DESCRIPTION

ANTICANCER AGENT FREE OF ADVERSE REACTIONS

TECHNICAL FIELD

[0001]

The present invention relates to a pharmaceutical composition and a method for treating cancers, characterized by administration and dosage of an anticancer agent of a type in which an anticancer active substance SN-38 and 4-arm PEG are covalently bonded to each other via a linker involved in a long-term controlled release of SN-38.

BACKGROUND ART

[0002]

Studies for pegylating a low-molecular pharmaceutical for increasing its stability in blood and enhancing its efficacy are in progress (Non Patent Literature 1). As such low-molecular pharmaceuticals, anticancer agents such as camptothecin, irinotecan, doxorubicin, SN-38, paclitaxel, docetaxel, cisplatin and gemcitabine have been developed in the form of pegylated pharmaceuticals.

[0003]

Among these anticancer agents, SN-38 (formal name: 7-ethyl-10-hydroxycamptothecin) is an active ingredient of Irinotecan (which is also called "CPT-11"). In that sense, Irinotecan is a prodrug of SN-38.

[0004]

Irinotecan is an antitumor drug synthesized by using, as a lead compound, camptothecin found in *Camptotheca acuminata* Nyssaceae, and is activated *in vivo*. It is converted to an active metabolite SN-38 by carboxyl esterases present in tissues of livers or the like. Irinotecan also exhibits an antitumor function by inhibiting type I DNA topoisomerase activity, and is widely used in cancer chemotherapy for lung cancer, cervical cancer, ovarian cancer, stomach cancer, colorectal cancer (or colon cancer), breast cancer, and malignant lymphoma. It is however said that this drug causes fatal side effects in patients having symptoms of myelosuppression, infection diseases, diarrhea, and/or intestinal obstruction (Patent Literature 1 and Non Patent Literature 2).

[0005]

There are proposals for improving the stability in blood and the resulting drug efficacy of Irinotecan or SN-38. For example, Patent Literature 2 describes a polymeric prodrug comprising 4-arm pegylated Irinotecan, and Patent Literatures 3 and 4 describe similar

polymeric prodrugs of SN-38. In all of these prodrugs, the OH group at the position 20 of Irinotecan or SN-38 and the 4-arm PEG are bonded to each other via an ester linkage (-O-CO-) which is easily enzymatically hydrolyzed by esterases or the like in blood.

[0006]

Besides, Patent Literature 5 describes a conjugate of a macromolecular carrier and a drug, which conjugate comprises a linker that can release a drug or prodrug via rate-controlled β-elimination, and specifically describes 4-arm pegylated SN-38 as an example. The binding site between the linker and SN-38 is a phenolic hydroxyl group of SN-38, and thus, a linkage different from the ester linkage is used.

[0007]

Multi-arm water-soluble polymeric pharmaceutical complexes, particularly polymer-based prodrugs, such as the prodrugs specifically mentioned above, are also described in Patent Literature 6, which describes that the binding portion between multi-arm polymer moiety and active substance moiety is desirable to be easily hydrolyzed *in vivo*, being generally a carboxylate ester, a carbonate ester, a phosphate ester, an anhydride, an acetal, a ketal, an acyloxyalkyl an ether, an imine, an ortho ester, an oligonucleotide, or the like, preferably a carboxylate ester or a carbonate ester.

PRIOR ART LITERATURE

PATENT LITERATURE

[0008]

[Patent Literature 1] Japanese Patent No. 4694776

[Patent Literature 2] Japanese Patent Publication (Kokai) No. 2011-213727

[Patent Literature 3] Japanese Patent Publication (Kohyo) No. 2013-500253

[Patent Literature 4] Japanese Patent Publication (Kohyo) No. 2012-524102

[Patent Literature 5] Japanese Patent Publication (Kohyo) No. 2013-528593

[Patent Literature 6] Japanese Patent Publication (Kohyo) No. 2007-505928

[Non Patent Literature]

[0009]

[Non Patent Literature 1] W. Li, Progress in Polymer Science (2013) 38: 421-444

[Non Patent Literature 2] S. Sawada et al., Chem. Pharm. Bull. (1991) 39(6): 1446-1454

SUMMARY OF THE INVENTION

PROBLEM TO BE SOLVED BY THE INVENTION

[0010]

The anticancer agent Irinotecan shows anticancer activity against a variety of cancers, but is known to have side effects such as digestive tube disorder accompanied by serious diarrhea, and severe leukopenia or neutropenia (myelosuppression).

[0011]

An object of the present invention is to provide a pharmaceutical composition and a method for treating cancer characterized by administration and dosage of an anticancer agent of a type in which SN-38 and 4-arm PEG are bonded to each other via a linker (which has a function capable of controlling the release of SN-38 for a long period of time), in which the composition can be repeatedly administered because there is no need to provide a drug withdrawal period for waiting for recovery of physical strength of a patient having been lowered by serious side effects, so that a strong anticancer effect can be provided without largely wasting the physical strength of the patient.

MEANS FOR SOLVING PROBLEM

[0012]

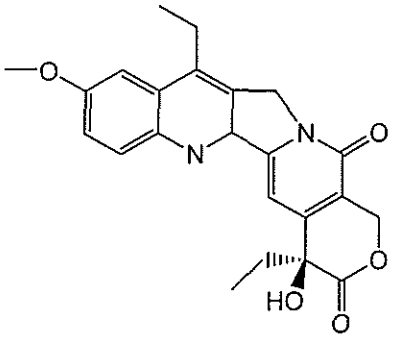
In short, the present invention has the following features.

[0013]

(1) A pharmaceutical composition for use in cancer treatment containing, as an active ingredient, a compound represented by the following formula I:

$C\text{-}[\text{-}CH_2\text{-}O\text{-}(\text{-}CH_2CH_2\text{-}O\text{-})_n\text{-}X^1\text{-}CHR^1\text{-}O\text{-}CO\text{-}NR^2\text{-}CH_2\text{-}X^2]_4$ (I)

wherein X^1-CHR^1-O-CO-NR^2 represents a linker, X^1 represents a spacer, R^1 represents a C1-C4 alkyl group that may be substituted, R^2 represents a phenyl group or a substituted phenyl group, X^2 is a group represented by the following formula II:



(II)

and n is an integer of 200 to 1,000,

[0014]

wherein the pharmaceutical composition is usable with administration and dosage in which a single dose of about 0.01 μmol/kg body weight to about 11 μmol/kg body weight is parenterally administered to a subject having cancer at a frequency of once a week, every

other week over at least 2 weeks, and has no Irinotecan-like side effects and provides a cancer growth inhibiting activity equivalent to or greater than that of Irinotecan when used at a dose as low as 1/10 (in a molar ratio) or less as compared with Irinotecan.

[0015]

(2) The composition according to (1), wherein R^1, R^2, X^2 and n are as defined above and X^1 is $(CH_2)_{1\text{-}5}$-CONH-$(CH_2)_{1\text{-}20}$ or $(CH_2)_{1\text{-}5}$-NHCO-$(CH_2)_{1\text{-}20}$ in the compound of the formula I.

[0016]

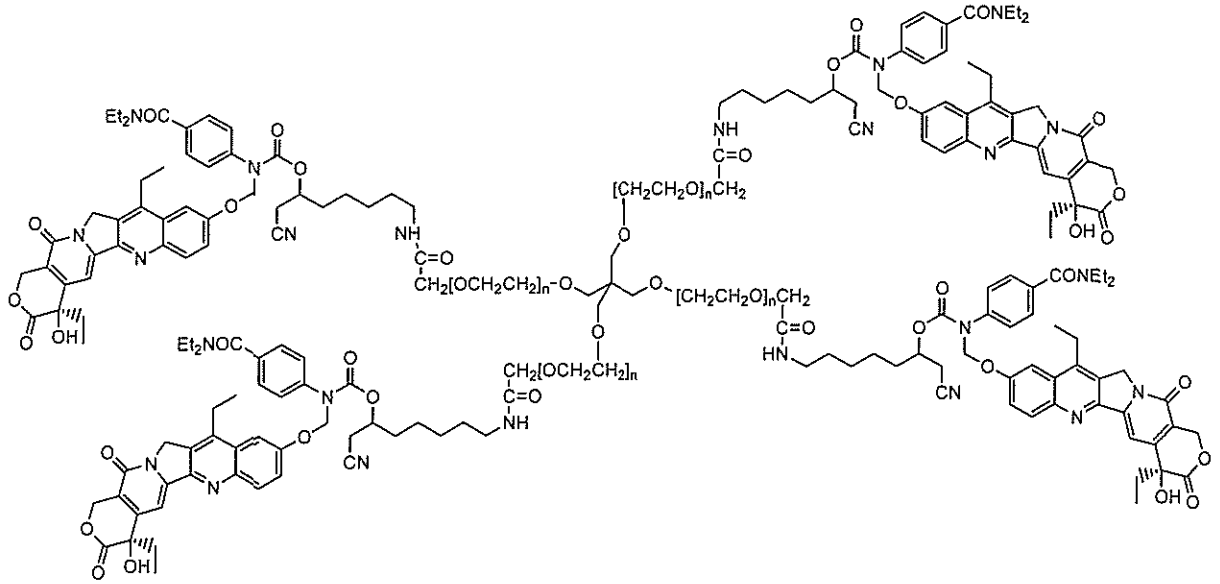
(3) The composition according to (1) or (2), wherein X^1, X^2 and n are as defined above, R^1 is a methyl group, an ethyl group, a substituted methyl group, or a substituted ethyl group, and R^2 is a substituted phenyl group in the compound of the formula I.

[0017]

(4) The composition according to (2) or (3), wherein X^1 and X^2 are as defined above, R^1 is a substituted methyl group, R^2 is a 4-substituted phenyl group, and n is an integer of 200 to 800 in the compound of the formula I.

[0018]

(5) The composition according to (4), wherein the compound of the formula I is a compound represented by the following formula III:



(III)

where n is as defined above.

[0020]

(6) The composition according to any one of (1) to (5), wherein the cancer is selected from the group consisting of colon cancer, breast cancer, ovarian cancer, cervical cancer,

uterine cancer, lung cancer, stomach cancer, pancreas cancer, esophageal cancer, head and neck cancer, hepatocellular carcinoma, gallbladder cancer, biliary cancer, sarcoma, and malignant lymphoma.

[0021]

(7) A method for treating a cancer comprising parenterally administering the composition according to any one of (1) to (5) to a subject having cancer at a single dose of the compound of about 0.01 μmol/kg body weight to about 11 μmol/kg body weight at a frequency of once a week, every other week over at least 2 weeks, in which no Irinotecan-like side effects including serious diarrhea and leukopenia and/or neutropenia are seen and a cancer growth inhibiting activity equivalent to or greater than that of Irinotecan is provided when the dose is as low as 1/10 (in a molar ratio) or less as compared with Irinotecan.

[0022]

(8) The method according to (7), wherein the cancer is selected from the group consisting of colon cancer, breast cancer, ovarian cancer, cervical cancer, uterine cancer, lung cancer, stomach cancer, pancreas cancer, esophageal cancer, head and neck cancer, hepatocellular carcinoma, gallbladder cancer, biliary cancer, sarcoma, and malignant lymphoma.

[0023]

(9) The method according to (7) or (8), wherein the parenteral administration is intravenous administration.

[0024]

(10) The method according to (9), wherein the intravenous administration is infusion or bolus injection.

[0025]

The present description comprises the contents described in the description and/or drawings of Japanese Patent Application No. 2013-237219 and No. 2013-209574, to which the present application claims priority.

EFFECT OF THE INVENTION

[0026]

The pharmaceutical composition and the treatment method of the present invention are characterized by administration conditions for an anticancer agent of a type in which SN-38 and 4-arm PEG are covalently bonded to each other via a linker (which has a function capable of controlling the release of SN-38 for a long period of time), in which the anticancer agent can be repeatedly administered because there is no need to provide a drug withdrawal period for waiting for recovery of physical strength of a patient having been lowered by serious side

effects, so that a strong anticancer function can be provided without largely wasting the physical strength of the patient. The composition can be parenterally administered to a subject, such as human, at a dosage in predetermined range at a frequency of once a week, every other week over at least 2 weeks, and side effects as those caused by Irinotecan (namely, Irinotecan-like side effects) such as serious diarrhea are not caused and hence it can be repeatedly administered continuously every other week. Therefore, the efficacy can be continuously kept, and as a result, a strong anticancer effect can be expected.

BRIEF DESCRIPTION OF DRAWINGS

[0027]

[Fig. 1] This figure shows changes in body weight of rats determined in efficacy tests. The tests were performed by using, as test samples, DFP-13318 (at a dose of 50 mg/kg, 100 mg/kg or 200 mg/kg) or Irinotecan (at doses of 70 mg/kg (for the first administration) and 50 mg/kg (for the second administration)). A vehicle was used as a control.

[Fig. 2] This figure shows changes in mean body weight of rat groups during the efficacy tests. A test was performed by using, as test articles, DFP-13318 (at a dose of 50 mg/kg, 100 mg/kg or 200 mg/kg) or Irinotecan (at doses of 70 mg/kg (for the first administration) and 50 mg/kg (for the second administration)). A vehicle was used as a control. ** $0.001 < P \leq 0.01$; P value: Irinotecan vs. vehicle = 0.006.

[Fig. 3] This figure shows antitumor activities determined in the treatment of a rat model, in which HT-29 human colon cancer cell line was subcutaneously implanted, using DFP-13318 (at a dose of 50 mg/kg, 100 mg/kg or 200 mg/kg) or Irinotecan (at doses of 70 mg/kg (for the first administration) and 50 mg/kg (for the second administration)). A vehicle was used as a control. Each test sample was intravenously administered on day 9 and on day 16 after the tumor implantation.

[Fig. 4] This figure indicates a dose-dependent accumulation of DFP-13318 in HT-29 tumor tissue after intravenous administration of DFP-13318.

[Fig. 5] This figure indicates a dose-dependent accumulation of SN-38 in HT-29 tumor tissue after intravenous administration of DFP-13318.

MODE FOR CARRYING OUT THE INVENTION

[0028]

The present invention will be described in more details.

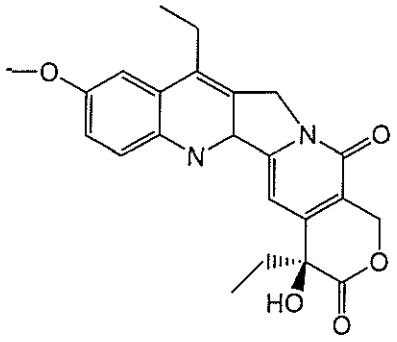
[0029]

<Anticancer compound>

The compound used as an active ingredient in the pharmaceutical composition or the treatment method of the present invention is represented by the following formula I:

$C\text{-}[\text{-}CH_2\text{-}O\text{-}(\text{-}CH_2CH_2\text{-}O\text{-})_n\text{-}X^1\text{-}CHR^1\text{-}O\text{-}CO\text{-}NR^2\text{-}CH_2\text{-}X^2]_4$ (I)

wherein X^1-CHR^1-O-CO-NR^2 represents a linker, X^1 represents a spacer, R^1 represents a C1-C4 alkyl group that may be substituted, R^2 represents a phenyl group or a substituted phenyl group, X^2 is a group represented by the following formula II:



(II)

[0030]

and n is an integer of 200 to 1,000.

[0031]

This compound is characterized by having a group represented by CHR^1-O-CO-NR^2-CH_2 between the group X^1 and the group X^2. A group similar to CHR^1-O-CO-NR^2-CH_2 is described in Japanese Patent Publication (Kohyo) No. 2013-528593 A (or WO 2011/140393), and it is presumed that such a group is dissociated, in tumor, into a polymer portion and an active substance through β-elimination caused by abstraction of a hydrogen atom (H) on a carbon atom of a group adjacent to the -CHR^1 group, or through cleavage caused under acidic environment, and that the active substance works tumor-selectively. For advantageously causing the β-elimination, the group R^1 is preferably an electron-withdrawing group. Examples of the electron-withdrawing group include a cyano group, a methane sulfonyl group, a trifluoromethyl group, a nitro group, an acetoxy group and a C1-C4 alkoxycarbonyl group (e.g., methoxycarbonyl or ethoxycarbonyl).

[0032]

The group X^2 is the SN-38 moiety of the formula II, and is bonded between the phenolic hydroxyl group of SN-38 and the CH_2 of X^1-CHR^1-O-CO-NR^2-CH_2 via an ether linkage (-O-). Thus, this ether linkage is different from the ester linkage that is said to be easily cleaved *in vivo*.

[0033]

Where the group R^2 is a substituted phenyl group, examples of the substituent include halogen (such as fluoro, chloro, bromo or iodo), a C1-C4 alkoxycarbonyl group (such as methoxycarbonyl or ethoxycarbonyl), N,N-di-C1-C4 alkyl carboxamide (such as N,N-diethyl

carboxamide), morphorinocarbonyl, and morphorinosulfonyl. The number of substituents and the position of substitution are not limited, and the number of substituents is 1 to 3, preferably 1, and the position of substitution is *o*-, *p*- or *m*-position, preferably *p*-position (which is also designated as "4-position").

[0034]

The group X^1 is a spacer for binding a PEG moiety to another moiety as in the formula I and, for example, it may be a linker containing one or more methylene groups and a linkage group such as CONH or NHCO, and specifically it includes $(CH_2)_{1-5}$-CONH-$(CH_2)_{1-20}$, $(CH_2)_{1-5}$-NHCO-$(CH_2)_{1-20}$, or CO-$(CH_2)_{1-5}$-CONH-$(CH_2)_{1-20}$.

[0035]

The compound of the formula I can be produced by reacting a derivative having any of various functional groups added to a terminal of 4-arm PEG, with (X^1 moiety having a terminal functional group)-CHR^1-O-CO-NR^2-CH_2-X^2.

[0036]

The derivative having any of various functional groups added to a terminal of 4-arm PEG can be obtained by binding a PEG derivative having any of different weight average molecular weights to, for example, a pentaerythritol nucleus via a spacer if necessary. The 4-arm PEG derivative is available from, for example, JenKem Technology Co., Ltd.

[0037]

In the PEG polymer moiety of the compound of the formula I, n is unlimitedly about 65 or more, for example about 100 to about 2,300, preferably about 200 to about 1,000, and the weight average molecular weight of the polymer is about 3 kDa to about 100 kDa, for example about 4 kDa to about 80 kDa, about 5 kDa to about 60 kDa, about 8 kDa to about 40 kDa, or about 10 kDa to about 20 kDa. In consideration of the influence of viscosity or toxicity of the 4-arm PEG derivative, the weight average molecular weight is preferably about 10 kDa to about 20 kDa.

[0038]

Examples of the terminal functional group of the 4-arm PEG derivative include, but are not limited to, groups to be linked to CH_2-O-(-CH_2CH_2-O-)$_n$- of the derivative, such as -CH_2-COO-NHS, -$CH_2CH_2CH_2CH_2CH_2$-COO-NHS, -CH_2CH_2-CHO, -$CH_2CH_2CH_2NH_2$, -$CH_2CH_2CH(OC_2H_5)_2$, -$CH_2CH_2CH_2NHCOCH_2CH_2$-maleimide, and -CH_2CH_2SH, where NHS represents N-hydroxysuccinimide.

[0039]

On the other hand, examples of the terminal functional group of the X^1 moiety reactive with the terminal functional group of the PEG moiety include, but are not limited to, a NH_2,

OH or SH group reactive with NHS group, a NH_2 group reactive with CHO group, a COOH group reactive with NH_2 group, a SH, maleimide or COOH group reactive with SH group, a NH_2 group reactive with $(OC_2H_5)_2$ group, and a SH group reactive with maleimide group.

[0040]

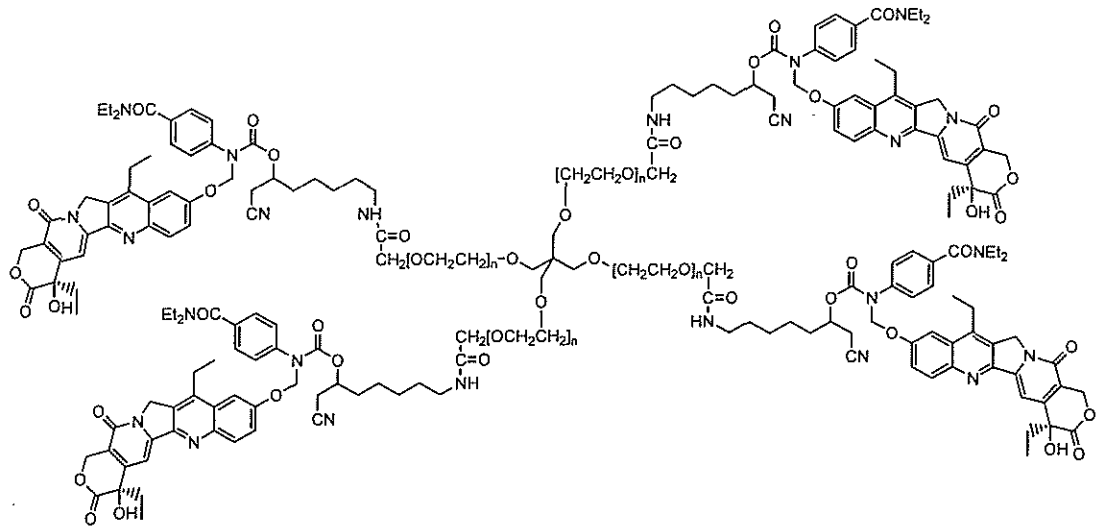
Preferably, in the compound of the formula I, X^1, X^2 and n are as defined above, R^1 is a methyl, ethyl, substituted methyl, or substituted ethyl group, and R^2 is a substituted phenyl group. Here, examples of the substituents are those exemplified above.

[0041]

More preferably, in the compound of the formula I, X^1 and X^2 are as defined above, R^1 is a substituted methyl group, R^2 is a 4-substituted phenyl group, and n is an integer of 200 to 800. Here, examples of the substituents are those exemplified above.

[0042]

Specifically, the compound of the formula I is a compound represented by the following formula III:



(III)

[0043]

wherein n is as defined above.

[0044]

The above-described compound used as the active ingredient of the present invention has a stability in blood of ≥ about 50,000-fold higher than that of its metabolite, SN-38 or SN-38G (which is SN-38 glucuronide), as estimated from the area under blood concentration-time curve (AUC) described in Examples later. Even when the compound is parenterally administered at a single dose of approximately 0.01-11μmol/kg body weight at a frequency of once a week, the anticancer active ingredient SN-38 is released in a controlled manner over a

long period of 1 week. As such, this compound can exhibit the pharmaceutical efficacy without causing serious Irinotecan-like side effects.

[0045]

As used herein, the term "Irinotecan-like side effects" refers to side effects including symptoms of myelosuppression such as leukopenia or neutropenia, and diarrhea.

[0046]

Furthermore, since the above-described compound used as the active ingredient of the present invention does not cause the serious side effects such as myelosuppression like leukopenia or neutropenia, and diarrhea when used under the aforementioned administration conditions, the compound can be parenterally administered repeatedly to a subject in the aforementioned dosage range for at least 2 weeks, preferably at least 4 weeks or at least 6 weeks without taking a drug withdrawal period. It has been suggested that the serious diarrhea caused by SN-38 occurs because SN-38G excreted from bile is converted to SN-38 in the intestinal tract and this pharmaceutical active ingredient, i.e. SN-38, injures the intestinal mucosa to induce diarrhea (K. Takasuna et al., Cancer Chemother. Pharmacol. 42: 280-286, 1998). The compound of the formula I used under the administration conditions of the present invention produces SN-38G *in vivo* in an amount as small as 1/100 or less of that produced by, for example, Irinotecan (see Table 10 described below). This is the reason why the side effect of serious diarrhea does not occur.

[0047]

Moreover, the compound of the present invention can provide a cancer growth inhibiting activity equivalent to or greater than that of Irinotecan (also designated as CPT-11) when used at a dose as small as 1/10 (in a molar ratio) or less as compared with Irinotecan (see Table 6 described below) (see Fig. 3).

[0048]

The compound of the formula I has been confirmed of the anticancer activity by an intravenous administration test performed in tumor-bearing rats (Fig. 3), and hence, its retention time in blood is extremely long, and as a result, the probability for the compound of coming into contact with a tumor site is increased. In addition, since the compound is a polymer substance, it probably shows its efficacy *in vivo* through stages of being incorporated into tumor cells by endocytosis and freeing the active substance SN-38 within the tumor cells. Since tumor cells have properties that they easily incorporate a polymer substance as compared with normal cells, they could incorporate the compound of the formula I of the present inventions having a high molecular weight.

[0049]

<Pharmaceutical composition>

The present invention also provides a pharmaceutical composition for use in treatment of cancers comprising the above-described compound as an active ingredient. The pharmaceutical composition is usable with administration and dosage in which a single dose of the compound of approximately 0.01-11 μmol/kg body weight is parenterally administered to a subject having cancer at a frequency of once a week, every other week over at least 2 weeks, and has no Irinotecan-like side effects and provides a cancer growth inhibiting activity equivalent to or larger than that of Irinotecan when used at a dose as low as 1/10 (in a molar ratio) or less as compared with Irinotecan.

[0050]

A dose per administration of the compound varies depending upon the sex, age, body weight, symptom, seriousness or the like of the subject, and is suitably approximately 0.01-11 μmol/kg body weight, and for example, approximately 1-11 μmol/kg body weight, approximately 3.5-7 μmol/kg body weight, approximately 4-6 μmol/kg body weight, approximately 0.1-1.8 μmol/kg body weight, or approximately 0.4-1 μmol/kg body weight. In the Examples described later, an effective dose was found through experiments using rats, and when an effective dose (mg/kg) for rat is converted into a dose (mg/kg) for human, the effective dose (mg/kg) for human is known to be about 1/100 to about 1/6 (or about 1/10) of the dose for rat. If the compound is, for example, the compound of the formula III (DFP-13318, MW 45,700), the dose is suitably approximately 0.5-450 mg/kg body weight, and for example, is approximately 50-450 mg/kg body weight, approximately 100-300 mg/kg body weight, approximately 150-300 mg/kg body weight, approximately 170-250 mg/kg body weight, approximately 0.5-75 mg/kg body weight, or approximately 17-42 mg/kg body weight. The compound of the formula I of the present invention contains, per molecule, 4 molecules of SN-38 (MW 392), the active component.

[0051]

As illustrated as tumor growth inhibition by the intravenous administration of the pharmaceutical in Fig. 3, when the pharmaceutical was administered at a frequency of once a week, every other week over 2 weeks, even when DFP-13318 was administered at a dose (200 mg/kg) as small as 1/25 (in a molar ratio) as compared with Irinotecan, stronger antitumor activity than that of Irinotecan was shown, and a tumor growth inhibition (TGI) value was 61% ($P = 0.030$).

[0052]

As described in Example 2 later, in animal experiments using rats, following intravenous administration of DFP-13318 at a dose of 225 mg/kg, mean Tmax and Cmax

values were respectively determined to be 2 hours and 3.05 mg/mL for DFP-13318, 1 hour and 97.6 ng/mL for SN-38, and 1 hour and 12.7 ng/mL for SN-38G. Furthermore, DFP-13318 had mean plasma half-life $T_{1/2}$ of 36.8 hours. Mean values of clearance and MRT_{last} of DFP-13318 were respectively determined to be 2.02 mL/hr/kg and 28.8 hours. The mean volume of distribution (Vss) was determined to be 92.2 mL/kg, and values of AUC_{last} and AUC_{inf} of DFP-13318 were respectively determined to be 92.6 hr·mg/mL and 117 hr·mg/mL. Besides, the mean value of AUC_%Extrap was 24.0%.

[0053]

The aforementioned results indicate that the retention time in blood of DFP-13318 is extremely long and hence its stability in the blood is extremely high. This characteristic could apply to any compounds represented by the formula I of the present invention including DFP-13318.

[0054]

The pharmaceutical composition comprising the compound of the formula I as the active ingredient is formulated into a parenteral administration preparation. Examples of the parenteral route include intravenous, intraarterial, intraperitoneal, subcutaneous, intracutaneous, intramuscular, intracranial, nasal, and transmucosal routes, and the preferable route is the intravenous route. Therefore, the composition is formulated preferably into a preparation for injection, (instillation) infusion or bolus injection. The formulation can be performed by, for example, a method described in REMINGTON; THE SCIENCE AND PRACTICE OF PHARMACY (20th Ed) or the like, or by using an excipient, a diluent, an additive or the like. The preparation for injection, (instillation) infusion or bolus injection can be formulated by dissolving the aforementioned effective dose of the compound of the formula I or III in a diluent such as saline, a buffer or a Ringer's solution, and allowing the resultant solution to pass through a sterilizing filter. An additive, such as solvent or preservative, may be added where needed.

[0055]

In the present invention, examples of cancer to be treated include all cancers clinically treated with Irinotecan, such as colon cancer, breast cancer, ovarian cancer, cervical cancer, uterine cancer, lung cancer, stomach cancer, pancreas cancer, esophageal cancer, head and neck cancer, hepatocellular carcinoma, gallbladder cancer, biliary cancer, sarcoma and malignant lymphoma. Since the composition of the present invention does not cause the Irinotecan-like side effects such as serious diarrhea and leukopenia or neutropenia, it is regarded that the composition can be also effectively used against a cancer other than the aforementioned cancers.

[0056]

Furthermore, examples of the subject to be treated for cancer include animals including a human, and the subject is preferably a mammal, such as a human or a pet animal (like a dog or a cat). The animal is preferably a human.

[0057]

<Treatment method>

The present invention further provides a method of treating cancers comprising parentally administering the compound of the formula I or the formula II to a subject having cancer at a single dose of approximately 0.01-11 μmol/kg body weight at a frequency of once a week, every other week over at least 2 weeks, at least 4 weeks, or at least 6 weeks, in which no Irinotecan-like side effects including serious diarrhea and leukopenia and/or neutropenia are seen or observed, and the cancer growth inhibiting activity equivalent to or greater than that of Irinotecan is provided when the dose is as low as 1/10 (in a molar ratio) or less as compared with Irinotecan.

[0058]

The compound and the pharmaceutical are the same as those described above.

[0059]

The compound used in the treatment method of the present invention has characteristics that it is dose-dependently accumulated within tumor after the administration by, for example, intravenous administration, and in addition, it releases the activator SN-38 in the tumor dose-dependently and continuously (Figs. 4 and 5). On the contrary, when Irinotecan was administered under the same conditions, no SN-38 was detected in the tumor (see Example 3).

[0060]

The cancer to be treated and the subject to be treated are also the same as those described above. Preferable examples of the cancer include, but are not limited to, colon cancer, breast cancer, ovarian cancer, cervical cancer, uterine cancer, lung cancer, stomach cancer, pancreas cancer, esophageal cancer, head and neck cancer, hepatocellular carcinoma, gallbladder cancer, biliary cancer, sarcoma and malignant lymphoma. Besides, a preferable example of the subject is a human.

[0061]

In the treatment method of the present invention, the parenteral administration is employed, and a preferable example of the route is the intravenous route as described above, and preferable examples of the administration include (instillation) infusion and bolus injection.

EXAMPLES

[0062]

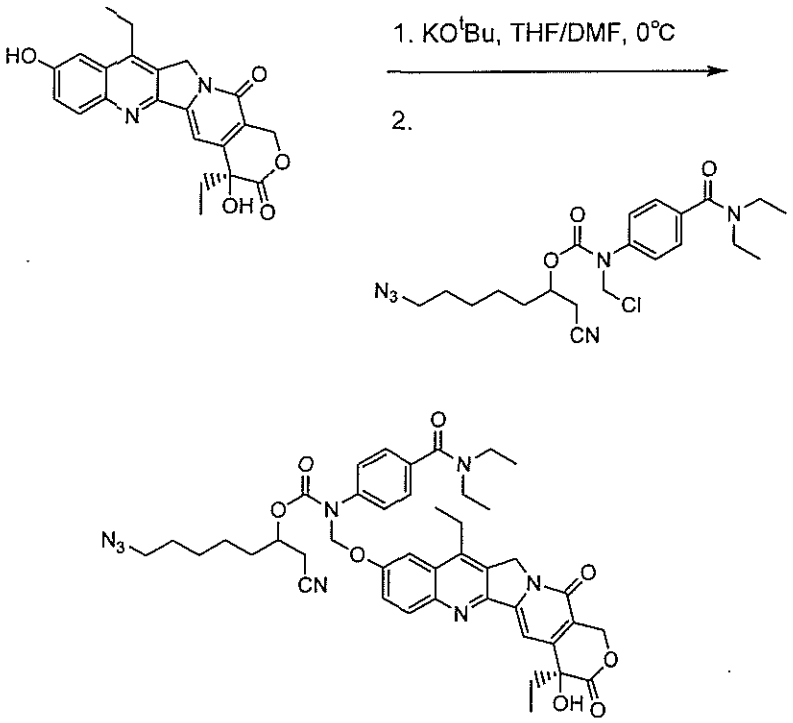
The present invention will now be described more specifically with reference to the following Examples, and it is contemplated that the technical scope of the present invention is not limited to those Examples.

[0063]

[Example 1]

<Production of compound DFP-13318>

1. Production of azido-linker-SN38



[0064]

SN38 (1.00 g, 2.55 mmol; Haorui) was suspended in 10 mL of anhydrous pyridine, then concentrated to dryness under vacuum at 50°C. This was repeated with 10 mL of anhydrous THF. The resulting pale yellow solid was dissolved in 50 mL of anhydrous THF and 50 mL of anhydrous DMF under N_2 atmosphere, then cooled on ice. A 1.0 M solution of potassium *tert*-butoxide in THF (2.55 mL, 2.55 mmol) was added forming an initial dark green color that changed to a thick orange suspension. After 15 min, a THF solution of the 7-azido-1-cyano-2-hexyl N-(chloromethyl)-4-(N,N-diethylcarboxamide)-phenylcarbamate (7.5 mL, 2.8 mmol) was added. After 15 min at 4°C, the light orange mix was allowed to warm to room temperature. After 1 hr, HPLC analysis (5 µL of sample + 1 mL of CAN/TFA) indicated product/SN38=86/14. The pale yellow mixture was diluted with 200 mL of ethyl acetate, washed 2x100 mL of water, 100 mL of saturated aq. NaCl, dried over $MgSO_4$,

filtered, and evaporated. Excess DMF was removed by trituration of the oily residue with water, and the residue was dissolved in 50 mL of acetonitrile, filtered, and evaporated to yield 2.96 g of yellow glass. The residue was chromatographed on SiO_2 (80 g) using a stepwise gradient of 200 mL each of hexane, 20%, 40%, 60%, 80%, and 100% acetone in hexane, providing the purified azido-linker-SN38 (1.66 g, 81%).

[0065]

This material was dissolved in 50 mL of acetone, and 45 mL of 0.1% acetic acid in water was added dropwise with stirring until the mixture became cloudy. Upon stirring, a solid material separated. An additional 5 mL of 0.1% acetic acid in water was then added to complete the precipitation. After stirring for 2 h, the solid was collected by vacuum filtration, washed with water, and dried to provide 1.44 g (70%) of pale yellow powder.

[0066]

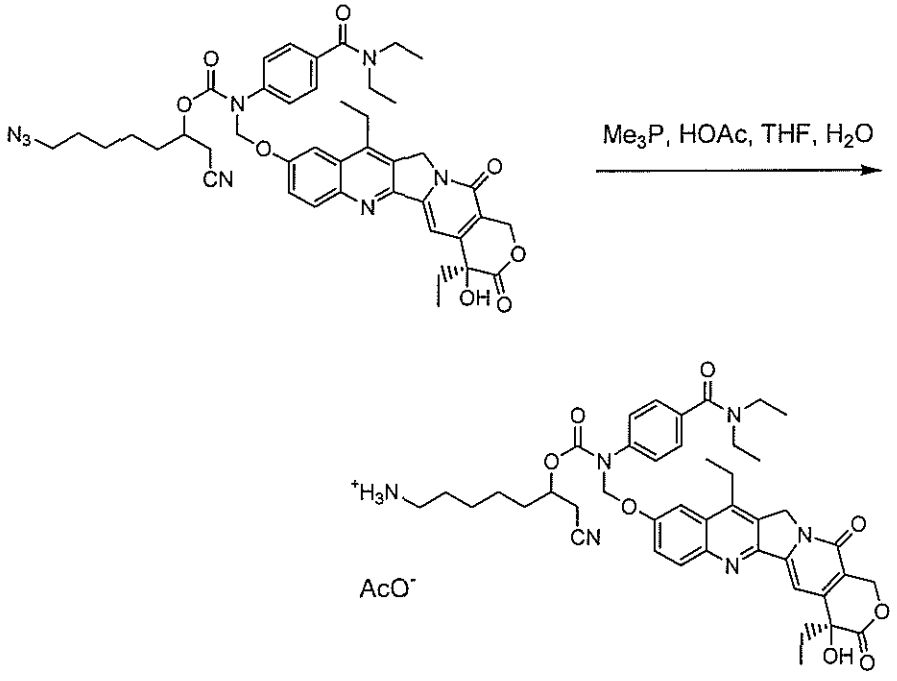
The analysis values are as follows.

[0067]

^{1}H-NMR(400 MHz,$CDCl_3$): δ 8.15 (1H, d, J = 9.2 Hz), 7.60 (1H, s), 7.48 (1H, dd, J = 2,9 Hz), 7.40 (4H, m), 7.25 (1H, d, J = 2), 5.75 (2H, br), 5.73 (1H, d, J = 16 Hz), 5.28 (1H, d, J = 16 Hz), 5.22 (2H, s), 4.99 (1H, m), 3.84 (1H, s), 3.53 (2H, br), 3.53 (2H, br), 3.17 (2H, t, J = 7 Hz), 3.12 (2H, q, J = 7 Hz), 2.74 (1H, dd, J = 1, 17 Hz), 2.54 (1H, dd, J = 5, 17), 1.86 (2H, m), 1.6 (1H, m), 1.46 (1H, m), 1.37 (3H, t, J = 7 Hz), 1.25 (6 H, m), 1.12 (4 H, m), 1.02 (3H, t, J = 7.3 Hz)

LC-MS: [M+H]+ = 805.3 (calculation value of $C_{44}H_{51}N_8O_8$ = 805.3)

2. Production of amino-linker-SN38 acetate salt

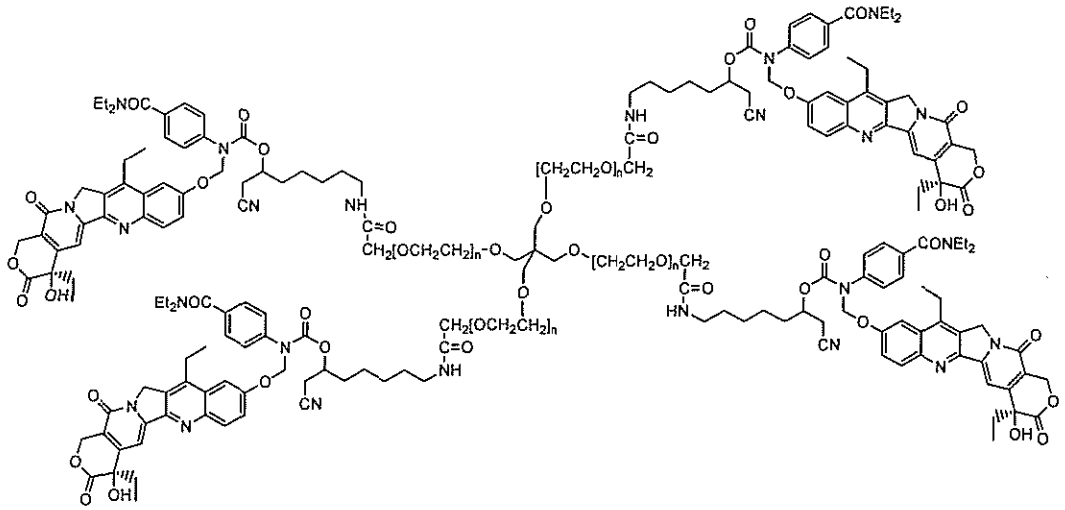


[0068]

A 1 M solution of trimethylphosphine in THF (2.9 mL, 2.9 mmol) was added to a solution of the azido-linker-SN38 (1.13 g, 1.4 mmol) and acetic acid (0.19 mL, 3.3 mmol) in 10 mL of THF. After stirring for 2 h, water (1.0 mL) was added and the mixture was stirred for an additional 1 h. The residue was partitioned between ether and water. The water phase was washed once with ethyl acetate, and the resulting clear yellow aqueous phase was evaporated to provide 800 mg of yellow foam. This was dissolved in THF, filtered, and quantitated by UV absorbance to provide a solution containing 1.2 μmol (86%) of product. C18 HPLC showed a single peak, and LC-MS showed [M+H]+ = 779.3 (calculated779.4).

[0069]

3. Production of compound DFP-13318 (molecular weight: approximately 45,700)



[0070]

4-arm tetra-(succinimidyl-carboxymethyl)-PEG (JenKem Technology; 10.0 g, 1.0 mmol HSE), the amino-linker-SN38 acetate salt of Example 1 (1.2 mmol), and N,N-diisopropylethylamine (0.21 mL, 1.2 mmol) were mixed in 75 mL of THF and left to stand at room temperature. Coupling progress was monitored by HPLC, which indicated completion of reaction by 90 minutes. After a total of 2 h, the mixture was filtered into 500 mL of stirred *tert*-butyl methyl ether (MTBE). The precipitate was collected by vacuum filtration, washed with MTBE, and dried under vacuum to provide the conjugate as a waxy pale yellow solid (10.1 g, 95%). Spectrophotometric analysis of a 2.0 mg sample in 1.0 mL of water indicated 0.17 mM SN-38 (calculated from weight, 0.175 mM SN38), yield 96%. C18-HPLC analysis indicated a single major peak (98% of total peak area at 363 nm; 97% at 256 nm), with 0.6 mol% of free SN38 as a contaminated amount.

[0071]

[Example 2]

<Antitumor properties of compound DFP-13318>

1. Test conditions

Studies for toxicity, pharmacokinetics and efficacy were performed by intravenous injection (iv) of each test sample to tumor-bearing rats under conditions shown in Tables 1 and 2.

[Table 1]

Rat groups and treatment for efficacy study

Group	n*	Treatment	Dosage (mg/kg)**	Route/schedule
1	5	Vehicle control***	--	iv, qwk x 2 wks
2	5	DFP-13318	50	iv, qwk x 2 wks
3	5	DFP-13318	100	iv, qwk x 2 wks
4	5	DFP-13318	200	iv, qwk x 2 wks
5	5	Irinotecan	70/50	iv, qwk x 2 wks

Note: *n: the number of animals, **Dosage: adjusted based on body weight (10 μL/g), ***Vehicle control: 10 mM acetate buffer, pH 5

[Table 2]

Satellite group for pharmacokinetics (PK) evaluation

Group	n*	Treatment	Dosage (mg/kg)**	Route/schedule
-	3	DFP-13318	200	iv, Single bolus injection

Note: *n: the number of animals, **Dosage: adjusted based on body weight (10 μL/g)

[0072]

2. Test animals

The following rats were used as test animals.

[0073]

Species: Rattus Norvegicus

Strain: Nude rat

Age: 6 to 8 weeks old

Sex: Female

Body weight: 140 to 180 g

The number of animals: 25 rats + 40% extra rats

Animal supplier: Vital River Laboratory Animal Technology, Co., Ltd.

3. Cell line

The human colon cancer cell line HT-29 was maintained as a monolayer culture in a McCoy 5a modified medium (containing 10% heat inactivated fetal calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin, and L-glutamine (2 mM)) at 37°C in an atmosphere of 5% CO_2/air. The tumor cells were routinely subcultured twice a week by trypsin-EDTA

treatment. The cells in an exponential growth phase were harvested and counted for tumor implantation.

[0074]

4. Tumor implantation

Each nude rat was implanted subcutaneously at the flank with HT-29 tumor cells (5 x 10^6) in 0.1 ml of PBS for tumor development. The treatment started on day 9 after tumor implantation when the mean tumor size reached about 120 mm^3. Each group consisted of 5 tumor-bearing nude rats for the efficacy study and for the pharmacokinetics (PK) study. The test samples were administered intravenously twice, once a week from day 9 after tumor implantation to the rats for the efficacy study, and once to the rats of the satellite group for the PK evaluation.

[0075]

5. Preparation of test article formulations

Preparation of test sample formulations is shown in Table 3.

[Table 3]

Compound	Preparation [a]	Concentration[b] (mg/ml)	Storage
Vehicle (10 mM acetate buffer)	A 10 mM acetate buffer (pH 5) was prepared by dissolving 246 mg of sodium acetate in 300 mL of saline, followed by well mixing.	--	4°C
DFP-13318	DFP-13318 in an amount of 420 mg was dissolved in 21 mL of vehicle, followed by well mixing.	20	-20°C, protected from light
	DFP-13318 (20 mg/ml) was dissolved in 9 mL of vehicle, followed by well mixing.	10	
	DFP-13318 (10 mg/ml) was dissolved in 6 mL of vehicle, followed by well mixing.	5	
Irinotecan	Irinotecan in an amount of 80 mg was dissolved in 11.43 mL of saline, followed by well mixing.	7	4°C, protected from light
Irinotecan	Irinotecan in an amount of 80 mg was dissolved in 16 mL of saline, followed by well mixing.	5	4°C, protected from light
DFP-13318 (for PK profiling)	DFP-13318 in an amount of 240 mg was dissolved in 12 mL of vehicle, followed by well mixing.	20	-20°C, protected from light
1M Citrate buffer/ 0.1% Pluronic F68	Sodium citrate in an amount of 588.20 mg was dissolved in 2 mL of WFI, and 2 mg of Pluronic F68 was added thereto, followed by well mixing (pH 4.5).	--	4°C

Note: a. A fresh formulation was prepared before every use; b. Each of the shown concentrations was based on the weight of the compound.

[0076]

6. Tumor measurement and endpoint

The major endpoint was to see if the tumor growth could be delayed or tumors disappeared. The body weight of each rat was measured twice a week, and the difference in tumor size was measured twice a week in two dimensions using a caliper. The tumor volume was expressed in mm^3 using a formula: $V = a \times b^2/2$ (where a and b represent long and short diameters of a tumor, respectively). The tumor volumes were used for tumor growth inhibition (TGI) values to evaluate the antitumor effectiveness. The TGI value was calculated in accordance with a formula: $TGI = (1- T/C) \times 100\%$ (where C and T represent the mean volumes of tumors in control and treated groups, respectively, on a given day). Tolerability was evaluated based on % body weight difference during the treatment period.

Tumor fragments with sizes of 200 to 400 mm^3 were collected from the efficacy study groups two weeks after the second dosing and were stored at -80°C for bioanalysis.

[0077]

7. Pharmacokinetics (PK) study

Blood samples were taken for the PK study at 0 (pre-dose), 1, 72 and 120 hours post dosing from the satellite group. At each time interval, whole blood samples of 0.5 mL each were drawn from the retro-orbital plexus of each tumor-bearing nude rat, and directly added to 50 μL of 1 M citrate buffer/0.1% Pluronic F68 solution (pH 4.5) to reduce the sample pH to stabilize the remaining intact conjugate of the test sample as well as to block coagulation. The blood samples collected within 60 minutes were centrifuged at about 1,500 x g for 10 minutes at 4°C to collect 200 to 250 μL plasma. The plasma was then divided into 2 aliquots in syringe vials and stored in a -80°C freezer, and then transferred to a bioanalysis group for the PK analysis (12 samples in total). Concentrations of DFP-13318, SN-38 and SN-38G in the plasma samples were analyzed by using HPLC/FLD and LC-MS/MS methods.

[0078]

8. Statistical analysis

Data including the mean, the standard error of the mean (SEM), the tumor volume at each time point, and the TGI were summarized. Statistical analyses of a difference in tumor volume among groups were conducted by using the data obtained at time points of the best antitumor activity measured on day 27 after the tumor implantation or on day 18 after starting the treatment. One-way ANOVA of analysis of variance was performed to compare the body weights and the tumor volumes between groups, and all data were analyzed using software SPSS 17.0. $P \leq 0.05$ is regarded statistically significant.

[0079]

9. Side effect reduction by compound DFP-13318

The drug tolerability study was performed by using the test animals.

[0080]

Measured results of changes in body weight of the tumor-bearing nude rats having been treated with DFP-13318 (at a dose of 50 mg/kg, 100 mg/kg or 200 mg/kg) or Irinotecan (at a dose of 70/50 mg/kg) in the efficacy study are shown in Figs. 1 and 2.

[0081]

10. Tumor growth inhibiting activity of compound DFP-13318

Changes in tumor volume and inhibition of tumor growth in the tumor-bearing rat groups having been treated with DFP-13318 and Irinotecan were studied.

[0082]

Results are shown in Tables 4 and 5, and tumor growth curves are depicted in Fig.3.

[Table 4]

Tumor volumes (rat model in which human colon cancer cell line HT-29 was implanted)

Tumor volume (mm^3)[a]					
Day	Vehicle control	DFP-13318 50 mg/kg	DFP-13318 100 mg/kg	DFP-13318 200 mg/kg	Irinotecan 70/50 mg/kg
9	115 ± 16	113 ± 11	116 ± 17	116 ± 17	114 ± 14
13	355 ± 43	308 ± 20	255 ± 32*	178 ± 15***	168 ± 14***
16	540 ± 62	468 ± 47	342 ± 41**	244 ± 23***	258 ± 30***
20	964 ± 123	810 ± 61	640 ± 80*	407 ± 45***	518 ± 78***
23	1,352 ± 188	1,178 ± 109	871 ± 72	572 ± 55	727 ± 92
27	1,914 ± 223	1,676 ± 76	1,233 ± 79	756 ± 82*	1,037 ± 112
30	2,577 ± 299	2,109 ± 78	1,697 ± 159	1,004 ± 93*	1,453

Note: a. Mean ± SEM. * $0.01 < P \leq 0.05$, ** $0.001 < P \leq 0.01$, ***$P \leq 0.001$

[Table 5]

Tumor growth inhibition (rat model in which human colon cancer cell line HT-29 was implanted)

Treatment	Tumor volume (Day 27 after implanted, mm^3)[a]	TGI (Day 27 after implanted, %)	P value[b]
G1: Vehicle control	1,914 ± 223	--	--
G2: DFP-13318 50 mg/kg	1,676 ± 76	13	0.948
G3: DFP-13318 100 mg/kg	1,233 ± 79	35	0.204
G4: DFP-13318 200 mg/kg	756 ± 82	61	0.030
G5: Irinotecan 70/50 mg/kg	1,037 ± 112	45	0.089

Note: a. Mean ± SEM; b. vs. vehicle control

P value: G2 vs. G3 = 0.031; G2 vs. G4 < 0.001; G2 vs. G5 = 0.017; G3 vs. G4 = 0.025; G3 vs. G5 = 0.784; G4 vs. G5 = 0.452

[0083]

Molar ratios in the respective test samples administered are shown in Table 6.

[Table 6]

Test sample	Dosage (mg/kg)	Dosage (μmol/kg)	Dosage in terms of SN-38 (μmol/kg)	Molar ratio (%) of DFP-13318 to CPT-11
DFP-13318	50	1.16	0.04	1.0
DFP-13318	100	2.33	0.08	1.9
DFP-13318	200	4.65	0.17	3.9
Irinotecan	70	119.32	119.32	100

[0084]

In this study, the antitumor activity and drug tolerability of DFP-13318, which is a test compound, at the three dose levels of 50, 100 and 200 mg/kg were evaluated, and compared with those of Irinotecan (70/50 mg/kg) attained in the treatment of a nude rat model in which the human colon cancer cell line HT-29 was implanted. The tumor volumes in the 5 rats of each treatment group at different time points are shown in Tables 4 and 5 and Fig. 3.

[0085]

The tumor volume of the vehicle treated group reached 1,914 mm^3 on day 27 after the tumor implantation, and the dose (200 mg/kg) of DFP-13318 as low as 1/25 (in a molar ratio) of that of Irinotecan showed stronger antitumor activity than Irinotecan with the tumor growth inhibition (TGI) value of 61% (P = 0.030). The TGI values attained in the groups receiving doses of 50 mg/kg and 100 mg/kg of DFP-13318 (respectively corresponding to 1/100 and 1/50 (in a molar ratio) of that of Irinotecan) were 13% and 35%, respectively. Irinotecan dosed at 70/50 mg/kg showed moderate antitumor activity with a TGI value of 45%. The antitumor activity of DFP-13318 is attributed to the released SN-38, which is less than 4% of the total weight from its PEG conjugate, and therefore, the dosages (mg/kg) of the test samples should be converted to molar ratios between DFP-13318 and Irinotecan as shown in Table 6 for the fair comparison. When thus compared, it is apparent that the antitumor activity of DFP-13318 is very potent as compared with that of Irinotecan.

[0086]

Regarding the safety profile, DFP-13318 at the three dose levels was well tolerated in the tumor-bearing rats. The body weight losses were less than 2.0% at the most, and no diarrhea was observed in the groups treated with DFP-13318. This is probably because the production level of SN-38G causing serious diarrhea is extremely low in DFP-13318 and is as low as 1/100 or less of that in Irinotecan. Specifically, based on results shown in Tables 10 and 11 described later, production ratios of SN-38G to SN-38 were 0.07-fold and 0.06-fold respectively at the doses of DFP-13318 of 225 mg/kg and 450 mg/kg, but in using Irinotecan, the production ratio of SN-38G to SN-38 was 8.7-fold (Table 1 of Cancer Chemother. Pharmacol. 42(4): 280-286, 1998). On the other hand, when Irinotecan was administered at an initial dose of 70 mg/kg, severe body weight loss (16%) occurred, and hence the dose was reduced to 50 mg/kg at the second dosing.

[0087]

11. Pharmacokinetics (PK) study for compound DFP-13318

SD rats (female) of 8 to 12 weeks old (Vital River Laboratory Animal Technology, Co., Ltd.) were randomly divided by 3 rats into a first group (single bolus iv injection) and a

second group (10-minute iv injection) to be used for the PK study. Each animal received single-dose administration on day 1 as shown in Table 7.

[Table 7]

Test animal groups

Group	Treatment	Dose level (mg/kg)	Dose volume (mL/kg)	Concentration (mg/mL)	Route	The number of animals
1	DFP-13318	225	5	45	iv bolus injection	3
2	DFP-13318	450	10	45	10-min iv injection	3

[0088]

On the day of the administration, a fresh solution of 45 mg/mL DFP-13318 was prepared by dissolving a predetermined amount of DFP-13318 in a 10 mM sodium acetate buffer (pH 5.0), and before the administration, the resulting solution was allowed to pass through a 0.22 μm PVDF filter for sterilization.

[0089]

The schedule for collection of blood samples for PK profiling is shown in Table 8.

[Table 8]

Blood collection schedule

Animal		All rats
Time points of blood collection	First group[a]	0 (pre-dose), 1, 2, 4, 8, 10, 12, 24, 48, 72 and 120 hours post dosing (11 time points)
	Second group	0 (after injection), 1, 2, 4, 8, 10, 12, 24, 48, 72 and 120 hours post dosing (11 time points)
Blood collection site		Collected from jugular by a catheter.
Blood collection amount		0.4 mL (whole blood) at each time point
Blood collection tube		Tube containing 40 μL of 1 M citrate/0.1% Pluronic F68 solution (pH 4.5)

a. Each time point refers to time post dosing except for "pre-dose" for which a sample was collected 14 minutes before dosing.

[0090]

The HPLC/FLD method was developed and used for analyzing DFP-13318, and the LC-MS/MS method was established and used for quantifying SN-38 and SN-38G. The concentrations of DFP-13318 and its metabolites SN-38 and SN-38G in plasma were used for calculating PK parameters by employing noncompartmental analysis (Phoenix (TM) WinNonlin (registered trademark), version 6.1, model Plasma (200-202)). Plasma

concentrations below *LLOQ* (*BLLOQ*) were excluded in the calculation of the PK parameters. Mean values of the PK parameters were calculated by using Microsoft Excel.

[0091]

Table 9 shows the measured results of the plasma concentrations of DFP-13318, Table 10 shows the measured results of the plasma concentrations of SN-38, and Table 11 shows the measured results of the plasma concentrations of SN-38G. Besides, Table 12 shows the calculated results of the PK parameters of DFP-13318, Table 13 shows the calculated results of the PK parameters of SN-38, and Table 14 shows the calculated results of the PK parameters of SN-38G.

[Table 9]

Plasma concentrations (mg/mL) of DFP-13318 after intravenous administration of DFP-13318

Group No.	Dosage	Route	Animal No.	Time point											
				Pre-dose	0 hr	1 hr	2 hr	4 hr	8 hr	10 hr	12 hr	24 hr	48 hr	72 hr	120 hr
1	225 mg/kg	IV bolus injection	78979	BLLOQ	-	2.88	3.03	2.74	2.30	1.85	1.76	1.40	0.954	0.577	0.264
			78980	BLLOQ	-	3.00	3.10	2.92	2.58	2.12	1.80	1.42	0.983	0.579	0.310
			78981	BLLOQ	-	2.92	3.03	2.68	2.27	1.88	1.71	1.19	NS	NS	NS
			Mean	-	-	2.93	3.05	2.78	2.38	1.95	1.76	1.33	0.969	0.578	0.287
			SD	-	-	0.061	0.043	0.125	0.169	0.147	0.045	0.129	0.021	0.001	0.033
2	450 mg/kg	10-min. IV injection	78982	-	9.20	5.91	5.59	6.61	3.53	3.46	3.27	2.33	1.15	0.724	0.281
			78983	-	5.66	5.47	4.94	6.18	5.17	3.44	3.23	2.20	1.15	0.730	0.287
			78984	-	6.86	6.27	6.22	4.80	4.50	4.17	3.80	2.81	1.28	0.636	0.271
			Mean	-	7.24	5.88	5.58	5.86	4.40	3.69	3.43	2.44	1.19	0.697	0.280
			SD	-	1.80	0.401	0.640	0.946	0.825	0.413	0.318	0.321	0.075	0.053	0.008

BLLOQ = below the lower limit of quantification (LLOQ = 0.01 mg/mL)

- = not measured

NS = no samples

[Table 10]

Plasma concentrations (ng/mL) of SN-38 after intravenous administration of DFP-13318

Group No.	Dosage	Route	Animal No.	Time point											
				Pre-dose	0 hr	1 hr	2 hr	4 hr	8 hr	10 hr	12 hr	24 hr	48 hr	72 hr	120 hr
1	225 mg/kg	IV bolus injection	78979	BLLOQ	-	42.1	36.3	43.4	54.2	23.8	22.0	20.1	10.9	6.99	4.20
			78980	BLLOQ	-	51.6	45.4	48.9	44.1	26.9	24.2	25.8	13.9	6.78	5.57
			78981	BLLOQ	-	199	103	99.0	99.2	80.8	79.3	28.3	NS	NS	NS
			Mean	-	-	97.6	61.6	63.8	65.8	43.8	41.8	24.7	12.4	6.89	4.89
			SD	-	-	88.0	36.2	30.6	29.3	32.1	32.5	4.20	2.12	0.148	0.969
2	450 mg/kg	10-min. IV injection	78982	-	324	379	380	193	95.0	63.5	58.0	67.7	32.7	23.3	10.7
			78983	-	270	186	232	119	94.9	129	59.6	82.4	33.7	51.4	5.43
			78984	-	336	381	324	316	255	179	203	229	30.6	11.2	9.23
			Mean	-	310	315	312	209	148	124	107	126	32.3	28.6	8.45
			SD	-	35.2	112	74.7	99.5	92.4	57.9	83.3	89.2	1.58	20.6	2.72

BLLOQ = below the lower limit of quantification (LLOQ = 2.00 ng/mL)

- = not measured

NS = no samples

[Table 11]

Plasma concentrations (ng/mL) of SN-38G after intravenous administration of DFP-13318

Group No.	Dosage	Route	Animal No.	*Time point*											
				Pre-dose	0 hr	1 hr	2 hr	4 hr	8 hr	10 hr	12 hr	24 hr	48 hr	72 hr	120 hr
1	225 mg/kg	IV bolus injection	78979	BLLOQ	-	15.3	5.10	4.72	6.18	5.47	4.44	4.62	BLLOQ	BLLOQ	BLLOQ
			78980	BLLOQ	-	14.5	6.42	6.13	5.70	3.40	2.90	3.84	BLLOQ	BLLOQ	BLLOQ
			78981	BLLOQ	-	8.25	2.81	3.30	2.31	4.33	3.18	3.60	NS	NS	NS
			Mean	-	-	12.7	4.78	4.72	4.73	4.40	3.51	4.02	-	-	-
			SD	-	-	3.86	1.83	1.42	2.11	1.04	0.820	0.533	-	-	-
2	450 mg/kg	10-min. IV injection	78982	-	60.8	20.6	14.2	8.23	10.1	8.26	7.91	4.43	BLLOQ	BLLOQ	BLLOQ
			78983	-	46.3	9.95	4.79	5.70	7.71	7.81	7.84	9.48	3.50	3.29	BLLOQ
			78984	-	51.8	23.9	7.37	6.13	8.64	13.6	11.9	19.6	4.66	BLLOQ	BLLOQ
			Mean	-	53.0	18.2	8.79	6.69	8.82	9.89	9.22	11.2	4.08	-	-
			SD	-	7.32	7.29	4.86	1.35	1.20	3.22	2.32	7.72	0.820	-	-

BLLOQ = below the lower limit of quantification (LLOQ = 2.00 ng/mL)

- = not measured

NS = no samples

[Table 12]

Pharmacokinetics parameters of DFP-13318 after intravenous administration of DFP-13318

Group No.	Dosage	Animal No.	T_{max} hr	C_{max} (mg/mL)	Cl_obs (mL/h/kg)	$T_{1/2}$ (hr)	AUC_{last} (hr*mg/mL)	AUC_{inf} (hr*mg/mL)	AUC_%Extrap (%)	MRT_{last} (hr)	AUC_{last}/D (hr*kg/mL)	V_z_obs (mL/kg)	V_{ss}_obs (mL/kg)	Rsq
1	225 mg/kg	78979	2	3.03	1.75	40.7	113	129	12.0	38.2	0.503	103	96.3	0.9974
		78980	2	3.10	1.61	48.0	118	140	15.4	38.3	0.526	111	99.0	0.9858
		78981	2	3.03	2.70	21.7	46.4	83.5	44.5	9.94	0.206	84.4	81.2	0.9974
		Mean	2	3.05	2.02	36.8	92.6	117	24.0	28.8	0.412	99.5	92.2	-
		SD	-	-	0.591	13.6	40.2	29.8	17.8	16.3	0.179	13.8	9.57	-
2	450 mg/kg	78982	0	9.20	2.35	35.3	177	192	7.48	31.9	0.394	120	99.5	1.000
		78983	4	6.18	2.36	35.9	176	191	7.78	32.2	0.391	122	102	1.000
		78984	0	6.86	2.23	36.1	188	202	6.99	31.0	0.418	116	91.0	0.9896
		Mean	0	7.24	2.31	35.8	180	195	7.42	31.7	0.401	119	97.3	-
		SD	-	-	0.0741	0.403	6.65	6.36	0.400	0.620	0.0148	3.02	5.59	-

Note: $T_{1/2}$ was calculated by using WinNonlin based on concentrations at last three time points. It was reported that $T_{1/2}$ was NC (not calculable) when a correlation coefficient (Rsq) was less than 0.85.

-: not measured; T_{max}: time corresponding to the maximum (peak) concentration after administration; C_{max}: maximum concentration; Cl_obs: clearance; $T_{1/2}$: final half-life; AUC_{last}: an area under the concentration-time curve from zero to the last quantifiable concentration time; AUC_{inf}: an area under the concentration-time curve from zero to infinity; AUC_%Extrap: % of an area under the curve that has been derived after extrapolation of % residual area; MRT_{last}: mean residence time from zero to the last time point; AUC_{last}/D: a ratio AUC_{last} to dosage; V_z_obs: an apparent volume of distribution; V_{ss}_obs: a steady-state volume of distribution

[Table 13]

Pharmacokinetics parameters of SN-38 after intravenous administration of DFP-13318

Group No.	Dosage	Animal No.hr	T_{max} (ng/mL)	C_{max} (hr)	$T_{1/2}$ (hr*ng/mL)	AUC_{last} (hr*ng/mL)	AUC_{inf} (%)	AUC_%Extrap (hr)	MRT_{last}	Rsq
1	225 mg/kg	78979	8	54.2	57.7	1567	1916	18.3	36.4	0.9828
		78980	1	51.6	NC	1799	2525	28.7	36.8	0.6678
		78981	1	199	8.71	1959	2315	15.4	8.56	0.9943
		Mean	1	97.6	33.2	1775	2252	20.8	27.2	-
		SD	-	-	34.7	197	309	7.03	16.2	-
2	450 mg/kg	78982	2	380	43.8	5464	6140	11.0	31.7	0.9994
		78983	0	270	24.1	5740	5929	3.19	37.4	0.9279
		78984	1	381	NC	9206	10428	11.7	23.2	0.5408
		Mean	1	315	34.0	6803	7499	8.64	30.8	-
		SD	-	-	13.9	2085	2539	4.74	7.19	-

Note: $T_{1/2}$ was calculated by using WinNonlin based on concentrations at last three time points. It was reported that $T_{1/2}$ was NC (not calculable) when a correlation coefficient (Rsq) was less than 0.85.

-: not measured; T_{max}: time corresponding to the maximum (peak) concentration after administration; C_{max}: maximum concentration; Cl_obs: clearance; $T_{1/2}$: final half-life; AUC_{last}: an area under the concentration-time curve from zero to the last quantifiable concentration time; AUC_{inf}: an area under the concentration-time curve from zero to infinity; AUC_%Extrap: % of an area under the curve that has been derived after extrapolation of % residual area; MRT_{last}: mean residence time from zero to the last time point

[Table 14]

Pharmacokinetics parameters of SN-38G after intravenous administration of DFP-13318

Group No.	Dosage	Animal No. hr	T_{max} (ng/mL)	C_{max} (hr)	$T_{1/2}$ (hr*ng/mL)	AUC_{last} (hr*ng/mL)	AUC_{inf} (%)	AUC_%Extrap (hr)	MRT_{last}	Rsq
1	225 mg/kg	78979	1	15.3	NC	144	1069	86.5	9.54	0.1339
		78980	1	14.5	NBC	124	NBC	NBC	8.76	NBC
		78981	1	8.25	NC	91.6	2827	96.8	10.6	0.004389
		Mean	1	12.7	-	120	1948	91.6	9.65	-
		SD	-	-	-	26.7	1243	7.26	0.937	-
2	450 mg/kg	78982	0	60.8	15.0	219	315	30.5	8.69	0.9973
		78983	0	46.3	NC	428	708	39.5	28.4	0.4812
		78984	0	51.8	22.8	575	728	21.0	21.6	0.8482
		Mean	0	53.0	18.9	407	584	30.3	19.5	-
		SD	-	-	5.5	179	233	9.24	10.0	-

Note: $T_{1/2}$ was calculated by using WinNonlin based on concentrations at last three time points. It was reported that $T_{1/2}$ was NC (not calculable) when a correlation coefficient (Rsq) was less than 0.85.

-: not measured; T_{max}: time corresponding to the maximum (peak) concentration after administration; C_{max}: maximum concentration; Cl_obs: clearance; $T_{1/2}$: final half-life; AUC_{last}: an area under the concentration-time curve from zero to the last quantifiable concentration time; AUC_{inf}: an area under the concentration-time curve from zero to infinity; AUC_%Extrap: % of an area under the curve that has been derived after extrapolation of % residual area; MRT_{last}: mean residence time from zero to the last time point

[0092]

Following intravenous administration of DFP-13318 to the female rats at a dose of 225 mg/kg, the mean Tmax and Cmax values were respectively determined to be 2 hours and 3.05 mg/mL for DFP-13318, 1 hour and 97.6 ng/mL for SN-38, and 1 hour and 12.7 ng/mL for SN-38G. Furthermore, DFP-13318 had mean plasma half-life $T_{1/2}$ of 36.8 hours. Mean values of clearance and MRT_{last} of DFP-13318 were determined to be 2.02 mL/hr/kg and 28.8 hours, respectively. The mean volume of distribution (Vss) was determined to be 92.2 mL/kg, and values of AUC_{last} and AUC_{inf} of DFP-13318 were determined to be 92.6 hr·mg/mL and 117 hr·mg/mL, respectively. Besides, the mean value of AUC_%Extrap was 24.0%.

[0093]

On the other hand, as for the metabolites SN-38 and SN-38G, the mean plasma half-life $T_{1/2}$ was determined to be 33.2 hours for SN-38, and not calculable (NC/NBC) for SN-38G because a measurement coefficient (r^2) was too low. The mean values of MRT_{last} of SN-38 and SN-38G were determined to be 27.2 hours and 9.65 hours, respectively. Besides, the mean values of AUC_{last} and AUC_{inf} were determined to be 1,775 hr·ng/mL and 2,252 hr·ng/mL for SN-38, and 120 hr·ng/mL and 1,948 hr·ng/mL for SN-38G. The mean values of AUC_%Extrap of SN-38 and SN-38G were respectively 20.8% and 91.6%.

[0094]

During the study, one of rats of the first group (Animal No.: 78981) was found to have died from bleeding after collecting a sample blood at 24 hours post dosing. No abnormal clinical sign was shown before the sample collection. The other two rats of this group showed no abnormal sign, and all the sample blood could be collected up to the final time point. Furthermore, during the whole survival time, no abnormal clinical sign was observed in the rat group administered with DFP-13318 at a high dose. Accordingly, it was concluded that the death of the rat was caused by bleeding and not related to the test article.

[0095]

Following intravenous injection of DFP-13318 to the female rats at a dose of 450 mg/kg, the mean Tmax and Cmax values were determined to be 0 hour and 7.24 mg/mL for DFP-13318, 1 hour and 315 ng/mL for SN-38, and 0 hour and 53.0 ng/mL for SN-38G, respectively. Furthermore, DFP-13318 had mean plasma half-life $T_{1/2}$ of 35.8 hours. Mean values of clearance and MRT_{last} of DFP-13318 were respectively determined to be 2.31 mL/hr/kg and 31.7 hours. The mean volume of distribution (Vss) was determined to be 97.3 mL/kg, and values of AUC_{last} and AUC_{inf} of DFP-13318 were respectively determined to be 180 hr·mg/mL and 195 hr·mg/mL. Besides, the mean value of AUC_%Extrap was 7.42%.

[0096]

On the other hand, as for the metabolites SN-38 and SN-38G, the mean plasma half-life $T_{1/2}$ was determined to be 34.0 hours for SN-38, and 18.9 hours for SN-38G. The mean values of MRT_{last} of SN-38 and SN-38G were respectively determined to be 30.8 hours and 19.55 hours. Besides, the mean values of AUC_{last} and AUC_{inf} were respectively determined to be 6,803 hr·ng/mL and 7,499 hr·ng/mL for SN-38, and 407 hr·ng/mL and 584 hr·ng/mL for SN-38G. The mean values of AUC_%Extrap of SN-38 and SN-38G were respectively 8.64% and 30.3%.

[0097]

When DFP-13318 was administered by iv bolus injection at a dose of 225 mg/kg or by iv infusion at a dose of 450 mg/kg, the values of C_{max} and AUC_{last} for DFP-13318 were generally increased in proportion to the dose. As for SN-38 and SN-38G, however, the degree of increase was larger than the proportion to the dose. The similar pharmacokinetics profiles of DFP-13318, such as $T_{1/2}$, CL and Vss, were observed after administering DFP-13318 to the animals by iv bolus injection or iv infusion.

[0098]

[Example 3]

<*Confirmation of DFP-13318 accumulation in tumor*>

This example was performed for confirming that DFP-13318 and its metabolite SN-38 are accumulated in tumor tissues collected from a HT-29 tumor-bearing nude rat model after intravenously administering DFP-13318 for PK/PD (pharmacokinetics/pharmacodynamics) study.

[0099]

In the PK/PD study, 25 nude rats were used as shown in Table 15.

[Table 15]

Rat groups and treatments

Group	n*	Treatment	Dosage (mg/kg)**	Route/schedule
1	5	Vehicle control ***	--	iv, qwk x 2 wks
2	5	DFP-13318	50	iv, qwk x 2 wks
3	5	DFP-13318	100	iv, qwk x 2 wks
4	5	DFP-13318	200	iv, qwk x 2 wks
5	5	Irinotecan	70/50	iv, qwk x 2 wks

Note: *n: the number of animals; **Dosage: adjusted based on body weight (10 μL/g);

***Vehicle control: 10 mM acetate buffer, pH 5

[0100]

Of 25 tumor fragments (with sizes of 200 to 400 mm^3) collected two weeks after the second dosing and stored at -80°C, 15 samples from groups 2 to 4 were used for the quantification of both DFP-13318 and SN-38, 5 samples from group 5 for SN-38 alone. Five samples from group 1 were used for creating calibration curves and for preparing samples.

[0101]

The HPLC/FLD and LC-MS/MS bioanalytical methods were employed for quantifying DFP-13318 and SN-38 in each tumor sample, respectively. The bioanalytical methods were performed as follows:

[0102]

(1) About 200 mg blank (or sample) was weighed into a tube, 0.8 mL of an acetate buffer (10 mM, pH 5) was added thereto at a ratio of 1:4, and the resultant was subjected to ultrasonic disruption.

[0103]

(2) The resulting sample was transferred to a 1.1 mL plastic tube.

[0104]

(3) Each tube was vortexed for well mixing the sample, shook for about 10 minutes, and centrifuged at 1109 x g for 10 minutes at 4°C.

[0105]

(4) A supernatant in an amount of 280 μL was transferred to another 1.1 mL tube and was dried at 40°C by blowing a nitrogen gas toward it.

[0106]

(5) The residue remaining in the tube was reconstituted by using a 10 mM acetate buffer (pH 5), and the resultant was vortexed for about 1 minute.

[0107]

(6) Twenty μL of the resultant sample solution was injected into HPLC.

[0108]

The HPLC conditions were as follows:

[0109]

Instrument: Agilent 1200 HPLC system

Analytical column: Agilent ZORBAX C18 column (5 μm, 3.0 mm x 50 mm column)

Column temperature: room temperature

Analysis time: 2.0 minutes

Mobile phase A: 20 mM ammonium acetate (pH 5.0), B: acetonitrile

Injection volume: 10 μL

Retention time: SN-38: 0.46 minute, internal standard: 0.49 minute

Gradient program: time of 0 to 2.00 minutes: A50% and B50%, flow rate of 0.600 mL/min., time of 2.00 minutes and thereafter: A50% and B50%, flow rate of 0.600 mL/min.

Autosampler temperature: 5 ± 3°C

Mass spectrometer conditions were as follows:

[0110]

Instrument: Applied Biosystems/AB Sciex API 4000 triple quadrupole mass spectrometer, Analyst 1.5.2 software

Source: ESI

Ionization mode: positive

Monitoring mode: MRM

Capillary: 5500 V

Gas temperature: 500°C

Peak integration: peak area

Calibration curves for DFP-13318 ranged from 0.0100 to 1.00 mg/mL with the linear regression coefficient equal to 0.9957 (HPLC/FLD). The individual accuracy of calibration standards ranged from 91.0% to 108%. Calibration curves for SN-38 ranged from 2.00 to 1,000 ng/mL with the linear regression coefficient equal to 0.9961. The individual accuracy of calibration standards ranged from 89.8% to 107%. In accepted batches, the accuracy of at least 75% of calibration standards met the acceptance criteria (within 85% to 115% of the nominal values except LLOQ where it should be within 80% to 120% of the nominal values). Concentrations of calibration standards and calibration curve parameters in tumor samples tested were calculated.

[0111]

The individual accuracy of samples ranged from 94.0% to 111% for DFP-13318, and 98.5% to 112% for SN-38, all of which met the acceptance criteria (that the accuracy of at least 67% of total samples is within 80% to 120% of the nominal values, and at least 50% of samples at each concentration level (low, middle and high) are within 80% to 120% of the nominal values). The results are summarized in Table 16.

[0112]

The precision of the samples at the low, middle and high concentration levels was ranged from 1.52% to 3.86% for DFP-13318, and 1.42% to 6.00% for SN-38, respectively.

[0113]

Contents of DFP-13318 and its metabolite SN-38 in individual tumor tissues were summarized in Tables 17 and 18, respectively. Figs. 4 and 5 show the dose-dependency of the DFP-13318 and SN-38 contents in tumor tissues.

[0114]

When DFP-13318 was intravenously administered at doses of 50 mg/kg, 100 mg/kg and 200 mg/kg, the contents of DFP-13318 and its metabolite SN-38 in HT-29 tumor tissues increased in a dose-dependent manner. No SN-38 was detected in group 5 when Irinotecan (prodrug of SN-38) was administered, however. As shown in Table 19, the SN-38 contents in HT-29 tumor were consistently around 1% of the content of DFP-13318, and the parental compound (4-arm PEG with 4 molecules of SN-38) was accumulated in the tumor tissues after intravenously administering the compound at doses of 50, 100 and 200 mg/kg (Table 17 and Fig. 4, respectively). It is suggested that the long lasting SN-38 level can be maintained by conversion from the parental compound.

[Table 16]

Results of samples

Batch ID	DFP-13318 Concentration (mg/mL)					
	LQC		MQC		HQC	
	0.0300	Accuracy (%)	0.0800	Accuracy (%)	0.800	Accuracy (%)
DFP-13318 _100813	0.0282	94.0	0.0817	102	0.827	103
	0.0306	102	0.0797	100	0.813	102
	0.0301	100	0.0795	99.4	0.858	107
	0.0300	100	0.0620	77.5	0.886	111
N	4	NA	3	NA	4	NA
Mean (mg/mL)	0.0297		0.0803		0.846	
SD	0.00105		0.00122		0.0326	
Precision (%CV)	3.53		1.52		3.86	
Accuracy (%)	99.1		100		106	
Batch ID	SN-38 Concentration (ng/mL)					
	LQC		MQC		HQC	
	6.00	Accuracy (%)	80.0	Accuracy (%)	800	Accuracy (%)
SN-38_rat tissue _09292013	5.96	99.3	81.6	102	898	112
	6.73	112	78.8	98.5	843	105
	6.06	101	80.3	100	806	101
	5.96	99.3	80.3	100	840	105
N	4	NA	4	NA	4	NA
Mean	6.18		80.3		847	
Accuracy (%)	103		100		106	
CV (%)	6.00		1.42		4.50	

Underlined: The accuracy deviation of values was 20% or more of the nominal value for HPLC-FLD method.

NA: Not applicable

[Table 17]

Concentrations (mg/mL) of DFP-13318 in HT-29 tumor after intravenous administration of DFP-13318

Group No.	Animal No.	Concentration (mg/mL)	Weight of sample (g)	Volume of sample (μL)	Volume of sample (mL)	Content [1] (mg/g)	Content [2] (nmol/g)
2	2	0.0280	0.1114	446	0.446	0.140	3.06
	16	0.0107	0.1117	447	0.447	0.0535	1.17
	18	0.0156	0.1142	457	0.457	0.0780	1.71
	20	0.0193	0.1244	498	0.498	0.0966	2.11
	23	0.0146	0.0952	381	0.381	0.0730	1.60
	N	5	5	5	5	5	5
	Mean	0.0176	0.111	446	0.446	0.0882	1.93
	SD	0.00655	0.0105	42.0	0.0420	0.0328	0.715
3	11	0.0571	0.1146	458	0.458	0.285	6.24
	15	0.0751	0.1307	523	0.523	0.376	8.23
	27	0.0450	0.1244	498	0.498	0.225	4.92
	28	0.0271	0.1089	436	0.436	0.136	2.98
	31	0.0250	0.1397	559	0.559	0.125	2.74
	N	5	5	5	5	5	5
	Mean	0.0459	0.124	495	0.495	0.229	5.02
	SD	0.0210	0.0123	49.3	0.0493	0.105	2.30
4	1	0.0783	0.1308	523	0.523	0.391	8.56
	6	0.0507	0.0998	399	0.399	0.253	5.54
	8	0.0629	0.0909	364	0.364	0.315	6.89
	17	0.0801	0.1036	414	0.414	0.400	8.75
	19	0.0813	0.1163	465	0.465	0.406	8.88
	N	5	5	5	5	5	5
	Mean	0.0707	0.108	433	0.433	0.353	7.72
	SD	0.0134	0.0156	62.1	0.0621	0.0669	1.46

MW for DFP-13318: 45,700 daltons;

Content[2] = Content[1] x 1,000,000/DFP-13318 MW

[Table 18]

Concentrations (ng/mL) of SN-38 in HT-29 tumor after intravenous administration of DFP-13318

Group No.	Animal No.	Concentration (ng/mL)	Weight of sample (g)	Volume of sample (μL)	Volume of sample (mL)	Content [1] (ng/g)	Content [2] (nmol/g)
2	2	2.27	0.1111	444	0.444	11.3	0.0288
	16	2.03	0.1251	500	0.500	10.1	0.0258
	18	2.17	0.1088	435	0.435	10.8	0.0276
	20	2.14	0.1296	518	0.518	10.7	0.0273
	23	BLLOQ	0.1170	468	0.468	NA	NA
	N	4	5	5	5	4	4
	Mean	2.15	0.118	473	0.473	10.7	0.0274
	SD	0.0988	0.00891	35.6	0.0356	0.492	0.00123
3	11	BLLOQ	0.1267	507	0.507	NA	NA
	15	4.21	0.1334	534	0.534	21.1	0.0538
	27	2.15	0.1169	468	0.468	10.8	0.0276
	28	2.75	0.1200	480	0.480	13.8	0.0352
	31	3.05	0.1350	540	0.540	15.3	0.0390
	N	4	5	5	5	4	4
	Mean	3.04	0.126	506	0.506	15.3	0.0389
	SD	0.865	0.00797	31.9	0.0319	4.33	0.0110
4	1	4.35	0.1274	510	0.510	21.8	0.0556
	6	9.90	0.1372	549	0.549	49.5	0.1263
	8	5.33	0.1110	444	0.444	26.7	0.0681
	17	3.56	0.1123	449	0.449	17.8	0.0454
	19	4.21	0.0984	394	0.394	21.1	0.0538
	N	5	5	5	5	5	5
	Mean	5.47	0.117	469	0.469	27.4	0.0698
	SD	2.56	0.0152	60.7	0.0607	12.8	0.0326
5	5	BLLOQ	0.1003	401	0.401	NA	NA
	12	BLLOQ	0.1133	453	0.453	NA	NA
	22	BLLOQ	0.1059	434	0.434	NA	NA
	24	BLLOQ	0.0994	398	0.398	NA	NA
	29	BLLOQ	0.1061	424	0.424	NA	NA

MW for SN-38: 392 daltons; Content^2 = Content^1/SN-38 MW

NA: Not applicable

BLLOQ: Below the lower limit of quantification

[Table 19]

Summary of tumor uptake of DFP-13318 and SN-38

Group No.	Group	Dose (mg/kg)	DFP-13318 content (nmole/g tumor)	SN-38 content (pmole/g tumor)	Ratio (SN-38/DFP-13318)
2	DFP-13318	50	1.93	27.4	1.42%
3	DFP-13318	100	5.02	38.9	0.78%
4	DFP-13318	200	7.72	69.8	0.90%
5	CPT-11	70	NA	BLLOQ	NA

NA: Not applicable; BLLOQ: Below the lower limit of quantification; MW for DFP-13318: 45,700 daltons; MW for SN-38: 392 daltons

INDUSTRIAL APPLICABILITY

[0115]

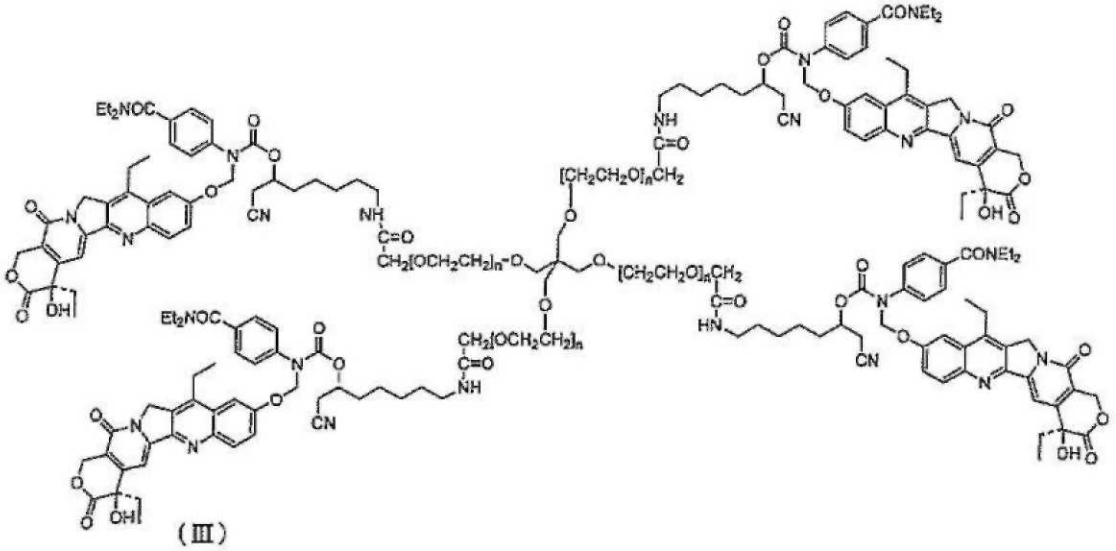
The present invention can realize the cancer treatment free from side effects in which excellent efficacy without accompanying serious side effects such as diarrhea can be provided by repeatedly intravenously administering a drug related to Irinotecan and SN-38 derivatives at a low dose at a frequency of once a week, and hence, the present invention is extremely useful in the pharmaceutical industry.

[0016]

All publications, patents and patent applications cited herein are incorporated herein by reference in their entirety.

CLAIMS

1. Use of a pharmaceutical composition in the manufacture of a medicament for the treatment of cancer, the pharmaceutical composition comprising, as an active ingredient, a compound represented by the following formula III:



(III)

(wherein n is an integer of 200 to 1,000),

wherein the compound is used with administration and dosage in which a single dose of 0.01 μmol/kg body weight to 11 μmol/kg body weight is to be parenterally administered to a subject having cancer and the compound can be administered at a frequency of once a week, every other week over at least 2 weeks, and has no Irinotecan-like side effects and provides a cancer growth inhibiting activity equivalent to or larger than that of Irinotecan when used at a dose as low as 1/10 (in a molar ratio) or less as compared with Irinotecan.

2. The use according to claim 1, wherein n is an integer of 200 to 800 in the compound of the formula III.

3. The use according to claim 1 or 2, wherein the cancer is selected from the group consisting of colon cancer, breast cancer, ovarian cancer, cervical cancer, uterine cancer, lung cancer, stomach cancer, pancreas cancer, esophageal cancer, head and neck cancer, hepatocellular carcinoma, gallbladder cancer, biliary cancer, sarcoma, and malignant lymphoma.

4. The use according to any one of claims 1 to 3, wherein the parenteral administration is the intravenous administration.

5. The use according to claim 4, wherein the intravenous administration is infusion or bolus injection.