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Liposomal Sustained-Release Delivery Systems for Intravenous Injection. V.¹⁾ Biological Disposition of Liposome-Entrapped Lipophilic Prodrug of 1- β -D-Arabinofuranosylcytosine

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A lipophilic prodrug of 1- β -D-arabinofuranosylcytosine (Ara-C), N^4 -[N -(cholesteryloxy-carbonyl)glycyl]-Ara-C (COCG-Ara-C) was entrapped in unilamellar liposomes or solubilized in pH 7.4 phosphate-buffered saline with hydrogenated castor oil polyethylene glycol ether (HCO-60), and its biological disposition was studied in mice. Regardless of dosage form, COCG-Ara-C was accumulated in the liver after intravenous (i.v.) injection, but not in the lung or kidney. In contrast, the blood clearance and spleen distribution of COCG-Ara-C varied with dosage form: slow blood clearance and increased spleen levels of the prodrug were observed for the liposome-entrapped form relative to the detergent-solubilized form. Both COCG-Ara-C preparations successfully maintained the plasma levels of Ara-C compared with Ara-C aqueous solution. However, the plasma Ara-C concentration-time profiles were markedly different between the preparations. After i.v. injection of liposome-entrapped COCG-Ara-C, plasma Ara-C levels increased slowly for the first 16 h and subsequently decreased gradually. On the other hand, rapid appearance of Ara-C in the circulation and subsequently gradual elimination were observed after injection of the detergent-solubilized prodrug. The relative bioavailabilities obtained with respect to Ara-C aqueous solution were 592% and 129% for the liposome-entrapped and detergent-solubilized forms, respectively. The extremely high bioavailability of liposome-entrapped COCG-Ara-C could be explained in part by the finding that the liposome-entrapped prodrug reduced the plasma clearance of the parent drug Ara-C. These results indicate the potential utility of COCG-Ara-C-bearing liposomes as a sustained-release delivery system of Ara-C applied by i.v. injection.

Keywords—1- β -D-arabinofuranosylcytosine; lipophilic prodrug; liposome; dosage form; intravenous injection; sustained-release; disposition

Liposomes have been used as biological carriers for a variety of drugs, including anticancer agents.²⁾ Several investigators have demonstrated that encapsulation of 1- β -D-arabinofuranosylcytosine (Ara-C) in liposomes enhances its antitumor effects against L 1210 leukemia and some solid tumors.³⁾ Liposomes may play an important role in the prevention of premature enzymatic degradation of Ara-C to 1- β -D-arabinofuranosyluracil (Ara-U),⁴⁾ and could act as a depot to release active Ara-C over a prolonged time. Thus, liposome encapsulation of Ara-C might be utilized to replace a clinical long-term infusion schedule for Ara-C.

However, practical use of liposome-encapsulated Ara-C is severely limited due to some disadvantages: *i.e.*, low liposome entrapment of Ara-C, the troublesome process of separation of encapsulated Ara-C from unencapsulated (free) drug, leakage of Ara-C from liposome carriers during storage, *etc.*^{3c,5)} To circumvent these difficulties and to design a more practical liposomal delivery system of Ara-C, we synthesized a lipophilic derivative of Ara-C, N^4 -[N -(cholesteryloxy-carbonyl)glycyl]-Ara-C (COCG-Ara-C), shown in Fig. 1, by coupling Ara-C

Fig. 1. Structure of COCG-Ara-C

to cholesterol through *N*-oxycarbonylglycine as a spacer.¹⁾ COCG-Ara-C was almost completely incorporated into liposomes. *In vitro* stability experiments revealed that the derivative is converted to Ara-C by chemical and enzymatic hydrolysis.¹⁾ Liposome-entrapped COCG-Ara-C showed a chemotherapeutic effect superior to that of Ara-C aqueous solution when used against mouse L 1210 leukemia and human lung adenocarcinoma A 549. These findings suggested the possibility that liposome-entrapped COCG-Ara-C might act as a slow-release reservoir *in vivo* to release active Ara-C over a prolonged period of time.

In the present study, the biological disposition of liposome-entrapped COCG-Ara-C was investigated to assess the utility of prodrug-bearing liposomes as an intravenously injectable liposomal sustained-release delivery system of Ara-C, and its disposition characteristics were compared with those of detergent-solubilized COCG-Ara-C, which exhibited a less potent antitumor effect than the liposome-entrapped derivative.¹⁾

Experimental

Chemicals—Ara-C and Ara-U were purchased from Yamasa Shoyu Co. COCG-Ara-C was synthesized according to the method previously described.¹⁾ Egg phosphatidylcholine (PC) and egg sphingomyelin (SM) were obtained from Sigma Chemical Co. Hydrogenated castor oil polyethylene glycol ether (HCO-60) was from Nikko Chemicals Co. All other chemicals were of reagent grade or better.

COCG-Ara-C Preparations—COCG-Ara-C-bearing unilamellar liposomes composed of PC, SM and COCG-Ara-C in a molar ratio of 7:3:2 were prepared as described previously.¹⁾ Their final phospholipid concentration was approximately 50 mM. Detergent-solubilized COCG-Ara-C was prepared as follows. COCG-Ara-C (125 μ mol) and HCO-60 (2.5 g) were dissolved in chloroform-methanol (2:1, v/v) and the organic solvents were evaporated off. The dried film was taken up in 10 ml of pH 7.4 phosphate-buffered saline (PBS) and the mixture was agitated by hand, yielding a clear solution.

All preparations were sterilized by filtration through a Millex GV (0.22 μ m, Millipore). The concentration of COCG-Ara-C was determined by high performance liquid chromatography (HPLC). The preparations were diluted with sterile PBS to obtain the desired concentration (10 mM) and kept under nitrogen at 4°C until use.

Determination of Vesicle Size—Liposome size was determined by using a laser light scattering instrument (Coulter, model N4).

Animal Experiments—Female ICR mice weighing 22–24 g were used. For evaluating the disposition of COCG-Ara-C administered in either liposome-entrapped or detergent-solubilized (free) form, the animals were injected with 0.2 ml of preparations at a dose of 2 μ mol drug/mouse into the tail vein. At various times after injection, mice were anesthetized with ether and blood was obtained by cardiac puncture. Subsequently, organs such as the liver, spleen, kidney and lung were excised. For determination of COCG-Ara-C, the organs and an aliquot (0.2 ml) of the blood were immediately frozen in liquid nitrogen to avoid postcollection degradation of the prodrug, and lyophilized. A residual aliquot of the blood was centrifuged to obtain plasma for assay of Ara-C and Ara-U. To avoid postcollection generation of Ara-C and Ara-U, an aliquot (0.1 ml) of the plasma was immediately frozen in liquid nitrogen and stored in a freezer until assay.

For evaluating the effect of COCG-Ara-C preparations on the blood disposition of Ara-C, mice were intravenously injected with 0.2 ml of COCG-Ara-C preparations containing Ara-C at doses of 2 μ mol/mouse for the prodrug and 2 μ mol/mouse for Ara-C. At various times after injection, blood was obtained as described above. The plasma samples after centrifugation were subjected to analysis for Ara-C and Ara-U.

Extraction Procedure—The freeze-dried biological samples were homogenized in 2 or 5 ml of ethanol with a Polytron homogenizer (Kinematica, GmbH). The supernatant after centrifugation was supplied for assay of COCG-Ara-C.

For simultaneous analysis of Ara-C and Ara-U, the frozen plasma samples (0.1 ml) were deproteinized by addition of cold acetonitrile (0.7 ml) at 0 °C soon after thawing in 0.4 ml of cold distilled water. After centrifugation, the supernatant was applied to a Sep-pak C₁₈ cartridge (Waters) to remove the prodrug. After applying 2 ml of cold acetonitrile-distilled water (1:1, v/v), the eluent was evaporated. The residue was dissolved in distilled water (0.2 ml) for analysis of Ara-C and Ara-U.

HPLC Assay—COCG-Ara-C, Ara-C and Ara-U were determined by HPLC as described previously.¹⁾

Results

Liposome Size

The mean diameters of the liposomes used in this study are summarized in Table I. All liposome preparations were almost equal in size and showed a fairly narrow size distribution.

Tissue Distribution of COCG-Ara-C

The tissue levels of COCG-Ara-C after i.v. injection of the prodrug in the liposome-entrapped and detergent-solubilized forms are summarized in Tables II and III, respectively. COCG-Ara-C was predominantly accumulated in the liver up to 16 h after injection and no significant difference was observed between the two preparations. Thereafter, COCG-Ara-C levels in the liver continuously increased for the liposome-entrapped form, while a gradual decrease of liver distribution was observed for the detergent-solubilized form. The amounts of COCG-Ara-C associated with the liver at 48 h after injection were 46.6% for the liposome-entrapped prodrug and 20.0% for the detergent-solubilized prodrug. COCG-Ara-C was not accumulated in the kidney or lung. The major difference between the two preparations was observed in the spleen. Maximum levels of the prodrug in the spleen were 8.5% for the

TABLE I. Size of Liposomes

Preparation ^{a)}	COCG-Ara-C conc. (mM)	Diameter ^{b)} (nm)
A	10	124 ± 23
B	0	114 ± 22
C	10	118 ± 23

^{a)} Preparation A was used in the biological disposition study of liposome-entrapped COCG-Ara-C. Preparations B and C were used in simultaneous administration experiments. Phospholipid concentration of all preparations was approximately 50 mM. ^{b)} Liposome size was determined by dynamic laser light scattering and is expressed as the mean ± S.D.

TABLE II. Tissue Distribution of COCG-Ara-C in Mice after i.v. Injection in Liposome-Entrapped Form

Tissue ^{a)}	1 h	4 h	8 h	16 h	24 h	48 h
Blood	42.7 ± 1.5	32.3 ± 3.5	25.2 ± 1.5	11.1 ± 1.5	6.8 ± 1.8	1.7 ± 0.8
Liver	7.4 ± 0.5	16.4 ± 2.2	23.9 ± 6.2	26.7 ± 2.5	29.7 ± 3.7	32.0 ± 6.5
	9.5 ± 1.7	21.1 ± 3.4	27.2 ± 7.1	39.6 ± 3.6	44.9 ± 4.4	46.6 ± 5.5
Spleen	59.2 ± 11.0	81.5 ± 17.6	89.5 ± 22.8	60.8 ± 10.9	60.2 ± 17.8	47.0 ± 15.7
	5.4 ± 0.8	7.3 ± 1.1	8.5 ± 2.6	5.3 ± 1.2	5.9 ± 2.3	6.3 ± 0.9
Lung	7.4 ± 0.5	7.3 ± 0.8	7.1 ± 0.8	5.0 ± 0.6	5.3 ± 0.8	4.6 ± 1.6
	1.2 ± 0.01	1.2 ± 0.2	1.1 ± 0.1	0.8 ± 0.2	0.9 ± 0.2	0.6 ± 0.1
Kidney	5.2 ± 0.5	5.1 ± 0.9	4.2 ± 0.4	3.9 ± 0.7	3.9 ± 1.1	2.9 ± 0.3
	1.6 ± 0.2	1.6 ± 0.4	1.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.3	1.0 ± 0.2

^{a)} Blood level is expressed as % of dose/ml. The first value listed for each tissue is percent of dose/g tissue. The second is percent of dose/total tissue. Each value is the mean ± S.D. of four mice.

TABLE III. Tissue Distribution of COCG-Ara-C in Mice after i.v. Injection in Detergent-Solubilized Form

Tissue ^{a)}	1 h	4 h	8 h	16 h	24 h	48 h
Blood	29.4 ± 1.5	18.8 ± 2.3	13.7 ± 2.6	5.3 ± 0.1	3.5 ± 0.2	1.8 ± 0.2
Liver	6.6 ± 1.8	16.7 ± 2.1	23.2 ± 0.9	24.1 ± 3.6	23.6 ± 1.6	14.1 ± 4.6
	8.2 ± 0.1	20.4 ± 2.2	27.4 ± 0.9	26.5 ± 1.4	35.0 ± 3.2	20.0 ± 5.9
Spleen	8.4 ± 0.7	14.3 ± 1.3	18.2 ± 1.4	14.6 ± 1.5	12.3 ± 0.9	7.7 ± 0.9
	0.8 ± 0.1	1.6 ± 0.3	1.6 ± 0.2	1.5 ± 0.2	1.1 ± 0.1	0.7 ± 0.1
Lung	6.8 ± 0.5	6.9 ± 0.4	7.5 ± 0.6	8.0 ± 0.6	6.6 ± 0.9	3.0 ± 0.7
	1.1 ± 0.1	1.2 ± 0.2	1.2 ± 0.3	1.2 ± 0.1	1.0 ± 0.2	0.5 ± 0.1
Kidney	5.6 ± 1.3	6.0 ± 1.0	6.3 ± 0.7	6.3 ± 0.5	5.0 ± 0.5	3.7 ± 0.4
	1.9 ± 0.4	1.9 ± 0.3	2.0 ± 0.2	2.2 ± 0.3	1.7 ± 0.2	1.2 ± 0.2

a) Data were calculated as in Table II. Each value is the mean ± S.D. of four mice.

Fig. 2. Blood Levels of COCG-Ara-C in Mice after i.v. Injection in Two Different Dosage Forms

Mice were injected with COCG-Ara-C (2 μmol/mouse) in liposome-entrapped (●) or detergent-solubilized (○) form. Each point is the mean ± S.D. of four mice.

Fig. 3. Plasma Levels of Ara-C in Mice after i.v. Injection of COCG-Ara-C Preparations and Ara-C Aqueous Solution

Mice were injected with liposome-entrapped COCG-Ara-C (●), detergent-solubilized COCG-Ara-C (○) or Ara-C aqueous solution (▲) at a dose of 2 μmol/mouse. Plasma Ara-C levels until 15 min after injection of Ara-C aqueous solution are not shown because of scaling problems. Refer to Fig. 4. Each point is the mean ± S.D. of four mice.

liposome-entrapped form and 1.6% for the detergent-solubilized form.

Blood Levels of COCG-Ara-C and Ara-C

Figure 2 depicts the blood levels of COCG-Ara-C after i.v. injection of the prodrug in the liposome-entrapped and detergent-solubilized forms. The liposome-entrapped form showed significantly higher blood levels of COCG-Ara-C than the detergent-solubilized form.

The plasma concentration-time profiles of regenerated Ara-C after i.v. injection of COCG-Ara-C preparations are shown in Fig. 3, together with the plasma Ara-C levels found after i.v. injection of an equimolar amount of Ara-C in an aqueous solution. Ara-C was rapidly cleared from the circulation with a half-life of 20 min in the terminal phase and an inactive metabolite, Ara-U, appeared in the plasma (data not shown) after injection of Ara-C

TABLE IV. *AUCs* and Relative Bioavailabilities of Ara-C after i.v. Injection of COCG-Ara-C Preparations

Preparation	<i>AUC</i> ₀ ^{48 a)} (nmol · h/ml)	Bioavailability ^{b)} (%)
Ara-C aqueous solution	32.8	100
Liposome-entrapped COCG-Ara-C	194.0	592
Detergent-solubilized COCG-Ara-C	43.2	129

a) *AUC* up to 48 h post administration was calculated by the trapezoidal method. b) Relative bioavailability = (*AUC*_{COCG-Ara-C preparation} / *AUC*_{Ara-C aqueous solution}) × 100.

Fig. 4. Effect of Simultaneous Administration of COCG-Ara-C Preparations on the Plasma Clearance of Ara-C

Mice were injected with Ara-C at a dose of 2 μmol/mouse and simultaneous treatment with COCG-Ara-C preparations (2 μmol/mouse) or plain liposomes was carried out. Δ, no treatment; ○, treatment with plain liposomes; ●, treatment with liposome-entrapped COCG-Ara-C; ▲, treatment with detergent-solubilized COCG-Ara-C. Each result is the mean ± S.D. of at least three mice.

Fig. 5. Effect of Simultaneous Administration of COCG-Ara-C Preparations on the Plasma Levels of Ara-U

Mice were treated as described in Fig. 4. Symbols are the same as in Fig. 4. Each point is the mean ± S.D. of at least three mice.

aqueous solution. Compared with Ara-C aqueous solution, both prodrug preparations showed well maintained plasma levels of the parent drug Ara-C. However, the plasma concentration-time profiles of regenerated Ara-C were markedly different between the preparations. After injection of detergent-solubilized COCG-Ara-C, Ara-C rapidly appeared in the circulation and was then gradually eliminated. On the other hand, the liposome-entrapped prodrug showed gradually increased plasma Ara-C levels during the first 16 h and Ara-C levels slowly decreased thereafter. Plasma Ara-U concentration was below the detectable level (0.1 μM) for both preparations.

For evaluating the relative bioavailabilities of COCG-Ara-C preparations, the areas under the mean plasma concentration-time curves (*AUCs*) of Ara-C were calculated by the trapezoidal method. Relative bioavailabilities were then obtained by comparison with the *AUC* of Ara-C aqueous solution. As shown in Table IV, the calculated relative bioavail-

TABLE V. *AUCs and Half-Lives of Ara-C and Ara-U after Simultaneous Administration of COCG-Ara-C Preparations*

Treatment	Compound	$AUC_0^{4\text{ h}}$ (nmol · h/ml)	<i>AUC</i> ratio ^{b)}	Half-life ^{c)} (min)
No treatment	Ara-C	32.8	1.0	20.0
	Ara-U	46.1	1.0	55.7
Plain liposomes	Ara-C	84.4	2.6	44.4
	Ara-U	50.0	1.1	38.0
Liposome-entrapped COCG-Ara-C	Ara-C	211.7	6.5	55.9
	Ara-U	58.4	1.3	54.1
Detergent-solubilized COCG-Ara-C	Ara-C	28.8	0.9	22.7
	Ara-U	42.9	0.9	44.1

a) *AUC* up to 4 h post administration was calculated by the trapezoidal method. Each Ara-C level after simultaneous administration of COCG-Ara-C preparations was corrected for the Ara-C regenerated from the prodrug. b) *AUC* ratio = $AUC_{\text{treatment}}/AUC_{\text{no treatment}}$. c) Half-life in the terminal phase.

abilities were 129% for the detergent-solubilized form and 592% for the liposome-entrapped form.

Effect of COCG-Ara-C Preparations on the Blood Disposition of Ara-C

For evaluating the effect of COCG-Ara-C preparations on the plasma clearance of the parent drug Ara-C, simultaneous administration of prodrug preparations was carried out. Figures 4 and 5 show the plasma concentration–time profiles of Ara-C and Ara-U, respectively. Simultaneous administration of the liposome-entrapped prodrug markedly decreased the plasma clearance of Ara-C, but the detergent-solubilized prodrug did not. Plain liposomes also prolonged the plasma clearance of Ara-C, but the effect was only moderate (Fig. 4). The plasma levels of Ara-U were not affected by simultaneous administration of these preparations (Fig. 5).

Table V summarizes the *AUCs* and half-lives (the terminal phase) of Ara-C and Ara-U, calculated from the mean plasma concentration–time courses. In the case of simultaneous administration of COCG-Ara-C preparations, each Ara-C level was corrected for the Ara-C regenerated from the prodrug. Simultaneous administration of liposome-entrapped COCG-Ara-C caused a 6.5-fold increase in the *AUC* and a 2.8-fold increase in the half-life of Ara-C. In contrast, when detergent-solubilized COCG-Ara-C was simultaneously injected, no remarkable overall change in the *AUC* and half-life of Ara-C was observed. In all the experiments, no marked differences in the *AUC* and half-life of Ara-U were observed.

Discussion

Ara-C is a potential antimetabolite used in the treatment of acute myelogenous leukemia.⁶⁾ Because of the S-phase specificity of its antitumor action,⁷⁾ the efficacy of Ara-C depends on the maintenance of therapeutic levels of the agent in the blood and tissues for a time long enough to inhibit DNA synthesis of dividing tumor cells.⁸⁾ However, after i.v. injection Ara-C is rapidly cleared from the circulation by deamination to an inactive metabolite, Ara-U. As a result, Ara-C therapy requires a very complex and precise dosage schedule.^{8,9)} To overcome these scheduling problems, a number of Ara-C derivatives have been synthesized.¹⁰⁾ Some derivatives have already been incorporated into liposomes and their efficacy has been proved to be enhanced by this type of modification.¹¹⁾ To our knowledge, however, little has been reported so far on the disposition of Ara-C derivatives entrapped in liposomes.

In a disposition study on liposome-entrapped drugs, it is necessary to use homogeneous preparations to obtain reproducible results.¹²⁾ As regards the size distribution of the vesicles, the liposomes used in this study were fairly homogeneous (Table I).

The major difference in the tissue distribution of COCG-Ara-C between the liposome-entrapped and detergent-solubilized forms was observed in the spleen (Tables II and III). Extremely high spleen levels of the prodrug after i.v. injection in the liposome-entrapped form can be explained by the preferential uptake of the liposome carriers by this tissue (unpublished data). Although the liposomes used in this study were slowly but predominantly accumulated in the liver, no significant difference in liver distribution of the prodrug for the first 16 h was observed between the two preparations. This finding suggests that detergent-solubilized (free) COCG-Ara-C is inherently distributed in the liver. It has been shown that *N*⁴-behenoyl-Ara-C, a highly lipophilic prodrug of Ara-C, is also accumulated in the liver when administered in the detergent-solubilized form.¹³⁾

As previously reported,¹⁴⁾ the liposome carriers used in this study are fairly stable to high density lipoprotein (HDL) attack and have a long life-time in the circulation. Also, COCG-Ara-C is firmly associated with liposomes even in the circulation (unpublished data). This is one reason why liposome-entrapped COCG-Ara-C was retained in the circulation for a long time relative to the detergent-solubilized prodrug (Fig. 2).

In contrast to Ara-C aqueous solution, COCG-Ara-C preparations showed well maintained plasma levels of Ara-C (Fig. 3). This finding suggests that the derivative may act in the body as a sustained-release reservoir of Ara-C. The extremely high antitumor effect of COCG-Ara-C preparations compared to Ara-C aqueous solution reported previously¹⁾ could be explained by the sustained plasma levels of Ara-C. In addition, the more successfully prolonged plasma levels of regenerated Ara-C after i.v. injection of liposome-entrapped COCG-Ara-C indicate the potential utility of the liposomes as a dosage form of the derivative. As previously reported,¹⁾ liposome-entrapped COCG-Ara-C showed a significantly more potent antileukemic activity compared with the detergent-solubilized prodrug.

As shown in Table IV, an extremely high bioavailability value of Ara-C (592%) was obtained for liposome-entrapped COCG-Ara-C. One possible explanation of this result is that liposome-entrapped COCG-Ara-C prolongs the plasma clearance of regenerated Ara-C.

This possibility was confirmed by simultaneous injection experiments. As shown in Fig. 4, simultaneous injection of liposome-entrapped COCG-Ara-C markedly decreased the plasma clearance of Ara-C. Compared with non-treatment, simultaneous injection of the liposome-entrapped prodrug caused 2.8-fold and 6.5-fold increases in the half-life and *AUC* of Ara-C, respectively (Table V). In contrast, detergent-solubilized COCG-Ara-C had no effect on the plasma clearance of Ara-C. Plain liposomes also showed a similar prolonging effect, but it was rather moderate. Therefore, the effect of the liposome-entrapped derivative on the plasma clearance of Ara-C cannot be completely explained only in terms of the effect of the carrier vehicle. Liposome-entrapped COCG-Ara-C may affect the biological disposition (metabolism, excretion, etc.) of Ara-C.

Similar delayed blood clearance of Ara-C by the prior administration of tetrahydro-uridine (THU) has been reported by Dareer *et al.*¹⁵⁾ THU is a potent inhibitor of cytidine deaminase, and inhibits the *in vivo* deamination process of Ara-C to Ara-U.¹⁶⁾ Consequently, plasma Ara-C levels were maintained while plasma Ara-U levels were decreased. In the present study, however, decrease in the plasma clearance of Ara-C by simultaneous injection of liposome-entrapped COCG-Ara-C was not accompanied with a decrease in plasma Ara-U levels (Fig. 5 and Table V). Therefore, decreased plasma clearance of Ara-C (Fig. 4) cannot be explained in terms of inhibition of the *in vivo* deamination process. Liposome-entrapped COCG-Ara-C might affect other disposition processes.

Although the detailed mechanism of the decreased plasma clearance of Ara-C remains

uncertain, it is apparent that liposome-entrapped COCG-Ara-C does reduce plasma clearance of Ara-C. This in part explains the extremely high bioavailability of Ara-C obtained after i.v. injection of liposomal COCG-Ara-C. Further studies are under way to investigate the effect of liposomal prodrug on the biological disposition of Ara-C in order to clarify the mechanism of decreased plasma clearance of the parent drug.

On the basis of the present results, liposome-entrapped COCG-Ara-C administered intravenously acts as a slow-release reservoir to supply the active parent drug over a prolonged period of time. Also, liposome-entrapped COCG-Ara-C seems to reduce the clearance of regenerated Ara-C, tending to maintain the plasma levels of active Ara-C.

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