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(54) Title: COMPOSITIONS AND METHOD FOR SOFT TISSUE TUMORS

(57) Abstract

Compositions and method for therapeutic and/or diagnostic treatment of soft tissue tumors in mammals using certain metal ions or particle-emitting radionuclides complexed with a phenolic carboxylate ligand is described.

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COMPOSITIONS AND METHOD FOR SOFT TISSUE TUMORS

This invention concerns compositions, and a method of treating and/or diagnosing soft tissue tumors in mammals with metal-phenolic carboxylate ligand complexes and their formulations.

Metal ligand complexes are routinely used for medicinal applications. For example, gadolinium complexes (gadolinium-diethylenetriaminepentaacetic acid, Gd-DTPA) are used to enhance the quality of magnetic resonance imaging. Gd-DTPA has been utilized in studying abnormalities of the gastrointestinal tract, liver, and kidneys as well as visualizing heart infarcts. [See I. K. Adzaml., *J.Nucl.Med.* 32, 139 (1989).] When radioactive metal ions are used, diagnostic imaging or therapy can be the end objective. Thus ^{99m}Tc, a pure gamma emitter, in the form of a metal ligand complex is routinely used as a diagnostic agent. In some cases, such as the use of ^{99m}Tc-DTPA, injection of the complex into the bloodstream does not result in the radionuclide localizing in any tissue. Instead, the radionuclide is eliminated from the body by the kidneys into the urine. In other cases, the radionuclide does localize in desired specific organs or tissues. Thus specific ^{99m}Tc-phosphonic acid complexes localize in bone [*Radiology* 149, 823-828 (1983)] and one of the uses of ^{99m}Tc-phosphonic acid complexes is the detection of calcific tumors.

More recently, similar chemistry has been used to deliver particle emitting radionuclides to calcific tumors. The aim of these agents is to deliver a therapeutic radiation dose to the site of the tumor. This type of agent takes advantage of fast bone turnover for its localization. Thus Deutsch et al. [*Radiology* 166, 501-507 (1988)] have proposed a rhenium-diphosphonate for the treatment of bone cancers and Simon et al. (U. S. Patent 4,898,724) have taught the use of rare earth radionuclides with aminophosphonic acids towards the same objective.

The specific delivery of metals to soft tissue (i.e. non-calcific) tumors has also been an objective for scientists. Anghilery in *Nuklearmedizin* 23, 9-14 (1984) describes the difficulty in achieving this objective when he states that "there are no fundamental qualitative differences in the structural, biochemical and functional characteristics of a tumor compared to the normal cell." With the advent of monoclonal antibodies, a plethora of activity has emerged using these proteins to deliver radionuclides to soft tissue tumors [e.g. A. R. Fritzberg et al., *Pharm.Res.* 5(6), 325 (1988)]. Bifunctional chelating agents were developed to bind the metal ions to the monoclonal antibody through a chelating agent (the metal-ligand-antibody moiety is termed a "conjugate") and many such conjugates have emerged. Some conjugates use gamma emitters such as ^{99m}Tc or ^{111}In for imaging (see for example U.S. Patents 4,454,106, 3,994,966, 4,662,420 and 4,479,930); and others propose a conjugate with particle emmiters such as ^{67}Cu [see for example J.-C. Roberts et al., *Appl. Rad. Isotopes* 40(9), 775 (1989)] or ^{90}Y [see for example *J. Nucl. Med.* 26(5), 503 (1985)] for therapy. It was believed that the use of the conjugates provided the answer to the site specific delivery of a radioactive metal ion to soft tissue tumors. However, in the practice of the use of these conjugates a series of problems has been observed. For example, problems have been noted involving the fragile nature of the antibody, the slow clearance of the radioactivity from the blood stream, the uptake of radioactivity in non-target tissues such as liver and kidney, and the potential of an immune response of the patient to the injected protein.

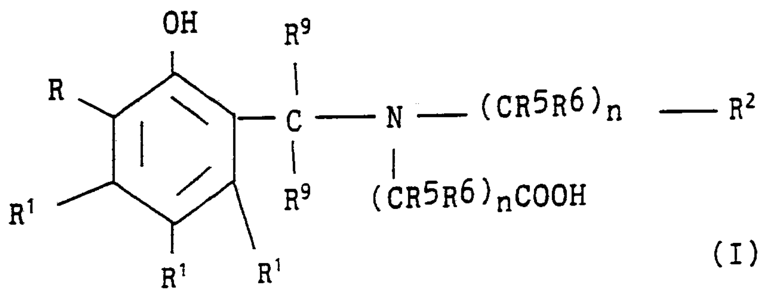
Another approach to delivering metals to soft tissue tumors is by means of a metal ligand complex. Although this approach has not been pursued in the recent literature, it has received extensive attention in earlier literature. The recognition by Andrews et al. in *Radiology* 61, 570-599 (1953) that Ga^{+3} had a tendency to localize in soft tissue tumors led to the development of ^{67}Ga-citrate as a tumor imaging agent [R. L. Hayes, *Int. J. Nucl. Med. Biol.* 10(4), 257-251 (1983)]. Although ^{67}Ga-citrate is presently used for detecting abscesses more than for tumor diagnosis, many clinicians prefer to use it over the monoclonal antibodv conjugates for diagnosis. Even though ^{67}Ga-citrate is widely used, it has various disadvantages. For example, the rate of blood clearance is slow so that images are taken as much as 48 hours post injection with ^{67}Ga-citrate [see *Int. J. Appl. Nucl. Med. Biol.* 8, 249-255 (1984)]. In addition, high uptake of the ^{67}Ga-citrate in non-target tissues make images difficult to interpret [see *Curr. Concepts in Diagn. Nucl. Med.* 1(4), 3-12 (1984)].

In attempts to obtain more useful complexes for delivery of metal ions to soft tissue tumors, certain aminocarboxylic acid complexes have been used. For example, Karube et al. in *Chem. Pharm. Bull.* 30(7), 2529-2533 (1982) found that ^{99m}Tc-ethylenediaminediacetic acid (EDDA) and ^{57}Co-EDDA could be used to image tumors in experimental aminals bearing Ehrlich tumors. However, ^{99m}Tc complexes with other ligands were less effective. Some of the ligands tested with ^{99m}Tc were iminodiacetic acid (IDA), methyliminodiacetic acid (MIDA), nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), and hydroxyethylethylenediaminetriacetic acid (HEDTA). Woolfenden et al. in *Int. J. Nucl. Med.* 10(4), 251-256 (1983) found that ^{153}Sm-citrate and ^{153}Sm-chloride had a high liver uptake and suggested the use of higher stability chelates, such as ^{153}Sm-EDTA, could improve the tumor to liver ratio. More recently, J. Harvey Turner in *Eur. J. Nucl. Med.* 13, 432-438 (1987) studied ^{153}Sm chelates including HEDTA. The ^{153}Sm-HEDTA chelates used a 20 to 1 HEDTA to Sm molar ratio and found tumor uptake to be significantly less than that of ^{67}Ga-citrate. An unacceptably large liver uptake was noted when using ^{153}Sm-HEDTA at these ratios. He concluded that "it is unlikely that effective therapy doses of Sm-153 can be delivered to melanoma tumors by these and similar chelates." He suggested the use of monoclonal antibodies with ^{153}Sm. Another attempt to have complexes deliver metal ions to soft tissue tumors was made by Tse et al. in *J. Nucl. Med.* 30, 202-208 (1989) where they studied ^{153}Sm-EDTA at a 10 to 1 ligand to metal molar ratio. These researchers proved that the complex was stable and compared the use of high specific activity ^{153}Sm (1.7 Ci/mG) to low specific activity ^{153}Sm (1.1 mCi/mG) in mice bearing Lewis lung carcinoma. They propose using the complex as an imaging agent using the high specific activity ^{153}Sm. However, as J. Harvey Turner had reported for ^{153}Sm-HEDTA, these researchers also found significant uptake in the liver as shown by their biodistribution and images.

Therefore, there is still a need for an adaquate system to deliver radionuclides selectively to soft tissue tumors. Surprisingly, it has now been found that various metal-ligand complexes wherein the ligand is an aminocarboxylate containing a phenolic moiety, particularly the metal-Bis-IDA complex, preferably with a high ligand to metal ratio, such as at least 50:1, give good soft tissue tumor localization with no significant liver uptake and can be used as diagnostic or therapeutic agents.

The present invention concerns metal-ligand complexes wherein the ligand is an aminocarboxylate containing a phenolic moiety, their formulations, and a method for the therapeutic and/or diagnostic treatment of a mammal having soft tissue tumors.

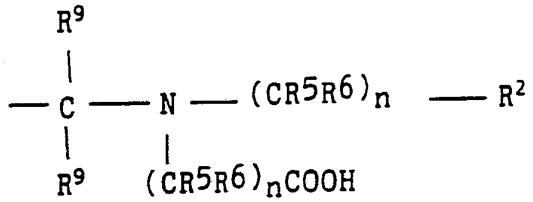
The metal-phenolic carboxylate ligand complex of the present invention has as the metal ion ^{153}Sm, ^{166}Ho, ^{90}Y, ^{159}Gd, ^{177}Lu, ^{111}In, ^{115m}In, ^{175}Yb, ^{47}Sc, ^{165}Dy, ^{52}Fe, ^{72}Ga, ^{67}Ga, ^{68}Ga, Gd, or Fe and has as the ligand a compound of the formula



(I)

wherein:

R represents hydrogen, -COOH, R^1 or



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R^1 independently represents hydrogen, C_1-C_{18} alkyl, -Cl, -Br, -I, $-N(R^4)_2$, $-NR^8C(O)CR^5R^6R^7$, $-N(OR^5)C(O)R^6$, $-N(O)R^5R^6$, $-NR^5-NR^6R^7$, $-NR^5C(O)NR^6R^7$, $-NO_2$, $-OR^5$, $-OC(O)R^5$, $-OC(O)OR^5$, -CN, $-SO_3H$, $-SO_2NR^5R^6$, $-NR^5SO_2R^6$, $-NR^5-OR^7$, $-C=NR^5$, $-N=CR^5R^6$, -NCS, -N=O, or $-N^+R^5R^6R^7$ and where R^1 can be interconnected with another R^1 to form a saturated or unsaturated ring;

where R^4 represents hydrogen, C_1-C_{18} alkyl, or $-CO(CH_2)_yCH_3$, where y is an interger from 0 to 17;

R^5, R^6, R^7 and R^8 independently represent hydrogen, C_1-C_{18} alkyl, aryl or aryl-C_1-C_4 alkyl;

R^2 represents -COOH, $-CH_2OH$ or $-CH(CH_3)OH$;

R^9 independently represents hydrogen, -COOH, C_1-C_{18} alkyl, aryl or aryl-C_1-C_4 alkyl;

n is an intereger of 1, 2 or 3;

or a pharmaceutically acceptable salt thereof.

The formulations have the above complexes with a physiologically acceptable liquid carrier.

The method of the present invention concerns the use of the formulations for the therapeutic and/or diagnostic treatment of a mammal having a soft tissue carcinoma.

The method of this invention is used for the therapeutic and/or diagnostic treatment of a mammal having soft tissue tumors. The compositions used in the method have a radionuclide or metal complexed with a chelating agent. As will be more fully discussed later, the properties of the radionuclide, of the chelating agent and of the complex formed therefrom are important considerations in determining the effectiveness of any particular composition employed for such treatment.

For the purposes of this invention, the term "tumor" shall denote a neoplasm, a new abnormal growth of tissue that is not inflammatory, which arises without obvious cause from cells of preexistent tissue, ana generally possesses no physiologic function. Examples may include "carcinomas" which originate from epithelial cells, "sarcomas" of mesodermal (connective tissue) orgin, and lymphomas from the lymphatic system. The origin of the neoplasm is not critical to this invention.

The term "alkyl" includes the straight or branched-chain alkyl moieties, such as methyl, ethyl, isopropyl, n-propyl, n-butyl, t-butyl, n-decyl as well as others. The "aryl" includes phenyl, naphthyl, or benzyl. The term "aryl-C1-C4 alkyl" includes tolyl, or 2,4-dimethylphenyl. The R^1 joined "saturated or unsaturated ring" has a total of six carbon atoms and is a phenyl or cyclohexyl moiety having one common side of the ring with the phenolic ring.

As used herein, "complex" refers to a chelating agent complexed with a metal ion, preferrably a +3 metal ion, especially a radioactive rare-earth type metal ion, wherein at least one metal atom is chelated or sequestered; "radioactive" when used in conjunction with the word "metal ion" refers to one or more isotopes of the rare-earth type elements that emit particles and/or photons. The term "radionuclide" or "metal" indicates the metal ion. When the ligand to metal ratio is discussed, the ratio is molar. The metal ligand complexes of this invention can consist of a formulation having the combination of 1 metal with 1 ligand in the form of a complex and having one or more complexes comprised of a different metal and/or different ligand, present in the same formulation. An example of this would be combining one metal ion that is a gamma emitting radionuclide for imaging with a ligand and also having present another metal that is a particle emitter for therapy with the same or different ligand. The combination of radionuclides may be more efficaious than either radionuclide alone. These combinations of complexes may be prepared by administrating two complexes at about the same time to the mammal, or making each complex separately and mixing them prior to use, or mixing the two metal ions with the same ligand and preparing the two or more complexes concurrently.

The radionuclide used in the complex of the present invention may be suitable for therapeutic, diagnostic or both therapeutic and diagnostic purposes. Examples of the radionuclide used for diagnostic purposes are Fe, Gd, ^{111}In, ^{67}Ga, or ^{68}Ga, especially preferred are ^{111}In, or ^{67}Ga. Examples of the radionuclide used for therapeutic purposes are ^{166}Ho, ^{165}Dy, ^{90}Y, ^{115m}In, ^{52}Fe, or ^{72}Ga, with ^{166}Ho and ^{90}Y being preferred. Examples of the radionuclides used for both therapeutic and diagnostic purposes the radionuclide used is ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{175}Yb, or ^{47}Sc, with ^{153}Sm, ^{177}Lu, ^{175}Yb, ^{159}Gd being preferred.

Radionuclides can be produced in several ways. In a nuclear reactor, a nuclide is bombarded with neutrons to obtain a radionuclide, e.g.

$$\text{Sm-152} + \text{neutron} \longrightarrow \text{Sm-153} + \text{gamma.}$$

Another method of obtaining radionuclides is by bombarding nuclides with linear accelerator or cyclotron-produced particles. Yet another way of obtaining radionuclides is to isolate them from fission product mixtures. The method of obtaining the radionuclide is not critical to the present invention.

To irradiate Sm_2O_3 for production of Sm-153, the desired amount of target is first weighed into a quartz vial, the vial is flame sealed under vacuum and welded into an aluminum can. The can is irradiated for the desired length of time, cooled for several hours and opened remotely in a hot cell. The quartz vial is removed and transferred to a glove box, crushed into a glass vial which is then sealed with a rubber septum and an aluminum crimp cap. One milliliter of 1-4 M HCl is then added to the vial via syringe to dissolve the Sm_2O_3. Once dissolved, the solution is diluted to the appropriate volume by addition of water. The solution is removed from the original dissolution vial which contains shards of the crushed quartz vial and transferred via syringe to a clean glass serum vial. This solution is then used for complex preparation. Similar procedures are used to prepare ^{177}Lu, ^{159}Gd, and ^{166}Ho. All radionuclides for this invention are either available commercially or are available from the reactor at the University of Missouri at Columbia.

When aqueous solutions of metal ions are mixed with solutions containing the ligand of Formula I, a complex between the metal ion and the ligand can be formed as shown by the equation below.

$$M + L \rightleftharpoons M \cdot L$$

The reaction is believed to be in equilibrium such that the concentrations of metal (M) and complexing agent, or ligand (L), can affect the concentration of species present in solution. Competing side reactions, such as metal hydroxide formation, can also occur in aqueous solution, thus

$$xM + yOH^{-} \longrightarrow M_x(OH)_y.$$

The OH^- concentration in solution, which is related to pH is, therefore, an important parameter to be considered. If the pH is too high, the metal tends to form metal hydroxides rather than complexes. The complexing agents may also be adversely affected by low pH. Complexation may require the loss of proton(s); therefore at low pH, conditions may not be favorable for complexation to occur. Consideration must be given to the solubility characteristics of the ligand, radionuclide, and complex. Although not limited thereto, a pH in the range of from 5 to 11 is preferred for complexation.

The chelating agent is a compound of Formula I as defined above, or a pharmaceutically acceptable salt thereof. The compounds of Formula I may be prepared readily by methods known to those skilled in the art of organic synthesis, such as the process shown in published European application 0,367,223 on May 9, 1990.

For the purpose of the present invention, the complexes described herein and physiologically acceptable salts thereof are considered equivalent in the therapeutically effective compositions. Physiologically acceptable salts refer to the acid addition salts of those bases which will form a salt with at least one acid group of the ligand employed and which will not cause a significant adverse physiological effect when administered to a mammal at dosages consistent with good pharmacological practice. Suitable bases include, for example, the alkali metal and alkaline earth metal hydroxides, carbonates, and bicarbonates such as sodium hydroxide, potassium hydroxide, calcium hydroxide, potassium carbonate, sodium bicarbonate, magnesium carbonate and the like, ammonia, primary, secondary and tertiary amines and the like. Physiologically acceptable salts may be prepared by treating the acid with an appropriate base.

The metal and ligand may be combined under any conditions which allow the two to form a complex. Generally, mixing in water at a controlled pH (the choice of pH is dependent upon the choice of metal) is all that is required. Most of the complexes employed in this invention were prepared as follows: the desired amount of ligand of Formula I was placed in a vial and dissolved by addition of water. At some higher ligand concentrations, it was necessary to add base in order to completely dissolve the ligand. Heating was also found in some cases to be useful for dissolving the ligands. The appropriate amount of the samarium, or other radionuclide, in the stock solution described above was then added to the ligand solution. The pH of the resulting solution was then adjusted to the appropriate level (usually 7-8). Additionally, the complex used in this invention may be a mixture of the different metals as described under the complex term before.

In the method of this invention, it is preferred to employ the complex in the presence of an excess of ligand. The ligand to metal ratio (L:M) of the ligand to radionuclide or metal is at least 1:1. The upper limit of L:M depends on the toxicity of the ligand or the specific activity of the radionuclide. The preferred range for the L:M ratio is at least 1:1, preferrably from 1:1 to about 600:1, more preferably from 1:1 to about 300:1, most preferrably from about 100:1 to about 300:1. When the radionuclide is used in the no carrier added form, then the upper L:M range could be significantly higher, such as $5X10^7$:1.

As used herein, the term "mammal" means animals that nourish their young with milk secreted by mammary glands, preferably warm blooded mammals, more preferably humans.

As used herein, "pharmaceutically acceptable salt" means any salt of a compound of formula (I) which is sufficiently non-toxic to be useful in therapy or diagnosis of mammals. Thus, the salts are useful in accordance with this invention. Representative of those salts, which are formed by standard reactions, from both organic and inorganic sources include, for example, sulfuric, hydrochloric, phosphoric, acetic, succinic, citric, lactic, maleic, fumaric, palmitic, cholic, palmoic, mucic, glutamic, d-camphoric, glutaric, glycolic, phthalic, tartaric, formic, lauric, steric, salicylic, methanesulfonic, benzenesulfonic, sorbic, picric, benzoic, cinnamic acids and other suitable acids. Also included are salts formed by standard reactions from both organic and inorganic sources such as ammonium, alkali metal ions, alkaline earth metal ions, and other similar ions. Particularly preferred are the salts of the compounds of formula (I) where the salt is potassium, sodium, ammonium, or mixtures thereof.

The formulations of the present invention are in the solid or liquid form containing the active radionuclide complexed with the ligand. These formulations may be in kit form such that the two components (i.e. ligand and metal) are mixed at the appropriate time prior to use. Whether premixed or as a kit, the formulations usually require a pharmaceutically acceptable carrier.

Injectable compositions of the present invention may be either in suspension or solution form. In the preparation of suitable formulations it will be recognized that, in general, the water solubility of the salt is greater than the acid form. In solution form the complex (or when desired the separate components) is dissolved in a physiologically acceptable carrier. Such carriers comprise a suitable solvent, preservatives such as benzyl alcohol, if needed, and buffers. Useful solvents include, for example, water, aqueous alcohols, glycols, and phosphate or carbonate esters. Such aqueous solutions contain no more than 50 percent of the organic solvent by volume.

Injectable suspensions are compositions of the present invention that require a liquid suspending medium, with or without adjuvants, as a carrier. The suspending medium can be, for example, aqueous polyvinylpyrrolidone, inert oils such as vegetable oils or highly refined mineral oils, or aqueous carboxymethylcellulose. Suitable physiologically acceptable adjuvants, if necessary to keep the complex in suspension, may be chosen from among thickeners such as carboxymethylcellulose, polyvinylpyrrolidone, gelatin, and the alginates. Many surfactants are also useful as suspending agents, for example, lecithin, alkylphenol, polyethylene oxide adducts, napthalenesulfonates, alkylbenzenesulfonates, and the polyoxyethylene sorbitan esters.

Many substances which affect the hydrophilicity, density, and surface tension of the liquid suspension medium can assist in making injectable suspensions in individual cases. For example, silicone antifoames, sorbitol, and sugars are all useful suspending agents.

An "effective amount" of the formulation is used for therapy. The dose will vary depending on the disease being treated. Although *in vitro* diagnostics can be performed with the formulations of this invention, *in vivo* diagnostics are also contemplated using formulations of this invention. The invention described herein provides a means of delivering a therapeutic amount of radioactivity to soft tissue tumors. However, it may also be desirable to administer a "sub-therapeutic" amount to determine the fate of the radionuclide using a scintillation camera prior to administering a therapeutic dose or if diagnostic images are the desired result. Therapeutic doses will be administered in sufficient amounts to reduce pain and/or inhibit tumor growth and/or cause regression of tumors and/or kill the tumor. Amounts of radionuclide needed to provide the desired therapeutic dose will be determined experimentally and optimized for each particular composition. The amount of radioactivity required to deliver a therapeutic dose will vary with the individual composition employed. The composition to be administered may be given in a single treatment or fractionated into several portions and administered at different times. Administering the composition in fractionated doses may make it possible to minimize damage to non-target tissue. Such multiple dose administration may be more effective.

The compositions of the present invention may be used in conjunction with other active agents and/or ingredients that enhance the therapeutic effectiveness of the compositions and/or facilitate easier administration of the compositions.

Studies to determine the qualitative biodistribution of the various radionuclides were conducted by injecting the compositions into miniature pigs having melanotic lesions, which lesions occur spontaneously. ^{67}Ga-citrate was used as the control and was given by the same route of administration as the test samples.

While not wishing to be bound by theory, it is believed that the advantageous results of the present invention are obtained because of the possible uptake preferentially in the tumor. The mechanism of uptake of the radionuclide by neoplastic tissue is not clear. Some suggested mechanisms are:

a) An imbalance between arterial blood supply to the tumor and venous drainage from the tumor. A reduced venous drainage would result in an increase in concentration of the material within the tumor mass.

b) Lymphatic drainage from a tumor may be decreased.

c) Non-specific binding to protein within the tumor may occur.

d) Because inflammatory reaction is usually present near a tumor, this may result in the differential concentration of radiolabel within the tumor.

e) Metallothionein, a protein binder of heavy metals, may occur with some tumors.

f) Several mechanisms may be involved.

Although the theory for the mechanism of action of a complex is still unknown, the present invention provides a complex which allows a radionuclide to localize in the tumor and displays low radionuclide uptake in other tissues, e.g. liver.

The following definitions are provided for some terms that are used throughout this text.

Glossary:

Conc. = concentrated
G = grams
mG = milligrams
mCi = milliCuries
IDA = iminodiacetic acid
Bis-IDA = 2,6-bis[N,N-bis(carboxymethyl)-aminomethyl]-4-(acetamido)phenol
Bis-IDA-NH_2 = 2,6-bis[N,N-bis(carboxymethyl)-aminomethyl]-4-(amino)phenol
Mono-IDA = 2-[N,N-bis(carboxymethyl)-aminomethyl]-4-(acetamido)phenol
Sm = Samarium
Ho = Holmium
Yb = Ytterbium
Y = Yttrium
Gd = Gadolinium
Lu = Lutetium
In = Indium
Sc = Scandium
Fe = iron

Ga = Gallium
chelant is equivalent to ligand
complex is equivalent to chelate, and
L:M = ligand to metal molar ratio.

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

Preparation of Starting Materials

Example A : Preparation of Bis-IDA

Into a flask equipped with a water-cooled reflux condenser, mechanical stirrer, therometer with a temperature controller, and addition funnel, was added 50 G of deionized water, 64.0 G (0.47 mole) of 89% IDA, and 60.1 G of 50% aqueous NaOH. The mixture was heated, with stirring, to a temperature of 55°C. Aqueous 37% formaladehde solution, 35.0 G (0.43 mole), was placed in the addition funnel and added to the mixture over 30 minutes. The mixture was heated at 55°C for one hour, cooled and transferred to an addition funnel. To a 500 mL flask, equiped as before, was added 31.0 G (0.20 mole) of 98% 4-acetamidophenol, 50 G of deionized water, and 15.5 G of 50% aqueous NaOH solution. The mixture was heated, with stirring, to a temperature of about 65°C, and the formaldehyde-IDA adduct solution added over one hour. The reaction mixture was heated at 65°C for an additional 12 hours and cooled. Conc. HCl, 96 G, was added and the reaction mixture stirred for one hour. The solution was allowed to stand for serveral weeks and the crystalline solids filtered, washed with deionized water and dried in a vacuum oven at 65°C for several hours. Approximately 48 G of bis-IDA were recovered which structure was confirmed by proton and carbon NMR.

Example B: Preparation of Mono-IDA

Into a flask equipped with a water-cooled reflux condenser, mechanical stirrer, therometer with a temperature controller, and addition funnel, was added 35.3 G of deionized water, 35.3 G (0.26 mole) of 89% IDA, and 39.9 G of 50% aqueous NaOH. The mixture was heated, with stirring, to a temperature of 55°C. Aqueous 37% formaladehde solution, 21.5 G (0.27 mole), was placed in the addition funnel and added to the mixture over 15 minutes. The mixture was heated at 55°C for 45 minutes, cooled and transferred to an addition funnel. To a 500 mL flask, equiped as before, was added 38.7 G (0.25 mole) of 98% 4-acetamidophenol, 35.3 G of deionized water, and 12.2 G of 50% aqueous NaOH solution. The mixture was heated, with stirring, to a temperature of about 65°C, and the formaldehyde-IDA adduct solution added over 30 minutes. The reaction mixture was heated at 65°C for an additional 12 hours and cooled. Conc. HCl, 55.5 G, was added and the reaction mixture stirred for one hour. The solution was allowed to stand for serveral weeks and the crystalline solids filtered, washed with deionized water, and dried in a vacuum oven at 65°C for several hours. Approximately 17.5 G of mono-IDA were recovered which structure was confirmed by proton NMR.

Example C : Preparation of Bis-IDA-NH_2

Into a flask equipped with a water-cooled condenser, mechanical stirrer, and heating mantel, was added 5.10 G of Bis-IDA, prepared in Example A. To the flask was added 21.0 G of conc. HCl. The resulting solution was refluxed with stirring for one hour. The reaction mixture was evaporated to dryness *in vacuo* to yield 5.78 G, as pale yellow crystals, of Bis-IDA-NH_2 as the hydrochloride salt. The structure was confirmed by proton and carbon NMR.

Preparation of Final Products

Example 1

Bis-IDA, 43.0 mG, was weighed into a 5 mL glass vial and 2.38 mL of a $3X10^{-4}$M solution of $SmCl_3$ in 0.1M HCl and 0.62 mL of a $3X10^{-4}$M solution of $^{153}SmCl_3$ in 0.1M HCl was added. The pH was adjusted to 7-8 using 50% NaOH. The activity of the final solution was about 3.5 mCi in about 3.0 mL with a ligand to metal ratio of about 300:1.

Example 2

Mono-IDA, 90.0 mG, was weighed into a 5 mL glass vial and 2.5 mL of a $3X10^{-4}$M solution of $SmCl_3$ in 0.1M HCl and 0.5 mL of $3X10^{-4}$M solution of $SmCl_3$ in 0.1M HCl was added. The pH was adjusted to 7-8 using 50% NaOH. The activity of the final solution was about 3.9 mCi in about 3.0 mL with a ligand to metal ratio of about 300:1.

Example 3

Bis-IDA, 28.6 mG, was weighed into a 5 mL glass vial and 2.0 mL of a $3X10^{-4}$M solution of $SmCl_3$ in 0.1M HCl and containing a tracer amount of ^{153}Sm. The pH was raised to 13-14 using 50% NaOH. The pH was then adjusted to 7-8 using HCl. The activity of the final solution was about 2 mCi/mL with a ligand to metal ratio of 100:1.

Example 4

Bis-IDA, 5.7 mG, was weighed into a 5 mL glass vial and 2.0 mL of a $3X10^{-4}M$ solution of $SmCl_3$ in 0.1M HCl and and containing a tracer amount of ^{153}Sm. The pH was adjusted to 7-8 by the procedure described in Example 3. The activity of the final solution was about 4 mCi in about 2 mL with a ligand to metal ratio of 20:1.

Example 5

Bis-IDA-NH_2, 117.7 mG, was weighed into a 5 mL glass vial and 2.0 mL of a $3X10^{-4}M$ solution of $SmCl_3$ in 0.1M HCl and and containing a tracer amount of ^{153}Sm. The pH was adjusted to 7 using NaOH solution (85 µL of 50% NaOH). The activity of the final solution was about 2 mCi in about 1 mL with a ligand to metal ratio of 300:1.

Example 6

Bis-IDA, 5.458 G, was weighed into a 100 mL beaker and 44.95 mL of a 1.0M solution of NaOH was added with stirring. After the solid was completely dissolved, the yellow solution was quantitatively transferred to a 50 mL volumetric flask and brought to volume with deionized water. The solution was filtered through a 0.22 µm filter into a 100 mL beaker. This solution was dispensed ionto 10 mL serum vials (2.3 mL in each vial). Each vial contained 0.54 mmol of Bis-IDA and 2.043 mmol of NaOH. Twenty vials were filled, the contents frozen and freeze-dried, then sealed under vacuum to form kits.

To reconstitute a kit, 6.0 mL of a $3X10^{-4}M$ solution of $SmCl_3$ in 0.1M HCl and containing the desired amount of ^{153}Sm, typically 20-30 mCi, is added via syringe. The vial is shaken to assure dissolution and the final pH is verified to be 7 to 8. The kit is then ready for injection into the animal.

Biological Examples

Example I

The solutions prepared in Examples 1-6 were injected I.V. into miniature pigs with naturally occurring melanotic lesions. The sample solution used for injection was from 0.5-1mL containing 1-2 mCi of ^{153}Sm. The pigs had whole body counts immediately after injection and again at 24, 48 and 72 hours. Images (right lateral, left lateral and dorsal) were made at 4, 24, 48 and 72 hours.

The 24 hour images were evaluated independently by 3 investigators for the uptake of ^{153}Sm in bone, liver and tumor. The following scheme was used for scaling the uptake of ^{153}Sm in the various tissues:

0 - No discernable uptake
1 - Slight uptake (negligible)
2 - Moderate uptake (intermediate)
3 - Definite uptake (high)

The average of the 3 investigators independent scores were used in the following table.

TABLE I

Compound Example	Ligand/ Metal Ratio	% Whole Body Retention (24 hrs.)	Bone Up-take	Liver Up-take	Tumor Uptake (Location)
4[a]	20	55.2	2.67	3.00	0.00 (Left carpus) 0.00 (Left stifle)
4[a]	20	48.2	1.67	2.00	2.00 (Right ear)
3[a]	100	42.9	2.67	1.33	0.00 (Right stifle)
3[a]	100	37.1	2.67	0.00	3.00 (Left sacrum) 2.00 (Right nasal)
1[a]	300	29.5	3.00	0.67	3.00 (Dorsal neck) 2.00 (Right thorax) 2.00 (Left stifle)
1[a]	300	40.1	2.67	0.67	0.67 (Right head)
2[b]	300	60.7	1.33	2.00	0.00 right head 0.00 draining node
2[b]	300	46.6	2.33	1.00	3.00 (Dorsal neck) 0.00 (Right thorax) 2.00 (Left stifle)

a = Bis-IDA ligand with ^{153}Sm.
b = Mono-IDA ligand with ^{153}Sm.
c = Bis-IDA kit, reconstituted
% = percentage of injected dose.

TABLE I Cont'd

Compound Example	Ligand/ Metal Ratio	% Whole Body Retention (24 hrs.)	Bone Up-take	Liver Up-take	Tumor Uptake (Location)
5	300	46.7	2.75	0.50	2.00 (Midline, lumbar area)
5	300	46.5	2.75	0.50	2.00 (Head) 0.50 (Inside left hock)
5	300	48.9	2.75	0.25	2.00 (Midline, scapula area)
6[c]	300	40.0	2.50	0.50	0.75 (Right ear)
6[c]	300	33.5	2.50	0.25	2.25 (Midline, cervical area)
6[c]	300	42.5	2.75	0.00	0.50 (Right elbow)

a = Bis-IDA ligand with ^{153}Sm.
b = Mono-IDA ligand with ^{153}Sm.
c = Bis-IDA kit, reconstituted
% = percentage of injected dose.

The above data shows that for the complexes of the present invention, the radionuclide will localize in the tumor. Particulary, when the ligand/metal ratio is high, then the whole body retention and the liver uptake significantly drop. Because of these uptake differences, the images are vastly improved for the higher ligand to metal ratio injections.

Comparative Example A

When ^{67}Ga-citrate (purchased from Syncor) was used in a procedure similar to Example 5, the results obtained are shown in the following table:

TABLE A

Example	%Whole Body Retention (72 hrs.)	Bone Uptake (72 hr)	Liver Uptake (72 hrs)	Tumor Uptake (Location)
A	98.5	1.00	3.00	0.00 (Left sacrum)
A	96.7	1.00	2.67	0.33 (Right head) 1.67 (Draining node)
A	97.1	1.00	3.00	3.00 (Left head) 3.00 (Right thorax) 2.00 (Right stifle)

% = the percentage of injected dose.

^{67}Ga-citrate never cleared the extracellular fluid and had an unacceptably large liver uptake. Although tumor uptake was noted, the degree of uptake was similar to the degree of uptake in non-targret tissue which made the tumor image almost indistinguishable from the high background activity.

Example II

A poodle dog with an oral melanoma on the left mandible that had metastasized to a regional lymph node was treated with about 3 mCi of ^{153}Sm from a reconstituted kit of Example 6. Gamma images clearly show uptake of ^{153}Sm in the lymph node.

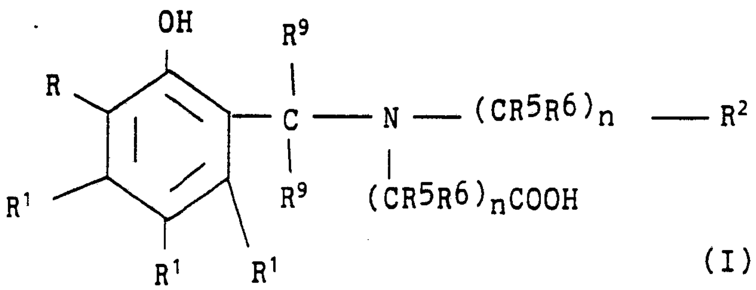
Example III

A Scottie dog with a surgically removed recurrent melanoma on the left side was treated with about 3 mCi of ^{153}Sm from a reconstituted kit of Example 6. Gamma images clearly show uptake of ^{153}Sm at the surgical site.

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.

WHAT IS CLAIMED IS:

1. A metal-phenolic carboxylate ligand complex wherein the metal ion is ^{153}Sm, ^{166}Ho, ^{90}Y, ^{165}Dy, ^{159}Gd, ^{177}Lu, ^{111}In, ^{115m}In, ^{175}Yb, ^{47}Sc, ^{52}Fe, ^{72}Ga, ^{67}Ga, ^{68}Ga, Gd, or Fe and the ligand is of the formula



(I)

wherein:

R represents hydrogen, -COOH, R^1 or

$$-\overset{\displaystyle R^9}{\underset{\displaystyle R^9}{\overset{|}{\underset{|}{C}}}}-\underset{\displaystyle (CR^5R^6)_nCOOH}{\underset{|}{N}}-(CR^5R^6)_n-R^2 \quad ;$$

R^1 independently represents hydrogen, C_1-C_{18} alkyl, -Cl, -Br, -I, $-N(R^4)_2$, $-NR^8C(O)CR^5R^6R^7$, $-N(OR^5)C(O)R^6$, -$N(O)R^5R^6$, $-NR^5-NR^6R^7$, $-NR^5C(O)NR^6R^7$, $-NO_2$, $-OR^5$, -$OC(O)R^5$, $-OC(O)OR^5$, -CN, $-SO_3H$, $-SO_2NR^5R^6$, $-NR^5SO_2R^6$, $-NR^5-OR^7$, $-C{=}NR^5$, $-N{=}CR^5R^6$, -NCS, -N=O, or $-N^+R^5R^6R^7$ and where R^1 can be interconnected with another R^1 to form a saturated or unsaturated ring;

where R^4 represents hydrogen, C_1-C_{18} alkyl, or $-CO(CH_2)_yCH_3$, where y is an interger from 0 to 17;

R^5, R^6, R^7 and R^8 independently represent hydrogen, C_1-C_{18} alkyl, aryl or aryl-C_1-C_4 alkyl;

R^2 represents -COOH, $-CH_2OH$ or $-CH(CH_3)OH$;

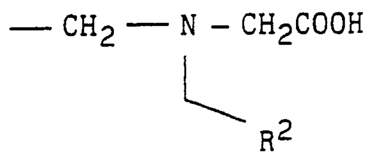
R^9 independently represents hydrogen, -COOH, C_1-C_{18} alkyl, aryl or aryl-C_1-C_4 alkyl;

n is an intereger of 1, 2 or 3;

or a pharmaceutically acceptable salt thereof.

2. A complex of Claim 1 wherein R^1 represents $-N(R^4)_2$, $-NHCOCH_3$ or C_1-C_{18} alkyl.

3. A complex of Claim 1 wherein R represents

 ;

wherein R^2 is defined as in Claim 1.

4. The complex of Claim 3 wherein R^1 is in the 4 position and is $-NHCOCH_3$, and R^2 is $-COOH$, or a pharmaceutically acceptable salt thereof.

5. The complex of Claim 3 wherein R^1 is in the 4 position and is $-NH_2$, and R^2 is $-COOH$, or a pharmaceutically acceptable salt thereof.

6. A complex of Claim 1 wherein R represents hydrogen.

7. The complex of Claim 6 wherein R^1 is in the 4 position and is $-NHCOCH_3$, and R^2 is $-COOH$, or a pharmaceutically acceptable salt thereof.

8. A complex of any one of Claims 1 to 7 wherein the ligand to metal ratio is at least 1:1.

9. A complex of Claim 8 wherein the ligand to metal ratio is from 1:1 to 600:1.

10. A complex of Claim 9 wherein the ligand to metal ratio is from 1:1 to 300:1.

11. A complex of Claim 10 wherein the ligand to metal ratio is from 100:1 to 300:1.

12. A complex of any one of Claims 1 to 7 wherein the metal used is in the no carrier added form.

13. A complex of Claim 12 wherein the ligand to metal ratio is $5X10^7$:1.

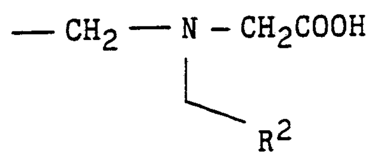
14. A complex of any one of Claims 1 to 7 wherein the metal is ^{153}Sm, ^{166}Ho, ^{177}Lu, ^{111}In, ^{90}Y, ^{175}Yb, ^{67}Ga, Gd, or Fe.

15. The complex of Claim 14 wherein the metal is ^{153}Sm.

16. A pharmaceutical formulation comprising a complex as claimed in any one of Claims 1 to 7 with a physiologically acceptable liquid carrier.

17. A formulation of Claim 16 wherein the physiologically acceptable liquid carrier is water and the resulting solution is adjusted to have a pH of about 7 to about 8.

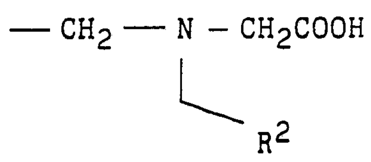
18. The formulation of Claim 17 wherein the complex comprises a metal with a ligand of Formula I as claimed in Claim 1 wherein R represents



;

R^1 is $-NHCOCH_3$, and R^2 is $-COOH$, or a pharmaceutically acceptable salt thereof.

19. The formulation of Claim 17 wherein the complex comprises a metal with a ligand of Formula I as claimed in Claim 1 wherein R represents

 ;

R^1 is $-NH_2$, and R^2 is -COOH, or a pharmaceutically acceptable salt thereof.

20. The formulation of Claim 17 wherein the composition administered to a mammal may contain 2 or more different radioisotopes.

21. The formulation of Claim 17 wherein the composition administered to a mammal may be in the form of a reconstituted kit.

22. A method for the therapeutic and/or diagnostic treatment of a mammal having a soft tissue tumor which comprises administering to said mammal an effective amount of a formulation as claimed in any one of Claims 16 through 21.

23. A method of Claim 22 for therapeutic treatment.

24. The method of Claim 23 wherein the metal ion is ^{166}Ho, or ^{90}Y.

25. A method of Claim 22 for diagnostic treatment.

26. The method of Claim 25 wherein the metal ion is ^{111}In, ^{67}Ga, Gd, or Fe.

27. A method of Claim 22 for therapeutic and diagnostic treatment.

28. The method of Claim 27 wherein the metal ion is ^{153}Sm, ^{177}Lu, or ^{175}Yb.

29. The method of Claim 28 wherein the metal ion is ^{153}Sm.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/00682

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

Int.Cl.5 C 07 C 229/16 A 61 K 49/02 C 07 C 233/43
C 07 B 59/00 A 61 K 43/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 B 59/00 C 07 C 229/00 C 07 C 233/00 A 61 K 49/00

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 °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	EP,A,0367223 (DOW CHEMICAL) 9 May 1990, see the abstract; claims; table 1, examples 10,14; examples XXIV-XXVII,XXIX (cited in the application) ---	1,2,6-12,14-17
X	FR,A,1242901 (CIBA) 29 August 1960, see examples 1,3 ---	1-3,8-10,12,14
A	Journal of Nuclear Medicine, vol. 30, no. 2, 30 February 1989, (New York, US), J.W. TSE et al.: "High specific activity [samarium-153] EDTA for imaging of experimental tumor models", pages 202-208, see the complete article (cited in the application) --- -/-	16-29

° 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
18-05-1992	17 JUN 1992
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MISS T. TAZELAAR

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATION 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. ☒ Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

   Remark: Although claims 22-29 are directed to a method of treatment of, or diagnostic method practised on the human/ animal body the search has been carried out and based on the alleged effects of the composition.

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.

International Application No PCT/US 92/00682

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.
A	WO,A,8906980 (UNIVERSITY OF QUEENSLAND) 10 August 1989, see the claims; tables 1-6 -----	16-29

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9200682 SA 57015

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 11/06/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0367223	09-05-90	AU-A- 4391689 CA-A- 2001765 JP-A- 2196761	04-10-90 30-04-90 03-08-90
FR-A- 1242901		OA-A- 1203	25-01-69
WO-A- 8906980	10-08-89	None	

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82