

[Chem. Pharm. Bull.]
30(6)2245-2247(1982)

Oral Administration of Liposomally-Entrapped Heparin to Beagle Dogs

MASAHARU UENO,^{*,a} TAKASHI NAKASAKI,^a ISAMU HORIKOSHI,^a
and NOBUO SAKURAGAWA^b

*Department of Hospital Pharmacy, Toyama Medical and Pharmaceutical University,^a
Sugitani, Toyama-shi 930-01, Japan and Central Clinical Laboratory, Toyama
Medical and Pharmaceutical University,^b Sugitani,
Toyama-shi 930-01, Japan*

(Received November 30, 1981)

The oral administration of heparin alone or entrapped in liposomes was investigated. The prolongation of clotting time (a-PTT) was observed after administration of heparin and attributed to an increase of heparin activity in the blood as a result of effective absorption from the intestine. The increase of heparin activity was promoted by entrapping heparin in liposomes, but not by mere addition of empty liposomes.

Keywords—oral administration; heparin; liposome; a-PTT; intestine

Several applications of liposomes as a drug carrier have been investigated. These include targeting of drugs to the active site,^{1,2)} enzyme-replacement therapy^{3,4)} and immunologic adjuvants.⁵⁾ Recently, oral administrations of some drugs, such as insulin^{6,7)} and antihemophilic drugs,^{8,9)} which are poorly absorbed from the gastrointestinal tract, were tried by the use of liposomes. The results suggest that such a drug, entrapped in liposomes, can be absorbed from the intestine, although the extent of the absorption is small. In this report, we studied the oral administration of heparin alone or entrapped in liposomes and compared the results.

Heparin is a typical anti-coagulant drug, whose mode of action is to accelerate the rate of neutralization of activated serine proteases by antithrombin III. Heparin is usually active only when it is administered intravenously or subcutaneously. Recently, Sezaki *et al.* reported that the absorption of heparin from the intestine was promoted by addition of appropriate adjuvants, such as monoolein-sodium taurocholate,^{10,11)} but their administration method was direct introduction of heparin into the intestinal loop, and not oral. Therefore loss of activity of heparin through the gastrointestinal tract was not considered. In this work, we administered heparin orally to beagle dogs and measured the clotting time and heparin activity in the blood.

Pure heparin was supplied by Nikken Chemicals. Egg lecithin (from Asahi Kasei Co. Ltd.) and stearylamine (from Sigma Co. Ltd.) were used for the preparation of liposomes.

Preparation of Heparin-entrapped Liposomes: The inner surface of a 1 l round-bottomed flask was coated with 1 g of lecithin containing 5% stearylamine by solvent evaporation. Then 2.5 g (corresponding to 400000 units) of heparin was dissolved in 10 ml of 0.1 M phosphate buffer (pH 7.0), and poured into the lecithin-coated flask. The liposome suspension was centrifuged for 30 min at 27000 *g* at 4°C. The aqueous phase was removed with a syringe and used to make a second liposome preparation. All liposome preparations were pooled and washed once with isotonic saline followed by centrifugation at 40000 *g* for 10 min. The amount of heparin entrapped in liposomes was measured to be 940 mg (corresponding to 150000 units) by the toluidine blue method.¹²⁾

Administration: Case I; heparin (150000 units) was dispersed in 200 ml of milk and orally administered to two beagle dogs (♀, 10 kg each). Case II; heparin (150000 units) entrapped in liposomes was dispersed in 200 ml of milk and orally administered to the beagle dogs. Case III; heparin (150000 units) and empty liposomes (entrapping normal saline) were simultaneously dispersed in 200 ml of milk and orally administered to the beagle dogs.

Clotting Study and Heparin Assay: Blood was taken from a bronchial vein before administration and 1, 2, 4, 6 and 24 h after oral administration. It was prevented from coagulating with 3.8% sodium citrate solution. Test plasmas were separated by centrifugation at 3000 rpm for 15 min at 4°C. The a-PTT (activated partial thromboplastin time) was measured by using test reagents from DADE Co. Ltd. Heparin activity was measured as follows: diluted pooled normal plasma was added to a heparinized plasma sample to eliminate the effects of possible variations in test plasma antithrombin III levels; thrombin was added and the mixture was incubated; residual thrombin activity was determined kinetically by measuring the release of the fluorescent molecule, 5-aminoisophthalic acid dimethyl ester, from the substrate, D-phenylalanine-proline-arginine-5-amino-isophthalic acid dimethyl ester. The details were presented in related papers.^{13,14)}

Time dependences of a-PTT after oral administration of heparin are shown in Fig. 1. The extent of the difference in a-PTT between the two dogs is 1–5 s. In each case in Fig. 1, the a-PTT was prolonged compared to the control. The prolongation of a-PTT after oral administration of heparin entrapped in liposomes was the greatest. The a-PTT reached a maximum of 64 s 4 h after administration. In the case of administration of heparin alone (without liposomal entrapment), the maximum of a-PTT was observed 2 h after administration and was 40 s. The extent of prolongation of a-PTT after simultaneous administration of heparin and empty liposomes was less than that of heparin entrapped in liposomes and was rather similar to that of heparin alone. In Fig. 2, the time dependence of heparin activity in blood is shown. The prolongation of a-PTT (in Fig. 1) and the increase of heparin activity in the blood (in Fig. 2) showed similar time dependences. We concluded that the prolongation of a-PTT after oral administration of heparin was attributable to the increase of heparin activity in the blood due to effective absorption of heparin from the intestine and that the increase of heparin activity

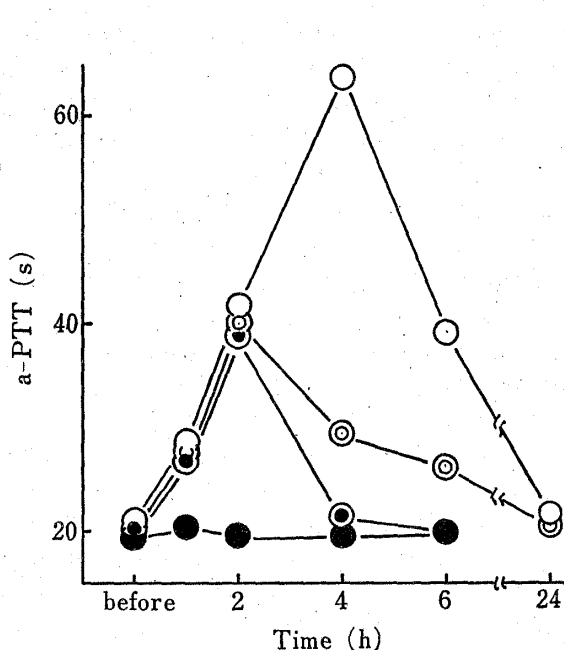


Fig. 1. Time Dependences of a-PTT after Oral Administration of Heparin

- : heparin (150000 units) entrapped in liposomes.
- ⊙: heparin (150000 units) alone.
- ⊙: heparin (150000 units) + normal saline-containing liposomes.
- : normal saline-containing liposomes as a control.

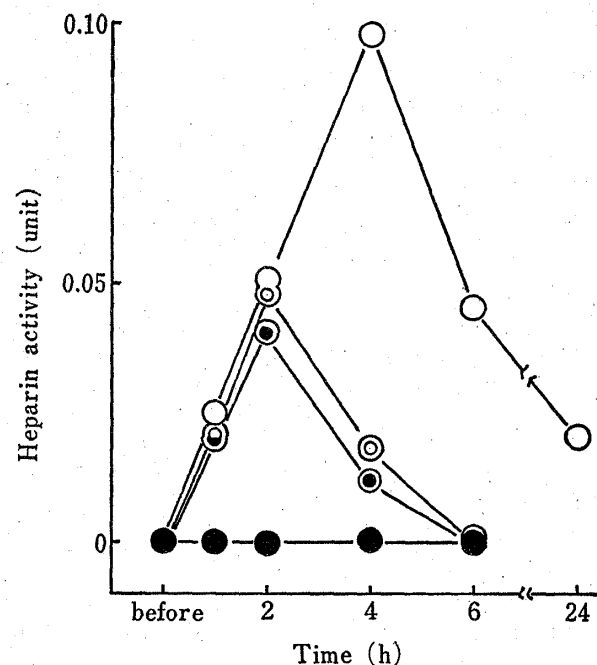


Fig. 2. Time Dependences of Heparin Activity in the Blood after Oral Administration of Heparin

- : heparin (150000 units) entrapped in liposomes.
- ⊙: heparin (150000 units) alone.
- ⊙: heparin (150000 units) + normal saline-containing liposomes.
- : normal saline-containing liposomes as a control.

was promoted by entrapping heparin in liposomes, though not by mere addition of empty liposomes. It was not established whether inactivation of heparin by acidic gastric juices and various enzymes in the gastrointestinal tract was depressed by entrapping the heparin in liposomes or whether absorption of heparin from the intestine was promoted by membrane fusion between intestinal cell membranes and liposomal membranes, or both.

References

- 1) Y.E. Rahman, W.E. Kisielleski, E.M. Buess, and E.A. Cerny, *Eur. J. Cancer* **11**, 883 (1975).
- 2) D. Papahadjopoulos, G. Poste, W.J. Vail, and J.L. Biedler, *Can. Res.*, **36**, 2988 (1976).
- 3) G. Gregoriadis and R.A. Buckland, *Nature*, **244**, 170 (1973).
- 4) R.O. Brady, P.G. Pentchev, and A.E. Gal, *Fed. Proc.*, **34**, 1310 (1975).
- 5) A.C. Allison and G. Gregoriadis, *Nature*, **252**, 252 (1974).
- 6) G. Gregoriadis, *N. Engl. J. Med.*, **295**, 704 (1976).
- 7) H.M. Patel and B.E. Ryman, *FEBS Lett.*, **62**, 60 (1976).
- 8) H.C. Hemker, W. Th. Hermens, A.D. Muller, and R.F.A. Zwall, *Lancet*, **8159**, 70 (1980).
- 9) M. Ueno, I. Horikoshi, K. Takahashi, and N. Sakuragawa, *Yakugaku Zasshi*, **102**, 202 (1982).
- 10) Y. Tokunaga, S. Muranishi, and H. Sezaki, *J. Pharm. Dyn.*, **1**, 28 (1978).
- 11) K. Taniguchi, S. Muranishi, and H. Sezaki, *Int. J. Pharm.*, **4**, 219 (1980).
- 12) D.T. Graham and A.R. Pomeroy, *Thrombos. Haemostas.* **42**, 1598 (1979).
- 13) A.N. Teien and M. Lie, *Thromb. Res.* **10**, 399 (1977).
- 14) J. Estes and P. Poulin, *Thromb. Diath. Haemorrh.* **33**, 26 (1974).