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Bioavailability of Desmin, a low molecular weight dermatan sulfate, after subcutaneous administration to healthy volunteers

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Abstract The bioavailability of two different s.c. doses of Desmin (a new low molecular weight dermatan sulfate) was evaluated in 12 healthy volunteers (6 men, 6 women aged 22–45 years) who were injected, on 3 separate days and with a wash-out period of at least 21 days between each administration, with 200 and 300 mg of Desmin by the s.c. route and 200 mg by the i.v. route. Immediately before injection and at various times thereafter (after 15 min and 30 min for i.v. only and after 1, 2, 3, 4, 6, 8, 12, and 24 h for both s.c. and i.v. dosing), blood samples were drawn to investigate bioavailability by measuring several coagulation parameters: activated partial thromboplastin time, thrombin time, inhibition of factor Xa, Heptest, and heparin cofactor II. Furthermore the local tolerance of the s.c. and i.v. injections were investigated. The s.c. administration of the two Desmin doses had a negligible effect on the activated partial thromboplastin time and a very small effect on the thrombin time, measured with human thrombin; in contrast, Heptest, heparin cofactor II, and anti-Xa activities increased, with a good drug bioavailability (more than 100%). The plasma effects of Desmin were dose dependent only when measured by Heptest, which also gave a greater response after the s.c. administrations. There were no symptoms of intolerance or pain at the injection site after single i.v. and s.c. Desmin administrations.

Key words Low molecular weight dermatan sulfate · Desmin · Healthy volunteers · Bioavailability · Coagulation

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Introduction

Dermatan sulfate is a natural glycosaminoglycan which enhances the inactivation of thrombosis by heparin cofactor II (HC II) [1]. Desmin is a low molecular weight dermatan sulfate obtained from native dermatan sulfate by free radical depolymerization with subsequent chelating resin chromatographic purification [2, 3]. In animals, Desmin has a marked antithrombotic activity at dosages that cause only minor alterations in plasma clotting tests and bleeding time [4]. As natural dermatan sulfate, Desmin shows nearly the same antithrombotic efficacy after i.v. administration, but, due to its higher bioavailability [5], it is twice as effective when given by the s.c. route [4]. In addition to preventing thrombus formation, Desmin also considerably reduces the weight of performed thrombi; this effect seems to be primarily due to an anticoagulant-independent mechanism, probably involving local fibrinolysis [6–8].

Previous pharmacodynamic studies in man have confirmed the anticoagulant activity shown in animals by different routes of administration. Moreover, studies in humans have shown that Desmin has a good local and general tolerability [9–13]. The aim of our study was to evaluate the bioavailability of Desmin after the single s.c. administration of 200 and 300 mg in healthy volunteers, by comparing the effects on blood coagulation with those of i.v. administration.

Materials and methods

Desmin is produced by Opocrin (Modena, Italy) but is not marketed and was provided as sterile injectable ampoules by Alfa Wassermann (Bologna, Italy). Its mean molecular weight is 5,500 daltons, as determined by HPLC; specific *in vitro* activities have been calculated, against the fourth international standard for heparin, as 89 U/mg in the presence of HC II (Stachrom D.S. test), 12 U/mg by Heptest, and 5 U/mg by anti-Xa chromogenic assay [3].

The study was performed according to Good Clinical Practice guidelines and the study protocol was cleared by the ethics committee of our hospital. Subjects were judged healthy based on a thorough physical examination and medical history, as well as measurement

of blood pressure and heart rate and ECG, hematology, hematochemistry, and urinalysis tests. Subjects provided written and informed consent and were included in the study according to the following criteria: good psycho-physical state of health; age range 18–45 years; weight range, females 50–65 kg and male 60–80 kg. Exclusion criteria were: pregnancy or breast-feeding; confirmed or suspected hypersensitivity to extractive mucopolysaccharides; contemporary intake of drugs that might interfere with the product to be studied.

All volunteers underwent the same study phases: following a cross-over design, on 3 separate days, and with a wash-out period of at least 21 days between drug administrations. All received, while fasting, a single morning i.v. infusion of 200 mg of Desmin and single s.c. injections of 200 mg and 300 mg of Desmin. On the week preceding the study, volunteers were not allowed to take any drugs without the authorization of the research physician.

Citrated blood samples (9 ml) were drawn from each volunteer prior to each drug injection (T_0). Desmin (200 mg) was administered by i.v. bolus injection into the median antecubital vein and blood samples were then taken at 15, 30, and 60 min and 2, 3, 4, 6, 8, 12 and 24 h, to obtain reference curves. On the 2 subsequent study days (after wash-out periods), each subject was injected s.c. in the paraumbilical area with 200 mg (*phase 2*) and 300 mg (*phase 3*) of Desmin. Blood samples were taken at 1, 2, 3, 4, 6, 8, 12, and 24 h after injection.

All samples were immediately centrifuged at 5,000 rpm for 10 min. The (platelet-poor) plasma obtained was stored in small aliquots, immediately frozen at −80 °C, and kept at this temperature until assay. The following coagulation parameters were monitored: activated partial thromboplastin time (aPTT) (PTT reagent Diagnostica Stago, France); thrombin time (TT), using 2 U/ml human thrombin (Ortho, Milan, Italy) (human thrombin was chosen since it was more sensitive to the action of Desmin, even when the drug was added in vitro); anti-Xa (factor Xa inhibition test) (chromogenic substrate from Heparin Coatest, Ortho); Heptest (Haemachem, USA); and HC II activity (Stachrom D.S. – Diagnostica Stago, France).

The local tolerability of s.c. injections was assessed by recording the presence/absence of erythema, pain, burning, and bleeding at the injection site, scored as 0 = absent, 1 = slight and tolerable, 2 = mild, 3 = severe. Moreover, if pain was present, subjects were asked to quantify (after 0, 5, 10, 15, 20, 30, and 60 min) its severity by means of a Visual Analogue Scale (VAS) [14, 15], consisting of a straight vertical line of 10 cm, where the intensity of pain was marked as a point between the bottom (= no pain) and the top (= maximum tolerable pain). Systemic tolerance of Desmin was monitored by routine laboratory tests on the first and last study days. On each phase day of the study, subjects were also monitored for any adverse event, while they were asked to report any unexpected reaction immediately during the wash-out periods.

The pharmacokinetic parameters were calculated by SIPHAR software (Simed, France), according to a single, open-compartment model, using the Powell algorithm for curve fitting and analyzing separately the data from each volunteer. The following parameters were calculated: maximum plasma concentrations (C_{max}), the corresponding times (t_{max}), and elimination half-life ($t_{1/2}$). The area under curve (AUC) was calculated by the trapezoidal method between the first and last experimental points (AUC_{0-24}). The availability (F) of the s.c. compared with the i.v. administration was calculated as the ratio of the respective AUCs and expressed as a percentage. The kinetic parameters were statistically compared by analysis of variance, taking a P value <0.05 as statistically significant; the test was completed by Bartlett's test for homogeneity of variances among groups. When the test was significant, a non-parametric analysis was performed (Kruskal-Wallis test).

Results

Twelve healthy volunteers were admitted to the study, 6 males and 6 females, mean 33 years (range 22–45 years). Subjects 3 and 5 were not included in the pharmacokinetic evaluation of the i.v. route, as they did not receive the whole Desmin dose. In both subjects washing of the butterfly needle tube was impossible due to the rupture of the cubital vein.

The aPTT (normal values 30–40 s) showed very small variations and remained within the normal range, both for the i.v. and the s.c. injections. The absence of any variation after Desmin administration ruled out a pharmacokinetic evaluation. Our data indicate, in accordance with other reported studies [10–14], that aPTT is not influenced by single i.v. or s.c. administration of the doses tested.

The TT (normal values 15–21 s), measured using 2 U/ml human thrombin, showed, after i.v. administration of 200 mg of Desmin, an almost immediate and quite marked activity peak, producing an incoagulable (>600 s) sample. After 30 min values returned to normal. The s.c. injection of 200 mg induced a slight variation in TT, reaching a plateau of about 21 s, which was maintained for up to 8–12 h. The s.c. injection of 300 mg produced the mean peak of activity (28.3 s) after 2 h, with return to the normal range in approximately 12 h (Fig. 1A). Pharmacokinetic evaluation of these data was not possible, due to the short-lasting effect of the drug, which failed to influence this parameter under our experimental conditions.

The activity of Heptest was calculated as micrograms per milliliter of Desmin using a reference curve obtained by in vitro addition of known amounts of Desmin to normal plasma. This parameter was clearly influenced both by the i.v. and the s.c. (200 and 300 mg) administration of the drug (Fig. 1B, Table 1). After i.v. administration, 200 mg of Desmin induced significant increases of Heptest, with return towards baseline in about 6 h. After the s.c. injection of 200 and 300 mg the C_{max} was half that recorded after i.v. infusion (10.30 and 13.83 μg/ml, respectively); this was recorded after 3 h with values returning to normal in about 24 h. Taking into account the AUC, the plasma effects measured by Heptest were dose dependent, while the peak times (reached for both dosages in about 3 h) were independent of dose; the half-lives were longer after s.c. than i.v. injection and were dose dependent ($P<0.05$). When compared with i.v. administration, the mean availability of the single 200-mg s.c. dose was more than 100% (167%).

HC II activity (Stachrom D.S.) was calculated as micrograms per milliliter by means of a reference curve obtained by in vitro addition of known amounts of Desmin to normal plasma. HC II activity was clearly influenced by the single i.v. administration of 200 mg of Desmin: the first sample after injection, drawn at 15 min, showed a mean activity of 17.58 μg/ml; values returned to baseline in about 2 h. In contrast, s.c. injection of 200 and 300 mg produced, at the same peak time (around 120 min), a mean C_{max} value of 3.1 and 3.56 μg/ml, respectively, which was about fivefold less than after i.v. injection. Within 12–24 h values again returned to the normal range. The Stachrom D.S. activities disappeared with an average $t_{1/2}$ of around 2.5 h, at least twice as slowly as after i.v. injection (Fig. 1C, Table 1). When compared with i.v. administration, the mean availability of the single 200-mg s.c. injection was 136%.

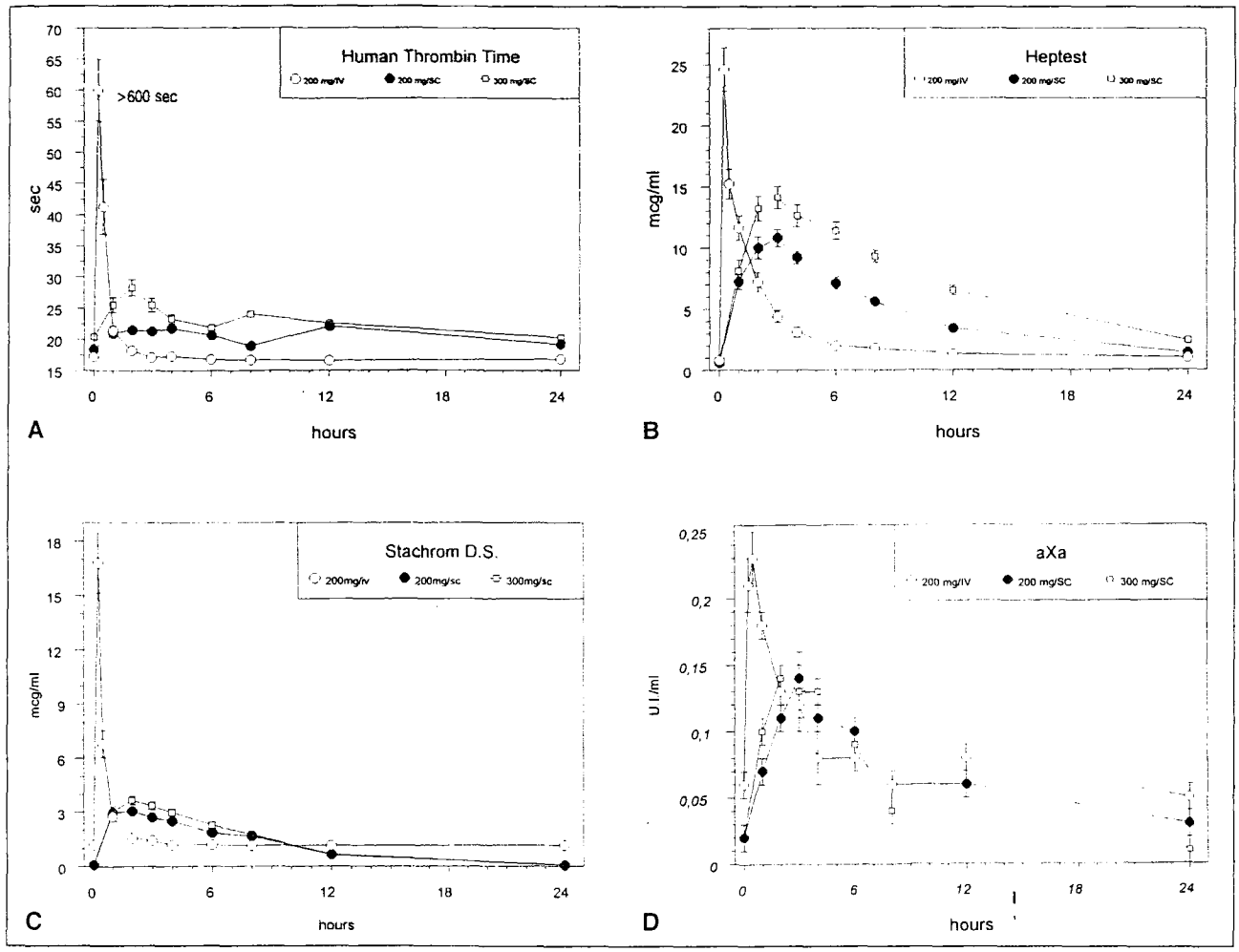

Fig. 1 Effect of the i.v. administration of 200 mg Desmin and of the s.c. administration of 200 and 300 mg Desmin on thrombin time (performed using human thrombin 2 U/ml) (**A**), on Heptest (**B**), on heparin cofactor II activity (Stachrom D.S.) (**C**), and on the inhibition of factor Xa (chromogenic aXa method) (**D**). Data are mean ± SEM

Anti-Xa activity was expressed as heparin international units per milliliter, calculated against reference curves obtained by diluting the fourth international heparin standard. After an i.v. bolus, a significantly elevated activity, with a mean peak value of 0.25 IU/ml, was recorded; after 200- and 300-mg s.c. doses the mean peaks were recorded at around 3 h, of about the same value (0.15 IU/ml). Normal values were reached within 8–10 h (Fig. 1D, Table 1). The kinetic behaviour was dose independent and the bioavailability of the 200-mg s.c. dose was 108%. The AUC of these two s.c. dosages was almost superimposable (P = NS).

No signs of local intolerance were recorded in the 12 volunteers. All subjects rated an absence of pain at the injection site by VAS, except subject no. 2, who complained, after the 200-mg dose, of a very painful reaction, not present after the 300-mg dose and clearly attributable to an excessive psychological reaction. No changes in laboratory parameters of systemic tolerance were detected at the end of the study compared with basal values. Furthermore, throughout the whole study period no adverse reactions were recorded.

Discussion

The single i.v. administration of 200 mg of Desmin in healthy volunteers has a very slight effect on some tests such as aPTT and TT, while the Heptest and Stachrom D.S. tests show specific drug effects. The single administration of the two s.c. doses tested confirms that these standard coagulation tests are not sensitive enough to Desmin to serve as useful indicators of circulating concentrations, particularly at the low levels generated by extravascular administration. In contrast, the Heptest and Stachrom D.S. assays were clearly influenced by the single s.c. administration of 200 and 300 mg of the drug: the greatest response being recorded for Heptest. A possible explanation of this is that while Stachrom D.S. measures only the affinity of Desmin for HC II, the drug influence on Heptest is the result of both anti-IIa and anti-Xa activities. It is generally

Table 1

	C_{max} (μg/ml)	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{(0-24)}$ (μg/ml per hour)	F (%)
Heptest					
200 i.v.	25.90 (1.57)	–	2.35 (0.78)	56.69 (6.38)	
200 s.c.	10.30 (0.73)	2.75 (0.13)	5.78 (0.51)	94.77 (6.50)	167
300 s.c.	13.83 (0.94)	3.08 (0.31)	7.08 (0.38)	154.66 (9.18)	
200 s.c. vs. 300 s.c.	$P<0.01$	NS	$P<0.05$	$P<0.001$	
Heparin cofactor II activity					
200 i.v.	17.58 (1.15)	–	0.88 (0.09)	17.65 (1.66)	
200 s.c.	3.10 (0.20)	2.17 (0.39)	2.36 (0.31)	24.02 (1.45)	136
300 s.c.	3.56 (0.22)	1.92 (0.15)	2.54 (0.33)	27.62 (1.38)	
200 s.c. vs. 300 s.c.	NS	NS	NS	NS	

[a] Mean (±SEM) Pharmacokinetic parameters of Desmin (C_{max} maximum plasma concentration, t_{max} time of C_{max}, $t_{1/2}$ half-life, *AUC* area under the curve, *F* availability)

accepted that Heptest measures predominantly the anti-Xa activity of glycosaminoglycans given at low concentrations, while at higher concentrations it is influenced by both the anti-Xa and the anti-IIa activities [16]. Furthermore, it was possible only with Heptest to register dose-related pharmacokinetics of a single dose, presumably due to different sensitivity of the two tests.

A very high bioavailability, always greater than 100%, was registered for the s.c. route of administration: this could be due to the low molecular weight of Desmin, which facilitates the absorption from the site of injection and the diffusion into the intravascular compartment. The bioavailability of natural dermatan sulfate at the same dose (200 mg) is much lower [17]. The calculated AUC of greater than 100% could be due to the incomplete correlation between the units recorded *in vivo* and the re-calculation *in vitro*; furthermore, the AUC calculated for the i.v. administration may be an underestimate, since the first blood sample after i.v. infusion was drawn after 15 min. Single administrations of Desmin were without adverse effects and were characterized by a very good local tolerability.

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