

Potential Absorption of Heparin from the Small Intestine and the Large Intestine in the Presence of Monoolein Mixed Micelles

Remarkable enhanced absorption of heparin was found by the administration of monoolein-bile salts mixed micelles solution in the small intestine and the large intestine of rats. This result suggested that monoolein mixed micelles was greatly useful as a safe adjuvant to potentiate absorption of heparin.

Keywords—mixed micelles; oral administration; rectal administration; heparin; high molecular weight compound; monoolein; enhanced absorption; safe adjuvant

Heparin is a high molecular weight compound which has been known to be hardly absorbed from the gastrointestinal tract.¹⁾ Since the oral and rectal administration of heparin are highly desirable for patients who are suffering from thrombosis and generally in the anticoagulant therapy, several works²⁻⁷⁾ had been done in order to promote its absorption from the gastrointestinal tract which is impermeable to it. It was found that the sodium salt of ethylenediaminetetraacetic acid (EDTA)^{2,3)} and oil in water (O/W) emulsions⁵⁾ possess the ability to affect the gastrointestinal absorption of heparin. But, although these adjuvants were successful in increasing the absorption of heparin to a certain extent, they have some undesirable effects which should be taken into consideration. For example EDTA is a substance known to cause mucosal damage in the intestine,⁸⁾ and in general O/W emulsions are not sufficiently stable.

The present communication describes the use of monoolein-bile salts micelles as a suitable and safe adjuvant which are not harmful to the mucosal membrane and at the same time affect the gastrointestinal absorption of heparin to an appreciable extent.

Drugs studied were heparin sodium (Nakarai Chemical Co.) and ³⁵S-labeled heparin sodium (New England Nuclear) with a specific activity of 12.4 μ Ci/mg. Adjuvants used were monoolein (Tokyo Kasei Co.), sodium glycocholate and taurocholate which were synthesized according to the method of Norman.⁹⁾ The purity of both bile salts was checked by thin-layer chromatography and infrared spectroscopy. Mixed micellar solutions were prepared by dissolving 390 mg monoolein in 24 ml of a solution containing 0.04M sodium glycocholate or taurocholate and 5.0 mg heparin sodium/ml, then this mixture was sonicated for 5 min with Ohtake sonicator (20 kW). The O/W emulsion was prepared by mixing 3.6 ml of trioctanoin (Tokyo Kasei Co.) with 20.4 ml of a solution of 1% bovine serum albumin fraction V (Sigma Co.) containing 120 mg heparin sodium and sonication for 5 min. Male Wistar rats weighing 200–250 g were anesthetized with pentobarbital. The intestine was exposed through a midline incision, then a closed loop of the small intestine (from the duodenum to the ileum) or the large intestine (the colon including the rectum) was prepared by ligation at the proximal and distal ends. Four ml or two ml of the test solution was introduced into the small or the large intestinal loop respectively. Blood samples were collected from the carotid artery. Determination of heparin was carried out by measuring the turbidity at 650 m μ according to the method of Koron.¹⁰⁾ The

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radioactivity of ^{35}S was estimated with a liquid scintillation counter by mixing 0.2 ml plasma and 0.1 ml 1 N HCl with 15 ml of a scintillation medium (1.0 liter of ethyleneglycol monoethyl-ether).

The effect of different adjuvants on the intestinal absorption of heparin was demonstrated. Measurement of the clearing factor activity in plasma samples at 30 min after administration was taken as a criterion of heparin absorption. The mean decrease in OD_{650} at 15 and 30 min

TABLE I. The Clearing Factor Activity in Plasma After the Administration of Heparin with Different Adjuvants^{a)}

Adjuvant	Decrease in $\text{OD}_{650} \pm \text{SE}$		
	15 min ^{b)}	30 min ^{b)}	
(A) Small intestine	Control ^{c)}	0.020 ± 0.003 (5) ^{d)}	0.035 ± 0.005
	NaGC	0.049 ± 0.007 (4)	0.109 ± 0.004
	NaGC+NaTC ^{e)}	0.046 ± 0.012 (3)	0.108 ± 0.006
	MO+NaGC	0.231 ± 0.021 (4)	0.378 ± 0.023
	MO+NaTC	0.229 ± 0.021 (4)	0.341 ± 0.024
	MO+NaGC+NaTC ^{e)}	0.217 ± 0.035 (4)	0.327 ± 0.042
	O/W TO emulsion	0.068 ± 0.010 (4)	0.113 ± 0.016
	MO+NaGC+5% glucose	0.222 ± 0.017 (3)	0.356 ± 0.028
(B) Large intestine	Control ^{c)}	0.019 ± 0.006 (6)	0.025 ± 0.003
	MO+NaGC	0.286 ± 0.035 (5)	0.433 ± 0.036
	MO+NaTC	0.276 ± 0.016 (4)	0.426 ± 0.014

a) NaGC=sodium glycocholate, NaTC=sodium taurocholate, MO=monoolein, TO=trioctanoin; final concentration: heparin, 5.0 mg/ml; bile salts and monoolein, 0.04 M; oil phase of emulsion, 15.0%

b) incubation time

c) control: aqueous solution of heparin was administered.

d) Figures in parentheses refer to the number of animals.

e) NaGC and NaTC were used in a concentration of 0.02 M.

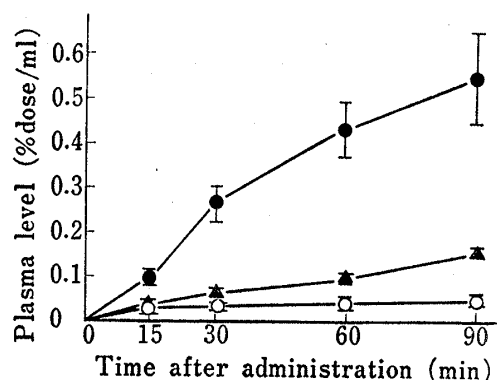


Fig. 1. Radioactivity Levels in Plasma After the Administration of ^{35}S -Heparin incorporated with Different Adjuvants into the Small Intestine^{a)}

a) Data points represent the average of animals; the vertical bars represent standard error of the mean. \circ aqueous solution (4), \blacktriangle sodium glycocholate solution (4), \bullet monoolein plus sodium glycocholate solution (8). Figures in parentheses refer to the number of animals.

after incubation at 27.5° is presented in Table I, (A). It is evident that in the presence of sodium taurocholate or glycocholate a two or three folds increase in the clearing factor activity (in comparison to the control) at 15 and 30 min respectively can be noted. However, when heparin was administered in a mixed micellar solution of monoolein either with taurocholate, glycocholate or both a marked and strong clearing factor response was elicited. Furthermore, the addition of 5% glucose to monoolein glycocholate mixed micellar solution showed no effect on the strong response of heparin. On the other hand, although O/W trioctanoin emulsion stabilized by bovine serum albumin fraction V caused a significant increase in the clearing factor activity, it is still very low compared to that of monoolein mixed micelles.

Then, experiments of the plasma level-time curves of ^{35}S -labeled heparin were done. The results are shown in Fig. 1 which indicate that the plasma radioactivity in the presence of glycocholate was increased about 2 folds, while in the presence of monoolein glycocholate mixed micelles it was about 10 folds increase compared to the control, and it was continued to increase up to 90 min. This is another evidence which confirms the enhancement effect of the monoolein mixed micelles on the intestinal absorption of heparin.

Moreover, the absorption of heparin from the large intestine was extremely promoted and this can be seen in Table I, (B) where a remarkable increase in the clearing factor activity by monoolein mixed micelles was occurred although the administered dose was half of that of the small intestine. Therefore, the gastrointestinal absorption of heparin which is practically not absorbed can be induced if the drug was administered in the form of monoolein bile salts mixed micelles.

Also, taurocholate and glycocholate had been found to be extremely less harmful to the gastrointestinal tract contrary to dihydroxy bile acids such as deoxycholate and chenodeoxycholate.¹¹⁾ Moreover, the presence of monoolein taurocholate mixture could modify a number of the toxic effect of deoxycholate on jejunal function.¹²⁾ Therefore, the adjuvants used in our experiment are considered to be safe for the gastrointestinal tract. The mechanism of action of the monoolein bile salts mixed micelles is not clear and our studied are proceeding forward to elucidate the real mechanism.

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Biosynthesis of Streptothricin Antibiotics. IV.¹⁾ On the Incorporation of L-Arginine into Streptolidine Moiety by *Streptomyces lavendulae* OP-2

Carbon atom from L-arginine-U-¹⁴C was preferentially incorporated into racemomycin-D which was produced by strain of *S. lavendulae* OP-2. 51% of the activity was located in the streptolidine moiety.

Keywords—*S. lavendulae*; *S. noursei*; Streptothricin; Nourseothricin; Racemomycin; streptolidine moiety; lysine pathway

Through the studies of streptothricin antibiotics, we have described previously the physico-chemical and biological properties of racemomycins A, B and C.²⁾

Recently, a strain OP-2 which belongs to *S. lavendulae* was found to produce a racemomycin-D as a main component.³⁾ Working with this strain, we examined the efficiencies of various amino acids on the fermentative production of racemomycin-D. It was confirmed that several amino acids stimulated the antibiotic production and only arginine was specifically incorporated into streptolidine moiety.

In order to investigate the influence of amino acids on the yield of antibiotic, the supplementation of the synthetic medium containing 3% glucose, 1% ammonium tartarate,

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3) Taxonomical respects of this strain and physico-chemical and biological properties of racemomycin-D will be published elsewhere.