death in such animals. For example, among cats (within Japan), the incidence of chronic renal failure rose markedly from 0.64% to 3.95% in the ten-year period starting in 1996, climbing from 28th place to 4th place in the ranking of diseases (Non-Patent Document 2). Human EPO preparations are being therapeutically used also to treat anemia in animals. However, because physiologically active proteins such as EPO have species-specific amino acid sequences, when a human-derived EPO preparation is administered to animals other than humans, there is a risk that it will induce the expression of anti-EPO antibodies. As a result, investigations are currently being carried out to develop EPO preparations for the treatment in cats or dogs, each of which has an amino acid sequence that is specific to the particular species.

[0007]

Patent Document 1: WO 02/32957

Patent Document 2: JP-A S62-89627

[0008]

Non-Patent Document 1: "Polyethylene glycol-conjugated pharmaceutical proteins," *PSTT*, Vol. 1, No. 8, 352-356 (1998).

Non-Patent Document 2: Tama Jūi Rinshō Kenkyūkai Chōsa Hōkoku (2006 Nendo) [2006 Report on Survey of Tama Veterinary Clinics Association].

SUMMARY OF THE INVENTION

[0009]

An object of the ~~invention~~disclosure is to provide a pharmaceutical composition that contains EPO as the active ingredient; has a high safety in vivo although having a high physiological activity; and has a feature that has, when administered in humans and/or animals, a hematopoietic effect that lasts for not less than seven days.

[0010]

The inventors have conducted careful investigations on the chemical modification of EPO with PEG, as a result of which they have found that, depending on the number of PEG molecules added and the PEG molecular weight or structure, the hematopoietic effect lasts for not less than seven days. Thus the ~~invention~~disclosure has been arrived at.

[0011]

Accordingly, the ~~invention~~disclosure relates to a pharmaceutical composition comprising an erythropoietin to which two or more water-soluble long-chain molecules are added, wherein an amount of said erythropoietin is not less than 50% of total erythropoietin.

[0012]

The ~~invention~~disclosure further relates to a pharmaceutical composition containing erythropoietin to which a water-soluble long-chain molecule is added, wherein the water-soluble long-chain molecule has a molecular weight of not less than 30 kDa.

[0013]

The ~~invention~~disclosure still further relates to a pharmaceutical composition containing erythropoietin to which a water-soluble long-chain molecule is added, wherein the water-soluble long-chain molecule has a branched chain.

[0014]

The water-soluble long-chain molecule is preferably not less than one compound selected from the group consisting of polyethylene glycol, polyamino acids and polypropylene glycol.

[0015]

The pharmaceutical composition preferably has a hematopoietic effect in humans and/or animals which lasts for not less than seven days, and the animals are preferably animals of the family Felidae and/or the family Canidae.

[0016]

The ~~invention~~disclosure further relates to a drug for treating anemia and a hematopoietic drug, each of which includes the foregoing pharmaceutical composition.

[0023]

By administering the pharmaceutical composition of the ~~invention~~disclosure in humans or animals, it is possible for the hematopoietic effect to last for not less than seven days. The pharmaceutical composition of the ~~invention~~disclosure, when the preparation is administered, places little strain on the patient, such as less pain following injection, and moreover has a long-lasting drug efficacy.

[0023a]

In a first aspect, the present invention provides a pharmaceutical composition comprising an erythropoietin to which two or more straight-chain polyethylene glycols are added, wherein an amount of said erythropoietin is not less than 50% of total erythropoietin, wherein the erythropoietin is the following (a) to (c): (a) a polypeptide having an amino acid sequence set forth in SEQ ID NO:1; (b) a polypeptide that has an amino acid sequence of not less than 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:1, and has an erythropoietin activity in a cell proliferation assay using BaF/EPOR cells; or (c) a polypeptide that has one or a plurality of amino acid substitutions, deletions, insertions and/or additions with respect to the amino acid sequence set forth in SEQ ID NO:1, and has an erythropoietin activity in a cell proliferation assay using BaF/EPOR cells, and wherein not less than one polyethylene glycol addition site is a lysine-78 residue.

[0023b]

In a second aspect, the present invention provides a drug for treating anemia, comprising the pharmaceutical composition disclosed herein.

[0023c]

In a third aspect, the present invention provides a hematopoietic drug, comprising the pharmaceutical composition disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024]

FIG. 1 compares the hematopoietic effects caused when rats were administered PEG-modified cat EPOs in which had been added various numbers of molecules of straight-chain PEG having a molecular weight of 20,000.

FIG. 2 compares the hematopoietic effects caused when rats were administered PEG-modified cat EPOs in which had been added various numbers of molecules of straight-chain PEG having a molecular weight of 5,000 (the control was a mono-PEGylated cat EPO in which had been added one molecule of straight-chain PEG having a molecular weight of 20,000).

FIG. 3 compares the hematopoietic effects caused when rats were administered PEG-modified cat EPOs in which had been added one or two molecules of straight-chain PEG having a molecular weight of 40,000 (the control was a mono-PEGylated cat EPO in which had been added one molecule of straight-chain PEG having a molecular weight of 20,000).

FIG. 4 compares the hematopoietic effects caused when rats were administered PEG-modified cat EPOs in which had been added one or two molecules of branched PEG having a molecular weight of 20,000 (the control was a mono-

CLAIMS

1. A pharmaceutical composition comprising an erythropoietin to which two or more water-soluble long-chain molecules are added, wherein an amount of said erythropoietin is not less than 50% of total erythropoietin.

2. A pharmaceutical composition comprising erythropoietin to which a water-soluble long-chain molecule is added, wherein the water-soluble long-chain molecule has a molecular weight of not less than 30 kDa.

3. A pharmaceutical composition comprising erythropoietin to which a water-soluble long-chain molecule is added, wherein the water-soluble long-chain molecule has a branched chain.

4. The pharmaceutical composition according to any one of claims 1 to 3, wherein the water-soluble long-chain molecule is not less than one compound selected from the group consisting of polyethylene glycol, polyamino acids and polypropylene glycol.

5. The pharmaceutical composition according to any one of claims 1 to 4, which has a hematopoietic effect in humans and/or animals which lasts for not less than seven days.

6. The pharmaceutical composition according to claim 5, wherein the animals are animals of the family Felidae and/or the family Canidae.

7. The pharmaceutical composition according to any one of claims 1 to 6, which is a solution or gel, the solution or gel having a pH of not less than 4 but not more than 8.

8. The pharmaceutical composition according to claim 7, wherein the solution is formulated to have substantially the same osmotic pressure and pH as those of a mammalian bodily fluid so as to give no pain when administered.

9. The pharmaceutical composition according to any one of claims 1 to 8, wherein the erythropoietin has a human-, cat- or dog-derived amino acid sequence.

10. The pharmaceutical composition according to any one of claims 1 to 9, wherein the erythropoietin is the following (a) to (c):

(a) a polypeptide having an amino acid sequence set forth in SEQ ID NO:1;

(b) a polypeptide that has an amino acid sequence of not less than 70% sequence identity to the amino acid sequence set forth in SEQ ID NO:1, and has an erythropoietin activity in a cell proliferation assay using BaF/EPOR cells; or

(c) a polypeptide that has one or a plurality of amino acid substitutions, deletions, insertions and/or additions with respect to the amino acid sequence set forth in SEQ ID NO:1, and has an erythropoietin activity in a cell proliferation assay using BaF/EPOR cells.

11. The pharmaceutical composition according to claim 10, wherein not less than one polyethylene glycol addition site is a lysine-78 residue.

12. The pharmaceutical composition according to any one of claims 1 to 11, wherein even when repeatedly administered, the pharmaceutical composition does not trigger the production of an antibody to erythropoietin.

~~13. A drug for treating anemia, comprising the~~ pharmaceutical composition according to any one of claims 1 to 12.

14. A hematopoietic drug, comprising the pharmaceutical composition according to any one of claims 1 to 12.

CLAIMS

1. A pharmaceutical composition comprising an erythropoietin to which two or more straight-chain polyethylene glycols are added,

wherein an amount of said erythropoietin is not less than 50% of total erythropoietin,

wherein the erythropoietin is the following (a) to (c):

(a) a polypeptide having an amino acid sequence set forth in SEQ ID N0:1;

(b) a polypeptide that has an amino acid sequence of not less than 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:1, and has an erythropoietin activity in a cell proliferation assay using BaF/EPOR cells; or

(c) a polypeptide that has one or a plurality of amino acid substitutions, deletions, insertions and/or additions with respect to the amino acid sequence set forth in SEQ ID NO:1, and has an erythropoietin activity in a cell proliferation assay using BaF/EPOR cells, and

wherein not less than one polyethylene glycol addition site is a lysine-78 residue.

2. The pharmaceutical composition according to Claim 1,

which is for administration to animals of the family Felidae and/or the family Canidae.

3. The pharmaceutical composition according to Claim 1 or 2,

which has a hematopoietic effect that lasts for not less than seven days in animals of the family Felidae and/or the family Canidae.

4. The pharmaceutical composition according to any one of Claims 1 to 3,

which is a solution or gel, the solution or gel having a pH of not less than 4 but not more than 8.

5. The pharmaceutical composition according to any one of Claims 1 to 4,

wherein even when repeatedly administered, the pharmaceutical composition does not trigger the production of an antibody to erythropoietin.

6. The pharmaceutical composition according to any one of Claims 1 to 5,

wherein the straight-chain polyethylene glycols have a molecular weight of 5 to 40 kDa.

7. A drug for treating anemia, comprising the pharmaceutical composition according to any one of Claims 1 to 6.

8. A hematopoietic drug, comprising the pharmaceutical composition according to any one of Claims 1 to 6.