International Journal of Pharmaceutics, 31 (1986) 185–191
Elsevier

IJP 01044

Research Papers

Rectal absorption and lymphatic uptake of Ara-C in rats

Toshiaki Nishihata [1], Suni Kim [2], Gary G. Liversidge * and Takeru Higuchi [2]

[1] *Faculty of Pharmaceutical Science, Osaka University, 133-1 Yamadakami, Suita, Osaka 565, Japan and*
[2] *Pharmaceutical Chemistry Department, University of Kansas, Lawrence, KS 66045 (U.S.A.)*

(Received October 4th, 1985)
(Modified version received January 23rd, 1986)
(Accepted February 14th, 1986)

Key words: Ara-C – lymphatic targeting – rectal absorption – adjuvants

Summary

Enhanced rectal absorption and lymphatic uptake of Ara-C is observed when administered in conjunction with salicylate adjuvants alone or in combination with any of the following adjuvants: glycerol, glycerol monooleate or peanut butter. Uptake into the lymphatic system is also enhanced when Ara-C is administered intravenously and glycerol monooleate is administered rectally. Increased lymphatic uptake was attributed to increased lymph flow and selective transport specificity of Ara-C in the rectal area caused by the adjuvants. Surgical effects of thoracic cannulation cause a decrease in serum Ara-C levels which may be attributed to a number of physiological factors.

Introduction

Ara-C (1-β-D-Arabinofuranosylcytosine) is a powerful antitumor agent (Evans et al., 1964), and is currently one of the major drugs for the treatment of acute granulocytic leukemia (Goodell et al., 1971; Chu and Fisher, 1962). Treatment with Ara-C is carried out by intravenous or intramuscular injection due to its poor absorption from the gastrointestinal tract (Hanka et al., 1970; Dedrick et al., 1972; Momparler et al., 1972).

Recently, it has been reported that drugs which are poorly absorbed in the rectum, due to either their high water solubility (Nishihata et al., 1982) or high molecular weight (Nishihata et al., 1981), can be improved by the presence of nons-surfactant adjuvants such as salicylates. Salicylates also enhance the uptake of compounds such as phenol red, cefoxitin and insulin into the lymphatic system when administered rectally (Caldwell et al., 1982).

In the present paper, we show the effects of rectal administration on the lymphatic transport of Ara-C. This route is more convenient than intravenous or intramuscular administration and provides a method of targeting Ara-C to the lymphatic system. This will be particularly advantageous for metastasis treatment through lymphatic pathways (Hashida et al., 1979).

Materials and Methods

Ara-C (Upjohn), sodium salicylate (Aldrich), sodium-5-methoxysalicylate (Aldrich), glycerol (Sigma), glycerol monooleate (Sigma) and tetrahy-

* *Present address to be used for correspondence*: G.G. Liversidge, Smith Kline & French F112, 1500 Spring Garden Street, Philadelphia, PA 19101, U.S.A.

TABLE 1

MICROENEMA FORMULATION OF ARA-C

Formulation	Ara-C (mg)	Content of microenema					Total volume	Purpose
		Adjuvant (mg)	Glycerine (μl)	Lipid I [a] (μl)	Lipid II [b] (μl)	H_2O		
I	15	–	–	–	–	q.s.	1.0	i.v. injection
II	15	15	–	–	–	q.s.	1.0	i.v. injection
III	15	designated amount	–	–	–	q.s.	1.0	rectal administration (RA)
IV	15	–	500	–	–	q.s.	1.0	(RA)
V	15	–	–	700	–	q.s.	1.0	(RA)
VI	15	–	–	–	700	q.s.	1.0	(RA)
VII	15	50	500	–	–	q.s.	1.0	(RA)
VIII	15	50	–	700	–	q.s.	1.0	(RA)
IX	15	50	–	–	700	q.s.	1.0	(RA)

[a] Lipid I was composed of glycerol/glycerine monooleate (7 : 3).

[b] Lipid II was composed of glycerol/natural peanut butter (7 : 3).

drouridine (THU) (Carbiochem) were used as obtained. Peanut butter was ground to a fine paste. The formulations used in this study are shown in Table 1.

Sprague–Dawley male rats, 200–225 g, were used. They were fasted for 16 h prior to experimentation. During the experiments, the rats were kept on a 38°C surface and anaesthetised with sodium pentabaritone (60 mg/kg of body weight).

In situ rectal loop studies were carried out in a manner similar to that reported by Levine et al. (1955) for small intestinal loops. About 4 cm of the rectal compartment (372 ± 33 mg, measured after the experiment) from the anus were employed for this study. The rectal loop was removed 1 h after administration of a microenema (1 ml/kg of body weight). The rectal contents were collected by washing with distilled water, then the amount of drug remaining in the loop was measured. Rats subjected to the above were termed "Rectal Loop Prepared Rats". Blood samples were taken from the jugular vein at designated times, then mixed with 1 mg of THU (to prevent degradation); and serum samples were then obtained.

For the in vivo studies, 1.0 ml of microenema/kg of body weight was administered at a 1 cm depth from the rats' anus. The anus was ligated to prevent leakage of the microenema. Rats subjected to this treatment were termed "Normal Rats". Blood samples were collected at designated time intervals, and serum was obtained as described above.

Lymph fluid was collected from the thoracic duct according to the method of Bollman et al. (1948) using Intramedic PE 50 Tubing, rats thus treated were termed "Cannulated Rats". The effects on the drug plasma levels of the surgical procedure required for thoracic cannulation was also studied. Rats were subjected to surgery with the exception of actual cannulation of the thoracic duct. Rats subjected to this treatment were termed "Surgically Prepared Rats". Serum levels were then monitored after either intravenous or rectal administration. Serum samples were analyzed according to the method of Liversidge et al. (1983).

Results and Discussion

Rectal absorption of Ara-C

Ara-C is poorly absorbed rectally when administered alone, without an adjuvant. However, co-administration of adjuvants such as sodium salicylate or sodium-5-methoxysalicylate increase absorption which is reflected in increased serum levels and increased disappearance of Ara-C from rectal loops (Table 2). Enhanced rectal absorption

TABLE 2

EFFECTS OF SURGERY, CONCENTRATION OF SODIUM SALICYLATE AND 5-METHOXYSALICYLATE ON THE PLASMA LEVELS OF ARA-C (AT 0.5 h) AND ON THE DISAPPEARANCE OF ARA-C FROM THE RECTAL LOOP (AT 1 h) OF "RECTAL LOOP RATS" AFTER ADMINISTRATION OF ARA-C IN FORMULATION III (1 ml/kg DOSE)

Concentration of adjuvant in microenema (mg/ml)	Plasma concentration (μg/ml)	Disappearance (%)
"Rectal Loop Rats"		
0	< 0.3	4.9 ± 5.1
Salicylate		
15	2.8 ± 0.7	22.3 ± 7.9
25	6.5 ± 0.9	53.1 ± 12.7
50	10.8 ± 1.6	83.5 ± 12.4
5-Methoxysalicylate		
15	3.9 ± 0.6	29.6 ± 10.3
25	7.1 ± 1.2	62.8 ± 9.5
50	10.3 ± 1.9	86.2 ± 9.8
"Rectal Loop Rats" surgically prepared for thoracic cannulation		
25	3.7 ± 0.6	32.8 ± 7.5
50	5.8 ± 1.1	58.3 ± 10.4

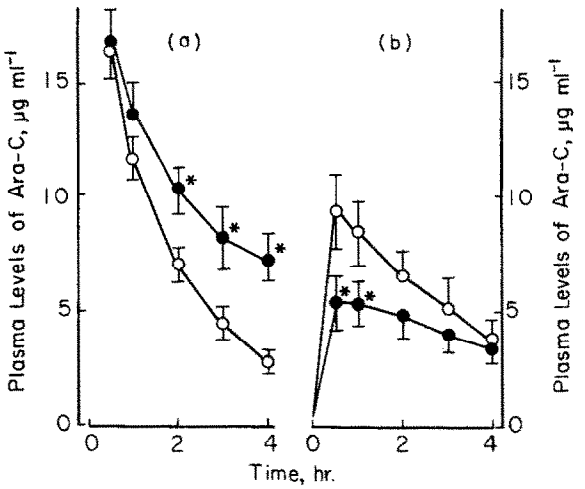

Fig. 1. Plasma Ara-C concentration after (a) intravenous administration and (b) rectal administration with sodium salicylate in normal rats (○) and in surgically prepared rats (●). Formulation I (see Table 1) was employed for intravenous administration at a dosage volume of 1.0 ml/kg. Formulation III (see Table 1) containing 50 mg of sodium salicylate/ml was employed for rectal administration at a dosage volume of 1.0 ml/kg. Each value shows mean ± S.D. ($n \geq 6$). * represents significant difference ($P < 0.001$ Student's t-test) under no surgical conditions.

of Ara-C depends on the dose of adjuvant (Table 2).

Prior to studying the lymphatic transport of Ara-C, surgical effects of thoracic cannulation on the plasma behavior and rectal absorption of Ara-C were determined. Actual cannulation was not performed, but all the surgery necessary was (termed "Surgically Prepared Rats", see Materials and Methods section). Elimination of Ara-C from the serum after intravenous administration in surgically prepared rats showed a delay compared to normal rats (Fig. 1). Similar delays between surgically and non-surgically prepared rats were shown when Ara-C was rectally administered in the presence of salicylate for both rectal loop prepared rats (Table 2) and normal rats (Fig. 1). These findings indicate that surgery may significantly change blood flow, renal elimination and other physiological conditions in rats. These changes are evidenced by a lowering of sera Ara-C levels in surgically prepared rats compared to non-surgically prepared rats after rectal administration of Ara-C in the presence of an adjuvant.

Lymphatic uptake of Ara-C

The following study was carried out on cannulated rats and we would expect to see similar behavior to that of surgically prepared rats. Lymphatic levels of Ara-C after intravenous administration were lower than serum levels at earlier periods; addition of salicylate did not effect the Ara-C levels in either fluid (Fig. 2a). However, rectal administration of Ara-C with salicylate in a microenema caused an increase in Ara-C lymphatic levels when compared to serum levels (Fig. 2b and c). A more significant increase in lymphatic levels was observed in cannulated rectal loop prepared rats than in the cannulated normal rats. At present, we have not elucidated the mechanism for this phenomenon.

Rectal administration of Ara-C with salicylate gave rise to higher lymphatic levels of Ara-C against serum levels in comparison to intravenous

administration. In a previous paper (Nishihata et al., 1983), it has been reported that injection of cefoxitin, a highly water-soluble drug, into rat rectal tissue showed high lymphatic levels compared to femoral muscle injection. Furthermore, lymphatic levels of cefoxitin after rectal tissue and femoral muscle injection were increased by the co-administration of 5-methoxysalicylate. Higher lymphatic levels of Ara-C after rectal administration with salicylate, in this study, may be due to selective specificity of lymphatic uptake in the presence of salicylate in rectal tissue compared to other sites such as muscle and blood.

Effect of lipids

The following study was carried out on cannulated normal rats. It is generally known that lipids after administration in the small intestine are well taken up into the lymph. For this study, peanut butter (Skippy brand) was selected as a good source of lipid materials that enjoy wide acceptability with minimal toxic effects. Furthermore, it has been reported that monoglycerides incorporated into liposomal membranes change the permeability of compounds through these membranes (Muranishi et al., 1981). In our work, glycerine monooleate and peanut butter were employed to study their effect on rectal absorption of Ara-C and lymphatic uptake of Ara-C after rectal administration. Glycerine was also studied and used as a dispersant for glycerol monooleate and peanut butter in microenemas.

Rectal absorption of Ara-C was enhanced by co-administration of glycerine (formulation IV, Fig. 3a), glycerine/glycerine monooleate (formulation V, Fig. 4a) and glycerine/peanut butter (formulation VI, Fig. 5a). Lymphatic levels of

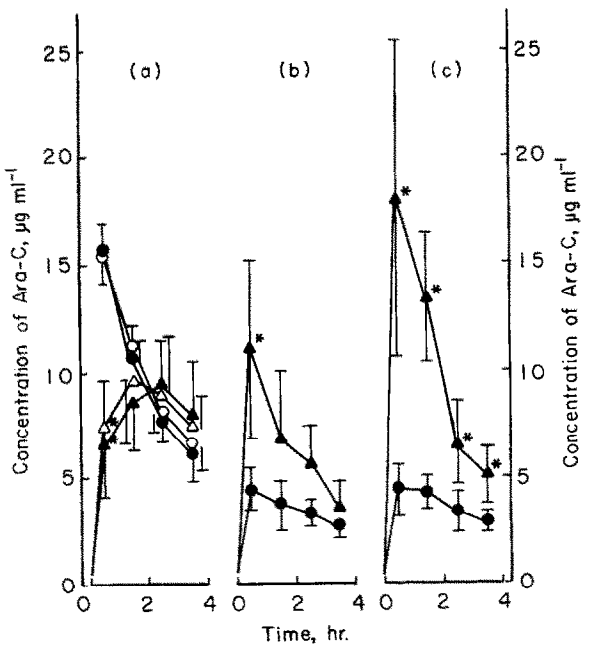

Fig. 2. Concentration of Ara-C in plasma (○ and ●) and in lymphatic fluid (△ and ▲) after (a) intravenous injection and rectal administration with sodium salicylate, (b) rectal administration to cannulated normal rats and (c) rectal administration to cannulated rectal loop prepared rats. Formulation I (○ and △) and formulation II (● and ▲) were employed for intravenous injection at a dosage volume of 1.0 ml/kg. Formulation III containing 50 mg of salicylate/ml was employed for rectal administration at a dosage volume of 1.0 ml/kg. Each value represents mean ± S.D. ($n \geqq 6$). * represents significant difference ($P < 0.001$ Student's t-test) against plasma Ara-C levels.

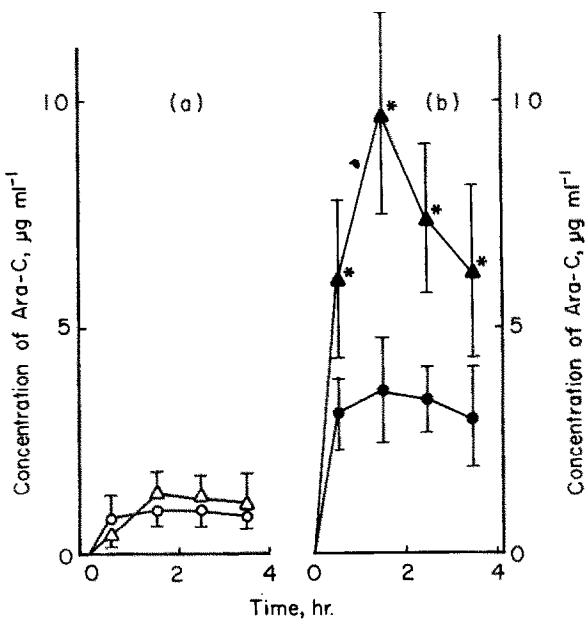

Fig. 3. Concentration of Ara-C in plasma (○ and ●) and in lymphatic fluid (△ and ▲) after rectal administration of (a) formulation IV containing glycerine (○ and △) and of (b) formulation VII containing glycerine and sodium salicylate (● and ▲). Each value represents mean ± S.D. ($n \geqq 3$). * represents significant difference ($P < 0.001$ Student's t-test) against plasma levels of Ara-C.

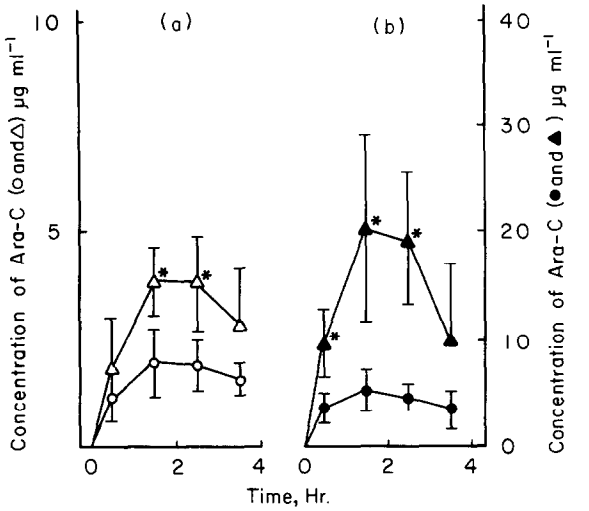

Fig. 4. Concentration of Ara-C in plasma (○ and ●) and in lymphatic fluid (△ and ▲) after rectal administration of (a) formulation V containing glycerine/glycerine monooleate (○ and △) and of (b) formulation VIII containing glycerine/glycerine monooleate and sodium salicylate (● and ▲). Each value represents mean ± S.D. ($n \geq 5$). * represents significant difference ($P < 0.001$ Student's t-test) against plasma levels of Ara-C.

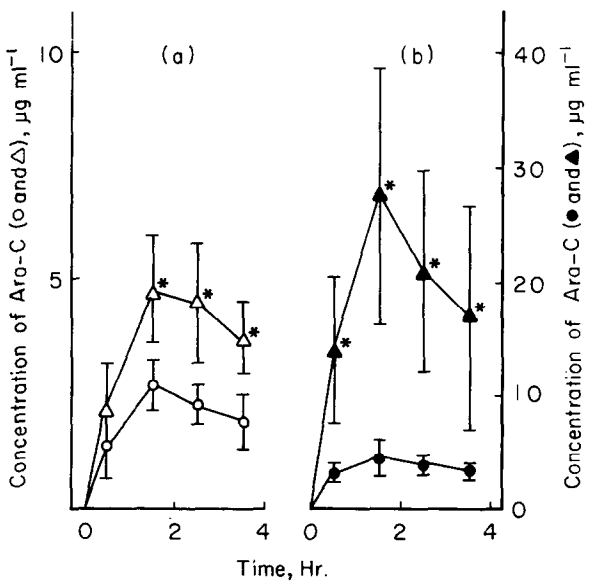

Fig. 5. Concentration of Ara-C in plasma (○ and ●) and in lymphatic fluid (△ and ▲) after rectal administration of (a) formulation VI containing glycerine/peanut butter (○ and △) and of (b) formulation IX containing glycerine/peanut butter and sodium salicylate (● and ▲). Each value represents mean ± S.D. ($n \geq 5$). * represents significant difference ($P < 0.001$ Student's t-test) against plasma levels of Ara-C.

TABLE 3

RECOVERY AND BIOAVAILABILITY OF ARA-C FROM THE THORACIC DUCT 4 h AFTER ADMINISTRATION

Formulation	Ara-C				
	(A)	(B)	(C)	(D)	(E)
i.v. injection					
I	8.4 ± 1.9	0.28	34.5 ± 4.6	1.00	0.28
II	9.1 ± 2.3	0.30	35.2 ± 2.8	1.01	0.30
rectal administration					
III	13.9 ± 5.2	0.46	12.7 ± 3.1	0.37	1.24
IV	1.2 ± 0.4	0.04	2.6 ± 1.7	0.075	0.53
V	6.1 ± 1.2	0.20	5.6 ± 1.1	0.16	1.25
VI	6.8 ± 1.7	0.23	7.2 ± 1.4	0.21	1.10
VII	12.3 ± 4.6	0.41	11.6 ± 2.3	0.34	1.21
VIII	34.5 ± 11.6	1.15	14.2 ± 3.0	0.41	2.80
IX	48.8 ± 16.8	1.62	15.1 ± 3.8	0.43	3.77

(A) = Recovery amount from thoracic duct (μg).

(B) = $\frac{(A)}{\text{Dose}} \times 100$.

(C) = $[AUC]_{0 \rightarrow 3.5\ h}$ in plasma (μg/h/ml) after rectal administration.

(D) = $\frac{(C)}{[AUC]_{i.v.}}$.

(E) = $\frac{(B)}{(D)}$.

Ara-C were significantly higher than plasma levels (Figs. 4a and 5a), except for formulation IV (Fig. 3a). The above findings indicate that glycerine monooleate and peanut butter enhance the uptake of Ara-C into the lymphatic system after rectal administration. Rectal absorption of Ara-C after administration of each of the above lipids was further enhanced by the presence of sodium salicylate in microenemas (Figs. 3b, 4b and 5b), and lymphatic uptake of Ara-C occurred to a much higher extent. The increased lymphatic level of Ara-C after rectal administration with lipid/sodium salicylate was more significant than after rectal administration of either sodium salicylate or lipid alone.

Recovery of Ara-C from thoracic ducts after administration with each formulation is given in Table 3. Recovery of Ara-C after rectal administration with sodium salicylate and each of the lipids, glycerine monooleate and peanut butter were significantly higher than for the other rectal formulations.

Although recovery of Ara-C from the thoracic duct after rectal administration with each lipid appeared lower than after intravenous injection, the bioavailability of Ara-C after rectal administration should be noted. As an indication of the bioavailability of Ara-C in serum, the AUC of Ara-C in sera were determined using cannulated rats (Table 3). When looking at the ratio of the percent of Ara-C recovered from the thoracic duct against bioavailability in serum (Table 3), the ratio after rectal administration with every formulation was significantly higher than after intravenous administration. These findings indicate that glycerol monooleate and peanut butter facilitate the uptake of Ara-C into the lymphatic system after rectal administration, and co-administration with sodium salicylate enhances further serum levels of Ara-C and the uptake of Ara-C into the lymphatic system.

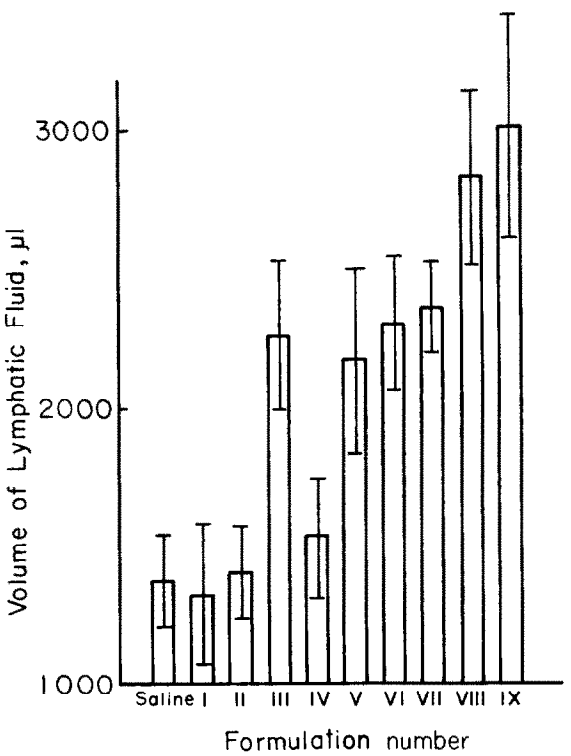

Fig. 6. Lymphatic fluid volume collected from thoracic duct during 4 h after intravenous administration with formulations I and II (see Table 1) and rectal administration with sodium and formulations III-IX (see Table 1). Each value represents mean ±S.D. (Experimental number is described in Figs. 2–5 for each formulation).

Effect of lymphatic fluid flow

The volume of lymph collected from the thoracic duct increased when Ara-C was administered rectally in a microenema containing salicylate/lipid, compared to an Ara-C microenema containing saline or Ara-C administered intravenously (Fig. 6). This increase in lymphatic flow may be one of the reasons why lymphatic uptake of Ara-C was increased after rectal administration of sodium salicylate and for lipids. Lymphatic uptake of Ara-C after intravenous injection was enhanced after increasing the lymphatic flow by rectally administering a microenema containing glycerol monooleate (Fig. 7). In a previous paper (Nishihata et al., 1985), it has been reported that the increase observed in concentration and total uptake of cefoxitin by the lymphatic system when injected into rectal tissue together with sodium-5-methoxysalicylate was reduced by the co-administration of ϵ-amino caproic acid. ϵ-Amino caproic acid causes a decrease in lymph flow. These findings indicate that sodium salicylate and lipid taken up by rectal tissue cause an increase in lymph flow at the rectal tissue and facilitate the transport of Ara-C into the lymphatic system.

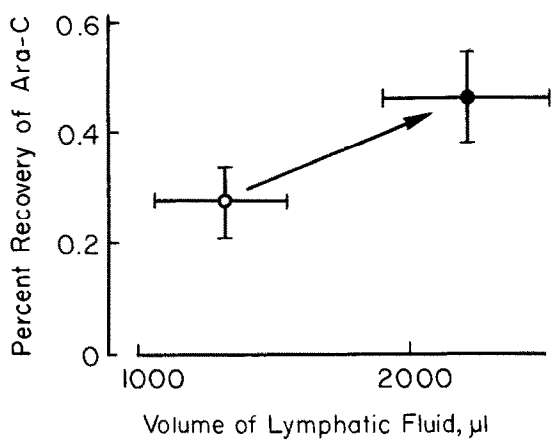

Fig. 7. Ara-C and lymphatic fluid volumes collected from thoracic duct 4 h after intravenous administration of Ara-C (formulation I) with (●) and without (○) rectal administration of microenemas containing 700 µl of glycerine/glycerine monooleate (7:3) per 1.0 ml at 5 min before intravenous injection of Ara-C.

Acknowledgements

This work was supported by a grant from INTER$_x$, Merck Sharp and Dohme, Lawrence, KS. The authors also acknowledge the support provided by the Center for Biomedical Research, The University of Kansas.

References

Bollman, J.L., Cain, J.C. and Grindlay, J.H., Techniques for the collection of lymph from the liver, small intestine or thoracic duct of the rat. J. Lab. Clin. Med., 33 (1948) 1349–1352.

Caldwell, L., Nishihata, T., Rytting, J.H. and Higuchi, T., Lymphatic uptake of water-soluble drugs after rectal administration. J. Pharm. Pharmacol., 34 (1982) 520–522.

Chu, M.Y. and Fischer, G.A., A proposed mechanism of action of 1-β-D-arabinofuranosyl-cytosine as an inhibitor of the growth of leukemic cells. Biochem. Pharmacol., 11 (1962) 423–430.

Dedrick, R.L., Forrester, D.D. and Ho, D.H.W., In-vitro–in vivo correlation of drug metabolism-deamination of 1-β-D-arabinofuranosylrosyleytosine. Biochem. Pharmacol., 21 (1972) 1–16.

Evans, J.S., Musser, E., Boturide, L. and Mengel, G.D., The effect of 1-β-D-arabinofuranosylcytosine hydrochloride on murine neoplasms. Cancer Res., 24 (1964) 1285–1293.

Goodell, B., Leventhal, B. and Henderson, E., Cytosine arabinoside in acute granulocytic leukemia. Clin. Pharmacol. Ther., 12 (1971) 606.

Hanka, E.J., Keuntzel, S.L. and Neil, G., Improved microbiological assay for cytosine arabinoside (NSC-63878). Cancer Chem. Rept., 54 (1970) 393–397.

Hashida, M., Muranishi, S., Sezaki, H., Tanigawa, N., Satomura, K. and Hisaka, Y., Increased lymphatic delivery of bleomycin by microsphere in oil emulsion and its effect on lymph node metastasis. Int. J. Pharm., (1979) 245–256.

Levine, R.M., Blair, M.R. and Clark, B.C., Factors influencing the intestinal absorption of certain monoquaternary antichloinergic compounds with special reference to benzomethamine [N-diethylaminoethyl-N′-methylbenzalamide methobromide (MC 3197). J. Pharmacol. Exp. Ther., 114 (1955) 78-86.

Liversidge, G.G., Nishihata, T., Higuchi, T., Schaffer, R. and Cortese, M., Simultaneous analysis of 1-β-D-arabinofuranosylcytosine, 1-β-D-arabinofuranosyluracil and sodium salicylate in biological samples by high performance liquid chromatography. J. Chromatogr. Biomed. Appl., 276 (1983) 375–383.

Momparler, R.L., Labitan, A. and Rosci, M., Enzymatic estimation and metabolism of 1-β-D-arabinofuranosylcytosine in men. Cancer Res., 32 (1972) 408–412.

Muranushi, N., Takagi, N., Muranishi, S. and Sezaki, H., Effect of fatty acids and monoglycerides on permeability of lipid bilayer. Chem. Phys. Lipids, 28 (1981) 269–279.

Nishihata, T., Rytting, J.H., Higuchi, T. and Caldwell, L., Enhanced rectal absorption of insulin and heparin in rats in the presence of non-surfactant adjuvants. J. Pharm. Pharmacol., 33 (1981) 334–335.

Nishihata, T., Rytting, J.H. and Higuchi, T., Effect of salicylate on the rectal absorption of lidocaine, levodopa and cefmetazole in rats. J. Pharm. Sci., 71 (1982) 869–872.

Nishihata, T., Kim, S., Kamada, A., Frederick, G., Dillsaver, M., Higuchi, T., Lymphatic transport of sodium cefoxitin in the presence of sodium 5-methoxysalicylate after injection into rat rectal connective tissue, femoral muscle and femoral vein. J. Pharm. Pharmacol., 37 (1985) 509–511.