NOVEL BLOCK COPOLYMER, MICELLE PREPARATION, AND ANTICANCER AGENT CONTAINING THE SAME AS ACTIVE INGREDIENT

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Appl. No.: 11/662,834
PCT Filed: Sep. 16, 2005
PCT No.: PCT/JP05/17127
§ 371 (c)(1), (2), (4) Date: May 18, 2007

Foreign Application Priority Data
Sep. 22, 2004 (JP) 2004-275625

Publication Classification
Int. Cl. A61K 31/337 (2006.01) A61K 9/14 (2006.01)
U.S. Cl. 424/486, 525/438; 514/449

ABSTRACT
A medicinal preparation is desired which has no harmful side effects such as hypersensitive reaction, heightens the water solubility of a sparingly water-soluble anticancer agent, maintains a high drug concentration in the blood, accumulates a drug in a tumor tissue at a high concentration, heightens the pharmacological effect of the sparingly water-soluble anticancer agent, and diminishes the side effects of the anticancer agent. Provided are: a novel block copolymer which can be a drug carrier having no harmful side effects such as hypersensitive reaction; a micelle preparation in which micelles are formed and which contains a sparingly water-soluble anticancer agent, especially paclitaxel, incorporated in the micelles in an amount necessary for a disease treatment without bonding it to the block copolymer and which can heighten the solubility of the drug in water; and an anticancer agent which comprises the micelle preparation as a medical ingredient, maintains a high concentration in the blood, has more potent drug activity, and is reduced in toxicity.
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TECHNICAL FIELD

The present invention relates to a novel block copolymer, a micelle preparation using the same, and an anticancer agent containing the micelle preparation as an active ingredient.

BACKGROUND ART

Many of drugs, particularly anticancer agents, are sparingly water-soluble hydrophobic compounds. When such drug is used to attain a desired therapeutic effect, the drug is usually solubilized and administered to a patient. Accordingly, solubilization of sparingly water-soluble drugs, particularly sparingly water-solubleanticancer agents, is important for oral or parenteral pharmaceutical preparations, particularly those for intravenous administration.

As one method of solubilizing a sparingly water-soluble anticancer agent, there is a method which comprises adding a surfactant, and it is known to use, for example, a polyoxyethylene castor oil derivative (Cremophor) in order to solubilize paclitaxel. As another method, a method of using a micelle-forming block copolymer as a drug carrier is described in, for example, JP-A-6-107565 (Patent Document 1), JP-A-6-206815 (Patent Document 2) or JP-A-11-335267 (Patent Document 3), and paclitaxel-encapsulated micelles are described in JP-A-2001-226294 (Patent Document 4).

SUMMARY OF INVENTION

In the above-described method of solubilization with a surfactant, there is a problem that harmful side effects such as hypersensitive reaction attributable to the surfactant are observed in some cases and the stability of a pharmaceutical preparation is reduced so that when a drug-containing solution is stored or left, the drug is precipitated to make its administration difficult.

A pharmaceutical preparation comprising a sparingly water-soluble anticancer agent such as a taxane anticancer agent with a block copolymer as a drug carrier, when intravenously administered, has never achieved retention of a higher concentration of the drug in blood, accumulation of the drug at a higher concentration in a tumor tissue, a higher pharmacological effect and lower side effects than when the drug is administered alone.

Accordingly, there is need for a medicinal preparation which has no harmful side effects such as hypersensitive reaction, increases the water solubility of a sparingly water-soluble anticancer agent, maintains a high drug concentration in blood, accumulates a drug at a high concentration in a tumor tissue, enhances the pharmacological effect of the sparingly water-soluble anticancer agent, and reduces the side effects of the anticancer agent.

The present inventors made extensive study to solve the problem described above, and as a result, they found a novel block copolymer, a micelle preparation using the copolymer, and an anticancer agent comprising the same as an active ingredient, and the present invention was thereby completed.

That is, the present invention relates to:

1) a block copolymer obtained by reacting a compound represented by the following general formula (1):

\[
R_1-(\text{OCH}_2\text{CH}_3)_m-O-R_2-[(\text{NHCOCH})_x-(\text{NHCO}-R_3-\text{CH}_2-\text{CONH})_{1-x}]\rightarrow NR_4
\]

wherein \( R_1 \) represents a hydrogen atom or a \((\text{C}_1 \text{ to } \text{C}_5)\) alkyl group, \( R_2 \) represents a \((\text{C}_1 \text{ to } \text{C}_5)\) alkylene group, \( R_3 \) represents a methylene group or an ethylene group, \( R_4 \) represents a hydrogen atom or a \((\text{C}_1 \text{ to } \text{C}_4)\) acyl group, \( R_5 \) represents a hydroxyl group, or an optionally substituted aryl \((\text{C}_1 \text{ to } \text{C}_8)\) alkoxy group or \(-N(R_6)-CO-\rightarrow NR_7\), \( R_6 \) and \( R_7 \) may be the same or different and each represents a \((\text{C}_3 \text{ to } \text{C}_6)\) cyclic alky group, or a \((\text{C}_1 \text{ to } \text{C}_5)\) alkyl group optionally substituted with a tertiary amino group; \( n \) represents 5 to 1000, \( m \) represents 2 to 300, \( x \) represents 0 to 300 and \( y \) represents 0 to 300, provided that the sum of \( x \) and \( y \) is 1 or more to 5 or less; and \( R_5 \) is a hydroxyl group at a ratio of 1-99% relative to \( m \), an optionally substituted aryl \((\text{C}_1 \text{ to } \text{C}_8)\) alkoxy group at a ratio of 1-99% relative to \( m \) and \(-N(R_6)-CO-\rightarrow NR_7\) at a ratio of 0-10% relative to \( m \), with a carbodiimide compound in an amount of \( m \) to 5 equivalents relative to the compound represented by the general formula (1) in a solvent at 30 to 60°C. for 2 to 48 hours;

2) a block copolymer obtained by reacting a compound represented by the following general formula (2):

\[
R_1-(\text{OCH}_2\text{CH}_3)_m-O-R_2-[(\text{NHCOCH})_x-(\text{NHCO}-R_3-\text{CH}_2-\text{CONH})_{1-x}]\rightarrow NR_4
\]

\[
R_3\rightarrow\text{COOH}
\]

wherein \( R_1 \) represents a hydrogen atom or a \((\text{C}_1 \text{ to } \text{C}_5)\) alkyl group, \( R_2 \) represents a \((\text{C}_1 \text{ to } \text{C}_5)\) alkylene group, \( R_3 \) represents a methylene group or an ethylene group, \( R_4 \) represents a hydrogen atom or a \((\text{C}_1 \text{ to } \text{C}_4)\) acyl group, \( n \) represents 5 to 1000, \( x \) represents 0 to 300 and \( y \) represents 0 to 300, provided that the sum of \( x \) and \( y \) is 2 to 300, with an optionally substituted aryl \((\text{C}_1 \text{ to } \text{C}_8)\) alkoxy alcohol or an optionally substituted aryl \((\text{C}_1 \text{ to } \text{C}_8)\) alkyl halide to give a product which is partially esterified in the carboxylic acid side chains, followed by reacting the product with a carbodiimide compound in an amount of \((x+y)\) to 5\((x+y)\) equivalents relative to the compound represented by the general formula (2) in a solvent at 30 to 60°C. for 2 to 48 hours;
3) the block copolymer according to the above-mentioned 1) or 2), wherein R1 is a methyl group, R2 is a trimethylene group, R3 is a methylene group, R4 is an acetyl group, n is 20 to 500, m is 10 to 100, x is 0 to 100, and y is 0 to 100;
4) the block copolymer according to any of the above-mentioned 1) to 3), wherein the carbodiimide compound is diethyl carbodiimide, disopropyl carbodiimide, dicyclohexyl carbodiimide or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide or an inorganic salt thereof;
5) the block copolymer according to any of the above-mentioned 1) to 3), wherein the carbodiimide compound is diisopropyl carbodiimide;
6) a block copolymer represented by the following general formula (3):

\[
\begin{align*}
R1 & \rightarrow \text{OCH}_2\text{CH}_2\text{O} \rightarrow \text{O} \rightarrow \text{R2} \rightarrow \text{[NHCOCH]x'} \rightarrow \text{(NHCO-R3CHy'} \rightarrow \text{(NCOCH)\text{m-x'y'}]} \rightarrow \text{NHR4} \\
& \rightarrow \text{COR5} \rightarrow \text{COR5} \rightarrow \text{COR5} \rightarrow \text{CO-R3}
\end{align*}
\]

wherein R1 represents a hydrogen atom or a (C1 to C5) alkyl group, R2 represents a (C1 to C5) alkylene group, R3 represents a methylene group or an ethylene group, R4 represents a hydrogen atom or a (C1 to C4) acyl group, R5 represents a hydroxyl group, an optionally substituted aryl (C1 to C8) alkoxy group or —NH(R6)-CO—NHR7, R6 and R7 may be the same or different and each represents a (C3 to C6) cyclic alkyl group or a (C1 to C5) alkylene group optionally substituted with a tertiary amino group; n represents 5 to 1000, m represents 2 to 300, x' represents 0 to 300 and y' represents 0 to 300, provided that the sum of x' and y' is 1 or more to m or less; and R5 is a hydroxyl group at a ratio of 0-88% relative to m, an optionally substituted aryl (C1 to C8) alkoxy group at a ratio of 1-89% relative to m, and —NH(R6)-CO—NHR7 at a ratio of 11-30% relative to m;
7) the block copolymer according to the above-mentioned 6), wherein R1 is a methyl group, R2 is a trimethylene group, R3 is a methylene group, R4 is an acetyl group, the optionally substituted aryl (C1 to C8) alkoxy group represented by R5 is a benzyloxy group or a 4-phenyl-1-butoxy group, each of R6 and R7 is an isopropyl group, n is 20 to 500, m is 10 to 10, x' is 0 to 100, and y' is 0 to 100;
8) the block copolymer according to the above-mentioned 6) or 7), wherein R5 is a hydroxyl group at a ratio of 0-75% relative to m, an optionally substituted aryl (C1 to C8) alkoxy group at a ratio of 10-80% relative to m, and —NH(R6)-CO—NHR7 at a ratio of 11-30% relative to m;
9) the block copolymer according to the above-mentioned 8), wherein R5 is a hydroxyl group at a ratio of 0% relative to m;
10) a micelle preparation formed from the block copolymer of any of the above-mentioned 1) to 9) and a sparingly water-soluble anticancer agent.
11) the micelle preparation according to the above-mentioned 10), wherein the sparingly water-soluble anticancer agent is a taxane-based anticancer agent;
12) the micelle preparation according to the above-mentioned 11), wherein the taxane-based anticancer agent is paclitaxel; and

13) an anticancer agent comprising the micelle preparation of any of the above-mentioned 10) to 12) as an active ingredient.

**EFFECT OF THE INVENTION**

**[0009]** The novel block copolymer of the present invention can be a drug carrier of less toxicity without showing harmful side effects such as hypersensitive reaction. The block copolymer can form micelles in an aqueous medium and incorporate a sparingly water-soluble anticancer agent, especially paclitaxel, into the micelles in an amount necessary for disease treatment without bonding it to the block copolymer, thereby increasing the water solubility of the drug. When an aqueous solution of the micelle preparation of the present invention having the drug incorporated into it with the block copolymer is left at room temperature, the micelle preparation containing the sparingly water-soluble anticancer agent is stable in an aqueous medium without observing aggregation of the micelles or release of the drug from the micelles for at least several hours. The micelle preparation can be clinically useful anticancer agent because it maintains a higher concentration in blood and exhibit more potent drug activity with reduced side effects than by administering the anticancer agent alone or by administering the anticancer agent solubilized with a conventional surfactant.

**BEST MODE FOR CARRYING OUT THE INVENTION**

**[0010]** The block copolymer of the present invention is obtained by reacting a compound having a polyethylene glycol (PEG) structural moiety and a polyamino acid structural moiety represented by the general formula (1) wherein R1 represents a hydrogen atom or a (C1 to C5) alkyl group, R2 represents a (C1 to C5) alkylene group, R3 represents a methylene group or an ethylene group, R4 represents a hydrogen atom or a (C1 to C4) acyl group, R5 represents a hydroxyl group, an optionally substituted aryl (C1 to C8) alkoxy group or —NH(R6)-CO—NHR7, R6 and R7 may be the same or different and each represents a (C3 to C6) cyclic alkyl group, or a (C1 to C5) alkylene group optionally substituted with a tertiary amino group; n represents 5 to 1000, m represents 2 to 300, x represents 0 to 300 and y represents 0 to 300, provided that the sum of x and y is 1 or more to m or less; and R5 is a hydroxyl group at a ratio of 1-99% relative to m, an optionally substituted aryl (C1 to C8) alkoxy group or —NH(R6)-CO—NHR7 at a ratio of 0-10% relative to m, with a carbodiimide compound in an amount of m to 5 m equivalents relative to the compound represented by the general formula (1) in a solvent at 30 to 60°C. for 2 to 48 hours.

**[0011]** R1 in the compound represented by the general formula (1) used in the present invention represents a hydrogen atom or a (C1 to C5) alkyl group among which the (C1 to C5) alkyl group is preferable. Specific examples of the (C1 to C5) alkyl group include a methyl group, ethyl group, n-propyl
group, isopropyl group, n-butyl group, s-butyl group, t-butyl group and n-pentyl group, etc., among which a methyl group is particularly preferable.

[0012] Specifically, the (C1 to C5) alkylene group represented by R2 includes a methylene group, ethylene group, trimethylene group and tetramethylene group, etc., and is preferably an ethylene group or a trimethylene group.

[0013] R3 represents a methylene group or an ethylene group, preferably a methylene group.

[0014] R4 represents a hydrogen atom or a (C1 to C4) acyl group, preferably a (C1 to C4) acyl group, and specific examples include a formyl group, acetyl group, propionyl group, butyroyl group etc., particularly preferably an acetyl group.

[0015] The aryl (C1 to C8) alkoxy group represented by R5 includes a linear or branched (C1 to C8) alkoxy group to which an aromatic hydrocarbon group such as a phenyl group or a naphthyl group was bonded, and specific examples include a benzyloxy group, phenethyloxy group, phenylpropoxy group, phenylbutyloxy group, phenylpentyloxy group, phenylethoxy group, phenylethylpropoxy group, phenylethylbutyloxy group and phenylethylpentyloxy group, etc.

[0016] The substituent on the optionally substituted aryl (C1 to C8) alkoxy group includes a lower alkoxy group such as a methoxy group, ethoxy group, isopropanoxy group, n-butoxy group and t-butoxy group, a halogen atom such as a fluorine atom, chlorine atom and bromine atom, a nitro group, a cyano group, etc. Although the number of substituents may be 1 to the maximum number of substituents of aryl (C1 to C8) alkoxy group is preferably not substituted.

[0017] The optionally substituted aryl (C1 to C8) alkoxy group is preferably an unsubstituted phenyl (C1 to C6) alkoxy group, and examples thereof include an unsubstituted benzyloxy group, an unsubstituted phenethyloxy group, an unsubstituted phenylpropoxy group, an unsubstituted phenylbutyloxy group, an unsubstituted phenylpentyloxy group, an unsubstituted phenylethoxy group, etc., among which an unsubstituted benzyloxy group and an unsubstituted phenylbutyloxy group are particularly preferable.

[0018] Specific examples of the (C3 to C6) cyclic alkyl group, or (C1 to C5) alkyl group which may be substituted with a tertiary amino group, represented by R6 or R7, include a cyclopropyl group, cyclopentyl group, cyclohexyl group, methyl group, ethyl group, isopropyl group, n-butyl group, 3-dimethylaminopropyl group and 5-dimethylaminopentyl group, etc., preferably an ethyl group, propyl group, cyclohexyl group and 3-dimethylaminopropyl group, particularly preferably an isopropyl group.

[0019] In the general formula (1), m means the number of polymerized amino acid structural units in the polyanino acid structural moiety. The polyanino acid structural moiety contains each structural unit wherein R5 in the general formula (1) is a hydroxyl group, an optionally substituted aryl (C1 to C8) alkoxy group or —N(R6)-CO—NHR7 and a structural unit having a cyclic imide structure.

[0020] The ratio at which R5 in the general formula (1) is a hydroxyl group is 1 to 99%, preferably 10 to 90%, more preferably 20 to 80%, relative to m, the ratio at which R5 is an optionally substituted aryl (C1 to C8) alkoxy group is 1 to 99%, preferably 10 to 90%, more preferably 20 to 80%, relative to m, and the ratio at which R5 is —N(R6)-CO—NHR7 is 0 to 10% relative to m.

[0021] In the compound represented by the general formula (1) used in the present invention, n is 5 to 1000, preferably 20 to 500, more preferably 80 to 400, m is 2 to 500, preferably 10 to 100, more preferably 15 to 60, x is 0 to 300, preferably 0 to 100, more preferably 5 to 60, y is 0 to 300, preferably 0 to 100, more preferably 5 to 60, and the sum of x and y is 1 or more to m or less.

[0022] In the polyamino acid structural moiety of the compound represented by the general formula (1) used in the present invention, the respective amino acid structural units may be bound at random or in a block form.

[0023] Now, the reaction of the compound represented by the general formula (1) with the carbodiimide compound is described.

[0024] This reaction is carried out in a solvent, and examples of the solvent used include, but are not limited to, polar solvents such as dimethylformamide (DMF), dimethylsulfoxide (DMSO), acetonitrile, tetrahydrofuran and dioxane, nonpolar solvents such as benzene, n-hexane and diethyl ether, and water and mixed solvents thereof. The amount of the solvent used is usually 1 to 500 parts by weight per part of the starting compounds.

[0025] The carbodiimide compound used in the reaction described above includes carbodiimide compounds having a (C3 to C6) cyclic alkyl group or a (C1 to C5) alkyl group which may be substituted with a tertiary amino group, and specific examples include diethyl carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC-HCl), dicyclohexyl carbodiimide (DCC), diisopropyl carbodiimide (DIPC1) etc., preferably DCC or DIPC1, particularly preferably DIPC1.

[0026] The amount of the carbodiimide compound used in the reaction, in terms of the number (m) of amino acid structural units polymerized, is m to 5 m equivalents, preferably m to 3 m equivalents, relative to the compound represented by the general formula (1). That is, the carbodiimide compound may be used in m- to 5 m-fold mol, preferably in m- to 3 m-fold mol, relative to the compound represented by the general formula (1).

[0027] A reaction assistant such as N-hydroxysuccinimide, 1-hydroxymontaziniole (HOBr), N-hydroxy-5-norbornene-2,3-dicarbonylic acid imide (HOBNI), 4-dimethylaminopiridin (DMAP), N,N-diisopropylthiourea or triethylamine may be allowed to be coexistent in the reaction, among which DMAP is preferable. When a reaction assistant is used, the amount thereof is about 0.1 m to 5 m equivalents, preferably about 0.2 m to 2 m equivalents, based on the compound represented by the general formula (1).

[0028] The reaction temperature is preferably 30 to 60°C, particularly preferably 30 to 40°C. The reaction time is 2 to 48 hours, preferably 6 to 36 hours.

[0029] The method for preparing the compound represented by the general formula (1) is not particularly limited; for example, there is a method in which the compound wherein R5 is an optionally substituted aryl (C1 to C8) alkoxy group is partially hydrolyzed with an acid or an alkali according to a method described in JP-A-11-335267 (Patent Document 3) or JP-A-2001-226294 (Patent Document 4) supra.

[0030] The compound represented by the general formula (1) can also be obtained by reacting the compound represented by the general formula (2) wherein R1 represents a hydrogen atom or a (C1 to C5) alkyl group, R2 represents a (C1 to C5) alkylene group, R3 represents a methylene group
or an ethylene group, R4 represents a hydrogen atom or a (C1
to C4) acyl group, n represents 5 to 1000, x represents 0 to 360
and y represents 0 to 300, provided that the sum of x and y is
2 to 300, with an optionally substituted aryl (C1 to C8) alkyl
alcohol or an optionally substituted aryl (C1 to C8) alkyl
halide.

[0031] In the compound of the general formula (2), R1, R2,
R3 and R4 each represent the same group as in the general
formula (1), and the preferable group is also the same as in
the general formula (1).

[0032] In the compound of the general formula (2), n, x and
y are also preferably the same as in the general formula (1).

[0033] The reaction of the compound represented by the
general formula (2) with the optionally substituted aryl (C1 to
C8) alkyl alcohol is specifically a dehydration condensation
reaction in the presence of a carbodiimide compound in a
solvent.

[0034] The optionally substituted aryl (C1 to C8) alkyl
alcohol is an alcohol corresponding to the optionally substi-
tuted aryl (C1 to C8) alkoxyl group.

[0035] The amount of the aryl (C1 to C8) alkyl alcohol used
in this reaction is 0.01 to 5 equivalents, preferably 0.1 to 3
equivalents, more preferably 0.15 to 2 equivalents, based on
the amount of carboxyl groups (that is, the sum of x and y) in
the general formula (2).

[0036] The solvent used in this reaction is the same as used
in the reaction of the compound represented by the general
formula (1) with the carbodiimide compound, and the amount
of the solvent used is also the same as defined therein.

[0037] The carbodiimide compound used in this reaction
can also be the same as defined therein, and the amount of
the carbodiimide compound used may be the same as defined
therein. The reaction assistant used may be the same as
defined above, and the amount of the reaction assistant used
may be the same as defined above.

[0038] The temperature is preferably 5 to 35°C, more
preferably 15 to 30°C. The reaction time is 2 to 48 hours,
preferably 6 to 36 hours.

[0039] The reaction of the compound represented by the
general formula (2) with the optionally substituted aryl (C1 to
C8) alkyl halide includes a halogen reaction by nucleophilic
substitution in the presence of a base in a solvent.

[0040] The optionally substituted aryl (C1 to C8) alkyl
halide is the same compound as the optionally substituted aryl
(C1 to C8) alkyl alcohol described above except that a halogen
atom is present in place of the hydroxyl group of the latter
compound.

[0041] The halogen atom in the optionally substituted aryl
(C1 to C8) alkyl halide includes a fluorine atom, chlorine
atom, bromine atom and iodine atom, preferably a bromine
atom or iodine atom.

[0042] The amount of the aryl (C1 to C8) alkyl halide used
in this reaction is 0.01 to 5 equivalents, preferably 0.1 to 3
equivalents, more preferably 0.15 to 2 equivalents, relative to
the amount (the sum of x and y) of carboxyl groups in the
general formula (2).

[0043] The solvent used in this reaction is the same as in
the reaction of the compound represented by the general formula
(1) with the carbodiimide compound, and the amount of
the solvent used is also the same as defined therein.

[0044] The base used in this reaction includes, for example,
tertiary amines such as triethylamine, N,N-diisopropylethyl-
lamine, 1.8-diazabicyclo[5.4.0]undec-7-ene (DBU), among
which N,N-diisopropylethylamine and DBU are particularly
preferable.

[0045] The amount of the base used is about 0.1 to 5 equiva-
lents, more preferably 0.2 to 2 equivalents, relative to the
amount (the sum of x and y) of carboxyl groups in the
compound represented by the general formula (2).

[0046] This reaction is carried out preferably at 5 to 60°C,
more preferably 15 to 40°C.

[0047] The reaction time is 2 to 48 hours, preferably 6 to 36
hours.

[0048] The optionally substituted aryl (C1 to C8) alkyl
alcohol or the optionally substituted aryl (C1 to C8) alkyl
halide may be a commercially available compound or a com-
 pound prepared by a known organic synthesis method or a
 compound prepared by using a known organic reaction.

[0049] The optionally substituted aryl (C1 to C8) alkyl
alcohol or the optionally substituted aryl (C1 to C8) alkyl
halide include those compounds which correspond to the
optionally substituted aryl (C1 to C8) alkoxyl group described
above, and preferable compounds thereof are also the same as
defined therein.

[0050] Preferable examples of the optionally substituted
aryl (C1 to C8) alkyl alcohol or the optionally substituted aryl
(C1 to C8) alkyl halide include unsubstituted benzyl alcohol,
unsubstituted phenethyl alcohol, unsubstituted phenyl pro-
panol, unsubstituted phenyl butanol, unsubstituted phenyl
pentanol, unsubstituted phenyl hexanol, unsubstituted benzyl
bromide, unsubstituted phenethyl bromide, unsubstituted
phenyl propyl bromide, unsubstituted phenyl butyl bromide,
unsubstituted phenyl pentyl bromide etc., and particularly
preferable examples include unsubstituted benzyl alcohol,
unsubstituted phenyl butanol and unsubstituted benzyl bro-
mide.

[0051] A block copolymer obtained by reacting the
compound represented by the general formula (2) with an
optionally substituted aryl (C1 to C8) alkyl alcohol or an
optionally substituted aryl (C1 to C8) alkyl halide to give a
product which is partially esterified in the carboxylic acid side
chains, followed by reacting the product with a carbodiimide
compound in an amount of (x + y) to 5(x + y) equivalents relative
to the compound represented by the general formula (2) in a
solvent at 30 to 60°C, preferably 30 to 40°C, for 2 to 48
hours, that is, a block copolymer obtained in 2-stage reaction
from the compound represented by the general formula (2),
also falls under the scope of the invention.

[0052] The reaction may be carried out in the same solvent
under the same reaction conditions as in the reaction of
the compound represented by the general formula (1) with
the carbodiimide compound, and the preferable reaction condi-
tions are also the same as defined therein. That is, the
amount of the carbodiimide compound is (x + y) to 5(x + y)
equivalents, preferably (x + y) to 3(x + y) equivalents, based on
the compound represented by the general formula (2).

[0053] The method for preparing the compound of the
general formula (2) includes, for example, a method described

[0054] The present invention also encompasses a block
copolymer represented by the general formula (3) wherein
R1 represents a hydrogen atom or a (C1 to C5) alkyl group,
R2 represents a (C1 to C5) alkylene group, R3 represents a meth-
ylene group or an ethylene group, R4 represents a hydrogen
atom or a (C1 to C4) acyl group, R5 represents a hydroxyl
group, an optionally substituted aryl (C1 to C8) alkoxyl group
or \(-\text{N}(R6)\text{-CO} - \text{NHR7}\), R6 and R7 may be the same or different and each represents a (C3 to C6) cyclic alkyl group, or a (C1 to C5) alkyl group optionally substituted with a tertiary amino group; \(n\) represents 5 to 1000, \(m\) represents 2 to 300, \(x\) represents 0 to 300 and \(y\) represents 0 to 300, provided that the sum of \(x\) and \(y\) is 1 or more to \(m\) or less; and \(R5\) is a hydroxyl group at a ratio of 88% relative to \(m\), an optionally substituted aryl (C1 to C8) alkoxyl group at a ratio of 1-89% relative to \(m\), and \(-\text{N}(R6)\text{-CO} - \text{NHR7}\) at a ratio of 11-30% relative to \(m\). The compound represented by the general formula (3) also includes a block copolymer obtained by reacting the compound represented by the general formula (1) with a carbodiimide compound.

In the compound of the general formula (3), \(m\) has the same meaning as defined in the general formula (1), and preferable groups are also the same as defined therein. That is, the compound of the general formula (3) is preferably a block copolymer wherein \(R1\) is a methyl group, \(R2\) is a trimethylene group, \(R3\) is a methylene group, \(R4\) is an acetyl group, the optionally substituted aryl (C1 to C8) alkoxyl group represented by \(R3\) is a benzyl group or a 4-phenyl-1-butoxy group, and \(R6\) and \(R7\) each represent an isopropyl group.

In the compound of the general formula (3), the ratio at which \(R5\) is a hydroxyl group is 0 to 88%, preferably 0 to 75%, more preferably 0 to 50%, relative to \(m\), the ratio at which \(R5\) is an aryl (C1 to C8) alkoxyl group is 1 to 89%, preferably 10 to 80%, more preferably 20 to 70%, relative to \(m\), and the ratio at which \(R5\) is \(-\text{N}(R6)\text{-CO} - \text{NHR7}\) is 11 to 30% relative to \(m\).

In the compound of the general formula (3), the ratio at which \(R5\) is a hydroxyl group is particularly preferably 0% relative to \(m\). The fact that the ratio at which \(R5\) is a hydroxyl group is 0% relative to \(m\) means that the compound of the general formula (3) does not have properties of carboxylic acid, and specifically this is revealed by the fact that in an analysis with high performance liquid chromatography on an anion exchange column, the compound is not retained on the column.

The present invention also encompasses a micelle preparation formed from the block copolymer and a sparingly water-soluble anticancer agent.

When the block copolymer has carboxyl groups, the block copolymer contained in the micelle preparation may be in the form of a salt formed by ionic dissociation of a part or all of the carboxyl groups. The salt includes an alkali metal salt, an alkaline earth metal salt, an ammonium salt and an organic ammonium salt, etc., and specific examples include a sodium salt, a potassium salt, a calcium salt, an ammonium salt and a triethylammonium salt, etc.

The sparingly water-soluble anticancer agent refers to an anticancer agent which is substantially not dissolved in an equal amount of water in an environment at room temperature, at ordinary pressure etc. or is partitioned preferentially into a chloroform phase in a solvent system consisting of water and chloroform in equal amounts. Such anticancer agent can include, for example, anthracycline-based anticancer agents such as adriamycin, taxane-based anticancer agents such as paclitaxel and docetaxel, vinca alkaloid-based anticancer agents such as vincristine, methotrexate or derivatives thereof; particularly taxane-based anticancer agents, especially paclitaxel, can be mentioned. The water solubility of paclitaxel is not higher than 1 \(\mu\)g/mL.

In the micelle preparation of the present invention, the block copolymer sparingly water-soluble anticancer agent ratio by weight is 1000:1 to 1:1, preferably 100:1 to 1:5:1, more preferably 20:1 to 2:1. However, when the micelle preparation is water-soluble, the sparingly water-soluble anticancer agent may be contained in an amount as large as possible.

The micelle preparation can be prepared for example by any of the following methods.

Method a: Method of Encapsulating the Drug by Stirring

The sparingly water-soluble anticancer agent is dissolved if necessary in a water-immiscible organic solvent and then mixed under stirring with an aqueous dispersion of the block copolymer. The mixture when mixed under stirring may be heated.

Method b: Solvent Volatilization Method

A solution of the sparingly water-soluble anticancer agent in a water-immiscible organic solvent is mixed with an aqueous dispersion of the block copolymer, followed by volatilization of the organic solvent under stirring.

Method c: Dialysis Method

The sparingly water-soluble anticancer agent and the block copolymer are dissolved in a water-immiscible organic solvent and the resulting solution is dialyzed against a buffer solution and/or water.

Method d: Other Method

The sparingly water-soluble anticancer agent and the block copolymer are dissolved in a water-immiscible organic solvent, and the resulting solution is mixed with water and stirred to form an oil-in-water (O/W) emulsion followed by volatilizing the organic solvent.

Specifically, the method of preparing micelles by Method c is described in, for example, JP-A-6-107565 (Patent Document 1) supra.

Now, the methods b and d which involve volatilization of the organic solvent are described in more detail. The water-immiscible organic solvent refers to a solvent with a concept opposed to DMF, DMSO, acetonitrile etc. which are substantially freely miscible with water used in formation of polymer micelles in JP-A-11-335267 (Patent Document 3) supra, and non-limiting examples of the water-immiscible organic solvent can include chloroform, methylene chloride, toluene, xylene and n-hexane, etc., or mixed solvents thereof.

The water-immiscible organic solvent is mixed with an aqueous medium, that is, water (including purified water or deionized water) or an isotonic or buffered aqueous solution containing sugars, a stabilizer, common salt, a buffer etc. In this case, a small amount of a water-miscible organic solvent and other inorganic salts (for example, sodium sulfate etc.) may be contained unless they adversely influence formation of O/W emulsion.

Usually, the water-immiscible organic solvent and the aqueous medium are mixed at a volume ratio of 1:100, preferably 1:10. This mixing means can be any means used customarily in forming various emulsions, such as a mechanical stirrer, a shaking apparatus and an ultrasonic irradiator. The operation temperature is not limited, but in consideration of the temperature stability of the drug, the boiling point of
the solvent, etc., the temperature is preferably set in the range of about −5° C. to about 40° C.

[0072] Subsequently, the mixing operation is continued in an open system or the organic solvent is removed by evaporation (or removed by volatilization) under stirring under reduced pressure.

[0073] The aqueous solution of the solution preparation may be used as it is or when the solution preparation may have been associated or aggregated, the preparation may be subjected to ultrasonication and then filtered to remove insolubles or precipitates. The filter membrane used is not particularly limited, and is preferably a membrane having a pore diameter of about 0.1 to 1 μm.

[0074] The micelle preparation of the present invention is stable in an aqueous medium, and the drug concentration of the anticancer agent in an aqueous medium can be increased by the present invention.

[0075] For further increasing the concentration of the micelle preparation in an aqueous medium, the preparation can be concentrated under reduced pressure or subjected to ultrafiltration or lyophilization.

[0076] The concentration of the sparingly water-soluble antigen agent in the micelle preparation is 0.1 to 50 wt %, preferably 1 to 40 wt %, more preferably 5 to 35 wt %, based on the total weight of the sparingly water-soluble antigen agent and the block copolymer, and the amount of the drug can be about 0.01 mg or more, preferably about 0.1 mg or more, more preferably about 1 mg or more, per mL of the aqueous solution of the micelle preparation.

[0077] The micelle preparation of the present invention is micelles having polyethylene glycol structural moieties directed outside in an aqueous medium and including the sparingly water-soluble antigen agent in hydrophobic moieties inside the micelles. The particle diameter of the micelles can be measured with a commercial light scattering particle size measuring device, and the average particle diameter is preferably 10 to 200 nm, particularly preferably 20 to 120 nm.

[0078] The present invention also encompasses an anticancer agent comprising the micelle preparation containing the sparingly water-soluble antigen agent as an active ingredient. When the micelle preparation is administered as a pharmaceutical preparation, the dose varies depending on the age, weight, medical condition, therapeutic purpose etc. of patients, and is roughly 10 to 500 mg/body/day. The pharmaceutical preparation to be administered may contain a pharmacologically acceptable additive, and may be dissolved in a pharmaceutically acceptable solvent prior to administration. The present invention also encompasses a lyophilized product of the micelle preparation.

EXAMPLES

[0079] Hereinafter, the present invention is described in more detail by reference to the Examples, but the present invention is not limited to the following examples. In the Examples, HPLC means high performance liquid chromatography, NMR means hydrogen nuclear magnetic resonance spectrum, and NMR was measured with sodium 2,2,3,3-deuterated-3-(trimethylsilyl)propionate as an internal standard in a solvent shown below with an apparatus (400 MHz) manufactured by BRUKER.

Example 1

Production of Block Copolymer 2

[0080] DMF (630 mL) was added to 42.00 g of PEG (average molecular weight 12000)·p-Asp (polyspartic acid; average polymerization degree 40)·Ac (represented by the general formula (2) wherein R1 is a methyl group, R2 is a trimethylene glycol group, R3 is a methylene group, R4 is an acetyl group, n is about 272, x is about 10, y is about 30; abbreviated hereinafter as PEG·p-Asp·Ac) produced by a method described in JP-A-6-206815 (Patent Document 2) supra, and PEG·p-Asp·Ac was dissolved at 25° C., and DMAP (9.90 g), 4-phenyl-1-butanol (10.93 mL) and DIPC (15.86 mL) were added thereto and reacted at the same temperature for 24 hours. 1.58 L of ethyl acetate and then 4.73 L of hexane were added to the reaction liquid, and precipitates were collected by filtration and dried under reduced pressure to give 49.56 g crude crystals. The crude crystals were dissolved in acetonitrile containing 50% water (hereinafter referred to as “50% acetonitrile”), then passed through 300 mL of cation-exchange resin Dowex 50w×8 (manufactured by Dow Chemical Company) and washed with 50% acetonitrile. The eluent was concentrated under reduced pressure and lyophilized to give 48.25 g of block copolymer 1.

[0081] The block copolymer 1 (19.5 mg) was dissolved in 2 mL of acetonitrile, and 2 mL of 0.5 N aqueous sodium hydroxide solution was added thereto, and the solution was stirred at room temperature for 20 minutes to hydrolyze its ester linkages, then neutralized with 0.5 mL of acetic acid, and prepared to a volume of 25 mL with 50% acetonitrile. The prepared solution was quantified for free 4-phenyl-1-butanol by reverse HPLC. The result indicated that 4-phenyl-1-butanol bound via an ester linkage was 54% relative to m (number of polymerized aspartic acid structural units in the polyspartic acid structural moieties of the block copolymer) in the general formula (1).

[0082] When the block copolymer 1 was measured by anion exchange HPLC under conditions as described below, a peak was detected at a retention time of 17.4 minutes.

Measurement conditions for anion exchange HPLC

Column: TSKgel DEAIE-5PW (manufactured by Tosoh Corporation)
Sample concentration: 10 mg/mL
Injection volume: 20 μL
Column temperature: 40° C.
Mobile phases
(A) 20 mM Tris-Cl buffer (pH 8.0): acetonitrile=80:20
(B) 20 mM Tris-Cl buffer+1 M aqueous sodium chloride solution (pH 8.0): acetonitrile=80:20
Flow rate: 1 mL/min
Gradient condition B % (min): 10 (0), 10 (5), 100 (40), 10 (40.1), stop (50.1)
Detector: UV-visible spectrophotometric detector (detection wavelength 260 nm)

[0084] The block copolymer 1 was dissolved in a mixed solution of deuterated sodium hydroxide (NaOD)·heavy water (D2O)·deuterated acetonitrile (CD3CN), and measured by NMR, indicating that the partial structure of —N(i-Pr)—CO—N(i-Pr)—(that is, a structure of the —N(R6)—CO—
NHR7 in the general formula (1) wherein each of R6 and R7 is an isopropyl group) was 6% relative to m.

[0095] 946 mL of DMF was added to the block copolymer 1 (47.37 g) obtained above to dissolve it at 35°C, and DMAP (7.23 g) and DIPC (14.37 mL) were added thereto and reacted at the same temperature for 20 hours. 2.4 L of ethyl acetate and then 7.1 L of hexane were added to the reaction liquid, and precipitates were collected by filtration and dried under reduced pressure to give 44.89 g of crude crystals. The crude crystals were dissolved in 50% hydrous acetonitrile, then passed through cation-exchange resin Dowex 50w8 (300 mL) and washed with 50% hydrous acetonitrile. The eluent was concentrated under reduced pressure and lyophilized to give 43.54 g of block copolymer 2 of the present invention.

[0096] The block copolymer 2 (27.6 mg) was hydrolyzed by the same method as described above and measured by reverse phase HPLC, indicating that 4-phenyl-1-butanol bound via an ester linkage was 49% relative to m.

[0097] When the block copolymer 2 was measured by anion exchange HPLC under the same conditions as described above, no peak retained on the column was detected.

[0098] The block copolymer 2 was measured by NMR under the same conditions as described above, indicating that the partial structure of —N(i-Pr)—CO—NH(i-Pr) was 14% relative to m.

Comparative Example 1

Production of Block Copolymer 3

[0099] 200 mL of DMF was added to PEG-pAsp-Ac (10.00 g) produced by a method described in JP-A-6-206815 (Patent Document 2), to dissolve it at 35°C, and DMAP (2.20 g), 4-phenyl-1-butanol (3.47 mL) and DIPC (3.70 mL) were added thereto and reacted at the same temperature for 20 hours. 0.5 L of ethyl acetate and then 1.5 L of hexane were added to the reaction liquid, and precipitates were collected by filtration and dried under reduced pressure to give 11.67 g of crude crystals. The crude crystals were dissolved in 50% hydrous acetonitrile, then passed through cation-exchange resin Dowex 50w8 (100 mL) to remove DMAP etc., and washed with 50% hydrous acetonitrile. The eluent was concentrated under reduced pressure and lyophilized to give 11.35 g of block copolymer 3.

[0100] The block copolymer 3 (29.7 mg) was hydrolyzed by the same method as described in Example 1 and measured by reverse phase HPLC, indicating that 4-phenyl-1-butanol bound via an ester linkage was 49% relative to m.

[0101] When the block copolymer 3 was measured by anion exchange HPLC under the same conditions as described in Example 1, a peak was detected at a retention time of 13.8 minutes.

[0102] When the block copolymer 3 was measured by NMR under the same conditions as in Example 1, the partial structure of —N(i-Pr)—CO—NH(i-Pr) was 7% relative to m.

Example 2

Production of Block Copolymer 5

[0103] PEG-pAsp-Ac (3.0 g) produced by a method described in JP-A-6-206815 (Patent Document 2) was dissolved in DMF (120 mL), and benzyl bromide (0.60 mL) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.75 mL) were added thereto and reacted at 35°C for 17 hours. This reaction liquid was added dropwise to a mixed solvent (1.2 L) consisting of diisopropyl ether:ethanol (4:1), and precipitates were recovered by filtration and dried under reduced pressure to give 3.17 g of crude crystals. The crude crystals were dissolved in 30% aqueous acetonitrile solution and then passed through cation-exchange resin Dowex 50w8 (40 mL) and washed with the 30% aqueous acetonitrile. The eluent was concentrated under reduced pressure and lyophilized to give 2.99 g of block copolymer 4.

[0104] The block copolymer 4 (19.5 mg) was hydrolyzed by the same method as in Example 1 and measured by reverse phase HPLC, indicating that benzyl alcohol bound via an ester linkage was 32% relative to m.

[0105] When the block copolymer 4 was measured by anion exchange HPLC under the same conditions as described in Example 1, a peak was detected at a retention time of 22.9 minutes.

[0106] When the block copolymer 4 was measured by NMR under the same conditions as in Example 1, the partial structure of —N(i-Pr)—CO—NH(i-Pr) was not detected.

[0107] DMF (6 mL) was added to the block copolymer 4 (300 mg) obtained above, to dissolve it at 35°C, and DMAP (63.9 mg) and DIPC (102 mL) were added thereto and reacted at the same temperature for 24 hours. 30 mL of ethyl acetate and then 90 mL of hexane were added to the reaction liquid, and precipitates were collected by filtration and dried under reduced pressure to give 299 mg of crude crystals. The crude crystals were dissolved in 50% hydrous acetonitrile, then passed through cation-exchange resin Dowex 50w8 (15 mL) and washed with 50% hydrous acetonitrile. The eluent was concentrated under reduced pressure and lyophilized to give 284 mg of block copolymer 5 of the present invention.

[0108] The block copolymer 5 (19.8 mg) was hydrolyzed by the same method as in Example 1 and measured by reverse phase HPLC, indicating that benzyl alcohol bound via an ester linkage was 21% relative to m.

[0109] When the block copolymer 5 was measured by anion exchange HPLC under the same conditions as described in Example 1, a peak was detected at a retention time of 13.5 minutes.

[0110] When the block copolymer 5 was measured by NMR under the same conditions as in Example 1, the partial structure of —N(i-Pr)—CO—NH(i-Pr) was 15% relative to m.

Example 3

Production of Block Copolymer 7

[0111] DMF (30 mL) was added to PEG-pAsp-Ac (2.0 g) produced by a method described in JP-A-6-206815 (Patent Document 2), to dissolve it at 25°C, and DMAP (0.472 g), benzyl alcohol (499 mL) and DIPC (755 mL) were added thereto and reacted at the same temperature for 21 hours. 75 mL of ethyl acetate and then 225 mL of hexane were added to the reaction liquid, and precipitates were collected by filtration and dried under reduced pressure to give 2.28 g of crude crystals. The crude crystals were dissolved in 50% hydrous acetonitrile, then passed through cation-exchange resin Dowex 50w8 (30 mL) and washed with 50% hydrous acetonitrile. The eluent was concentrated under reduced pressure and lyophilized to give 2.10 g of block copolymer 6.

[0112] The block copolymer 6 (35.5 mg) was hydrolyzed by the same method as in Example 1 and measured by reverse
phase HPLC, indicating that benzyl alcohol bound via an ester linkage was 60% relative to m.

[0113] When the block copolymer 6 was measured by anion exchange HPLC under the same conditions as in Example 1, a peak was detected at a retention time of 17.2 minutes.

[0114] When the block copolymer 6 was measured by NMR under the same conditions as in Example 1, the partial structure of —N(i-Pr)CO—NH(i-Pr) was 5% relative to m.

[0115] The block copolymer 6 (300 mg) produced above was dissolved in DMF (6 mL), and DMAP (60.9 mg) and DIPCI (97.6 μL) were added thereto at 35°C, and reacted for 18 hours. 30 mL of ethyl acetate and then 90 mL of hexane were added to the reaction liquid, and precipitates were collected by filtration and dried under reduced pressure to give 290 mg of crude crystals. The crude crystals were dissolved in 50% hydrous acetonitrile, then passed through cation-exchange resin Dowex 50w8 (5 mL) and washed with 50% hydrous acetonitrile. The eluent was concentrated under reduced pressure and lyophilized to give 282.5 mg of block copolymer 7 of the present invention.

[0116] The block copolymer 7 (36.1 mg) was hydrolyzed by the same method as in Example 1 and measured by reverse phase HPLC, indicating that benzyl alcohol bound via an ester linkage was 37% relative to m.

[0117] When the block copolymer 7 was measured by anion exchange HPLC under the same conditions as in Example 1, no peak retained on the column was detected.

[0118] When the block copolymer 7 was measured by NMR under the same conditions as in Example 1, the partial structure of —N(i-Pr)CO—NH(i-Pr) was 12% relative to m.

[0119] The results of the block copolymers obtained in Examples 1 to 3 and Comparative Example 1 are summarized in Table 1.

<table>
<thead>
<tr>
<th>Block copolymer</th>
<th>Ester linkage</th>
<th>Anion exchange HPLC retention time</th>
<th>—N(i-Pr)CO—NH(i-Pr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54%</td>
<td>17.4 min</td>
<td>6%</td>
</tr>
<tr>
<td>2 (Example 1)</td>
<td>49%</td>
<td>not detected</td>
<td>14%</td>
</tr>
<tr>
<td>3 (Comparative Example 1)</td>
<td>49%</td>
<td>13.8 min</td>
<td>7%</td>
</tr>
<tr>
<td>4</td>
<td>32%</td>
<td>22.9 min</td>
<td>0%</td>
</tr>
<tr>
<td>5 (Example 2)</td>
<td>21%</td>
<td>not detected</td>
<td>15%</td>
</tr>
<tr>
<td>6</td>
<td>50%</td>
<td>17.2 min</td>
<td>5%</td>
</tr>
<tr>
<td>7 (Example 3)</td>
<td>37%</td>
<td>not detected</td>
<td>12%</td>
</tr>
</tbody>
</table>

[0120] The notation “not detected” in anion exchange HPLC indicates that no retained peak was detected.

[0121] As shown in Table 1, the percentage of ester linkages of the block copolymers 2, 5 and 7 is lower than in the block copolymers 1, 4 and 6, and in measurement by anion exchange HPLC, these copolymers were not retained on the column. The block copolymer 3 (Comparative Example 1), on the other hand, showed a peak retained on the column in measurement by anion exchange HPLC. No retention of the block copolymers 2, 5 and 7 in anion exchange HPLC indicates that these block copolymers are substantially free of a carboxylic acid structure. The result in NMR measurement indicates that the percentage of the partial structure —N(i-Pr)CO—NH(i-Pr) in the block copolymers 2, 5 and 7 is higher than in the block copolymers 1, 4 and 6, and the percentage of the partial structure —N(i-Pr)CO—NH(i-Pr) in the block copolymer 2 in Example 1 is higher by 7% than in Comparative Example 1.

Example 4
Production of a Micelle Preparation (Drug: Paclitaxel)

[0122] 300 mg of the block copolymer 2 in Example 1 was weighed out and placed in a screw tube, and 30 mL of 40 mg/mL aqueous maltose solution was added to it to form a dispersion under stirring which was then cooled to 4°C under stirring. 3 mL of the solution of 30 mg/mL paclitaxel in dichloromethane was added to the tube and stirred for 16 hours in a refrigerator without capping the tube and then sonicated (130 W, 10 minutes) to give a micelle preparation. The paclitaxel concentration was 2.2 mg/mL. The average particle diameter thereof determined by a light scattering particle measuring device (manufactured by Particle Sizing System) was 57.8 nm.

Test Example 1
Fluctuation in Body Weight of Mouse Upon Administration of the Block Copolymer

[0123] The block copolymer 1 or block copolymer 2 was dissolved in 5% glucose injection and administered via a mouse caudal vein to female CDF1 mice in a dose of 333 mg/kg, and a fluctuation in the body weight was measured on Day 1 after administration. As the control group, the same amount of physiological saline was administered. The results are shown in Table 2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fluctuation in body weight</th>
<th>(Coefficient of fluctuation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (physiological saline)</td>
<td>+0.47 g</td>
<td>(+2.2%)</td>
</tr>
<tr>
<td>Block copolymer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-1.23 g</td>
<td>(-5.7%)</td>
</tr>
<tr>
<td>2</td>
<td>+0.60 g</td>
<td>(+2.7%)</td>
</tr>
</tbody>
</table>

[0124] As shown in Table 2, the body weight of the group which was given the block copolymer 1 was decreased by 5% or more on Day 1 after administration, while the group which was given the block copolymer 2 showed an increase in body weight, similar to the group which was given physiological saline. From this result, it was revealed that the block copolymer of the present invention had reduced toxicity in the mice.

Test Example 2
In Vivo Antitumor Effect on Colon 26

[0125] Mouse colon cancer Colon 26 cells were transplanted subcutaneously in the back of female CDF1 mouse, and after the volume of the tumor reached about 100 mm, the micelle preparation of Example 4, or paclitaxel alone as the control drug, was administered via a mouse caudal vein into the mouse 3 times at 4-day intervals, to examine the effect thereof on advanced cancer. The micelle preparation had been diluted with 5% glucose solution to form a solution contain-
ing paclitaxel at a concentration of 3 mg/mL. Paclitaxel for use as the sole regimen was dissolved in ethanol and mixed with an equal volume of Cremophor (manufactured by Sigma) to prepare a solution containing paclitaxel at a concentration of 30 mg/mL, and the resulting preparation was diluted with physiological saline to 3 mg/mL just before administration. The antitumor effect of each drug was judged in percentage (T/C %) of the average tumor volume of the group which was given the drug on Day 11 after administration, relative to the average tumor volume of the group which was not given the drug. A lower numerical value is indicative of higher effect. The results are shown in Table 3.

<table>
<thead>
<tr>
<th>TABLE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
</tr>
<tr>
<td>Micelle preparation</td>
</tr>
<tr>
<td>(the invention)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Paclitaxel alone</td>
</tr>
<tr>
<td>(control drug)</td>
</tr>
</tbody>
</table>

[0126] As is evident from Table 3, the groups which were given paclitaxel alone in daily doses of 100 and 50 mg/kg showed tumor volumes of 52.6 and 81.6% on Day 11 after administration respectively based on the group which was not given the drug, while the groups which were given the micelle preparation of the present invention in daily doses of 100, 75 and 50 mg/kg showed tumor volumes of 8.4, 22.1 and 30.7% respectively, indicating that the micelle preparation of the present invention had high antitumor effect.

Test Example 3
Fluctuation in Paclitaxel Levels in Mouse Plasma and in Tumor

[0127] Each drug was prepared according to the same method as in Test Example 2 (in vivo antitumor effect on Colon 26). A micelle preparation containing paclitaxel, or paclitaxel alone, each at a dose level of 50 mg/kg, was administered via a mouse caudal vein into female CDF1 mice transplanted with mouse colon cancer Colon 26 in the back, and after a predetermined time, whole blood was collected through an armpit artery. 0.01 mL of plasma obtained by centrifugation was deproteinized (3 times) with 0.2 mL of water and 1 mL of acetonitrile and then subjected to liquid/liquid extraction by adding 2 mL of t-butyl methyl ether. The organic layer was recovered, evaporated into dryness, dissolved in 0.4 mL of dissolving liquid for HPLC, and measured for its paclitaxel concentration by HPLC. Separately, the tumor was homogenized with 0.5% acetic acid to prepare 1% tumor homogenate, and 0.1 mL of 1% tumor homogenate was deproteinized (3 times) with 0.1 mL of water and 1 mL of acetonitrile and subjected to liquid/liquid extraction by adding 2 mL of t-butyl methyl ether. The organic layer was concentrated and dissolved in 0.4 mL of dissolving liquid for HPLC and measured for its paclitaxel concentration by HPLC. The results are shown in Tables 4 and 5.

<table>
<thead>
<tr>
<th>TABLE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel concentration in mouse plasma (μg/mL)</td>
</tr>
<tr>
<td>Time for blood collection (hours)</td>
</tr>
<tr>
<td>0.083</td>
</tr>
<tr>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>24</td>
</tr>
<tr>
<td>72</td>
</tr>
</tbody>
</table>

[0128] As is evident from Table 4, the micelle preparation of the present invention was recognized to maintain a higher concentration in plasma for a long time than when paclitaxel was administered alone.

[0129] As is evident from Table 5, the concentration of paclitaxel in the tumor was kept higher for a long time by administering the micelle preparation of the invention than by administering paclitaxel alone, indicating that paclitaxel was accumulated in the tumor by the micelle preparation of the present invention.

Test Example 4
Observation of Peripheral Nerve Damage to Mice (Stretch Reflex)

[0130] The micelle preparation of the present invention, or paclitaxel alone, was administered via a mouse caudal vein to female CDF1 mice for 5 consecutive days, and the stretch reflex of the mouse hind limb was observed as an indicator of the peripheral nerve damage caused by paclitaxel. Each drug was prepared in the same manner as in Test Example 2 (in vivo antitumor effect on Colon 26). The dose was 30 mg/kg in terms of paclitaxel. The results are shown in Table 6.

<table>
<thead>
<tr>
<th>TABLE 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation of peripheral nerve damage to mice (stretch reflex)</td>
</tr>
<tr>
<td>Administered drug</td>
</tr>
<tr>
<td>Micelle preparation</td>
</tr>
<tr>
<td>Paclitaxel alone</td>
</tr>
</tbody>
</table>

[0131] As is evident from Table 6, the group which was given paclitaxel alone at a dose of 30 mg/kg was recognized to lose stretch reflex in every mouse. On the other hand, the group which was given the micelle preparation at a dose of 30 mg/kg was not recognized to lose stretch reflex in every mouse. The micelle preparation of the present invention, as
compared with paclitaxel used as a sole regimen, reduced peripheral nerve toxicity as a side effect of paclitaxel.

1. A block copolymer obtained by reacting a compound represented by the following general formula (1):

\[
R_1\text{(OCH}_2\text{CH}_2)_n\text{O}\text{-}R_2\text{[NHCOCH}_x\text{]}^\text{x}\text{-}\text{NHCO}\text{-}R_3\text{CH}_y\text{-}\text{NH}_{R_4}\text{CO-R}_5
\]

wherein \(R_1\) represents a hydrogen atom or a \((C1\) to \(C5)\) alkyl group, \(R_2\) represents a \((C1\) to \(C5)\) alkylene group, \(R_3\) represents a methylene group or an ethylene group, \(R_4\) represents a hydrogen atom or a \((C1\) to \(C4)\) acyl group, \(R_5\) represents a hydroxyl group, an optionally substituted acyl \((C1)\) to \(C8)\) alkoxy group or \(-\text{N(R6)-CO-}\text{NHR}_7\), \(R_6\) and \(R_7\) may be the same or different and each represents a \((C3\) to \(C6)\) cyclic alkyl group or a \((C1\) to \(C5)\) alkyl group optionally substituted with a tertiary amino group; \(n\) represents 5 to 1000, \(m\) represents 2 to 300, \(x\) represents 0 to 100 and \(y\) represents 0 to 300, provided that the sum of \(x\) and \(y\) is 1 or more to \(m\) or less; and \(R_5\) is a hydroxyl group at a ratio of 1-99\% relative to \(m\), an methylene group, \(R_4\) is an acetyl group, \(n\) is 20 to 500, \(m\) is 10 to 100, \(x\) is 0 to 100, and \(y\) is 0 to 100.

2. A block copolymer obtained by reacting a compound represented by the following general formula (2):

\[
R_1\text{(OCH}_2\text{CH}_2)_n\text{O}\text{-}R_2\text{[NHCOCH}_x\text{]}^\text{x}\text{-}\text{NHCO}\text{-}R_3\text{CH}_y\text{-}\text{COOH}
\]

wherein \(R_1\) represents a hydrogen atom or a \((C1\) to \(C5)\) alkyl group, \(R_2\) represents a \((C1\) to \(C5)\) alkylene group, \(R_3\) represents a methylene group or an ethylene group, \(R_4\) represents a hydroxyl group or a \((C1\) to \(C4)\) acyl group, \(n\) represents 5 to 1000, \(x\) represents 0 to 300 and \(y\) represents 0 to 300 provided that the sum of \(x\) and \(y\) is 2 to 300, with an optionally substituted acyl \((C1)\) to \(C8)\) alkyl alcohol or an optionally substituted alkyl \((C1)\) to \(C8)\) alkyl halide to give a product which is partially esterified in the carboxylic acid side chains, followed by reacting the product with a carbodiimide compound in an amount of \((x+y)\) to \(5(x+y)\) equivalent to the compound represented by the general formula (2) in a solvent at 30 to 60° C. for 2 to 48 hours.

3. The block copolymer according to claim 1 or 2, wherein \(R_1\) is a methyl group, \(R_2\) is a trimethylene group, \(R_3\) is a

optionally substituted aryl \((C1\) to \(C8)\) alkoxy group at a ratio of 1-99\% relative to \(m\), and \(-\text{N(R6)-CO-}\text{NHR}_7\) at a ratio of 0-10\% relative to \(m\), with a carbodiimide compound in an amount of \(m\) to 5 \(m\) equivalents relative to the compound represented by the general formula (1) in a solvent at 30 to 60° C. for 2 to 48 hours.

4. The block copolymer according to any of claims 1 to 3, wherein the carbodiimide compound is diethyl carbodiimide, disopropyl carbodiimide, dicyclohexyl carbodiimide or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide or an inorganic salt thereof.

5. The block copolymer according to any of claims 1 to 3, wherein the carbodiimide compound is disopropyl carbodiimide.

6. A block copolymer represented by the following general formula (3):

\[
R_1\text{(OCH}_2\text{CH}_2)_n\text{O}\text{-}R_2\text{[NHCOCH}_x\text{]}^\text{x}\text{-}\text{NHCO}\text{-}R_3\text{CH}_y\text{-}\text{NH}_{R_4}\text{CO-R}_5
\]

wherein \(R_1\) represents a hydrogen atom or a \((C1\) to \(C5)\) alkyl group, \(R_2\) represents a \((C1\) to \(C5)\) alkylene group, \(R_3\) represents a methylene group or an ethylene group, \(R_4\) represents a hydrogen atom or a \((C1\) to \(C4)\) acyl group, \(R_5\) represents a hydroxyl group, an optionally substituted aryl \((C1)\) to \(C8)\) alkoxy group or \(-\text{N(R6)-CO-}\text{NHR}_7\), \(R_6\) and \(R_7\) may be the same or different and each represents a \((C3\) to \(C6)\) cyclic alkyl group or a \((C1\) to \(C5)\) alkyl group optionally substituted with a tertiary amino group; \(n\) represents 5 to 1000, \(m\) represents 2 to 300, \(x\) represents 0 to 300 and \(y\) represents 0 to 300, provided that the sum of \(x\) and \(y\) is 1 or more to \(m\) or less; and \(R_5\) is a hydroxyl group at a ratio of 0-88\% relative to \(m\), an optionally substituted aryl \((C1)\) to \(C8)\) alkoxy group at a ratio of 1-89\% relative to \(m\), and \(-\text{N(R6)-CO-}\text{NHR}_7\) at a ratio of 11-30\% relative to \(m\).

7. The block copolymer according to claim 6, wherein \(R_1\) is a methyl group, \(R_2\) is a trimethylene group, \(R_3\) is a methylene group, \(R_4\) is an acetyl group, the optionally substituted aryl \((C1)\) to \(C8)\) alkoxy group represented by \(R_5\) is a benzyloxy group or a \(4\)-phenyl-\(1\)-butoxy group, each of \(R_6\) and \(R_7\) is an isopropyl group, \(n\) is 20 to 500, \(m\) is 10 to 100, \(x\) is 0 to 100, and \(y\) is 0 to 100.

8. The block copolymer according to claim 6 or 7, wherein \(R_5\) is a hydroxyl group at a ratio of 0-75\% relative to \(m\), an optionally substituted aryl \((C1)\) to \(C8)\) alkoxy group at a ratio of 10-80\% relative to \(m\), and \(-\text{N(R6)-CO-}\text{NHR}_7\) at a ratio of 11-30\% relative to \(m\).

9. The block copolymer according to claim 8, wherein \(R_5\) is a hydroxyl group at a ratio of 0\% relative to \(m\).
10. A micelle preparation formed from the block copolymer of any of claims 1 to 9 and a sparingly water-soluble anticancer agent.

11. The micelle preparation according to claim 10, wherein the sparingly water-soluble anticancer agent is a taxane-based anticancer agent.

12. The micelle preparation according to claim 11, wherein the taxane-based anticancer agent is paclitaxel.

13. An anticancer agent comprising the micelle preparation of any of claims 10 to 12 as an active ingredient.

* * * * *