DESCRIPTION

TITLE OF THE INVENTION: EFFICACY ENHANCING AGENT FOR INFLAMMATORY DISEASE TREATMENT DRUG, AND ACCUMULATION-PROMOTING AGENT FOR PROMOTING ACCUMULATION OF CONTRAST MEDIUM IN INFLAMMATION SITE

TECHNICAL FIELD

[0001]

The present invention relates to an enhancing agent for enhancing the efficacy of a drug for treating an inflammatory disease. The present invention also relates to a set for treating an inflammatory disease using the enhancing agent. Furthermore, the present invention relates to an accumulation-promoting agent that promotes accumulation of a contrast medium in an inflammation site to enable imaging of the inflammation site. The present invention also relates to a set for imaging an inflammation site using the accumulation-promoting agent.

BACKGROUND ART

[0002]

Various drugs for treating an inflammatory disease represented by rheumatoid arthritis and the like have been developed by recent medical advances. For example, as drugs for treating rheumatoid arthritis, TNF inhibitors such as etanercept, infliximab, adalimumab, golimumab, certolizumab pegol, and the like; anti-IL-6 receptor antibodies such as tocilizumab, sarilumab, and the like; T cell-selective costimulatory modulators such as abatacept and the like; and other drugs have been put into practical use. However, while a certain effectiveness has been confirmed in conventional drugs for treating inflammatory diseases, there are still cases where a sufficiently satisfactory efficacy is not recognized. Therefore, there is a demand for the development of a drug

that enhances the efficacy of a drug for treating an inflammatory disease.

[0003]

In addition, when diagnosing an inflammatory disease or planning a treatment plan, it is important to perform imaging diagnosis of an inflammation site and accurately specify the location and size of the inflammation site. Therefore, development of a technique capable of efficiently imaging an inflammation site is also required.

[0004]

On the other hand, it has been reported that carbonated apatite has an action of enhancing an antitumor effect of an anticancer drug, an action of promoting accumulation of a contrast medium in a tumor, and the like (Patent Documents 1 and 2). It has also been reported that composite particles in which miR-29a and/or miR-29b is/are carried on carbonated apatite particles are effective for treating inflammatory bowel disease (Patent Document 3). However, in the treatment of inflammatory diseases, it has not been reported heretofore that carbonated apatite is administered without being complexed with a drug for treating an inflammatory disease, or carbonated apatite is used in imaging of an inflammation site.

PRIOR ART DOCUMENTS

PATENT DOCUMENTS

[0005]

Patent Document 1: International Publication WO2015/125934

Patent Document 2: Japanese Patent Laid-open Publication No. 2015-151377

Patent Document 3: International Publication WO2018/199121

SUMMARY OF THE INVENTION

PROBLEMS TO BE SOLVED BY THE INVENTION

[0006]

An object of the present invention is to provide a drug capable of enhancing the efficacy of a drug for treating an inflammatory disease. In addition, an object of the present invention is to provide a treatment set for treating an inflammatory disease using the drug.

[0007]

Furthermore, another object of the present invention is to provide an accumulation-promoting agent that enables imaging of an inflammation site by accumulating a contrast medium in the inflammation site by a simple method. Still another object of the present invention is to provide a set for imaging an inflammation site using the accumulation-promoting agent.

MEANS FOR SOLVING THE PROBLEM

[0008]

The present inventors have made intensive studies to solve the above problems, and have found that administering carbonated apatite that does not contain a drug for treating an inflammatory disease, in addition to administering a drug for treating an inflammatory disease, to a patient with an inflammatory disease makes it possible to dramatically enhance the effect of treating the inflammatory disease. Furthermore, the present inventors have found that by administering carbonated apatite not containing a contrast medium in addition to the administration of the contrast medium, accumulation of the contrast medium in an inflammation site can be promoted, and highly accurate imaging of the inflammation site becomes possible. The present invention has been completed by further conducting studies based on this finding.

[0009]

That is, the present invention provides inventions of the following aspects.

Item 1. An agent for enhancing the efficacy of a drug for treating an inflammatory disease, comprising as an active ingredient carbonated apatite not containing t a drug for treating an inflammatory disease.

Item 2. The agent according to item 1, which is used for enhancing the efficacy of a drug for treating rheumatoid arthritis.

Item 3. The e agent according to item 2, wherein the drug for treating rheumatoid arthritis is a TNF inhibitor.

Item 4. A set for treating an inflammatory disease, comprising a first preparation containing a drug for treating an inflammatory disease and a second preparation containing the agent according to any one of items 1 to 3,

wherein the first preparation and the second preparation are separately administered.

Item 5. An agent for promoting accumulation of a contrast medium in an inflammation site, containing as an active ingredient carbonated apatite not containing the contrast medium.

Item 6. The agent according to item 5, wherein the contrast medium is a fluorescent contrast medium.

Item 7. The agent according to item 6, wherein the fluorescent contrast medium is indocyanine green.

Item 8. A set for imaging an inflammation site, containing a first preparation containing a contrast medium and a second preparation containing the agent according to any one of items 5 to 7,

wherein the first preparation and the second preparation are separately administered.

Item 9. A method for treating an inflammatory disease, containing a step of

separately administering a first preparation containing a drug for treating an inflammatory disease and a second preparation containing the agent according to any one of items 1 to 3 to a patient with the inflammatory disease.

Item 10. Use of carbonated apatite not containing a drug for treating an inflammatory disease for the production of an agent for enhancing the efficacy of a drug for treating an inflammatory disease.

Item 11. Carbonated apatite not containing a drug for treating an inflammatory disease, which is used in a treatment for enhancing the efficacy of a drug for treating an inflammatory disease.

Item 12. A method for imaging an inflammation site, comprising a step of separately administering a preparation I containing a contrast medium and a preparation II containing the agent according to any one of items 5 to 7 to a subject in need of imaging the inflammation site.

Item 13. Use of carbonated apatite not containing a contrast medium for the production of an agent for promoting accumulation of a contrast medium in an inflammation site.

Item 14. Carbonated apatite not containing a contrast medium, which is used in a treatment for promoting accumulation of a contrast medium in an inflammation site.

ADVANTAGES OF THE INVENTION

[0010]

According to one aspect of the present invention, the efficacy of a drug for treating an inflammatory disease can be remarkably enhanced. Therefore, according to the present invention, it is expected to enhance the therapeutic effect on inflammatory diseases and to bring good news to patients with inflammatory diseases.

[0011]

In addition, according to another embodiment of the present invention, accumulation of a contrast medium in an inflammation site can be promoted by a simple technique of administering carbonated apatite, and thereby the inflammation site can be visualized and imaged. Therefore, according to the present invention, diagnosis of an inflammatory disease, planning of a treatment plan for an inflammatory disease, detection of an inflammation site in a test animal, and the like can be performed with high accuracy by a simple method, which can greatly contribute to technical progress in the field of diagnosis and treatment of inflammatory diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012]

Fig. 1 shows the results of observation and scoring of swelling of ankle joints in spontaneous rheumatoid arthritis models and rheumatoid arthritis non-onset/mild onset models, in Test Example 1.

Fig. 2 shows the results of observation of ankle joints after 5 hours and 8 hours after intraperitoneal administration of indocyanine green (ICG) (ICG (i.p.)), or intravenous administration of carbonated apatite (sCA) and intraperitoneal administration of ICG (sCA (i.v.) + ICG (i.p.)), to spontaneous rheumatoid arthritis models and rheumatoid arthritis non-onset models, in Test Example 1. The upper image is an image obtained by observing the ankle joint, and the lower image is an image obtained by fluorescence imaging of the ankle joint.

Fig. 3 shows the results of quantifying the fluorescence signal of ankle joints after 5 hours and 8 hours after intraperitoneal administration of ICG (ICG (i.p.)), or intravenous administration of sCA and intraperitoneal administration of ICG (sCA (i.v.) + ICG (i.p.)), to spontaneous rheumatoid arthritis models, in Test Example 1.

Fig. 4a shows the results of RNA analysis of tissues around joints of both legs

after 1 hour and 2 hours after intravenous administration of sCA to spontaneous rheumatoid arthritis models, in Test Example 2. Fig. 4b shows the results of performing Upstream analysis of IPA on 845 gene sets in which a variation was observed in Fig. 4a.

Fig. 5 shows the results of fluorescence imaging of an inflammation site after 30 minutes, 60 minutes, 90 minutes, and 150 minutes after intraperitoneal administration of ICG (ICG i.p.), or intravenous administration of freeze-dried sCA and intraperitoneal administration of ICG (sCA i.v. + ICG i.p.), to spontaneous rheumatoid arthritis models, in Test Example 3.

Fig. 6 shows the results of quantifying the fluorescence signal of an inflammation site after 30 minutes, 60 minutes, 90 minutes, and 150 minutes after intraperitoneal administration of ICG (ICG i.p.), or intravenous administration of freeze-dried sCA and intraperitoneal administration of ICG (sCA i.v. + ICG i.p.), to spontaneous rheumatoid arthritis models, in Test Example 3.

Fig. 7 shows the results of quantifying over time the fluorescence signal of ankle joints in a case where ICG was intraperitoneally administered (ICG i.p.), or in a case where sCA was intravenously administered and ICG was intraperitoneally administered 100 minutes after the intravenous administration (sCA i.v. (-100 min) + ICG i.p.), to spontaneous rheumatoid arthritis models, in Test Example 4.

Fig. 8 shows the results of quantifying over time the fluorescence signal of ankle joints after by intraperitoneal administration of ICG (ICG i.p.), or intravenous administration of freeze-dried sCA and intraperitoneal administration of ICG (sCA i.v. + ICG i.p.), to spontaneous rheumatoid arthritis models, in Test Example 5.

Fig. 9a shows the results of quantifying over time the fluorescence signal of ankle joints in a case where ICG was intraperitoneally administered (ICG i.p.), or in a

case where freeze-dried sCA was intravenously administered and ICG was intraperitoneally administered 100 minutes after the intravenous administration (1/2 sCA i.v. (-100 min) + ICG i.p.), to spontaneous rheumatoid arthritis models, in Test Example 6. Fig. 9b shows the results of quantifying over time the fluorescence signal of ankle joints after ICG alone was re-administered 103.5 hours after the first ICG administration in the ICG i.p. group and the 1/2 sCA i.v. (-100 min) + ICG i.p. group, in Test Example 6.

Fig. 10a is an image obtained by observing the hind paw of a mouse having developed arthritis, in Test Example 7. Fig. 10b is a view showing the results of measuring the width of the foot having developed arthritis over time by dividing the mice having developed arthritis into an untreated group (Con), an etanercept administration group (Etanercept i.p.), an sCA administration group (sCA i.v.), and a combined use group of sCA and etanercept (sCA i.v. + Etanercept i.p.), in Test Example 7. The arrow in Fig. 10b refers to the day on which the drug was administered.

Fig. 11 is a view showing the results of observing the degree of symptoms of rheumatoid arthritis 98 days after the first administration day of a drug (day 0), for an untreated group of rheumatoid arthritis non-onset mice (RA (-)), an untreated group of rheumatoid arthritis onset mice (RA (+)), an etanercept administration group of rheumatoid arthritis onset mice (RA (+) Etanercept i.p.), a combined use group of sCA and etanercept of rheumatoid arthritis onset mice (RA (+) sCA i.v. + Etanercept i.p.), and an sCA administration group of rheumatoid arthritis onset mice (RA (+) sCA i.v.), in Test Example 8.

EMBODIMENTS OF THE INVENTION

[0013]

1. Agent for enhancing efficacy of a drug for treating an inflammatory

disease

The efficacy enhancing agent of the present invention is used for the purpose of enhancing the efficacy of a drug for treating an inflammatory disease, and contains as an active ingredient carbonated apatite not containing a drug for treating an inflammatory disease. Hereinafter, the efficacy enhancing agent of the present invention will be described in detail.

[0014]

Carbonated apatite

The carbonated apatite has a structure in which a part of the hydroxyl group of the hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is substituted with CO_3, and is a compound represented by the general formula $Ca_{10-m}X_m(PO_4)_6(CO_3)_{1-n}Y_n$. Here, X is an element capable of partially substituting Ca in carbonated apatite, and examples thereof include Sr, Mn, rare earth elements, and the like. m is a positive number of usually 0 or more and 1 or less, preferably 0 or more and 0.1 or less, more preferably 0 or more and 0.01 or less, and still more preferably 0 or more and 0.001 or less. Y is a group or element capable of partially substituting CO_3 in carbonated apatite, and examples thereof include OH, F, Cl, and the like. n is a positive number of usually 0 or more and 0.1 or less, preferably 0 or more and 0.01 or less, more preferably 0 or more and 0.001 or less, and still more preferably 0 or more and 0.0001 or less.

[0015]

The average particle diameter of the carbonated apatite used in the present invention is not particularly limited as long as it is a size that allows the carbonated apatite to be administered into a living body and to migrate into cells, but it is usually more than 50 nm (for example, more than 50 nm and 3000 nm or less), preferably 100 to 3000 nm, more preferably 100 to 2000 nm, more preferably 200 to 2000 nm or 400 to

3000 nm, and particularly preferably 400 to 2000 nm.

[0016]

The average particle diameter of the carbonated apatite is a value measured by dynamic light scattering particle measurement (DLS). If there are huge particles (for example, the particle size is 5 μm or more) that are not suitable for measurement using DLS, they are removed from the measurement target range. In addition, in the present specification, the particle size means a particle size of independent particles that can be recognized as separate particles when measured with a scanning probe microscope. Therefore, when a plurality of particles are aggregated, an aggregate thereof is determined as one particle.

[0017]

Since the efficacy enhancing agent of the present invention is administered separately from the drug for treating an inflammatory disease, carbonated apatite not containing a drug for treating an inflammatory disease is used in the efficacy enhancing agent of the present invention. Here, "carbonated apatite not containing a drug for treating an inflammatory disease " refers to carbonated apatite not containing a drug for treating an inflammatory disease inside the particle and having no drug for treating an inflammatory disease attached or bonded to the particle surface.

[0018]

The carbonated apatite can be obtained according to a known method. For example, it can be obtained by allowing calcium ions, phosphate ions and hydrogen carbonate ions to coexist in an aqueous solution. Each ion concentration in the aqueous solution is not particularly limited as long as carbonated apatite is formed, and can be appropriately set with reference to the following.

[0019]

The concentration of calcium ions in the aqueous solution is usually 0.1 to 1000 mM, preferably 0.5 to 100 mM, and further preferably 1 to 10 mM.

[0020]

The phosphate ion concentration in the aqueous solution is usually 0.1 to 1000 mM, preferably 0.5 to 100 mM, and further preferably 1 to 10 mM.

[0021]

The hydrogen carbonate ion concentration in the aqueous solution is usually 1.0 to 10000 mM, preferably 5 to 1000 mM, and further preferably 10 to 100 mM.

[0022]

The source of calcium ions, phosphate ions, and hydrogen carbonate ions is not particularly limited as long as these ions can be supplied into the aqueous solution, and examples thereof include water-soluble salts of these ions. Specifically, $CaCl_2$ can be used as a calcium ion source, $NaH_2PO_4 \cdot 2H_2O$ can be used as a phosphate ion source, and $NaHCO_3$ can be used as a carbonate ion source.

[0023]

The aqueous solution for preparing carbonated apatite may contain components other than the above-described ion sources and other substances as long as carbonated apatite is formed. For example, by adding a fluorine ion, a chlorine ion, Sr, Mn, polyethylene glycol (PEG) or the like to the composition in an aqueous solution, Ca, CO_3 or the like in carbonated apatite may be partially substituted or modified. However, the addition amount of a fluorine ion, chlorine ion, Sr, Mn, PEG, or the like is preferably within a range that does not significantly affect the pH-dependent solubility and particle size range of the composite particles to be formed. As the aqueous solution for preparing carbonated apatite, water may be used as a base, but various media for cell culture, buffers, and the like may be used.

[0024]

In the preparation of the carbonated apatite used in the present invention, the mixing order of each ion source and other substances into the aqueous solution is not particularly limited, and the aqueous solution may be prepared in any mixing order as long as the intended carbonated apatite is obtained. For example, a first solution containing calcium ions and other substances is prepared, and a second solution containing phosphate ions and hydrogen carbonate ions is separately prepared, and the first solution and the second solution are mixed to prepare an aqueous solution.

[0025]

The carbonated apatite can be obtained by adjusting the pH of the aqueous solution containing each ion to a range of 6.0 to 9.0 and leaving (incubating) the aqueous solution for a certain period of time. The pH of the aqueous solution for forming carbonated apatite is, for example, 7.0 to 8.5, preferably 7.1 to 8.5, more preferably 7.2 to 8.5, still more preferably 7.3 to 8.5, particularly preferably 7.4 to 8.5, and most preferably 7.5 to 8.0.

[0026]

The temperature condition of the aqueous solution for forming carbonated apatite is not particularly limited as long as carbonated apatite is formed, but it is usually 4°C or higher, preferably 25 to 80°C, and further preferably 37 to 70°C or higher.

[0027]

The incubation time of the aqueous solution for forming carbonated apatite is not particularly limited as long as carbonated apatite is formed, but it is usually 1 minute to 24 hours, and preferably 5 minutes to 1 hour. The presence or absence of particle formation can be confirmed, for example, by observation under a microscope.

[0028]

The method for controlling the average particle diameter of carbonated apatite within the above range is not particularly limited, and examples thereof include a method in which carbonated apatite formed in the aqueous solution is subjected to an ultrasonic vibration treatment. The ultrasonic vibration treatment includes, specifically, a treatment of bringing an ultrasonic vibrator such as an ultrasonic crusher or the like into direct contact with a sample and applying an ultrasonic wave; a treatment in which a liquid (for example, water) is put in a water tank using an ultrasonic cleaner provided with an ultrasonic transducer and the water tank (cleaning tank), a container (for example, a plastic tube) containing carbonated apatite is floated thereon, and an ultrasonic wave is applied to an aqueous solution containing carbonated apatite via the liquid; and the like. By such an ultrasonic vibration treatment, the particle size of the carbonated apatite can be easily and efficiently refined to the above range. Furthermore, the particle size of the carbonated apatite can be adjusted to the above range by separating the carbonated apatite having a desired particle size with a filter having a predetermined pore size after the ultrasonic treatment.

[0029]

The conditions for the ultrasonic vibration treatment are not particularly limited as long as the particle diameter can be controlled within a predetermined range. For example, in the case of performing using an ultrasonic cleaner including an ultrasonic vibrator and a water tank (cleaning tank), the following conditions are exemplified.

Water tank temperature: For example, it is 5 to 45°C, preferably 10 to 35°C, and more preferably 20 to 30°C.

High frequency output: For example, it is 10 to 500 W, preferably 20 to 400 W, further preferably 30 to 300 W, and more preferably 40 to 100 W.

Oscillation frequency: For example, it is 10 to 60 Hz, preferably 20 to 50 Hz, and more preferably 30 to 40 Hz.

Treatment time: For example, it is 30 seconds to 30 minutes, preferably 1 to 20 minutes, and more preferably 3 to 10 minutes.

[0030]

The type of the container containing the carbonated apatite to be used for the ultrasonic vibration treatment is not limited as long as the particles can be refined to a predetermined particle diameter range, and can be appropriately selected according to the capacity of the aqueous solution and the intended use. For example, a 1 to 1000 ml volume plastic tube can be used.

[0031]

The ultrasonic vibration treatment is preferably performed in the presence of a dispersant (that is, a state in which the dispersant is added to an aqueous solution containing carbonated apatite.). This is because by performing the ultrasonic vibration treatment in an environment where the dispersant and the carbonated apatite coexist, carbonated apatite nano having a finer particle size can be obtained, and the re-aggregation of particles can also be suppressed. The type of the dispersant is not particularly limited as long as the carbonated apatite can be dispersed, and any dispersant generally added to a pharmaceutical product may be used, and an example thereof is albumin. The dispersant may be used singly or in combination of two or more kinds thereof. The concentration of the dispersant in the aqueous solution containing carbonated apatite is not particularly limited as long as the effect of refinement and/or suppressing re-aggregation is obtained, but for example, it is about 0.1 to 500 mg/ml, preferably 1 to 100 mg/ml, and more preferably 1 to 10 mg/ml, alternatively, about 0.001 to 10 wt%. As described above, the dispersant added for

micronizing the carbonated apatite particles can be administered in vivo together with the carbonated apatite particles in a state of being contained in the efficacy enhancing agent of the present invention.

[0032]

The efficacy enhancing agent of the present invention is used in the form of a dispersion liquid in which carbonated apatite is dispersed in a solvent such as physiological saline or the like. The efficacy enhancing agent of the present invention may be provided as a dispersion liquid of carbonated apatite, alternatively carbonated apatite may be provided in a dry state such as freeze-drying and the like, and used by being dispersed in a solvent such as physiological saline or the like at the time of use.

[0033]

Usage and dosage

The efficacy enhancing agent of the present invention is used for the purpose of enhancing the efficacy of a drug for treating an inflammatory disease. In the present invention, the "efficacy of a drug for treating an inflammatory disease " refers to a prophylactic, alleviating, ameliorating or therapeutic effect on an inflammatory disease exhibited by a drug for treating an inflammatory disease.

[0034]

Examples of the inflammatory diseases to which the efficacy enhancing agent of the present invention is applied (that is, the target disease of the drug for treating an inflammatory disease to be enhanced) include chronic inflammatory diseases, autoimmune diseases, allergic diseases, and the like. Among these inflammatory diseases, an inflammatory autoimmune disease is preferable.

[0035]

Specific examples of the inflammatory disease include rheumatoid arthritis,

inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, autoimmune hepatitis, primary biliary cirrhosis, Guillain-Barre syndrome, Basedow's disease, Harada's disease, pemphigus, multiple sclerosis, systemic lupus erythematosus, Sjogren's syndrome, systemic scleroderma, dermatomyositis, psoriasis, vitiligo vulgaris, bullous pemphigoid, idiopathic dilated cardiomyopathy, type 1 diabetes mellitus, Hashimoto's disease, myasthenia gravis, IgA nephropathy, membranous nephropathy, pernicious anemia, anti-phospholipid antibody syndrome, polymyositis, dermatomyositis, scleroderma, IgG4-related disease, vasculitis syndrome, mixed connective tissue disease, inflammatory bowel disease, atopic dermatitis, and the like.

[0036]

Among these inflammatory diseases, the subject to which the efficacy enhancing agent of the present invention is applied is preferably rheumatoid arthritis.

[0037]

In the efficacy enhancing agent of the present invention, the type of the drug for treating an inflammatory disease whose efficacy is to be enhanced is not particularly limited, and is appropriately selected according to the type of inflammatory disease. For example, drugs for treating rheumatoid arthritis include TNF inhibitors such as etanercept, infliximab, adalimumab, golimumab, certolizumab pegol, and the like; anti-IL-6 receptor antibodies such as tocilizumab, sarilumab, and the like; T cell selective costimulatory modulators such as abatacept and the like; etc. Among these drugs for treating rheumatoid arthritis, the efficacy enhancing agent of the present invention is particularly suitably used for the purpose of enhancing the efficacy of a TNF inhibitor (particularly, etanercept).

[0038]

The efficacy enhancing agent of the present invention is administered by

systemic administration. Specific examples of the systemic administration include intravascular (intraarterial or intravenous) administration, subcutaneous administration, intraperitoneal administration, and the like, and the systemic administration is preferably intravascular administration, and more preferably arteriovenous administration. The intravascular administration includes not only intravascular injection but also continuous infusion. The method for administering the efficacy enhancing agent of the present invention may be the same as or different from the method for administering a drug for treating an inflammatory disease whose efficacy is to be enhanced.

[0039]

In addition, the administration method of the a drug for treating an inflammatory disease for enhancing the efficacy by the efficacy enhancing agent of the present invention is also not particularly limited, and may be appropriately set according to the type of the drug for treating an inflammatory disease or the like, and may be the same as or different from the administration method of the efficacy enhancing agent of the present invention. Specific examples of the administration method of the drug for treating an inflammatory disease include intraperitoneal administration, intravascular (intraarterial or intravenous) administration, subcutaneous administration, and oral administration, and the like. The intravascular administration includes not only intravascular injection but also continuous infusion.

[0040]

The dose of the efficacy enhancing agent of the present invention is appropriately determined depending on the type of the drug for treating an inflammatory disease for which the efficacy is to be enhanced, the sex, age, symptom, and the like of the patient, and thus cannot be generally determined, and for example, the dose may be

about 10 mg to 1 g/kg (body weight) per dose in terms of carbonated apatite amount.

[0041]

In addition, the dose of the drug for treating an inflammatory disease whose efficacy is to be enhanced by the efficacy enhancing agent of the present invention may be any therapeutically effective amount, and may be appropriately determined according to the type of the inflammatory disease to be treated, the type of the drug for treating an inflammatory disease to be used, the sex, age, symptom and the like of the patient, and for example, may be about 1 to 1500 mg or about 10 to 1200 mg per adult.

[0042]

The efficacy enhancing agent of the present invention is administered separately from the drugs for treating an inflammatory disease. In the present invention, the phrase "administered separately from a drug for treating an inflammatory disease" means that the efficacy enhancing agent of the present invention and a drug for treating an inflammatory disease are not administered in the same preparation (in the form of a mixture). The administration timings of the efficacy enhancing agent of the present invention and the drug for treating an inflammatory disease may be the same, but in this case, the efficacy enhancing agent of the present invention is administered separately from the drug for treating an inflammatory disease.

[0043]

In addition, the administration timing of the efficacy enhancing agent of the present invention is not particularly limited, but may be, for example, at the same time as the administration of the drug for treating an inflammatory disease of which efficacy to be enhanced or within about 100 hours before and after the administration, and is preferably at the same time as the administration of the drug for treating an inflammatory disease or within 24 hours before and after the administration, more

preferably at the same time as the administration of the drug for treating an inflammatory disease or within 6 hours before and after the administration, and still more preferably at the same time as the administration of the drug for treating an inflammatory disease or within 2 hours before and after the administration.

[0044]

2. Set For Treating Inflammatory Disease

The set for treating an inflammatory disease of the present invention is a set of a drug used for treating an inflammatory disease, and includes a first preparation containing a drug for treating an inflammatory disease and a second preparation containing the efficacy enhancing agent, wherein the first preparation and the second preparation are administered separately.

[0045]

In the set for treating an inflammatory disease of the present invention, the above-described efficacy enhancing agent and the drug for treating an inflammatory disease are provided as a set. In the set for treating an inflammatory disease of the present invention, the type of the inflammatory disease to be treated, the type, administration method, dosage, and the like of the first preparation (drug for treating an inflammatory disease) and the second preparation (efficacy enhancing agent) are as shown in the column of "1. Agent for enhancing efficacy of a drug for treating an inflammatory disease " above. As described above, since the efficacy enhancing agent is administered separately from the drug for treating an inflammatory disease, the second preparation does not contain the drug for treating an inflammatory disease.

[0046]

3. Agent for promoting accumulation of contrast medium in inflammation site

The accumulation-promoting agent of the present invention is used for the purpose of promoting accumulation of a contrast medium in an inflammation site, and contains as an active ingredient carbonated apatite not containing the contrast medium. Hereinafter, the accumulation-promoting agent of the present invention will be described in detail.

[0047]

Carbonated apatite

The average particle diameter and production method of the carbonated apatite used in the present invention are as described in the column of "1. Agent for enhancing efficacy of a drug for treating an inflammatory disease " above.

[0048]

Since the accumulation-promoting agent of the present invention is administered separately from a contrast medium, carbonated apatite not containing the contrast medium is used in the accumulation-promoting agent of the present invention. Here, "carbonated apatite not containing a contrast medium" refers to carbonated apatite containing no contrast medium inside the particle and having no contrast medium attached or bonded to the particle surface.

[0049]

Usage and dosage

The accumulation-promoting agent of the present invention is used for the purpose of promoting accumulation of the contrast medium in an inflammation site occurring in an inflammatory disease. In the present invention, the type of the contrast medium that promotes accumulation in the inflammation site is not particularly limited as long as there is no cytotoxicity and the tissue in the living body can be imaged, and it does not matter whether or not the contrast medium itself can accumulate in the

inflammation site. Even if the contrast medium itself is not capable of accumulating in an inflammation site, the accumulation-promoting agent of the present invention can accumulate the contrast medium in the inflammation site to image the inflammation site.

[0050]

In the accumulation-promoting agent of the present invention, specific examples of the contrast medium to be accumulated at the inflammation site include a fluorescent contrast medium, an X-ray contrast medium, a contrast medium for MRI, a contrast medium for PET, a contrast medium for SPECT, and the like. Among these contrast media, the fluorescent contrast medium is suitable as an application target of the accumulation-promoting agent of the present invention because it has a high accumulation promoting effect in an inflammation site and a small burden on a subject.

[0051]

Specific examples of the fluorescent contrast medium include indocyanine green (ICG), fluorescein, quantum dots, near-infrared fluorescent dyes (Cy5.5, Cy7, AlexaFluoro, etc.), other fluorescent substances, and the like. Indocyanine green is a fluorescent contrast medium that receives excitation light having a wavelength of around 805 nm and emits fluorescence having a wavelength of around 835 nm. 5-aminolevulinic acid is a fluorescent contrast medium that receives excitation light having a wavelength of around 380 nm and emits fluorescence having a wavelength of around 620 nm. Fluorescein is a fluorescent contrast medium that receives excitation light having a wavelength of around 494 nm and emits fluorescence having a wavelength of around 521 nm. Among these fluorescent contrast media, indocyanine green is preferable from the viewpoint of more efficiently promoting accumulation in a tumor.

[0052]

In addition, preferred examples of inflammatory diseases to which the accumulation-promoting agent of the present invention is applied include inflammatory autoimmune diseases. Examples of the inflammatory autoimmune disease include rheumatoid arthritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, autoimmune hepatitis, primary biliary cirrhosis, Guillain-Barre syndrome, Basedow's disease, Harada's disease, pemphigus, and the like. Among these inflammatory diseases, the application target of the accumulation-promoting agent of the present invention is preferably rheumatoid arthritis.

[0053]

The accumulation-promoting agent of the present invention is administered by systemic administration. Specific examples of the systemic administration include intravascular (intraarterial or intravenous) administration, subcutaneous administration, intraperitoneal administration, and the like, and the systemic administration is preferably intravascular administration, and more preferably arteriovenous administration. The intravascular administration includes not only intravascular injection but also continuous infusion. The administration method of the accumulation-promoting agent of the present invention may be the same as or different from the administration method of the contrast medium.

[0054]

In addition, the method for administering the contrast medium to be accumulated at the inflammation site by the accumulation-promoting agent of the present invention is not particularly limited, and may be appropriately set according to the type of the contrast medium, the inflammation site, and the like, and may be the same as or different from the method for administering the accumulation-promoting agent of the present invention. Even when the method for administering the contrast

medium is different from the method for administering the accumulation-promoting agent of the present invention, accumulation of the contrast medium in an inflammation site can be promoted by the accumulation-promoting agent of the present invention. Specific examples of the method for administering the contrast medium include intraperitoneal administration, intravascular (intraarterial or intravenous) administration, subcutaneous administration, and the like, and intraperitoneal administration is preferable. The intravascular administration includes not only intravascular injection but also continuous infusion.

[0055]

The dose of the accumulation-promoting agent of the present invention is appropriately determined depending on the type of the contrast medium whose accumulation in the inflammation site is to be promoted, the sex, age, symptom, and the like of the patient, and thus cannot be generally determined, but may be, for example, about 10 mg to 1 g/kg (body weight) per dose in terms of carbonated apatite amount.

[0056]

In addition, the dose of the contrast medium to be accumulated in the inflammation site by the accumulation-promoting agent of the present invention may be appropriately determined according to the inflammation site, the sex, age, symptom, and the like of the patient, and may be, for example, about 5 to 30 mg per adult.

[0057]

The accumulation-promoting agent of the present invention is administered separately from the contrast medium. In the present invention, the phrase "administered separately from the contrast medium" means that the accumulation-promoting agent of the present invention and the contrast medium are not administered in the same preparation (mixture). The timing of administration of the accumulation-

promoting agent of the present invention and the contrast medium may be the same, but in this case, the accumulation-promoting agent of the present invention is administered separately from the contrast medium.

[0058]

In addition, the administration timing of the accumulation-promoting agent of the present invention is not particularly limited, but may be, for example, at the same time as the administration of the contrast medium or within 100 hours before and after the administration, and is preferably at the same time as the administration of the contrast medium or within 24 hours before and after the administration, more preferably at the same time as the administration of the contrast medium or within 6 hours before and after the administration, and still more preferably at the same time as the administration of the contrast medium or within 2 hours before and after the administration.

[0059]

By separately administering the accumulation-promoting agent of the present invention and the contrast medium, the contrast medium is accumulated in the inflammation site. Thereafter, the inflammation site can be imaged by a known method according to the type of the contrast medium used. For example, in a case where a fluorescent contrast medium is used, fluorescence is emitted from the fluorescent contrast medium by irradiation with excitation light, and thus, it is possible to image an inflammation site by observing the fluorescence.

[0060]

Imaging of an inflammation site using the accumulation-promoting agent of the present invention is suitably used for diagnosis of an inflammatory disease, confirmation of the degree of progression, confirmation of an inflammation site in a

laboratory animal, and the like.

[0061]

4. Set For Imaging Inflammation Site

The set for imaging an inflammation site of the present invention is a set of drugs used for imaging an inflammation site, and contains a preparation I containing a contrast medium and a preparation II containing the accumulation-promoting agent, wherein the preparation I and the preparation II are separately administered.

[0062]

In the set for imaging an inflammation site of the present invention, the accumulation-promoting agent and the contrast medium are provided as a set. In the set for imaging an inflammation site of the present invention, the type of an inflammatory disease to be imaged, the type, administration method, dosage, and the like of the preparation I (contrast medium) and the preparation II (accumulation-promoting agent) are as shown in the column of "3. Agent for promoting accumulation of contrast medium in inflammation site". Further, as described above, since the accumulation-promoting agent is administered separately from the contrast medium, the preparation II does not contain the contrast medium.

EXAMPLES

[0063]

Hereinafter, the present invention will be described with reference to Examples. However, the present invention is not to be construed as being limited to the following Examples.

[0064]

Reference Example 1: Production of carbonated apatite (sonicated carbonate apatite: sCA)

First, an inorganic aqueous solution ($NaHCO_3$; 44 mM, NaH_2PO_4; 0.9 mM, $CaCl_2$; 1.8 mM, pH 7.5) was prepared and incubated at 37°C for 30 minutes. Thereafter, the mixture was centrifuged at 12,000 rpm × 3 minutes to obtain a pellet of carbonated apatite (sCA). Physiological saline (containing 0.5 wt% albumin) was added to the obtained pellet so as to be 200 μL and dispersed, and this was subjected to ultrasonic vibration treatment (38 kHz, 80 W) over 10 minutes, and immediately used for the test, or this was freeze-dried. The obtained sCA was confirmed to have an average particle size of 400 to 2000 nm by dynamic light scattering particle measurement (DLS) using Zetasizer Nano ZS (Malvern Panalytical Ltd.).

[0065]

Test Example 1: Imaging of fluorescent substance in inflammation site using rheumatoid model

To 8 week old SKG/Jcl mice (Female, purchased from CLEA Japan, Inc.), mannan (purchased from sigma) was intraperitoneally administered (20 mg/500 μl physiological saline/mouse), and several weeks later, autoimmune arthritis that immunopathologically closely resembles human rheumatoid arthritis (RA) was developed to establish a spontaneous rheumatoid arthritis model. The degree of swelling of the ankle joint of a spontaneous rheumatoid arthritis model and a mouse not administered with mannan (rheumatoid arthritis non-onset model) was scored according to the following criteria. Also in the test examples described later, the inflammation score conforms to the following criteria. The sum of the inflammation scores is the sum of the inflammation scores of each swelling site observed in the mouse.

<Criteria for Ankle Joint Swelling Degree Scoring (Inflammation score, RA score)>

0: No swelling

0.5: Moderate swelling

1: Severe Swelling

[0066]

Fig. 1 shows the results of observing and scoring the swelling of the ankle joints of the spontaneous rheumatoid arthritis model and the rheumatoid arthritis non-onset model.

[0067]

200 μl of physiological saline containing 1.25 mg/ml of indocyanine green (ICG) was intraperitoneally administered (i.p.) to the spontaneous rheumatoid arthritis model and the rheumatoid arthritis non-onset model established above, and after 5 hours and 8 hours, the ankle joint was imaged by fluorescence imaging using IVIS Spectrum (ICG (i.p.) group). Furthermore, 3 days later, physiological saline containing sCA (physiological saline containing sCA in an amount corresponding to the amount prepared from 50 ml of the inorganic aqueous solution in Reference Example 1) was intravenously administered (i.v.), and 200 μl of physiological saline containing 1.25 mg/ml of ICG was intraperitoneally administered (i.p.), and after 5 hours and 8 hours, the ankle joint was imaged by fluorescence imaging using IVIS Spectrum (sCa (i.v.) + ICG (i.p.) group).

[0068]

Fig. 2 shows the results of detecting ICG by fluorescence imaging using IVIS Spectrum. As a result, in the spontaneous rheumatoid arthritis model with an inflammation score of 2, it was found that the fluorescence signal intensity in the ankle joint was stronger in the sCA (i.p.) + ICG (i.v.) group than in the ICG (i.p.) group. In addition, Fig. 3 shows the results of quantifying the fluorescence signal of the ankle joint in the spontaneous rheumatoid arthritis model. As a result, it was confirmed that

the fluorescence intensity was significantly higher in the sCA (i.v.) + ICG (i.p.) group than in the ICG (i.p.) group.

[0069]

Test Example 2: Mechanism of effect of enhancing accumulation of drug in inflammation site by sCA

A spontaneous rheumatoid arthritis model (total inflammation score: 2) was prepared in the same manner as in Test Example 1. Physiological saline containing sCA (physiological saline containing sCA in an amount corresponding to the amount prepared from 50 ml of the inorganic aqueous solution in Reference Example 1) was intravenously administered (i.v.) to a spontaneous rheumatoid arthritis model, and 1 hour and 2 hours after the administration, tissues around both ankle joints of the same mouse were collected after sacrificed. In addition, the same amount of physiological saline was intravenously administered without administering sCA, and 1 hour and 2 hours after the administration, tissues around both ankle joints of the same mouse were collected after sacrificed (untreated group).

[0070]

For each group of the untreated group (mouse n = 4), the group 1 hour after intravenous administration of sCA (mouse n = 3), and the group 2 hours after intravenous administration of sCA (mouse n = 3), RNA analysis of collected tissues around both ankle joints was performed. The results are shown in Fig. 4a. As a result, compared with the untreated group, in the group 2 hours after the intravenous administration of sCA, 845 genes with fold changes of ± 2 and $p < 0.05$ were observed, and it was found that these genes fluctuate over time. Further, the result of performing Upsteam analysis of Ingenuity Pathways Analysis (IPA) on the 845 gene sets is shown in Fig. 4b. As a result, it was predicted that upstream regulators such as H_2O_2, NFkB,

EGF, VEGF, prostaglandin E2, reactive oxygen species, TNF, PTGS2, NOS1, and the like were activated by intravenous administration of sCA. These factors are known to enhance vascular permeability.

[0071]

Test Example 3: Effect of enhancing accumulation of fluorescent substance in inflammation site by freeze-dried sCA: - Temporal imaging by simultaneous administration of sCA -

A spontaneous rheumatoid arthritis model (total inflammation score: 2) was prepared in the same manner as in Test Example 1. The spontaneous rheumatoid arthritis model used in this test had a plurality of joints also in the tail part, and these were evaluated as an inflammation site.

[0072]

500 μl of physiological saline containing 0.83 mg/ml of ICG was intraperitoneally administered (i.p.) to the spontaneous rheumatoid arthritis model, and 30 minutes, 60 minutes, 90 minutes, and 150 minutes after the administration, ankle joints were imaged by fluorescence imaging using IVIS Spectrum (ICG i.p. group). To the spontaneous rheumatoid arthritis model, physiological saline (physiological saline containing sCA in an amount corresponding to the amount prepared from 30 ml of the inorganic aqueous solution in Reference Example 1) containing freeze-dried sCA was intravenously administered (i.v.), and at the same time, 500 μl of physiological saline containing 0.83 mg/ml of ICG was intraperitoneally administered (i.p.), and 30 minutes, 60 minutes, 90 minutes, and 150 minutes after the administration, imaging of the inflammation site was performed by fluorescence imaging using IVIS Spectrum (sCA i.v. + ICG i.p. group).

[0073]

Fig. 5 shows the results of detecting ICG by fluorescence imaging using IVIS Spectrum. As a result, in the spontaneous rheumatoid arthritis model, it was found that the fluorescence signal intensity in the inflammation site was stronger in the sCA i.v. + ICG i.p. group than in the ICG i.p. group. In addition, Fig. 6 shows the results of quantifying the fluorescence signal at the inflammation site. As a result, it was confirmed that the fluorescence signal intensity at the inflammation site was significantly higher in the sCA i.v. + ICG i.p. group than in the ICG i.p. group.

[0074]

Test Example 4: Effect of enhancing accumulation of fluorescent substance in inflammation site by freeze-dried sCA: - Temporal imaging by 100 min pre-administration of sCA -

A spontaneous rheumatoid arthritis model (total inflammation score: 2) was prepared in the same manner as in Test Example 1.

[0075]

500 μl of physiological saline containing 0.83 mg/ml of ICG was intraperitoneally administered (i.p.) to the spontaneous rheumatoid arthritis model, the ankle joint was imaged by fluorescence imaging using IVIS Spectrum over time until 70 hours after the administration, and the fluorescence signal intensity in the ankle joint was quantified (ICG i.p. group). To the spontaneous rheumatoid arthritis model, physiological saline (physiological saline containing sCA in an amount corresponding to the amount prepared from 30 ml of the inorganic aqueous solution in Reference Example 1) containing freeze-dried sCA was intravenously administered (i.v.), and 100 minutes after the administration of sCa, 500 μl of physiological saline containing 0.83 mg/ml of ICG was intraperitoneally administered (i.p.), and the ankle joint was imaged by fluorescence imaging using IVIS Spectrum over time until 70 hours after the

administration of ICG, and the fluorescence signal intensity in the ankle joint was quantified (sCA i.v. (-100 min) + ICG i.p. group).

[0076]

The results are shown in Fig. 7. As a result, the relative fluorescence intensity of the ankle joint was higher in the sCA i.v. (-100 min) + ICG i.p. group than in the ICG i.p. group. That is, it was revealed that ICG can be efficiently accumulated in an inflammation site even when sCa is administered 100 minutes before administration of ICG.

[0077]

Test Example 5: Effect of enhancing accumulation of fluorescent substance in inflammation site by freeze-dried sCA: - Temporal imaging by simultaneous administration of sCA -

A spontaneous rheumatoid arthritis model (total inflammation score: 2) was prepared in the same manner as in Test Example 1.

[0078]

500 μl of physiological saline containing 0.83 mg/ml of ICG was intraperitoneally administered (i.p.) to the spontaneous rheumatoid arthritis model, and the ankle joint was imaged by fluorescence imaging using IVIS Spectrum over time until 45 hours after the administration, and the fluorescence signal intensity in the ankle joint was quantified (ICG i.p. group). To the spontaneous rheumatoid arthritis model, physiological saline containing freeze-dried sCA (physiological saline containing sCA in an amount corresponding to the amount prepared from 30 ml of the inorganic aqueous solution in Reference Example 1) was intravenously administered (i.v.), and at the same time, 500 μl of physiological saline containing 0.83 mg/ml of ICG was intraperitoneally administered (i.p.), and the ankle joint was imaged by fluorescence

imaging using IVIS Spectrum over time until 45 hours after the administration, and the fluorescence signal intensity in the ankle joint was quantified (sCA i.v. + ICG i.p. group).

[0079]

The results are shown in Fig. 8. As a result, the relative fluorescence intensity of the ankle joint was higher in the sCA i.v. + ICG i.p. group than in the ICG i.p. group.

[0080]

Test Example 6: Effect of enhancing accumulation of fluorescent substance in inflammation site by freeze-dried sCA: - Evaluation of sCA weight reduction and duration of enhancing action -

A spontaneous rheumatoid arthritis model (total inflammation score: 2) was prepared in the same manner as in Test Example 1.

[0081]

500 μl of physiological saline containing 0.83 mg/ml of ICG was intraperitoneally administered (i.p.) to the spontaneous rheumatoid arthritis model, and the ankle joint was imaged by fluorescence imaging using IVIS Spectrum over time, and the fluorescence signal intensity (first time) in the ankle joint was quantified (ICG i.p. group). Furthermore, in the ICG i.p. group, 103.5 hours after the first ICG administration, 500 μl of physiological saline containing 0.83 mg/ml of ICG was intraperitoneally administered (i.p.) again, and similarly, the fluorescence signal intensity (second time) in the ankle joint was quantified over time.

[0082]

To the spontaneous rheumatoid arthritis model, physiological saline (physiological saline containing sCA in an amount corresponding to the amount prepared from 15 ml of the inorganic aqueous solution in Reference Example 1; half of

sCa in Test Example 4) containing freeze-dried sCA was intravenously administered (i.v.), and 100 minutes after the administration of sCa, 500 μl of physiological saline containing 0.83 mg/ml of ICG was intraperitoneally administered (i.p.), and the ankle joint was imaged by fluorescence imaging using IVIS Spectrum over time, and the fluorescence signal intensity in the ankle joint was quantified (1/2 sCA i.v. (-100 min) + ICG i.p. group). Furthermore, in the 1/2 sCA i.v. (-100 min) + ICG i.p. group, 103.5 hours after the first ICG administration, 500 μl of physiological saline containing 0.83 mg/ml of ICG was intraperitoneally administered again (i.p.), and similarly, the fluorescence signal intensity (second) in the ankle joint was quantified over time.

[0083]

The results of measuring the fluorescence signal intensity in the ankle joint over time after the first ICG administration are shown in Fig. 9a, and the results of measuring the fluorescence signal intensity in the ankle joint over time after the second ICG administration are shown in Fig. 9b. From this result, it was confirmed that even when the dose of sCA was reduced, the effect of enhancing accumulation of ICG in the inflammation site by sCA was observed, and further, the effect of enhancing accumulation of ICG in the inflammation site by sCA was sustained for about 100 hours after the administration of sCA.

[0084]

Test Example 7: Study on action of enhancing treatment effect of sCA on antibody-induced collagen arthritis mouse model

Female DBA -1 mice (Charles River Laboratories Japan, Inc.) aged 8 weeks were intravenously administered, as a sensitizer, with a monoclonal antibody cocktail for inducing arthritis (Arthrogen-CIA arthritis-inducing monoclonal antibody, Chondrex) at a dose of 75 mg/kg, and after 6 days, LPS (Lipopolysaccharide) was

intraperitoneally administered at a dose of 1.25 mg/kg. After 7 days from the LPS administration, as shown in Fig. 10a, arthritis developed in the right foot of the mouse, and swelling was widely observed.

[0085]

Mice (Mice 7 days after LPS administration, Day 0) with onset of arthritis were divided into four groups shown in Table 1, drug administration was performed under each condition, and the width of the foot having developed arthritis was measured over time for 6 days from the start of administration of the drug (Day 0), and the therapeutic effect of each group was evaluated.

[0086]

[Table 1]

Group	Conditions for drug administration
Untreated group (Con) (foot n=6 from 3 mice)	No administration of etanercept and sCA
Etanercept administration group (Etanercept i.p.) (foot n=6 from 3 mice)	On Day 0 and Day 2, 1.5 mg/kg of etanercept was intraperitoneally administered, and on Day 4 and Day 5, 3.0 mg/kg of etanercept was intraperitoneally administered.
sCA administration group (sCA i.v.) (foot n=6 from 3 mice)	On Day 0, Day 2, Day 4 and Day 5, physiological saline containing freeze-dried sCA (physiological saline containing sCA corresponding to the amount prepared from 5 ml of the inorganic aqueous solution in Reference Example 1 above) was intravenously administered.
sCA and etanercept combination use group (sCA i.v. + etanercept i.p.) (foot n=6 from 3 mice)	On Day 0 and Day 2, 1.5 mg/kg of etanercept was intraperitoneally administered, and at the same time, physiological saline containing freeze-dried sCA (physiological saline containing sCA corresponding to the amount prepared from 5 ml of the inorganic aqueous solution in Reference Example 1 above) was intravenously administered. Furthermore, on Day 4 and Day 5, 3.0 mg/kg of etanercept was intraperitoneally administered, and at the same time, physiological saline containing freeze-dried sCA (physiological saline containing sCA corresponding to the amount prepared from 5 ml of the inorganic aqueous solution in Reference Example 1 above) was intravenously administered.

[0087]

The results are shown in Fig. 10b. As a result, in the combined use group of sCA and etanercept, the contraction tendency of the swollen foot was most remarkable, and the width was significantly smaller than other groups on Day 6. (One Way ANOVA with Tukey's multiple comparisons test: Con vs. sCA i.v. + Etanercept i.p. $p < 0.001$; Etanercept i.p. vs. sCA i.v. + Etanercept i.p. $p < 0.05$, sCA i.v. + Etanercept

i.p. vs. sCA i.v. $p < 0.05$).

[0088]

Test Example 8: Study on action of enhancing therapeutic effect of sCA on spontaneous rheumatoid arthritis model

SKG/Jcl mice (female, purchased from CLEA Japan, Inc.) of about 8 weeks of age were intraperitoneally administered with mannan (purchased from sigma) (20 mg/500 μl physiological saline/mouse), and 19 days after the administration of mannan (day 0), autoimmune arthritis that immunopathologically closely resembles human rheumatoid arthritis (RA) was developed to prepare rheumatoid arthritis onset mice that had a total inflammation score of 2 for swelling of the ankle joint. Separately, rheumatoid arthritis non-onset mice that did not develop autoimmune arthritis without administering mannan to SKG/Jcl mice aged about 8 weeks were also prepared.

[0089]

Rheumatoid arthritis onset mice and rheumatoid arthritis non-onset mice were divided into 5 groups shown in Table 2, and drug administration was performed under each condition, and the degree of symptoms of rheumatoid arthritis in each mouse was observed 98 days after the first administration day of the drug (day 0).

[0090]

[Table 2]

Group	Mouse used	Conditions for drug administration
RA (-) (n=3)	Rheumatoid arthritis non-onset mice	No administration of etanercept and sCA
RA (+) (n=3)	Rheumatoid arthritis onset mice	No administration of etanercept and sCA
RA (+) Etanercept i.p. (n=3)	Rheumatoid arthritis onset mice	On Day 0, Day 9, Day 12, Day 55 and Day 69, 1.5 mg /kg of etanercept was administered intraperitoneally.
RA (+) sCA i.v. + Etanercept i.p. (n=3)	Rheumatoid arthritis onset mice	On Day 0, Day 9, Day 12, Day 55, and Day 69, 1.5 mg/kg of etanercept was intraperitoneally administered, and at the same time, physiological saline containing freeze-dried sCA (physiological saline containing sCA corresponding to the amount prepared from 5 to 7.5 ml of the inorganic aqueous solution in Reference Example 1 above) was administered intravenously.
RA (+) sCA i.v. (n=3)	Rheumatoid arthritis onset mice	On Day 0, Day 9, Day 12, Day 55, and Day 69, physiological saline containing freeze-dried sCA (physiological saline containing sCA corresponding to the amount prepared from 5 to 7.5 ml of the inorganic aqueous solution in Reference Example 1 above) was administered intravenously.

[0091]

The results are shown in Fig. 11. As a result, in the RA (+) group, joint swelling, stiffness, deformation, and limb finger contracture were observed as compared with the RA (-) group. In addition, the symptoms of rheumatoid arthritis were not ameliorated in the three rheumatoid arthritis onset mice in the RA (+) Etanercept i.p. group. On the other hand, in the RA (+) sCA i.v. + Etanercept i.p. group, the symptoms of rheumatoid arthritis were significantly improved. That is, from the present results, it has become clear that sCa has an action of enhancing the therapeutic effect of etanercept. In addition, although it is usually required to administer 3.0 mg/kg of etanercept twice a week, a sufficient therapeutic effect was also observed by administering etanercept once a month by combining with sCa.

CLAIMS

1. An agent for enhancing the efficacy of a drug for treating an inflammatory disease, comprising as an active ingredient carbonated apatite not containing a drug for treating an inflammatory disease.

2. The agent according to claim 1, which is used for enhancing the efficacy of a drug for treating rheumatoid arthritis.

3. The agent according to claim 2, wherein the drug for treating rheumatoid arthritis is a TNF inhibitor.

4. A set for treating an inflammatory disease, comprising a first preparation containing a drug for treating an inflammatory disease and a second preparation containing the agent according to any one of claims 1 to 3,

wherein the first preparation and the second preparation are separately administered.

5. An agent for promoting accumulation of a contrast medium in an inflammation site, comprising as an active ingredient carbonated apatite not containing the contrast medium.

6. The agent according to claim 5, wherein the contrast medium is a fluorescent contrast medium.

7. The agent according to claim 6, wherein the fluorescent contrast medium is indocyanine green.

8. A set for imaging an inflammation site, comprising a first preparation containing a contrast medium and a second preparation containing the agent according to any one of claims 5 to 7,

wherein the first preparation and the second preparation are separately administered.

9. A method for treating an inflammatory disease, comprising a step of separately administering a first preparation containing a drug for treating an inflammatory disease and a second preparation containing the agent according to any one of claims 1 to 3 to a patient with the inflammatory disease.

10. Use of carbonated apatite not containing a drug for treating an inflammatory disease for the production of an agent for enhancing the efficacy of a drug for treating an inflammatory disease.

11. Carbonated apatite not containing a drug for treating an inflammatory disease, which is used in a treatment for enhancing the efficacy of a drug for treating an inflammatory disease.

12. A method for imaging an inflammation site, comprising a step of separately administering a preparation I containing a contrast medium and a preparation II containing the agent according to any one of claims 5 to 7 to a subject in need of imaging the inflammation site.

13. Use of carbonated apatite not containing a contrast medium for the production of an agent for promoting accumulation of a contrast medium in an inflammation site.

14. Carbonated apatite not containing a contrast medium, which is used in a treatment for promoting accumulation of a contrast medium in an inflammation site.