

## Rectal absorption of some glycosaminoglycan sulphates and heparin in rats

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A standardized extract of glycosaminoglycan sulphates containing heparin, with a low affinity for antithrombin III, and a commercial heparin were administered to rats, by the rectal route. When the glycosaminoglycan sulphates were given in oil emulsion with sodium laurylsarcosinate as surfactant, 1 mg kg<sup>-1</sup> and 3 mg kg<sup>-1</sup> were sufficient for the clearing and anticoagulant activities respectively. The rectal absorption of glycosaminoglycans after dosing with a suitable 'promoter' produced dose-dependent effects and their kinetics were comparable to those obtained after intramuscular administration. The oil emulsion improved the bioavailability of glycosaminoglycan sulphates at least 20 times.

Some controversy surrounds the gastrointestinal absorption of heparin and some other glycosaminoglycan sulphates (GAG). Recent studies (Sim 1978; Niada et al 1979) have demonstrated that when certain fractions of the GAG closely related to heparin are administered orally, they exert an antithrombotic action. The intestinal absorption of labelled GAG fractions after intraduodenal administration has also been demonstrated using fluorescein (Pescador et al 1980).

Rectal absorption of heparin has been described by Bianchini et al (1976) and by Kidron et al (1979), who observed significant modifications of the coagulation parameters after high doses of heparin (10 000 U kg<sup>-1</sup>).

It was our intention to improve the rectal absorption of heparin and other GAG by adding a surface-active agent to formulations containing them, and to evaluate differences in absorption between the different mixtures of GAG.

### *Material and equipment*

Commercial heparin with a titre of 156 I.U. mg<sup>-1</sup> = 40 LPL-RU† mg<sup>-1</sup>; 3GS (Radhakrishnamurthy et al 1978) with a titre of 74 I.U. mg<sup>-1</sup> = 20 LPL-RU mg<sup>-1</sup> (contained in VESSEL, Alfa Farmaceutici S.p.A). 3GS is a standard extract of GAG such as heparin, with a low affinity for antithrombin

\* Correspondence.

† LPL-RU = lipoprotein lipase releasing unit. One LPL-RU corresponds to the quantity of substances which, when injected intravenously to rats of 180 ± 10 g, provokes after 10' the release of a sufficient amount of clearing factor into the circulation, to induce a 50% decrease in vitro of absorbance of (Ediol is a triglyceride emulsion). a 1:2 Ediol-plasma mixture within 15' (Bianchini et al 1972).

III (Bianchini 1980), heparan sulphate, and dermatan sulphate; triacetin (Gianni); sodium laurylsarcosinate (Hoechst); Lipostrate (Calbiochem); PTT reagent for the determination of the partial thromboplastin time (Boehringer Mannheim GmbH Diagnostics); 'Thrombin' reagent for the determination of the thrombin time (Boehringer Mannheim GmbH Diagnostics); Schnitger and Gross coagulometer.

### *Method*

Groups of six female rats (S.D.), 150 ± 10 g, were treated with volumes 0.3 ml containing: 200, 100, or 50 LPL-RU in 0.9% NaCl (saline), equal to about 1300, 650, and 325 LPL-RU kg<sup>-1</sup> 3GS and 100, 50, 25, 12.5, 6.25, and 3.1 LPL-RU of 3GS and heparin, equal to about 650, 320, 160, 80, 40, and 20 LPL-RU kg<sup>-1</sup>, in an oil emulsion prepared by emulsifying the 3GS or heparin and an equal weight of 30% sodium laurylsarcosinate in a final volume of 1 ml of triacetin.

Administration was carried out using a sonde that enabled the various oil or aqueous suspensions to be deposited directly in the rectum, the anal orifice being immediately sealed with plaster to prevent expulsion of the injected emulsion.

The animals were anaesthetized with ether and killed, 5, 10, 20, 30, and 60 min after the dosing.

The lipoprotein lipase-inducing activity was determined in the plasma using the method of Bianchini et al (1972),‡ and the anticoagulant activity by deter-

‡ Based on the capacity of the plasma to 'clarify' an artificial lipid system.

mining the thrombin time and the partial thromboplastin time by the methods of Biggs & McFarlane (1962) and by Larrieu & Weiland (1957).

### RESULTS

Fig. 1. shows the effects of the absorption of 3GS administered in saline by the rectal route. There is a good correlation between the dose and the lipoprotein lipase-inducing effect. The advantage of

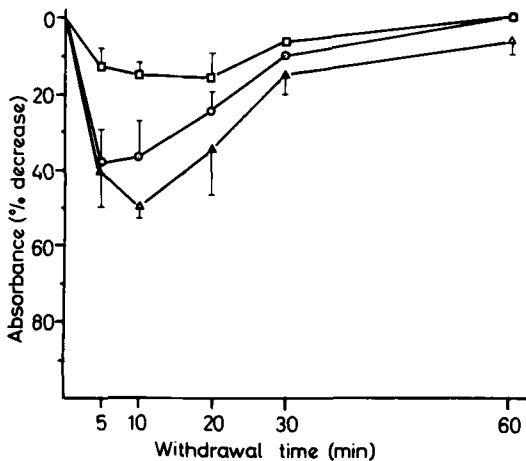


FIG. 1. Lipoprotein lipase-inducing action. Effect of administering 3GS in saline by the rectal route. 'Clarification' of the plasma-Ediol system after incubation for 15 min at 25 °C (ordinate), as a function of time after the administration.

△ 1300 LPL-RU kg<sup>-1</sup> = 64 mg kg<sup>-1</sup> of 3GS in saline  
○ 650 LPL-RU kg<sup>-1</sup> = 36 mg kg<sup>-2</sup> of 3GS in saline  
□ 325 LPL-RU kg<sup>-1</sup> = 16 mg kg<sup>-1</sup> of 3GS in saline

administering GAG in an oil emulsion, as opposed to administration in saline, is shown in Fig. 2. Tests not shown indicated that the vehicle itself was inactive. There was a good correlation between the clarifying effect and the dose administered, at least at the lower doses. The correlation between the dose and the effect for the doses of 160, 325, and 650 LPL-RU kg<sup>-1</sup> is not so good, possibly for two reasons. In the first place, the geometric function used to express the course of the percentage reduction of the absorption as a function of the dose, is sigmoidal, being described in good approximation by Hill's equation (Mimmo & Bauer 1977); it is therefore no longer linear at the higher concentrations. Secondly, above 80 LPL-RU kg<sup>-1</sup> the action of the drug becomes longer-lasting. This might suggest that there is a hypothetical saturation threshold of the drug, above which there is no longer a correspondence between the dose and the effect.

The anticoagulant activity of 3GS is transient when 60 LPL-RU kg<sup>-1</sup> administered, whereas it is persistent at a dose of 120 LPL-RU kg<sup>-1</sup> (Fig. 3).

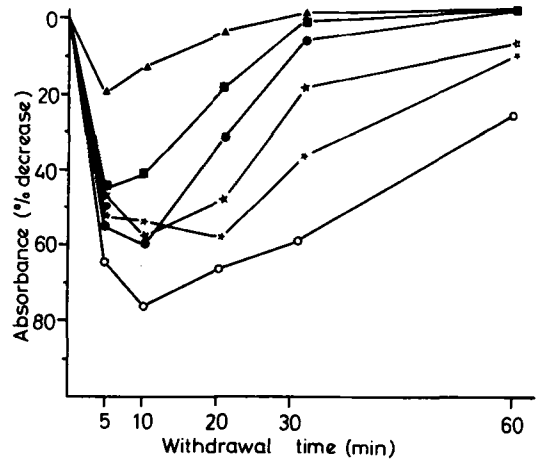


FIG. 2. Lipoprotein lipase-inducing action. Effect of the administration of 3GS by the rectal route in an oil suspension. 'Clarification' of the plasma-Ediol system after incubation for 15 min at 25 °C.

○ 650 LPL-RU kg<sup>-1</sup> = 32 mg kg<sup>-1</sup> of 3GS in an oil suspension  
\* 325 LPL-RU kg<sup>-1</sup> = 16 mg kg<sup>-1</sup> of 3GS in an oil suspension  
☆ 160 LPL-RU kg<sup>-1</sup> = 8 mg kg<sup>-1</sup> of 3GS in an oil suspension  
● 80 LPL-RU kg<sup>-1</sup> = 4 mg kg<sup>-1</sup> of 3GS in an oil suspension  
■ 40 LPL-RU kg<sup>-1</sup> = 2 mg kg<sup>-1</sup> of 3GS in an oil suspension  
▲ 20 LPL-RU kg<sup>-1</sup> = 1 mg kg<sup>-1</sup> of 3GS in an oil suspension

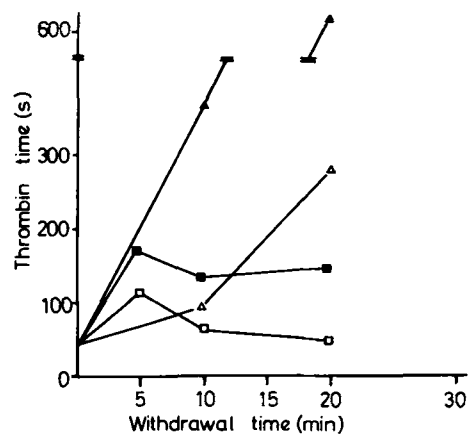


FIG. 3. Variation of thrombin time (TT) as a function of time after the administration of 3GS.

□ 60 LPL-RU kg<sup>-1</sup> with equal amounts of the surface-active agents.  
△ 120 LPL-RU kg<sup>-1</sup> with equal amounts of the surface-active agents.  
■ 60 LPL-RU kg<sup>-1</sup> with 4 times as much of the surface-active agents.  
▲ 120 LPL-RU kg<sup>-1</sup> with 4 times as much of the surface-active agents.

Clearly, the threshold at which the lipoprotein lipase action appears is lower (20 LPL-RU  $\text{kg}^{-1}$ , or about 1 mg  $\text{kg}^{-1}$ ) than the threshold for activation of the coagulation system (60 LPL-RU  $\text{kg}^{-1}$ , equal to about 3 mg  $\text{kg}^{-1}$ ).

In contrast, the rates at which the respective activities appear are about the same, and are comparable with the rate at which the lipoprotein lipase-inducing action appears when the product is administered intravenously (Intramuscular administration of 3GS to rats produces an activity with a maximum that occurs later than that obtained by the rectal route, Bianchini et al 1978.)

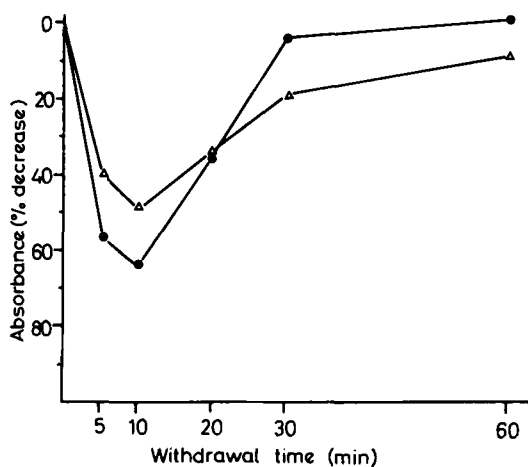


Fig. 4. Lipoprotein lipase-inducing action. Percentage reduction of the absorbance (ordinate) of the plasma-Ediol system after incubation for 15 min at 25 °C, as a function of the sampling time (abscissa). ● 80 LPL-RU  $\text{kg}^{-1}$  of 3GS in an oil suspension  $\Delta$  1300 LPL-RU  $\text{kg}^{-1}$  3GS in saline.

The activity by the rectal route is already very marked 5 min after the administration; it persists after 10 min, and begins to decrease after 20 min (Fig. 2).

The efficiency with which the GAG are transported by the oil emulsion is illustrated in Fig. 4, which shows that the bioavailability is about 20 times higher for the oil emulsion compared with the saline solution with respect to the lipoprotein lipase inducing action.

Fig. 5 shows the effects of the rectal absorption of heparin administered in an oil suspension in doses of 650, 320, 160, 80, 40, and 20 LPL-RU  $\text{kg}^{-1}$ . The results obtained are analogous to those obtained with 3GS, in terms of both the activity and the rate at which it appears.

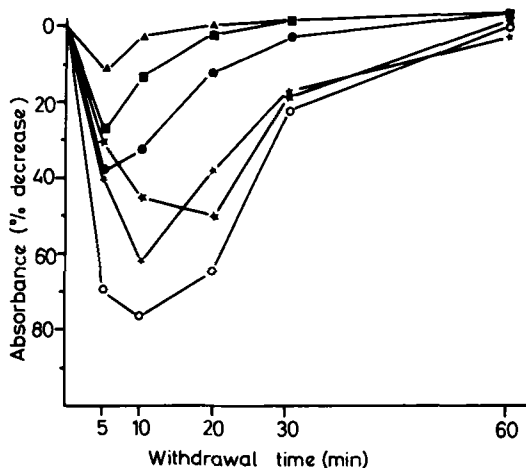


Fig. 5. Lipoprotein lipase-inducing action. Effect of administering heparin by the rectal route in an oil suspension. 'Clarification' of the plasma-Ediol system after incubation for 15 min at 25 °C.

○ 650 LPL-RU  $\text{kg}^{-1}$  = 32 mg  $\text{kg}^{-1}$  in an oil suspension  
 \* 325 LPL-RU  $\text{kg}^{-1}$  = 16 mg  $\text{kg}^{-1}$  in an oil suspension  
 ✱ 160 LPL-RU  $\text{kg}^{-1}$  = 8 mg  $\text{kg}^{-1}$  in an oil suspension  
 ● 80 LPL-RU  $\text{kg}^{-1}$  = 4 mg  $\text{kg}^{-1}$  in an oil suspension  
 ■ 40 LPL-RU  $\text{kg}^{-1}$  = 2 mg  $\text{kg}^{-1}$  in an oil suspension  
 ▲ 20 LPL-RU  $\text{kg}^{-1}$  = 1 mg  $\text{kg}^{-1}$  in an oil suspension

#### DISCUSSION

The results confirm that 3GS and heparin can be absorbed well through the mucosa of the colon, and better from an oil emulsion than from an aqueous solution.

The absorption is dose-dependent. The improved absorption caused by the vehicle used may be due to reduced ionization of the functional groups of the GAG (Sue et al 1976) and to higher permeability of the mucosa (Tokunaga et al 1978).

The rectal absorption of GAG transported by a vehicle produces effects with kinetics that are comparable, at least, to those obtained after intramuscular administration to rats.

Nishioka et al (1977) and Nishihata et al (1981) have shown that the rectal absorption of some drugs is improved by the simultaneous administration of sodium lauryl sulphate or ethylenediaminetetraacetic acid but there was some damage to the rectal membrane.

In preliminary toxicological studies, after the administration of the formulation we used to rats for eight days, we have found in a few animals signs of an initial irritation of the rectal mucous membrane.

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