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Original Article

The Antitumor Activity of NK012, an SN-38–Incorporating Micelle, in Combination With Bevacizumab Against Lung Cancer Xenografts

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BACKGROUND: It has been demonstrated that NK012, a novel 7-ethyl-10-hydroxycamptothecin (SN-38)-incorporating polymeric micelle, exerts significantly more potent antitumor activity against various human tumor xenografts than irinotecan (CPT-11) (a water-soluble prodrug of SN-38). Combination therapy of anticancer agents with bevacizumab (Bv), an anti-vascualr endothelial growth factor humanized monoclonal antibody, has more potently inhibited tumor growth than either agent alone. In the current study, the authors examined the antitumor effect of NK012 in combination with Bv against human lung cancer. **METHODS:** Nude mice bearing lung adenocarcinoma (PC-14 or A549 xenografts) were administered NK012 at SN-38-equivalent doses of 5 mg/kg or 30 mg/kg in combination with or without Bv at 5 mg/kg. CPT-11 at a dose of 66.7 mg/kg was administered with or without Bv at a dose of 5 mg/kg in the same experimental model. To evaluate interaction with Bv, the pharmacokinetics and microvessel density in tumors that were treated on each regimen were analyzed. **RESULT:** In vitro, the growth-inhibitory effect of NK012 was 50-fold more potent than that of CPT-11 and was almost equivalent to that of SN-38. In vivo studies revealed that the combination of NK012 plus Bv had significantly greater antitumor activity against human lung cancer xenografts compared with NK012 alone (PC-14, $P=.0261$; A549, $P < .001$). The pharmacokinetic profile of NK012 revealed that coadministration of Bv did not interfere with the accumulation of NK012. **CONCLUSIONS:** In this study, significant antitumor activity was noted with NK012 in combination with Bv against lung cancer cells. The current results warrant the clinical evaluation of NK012 in lung cancer. ***Cancer* 2010;116:4597–604.** *© 2010 American Cancer Society.*

KEYWORDS: NK012, drug-delivery system, lung cancer, lung adenocarcinoma, 7-ethyl-10-hydroxycamptothecin, SN-38, micelles, bevacizumab.

Lung cancer is the leading cause of cancer-related deaths worldwide, and nonsmall cell lung cancer (NSCLC), including adenocarcinoma, accounts for 75% to 80% of lung cancer cases.[1] Currently, cisplatin (CDDP)-based chemotherapy is the recommended first-line treatment for patients with advanced NSCLC.[2,3] Despite recent advances in the treatment of lung cancer, the prognosis for patients with NSCLC remains relatively poor, so attention currently is focused on finding novel agents, including new cytotoxic agents.

Irinotecan (CPT-11), a prodrug of 7-ethyl-10-hydroxycamptothecin (SN-38) (the active metabolite of irinotecan), which is a topoisomerase-I inhibitor, appears to be an effective agent against NSCLC when used as monotherapy or in combination with cisplatin.[4,5] Bevacizumab (Bv) is an antivascular endothelial growth factor (anti-VEGF) humanized monoclonal antibody. Bv reportedly is effective in various cancers, including colorectal cancer,[6] renal cell cancer,[7] and breast cancer.[8] Sandler et al reported that the addition of Bv to paclitaxel plus carboplatin in the treatment of NSCLC had a significant survival benefit.[9] In addition, Reck et al reported that the addition of Bv to gemcitabine plus cisplatin also had a significant clinical benefit in NSCLC.[10]

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NK012 is an SN-38-incorporating polymeric micelle and is categorized as a drug-delivery system. We previously demonstrated that NK012 accumulates more efficiently in various human tumor xenografts by using leaky tumor vessels and exerts significantly more potent antitumor activity against various human tumor xenografts compared with CPT-11.[11-17] Since the greater antitumor effect of NK012 may be attributed to its greater accumulation in the tumor using the leaky tumor vasculature, the addition of Bv to NK012 may hinder the efficient accumulation of NK012 in tumors because the permeability of tumor vasculature caused by VEGF is inhibited by Bv. In the current study, we evaluated the antitumor activity of NK012 administered in combination with Bv in experimental models.

MATERIALS AND METHODS

Drugs and Cells

NK012, an SN-38-incorporating polymeric micelle, and SN-38 were obtained from Nippon Kayaku Company, Ltd. (Tokyo, Japan), CPT-11 was purchased from Yakult Honsha Company, Ltd. (Tokyo, Japan), and Bv was purchased from Chugai Seiyaku Company, Ltd. (Tokyo, Japan). The human lung adenocarcinoma cell lines PC-14 and PC-9 kindly were provided by Dr. Y. Hayata (Tokyo Medical University, Tokyo, Japan). Human lung adenocarcinoma cell lines A549, NCI-H23, and NCI-H1975 were purchased from the American Type Culture Collection (Manassas, Va). These cell lines were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (Cell Culture Technologies, Gaggenau-Hoerden, Germany), penicillin (100 U/mL), streptomycin (100 μg/mL), and amphotericin B (25 μg/mL; all from Sigma, St. Louis, Mo) in a humidified, 5% CO_2 atmosphere at 37°C.

In Vitro Growth-Inhibition Assay

PC-14, A549, NCI-H23, and NCI-H1975 cells were seeded in 96-well plates at a density of 10,000 cells per well in a final volume of 100 μL. Twenty-four hours after seeding, the medium was removed, and a graded concentration of SN38, NK012, and CPT-11 was added to the wells. Cultures were maintained in a CO_2 incubator for an additional 72 hours. Then, cell growth inhibition was measured by using a tetrazolium salt-based proliferation assay (WST assay; Wako Chemicals, Osaka, Japan). After removal of the medium, WST-8 solution (10 μL) and medium (90 μL) were added to the wells, and the plates were incubated at 37°C for 1 hour. The absorbance of the formazan product formed was detected at 450 nm in a 96-well spectrophotometric plate reader. Cell viability was measured and compared with that of the control cells. Each experiment was carried out in triplicate. Data were averaged and normalized against the nontreated controls to generate dose-response curves.

In Vivo Growth-Inhibition Assay

The animal experimental protocols were approved by the Committee for Ethics of Animal Experimentation, and the experiments were conducted in accordance with the Guidelines for Animal Experiments from the National Cancer Center.

Female BALB/c mice, 6 weeks old, were obtained from SLC Japan (Shizuoka, Japan). These mice were maintained in a laminar air-flow cabinet and were inoculated subcutaneously with 5×10^6 PC-14 cells or with 5×10^6 A549 cells in the flank region. When tumor volumes (TVs) reached approximately 100 mm^3, the mice were divided randomly into test groups of 5 mice per group (Day 0). The length (a) and width (b) of the tumor mass were measured twice weekly, and the TV was calculated as follows: $TV = (a \times b^2)/2$. The relative TV (RTV) at Day n was calculated as follows: $RTV = TV_n/TV_0$, where TV_n is the TV at Day n, and TV_0 is the TV at Day 0.

Experiment 1: Evaluation of the Antitumor Effect of NK012 and CPT-11

By comparing the data between NK012 and CPT-11, we evaluated their effects as single agents against PC-14 or A549 xenografts. The maximum tolerated dose (MTD) of NK012 (30 mg/kg)[11] or the MTD of CPT-11 (66.7 mg/kg)[18] was administered by intravenous injection into the tail vein on Days 0, 4, and 8.

Experiment 2: Evaluation of the Antitumor Effect of NK012 Alone and NK012 With Bv

By comparing the data between NK012 alone and NK012 plus Bv, we evaluated the combined effect of NK012 plus Bv against PC-14 xenografts. NK012 at a dose of 5 mg/kg was administered intravenously into the tail vein on Days 0, 4, and 8 with or without Bv. In addition, we evaluated the combined effects against A549 xenografts (NK012 [30 mg/kg intravenously] with Bv). When Bv was coadministered with each anticancer agent, Bv was administered intraperitoneally at a dose of 5 mg/kg on Days 0, 4, and 8.

Table 1. Fifty Percent Inhibitory Concentration Values of 7-Ethyl-10-Hydroxycamptothecin (SN-38), the SN-38–Incorporating Polymeric Micelle NK012, and Irinotecan in Various Human Lung Adenocarcinoma Cell Lines

	IC_{50} (μmol/L)[a]		
Cell Line	SN-38	NK012	CPT-11
PC-14	0.050±0.003	0.053±0.002	9.688±1.187
A549	0.506±0.029	0.883±0.840	48.153±4.641
PC-9	0.028±0.011	0.059±0.005	21.782±2.145
NCI-H23	0.025±0.005	0.060±0.002	5.223±1.586
NCI-H1975	0.047±0.084	0.082±0.002	6.330±0.432

IC_{50} indicates 50% inhibitory concentration; CPT-11, irinotecan.
[a] All values shown are the mean values±standard deviation.

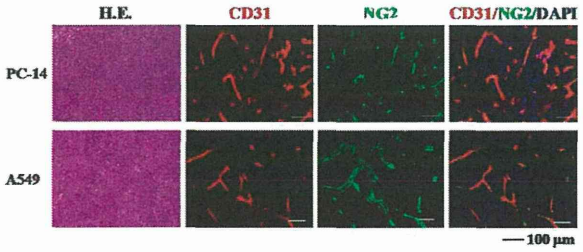

Figure 1. These photomicrographs are from the histologic examination of excised tumors from PC-14 and A549 xenografts that were stained with hematoxylin and eosin (H.E.) or analyzed by immunohistochemistry for cluster of differentiation molecule 31 (CD31) (also called platelet endothelial cell adhesion molecule 1) (red) for the chondroitin sulfate proteoglycan NG2 (green) and for 4′,6-diamidino-2-phenylindole (DAPI) (blue). Scale bars = 100 μm.

Distribution Studies of Free SN-38, CPT-11, and NK012 in Tumors by High-Performance Liquid Chromatography

When the PC-14 TV reached approximately 100 mm^3, NK012 (30 mg/kg) or CPT-11 (66.7 mg/kg) was administered intravenously with or without Bv (5 mg/kg intraperitoneally). Twenty-four hours after the injection of NK012 or CPT-11, each tumor was excised under anesthesia. In other experiments, NK012 (5 mg/kg) was administered intravenously with or without Bv (5 mg/kg intraperitoneally), and each tumor was excised under anesthesia at 12 hours, 24 hours, 3 days, 7 days, 10 days, and 14 days after the injection of NK012. The tumor tissues were rinsed with physiologic saline; mixed with 0.1 M glycine-HCl buffer, pH 3.0, in methanol at 5% (weight/weight); and homogenized. To detect free SN-38 and CPT-11, the tumor samples (100 μL) were mixed with 20 μL 1 mM phosphoric acid in methanol (1:1) and 40 μL ultrapure water, and camptothecin was used as the internal standard (10 ng/mL for free SN-38, 12 ng/mL for CPT-11). The samples were vortexed vigorously for 10 seconds and filtered through an Ultrafree-MC centrifugal filter device (Millipore, Bedford, Mass). Reverse-phase high-performance liquid chromatography (HPLC) was conducted at 35°C on a Mightysil RP-18 GP column (150 × 4.6 mm; Kanto Chemical, Tokyo, Japan). Then, the samples were injected into an Alliance Water 2795 HPLC system (Waters, Milford, Mass) equipped with a Waters 2475 multi-λ fluorescence detector. Fluorescence originating from SN-38 was detected at 540 nm with an excitation wavelength of 365 nm.

For the detection of polymer-bound SN-38, SN-38 was released from the polymer as described previously.[11] In brief, 100-μL tissue samples were diluted with 20 μL methanol (50% [weight/weight]) and 20 μL NaOH (0.7 M). The samples were incubated for 15 minutes at room temperature. After incubation, 20 μL HCl (0.7 M) and 60 μL of internal standard solution were added to the samples and the hydrolysate was filtered. The filtrate was applied to the HPLC system. Polymer-bound SN-38 was determined by subtraction of nonpolymer-bound SN-38 from the total SN-38 in the hydrolysate.

Immunofluorescence Study

At Day 14 after the injection of saline, Bv, NK012, or NK012 plus Bv, the PC-14 tumors were excised under anesthesia. Frozen sections of these tumors (10 μm) were fixed with 4% paraformaldehyde and washed with phosphate-buffered saline (PBS). After blocking with 5% skim milk (BD, Franklin Lakes, NJ) in PBS, the slides were incubated with anti-cluster of differentiation molecule 31 (anti-CD31) monoclonal antibody (1:100 dilution; PharMingen, San Diego, Calif) and anti-NG2 monoclonal antibody (1:1000 dilution; Chemicon, Temecula, Calif) for 1 hour. After washing with PBS, the slides were stained with Alexa 555-, Alexa 647-conjugated secondary antibodies, antirat (red) and antirabbit immunoglobulin G (green; 1:100 dilution; Invitrogen, Carlsbad, Calif), and 4′,6-diamidino-2-phenylindole (DAPI) for nuclear staining. Five areas were chosen randomly from each mouse (n = 2), and the fluorescence intensity was measured and analyzed with BZ-II ANALYZER software (Keyence, Osaka, Japan) for histologic quantification under fluorescence microscopy at 20-fold magnification.

Statistical Analysis

One-way fractional analyses of variance and multiple comparison tests (Scheffe and Bonferroni/Dunn)

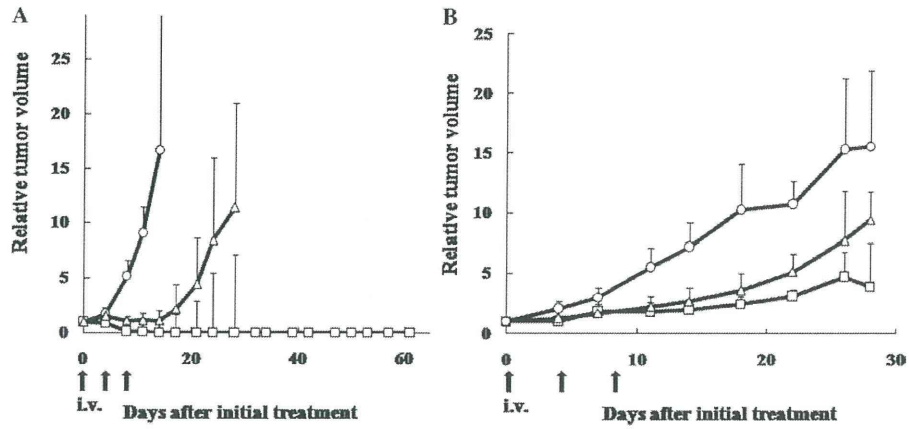

Figure 2. These graphs illustrate (A) the antitumor effects of the novel 7-ethyl-10-hydroxycamptothecin (SN-38)-incorporating polymeric micelle NK012 alone (5 mg/kg daily), bevacizumab (Bv) alone (5 mg/kg daily), and combined NK012 (5 mg/kg daily) plus Bv (5 mg/kg daily) against PC-14 tumor-bearing mice and (B) the effects of NK012 alone (30 mg/kg daily) and combined NK012 (30 mg/kg daily) plus Bv (5 mg/kg daily) against A549 tumor-bearing mice. Squares indicate NK012; open triangles, Bv; solid triangles, NK012 plus Bv; saline, circles. NK012 was administered intravenously (i.v.), and Bv was administered intraperitoneally (i.p.) on Days 0, 4, and 8. Each group included 5 mice. Points indicate mean values; bars, standard deviation.

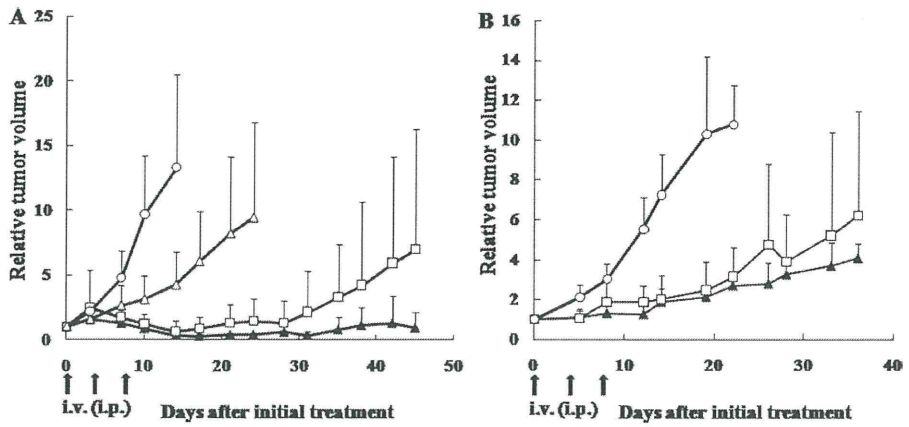

Figure 3. These graphs illustrate the antitumor effects of the novel 7-ethyl-10-hydroxycamptothecin (SN-38)-incorporating polymeric micelle NK012 alone or irinotecan (CPT-11) alone against (A) PC-14 (B) and A549 tumor-bearing mice. The treatment was initiated 11 days after PC-14 inoculation and 13 days after A549 inoculation. NK012 (30 mg/kg daily) (squares), CPT-11 (66.7 mg/kg daily) (triangles), or saline (circles) was administered intravenously (i.v.) on Days 0, 4, and 8. Each group included 5 mice. Points indicate mean values; bars, standard deviation.

conducted with StatView software (version 5.0; SAS Institute, Inc., Cary, NC) were used to compare the different treatment groups of xenografts. Data were expressed as the mean ± standard deviation. Data were analyzed with the Student *t* test when the groups had equal variance (*F* test) or with the Welch test when they had unequal variance (*F* test). *P* values <.05 were regarded as statistically significant. All statistical tests were 2-sided.

RESULTS

Sensitivity of Lung Cancer Cells to SN-38, NK012, and CPT-11

The 50% inhibitory concentration values of NK012 for the cell lines ranged from 0.059 μmol/L to 0.88 μmol/L. The growth-inhibitory effect of NK012 was 50-fold more potent than that of CPT-11 and was almost equivalent to that of SN-38 (Table 1).

Histologic Examination of PC-14 and A549 Xenografts

Hematoxylin and eosin staining of the tumors from PC-14 xenografts revealed that the tumors were poor in stroma, whereas the tumors from A549 xenografts appeared to be stroma-rich. Immunostaining of both tumor tissues with CD31 and NG2 indicated that vasculatures covered with pericytes were more abundant in the A549 xenografts than in the PC-14 xenografts (Fig. 1).

Antitumor Activity of NK012 and CPT-11 on Subcutaneous PC-14 and A549 Xenografts

Experiment 1: Comparison of the antitumor effect of NK012 and CPT-11

In PC-14 xenografts that were treated with NK012 at 30 mg/kg, the tumors started to shrink on Day 4, the tumors disappeared completely by Day 14, and there was no relapse during observation until 60 days after treatment (Fig. 2A). Comparison of the relative TV revealed that the antitumor activity of NK012 was significantly greater than that of CPT-11 ($P = .0267$). Conversely, the TV did not shrink in A549 tumor-bearing mice that were treated with NK012 (Fig. 2B). Although the antitumor activity of NK012 did not differ significantly from that of CPT-11 in A549 xenografts ($P = .0869$), a trend toward a superior antitumor effect against A549 tumors was observed in the NK012 treatment group.

Experiment 2: Comparison of the antitumor effect of NK012 alone and NK012 plus Bv

In PC-14 xenografts, the combination of 5 mg/kg NK012 with 5 mg/kg Bv resulted in a significantly greater inhibition of tumor growth compared with NK012 5 mg/kg alone ($P = .0261$) (Fig. 3A). Also in A549 xenografts, the combination of 30 mg/kg NK012 with 5 mg/kg Bv resulted in significant inhibition of tumor growth compared with NK012 30 mg/kg alone ($P < .0001$) (Fig. 3B).

Distribution Studies of Free SN-38, CPT-11, and NK012 in Tumors Using HPLC

In tumors that were obtained 24 hours after the injection of CPT-11 or NK012, the level of free SN-38 released from NK012 was significantly greater than the level of SN-38 converted from CPT-11 ($P = .003$) (Fig. 4A). Conversely, the level of free SN-38 released from treatment with NK012 plus Bv did not differ significantly from the level released from treatment with NK012 alone. The intratumor concentrations of polymer-bound SN-38 did not differ between NK012 plus Bv and NK012 alone

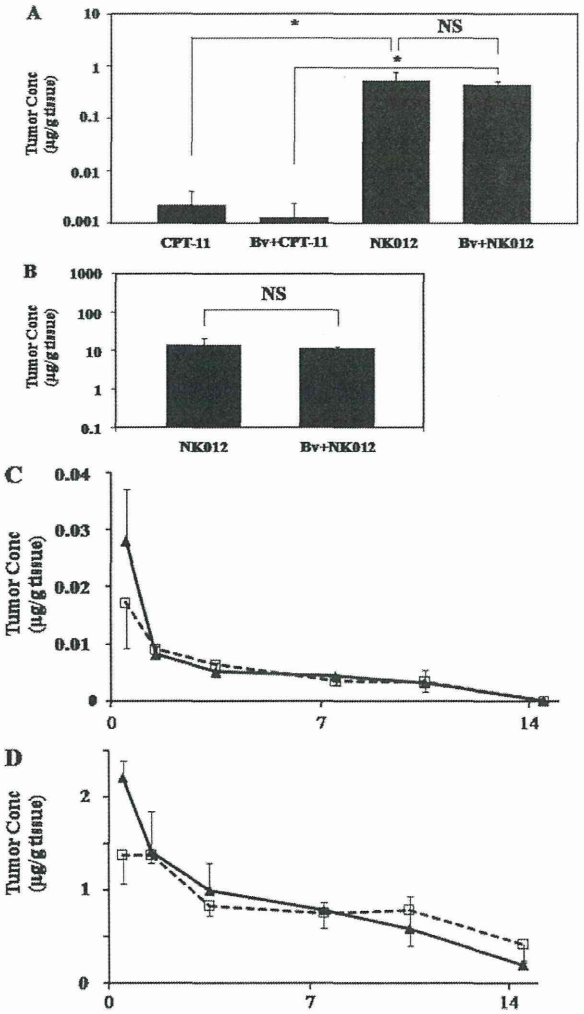

Figure 4. These charts illustrate pharmacokinetics in PC-14 tumor-bearing mice. (A) Polymer-unbound 7-ethyl-10-hydroxycamptothecin (free SN-38) in tumor was quantified by high-performance liquid chromatography (HPLC) 24 hours after the injection of irinotecan (CPT-11) (66.7 mg/kg), combined CPT-11 (66.7 mg/kg) plus bevacizumab (Bv) (5 mg/kg), the SN-38-incorporating micelle NK012 (30 mg/kg), or combined NK012 (30 mg/kg) plus Bv (5 mg/kg). (B) Polymer-bound SN-38 in tumor also was quantified by HPLC 24 hours after the injection of NK012 (30 mg/kg) or combined NK012 (30 mg/kg) plus Bv (5 mg/kg). Free SN-38 (C) and polymer-bound SN-38 (D) in tumor was quantified by HPLC at 12 hours, 24 hours, 3 days, 7 days, 10 days, and 14 days after the injection of NK012 (5 mg/kg daily) (squares) or combined NK012 (5 mg/kg daily) plus Bv (5 mg/kg daily) (triangles). Each group included 3 mice. Points indicate mean values; bars, standard deviation; asterisk, $P < .01$.

(Fig. 4B). At only 12 hours after injection, intratumor concentrations of polymer-bound SN-38 were significantly greater with NK012 alone than with NK012 plus Bv ($P = .015$). At this time point, however, there was no

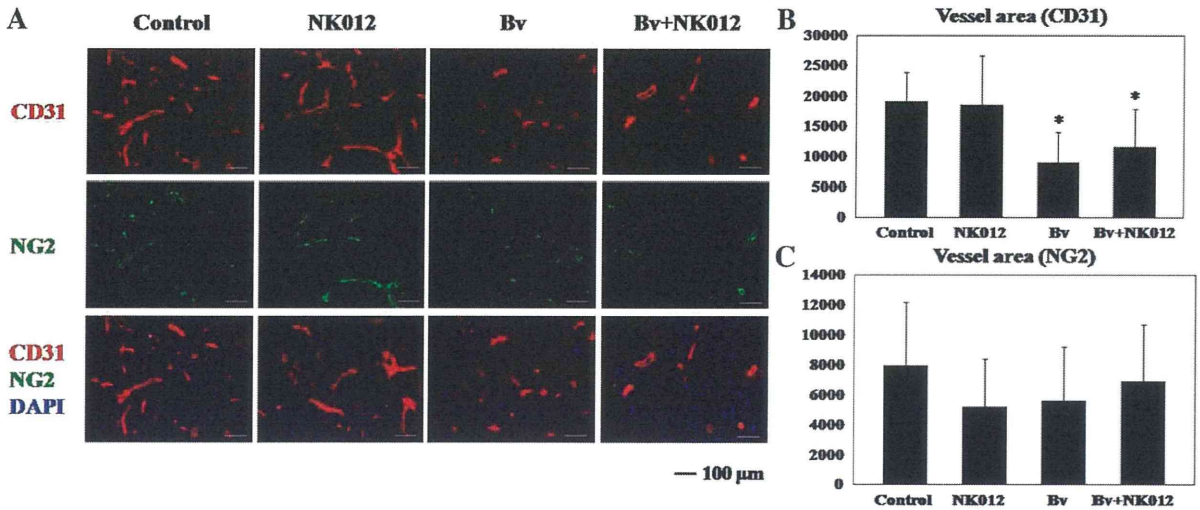

Figure 5. These photomicrographs reveal immunofluorescence staining of cluster of differentiation molecule 31 (CD31)-positive endothelial cells and the chondroitin sulfate proteoglycan NG2-positive pericytes. (A) Fourteen days after the injection of saline, bevacizumab (Bv), the novel 7-ethyl-10-hydroxycamptothecin (SN-38)-incorporating polymeric micelle NK012, or combined NK012 plus Bv, all tumors were excised from the mice. Frozen sections from these tumors (10 μm) were stained with anti-CD31 monoclonal antibody (red), anti-NG2 antibody (green), and 4′,6-diamidino-2-phenylindole (DAPI) (blue). Scale Bars = 100 μm. Histologic quantification under fluorescence microscopy at 20-fold magnification was performed (B) for CD31-positive areas and (C) for NG2-positive areas. Bars indicate standard deviation; asterisks, $P < .01$ compared with control.

difference in the intratumor concentration of free SN-38 between treatment with NK012 alone and treatment with NK012 plus Bv. Thereafter, the intratumor concentrations of both polymer-bound SN-38 and free SN-38 did not differ between treatment with NK012 alone and treatment with NK012 plus Bv (Fig. 4C,D).

Immunofluorescence Staining to Clarify the Antivascular Effect of Bv

Treatment with Bv in combination with or without NK012 significantly reduced the area of CD31-positive proliferating endothelial cells in the tumors compared with controls on Day 14 ($P < .01$) (Fig. 5A,B). Conversely, the area of NG2-positive pericytes was not significantly different between the groups (Fig. 5A,C).

DISCUSSION

The size of NK012 is approximately 20 nm in diameter, and NK012 is sufficiently large to avoid renal secretion. NK012 can evade nonspecific capture by the reticuloendothelial system in various organs, because the outer shell of NK012 is covered with polyethyleneglycol. Therefore, NK012 is expected to achieve a long plasma half-life, which permits large amounts of SN-38 to reach the tumor site through the enhanced permeability and retention effect.[19]

To date, we have reported that NK012 has significantly greater antitumor activity against various human tumor xenografts including, small cell lung cancer,[11,17] colorectal cancer,[14] renal cancer,[13] pancreatic cancer,[12] gastric cancer,[15] and malignant glioma,[16] compared with CPT-11. In the current study, NK012 also appeared to eradicate PC-14 xenografts completely, but not A549 xenografts. This difference may be because of differences in the sensitivity of each cell line to NK012 and in pericyte coverage on vasculatures. Less pericyte coverage reportedly to results in more leakiness of plasma substances; therefore, the degree of NK012 accumulation may be associated inversely with the degree of pericyte coverage.[20,21]

Angiogenesis, which permits tumors to grow and metastasize, plays a pivotal role in several pathologic disorders.[22] VEGF is 1 of the most potent positive regulators of angiogenesis[23] and is recognized as an attractive target in cancer therapy. Unlike normal vasculature, the microvessels of tumors are hyperpermeable to several substances, including macromolecules and nanoparticles. The permeability, interstitial fluid pressure, and numbers of microvessels are increased by VEGF-induced angiogenesis.[24,25] Anti-VEGF antibody administered in combination with chemotherapeutic agents, including doxorubicin,[26] topotecan,[27,28] paclitaxel,[29] and docetaxel,[30] resulted in more potent inhibition of tumor growth than either agent alone. However, it has not been clarified whether anti-VEGF antibody

administered in combination with drug-incorporating polymeric micelles has an additive effect. In the current study, we demonstrated that the combination of NK012 plus Bv had significantly greater antitumor activity against human lung adenocarcinoma cells (PC-14 and A549) compared with NK012 alone. The concentrations of either polymer-bound SN-38 or free SN-38 after the administration of NK012 plus Bv did not clearly differ from the concentrations after NK012 alone. In addition, after treatment with Bv, the area of vascular endothelial cells stained with CD31 was decreased significantly compared with controls. These results suggest that VEGF inhibition may not disturb NK012 accumulation in the tumors and that the direct effect of NK012 plus Bv produced an additional antitumor effect.

In the current study, we demonstrated that NK012 has significantly greater antitumor activity against human lung adenocarcinoma cells (PC-14 and A549) compared with CPT-11. Therefore, we believe that NK012 is a promising oncologic treatment for patients with NSCLC. In 2 individual phase 1 trials that were conducted in Japan and the United States, the toxic profile of NK012 was favorable, and the dose-limiting toxicity was neutropenia.[31,32] Diarrhea was mild; that is, even the worst diarrhea was grade 2 in the phase 1 setting.

In conclusion, the current study demonstrated the superior antitumor activity of NK012 against NSCLC cells compared with CPT-11. In patients with NSCLC, clinical trials of the combination of NK012 plus Bv may be warranted.

CONFLICT OF INTEREST DISCLOSURES

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Synergistic antitumor activity of the SN-38-incorporating polymeric micelles NK012 with S-1 in a mouse model of non-small cell lung cancer

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The combination therapy of CPT-11, a prodrug of SN-38, with S-1, a dihydropyrimidine dehydrogenase inhibitory fluoropyrimidine, shows a high clinical response rate in non-small cell lung cancer (NSCLC). However, this combination causes severe toxicities such as diarrhea. Here, we investigated the advantages of treatment with the SN-38-incorporating polymeric micelles NK012 over CPT-11 in combination with S-1 in mice bearing a NSCLC xenograft in terms of antitumor activity and toxic effects, particularly intestinal toxicity. *In vitro* cytotoxic effects were examined in human NSCLC cell lines (A549, PC-9, PC-14, EBC-1 and H520). *In vivo* antitumor effects were evaluated in PC-14- and EBC-1-bearing mice after NK012 or CPT-11 administration on Days 0 and 7 and S-1 administration on Days 0–13. Pathological changes in the small intestine were also investigated. The *in vitro* growth inhibitory effects of NK012 were 56.8- to 622-fold more potent than those of CPT-11. NK012/S-1 treatment showed significantly higher antitumor activity both in PC-14-bearing ($p = 0.0007$) and EBC-1-bearing mice ($p < 0.0001$) than CPT-11/S-1 treatment. The deformity and decrease in the density of intestinal villi were more severe in CPT-11/S-1-treated mice than in NK012/S-1-treated mice. NK012/S-1 combination is a promising candidate regimen against NSCLC without inducing toxicities such as severe diarrhea and therefore warrants clinical evaluation.

Lung cancer is the leading cause of death from malignancies worldwide in both men and women,[1] and accounted for 31% (male) and 26% (female) of all cancer deaths in 2008.[2] It is histologically classified into small-cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). The standard first-line chemotherapy for NSCLC is platinum-based regimens.[3] However, as shown in a randomized phase III study, the response rate to these regimens is only 30–33% and the 1-year survival rate is 48–59%, with a median survival period of 11–14 months for advanced NSCLC patients with PS 0 or 1.[4] Therefore, the development of new chemotherapeutic agents and combination regimens against NSCLC is urgently desired.

Irinotecan hydrochloride (CPT-11), an anticancer drug, is converted to its biologically active metabolite 7-ethyl-10-hydroxy-camptothecin (SN-38) by carboxylesterases, and SN-38 has been shown to be efficacious against various human cancers such as colorectal, lung and ovarian cancer.[5–8] Although SN-38 has 1,000-fold more potent cytotoxic activity against various cancer cell lines *in vitro* than CPT-11,[9] its conversion rate from CPT-11 to SN-38 is <10% of the original CPT-11 dose in the body.[10,11]

On the other hand, the SN-38-incorporating polymeric micelles NK012 appear to have the advantage of passive targeting of the drug delivery system (DDS). In this passive DDS targeting, the drug accumulates in tumor tissue by utilizing the enhanced permeability and retention (EPR) effect.[12–15] This EPR effect is based on several pathological mechanisms that include hypervascularity, secretion of tumor vascular permeability factors stimulating extravasation of macromolecules including nanoparticles such as liposomes and micelles, and the absence of an effective lymphatic

Key words: NK012, S-1, diarrhea, drug delivery system, non-small cell lung cancer

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