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Preclinical report

Biodistribution of NX211, liposomal lurtotecan, in tumor-bearing mice

John P Desjardins,[1] Elizabeth A Abbott,[1] David L Emerson,[1] Blake E Tomkinson,[1] Jeremy D Leray,[1] Eric N Brown,[1] Marta Hamilton,[1] Larry Dihel,[1] Mieke Ptaszynski,[1] Raymond A Bendele[1] and Frank C Richardson[1]

[1]Gilead Sciences, Inc, 2860 Wilderness Place, Boulder, CO 80301, USA.

Prolonging tumor exposure to topoisomerase I inhibitors has been correlated to enhance the efficacy of those agents. Lurtotecan, a water-soluble camptothecin analog, was formulated as a liposomal drug, NX211, to enhance the delivery of drug to tumors. Tumor-bearing mice were treated with either [^{14}C]NX211 containing [^{14}C]lurtotecan, [^{3}H]NX211 containing [^{3}H]phosphatidylcholine or [^{14}C]lurtotecan, euthanized at selected times post-injection, and tissues, plasma, urine and feces were collected. These studies demonstrated that KB tumors of [^{14}C]NX211-treated mice had approximately 70-fold greater concentrations of [^{14}C]lurtotecan at 24 h, respectively, compared to concentrations of [^{14}C]lurtotecan of the KB tumors of [^{14}C]lurtotecan-treated mice. The area under curve (AUC) from 0 to 48 h of [^{14}C]lurtotecan for the KB tumors of [^{14}C]NX211-treated animals was over 17-fold greater than the AUC of [^{14}C]lurtotecan for the tumors of [^{14}C]lurtotecan-treated animals. Treatment with [^{3}H]NX211 demonstrated that the lipid component continually accumulated over 24 h in the tissues. HPLC analysis of extracted material from tumors of [^{14}C]NX211-treated mice showed that more than 95% of the radioactive material was intact [^{14}C]lurtotecan. These findings are one of the keys justifying the development of a liposomal formulation of lurtotecan, which has the intent to increase tumor exposure and increase antitumor efficacy. [© 2001 Lippincott Williams & Wilkins.]

Key words: Biodistribution, camptothecin, liposomes, lurtotecan, radioactive.

Introduction

Topoisomerases play a critical role in the replication of DNA. Camptothecin and its analogs have been shown to inhibit topoisomerase I (Topo I).[1] The mechanism of inhibition has been shown to be the binding of camptothecin or analogs to the Topo I–DNA complex.[2,3] This binding prevents the religation step, necessary to reseal the parent strand of DNA leading to a double-strand break and attenuation of cell division. Other mechanisms such as induction of proteases or endonucleases may also play a part in the cytotoxicity of camptothecin and its analogs. Two camptothecins are commercially available, irinotecan (CPT-11, Camptosar®) and topotecan (Hycamtin®). Irinotecan is commercially available in the US and Europe for colorectal cancer, which has progressed on prior 5-fluorouracil (5-FU)-based regimens. In the US, it has recently also been approved for first-line treatment of colorectal cancer, in combination with 5-FU and LV. In addition, Irinotecan is used in Japan for non-small cell lung cancer (NSCLC). Topotecan is commercially available worldwide as a second-line therapy for patients with advanced ovarian cancer. Recently, topotecan has been recommended for approval by the FDA Oncology Drugs Advisory Committee as a second-line therapy for small cell lung cancer (SCLC). Irinotecan, topotecan and other camptothecin analogs are under intensive evaluation in several additional indications, as single agents and in combination with other cytotoxic drugs.

NX211 is the liposomal formulation of lurtotecan [GI147211C, 7(4-methylpiperazinomethylene)-10,11-ethylenedioxy-20-(*S*)-camptothecin dihydrochloride, also referred to as GG211], a water-soluble analog of camptothecin. Preclinical studies with lurtotecan administered as a single agent demonstrated that it is an effective inhibitor of mammalian DNA Topo I *in vitro* and *in vivo*, with at least similar potency compared to topotecan.[4–6] Pre-clinical and clinical development of lurtotecan has been undertaken by Glaxo-Wellcome, including phase I and II studies conducted in the US and Europe from 1994 to 1998. Like the other camptothecin analogs, it was shown to

Correspondence to JP Desjardins, Gilead Sciences, Inc, 2860 Wilderness Place, Boulder, CO 80301, USA.
Tel: (+1) 303 546-7760; Fax: (+1) 303 444-0672;
E-mail: jdesjardins@gilead.com

produce myelosuppression as its major dose-limiting effect. Phase II studies of lurtotecan showed it to produce responses in NSCLC, SCLC and ovarian cancer patients.[7–11]

Previous studies have examined alternative deliveries of camptothecins to tumor sites for increased efficacy and decreased toxicity.[12,13] Encapsulation of a drug into a liposomal formulation has been shown to provide greater delivery with enhanced efficacy against a variety of conditions.[14–16] Gilead Sciences has developed a liposomal preparation of lurtotecan, NX211, to possibly enhance the efficacy and reduce the side effects of lurtotecan. NX211, in comparison to the free lurtotecan, has increased plasma concentrations and residence times leading to a 1000-fold larger area under the curve (AUC) in rodents.[17,18] The half-life ($t_{½}$) for NX211 is in the range of 5–6 h, which is approximately 5-fold greater than the $t_{½}$ for lurtotecan in rodents. Other studies have examined the efficacy and dosing schedules of NX211 in xenograft tumor models.[19,20] These studies demonstrated that NX211 has greater potency and efficacy in these models compared to lurtotecan.

Studies were conducted with radiolabeled [^{14}C]lurtotecan, [^{14}C]NX211 or [^{3}H]NX211 (lipid labeled). These studies determined the effects of liposomal encapsulation of lurtotecan on the biodistribution in tumor-bearing mice. The distribution to different tumors histotypes, the effects of dose and fate of the lipid component were also examined. Lastly, radioactive material from tumors of [^{14}C]NX211-treated mice was analyzed for percent of radioactive material that was intact [^{14}C]lurtotecan.

Materials and methods

Animals

Female *nu/nu* mice (18–25 g) were obtained from Harlan Sprague-Dawley (Indianapolis, IN), housed in microisolator filtration racks, and maintained on filtered acidified water and sterile lab chow *ad libitum*. Animals were allowed to acclimatize to their new environment for 1 week prior to tumor cell implantation. For biodistribution studies, ES-2 (ovarian) or KB (head/neck) (both from ATCC, Manassas, VA) tumors were used. For analysis of radioactive material by HPLC, HT-29 (colon) (ATCC) tumors were used. All tumors were established by injecting harvested tumor cells in a single s.c. in the axillary region of the mice for the tumor distribution study. The tumors were grown until approximately 200 ± 50 mm^3 in size. The animals were then sorted according to body weight, with three animals per cage.

Material

Radiolabeled [^{14}C]lurtotecan (Figure 1) was obtained from Glaxo-Wellcome (Research Triangle Park, NC) and used in the preparation of [^{14}C]NX211 (Gilead Sciences, San Dimas, CA). The specific activity of [^{14}C]NX211 was 8.40 μCi/ml with a concentration of 0.33 mg lurtotecan/ml. [^{14}C]NX211 was mixed with non-radioactive NX211 (Gilead Sciences) to achieve total doses of 5.0 mg/kg with a final specific activity of approximately 4 μCi/mg. Sterile 5% w/v dextrose (McGaw, Irvine, CA) was used as a diluent. [^{3}H]NX211 (lot no. NA1022-54; Gilead Sciences) contained 0.49 mg lurtotecan/ml at 73.0 μCi/ml. The tritium label was a component of the lipid bilayer as L-α-dipalmitoyl-[2-palmitoyl-9,10-^{3}H(*N*)]-phosphatidylcholine (NEN, Boston, MA).

This methodology for liposome preparation yields small unilamellar liposomes under 100 nm with a 20:1 lipid:drug ratio and a lipid composition of 2:1 fully hydrogenated soy phosphotidylcholine:cholesterol.

Treatment of animals

Table 1 describes the treatment of animals with either radiolabeled lurtotecan or NX211 and the tumor histotype for the biodistribution studies. The total amount of radioactivity administered to each animal for each experiment was approximately 0.3–0.5 μCi for [^{14}C]NX211 or [^{14}C]lurtotecan and 3.0 μCi for [^{3}H]NX211. The compound was administered to the mice by bolus i.v. injection into the lateral tail vein.

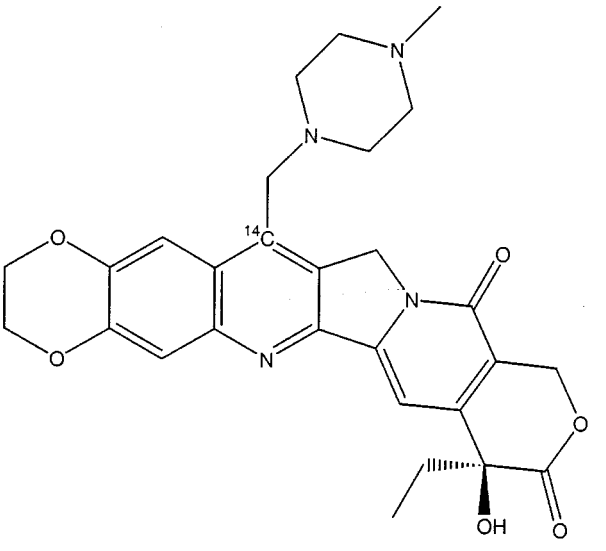

Figure 1. Structure of [^{14}C]lurtotecan.

Table 1. Treatment of animals with radiolabeled lurtotecan or NX211

Compound	Tumor	Dose (mg/kg)	Sample time (h)
[^{3}H]NX211	KB	1.3	1, 3, 6, 24
[^{14}C]NX211	KB	0.5	1, 3, 6, 12, 24
[^{14}C]NX211	KB	1.0	1, 3, 6, 24, 48
[^{14}C]NX211	KB	5.0	1, 3, 6, 12, 24, 48
[^{14}C]NX211	ES-2	5.0	1, 3, 6, 12, 24, 48
[^{14}C]Lurtotecan	KB	5.0	1, 3, 6, 12, 24, 48

Female *nu/nu* mice were implanted with either KB or ES-2 tumors prior to study. Animals were treated with either radiolabeled lurtotecan or NX211. The total amount of radioactivity administered to each animal for each experiment was approximately 0.3–0.5 μCi for [^{14}C]NX211 or [^{14}C]lurtotecan and 3.0 μCi for [^{3}H]NX211.

Following treatment, animals to be euthanized 1, 3 or 48 h post-dosing were housed in adequate sized mouse cages. Animals to be euthanized 6, 12 or 24 h post-dosing were housed in metabolic cages where urine and feces were collected. The animals were euthanized by exsanguination under anesthetic, and selected tissues were removed and weighed. Blood was collected in 1 cm^3 syringes containing 0.1 U heparin (Sigma, St Louis, MO). The blood was centrifuged at 3000 *g* for 10 min on a GS-15R centrifuge (Beckman Instruments, Palo Alto, CA) and plasma was placed in new tubes.

Mice bearing HT-29 tumors were administered 5.0 mg/kg [^{14}C]NX211 containing approximately 1 μCi radioactivity. The animals were euthanized by exsanguination 3 h post-injection. The tumor, liver and plasma were harvested for HPLC analysis.

Analysis of samples by a tissue oxidizer

Whole tissue samples, plasma aliquots, urine aliquots or feces were burned in a Packard tissue oxidizer model 307 (Packard Instrument, Meriden, CT). For analysis of ^{14}C, the resulting vials contained 8 ml Carbosorb E (Packard Instrument) and 12 ml Permafluor E (Packard Instrument). For analysis of ^{3}H, the resulting vials contained 2 ml Permafluor E and 15 ml Monophase S (Packard Instrument). The vials then were counted for radioactivity on a Packard Tri-Carb 2100RT scintillation counter. The raw c.p.m. data were recalculated to the 'true c.p.m.' to correct for efficiency of the tissue oxidizer and scintillation counter. The amount of radioactive material (μg/g or μg/ml) was then calculated by dividing true c.p.m. by the specific activity of that dose in that study. The level of detection for each experiment was 0.01 μg/g or μg/ml.

Analysis of samples by HPLC

Tumors were harvested, weighed, minced and then homogenized in a 4-fold weight to volume or volume to volume excess of 6.6% perchloric acid in a 2:1 water:methanol solution. The resulting homogenates were cleared by centrifugation and then separated by HPLC on a Zorbax Bonus RP C18 4.6 × 250 mm, 5 μm column equilibrated in a 60:40 mixture of phosphate buffer (pH 2.2) and methanol at a flow rate of 1 ml/min. Output signals were simultaneously generated by a Waters scanning fluorescence detector with a 100 μl flowcell and an In/Us Systems BetaRam radioactive detector with a 500 μl flowcell. The radioactive signal output was enhanced with the addition of Fisher ScintiSafe Plus scintilant at a rate of 1 ml/min. The resulting signals were then routed to Waters Millennium 32 software for analysis and display.

Statistical analysis

Mean and standard deviation (SD) were calculated using Microsoft Excel (Microsoft Excel 97, Microsoft, Redmond, WA). Data was graphed using GraphPad Prism software (version 3.00, San Diego, CA). Non-parametric tests and two-tailed *t*-tests were calculated using GraphPad Prism software. GraphPad Prism software was used to determine the area under the curve (AUC) of the concentration of drug (μg/g or μg/ml) over time (0–48 h for 1.0 and 5.0 mg/kg doses and 0–24 h for 0.5 mg/kg dose). The data from the studies with [^{3}H]NX211 were not included in statistical analysis since the radiolabel was associated with the lipid and did not guarantee presence of drug with the lipid.

Results

Tissue and plasma distribution of [^{14}C]NX211 and [^{14}C]lurtotecan

The tissues and plasma from [^{14}C]NX211-treated animals (5.0 mg/kg) contained significantly greater concentrations of radiolabeled material compared to the tissues and plasma of [^{14}C]lurtotecan-treated animals (5.0 mg/kg) at each selected time point examined. The apparent peak concentration in the KB tumor for [^{14}C]NX211-treated animals was at 3 h and was 3.87 ± 0.92 μg/g compared to 1.08 ± 0.11 μg/g in [^{14}C]lurtotecan-treated animals (Figure 2A). The KB tumor had 69.5- and 17.3-fold greater concentrations of radioactive material by 24 and 48 h, respectively, post-injection with [^{14}C]NX211 compared to [^{14}C]lurtote-

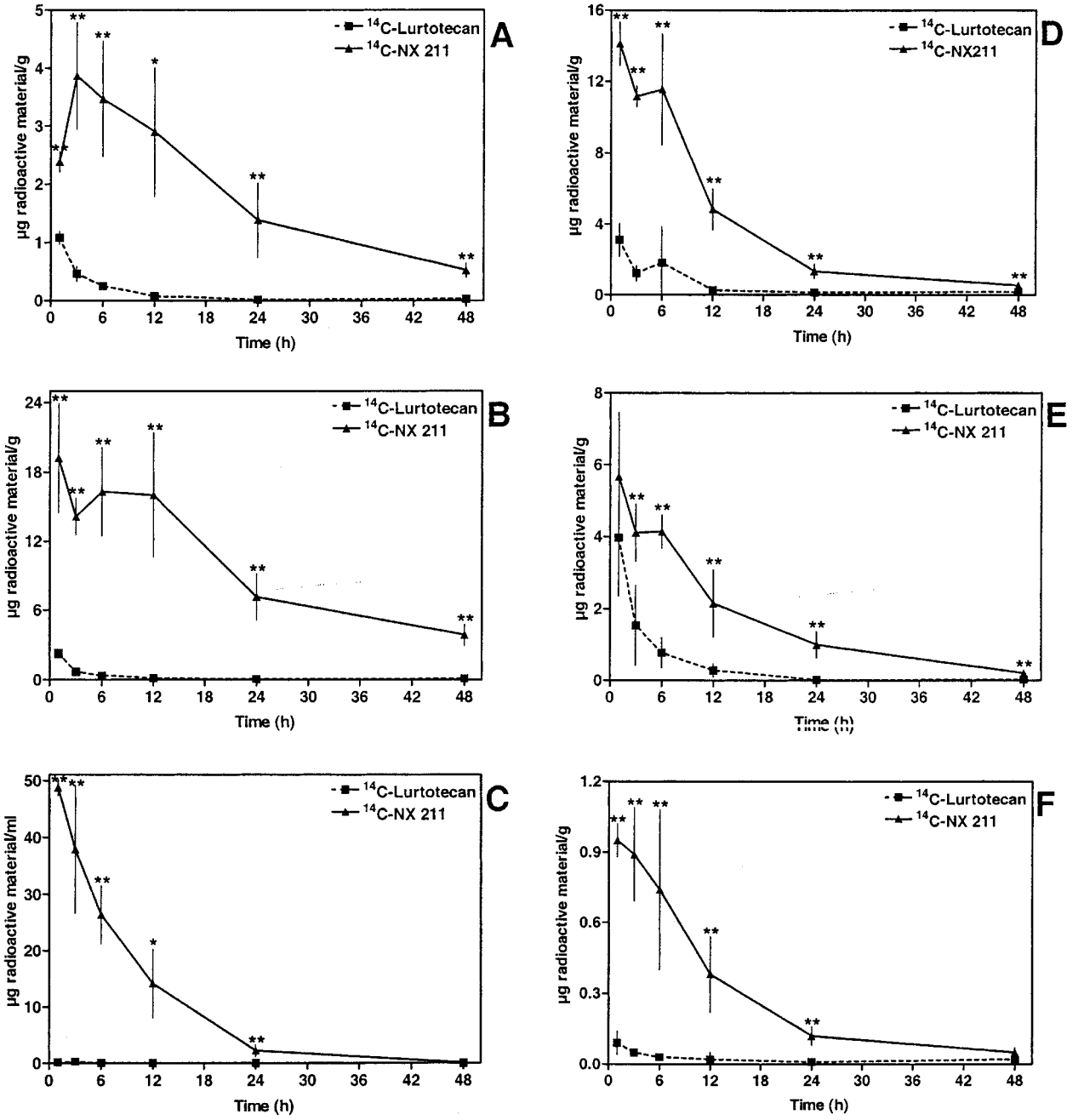

Figure 2. Biodistribution of [^{14}C]lurtotecan or [^{14}C]NX211 in selected tissues and plasma of KB tumor-bearing mice. Female *nu/nu* mice were implanted with KB tumors. Animals were treated with 5.0 mg/kg of either [^{14}C]lurtotecan (■) or [^{14}C]NX211 (▲). The samples are as follows: tumor (A), spleen (B), plasma (C), liver (D), intestine (E) and brain (F). Tissues and plasma were harvested at selected times. Samples were burned in a tissue oxidizer and radioactivity determined by liquid scintillation. Data is mean SD. Units are μg/g tissue or μg/ml plasma. Each point represents at least $n=3$. Statistical significance is represented by $^{*}p<0.05$ or $^{**}p<0.01$.

can. The AUCs over 48 h for the KB tumors were 85.0 (NX211) and 4.8 (lurtotecan) μg·h/g (Table 2). Therefore, exposure to the tumor was nearly 18-fold greater after treatment with [^{14}C]NX211 compared to treatment with [^{14}C]lurtotecan.

All tissues from [^{14}C]NX211-treated animals compared to the tissues from [^{14}C]lurtotecan-treated animals had greater concentrations of radioactive material at all time points examined (Figure 2A–F). The spleen contained the greatest concentration of

radioactive material. A maximal concentration of 19.21 ± 4.72 μg/g was observed at 1 h in the spleen of [^{14}C]NX211-treated animals compared to 2.26 ± 0.36 μg/g for the spleen of [^{14}C]lurtotecan-treated animals at 1 h (Figure 2B). The AUC from the spleens of [^{14}C]NX211-treated animals was 48.6-fold greater compared to the AUC of radioactive material of the spleens from [^{14}C]lurtotecan-treated animals (Table 2). The concentrations of radioactive material in the spleen and plasma were equivalent by 12 h. However, by 48 h, the spleen had an approximately 38-fold greater concentration of radioactive material compared to the concentration of radioactive material in the plasma. The AUCs of radioactive material for the liver, intestines and brain were 8.0-, 5.0- and 12.7-fold greater, respectively, after treatment with [^{14}C]NX211 compared to treatment with [^{14}C]lurtotecan (Table 2). These data demonstrated that liposomal encapsulation altered the primary site of uptake and the amount of material delivered to each tissue.

The plasma had the greatest initial concentration of radioactive material of any sample after treatment with [^{14}C]NX211 (Figure 2C). The peak concentration of radioactive material in the plasma of [^{14}C]NX211-treated animals was 48.7 ± 1.45 μg/ml, occurred at 1 h and accounted for nearly 80% of the injected dose (Figure 3C). The concentration of radioactive material at 1 h in [^{14}C]lurtotecan-treated animals was 0.19 ± 0.05 μg/ml. The concentration of [^{14}C]NX211 in the plasma continuously decreased over 48 h. The AUC of radioactive material in the plasma of [^{14}C]NX211-treated animals was over 269-fold greater than the AUC of radioactive material in the plasma of [^{14}C]lurtotecan-treated animals (Table 2). These data were consistent with other studies demonstrating liposomal drugs being restricted primarily to the plasma compartment.

Dose effects on tissue and plasma distribution

The effect of dose on biodistribution was examined for [^{14}C]NX211. As dose increased, greater concentrations of radioactive material were present in the tissues and plasma (Figure 3A–C). The tumor had apparent peak concentrations at 6 h post-dose with the 0.5 and 1.0 mg/kg doses of [^{14}C]NX211 (Figure 3A). The AUCs of radioactive material in the tumor were 6.2 and 12.5 μg·h/g for the 0.5 and 1.0 mg/kg doses, respectively (Table 2). The spleen had the greatest concentration of radioactive material of tissues examined with dose-dependent increases in AUCs and concentrations of radioactive material (Table 2 and Figure 3B). Plasma concentrations also increased dose dependently with AUCs (Table 2 and Figure 3C). The other tissues (liver, intestine and brain) all had similar increased AUCs as a result of increased dose (Table 2). These results demonstrated that saturation was not achieved at the doses examined. Further, increasing doses did not alter the biodistribution profile.

Tumor effects on tissue and plasma distribution in two different tumors

Vascularity differences between different tumors may dramatically affect liposomal accumulation. Thus, a second tumor (ES-2) was examined to determine the effects of different tumors on biodistribution after treatment with 5.0 mg/kg [^{14}C]NX211. No statistical difference was observed in concentrations of radioactive material between KB- or ES-2-bearing mice for the plasma or spleen at any time examined (Figure 4A–C). Further, the liver, intestines and brain also did not have any statistical differences between KB or ES-2-bearing mice at any time examined (data not shown). The concentration of radioactive material in the KB

Table 2. AUC for tissues and plasma after treatment with [^{14}C]lurtotecan or [^{14}C]NX211

	Dose (mg/kg)	Tumor	Liver	Spleen	Intestine	Brain	Plasma
Lurtotecan	5.0	4.8	21.1	9.2	14.5	1.0	1.6
NX211	0.5	6.2	14.1	48.0	6.8	1.0	31.2
NX211	1.0	12.5	23.8	95.6	14.6	1.0	74.6
NX211	5.0	85.0	168.1	446.9	74.3	12.7	430.5
Ratio of NX211/lurtotecan	0.5/5.0	1.3	0.7	5.2	0.5	1.0	19.5
Ratio of NX211/lurtotecan	1.0/5.0	2.6	1.1	10.4	1.0	1.0	46.6
Ratio of NX211/lurtotecan	5.0/5.0	17.7	8.0	48.6	5.1	12.7	269.1

Mice bearing KB tumors were injected bolus i.v. with 5.0 mg/kg [^{14}C]lurtotecan, or 0.5, 1.0 or 5.0 mg/kg [^{14}C]NX211. Selected tissues and plasma were harvested at selected times post-injection with radioactive material. Tissues or plasma were burned in a tissue oxidizer and radioactivity determined by liquid scintillation. AUCs were determined by GraphPad Prism over 0–48 h for 1.0 and 5.0 mg/kg doses and 0–24 h for 0.5 mg/kg dose. Data is in μg·h/g for tissues or μg·h/ml for plasma. The ratio of AUC of the NX211 sample over AUC of the lurtotecan sample was then calculated.

tumor (3.87 ± 0.92 μg/g) was statistically greater at 3 h compared to the ES-2 tumor (2.09 ± 0.45 μg/g). However, at 24 and 48 h, the concentrations of radioactive material in the ES-2 tumor (2.51 ± 0.86 and 1.14 ± 0.34 μg/g, respectively) were statistically greater compared to the KB tumor (1.39 ± 0.64 and

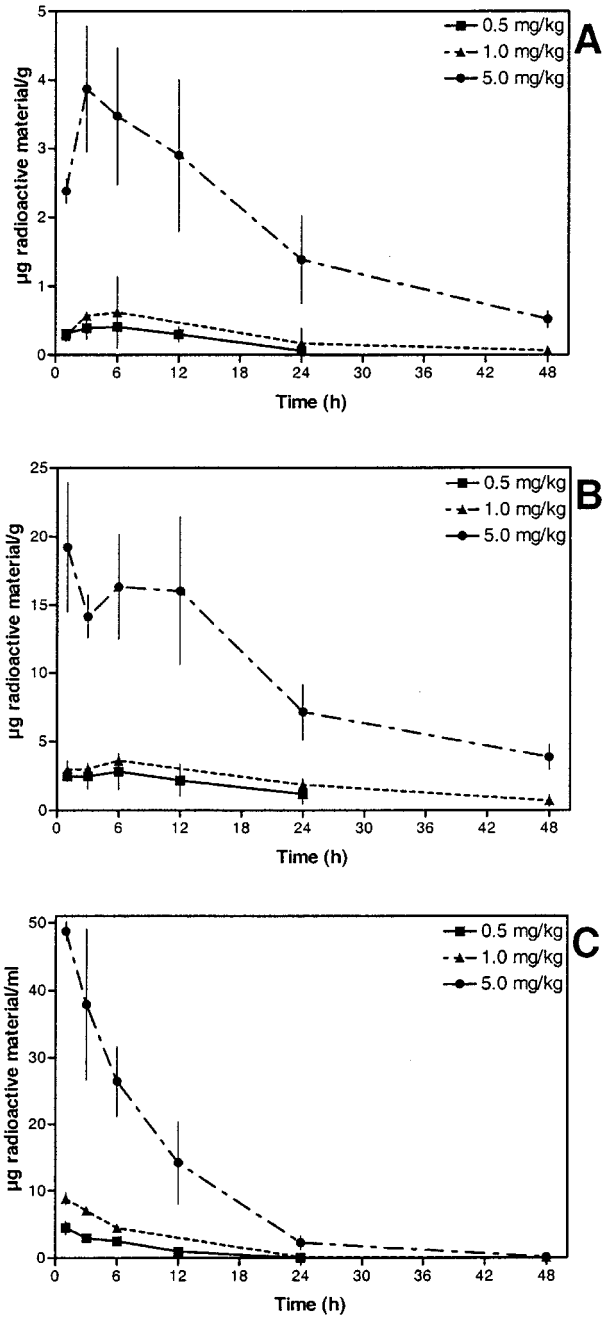

Figure 3. Biodistribution of different doses of [^{14}C]NX211 in tumor-bearing mice. Female *nu/nu* mice were implanted with KB tumors. Animals were treated with 0.5, 1.0 or 5.0 mg/kg of [^{14}C]NX211. Tumor (A), spleen (B) and plasma (C) were harvested at selected times. Samples were burned in a tissue oxidizer and radioactivity determined by liquid scintillation. Data is mean ± SD. Units are μg/g tissue or μg/ml plasma. Each point represents at least $n = 3$.

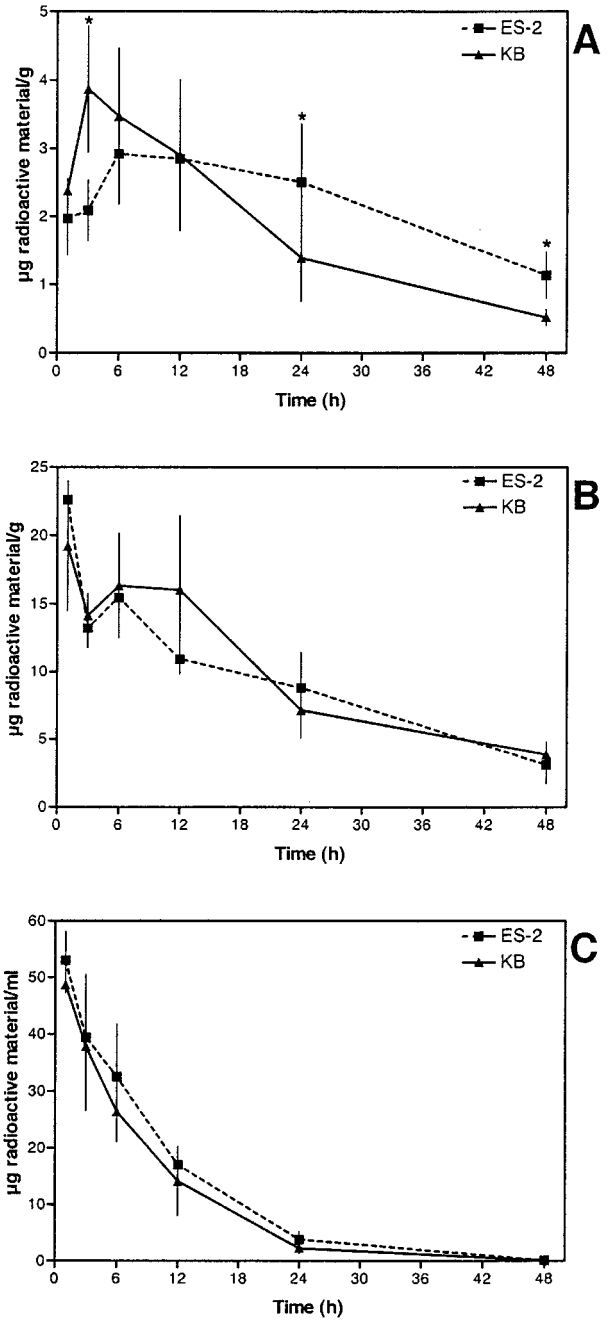

Figure 4. Biodistribution of [^{14}C]NX211 in tumor, spleen, and plasma in KB- or ES-2-bearing mice. Female *nu/nu* mice were implanted with either KB (▲) or ES-2 (■) tumors. Animals were treated with 5.0 mg/kg [^{14}C]NX211. Tumor (A), spleen (B) and plasma (C) were harvested at selected times. Samples were burned in a tissue oxidizer and radioactivity determined by liquid scintillation. Data is mean ± SD. Units are μg/g tissue or μg/ml plasma. Each point represents at least $n = 3$. Statistical significance is represented by $^*p < 0.05$.

0.52 ± 0.12 μg/g, respectively). The AUCs of radioactive material in the tumors were 104.8 (ES-2) and 85.0 (KB) μg·h/g. These data demonstrated that tumor histotype did not alter biodistribution to tissues. Also, biodistribution to the different tumor histotypes produced different profiles, but had nearly identical overall enhanced delivery.

Elimination of [^{14}C]NX211 and [^{14}C]lurtotecan

The amounts of radioactive material in the urine and feces are listed in Table 3. Previous work demonstrated the elimination of the lurtotecan was predominately in the feces via bilary excretion. The feces of [^{14}C]lurtotecan-treated or [^{14}C]NX211-treated mice contained approximately 51 and 40% of the administered dose by 24 h. The urine from [^{14}C]lurtotecan-treated mice contained approximately 17% of the administered dose by 24 h, whereas the urine from [^{14}C]NX211-treated mice contained approximately 10% by 24 h. Therefore, the primary route of excretion was not altered by the liposomal encapsulation of lurtotecan.

Biological fate of the lipid component

The biodistribution of the lipid component of NX211, [^{3}H]NX211, was examined and provided information on the fate of the lipid component. The KB tumor of [^{3}H]NX211-treated animals had an apparent 8-fold increased in concentration of radioactive material by 24 h compared to the concentration of radioactive material at 1 h (Figure 5A). The brain and intestine of [^{3}H]NX211-treated animals also increased in concentrations of radioactive material similar to the tumor by 24 h (data not shown). The spleen (Figure 5B) and liver (data not shown) of [^{3}H]NX211-treated animals, on the other hand, decreased in concentrations of radioactive material by 24 h. The plasma concentrations of [^{3}H]NX211-treated animals followed a similar elimination profile to the plasma of [^{14}C]NX211-treated animals (Figure 5C). The amount of the ^{3}H radioactive material in the urine and feces accounted for less than 10% of the administered dose (Table 3).

Analysis of tissues and plasma by HPLC

Figure 6 represents an HPLC chromatogram of extracted material from the tumor of [^{14}C]NX211-treated mice. The fluorescent peak (top trace) for [^{14}C]lurtotecan had the same retention time as the peak detected by radioactivity (bottom trace). Liver and plasma were also examined, and demonstrated chromatograms that had the same profile but of greater magnitudes (data not shown). These data indicated that the vast majority (greater than 95%) of radioactive material was intact lurtotecan. A small fluorescent peak with no corresponding radioactive peak was observed at a retention time of approximately 4.6 min. This peak was present in the dose solution and was considered a minor impurity. Extracted material from [^{14}C]lurtotecan-treated mice was also examined. The level of radioactive material present was below the detection level of HPLC system for analysis (data not shown).

Discussion

In vitro data as well as clinical experience suggest that prolonged tumor exposure may increase the cytotoxicity of camptothecins. Accordingly, continuous infusion schedules have been used with topotecan and lurtotecan to prolong tumor exposure while reducing the magnitude of peak plasma drug levels, with the rationale of improving the therapeutic index. Phase I studies have evaluated lurtotecan in several i.v. dosing schedules, including daily i.v. doses for 5 consecutive days,[21–23] continuous infusion for 72 h[23–26] and continuous infusion for 7, 14 or 21 days.[27] Lurtotecan

Table 3. Excretion of [^{14}C]lurtotecan, [^{14}C]NX211 or [^{3}H]NX211 into urine and feces

Collection time (h)	Urine			Feces		
	[^{14}C]Lurtotecan	[^{14}C]NX211	[^{3}H]NX211	[^{14}C]Lurtotecan	[^{14}C]NX211	[^{3}H]NX211
6	25.0	2.0	0.1	57.6	6.5	<0.1
12	50.7	5.8	n.d.	86.7	40.1	ND
24	63.6	34.8	2.0	190.4	150.4	0.5

Mice bearing KB tumors were injected bolus i.v. with 5.0 mg/kg [^{14}C]lurtotecan or [^{14}C]NX211 or 1.3 μg [^{3}H-NX211. Each group of animals was then placed in metabolic cages. Urine and feces were collected at 6, 12 or 24 h post-injection. Urine and feces were collected at selected times post-injection with radioactive material. Samples were burned in a tissue oxidizer and radioactivity quantified by liquid scintillation. Data is in summed total μg radioactive material/group to that time point. ND, not determined

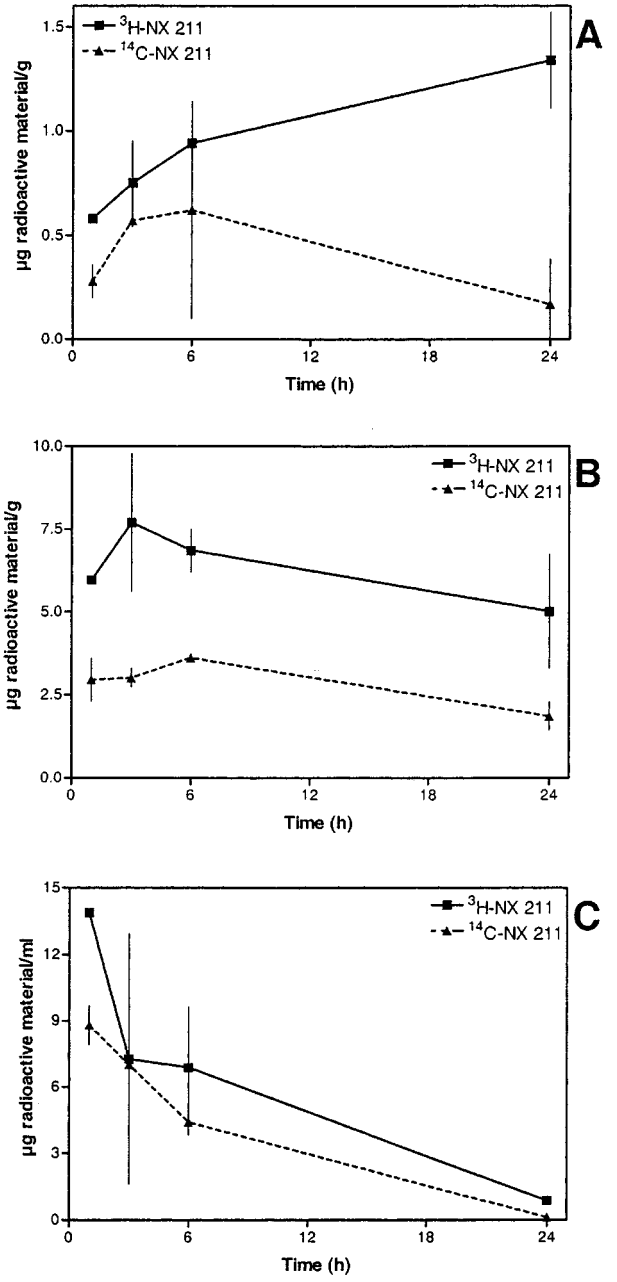

Figure 5. Biodistribution of [^{14}C]NX211 or [^{3}H]NX211 in tumor, spleen and plasma in KB-bearing mice. Female *nu/nu* mice were implanted with KB tumors. Animals were treated with 1.0 mg/kg [^{14}C]NX211 (▲) or 1.34 mg/kg [^{3}H]NX211 (■). Tumor (A), spleen (B) and plasma (C) were harvested at selected times. Samples were burned in a tissue oxidizer and radioactivity determined by liquid scintillation. Data is in mean ± SD Units are µg/g tissue or µg/ml plasma. Each point represents at least $n=3$ except for [^{3}H]NX211, for which $n=2$.

has a toxicity profile qualitatively similar to other camptothecin analogs, with myelotoxicity, especially neutropenia, being dose limiting and gastrointestinal symptoms being the most prominent side effects. Prolongation of the infusion did not increase the severity of neutropenia, but it led to more pronounced thrombocytopenia, suggesting the toxicity profile of lurtotecan might also be influenced by the schedule of administration. Tumor responses in these phase I studies were observed in the 3- and 21-day continuous infusion schedules, but not in the consecutive 5 day i.v. schedule. The number of patients was limited, but the results seem to suggest that the anti-tumor activity of lurtotecan was enhanced by continuous infusion. Current evidence also suggests that prolonged i.v. infusion of topotecan may increase tumor response.[28]

Previous studies have examined the potential of liposomal encapsulated drugs to enhance delivery of chemotherapeutics to tumors.[29–31] These studies demonstrated an increased exposure of tumor to the drug by treatment with NX211 compared to lurtotecan. Enhancement of exposure to drug was observed by comparison of treatment with 0.5 or 1.0 mg/kg [^{14}C]NX211 to treatment with 5.0 mg/kg [^{14}C]lurtotecan (Table 2). Further, the concentration of radioactive material by 24 and 48 h were 8.5- and 2.0-fold greater in tumors of animals treated with 1.0 mg/kg [^{14}C]NX211 compared to tumors of animals treated with 5.0 mg/kg [^{14}C]lurtotecan. Therefore, this greater delivery of [^{14}C]NX211 to tumors apparently contributed to its enhanced tumor efficacy.[32] The concentration of radioactive material in the tumor became greater than the plasma concentration of radioactive material between 24 and 48 h. These data suggested that mechanisms other than diffusion from the plasma were responsible for the enhanced delivery of NX211. One possible reason of the enhanced delivery by NX211 could be the altered vascularity of the tumor, which would entrap the liposomes in the smaller capillaries and release drug directly in the tumor.[33] Administration of [^{14}C]NX211 to mice bearing different tumor histotypes (KB versus ES-2) produced different distribution profiles over a 48 h period. However, the 1.2-fold difference in AUCs for radioactive material demonstrated that the enhanced delivery by the liposomal encapsulation of lurtotecan was not altered by tumor histotype. Lastly, HPLC analysis of the tumor extracts demonstrated that the liposomal formulation delivered the lurtotecan as an intact molecule. Although these studies did not determine the ratio of lactone (parent drug) to carboxylate (metabolite) of the radioactive material, current and previous work have demonstrated that liposomal encapsulation protects the camptothecin molecule in the active lactone species.[34]

Liposomal encapsulation of lurtotecan increased the concentrations and AUCs of radioactive material

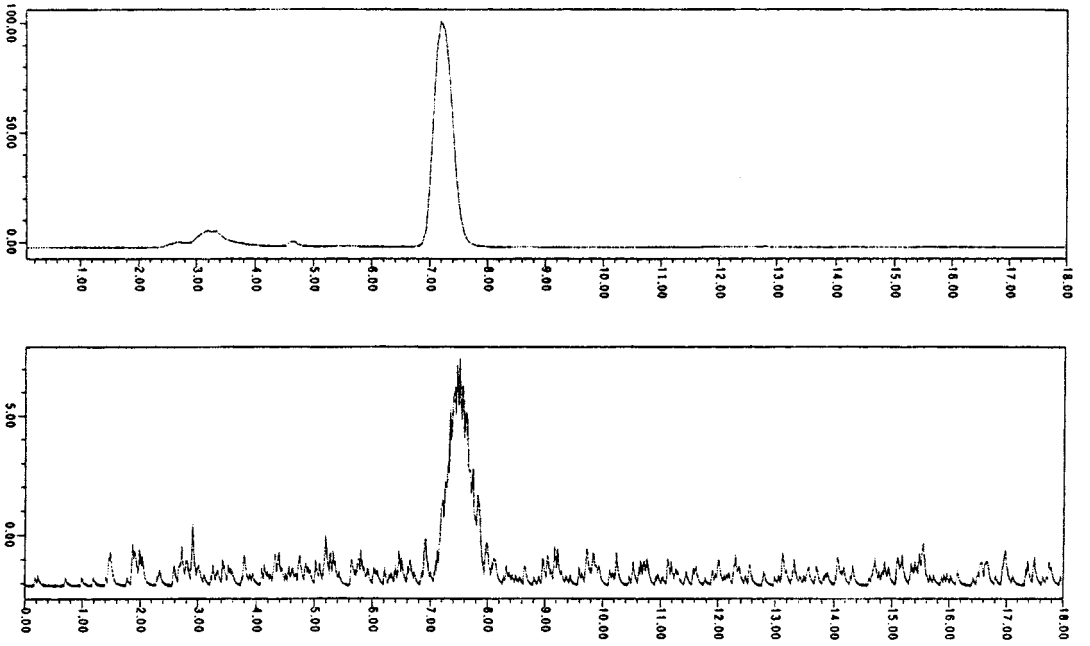

Figure 6. HPLC separation of radioactive material from tumor tissue. Tumor tissue from [^{14}C]NX211-treated mice was analyzed for the presence of radioactive material. The chromatograms represent the fluorescent (top) and radioactive (bottom) traces. The major peak corresponds to the parent compound [^{14}C]lurtotecan (not shown).

for the tissues and plasma of NX211-treated animals versus lurtotecan-treated animals. These data were in agreement with previous work that demonstrated increased uptake of liposomes into the spleen.[35,36] This accumulation in the spleen was a probable result of the uptake of the liposomal drug by the tissue-based macrophages in the spleen.[29,37] In comparison, the liver and intestines contained the greatest concentration of radioactive material of the tissues from [^{14}C]lurtotecan-treated animals. The plasma from NX211-treated animals contained nearly 80% of the total injected dose by 1 h. In comparison, the plasma from [^{14}C]lurtotecan-treated mice contained less than 0.4% of the administered dose by 1 h. These data agreed with previous observations that liposomal drugs have greater retention in the plasma.[29,38]

The feces contained the greatest amount of radioactive material of any sample examined from either lurtotecan- or NX211-treated animals. This observation would be expected since lurtotecan is primarily eliminated in the liver via bilary excretion into the intestinal tract. Nearly 50 and 40% of the injected radioactivity from lurtotecan- and NX211-treated animals, respectively, was eliminated into the feces by 24 h. The amount of drug in the urine after 24 h was 17% of the administered dose for lurtotecan and 10% of the administered dose for NX211. Therefore, the primary route of excretion of lurtotecan was not significantly altered by the liposomal formulation.

Treatment with [^{3}H]NX211 demonstrated that the lipid component continued to distribute throughout the body of the animal over the course of the study. The lipid accumulated in tissues with greater lipid content, such as the brain. This accumulation of [^{3}H]phospholipid in the tissues was reflected by the very low levels of radioactivity detected in the urine and feces. The L-α-dipalmitoyl-[2-palmitoyl-9,10^{3}H(*N*)]-phosphatidylcholine would require further metabolism for excretion. These data suggested that the lipid component of the liposomes is retained and utilized by the body.

Conclusions

NX211, a liposomal formulation of lurtotecan, has a different distribution behavior compared to the non-encapsulated drug. The hypothesis guiding development of NX211 is that liposomal encapsulation will prolong the plasma half-life of lurtotecan in human subjects, resulting in prolonged tumor exposure, enhanced efficacy and an improved therapeutic index. Preclinical data indicate that: (i) liposomal encapsulation markedly increases the plasma residence time of lurtotecan in animals, (ii) NX211 is more potent than

the free lurtotecan *in vivo* and (iii) NX211 has a greater therapeutic index than lurtotecan or topotecan in tumor models. In agreement with these observations, liposomally encapsulated lurtotecan accumulates significantly better than free lurtotecan in xenografted tumors. These key biodistribution data, described in this paper, confirm the development hypothesis that liposomal encapsulation of lurtotecan greatly increases tumor exposure to drug compared to the free drug. NX211 may therefore provide options for an effective and more convenient dosing schedule with less severe toxicity as compared to the free drug and possibly to topotecan.

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