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Effect of Some Non-ionic Surfactants on the Absorption of Enduracidin from the Muscles[1]

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Effect of some non-ionic surfactants on the muscular absorption of Enduracidin was investigated from both the urinary and blood level studies in the rat.

Addition of the non-ionic surfactants remarkably promoted the absorption of Enduracidin from the muscles of the rats and Nikkol HCO-50, hydrogenated castor oil polyoxyethylene ether, showed the most pronounced absorption-enhancing ability.

It was also found that there was no correlationship between the peak blood level and the concentration of Enduracidin and that the duration of blood level was dependent on the concentration of the antibiotic.

A rate-limiting process was proposed to the muscular absorption mechanism of Enduracidin from the result with EDTA.

Enduracidin, a new antibiotic isolated from *Streptomyces fungicidicus* in our Microbiological Research Laboratories is remarkably effective against the gram-positivie organisms *in vitro*[3] and it has been proved that Enduracidin is of clinical value from its protective effect against the experimental infections in animals.[4] Enduracidin is likely a basic polypeptide with molecular weight of about 2500 and readily forms a water-soluble salt with hydrochloric acid.[5]

In the course of developing its parenteral preparation from the biopharmaceutical standpoint, however, it was found that the aqueous solution of Enduracidin hydrochloride did not show the significantly high blood levels by the intramuscular injection to the rats.

For the purpose of enhancing the blood level and ensuring the stable injectable solution, the non-ionic surfactants were employed as the additives, as it has been known that they can often increase the transport of drugs across biological membrane.

The present paper describes the effect of some non-ionic surfactants on the muscular absorption of Enduracidin and the absorption characteristics of Enduracidin itself from the rat muscles, simultaneously.

Experimental

Enduracidin hydrochloride was supplied from our Microbiological Research Laboratories. As the non-ionic surfactants, Tween 80, Nikkol[6] HCO-30,[7] −50, and −120 were used. Drug solutions were prepared by dissolving Enduracidin hydrochloride to give its various concentrations in the solution containing up to 10% (w/v) of the surfactant and 5% (w/v) of inositol for isotonicity.

1) This work was presented at the 88th Annual Meeting of Pharmaceutical Society of Japan, Tokyo, April 1968.
2) Location: *Juso, Higashiyodogawa, Osaka, 532, Japan.*
3) E. Higashide, K. Hatano, M. Shibata, and K. Nakazawa, *J. Antibiotics* (Tokyo), **21**, 126 (1968).
4) K. Tsuchiya, M. Kondo, T. Yamazaki, and T. Ohishi, *J. Antibiotics* (Tokyo), **21**, 147 (1968).
5) M. Asai, M. Muroi, N. Sugita, H. Kawashima, K. Mizuno, and A. Miyake, *J. Antibiotics* (Tokyo), **21**, 138 (1968).
6) Trade name, Nikko Chemical Co., Tokyo.
7) Polyoxyethylene derivatives of hydrogenated castor oil. Numerical number following HCO represents the number of oxyethylene unit in one molecule of the surfactant.

Male Sprague-Dawley rats, weighing around 250 g, were used for absorption study. In the most experiments, 0.25 ml of drug solution was injected into the femoral muscles under ether anaesthesia.

For the urinary excretion experiment, the rats were confined in the special animal holders for 24 hours or over few days in order to permit urine collection. Food and water were always freely available to the rats.

In the blood level experiment, the rats were divided into four groups of four rats. Blood samples were withdrawn by heart puncture from respective groups at 4, 8, 12, and 24 hours after intramuscular injection.

Urine and blood specimens were assayed by the cup plate method with *Bacillus subtilis TCI* 219 as test organism in the Microbiological Research Laboratories.

Results

Effect of the Concentration of Surfactant

First of all, urinary excretion of Enduracidin was studied by employing 2.5% of Enduracidin hydrochloride solutions containing the various concentrations of HCO-50, one of the typical surfactants. The urinary excretion of Enduracidin in 24 hours following the injections of 0.25 ml solutions is illustrated in Fig. 1 as a function of HCO-50 concentration. Fig. 1 indicates that the surfactant had a pronounced effect on the muscular absorption of Enduracidin, that is, urinary excretion of Enduracidin increased remarkably by addition of the surfactant and that the increase in urinary excretion was especially prominent up to 1.5% of HCO-50 and the slight increase followed above this concentration in spite of the presence of larger amount of surfactant.

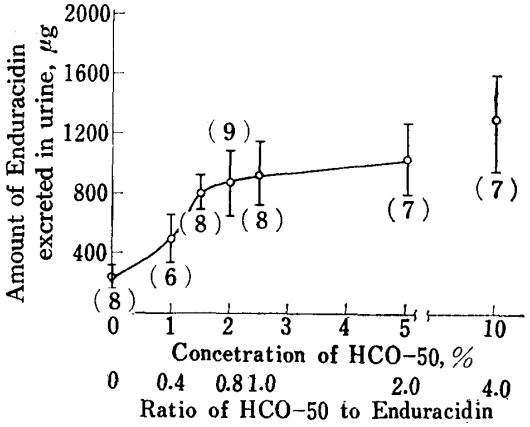

Fig. 1. Effect of HCO-50 on Urinary Excretion of Enduracidin Following Intramuscular Injections to Rats

concentration of Enduracidin; 2.5%
dose; 6.25 mg/rat (0.25 ml injected)
urine collection period; 24 hours
Number of animals used is given in parentheses.

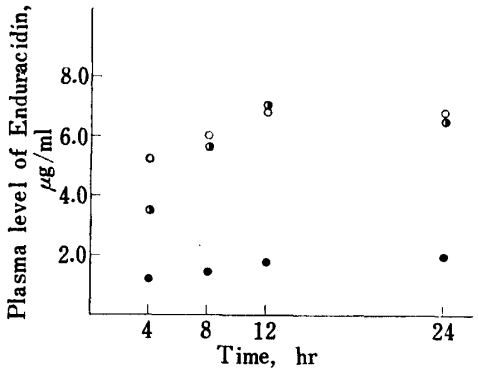

Fig. 2. Effect of HCO-50 on Blood Levels of Enduracidin Following Intramuscular Injections to Rats

concentration of Enduracidin; 2.5%
dose; 6.25 mg/rat (0.25 ml injected)
keys; ●: without HCO-50
○: with 2.5% HCO-50
◑: with 5% HCO-50
Each value is an average of four rats.

In case of 0.5 ml injections, the similar results to those of 0.25 ml injections were obtained and it was found that the urinary excretion was independent of the injected volume. It was suggested from these results that addition of the same or twice amount of the surfactant as that of the antibiotic was practically enough to potentiate the absorption of Enduracidin from the muscles.

Blood level study was carried out to verify the effect of the surfactant on the absorption following the injections of solutions containing 2.5% Enduracidin hydrochloride alone and with 2.5 and 5% of HCO-50, respectively. As shown in Fig. 2, addition of the surfactant surely produced blood levels of Enduracidin more than three fold higher than those obtained

with Enduracidin alone. And there was no significant difference between the blood levels obtained from 2.5 and 5% HCO-50 solutions as expected from the urinary excretion data.

In view of the results obtained in both the urinary and blood level experiments, it was proved that HCO-50 had a pronounced enhancing effect on the intramuscular absorption of Enduracidin.

Effect of the Type of Surfactants

To determine the surfactant having the most potent enhancing effect, HCO-30, -120 and Tween 80, besides HCO-50, were evaluated for their abilities to increase the blood level of Enduracidin. Table I indicates the blood levels after intramuscular injections of the solutions containing 2.5% Enduracidin hydrochloride and 5% of each surfactant.

Table I. Effect of Type of Surfactants on Blood Levels of Enduracidin Following Intramuscular Injections to Rats

Type of surfactant		Plasma level of enduracidin, μg/ml, mean ± s.d. 4 hr	8 hr	12 hr	24 hr	Area under blood level
HCO-30	HLB=11.1	3.0±0.2 (4)[a)]	3.6±0.4 (3)	4.4±0.3 (4)	3.8±0.5 (4)	0.63
HCO-50	HLB=13.4	3.5±0.8 (4)	5.6±0.7 (4)	7.0±0.4 (4)	6.5±1.5 (4)	1.00
HCO-120	HLB=17.3	3.1±0.7 (4)	4.5±0.5 (4)	4.9±1.0 (4)	4.7±0.9 (4)	0.73
Tween-80	HLB=15.0	2.9±0.5 (4)	4.0±0.2 (4)	4.5±0.5 (4)	4.3±0.4 (4)	0.67

a) Number of animals used is given in parentheses.
concentration of Enduracidin; 2.5% concentration of surfactant; 5.0% dose; 6.25 mg/rat (0.25 ml injected)

It was evident from Table I that all the surfactants showed the absorption-enhancing effect and HCO-50 was the most effective one among them in comparison of the areas under the 24-hours blood level curves.

Effect of the Concentration of Enduracidin

In order to investigate the relationship between the rate of absorption and the concentration of Enduracidin, the urinary excretion experiment was performed at its various concentrations from 0.5 up to 10%. Amount of HCO-50 was adjusted to give the same concentration as that of Enduracidin. The results are given in Fig. 3.

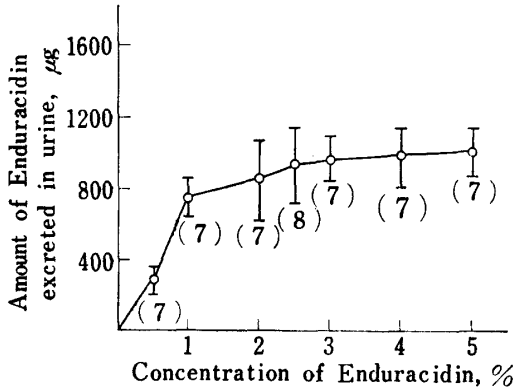

Fig. 3. Urinary Excretion of Enduracidin as a Function of Its Concentration Following Intramuscular Injections to Rats

ratio of HCO-50 to Enduracidin; 1:1
volume injected; 0.25 ml/rat
urine collection period; 24 hours
Number of animals used is given in parentheses.

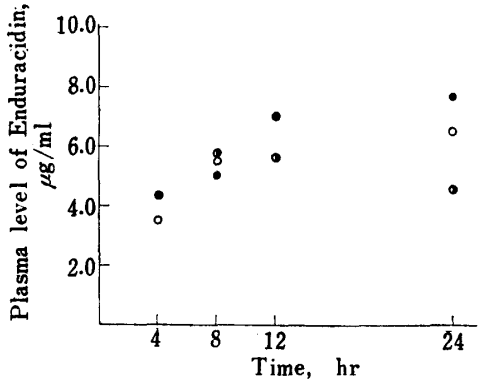

Fig. 4. Plasma Levels of Enduracidin as a Function of Its Concentration Following Intramuscular Injections to Rats

ratio of HCO-50 to Enduracidin; 2:1
volume injected; 0.25 ml/rat
keys: ◑: 1% Enduracidin solution
○: 2.5% Enduracidin solution
●: 5% Enduracidin solution
Each value is an average of four rats.

The urinary excretion of Enduracidin during 24 hours seemed to increase nearly in proportion to the concentration until its concentration was attained to 1%. At the higher concentration than 1%, however, the excreted amount increased only appreciably and the urinary recovery as a function of concentration decreased markedly as the concentration was increased. The similar results were obtained from the blood level experiment, in which 1, 2.5, and 5% Enduracidin solutions containing 2, 5, and 10% of HCO-50, respectively, were used. As illustrated in Fig. 4, there was no significant difference between the blood levels obtained at these three concentration levels for the first 8 hours after the injections. At the period of 12 hours, the blood levels of 2.5 and 5% solutions continued to increase, whereas the injection of 1% solution caused a small decline in the blood level. After 24 hours following the injections, a little increase was observed only in the case of 5% solution.

Furthermore, the corresponding rises did not occur in the peak blood levels with increasing the concentration and it was expected from these results that the higher concentration of Enduracidin probably resulted in the longer duration of comparatively high blood level. To investigate this possibility, frequent urine collection was performed during 24 hours following the injections of 0.5 and 5% Enduracidin solutions. As shown in Fig. 5, although the injection of 5% solution yielded a maximum excretion rate only twice higher than that of 0.5% solution, this high excretion rate was kept throughout from 6 hours, while the excretion rate from 0.5% solution fell into low level in 24 hours.

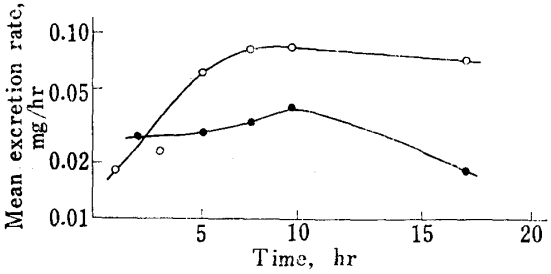

Fig. 5. Urinary Excretion Rates of Enduracidin Following Intramuscular Injections of 0.5 and 5 %Enduracidin Solutions to Rats

ratio of HCO-50 to Enduracidin; 2:1
injected volume; 0.25 ml/rat
keys; ●: 0.5% solution ○: 5% solution

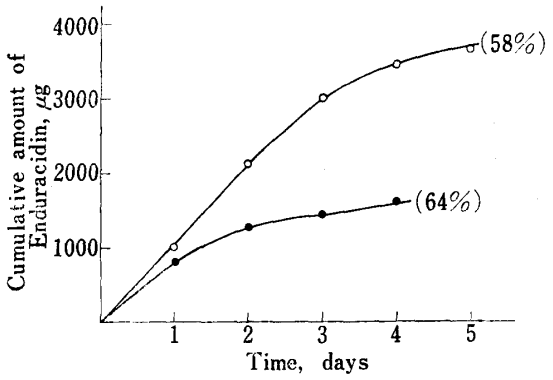

Fig. 6. Cumulative Amount of Enduracidin excreted in Urine Following Intramuscular Injections of 1 and 2.5% Enduracidin Solutions to Rats

ratio of HCO-50 to Enduracidin; 2:1
volume injected; 0.25 ml/rat
keys; ●: 1% solution ○: 2.5% solution

Accordingly, it was conceivable that the prolonged high excretion rate from 5% solution reflected the duration of the blood level. However, these phenomena also lead to one other possibility that absorbability of Enduracidin might be lowered with increasing a size of dose, because of deposition of Enduracidin at the injection site and/or deterioration of the muscles by Enduracidin, if any. To examine this, urine collection was carried out for several days after the injections of 1 and 2.5% of Enduracidin solutions and the urinary recoveries were compared. From the cumulative urinary excretion data shown in Fig. 6, it was found that the urinary excretion from 2.5% solution proceeded at an approximately constant rate of about 1 mg per day for the first three days, then decreased gradually and the total urinary recovery was raised up to 58% of the given dose by the 5th day following the injection, while the injection of 1% solution gave a relatively high excretion rate only in the first day and 64% of the dose was excreted by the 4th day after the injection. Therefore, it was apparent from these urinary recoveries that neither deposition nor deterioration was caused by the higher dose of Enduracidin.

Effect of Addition of EDTA

EDTA has been known to change the permeability of biological membrane and to enhance the absorption of some strong organic electrolytes[8] or water-soluble macromolecular substances[9] from the small intestine.

As Enduracidin is a macromolecular substance and its appearance in the blood stream is retarded, the urinary experiment was carried out by employing 2.5 and 5% Enduracidin solutions containing 2% of EDTA in addition to HCO-50, in order to investigate whether EDTA shows a similar effect on the muscular absorption of the antibiotic. The result in Fig. 7 shows that the urinary excretion of Enduracidin was significantly increased by adding EDTA and suggests that some limiting factors might be involved in the muscular absorption process of Enduracidin.

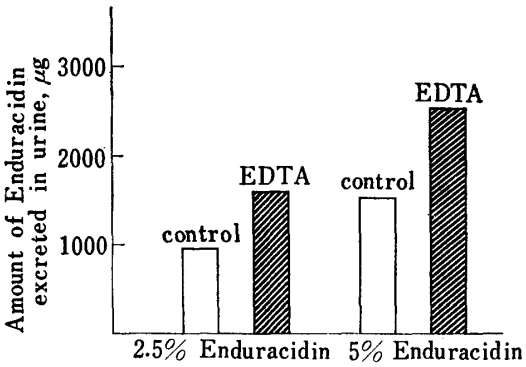

Fig. 7. Effect of EDTA on Muscular Absorption of Enduracidin in Rats Ratio of HCO-50 to Enduracidin; 2:1

concentration of EDTA•2Na; 2%
volume injected; 0.5 ml
urine collection period; 24 hours

Discussion

It can be concluded that the muscular absorption of Enduracidin is remarkably enhanced by the non-ionic surfactants, especially by HCO-50. However, the complicated mechanisms seem to be involved in the absorption-enhancing effect of surfactants.

When the surfactants affect the drug absorption, their effects are considered to be due to the actions on both the biological membrane and the drug. It is reasonable to suppose that one of the enhancing effects observed in the present study could be attributed to the surface tension lowering of the biological membrane, which allows the ready passage of Enduracidin through the muscles, though no attempt was made to obtain the direct evidence.

As for the effects on the drug, that is, on Enduracidin, it appears that one of their major effects is a sort of "protective effect" from the deposition of Enduracidin in the muscles. This is derived from the fact that Enduracidin hydrochloride alone solution was apt to cause precipitation by adding saline to it, whereas its solution with the surfactants did not reveal any changes with saline. Interestingly, a correlationship between the enhancing ability and HLB value of the non-ionic surfactants is not obtained in this study.

It is difficult to elucidate the reason why the urinary excretion from 2.5% Enduracidin solution was remarkably enhanced until the concentration of HCO-50 reached at 1.5% and it increased only slightly when the concentration became higher than 1.5%, since this small increase seems to be overlapped by the absorption characteristics of Enduracidin itself.

Concerning the muscular absorption process of Enduracidin, the fact that neither 24 hours urinary excretion nor the peak blood level did increase dependently on the concentration of Enduracidin and on the volume of injection leads to a doubt whether Enduracidin is not completely available from the intramuscular injection, due to its deposition or to some toxic effects on the muscles with increasing its concentration, that is, a size of dose. However, this doubt can be excluded from the excretion data in Fig. 5 and 6, which indicate that the concentration of Enduracidin is mainly related to the length of duration in the blood level. This duration of blood level suggests the following three possibilities on the bio-

8) L.S. Schanker and J.M. Johnson, *Biochem. Pharmacol.*, **8**, 421 (1961).
9) C.S. Tidball, *Am. J. Physiol.*, **206**, 243 (1964).

pharmaceutical aspect of Enduracidin. First, some rate-limiting factors are involved in either metabolic or excretory process of Enduracidin. Secondly, the antibiotic can not be easily transferred to the body tissues from the blood stream, owing to its plasma-protein binding. And finally, its penetration process through the muscular cell membrane is rate-limiting, probably due to its larger molecular volume and poor lipid solubility. The former two possibilities may be ruled out from the fact that excretion of Enduracidin was considerably rapid and about 60% of the given dose was excreted in 24 hours without losing its activity when it was administered to the rats by the intravenous route. A fact that addition of EDTA showed an absorption-enhancing effect is likely to provide one of the convincing evidences for the last possibility.

In conclusion, it is evident from all the experimental results that absorption of Enduracidin from the muscles is enhanced with the aid of the non-ionic surfactants, its appearance in the blood stream is delayed a little after the injection, its blood level is kept for a prolonged period once the high blood level is attained, and that the duration of the blood level is dependent on the size of dose, though unknown mechanisms on the effects of the non-ionic surfactant still remain to be investigated further. And it can be also expected that Enduracidin is available for clinical use in the form of intramuscular injection.

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