for a prolonged period of time as compared with iodinated ferritin or iodinated antibody protein (B-72.3), the iodinated proteins showing at least a

16-fold increase in the rate of leakage from the site of injection.

Other embodiments of the invention will be apparent to those skilled in the art from a consideration of this specification or practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

CLAIMS:

1. Use of a therapeutically effective amount of a pharmaceutical composition comprising a metal-binding protein, a therapeutic radionuclide bound to the protein, and a physiologically acceptable carrier, wherein the radionuclide is a beta-emitter with a half-life from 2 hours to 7 days and the protein has a molecular weight of greater than 50,000, for the treatment of rheumatoid arthritis in a subject in need of such treatment.

2. The use of claim 1, wherein the protein is ferritin, transferrin, hemoglobin, ceruloplasmin or hemocyanin.

3. The use of claim 1 or 2, wherein the radionuclide is Ho-166, Sm-153, Re-186, Y-90, La-140, Gd-159, Yb-175, Rh-105, Lu-177, In-115m, Dy-165, Re-188 or Sc-47.

4. The use of claim 1, 2 or 3, wherein the effective amount is a dose that delivers 5 Greys to 1,500 Greys.

5. The use according to any one of claims 1 to 4, wherein the pharmaceutical composition is for administration into the synovium of the subject.

6. Use of an effective amount of a pharmaceutical composition, which comprises a protein having a molecular weight of greater than 50,000, a bifunctional chelator covalently attached to the protein, a therapeutic radionuclide chelated to the bifunctional chelator, and a physiologically acceptable carrier, wherein the radionuclide is a beta-emitter having a half-life of 2 hours to 7 days, for treating rheumatoid arthritis in an animal in need of such treatment.

7. The use of claim 6, wherein the effective amount is a dose that delivers from 5 Greys to 1,500 Greys.

8. The use of claim 6 or 7, wherein the bifunctional chelator contains a polyaminocarboxylate or polyaminophosphonate chelating moiety.

9. The use of claim 6, 7 or 8, wherein the protein is ferritin, transferrin, hemoglobin, ceruloplasmin or hemocyanin.

10. The use of any one of claims 6 to 9, wherein the radionuclide is Ho-166, Sm-153, Re-186, Y-90, La-140, Gd-159, Yb-175, Rh-105, Lu-177, In-115m, Dy-165, Re-188 or Sc-47.

11. The use according to any one of claims 6 to 10, wherein the pharmaceutical composition is for administration into the synovium of the animal.

12. Use of a metal-binding protein and a therapeutic radionuclide capable of binding to the protein, wherein the radionuclide is a beta-emitter with a half-life from 2 hours to 7 days and the protein has a molecular weight of greater than 50,000, in the preparation of a medicament for the treatment of rheumatoid arthritis in a subject in need of such treatment.

13. The use of claim 12, wherein the protein is ferritin, transferrin, hemoglobin, ceruloplasmin or hemocyanin.

14. The use of claim 12 or 13, wherein the radionuclide is Ho-166, Sm-153, Re-186, Y-90, La-140, Gd-159, Yb-175, Rh-105, Lu-177, In-115m, Dy-165, Re-188 or Sc-47.

15. The use of claim 12, 13 or 14, wherein the medicament is used in a dose that delivers 5 Greys to 1,500 Greys.

16. The use according to any one of claims 12 to 15, wherein the medicament is for administration into the synovium of the subject.

17. Use of a protein having a molecular weight of greater than 50,000, a therapeutic radionuclide, and a bifunctional chelator capable of being covalently attached to the protein and chelated to the therapeutic radionuclide, wherein the radionuclide is a beta-emitter having a half-life of 2 hours to 7 days, in the preparation of a medicament for treating rheumatoid arthritis in an animal in need of such treatment.

18. The use of claim 17, wherein the medicament is used in a dose that delivers from 5 Greys to 1,500 Greys.

19. The use of claim 17 or 18, wherein the bifunctional chelator contains a polyaminocarboxylate or polyaminophosphonate chelating moiety.

20. The use of claim 17, 18 or 19, wherein the protein is ferritin, transferrin, hemoglobin, ceruloplasmin or hemocyanin.

21. The use of any one of claims 17 to 20, wherein the radionuclide is Ho-166, Sm-153, Re-186, Y-90, La-140, Gd-159, Yb-175, Rh-105, Lu-177, In-115m, Dy-165, Re-188 or Sc-47.

22. The use according to any one of claims 17 to 21, wherein the medicament is for administration into the synovium of the animal.

23. A pharmaceutical composition for the treatment of rheumatoid arthritis in a subject in need of such treatment, the composition comprising a metal-binding protein, a therapeutic radionuclide bound to the protein, and a physiologically acceptable carrier, wherein the radionuclide is a beta-emitter with a half-life from 2 hours to 7 days and the protein has a molecular weight of greater than 50,000.

24. The composition of claim 23, wherein the protein is ferritin, transferrin, hemoglobin, ceruloplasmin or hemocyanin.

25. The composition of claim 23 or 24, wherein the radionuclide is Ho-166, Sm-153, Re-186, Y-90, La-140, Gd-159, Yb-175, Rh-105, Lu-177, In-115m, Dy-165, Re-188 or Sc-47.

26. The composition of claim 23, 24 or 25, wherein the composition is used in a dose that delivers from 5 Greys to 1,500 Greys.

27. The composition according to any one of claims 23 to 26, which is for administration into the synovium of the subject.

28. A pharmaceutical composition comprising a protein having a molecular weight of greater than 50,000, a bifunctional chelator covalently attached to the protein, a therapeutic radionuclide chelated to the bifunctional chelator, and a physiologically acceptable carrier, wherein the radionuclide is a beta-emitter having a half-life of 2 hours to 7 days, for treating rheumatoid arthritis in an animal in need of such treatment.

29. The composition of claim 28, wherein the bifunctional chelator contains a polyaminocarboxylate or polyaminophosphonate chelating moiety.

30. The composition of claim 28 or 29, wherein the protein is ferritin, transferrin, hemoglobin, ceruloplasmin or hemocyanin.

31. The composition of claim 28, 29 or 30, wherein the radionuclide is Ho-166, Sm-153, Re-186, Y-90, La-140, Gd-159, Yb-175, Rh-105, Lu-177, In-115m, Dy-165, Re-188 or Sc-47.

32. The composition of any one of claims 28 to 31, wherein the composition is used in a dose that delivers from 5 Greys to 1,500 Greys.

33. The composition according to any one of claims 28 to 32, which is for administration into the synovium of the animal.

34. A kit for treating rheumatoid arthritis which comprises in a first compartment a radionuclide and in a second compartment a metal-binding protein wherein the radionuclide is a beta-emitter with a half-life from 2 hours to 7 days and the protein has a molecular weight of greater than 50,000.

35. The kit of claim 34, which further comprises a pharmaceutically acceptable carrier.

36. The kit of claim 34 or 35, wherein the protein is ferritin, transferrin, hemoglobin, ceruloplasmin or hemocyanin.

37. The kit of claim 34, 35 or 36, wherein the radionuclide is Ho-166, Sm-153, Re-186, Y-90, La-140, Gd-159, Yb-175, Rh-105, Lu-177, In-115m, Dy-165, Re-188 or Sc-47.