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BILE SALTS FACILITATE THE ABSORPTION OF HEPARIN FROM THE INTESTINE

E. ZIV,* A. ELDOR,† Y. KLEINMAN,* H. BAR-ON* and M. KIDRON‡

Department of Medicine B (and *Lipid Research Laboratory), †Hematology and ‡Clinical Biochemistry, Hadassah University Hospital, Jerusalem, Israel

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Abstract—Heparin was absorbed through the rectal mucosa of rodents and primates only when administered in solutions containing sodium cholate or sodium deoxycholate (DOC). The absorption of heparin was monitored by following the increase in plasma radioactivity after administration of [^{35}S]heparin, and by measurement of its biological effects: plasma lipase activity and prolongation of partial thromboplastin time (PTT). Following administration of the same concn of heparin in solutions lacking bile salts, there was almost no radioactivity in the blood, no prolongation of PTT and no release of plasma lipase activity. The PTT effect was found to be a less sensitive test of heparin absorption than the plasma lipase activity.

Heparin, a most potent anticoagulant, is widely used in the treatment and in the prevention of thromboembolism [1]. The drug has proved efficacious in the treatment of patients with venous or arterial thrombosis, when administered at high doses which prevent coagulation of the blood. It has also been shown that at doses insufficient to block coagulation, heparin is most effective in preventing deep postoperative venous thrombosis and significantly reduces the frequency of fatal pulmonary embolisms [2–6].

Heparin treatment is usually limited to hospitalized patients since the drug is given only by injection. Alternative routes have been attempted, and include an intrapulmonary spray or the administration of suppositories of heparin coupled with EDTA, acidic buffer, or sulfated and sulfonated surfactants, which facilitate its absorption through the gastrointestinal mucosa [7–9].

In a previous publication, we reported that heparin in solution with cetomacrogol 1000, a nonionic surfactant (detergent), is absorbed from the colon mucosa of rats, as evidenced by the prolongation of the partial thromboplastin time (PTT) [10]. The application of this method to human use was limited, however, owing to the possible adverse local effects of the poly-oxyethylene ether. Therefore the use of bile salts as physiological detergents was suggested [11].

We report here that heparin in solution with physiological concns of sodium cholate, or sodium deoxycholate (DOC), is absorbed through the colon mucosa of rodents and primates.

MATERIALS AND METHODS

Male rats of the Hebrew University strain, weighing 230–280 g, were fed with pelleted chow. A 10-year-old baboon, weighing 24 kg, was fed a fruit diet. In all the experiments, the animals were fasted overnight. Drug administration and blood sampling of the rats for PTT and lipase activity were performed under ether anesthesia; pentobarbital was used for the baboon.

Heparin was administered by enema: a solution of bile acids was mixed in saline to the desired concn. The rat enema vol. was 1 ml; the mixed solution was administered into the lower colon with a plastic injector inserted approximately 3 cm from the anus, and the anus was closed with a metal clip during the experiment. When the baboon was tested, the desired solution, in a vol. of 10–20 ml, was inserted into the colon, 30 cm from the anus, via a plastic injector with a plastic catheter. In both cases, control solutions containing either heparin or bile acids alone were also administered.

Two millilitre blood samples were taken from the rats, by cardiac puncture. Each animal was sampled once, either before, or 5 min after drug administration. In several experiments, 0.3 ml blood was collected from the tail vein 1 min before, and 5, 10, 15, 20 and 30 min after drug administration. Blood samples of 5 ml were taken from the leg veins of the baboon during the experiment.

The PTT of the plasma samples was determined as reported previously [12]. An *in vitro* calibration curve was constructed by the addition of known concns of heparin to the baboon plasma.

Plasma lipase activity was determined in the plasma samples according to Nilsson-Ehle and Schotz [13] under conditions which allowed full expression of both hepatic and lipoprotein lipase. Enzyme units were calculated using the same method.

The absorption of radioactive heparin was determined in rats as follows. Microenema were administered containing 2 μCi [^{35}S]heparin and 1000 I.U. heparin, DOC, if added, at a concn of 2 mg/ml (4.8 mM) and sodium cholate at a concn of 10 mg/ml (23 mM). Blood samples of 0.2 ml were taken

from the femoral artery, and the plasma was counted in vials containing scintillation fluid and 20% Triton X-100.

DOC, sodium cholate and glycerol trioleate were purchased from Sigma Chemical Co. (St. Louis, MO), [^{3}H]glycerol trioleate (500 mCi/mmole) and [*N*-sulphonate-^{35}S]heparin (9.56 mCi/g) were purchased from the Radiochemical Centre (Amersham, U.K.).

The results are expressed as mean values ± S.E.M.

RESULTS

Rectal administration of heparin in the rat

Heparin was absorbed through the rectal mucosa of the rat only when administered in solutions containing sodium cholate or DOC. This was evidenced by the gradual increase in plasma radioactivity following the rectal administration of 2 μCi [^{35}S]heparin (1000 I.U./animal) in a solution containing 2 mg/ml (4.8 mM) DOC or 10 mg/ml (23 mM) sodium cholate. A plateau of 12,000–16,000 cpm/ml plasma was reached 8–12 min after drug administration. Administration of the same amount of heparin, but without bile acids, resulted in almost no radioactivity in the blood (<1000 cpm/ml plasma).

Heparin absorption was also monitored by measuring its biological effects: its anticoagulant effect expressed by prolongation of PTT and the activation of plasma lipase. Five minutes after the administration of the heparin–bile acid solutions an increase in plasma lipase activity was observed. The dose-dependent effect of heparin on plasma lipase activity in the rat is described in Fig. 1(a) and (b). Heparin administered alone did not effect plasma lipase activity. The increase in plasma lipase activity following the absorption of heparin was logarithmically linear at heparin doses of 125–2000 I.U. Similar curves were obtained for heparin–sodium cholate solutions as well as for heparin–DOC solutions. Prolongation of PTT occurred only after the administration of high doses of heparin–bile acid solutions: 1000 I.U. heparin when mixed with sodium cholate and 2000 I.U. when mixed with DOC. There was no prolongation when the same concns of heparin were administered in the absence of bile acids.

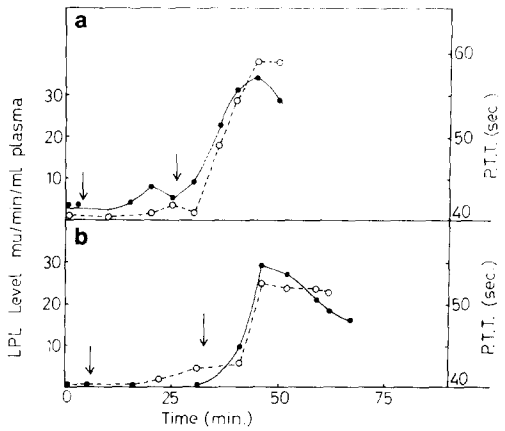

Fig. 2. Rectal administration of heparin to the baboon. (●– – –●) Plasma lipase activity. (○– – –○) PTT. (a) First arrow: administration of 1000 I.U. heparin in solution with 2 mg/ml DOC. Second arrow: administration of 20,000 I.U. heparin in solution with 2 mg/ml (4.8 mM) DOC. (b) First arrow: administration of 2000 I.U. heparin. Second arrow: administration of 45,000 I.U. heparin in solution with 10 mg/ml (23 mM) cholate.

Enteral administration of heparin in the baboon

In the baboon, heparin was absorbed through the rectal mucosa only when administered in solution with bile acids. The results of typical experiments are shown in Fig. 2(a) and (b); again heparin absorption was monitored by lipase activity and the prolongation of PTT. Lipase activity was found to be the more sensitive parameter for the detection of

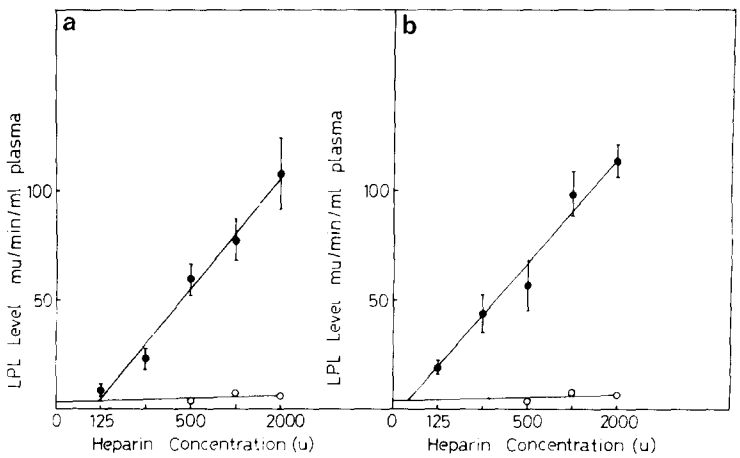

Fig. 1. Dose–response curves of heparin rectally administered to rats. Plasma lipase activity was measured in 10 and 20 μl plasma 5 min after heparin administration. (●– – –●) Heparin mixed with bile acids. (○– – –○) Heparin alone. (a) Heparin mixed with 2 mg/ml (4.8 mM) DOC. (b) Heparin mixed with 10 mg/ml (23 mM) sodium cholate. Each point represents the mean ± S.E.M. of five to nine observations.

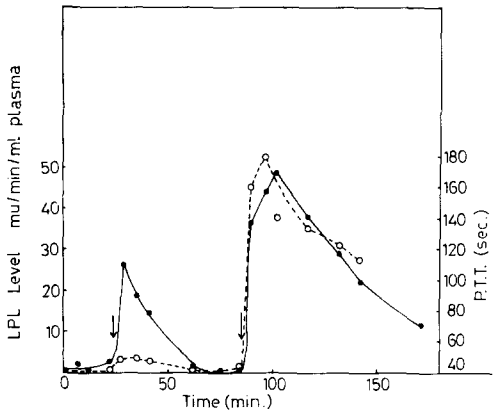

Fig. 3. Injection (i.v.) of heparin to the baboon. (●– – –●) Plasma lipase activity. (○– – –○) PTT. First arrow: i.v. injection of 100 I.U. heparin. Second arrow: i.v. injection of 1000 I.U. heparin.

heparin in the plasma. Doses of heparin as low as 100 I.U. given intravenously induced a substantial rise in plasma lipase activity together with minimal changes in PTT (Fig. 3). Larger doses of heparin [1000 I.U. i.v. or a high enema of 20,000 I.U. also containing 2 mg/ml (4.8 mM) DOC or 10 mg/ml (23 mM) sodium cholate] resulted in a parallel increase in both activities.

DISCUSSION

In this report we describe a new method for the administration of heparin through the intestinal tract of rodents and primates. The absorption of this high mol. wt glycoseaminoglycan was facilitated by the addition of naturally occurring detergents—the bile salts. The advantage in the use of bile salts over nonbiological detergents such as cetomacrogol 1000 is obvious. At concns of 2–4 mg/ml (4.8–9.6 mM) DOC did not cause any morphological changes in the rat jejunal mucosa cells observed by light microscopy. At concns of 10 mg/ml DOC only slight damage was detected in the microvilli using electron microscopic techniques [14].

Heparin absorption was monitored by the administration of [^{35}S]heparin mixed with cholate. Ten to 20 min following rectal administration 3–5% of the radioactivity appeared in the arterial circulation. The absorption of heparin from the intestine was also monitored by following two biological effects of the drug: plasma lipoprotein lipase activity and prolongation of the PTT. The results indicated that there is a linear correlation between the plasma lipase activity and the dose of heparin administered to the rat. The heparin-induced anticoagulant effect monitored by the prolongation of PTT was found to be a less sensitive test for the absorption of heparin (Table 1).

By extrapolation, calculating PTT and lipase activity vs concns of heparin mixtures administered, the percentage of heparin that was absorbed into the baboon's circulation was similar to the heparin absorbed into the rat blood circulation.

We have previously shown that the rectal administration of insulin in solution with bile salts facilitates the absorption of this macromolecule [11]. These results as well as the rectal absorption of heparin in solution with bile salts in the present experiments suggest that detergents in general, and bile salts in particular, modify membrane permeability, thus enabling the transport of macromolecules through the gut mucosa into the circulation.

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Table 1. Partial thromboplastin time (PTT) (sec) of rat plasma 5 min following heparin administration

Heparin doses administered (I.U.)	Enteral administration		
	Heparin + sodium cholate [10 mg/ml (23 mM)]	Heparin + DOC [2 mg/ml (4.3 mM)]	Control (heparin alone)
125	62 ± 3	45 ± 5.2	—
500	69 ± 3.7	50 ± 3.4	52 ± 5.4
1000	81 ± 8.4	51 ± 5.2	—
2000	163 ± 33	140 ± 25	56 ± 4.6

Each result represents mean ± S.E.M. of five to nine observations.

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