Effective Irinotecan (CPT-11)-containing Liposomes: Intraliposomal Conversion to the Active Metabolite SN-38

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Irinotecan hydrochloride (CPT-11) is a prodrug of SN-38, which is an active metabolite with anti-tumor activity and side toxicity. The activities of CPT-11 and SN-38 depend on the closed lactone ring form of SN-38. We have examined the tissue distributions of the closed and open forms of CPT-11 and SN-38 in Lewis lung carcinoma-bearing mice after the administration of liposomal CPT-11 (S-Lip) and polyethyleneglycol (PEG)-modified S-Lip (S-PEG). The plasma concentrations of closed CPT-11 and SN-38 were increased by liposomalization, and their blood circulation was prolonged by the PEG modification. The concentrations of closed CPT-11 and SN-38 in tumors were elevated by both the liposomalization and PEG modification. The closed/total ratio of SN-38 in the tumors of the S-PEG group was greater than that of the CPT-11 solution (Sol) group. Thus, SN-38 was thought to be generated in intact liposomes containing CPT-11. The bile concentration of closed SN-38, which is responsible for CPT-11-induced intestinal disorder, was decreased by liposomalization. In an *in vitro* experiment, the SN-38/CPT-11 ratio in the tumor cells of the S-Lip group was found to be higher than that of the Sol group, and the ratio of the closed form of SN-38 was increased by the liposomalization. Laser scanning confocal microscopy showed the generation of SN-38 in the liposomal membrane after the incubation of S-Lip with carboxylesterase. It is therefore considered that a part of CPT-11 is converted to SN-38 in the intact liposomes.

Key words: Antitumor activity — Irinotecan — SN-38 — Targeting — Conversion

Liposomes are used as models of cell membranes to examine drug permeability and as drug carriers for antitumor agents. Liposomes have also recently been found to be safe, non-viral vectors in gene targeting. Some antitumor agents encapsulated in liposomes have been used clinically and have been shown to be effective against Kaposi's sarcomas in patients with AIDS in the USA and Europe.[1] However, there is little information available on the efficacy of liposomal prodrugs.

Irinotecan hydrochloride (CPT-11) is a prodrug that can be converted to SN-38, an active metabolite with antitumor activity and side toxicity.[2] CPT-11 has strong activity against lung carcinomas clinically.[3] The activities of CPT-11 and SN-38 are known to depend on the closed lactone ring form of SN-38.[4] We previously reported that the use of liposomal CPT-11 improved the tissue distribution of CPT-11 and increased the antitumor activity of CPT-11 in Ehrlich ascites carcinoma-bearing mice.[5, 6] However, the effect of the liposomalization on the closed-open reaction of the lactone ring of CPT-11 or SN-38 is not clear. In this study, we examined the tissue distributions of the closed and open forms of CPT-11 and SN-38 in Lewis lung carcinoma-bearing mice after the administration of liposomal CPT-11. The conversion of CPT-11 to SN-38 in

the tumor is thought to result in an increase in antitumor activity and a decrease in the side toxicity of CPT-11; it is thus expected that SN-38 generation in tumors is necessary for effective antitumor activity of CPT-11. We therefore investigated SN-38 generation from CPT-11 encapsulated in liposomes *in vitro*.

MATERIALS AND METHODS

Drugs Irinotecan hydrochloride (100 mg/5 ml vial), used to prepare CPT-11 solution (Sol), was purchased from Daiichi Pharmaceutical Co., Tokyo. Distearoylphosphatidylcholine (DSPC) and dimyristoylphosphatidylglycerol (DMPG), used to prepare liposomes, were purchased from Nichiyu Liposome Co., Tokyo. CPT-11, used to prepare liposomes, was kindly provided by Yakult Honsha Co., Tokyo.   1-Monomethoxypolyethyleneglycol-2,3-dimyristoylglycerol (PEG-DMG) containing PEG with an average molecular weight of 2,000 was a gift from Nippon Oil & Fats Co., Tokyo. Carboxylesterase (EC 3.1.1.1) was obtained from Sigma Chemical Co. (St. Louis, MO).

Preparation of liposomes Liposome preparation was performed according to the method of Bangham *et al.*[7] Namely, DSPC/cholesterol/DMPG (100:100:60 μmol) and 10 mg of CPT-11 (15 μmol) were dissolved in a chloroform/methanol mixture (2:1, v/v). The chloroform and methanol were completely evaporated off under a stream

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of nitrogen gas. The resultant thin lipid film was evacuated in a desiccator, then hydrated with 8.0 ml of 9.0% sucrose in 10 mM lactate buffer (pH 4.0) in a water bath at 50–60°C for 10 min. The suspension was sonicated for 20 min above the phase transition temperature (Tc) after nitrogen gas bubbling. The liposome suspension was extruded through two stacked polycarbonate membrane filters with 0.2 μm pores, followed by extrusion five times through 0.1 μm pore filters above the Tc, to afford a homogeneously sized liposome suspension. PEG-modified S-Lip (S-PEG) and liposomal CPT-11 (S-Lip) were prepared by adding 2.0 ml of 9.0% sucrose in 10 mM lactate buffer (pH 4.0) with or without 5.0 mol% PEG-DMG, respectively, to 8.0 ml of the liposome suspension, followed by sonication of the mixture. The liposome suspension was dialyzed against 9.0% sucrose in 10 mM lactate buffer (pH 4.0) for 16 h. The trapping ratio of CPT-11 in all liposomes was above 90%.

Animal experiments Male CDF$_1$ mice (body weight, 20–25 g; 5 weeks old) were obtained from Japan SLC (Hamamatsu). Lewis lung carcinoma cells (5×10^5 cells/ animal) were subcutaneously transplanted onto the backs of the mice. In the distribution study, on the 21st day after transplantation, tumor-bearing mice were injected via a tail vein with Sol, S-Lip or S-PEG at a dose of 10 mg/kg as CPT-11. At 1, 2 and 8 h after injection, mice were killed by cervical dislocation, and blood was collected from the heart. The liver, gall bladder and tumor were immediately removed and washed. The CPT-11 and SN-38 concentrations in the plasma and each tissue were determined as follows. The tissues were homogenized in saline to obtain a 5.0% homogenate. One milliliter of tissue homogenate or 10% plasma was added to 0.5 ml of 0.1 M lactate buffer (pH 4.0) followed by mixing. This mixture was added to 3.0 ml of 1-butanol as an extracting solvent, and the whole was shaken in a vortex mixer then centrifuged at 1,200g for 15 min. After centrifugation, total CPT-11 and total SN-38 (i.e., open plus closed forms) in the upper layer were quantitated by fluorescence spectrophotometry (CPT-11, Ex: 374 nm, Em: 435 nm and SN-38, Ex: 380 nm, Em: 556 nm). The levels of closed CPT-11 and SN-38 were determined by the same procedure except for the addition of 0.1 M Hepes buffer (pH 7.5) in place of lactate buffer.[8] The level of each open form was obtained as total form minus closed form.

To study the effect of liposomalization on the efficacy of CPT-11 in Lewis lung carcinoma-bearing mice, male CDF$_1$ mice were divided into 4 groups, each consisting of 10 mice. Lewis lung carcinoma cells (5×10^5 cells/animal) were subcutaneously transplanted onto the backs of the mice. On the 15th, 18th and 21st days after inoculation, tumor-bearing mice were injected via a tail vein with Sol, S-Lip or S-PEG at a dose of 10 mg/kg/day×3 days. The numbers of live and dead animals were recorded daily.

Uptake of CPT-11 by tumor cells Ehrlich ascites carcinoma cells (1.0×10^6 cells/animal) were intraperitoneally transplanted into CDF$_1$ mice. The ascites fluid was collected on the 7th day after transplantation. The ascites carcinoma cells were washed twice and then resuspended (5.0×10^6 cells/ml) in Eagle's MEM medium containing 10% fetal bovine serum. Cell suspensions containing Sol, S-Lip or S-PEG (CPT-11 concentration : 30 nmol/ml (20 μg/ml)) were incubated at 37°C. After incubation, each cell suspension was cooled on ice and then centrifuged at 150g for 3 min. The cells were washed and then resuspended in ice-cold phosphate buffer (pH 7.8). The determination of CPT-11 was performed as described above.

Conversion of liposomal CPT-11 to SN-38 *in vitro* The reaction mixture contained Sol or S-Lip (CPT-11: 10 μg/ ml) and carboxylesterase (32.5 U/ml) in 0.1 M Tris-HCl buffer (pH 7.5). The generation of SN-38 was investigated in this reaction mixture at 37°C after 3 h incubation. In a preliminary experiment, we confirmed that the liposomes were not disrupted under these conditions. The CPT-11 and SN-38 closed-form and open-form concentrations were determined as described above. The level of SN-38 generation was calculated using a reaction mixture without the enzyme as a control. The location of SN-38 in the liposomes was examined by observation of these liposomes by laser scanning confocal microscopy (ACAS Ultima 575 UVC; Meridian Instruments Far East, Tokyo). For this microscopic observation, liposomes were prepared according to the above method without extrusion, so that the size of the liposomes was about 1–5 μm, which could be easily observed microscopically.

Statistical analysis Statistical analysis was carried out by using analysis of variance (ANOVA), Student's t test or Wilcoxon's test.

RESULTS

Tissue distribution of liposomal CPT-11
Plasma (Fig. 1): The concentrations of closed CPT-11 and closed SN-38 in plasma were increased by liposomalization, and PEG modification prolonged the circulation time in the blood. In particular, at 8 h after administration, SN-38 was not detected in the plasma of the Sol group mice, whereas in the S-PEG group, SN-38 was still detectable in the blood (closed SN-38 concentration; 0.34 μg/ml plasma).
Liver and bile: At 2 h after injection, the concentration of closed CPT-11 in the liver of the S-Lip group was 3.5-fold ($P<0.001$) higher than that of the Sol group. The concentration in the S-PEG group was similar to that of the Sol group. The changes in the concentration of closed SN-38 in the liver showed the same tendency as that of CPT-11. At 2 h after administration, the closed/total ratios of the SN-38 concentration in the liver in the Sol, S-Lip
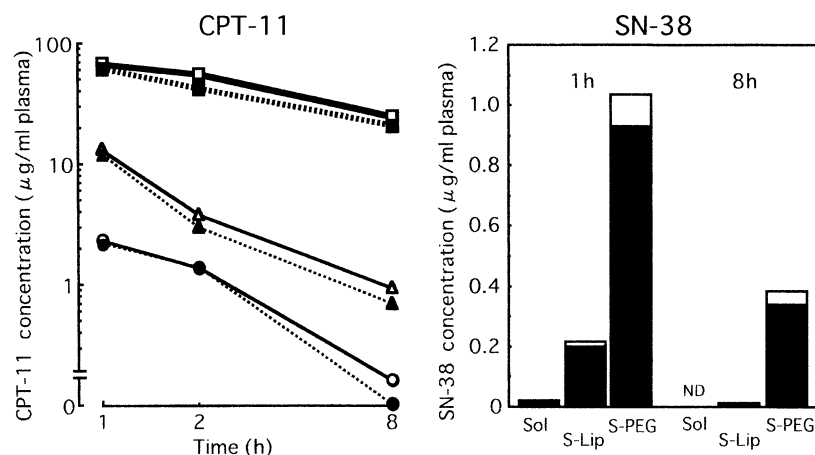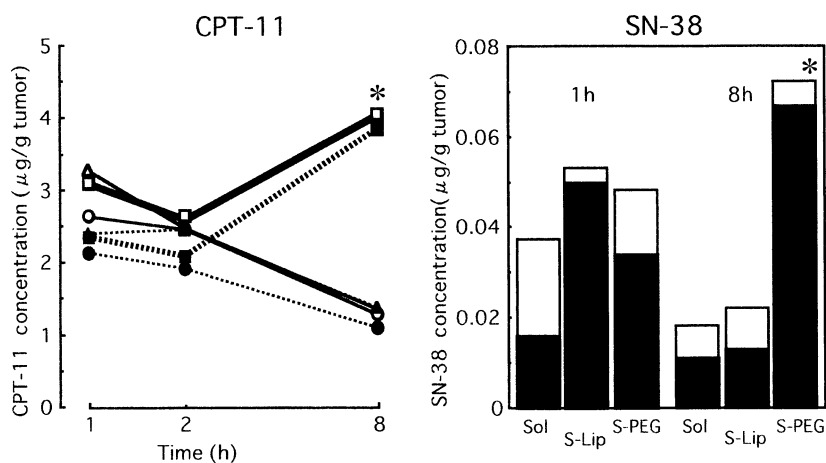
Fig. 1. CPT-11 and SN-38 concentrations in the plasma. Mice were injected with 10 mg/kg (i.v.) of CPT-11 in the form of Sol, S-Lip or S-PEG. The data represent the means for three mice, each with duplicate data with no more than 10% variation between them. Open and closed columns indicate open and closed SN-38, respectively. Total level is open level plus closed level. Each point and each column for the S-Lip and S-PEG groups showed significant differences ($P<0.001$) compared to the corresponding data for the Sol group. Total: ○ Sol, △ S-Lip, □ S-PEG; closed: ● Sol, ▲ S-Lip, ■ S-PEG.

Fig. 2. CPT-11 and SN-38 concentrations in the tumor. Mice were injected with 10 mg/kg (i.v.) of CPT-11 in the form of Sol, S-Lip or S-PEG. The data represent the means for three mice, each with duplicate data with no more than 10% variation between them. Open and closed columns indicate open and closed SN-38, respectively. Total level is open level plus closed level. A significant difference from the Sol level is indicated by ∗ $P<0.001$. Total: ○ Sol, △ S-Lip, □ S-PEG; closed: ● Sol, ▲ S-Lip, ■ S-PEG.

and S-PEG groups were 0.201, 0.674 and 0.451, respectively. Therefore, this ratio was elevated by the liposomalization (figure not shown).

In the bile, the CPT-11 and SN-38 concentrations were decreased by the liposomalization. The concentrations of closed SN-38 in the bile of the S-Lip and S-PEG groups were 60% and 52% ($P<0.01$) of that of the Sol group, respectively (figure not shown).

Tumor (Fig. 2): At 8 h after administration, the concentration of closed CPT-11 in the tumors of the S-PEG group was increased 3.5-fold ($P<0.001$) compared to that

of the Sol group. At the same time, the SN-38 concentration in the tumor was increased by the liposomalization and further increased by the PEG modification. In particular, the closed SN-38 concentration in the tumor at 8 h after S-PEG administration was 6.1-fold ($P<0.001$) higher than that in the Sol group. The closed/total ratio in the Sol group was 0.611, whereas that in the S-PEG group was 0.931.

Effect of liposomalization on the CPT-11-induced prolongation of survival in mice with Lewis lung carcinoma (Fig. 3) In mice inoculated with Lewis lung
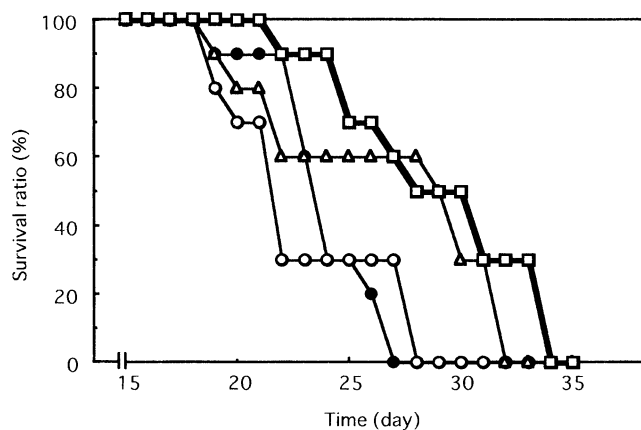
Fig. 3. Survival of tumor-bearing mice after administration of Sol, S-Lip or S-PEG. Lewis lung carcinoma cells (5×10^5 cells/animal) were subcutaneously transplanted onto the backs of the mice. On the 15th, 18th and 21st days after inoculation, tumor-bearing mice were injected via a tail vein with Sol, S-Lip or S-PEG at a dose of 10 mg/kg/day×3 days. Each group consisted of 10 mice. ● control, ○ Sol, △ S-Lip, □ S-PEG.

Fig. 4. Time course of uptake of CPT-11 by Ehrlich ascites carcinoma cells. The cell suspensions (5×10^6 cells/ml) in Eagle's MEM medium containing Sol, S-Lip or S-PEG (CPT-11 concentration: 20 μg/ml) were incubated at 37°C. After incubation, cells were collected and their CPT-11 level was determined. The data represent the means±SD for three experiments. ○ Sol, △ S-Lip, □ S-PEG.

carcinoma, the median number of survival days in the control group was 23.0 days. Sol administration had no effect on the survival period. In contrast, survival in the S-Lip and S-PEG groups was increased to 28.5 and 30.0 days ($P<0.01$ compared to the levels in the control and Sol groups), respectively. No significant difference between the S-Lip and S-PEG groups was observed.

Effect of liposomalization on the uptake of CPT-11 by tumor cells Regarding the uptake of Sol, S-Lip and S-PEG by Ehrlich ascites carcinoma cells, the CPT-11 concentrations in the tumor cells at 20 min after the addition of S-Lip and S-PEG were about half that in the Sol group (Fig. 4). As shown in Fig. 5A, with CPT-11 at 40 μg/ml, the CPT-11 concentration in the cells of the S-Lip group was half that of the Sol group, whereas in the case of CPT-11 at 5 μg/ml, the concentration in the S-Lip group was not significantly different from that in the Sol group. Thus, the cell uptake of CPT-11 in the Sol group increased with increase of CPT-11 concentration, whereas the elevation of the cell uptake in the S-Lip group showed a smaller concentraion dependence. However, the ratio of CPT-11 converted to SN-38 in the tumor cells was higher in the S-Lip than in the Sol group (Fig. 5B).

Conversion of liposomal CPT-11 to SN-38 *in vitro*
Incubation of S-Lip with carboxylesterase resulted in the generation of SN-38 without disruption of the liposomes (Fig. 6A). The closed/total ratio of CPT-11 or SN-38 was higher in the S-Lip than in the Sol group. In the S-Lip group, these ratios of CPT-11 and SN-38 were 100% and 89%, respectively (Fig. 6B). The results of observation of
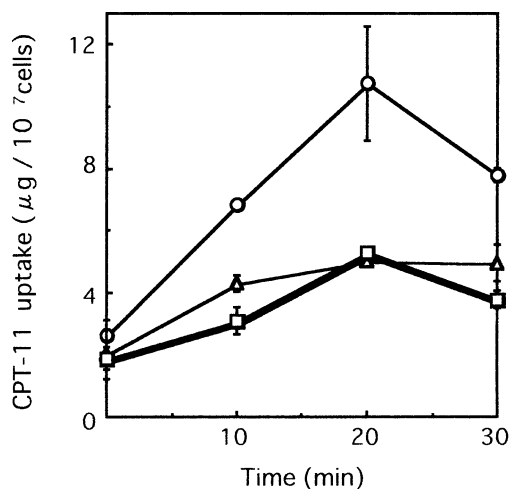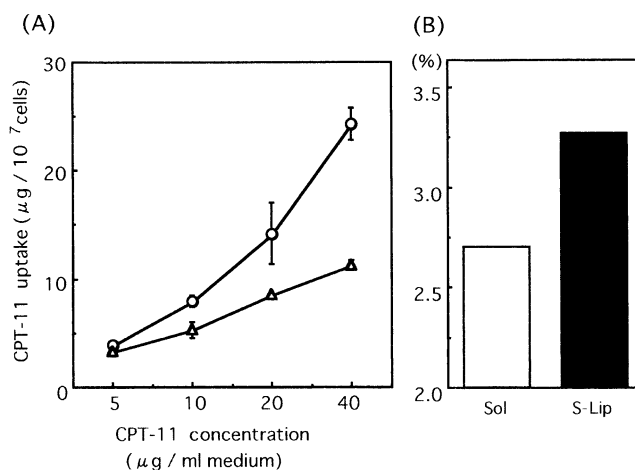

Fig. 5. Uptake of CPT-11 by Ehrlich ascites carcinoma cells. (A) Cell suspensions (5×10^6 cells/ml) in Eagle's MEM medium were exposed to Sol or S-Lip containing various CPT-11 concentrations for 20 min at 37°C. After the incubation, cells were collected and the CPT-11 level was determined. The data represent the means±SD for three experiments. ○ Sol, △ S-Lip. (B) SN-38/CPT-11 ratio with 40 μg/ml of CPT-11.

S-Lip by laser scanning confocal microscopy after incubation of S-Lip with carboxylesterase are shown in Fig. 7. In the photographs, the red coloration in the liposome indicates CPT-11, and the green indicates SN-38. Only the red color was observed in the liposomes incubated in
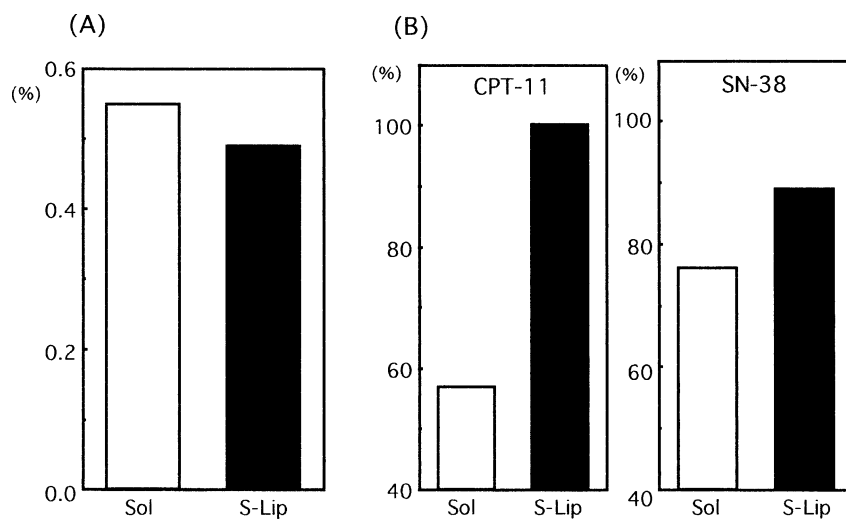
Fig. 6. The conversion of CPT-11 to SN-38 in liposomes. The reaction mixture contained Sol or S-Lip (CPT-11: 10 μg/ml) and carboxylesterase (32.5 U/ml) in 0.1 M Tris-HCl buffer (pH 7.5). The generation of SN-38 in this reaction mixture at 37°C was investigated after 3 h incubation. The CPT-11 and SN-38 closed-form and open-form concentrations were determined. (A) SN-38/CPT-11, (B) closed/total.
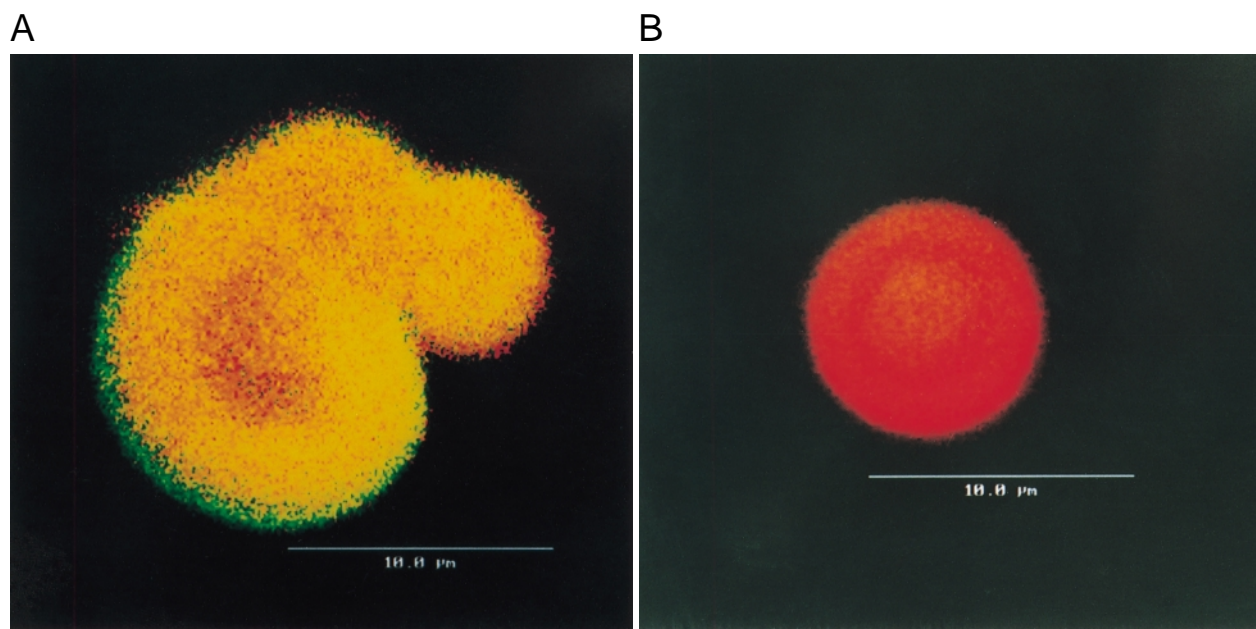

Fig. 7. Laser scanning confocal microscopic images of liposomal CPT-11 incubated with (A) or without (B) carboxylesterase. For this microscopic observation, liposomes were prepared as described in the text but without extrusion, so that their size was about 1–5 μm, which could be easily observed on microscopy.

buffer without carboxylesterase. Therefore, SN-38 did not exist in them. However, the liposomes incubated with carboxylesterase had green coloration on their surface. SN-38 is thus thought to have been generated from CPT-11 in the liposomal membrane in the latter case.

DISCUSSION

The lactone ring of CPT-11 is nonenzymatically hydrolyzed under neutral and basic conditions, and an equilibrium is reached between the open ring (carboxyl acid

form) and closed ring (lactone form) forms. The closed form of the lactone ring is essential for antitumor activity. In addition, the rate and equilibrium conditions of the open-closed reaction are changed on the binding of CPT-11 and SN-38 with serum albumin.[9–12] The closed lactone ring form of administered CPT-11 is cleaved gradually in the body. However, through encapsulation of CPT-11 in liposomes containing acidic water (pH 4.0), it was expected that the more stable lactone form of CPT-11 could be targeted to tumors. The concentrations of closed CPT-11 and SN-38 in the plasma of the Lewis lung carcinoma-bearing mice in the present study were increased by the liposomalization, and prolongation of circulation in the blood was produced by the PEG modification. In addition, the concentrations of closed CPT-11 and SN-38 in the tumors were elevated by both types of liposomes. Burke et al. reported that the lactone ring of camptothecin exists stably in the liposomal membrane.[13] Similarly, if the lactone ring of CPT-11 enters the liposomal membrane, the closed lactone ring could be stabilized. Since the closed/total ratio of SN-38 in the tumors of the present S-PEG group was larger than that of the Sol group, it is suspected that SN-38 was generated in the intact liposomes containing CPT-11. This effect, in conjunction with the targeting of loposomes to tumors, may enhance the antitumor activity while decreasing the intestinal side effect due to the closed form of SN-38. In fact, the closed SN-38 concentration in bile was decreased by the liposomalization of CPT-11 in the present study.

In the experiment involving Lewis lung carcinoma-bearing mice, the survival period of the Sol group was not prolonged, whereas those of the S-Lip and S-PEG groups were prolonged at the same dose. However, there was no significant difference in survival between the S-Lip and S-PEG groups. This experimental dose is chemotherapeutic and not toxic; namely, normal mice did not die or show decreased body weight, irrespective of the formulation. Therefore, the deaths of the mice were induced by the Lewis lung carcinomas, not by CPT-11-induced toxicity.

If CPT-11 is released from disrupted liposomes and then converted to SN-38, the closed/total ratio of SN-38 in the S-PEG group should be the same as that in the Sol group. However, increases in the closed SN-38 concentration and closed/total ratio of SN-38 in the tumors occurred upon liposomalization of CPT-11. This supports the idea that SN-38 is generated in intact liposomes.

The cellular uptake of CPT-11 in the Sol group increased depending on the CPT-11 concentration, whereas the concentration dependence was less in the S-Lip group. It is known that the amount of administered lipid affects the tissue distribution of liposomes[14, 15] and that liposome uptake by the liver is saturable.[16–18] With the use of 30–40 μg/ml of CPT-11 (this level is expected to be a lipid uptake saturation level), the lipid concentration was 78–104 mM, whereas with 5 μg/ml of CPT-11 (this level is below the uptake saturation level, and there was no difference in cellular uptake between the Sol and S-Lip groups), the lipid concentration was 13 mM. The CPT-11 concentration in the tumors *in vivo* was below 5 μg/g tumor, and the lipid concentration was below 13 mM. Namely, the lipid level did not reach the saturation level for cellular uptake *in vitro*, and there was no difference in the cellular uptake between the Sol and S-Lip groups. The CPT-11 concentration in the tumors of the S-Lip group was higher than that of the Sol group *in vivo*. Therefore, liposomal CPT-11 is more effective than a CPT-11 solution. In contrast, the CPT-11 concentration in the liver *in vivo* was about 30 μg/g liver and the lipid concentration was 78 mM, i.e., the uptake saturation level. Under these conditions, the liposomal uptake by the liver may be suppressed. In addition, the generation ratio of SN-38/CPT-11 in the tumor cells of the S-Lip group was higher than that of the Sol group. Thus, liposomalization resulted in significantly enhanced accumulation of SN-38 in the tumor relative to the liver.

CPT-11 is constituted from a hydrophilic piperidino-piperidinocarboxyl moiety and lipophilic SN-38, and is converted to SN-38 by carboxylesterase in the body, particularly in the liver.[19] We have confirmed that the concentration of closed SN-38 increases with the liposomalization of CPT-11. *In vitro*, under the absence of cells and without disruption of the liposomes, SN-38 was generated after the incubation of S-Lip with carboxylesterase despite the absence of CPT-11 in the medium (extra-liposomal fraction). As CPT-11 adsorbed on the surface of the liposomes was removed by NaCl, we suspect that SN-38
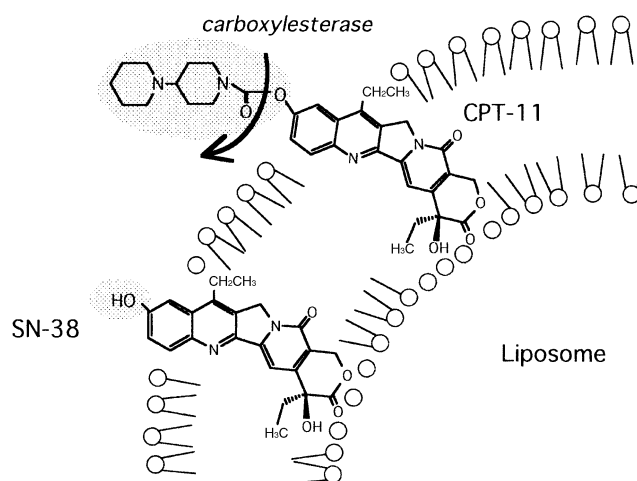

Fig. 8. A model of the conversion of CPT-11 to SN-38 in liposomes.

was generated from CPT-11 present in the liposomal membranes which were in contact with carboxylesterase in the medium. Furthermore, in the laser scanning confocal microscopy study, generation of SN-38 in the liposomal membranes was observed after the incubation of S-Lip with carboxylesterase. The structural change of the surface of liposomes following elimination of the piperidinopiperidinocarboxyl moiety is thought to result in a change in the ζ potentials of S-Lip during the incubation with carboxylesterase (data not shown). Based on these results, as shown in Fig. 8, the closed liposomal CPT-11 is speculated to enter, at least partly, the double lipid membranes of the liposomes in such a manner that the piperidinopiperidinocarboxyl moiety projects into the extra-liposomal fraction (medium). When these liposomes are distributed in some tissues, we suspect that CPT-11 in the membranes of the liposomes is exposed to carboxylesterase activity and generates closed SN-38 in the intact liposomes. Since the SN-38 thus generated exists in the membranes of liposomes, it is not affected by the physiological pH in the body. This closed SN-38 behaves like the liposomes, i.e., is preferentially distributed in the tumor. In mice and rats, carboxylesterase activity in the serum is high and the SN-38/CPT-11 ratio is also high compared with that in humans.[19] However, SN-38 generation by the carboxylesterase in the tumor of mice is similar to that of humans.[19] Therefore, the results in this paper have clinical relevance.

In conclusion, the liposomalization of CPT-11 increased the closed/total ratios of CPT-11 and SN-38 (i.e., favored the generation of closed SN-38) and elevated the antitumor activity of CPT-11 against Lewis lung carcinomas. In liposomal CPT-11, it is considered that a part of the prodrug CPT-11 is converted to SN-38 within the intact liposomes.

REFERENCES

1) Coukell, A. J. and Spencer, C. M. Polyethylene glycol-liposomal doxorubicin. *Drugs*, **53**, 520–538 (1997).

2) Kawato, Y., Aonuma, M., Hirota, Y., Kuga, H. and Sato, K. Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Res.*, **51**, 4187–4191 (1991).

3) Negoro, S., Fukuoka, M., Niitani, H., Suzuki, A., Nakabayashi, T., Kimura, M., Motomiya, M., Kurita, Y., Hasegawa, K. and Taguchi, T. A phase II study of CPT-11, a camptothecin derivative, in patients with primary lung cancer. *Jpn. J. Cancer Chemother.*, **18**, 1013–1019 (1991).

4) Potmesil, M. Camptothecins: from bench research to hospital wards. *Cancer Res.*, **54**, 1431–1439 (1994).

5) Sadzuka, Y., Hirotsu, S., Miyagishima, A., Nozawa, Y. and Hirota, S. The study of polyethyleneglycol-coated liposomes containing CPT-11. *J. Liposome Res.*, **7**, 241–260 (1997).

6) Sadzuka, Y., Hirotsu, S. and Hirota, S. Effect of liposomalization on the antitumor activity, side effect and tissue distribution of CPT-11. *Cancer Lett.*, **127**, 99–106 (1998).

7) Bangham, A. D., Standish, M. M. and Watkins, J. C. Diffusion of univalent ion across the lamellae of swollen phospholipids. *J. Mol. Biol.*, **13**, 238–252 (1965).

8) Sasaki, Y., Yoshida, Y., Sudoh, K., Hakusui, H., Fujii, H., Ohtsu, T., Wakita, H., Igarashi, T. and Itoh, K. Pharmacological correlation between total drug concentration and lactones of CPT-11 and SN-38 in patients treated with CPT-11. *Jpn. J. Cancer Res.*, **86**, 111–116 (1995).

9) Burke, T. G., Munshi, C. B., Mi, Z. and Jiang, Y. The important role of albumin in determining the relative human blood stabilities of the camptothecin anticancer drugs. *J. Pharm. Sci.*, **84**, 518–519 (1995).

10) Jaxel, C., Kohn, K. W., Wani, M. C., Wall, M. E. and Pommier, Y. Structure-activity study of the actions of camptothecin derivatives on mammalian topoisomerase I. *Cancer Res.*, **49**, 1465–1469 (1989).

11) Kurono, Y., Miyajima, M. and Ikeda, K. Interaction of camptothecin derivatives with human plasma proteins. *J. Pharm. Soc. Jpn.*, **113**, 167–175 (1993) (in Japanese).

12) Mi, Z., Malak, H. and Burke, T. G. Reduced albumin binding promotes the stability and activity of topotecan in human blood. *Biochemistry*, **34**, 13722–13728 (1995).

13) Burke, T. G., Staubus, A. E., Mishra, A. K. and Malak, H. Liposomal stabilization of camptothecin's lactone ring. *J. Am. Chem. Soc.*, **114**, 8318–8319 (1992).

14) Bosworth, M. E. and Hunt, C. A. Liposome disposition *in vivo* II. *J. Pharm. Sci.*, **71**, 100–104 (1982).

15) Sadzuka, Y. and Hirota, S. Does the amount of an antitumor agent entrapped in liposomes influence its tissue distribution and cell uptake? *Cancer Lett.*, **131**, 163–170 (1998).

16) Chow, D. D., Essien, H. E., Padki, M. M. and Hwang, K. J. Targeting small unilamellar liposomes to hepatic parenchymal cells by dose effect. *J. Pharmacol. Exp. Ther.*, **248**, 506–513 (1989).

17) Kume, Y., Maeda, F., Harashima, H. and Kiwada, H. Saturable, non-Michaelis-Menten type uptake of liposomes by the reticuloendothelial system. *J. Pharm. Pharmacol.*, **43**, 162–166 (1991).

18) Harashima, H., Kume, Y., Yamae, C. and Kiwada, H. Non-Michaelis-Menten type hepatic uptake of liposomes in the rat. *J. Pharm. Pharmacol.*, **44**, 707–712 (1992).

19) Kawato, Y., Aonuma, M., Matsumoto, K. and Sato, K. Production of SN-38, a main metabolite of the camptothecin derivative CPT-11, and its species and tissue specificities. *Xenobio. Met. Disp.*, **6**, 899–907 (1991).