Antitumor activity of IHL-305, a novel pegylated liposome containing irinotecan, in human xenograft models

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Abstract. The antitumor effect of IHL-305, a novel pegylated liposome containing irinotecan, was investigated in human xenograft models. After subcutaneous transplantation of several human cancer cell lines (colorectal, non-small cell lung, small cell lung, prostate, ovarian and gastric cancer cells) to nude mice, IHL-305 or CPT-11 was administered intravenously 3 times at 4-day intervals. In all xenograft models tested, IHL-305 showed superior antitumor activity to that of CPT-11 and a comparable tumor-growth-inhibitory effect at one-eighth or less of the dose of CPT-11, even against HT-29 colorectal and NCI-H460 non-small cell lung cancer cell lines, which show intrinsic resistance to CPT-11. A single injection or 2 injections of IHL-305 on several dosing schedules also resulted in a significant antitumor effect compared to that of vehicle control in a dose-dependent manner and showed comparable antitumor activity at about one-fifth the dose of the maximum tolerated dose of CPT-11. The analysis of the concentrations of irinotecan and SN-38, an active metabolite of CPT-11, in plasma and tumors revealed that irinotecan was maintained at high concentrations, and the prolonged presence of SN-38 in plasma and tumors in IHL-305 treated mice compared with CPT-11-treated mice. Therefore, the stronger tumor inhibitory effect of IHL-305, as compared to CPT-11, was associated with the difference in the concentration of irinotecan in plasma or tumors after each agent was administered and with the maintenance of a higher concentration of SN-38. These results indicate that IHL-305 demonstrated superior antitumor activity against a wide range of tumors at lower doses than CPT-11.

Introduction

Irinotecan hydrochloride (CPT-11) was semi-synthesized from 20(S)-Camptothecin which is an alkaloid isolated from Camptotheca acuminata (1). CPT-11 has been demonstrated to possess strong antitumor activities in preclinical studies against various kinds of experimental tumors both in vitro (2,3) and in vivo (4,5). In recent years, CPT-11 is being widely used clinically as one of the key drugs for standard chemotherapy against colorectal cancer due to its confirmed evidence of antitumor efficacy (6-8). Side effects such as myelosuppression or diarrhea, which are the dose-limiting toxicities of CPT-11, have however been reported, and have also been observed in clinical use (9,10). Therefore, many approaches have been tried to alleviate the side effects by reducing the dosage (11) or modifying the formulation of CPT-11 (12,13).

IHL-305 is a preparation of irinotecan encapsulated in polyethylene glycol (PEG)-modified liposomes (14). In general, liposome preparations are known to be selectively transported to tumor tissues due to their enhanced permeability and retention (EPR) (15-17), leading to the reduction of toxicity and augmentation of cytotoxicity in the target cells. For the past few years, numerous studies regarding liposome preparations have been conducted using anticancer agents such as doxorubicin (18-20), cisplatin (21), taxol (22,23) or camptothecin (24,25) in preclinical as well as in clinical trials. As one of constructed carriers, liposomes with a PEG coating have proved to be very successful as a drug carrier system. Recent pharmacokinetics and therapeutic studies reveal that sterically stabilized liposomes have considerable potential as drug carriers and the modification of some liposome preparations with PEG has improved their stability in the blood, resulting in better clinical results (12). For example, Doxil is a long-circulating pegylated liposomal preparation containing doxorubicin (DXR) which has been approved for clinical use in the treatment of cancers including phase I-II randomized trials (26,27). As one of their reported characteristics, liposomes exhibit reduced clearance or a prolonged plasma half-life due to the so-called stealth effect, by which they are not recognized by the reticuloendothelial systems (28). The potency of anticancer agents is thus expected to be improved by their modification using these carriers.

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The aim of this study was to evaluate the in vivo antitumor activities of a newly formulated pegylated liposomal preparation, IHL-305. In this study, the antitumor efficacy profiles of IHL-305 were examined in comparison with that of CPT-11 using nude mice subcutaneously transplanted with various human cancer cell lines. To elucidate the differences of the antitumor activity, the concentrations of irinotecan and SN-38, which is the active metabolite of CPT-11, was also determined in plasma and tumors after intravenous (i.v.) injection of IHL-305.

Materials and methods

Drugs. IHL-305 and irinotecan hydrochloride (CPT-11) were obtained from Terumo Corp. (Kanagawa, Japan) and Yakult Honsha Co., Ltd. (Tokyo, Japan), respectively. The components of IHL-305 are irinotecan, cholesterol, hydrogenated soybean phosphatidyl choline, PEG5000-DSPE, ammonium sulfate, sucrose, L-histidine, dilute hydrochloric acid, water for injection, and dehydrated alcohol. IHL-305 liposomes range in diameter from 70-120 nm. The dose of IHL-305 is represented as the amount of irinotecan encapsulated in liposomes.

Animals. Inbred specific pathogen-free 5-6-week-old male BALB/c nude mice were purchased from Japan Charles River Co., Ltd. (Yokohama, Japan) and CLEA Japan, Inc. (Tokyo, Japan). The mice were kept in plastic cages, given a standard diet (MF, Oriental Yeast Industry Co., Tokyo, Japan) and were allowed free access to water. The temperature and humidity were kept at 24±1°C and 55±10%, respectively.

Cells and culture. The human colorectal cancer cell lines (HCT116, HT-29), non-small lung cancer cell line (NCI-H460), small cell lung cancer cell line (NCI-H82), prostate cancer cell line (PC-3) and ovarian cancer cell line (ES-2) were purchased from American Type Culture Collection (ATCC, USA), NCI-H460 and PC-3 were maintained in Dulbecco’s modified Eagle’s medium (Sigma, MO, USA) containing 10% heat-inactivated fetal bovine serum (FBS). ES-2 was maintained in McCoy’s 5A medium (Sigma) containing 100 units/ml penicillin, 100 µg/ml streptomycin, 1.5 mM L-glutamine and 10% heat-inactivated FBS. NCI-H82 was maintained in RPMI-1640 medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 10% heat-inactivated FBS. The human gastric cancer cell line (MKN45) was purchased from Japan Health Sciences Foundation (JHSF, Tokyo, Japan) and was maintained in RPMI-1640 medium containing 100 units/ml penicillin, 100 µg/ml streptomycin and 10% heat-inactivated FBS. All cancer cell lines described above were subcultured serially in vitro and adjusted to the appropriate concentrations before use. The human non-small cell lung cancer cell line (QG-56), a gift from Daiichi Pharmaceutical Co., Ltd. was also used, and was maintained by in vivo passages.

Antitumor experiments. After transplanting human cancer cells or fragments subcutaneously to the inguinal region of nude mice, the mice were grouped (5-6 mice/group) on the day when the estimated tumor volume calculated by the following formula (A) reached about 40-220 mm³ (Day 0). IHL-305 or CPT-11 was administered intravenously (i.v.) 1-3 times at intervals of 4-14 days. Physiological saline or empty liposomes were administered as negative controls with the same administration schedule. Tumors were excised on Days 21 or 35, and tumor growth inhibition rate (IR %) formula (B) was calculated from tumor weights. During the study, the long and short diameters of the tumor and body weight of the mice were measured.

(A) Estimated tumor volume = 1/2 x long diameter x short diameter x short diameter.
(B) IR % = (1 - mean tumor weight in the IHL-305 or CPT-11 group/mean tumor weight in the control group) x 100.

All in vivo antitumor experiments were performed according to our internal and ethics committee regulations.

Sample preparation. Blood and tumors were collected at 1, 3, 6, 24, 48, 72 and 96 h after i.v. administration of IHL-305 (45 mg/kg) and CPT-11 (90 mg/kg) into nude mice. Blood was taken by cardiac puncture under ether anesthesia, after which the tumor was removed from the inguinal region. The blood sample was immediately centrifuged at 0°C for 30 sec at 15,000 rpm to separate the plasma. The plasma was diluted 5-fold with 0.15 M H₃PO₄ and divided into two aliquots. One aliquot of the plasma mixture was ultra-centrifuged at 10°C for 30 min at 100,000 x g, after which 0.05 ml of the upper layer was added to 0.45 ml of the internal standard (IS) solution (0.1 µg/ml camptothecin in 0.15 M H₃PO₄) (analytical sample for the determination of irinotecan and SN-38). The other aliquot of the plasma mixture was diluted with a 10-fold volume of methanol, and then 0.1 ml of the mixture was added to a 4-fold volume of the IS solution (analytical sample for the determination of irinotecan in the IHL-305 group). For each measurement, 0.1 ml was used. The tumors were minced and weighed, and then added to an equal volume of 0.15 M H₃PO₄ and an 8-fold volume of methanol, and then homogenized on ice with a teflon homogenizer. After centrifugation at 0°C for 15 min at 3,000 rpm, 50 µl of the supernatant was added to 0.45 ml of the IS solution (analytical sample for the determination of irinotecan and SN-38). For each measurement, 0.1 ml was used.

Measurement of irinotecan and SN-38 in plasma and tumors. According to the method of Kurita and Kaneda (29), each compound was measured with the HPLC system with a fully automated online solid-phase extraction system (PROSPEKT2; Spark Holland, Emmen, The Netherlands). Briefly, 0.1 ml of the plasma and tumor samples was used for the solid phase extraction with a Cartridge-C18 Analytichem (Spark Holland). A C18 reversed-phase column (Symmetry Column C18, 150x4.6 mm I.D., 5 µm, Waters, Milford, MA) was used at 50°C for chromatography. The fluorescence detector (470 scanning fluorescence detector; Waters) was set at 380 nm and 540 nm for 0-3.8 min for SN-38, and at 373 and 428 nm for 3.8-8 min for IS and irinotecan. The mobile phase consisted of 0.05 M KH₂PO₄:acetonitrile (70:30, v/v) containing 4 mM sodium 1-decanesulfonate (pH 3.5 with H₃PO₄) and the flow rate was 1.5 ml/min. The quantification limits of irinotecan and SN-38 were 5 and 1 ng/ml for plasma, and 50 and 5 ng/g for tumor, respectively.
Statistical analysis. Among the results obtained, the data of the tumor weight were analyzed statistically using Bonferroni’s or Dunnett’s multiple comparison test.

Ethics in animal experiments. All the in vivo experimental protocols were approved by the animal care committee of the Yakult Central Institute for Microbiological Research.

Results

Antitumor effect of IHL-305 against HT-29, PC-3 and QG-56. In a preliminary study, the antitumor effect of IHL-305 was compared with that of CPT-11 against HT-29, PC-3 and QG-56-bearing nude mice. Once tumors were established (mean tumor volume, 40-130 mm³) in mice transplanted subcutaneously with each tumor, IHL-305 (25, 50 or 100 mg/kg as irinotecan) or CPT-11 (25, 50 or 100 mg/kg) was administered intravenously (i.v.) 3 times at 4-days interval (Days 1, 5 and 9) to the mice. The inhibition rate (IR) of tumor growth was calculated from tumors excised and weighed on Day 21. Fig. 1A shows the growth curve of each tumor in mice treated with IHL-305 and CPT-11, and all tumors in the control group grew progressively. Changes in body weight are shown in Fig. 1B. As indicated in Tables I-III, IHL-305 showed significant (p<0.001) tumor growth inhibitory effects in all tumors tested at all doses used in this study. In particular, the IR for IHL-305 tested in PC-3-bearing mice was 99.2-99.5% with complete regression of tumor in 1 of 5 mice in the 100 mg/kg group, whereas the IR for CPT-11 was 35.5-67.2%. When comparisons were made at the same dose level, the tumor weight was significantly lower in the IHL-305 group than in the corresponding CPT-11 group.

Antitumor effect of IHL-305 on various cancer cell lines. In the second study, we assessed the antitumor effect of IHL-305 in various tumor-bearing mice. The highest dose of CPT-11 as the reference in this experiment was set at MTD dosing (total 270 mg/kg). IHL-305 was administered i.v. to various tumor-bearing mice and the subsequent tumor growth was monitored for 21 or 35 days. As indicated in Fig. 2A, IHL-305 effectively suppressed the tumor growth of HCT116-HT-29- and NCI-H460-bearing mice for 21 days. Moreover, IHL-305 showed strong inhibitory effects in MKN45-, NCI-H82- and ES-2-bearing mice for 35 days (Fig. 2B). Comparison of the IR between IHL-305 and CPT-11 at the same dose clearly demonstrated that IHL-305 also showed a higher IR and dose-dependent effect (Fig. 3).

Figure 1. Antitumor activity of IHL-305 and CPT-11 in HT-29-, PC-3- and QG-56-bearing nude mice. After HT-29, PC-3 or QG-56 cells were inoculated subcutaneously into nude mice, the mice were grouped (5-6 mice/group) on the day when their mean estimated tumor volume reached about 40-130 mm³ (Day 0). IHL-305 (◼, 25 mg/kg; ▲, 50 mg/kg; ●, 100 mg/kg) and CPT-11 (◻, 25 mg/kg; Δ, 50 mg/kg; ○, 100 mg/kg) was administered intravenously on Days 1, 5 and 9, and the long and short diameter of tumors were monitored. Control (+) was injected with saline. The tumors were excised on Day 21 and the inhibition rate (IR%) of tumor growth was calculated from tumor weights. (A) Estimated tumor volume, (B) body weight.
Antitumor effect of IHL-305 on various dosing schedules. The tumor growth inhibitory effect of IHL-305 on various dosing schedules was further investigated using HCT116-bearing nude mice (Table IV). The administration dose of IHL-305 was set at a total dose of 27 mg/kg as CPT-11, which is 1/10 of MTD dosing of CPT-11, so, that we could confirm the differ-
ences between the various dosing schedules. After grouping on Day 0, IHL-305 was administered i.v. to tumor-bearing mice on Days 1, 5, 9; Days 1, 8, 15; Days 1, 5; Days 1, 8; Days 1, 15; and Day 1. Control groups received physiological saline, empty liposomes or CPT-11 (total dose, 270 mg/kg; MTD) on Days 1, 5, 9, and the IR of tumor growth was calculated.
The inhibition rate (IR%) of tumor growth was calculated from tumor weights on Day 21 (A) or Day 35 (B).

Figure 3. Inhibition rate of IHL-305 and CPT-11 on several human xenografts. After HCT116, HT-29, NCI-H460, MKN-45, NCI-H82 or ES-2 (2-5x10^6 cells/mouse) was inoculated subcutaneously into nude mice, the mice were grouped (5-10 mice/group) on the day when their mean estimated tumor volume reached about 120-220 mm^3 (Day 0). IHL-305 (●, 16.875-135 mg/kg) and CPT-11 (○, 33.75-270 mg/kg) was administered intravenously on Days 1, 5 and 9, and the inhibition rate (IR%) of tumor growth was calculated from tumor weights on Day 21 (A) or Day 35 (B).

Figure 4. Measurement of CPT-11 and SN-38 in plasma and tumors. HT-29 (2x10^6 cells/mouse) was inoculated subcutaneously into nude mice on Day 0. The mice were grouped (3 mice/1 point/group) on Day 12 when the mean estimated tumor volume had reached about 430 mm^3 and IHL-305 (●, 45 mg/kg) and CPT-11 (○, 90 mg/kg) was administered intravenously. The plasma and tumors were collected for 96 h and the concentrations of CPT-11 and SN-38 were measured with HPLC as described in Materials and methods. Data represent the mean ± SD of 3 animals.

from tumors excised and weighed on Day 21. The IR of the tumor growth in animals which received empty liposomes was -5.1%, indicating no antitumor effect. However, the tumor weight in all IHL-305 groups differed significantly (p<0.001) compared with the control group, and the tumor growth IR was high (63.1-77.8%). When IHL-305 was administered 1, 2,
Concentrations of irinotecan and SN-38 in plasma and tumors. To examine the difference of the inhibitory effect between IHL-305 and CPT-11, the concentrations of irinotecan and SN-38 in plasma and tumors after i.v. injection of both drugs were measured. As indicated in Fig. 4A, the irinotecan concentration in plasma after IHL-305 injection (45 mg/kg) was maintained constantly until 3 h after injection, followed by an exponential decrease until 72 h, whereas the irinotecan concentration after CPT-11 injection (90 mg/kg) had decreased exponentially by 24 h although the dose in the irinotecan group was twice that of the CPT-11 group. The SN-38 concentration in plasma after IHL-305 injection was almost the same as that after CPT-11 injection at the maximum concentration, however, the disappearance of SN-38 in IHL-305 treated mice was apparently slower than that in CPT-11-treated mice and maintenance of SN-38 levels in the IHL-305 group was observed for 72 h after the injection. On the other hand, the intratumoral irinotecan concentration after IHL-305 injection peaked at 6 h after injection, and then decreased gradually until 96 h post-injection, whereas after CPT-11 injection it demonstrated constant maintenance until 3 h post-injection, followed by a rapid decrease by 24 h. The maximum post-injection intratumoral concentration of SN-38 of each drug was similar (Fig. 4B). The SN-38 concentration in tumors after IHL-305 injection was maintained at a certain level until 24 h after injection, and then decreased gradually by 48 h, whereas that after CPT-11 injection decreased consecutively after 1 h post-injection. SN-38 was still at detectable levels at 48 h after injection of IHL-305, but this was not the case with CPT-11.

Discussion

In this study, we examined the antitumor profile of a newly formulated pegylated liposome preparation, IHL-305, using nude mice subcutaneously inoculated with 8 kinds of human xenografts. Since CPT-11 has been reported to possess antitumor activity against human xenografts (30), it was expected that greater effects of IHL-305 would be shown in tumor-bearing mice. As the overall results demonstrated, IHL-305 showed successful antitumor activities against all xenografts tested.

In the first study, we compared the antitumor effect of IHL-305 to that of CPT-11 against human colorectal, prostate and non-small cell lung cancers over a 21-day period. The results showed that IHL-305 demonstrated significant tumor growth inhibitory effects in all tumors tested in spite of the quite low dosage compared with irinotecan. When comparisons were made at the same dose level in this study, tumor weight was significantly lower in the IHL-305 group than in the corresponding CPT-11 group. These results indicated that IHL-305 possesses a higher potential to suppress the tumor growth of xenografts at low doses compared to CPT-11. In particular, IHL-305 exhibited strong antitumor activity against the HT-29 colorectal cancer cell line which is recognized as having an intrinsic resistance to chemotherapeutic agents including CPT-11 (25,31). Although CPT-11 is reported to show antitumor activity against multidrug-resistant tumors both in vitro and in vivo (32), detailed investigations as to the overcoming of drug-resistance of IHL-305 are in progress. Moreover, neither drug had much impact on changes in body weight. These observations were in concordance with the reports that liposomes containing PEG have been shown to reduce toxic side effects such as body weight loss and can up-regulate the MTD of the drugs with a better therapeutic profile compared with CPT-11 (12,28).

Having confirmed the antitumor efficacy of IHL-305 over the 21-day period, we then planned further experiments over longer periods of observation with various human xenografts. In this series of experiments, the maximum dose of CPT-11 as the reference was set at the MTD so that an equal comparison with IHL-305 could be performed for the 21- or 35-day observation period. Comparison of the inhibition rate (IR) between IHL-305 and CPT-11 at the same dose clearly demonstrated that IHL-305 showed a higher IR and produced a comparable tumor growth inhibitory effect at doses from one-half to one-eighth that of CPT-11 even in HT-29- and NCI-H460-bearing mice, which are relatively resistant to CPT-11. These results demonstrated the long-lasting effect with a low dose of IHL-305 as one of the characteristics of liposome preparation, as described elsewhere (13,33).

Further examinations were made of the IHL-305 dosing schedules, which revealed that IHL-305 exhibited successful antitumor effect at all time periods tested, i.e. intervals of 4-14 days, indicating that IHL-305 will be easy to use or handle for various schedules. Liposome preparations are often used and evaluated with many dosing frequencies, such as 5 consecutive administrations, in animal experiments (13,18,25). However, our results suggest that IHL-305 could be used with a lower dosing frequency and at lower dosages compared with CPT-11.

To investigate the difference of the IR between IHL-305 and CPT-11 at the optimal dose in HT-29-bearing mice, we measured the concentrations of irinotecan and SN-38 in the plasma and tumors after i.v. injection of both drugs. The higher concentration of irinotecan in plasma in the IHL-305 group compared with the CPT-11 group lasted for 72 h after the injection of IHL-305, while the concentration of irinotecan in the CPT-11 group had reduced rapidly by 24 h after injection of CPT-11, even though the CPT-11 dosage was double that of IHL-305. On the other hand, the peak concentration level of SN-38 was almost the same in both groups just after the...
injection, but the levels of SN-38 in the IHL-305 group were maintained for 72 h after the injection. The same tendency as described above was observed in tumors after administration of each drug. From the pharmacokinetic analysis, it was speculated that the extension of the retention time of SN-38 in the tumor was due to the slow release of irinotecan from the IHL-305 liposomes distributed in the tumor, because the retention time of SN-38 in tumors was almost the same in both groups. These results indicated that the difference of the inhibitory effect between IHL-305 and CPT-11 depended on the maintenance of the concentrations of irinotecan and SN-38 in HT-29-bearing mice. However, further study will be required to clarify the detailed profile of the kinetics of IHL-305 in tumor-bearing mice.

In conclusion, we demonstrated the efficacy of a pegylated liposome preparation, IHL-305, in several human xenograft models. IHL-305 was shown to possess a wide range of antitumor activity against several human tumors. It was also indicated that the antitumor activity of IHL-305 exceeded that of CPT-11 in terms of animal experiments and could thus be expected to be used as a replacement for CPT-11. IHL-305 may provide a potential therapeutic tool for the treatment of human cancers. Clinical trial of IHL-305 is now in progress.

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References


