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Pharmacokinetics of sulodexide evaluated from labelled fast moving heparin and from labelled dermatan sulphate after single intravenous and oral administration

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Key words: Sulodexide, pharmacokinetics, oral administration, fast moving heparin, dermatan sulphate.

SUMMARY

The pharmacokinetics of Sulodexide from ^{125}I-labelled fast moving heparin fraction (FMH) and, separately, ^{125}I-labelled dermatan sulphate fraction (DS) were investigated in four groups of three patients undergoing radiotherapy after administration of 50 mg sulodexide given by a single intravenous bolus or by a single gastro-resistant oral capsule.

The concentration profiles could be fitted to a two-compartment model. A lag time of approximately 1.5 hours after oral dosing and a mean residence time of approximately 20 to 30 hours was found. The urinary excretion accounted for approximately 35% of the administered dose.

The absorption of sulodexide after oral dosing was equivalent when calculated from fast moving heparin or dermatan sulphate; the biological availability was almost equivalent after intravenous bolus and oral dosing. The elimination kinetics of fast moving heparin and dermatan sulphate, as well as the excretion rate, was almost equivalent and was not influenced by the administration route.

Sulodexide reached steady-state in four to six days. The oral administration of either 50 mg twice daily or 100 mg once daily provided comparable concentration profiles at steady-state. The absence of important peaks in comparison with the intravenous route, justifies a somewhat slower onset of pharmacodynamic (anti-thrombotic) action, and the absence of detectable anticoagulant effects.

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INTRODUCTION

We have already reported a preliminary pharmacokinetics investigation[1] on sulodexide†, a naturally occurring substance isolated and extracted from animal duodenum. It is a well characterised glucosaminoglycan fraction, containing two principal components [2] that account for over 90% of the product weight: a fast-moving or heparan-like fraction (FMH; approx. 80%) and a dermatan or chondroitin sulphate B fraction (DS; approx. 20%).

Our previous report monitored the radioactivity in blood, urine and faeces following the administration of ^{14}C labelled substance, but this approach may not fully elucidate the pharmacokinetic profile of sulodexide, since it fails to properly account for the two individual major components. Consequently, we extended our investigation to the assessment of the time-concentration profile of sulodexide, after individually labelling the FMH and DS fractions.

Sulodexide exerts a clinically well defined anti-thrombotic activity on both arterial and venous disorders [3 – 12]. It is thus possible that the profile in plasma only, as usually determined, is insufficient to explain its activity in pharmacokinetic terms. Therefore we separately investigated the profile in whole blood, in plasma and in the blood particulate. This may help to better understand the mechanism of action of the substance, thereby providing guidelines for the most appropriate dosage scheme to be applied in patients.

The treatment with sulodexide may be given both by parenteral and oral routes, but the prevalent trend in clinical practice is to resort to the oral treatment for the long-term periods necessary to exert an appropriate therapeutic action. Consequently this investigation was also aimed to assessing the relative utility – under the pharmacokinetic profile – of oral versus parenteral dosing, with the relevant extrapolations to the steady state.

PATIENTS AND METHODS

Twelve patients with cancer, undergoing radiotherapy, voluntarily gave written informed consent to take part into this study after being given appropriate information. The study was approved by an independent Ethics Committee before enrolment of patients began. Four groups of three subjects were treated with a single dose of 50 mg of sulodexide, labelled either on the FMH or DS fraction, administered either by intravenous or oral route (Table 1). All patients had been pre-treated during two days with potassium iodide, to avoid thyroidal uptake of ^{125}I.

To label the sulodexide components specifically and separately, the two fractions (FMH and DS) were separated from a pharmaceutical grade batch of raw material by fractional precipitation. Both fractions were thoroughly assayed to confirm identity and purity. Then, FMH was partly desulphated with a modification of the method of Levy and Petracek [13]. Similarly, DS was partly deacetylated with a modification of the method of Shaklee and Conrad [14]. These modified substances were labelled with ^{125}I according to Dawes and Pepper [15]. The compatibility of labelled with the native

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Table 1. Study sample.

Subject	Sex	Age	Pathology	Labelled	Route	Dose (μCi)
1	M	63	Tonsils neoplasm	FMH	IV	500
2	M	51	Lung metastases	FMH	IV	500
3	F	54	Supraclavear lymphoma	FMH	IV	500
4	F	82	Infiltrating ductural carcinoma	FMH	OS	1000
5	F	54	Infiltrating ductural carcinoma	FMH	OS	1000
6	M	80	Tongue neoplasm	FMH	OS	1000
7	F	76	Metastatic carcinoma	DS	IV	600
8	M	48	Cerebral neoplasm	DS	IV	600
9	M	52	Right lung lobe resection	DS	IV	600
10	F	51	Breast cancer with bone metastases	DS	OS	400
11	F	53	Uterine portio epidermoid cancer	DS	OS	400
12	M	81	Upper right alveolar edge cancer	DS	OS	400

substances was determined by testing the molecular weight distribution curve.

The labelled substances were introduced into vials for intravenous use (and subsequently tested for sterility and apyrogenicity) or into gastro-resistant capsules containing the appropriate amount of unlabelled sulodexide, to the total amount of 50 mg. The radioactivity amount, as shown in Table 1, exhibited a variability of not more than 10% between individual dosage units. The use of gastro–resistant capsules was necessary due to the evidence that the iodinated derivative is partly unstable [16] in the acidic gastric medium.

Each subject received a single administration of 50 mg of sulodexide labelled as indicated in Table 1, in the morning after an overnight fast, either as bolus intravenous injection or as oral capsule accompanied by a glass of plain water (approximately 120 ml). After oral dosing, no food nor drinks were allowed for the following two hours.

After 0.25, 0.5, 0.75, 1, 2, 3, 4, 8, 10 and 24 hours from the intravenous administration, or after 2, 4, 6, 8, 10, 24 and 48 hours after the oral dosing, approximately ten millilitres of blood were collected from the anterior cubital vein and distributed into two disposable tubes. One was directly stored at -80°C (blood); the other, citrated, was immediately centrifuged to 2000 g for 10 min at 4°C and the resulting plasma and particulate pellets were separately stored at -80°C until analysis.

Urine samples were collected over the period 0 – 6, 6 – 12, 12 – 24, 24 – 48 and 48 – 72 hours after dosing; the volume of sample was measured to the nearest whole millilitre, then a specimen frozen to -80°C until analysis.

All analyses were performed by scintillation, setting the parameters for the specific emission of ^{125}I.

Aliquote of plasma samples were also analysed by gel-permeation HPLC (column:

7.8 x 300 mm Supelco Progel TSK G3000 PWXL; mobile phase: 0.1 M NaCl; flow: 0.5 ml/min) using a flow radioactivity detector, to determine the proportion of total radioactivity bound to species with the same Molecular Weight profile as the native substance.

The obtained blood, plasma and particulate concentration data were processed according to the usual pharmacokinetic procedures [17, 18], on the assumption that the measured radioactivity is proportional to the amount of the parent substance. The bicompartmental model fitted to all data sets except in one case, in which the monocompartmental model had to be used.

The minimisation algorithm used was the Beal-modified Gauss-Newton method; the model had been fitted to data using the extended least squares algorithm with the error model: $var = f(Y_{calc})^{v(2)}$.

From the calculated parameters, the repeated-dose concentration profiles were extrapolated for the two most common dosage regimens, 50 mg sulodexide twice daily and 100 mg once daily.

The statistical processing was performed whenever possible by means of the multiple analysis of variance (MANOVA) for repeated measures using as categorical predictors the examined chemical species, the administration route and the compartment (blood, plasma, particulate). In this analysis the rejection limit was set at $p \leq 0.05$. In particular instances, the paired or unpaired t-test was also used, reducing the discriminant power according to the Bonferroni criterion.

Glossary of abbreviations

AUC_{exp}	exponential Area Under Curve.
Lag-time	time of delay.
MRT_{exp}	exponential Mean Residence Time.
C_{lapp}	apparent Clearance.
V_D	volume of distribution.
V_{DSS}	volume of distribution at the steady-state.
AUC_{model}	Area Under Curve calculated following the chosen pharmacokinetic model.
MRT_{model}	Mean Residence Time calculated following the chosen pharmacokinetic model.

RESULTS

The concentration-time data and the computed pharmacokinetic parameters for the two monitored components and the two administration routes are reported in Tables 2 (plasma), 3 (whole blood) and 4 (particulate). The urinary excretion is reported in Table 5.

The gel-permeation analysis confirmed that most of the radioactivity measured in plasma is accounted for by chemical species with the same Molecular Weight profile as that of the native substance (from over 80% in the first few hours, to approximately 70% after 24 to 48 hours from administration). These results shall be reported in a future paper.

To the effect of this report, this information means that the reported concentrations should be considered over-estimated by a factor of 0.2 to 0.3 relative to the actual concentration of the chemical entities constituting sulodexide.

Table 2. Concentrations of sulodexide (as FMH or DS) in plasma after intravenous and oral administration of 50 mg and relevant pharmacokinetic parameters (mean ± SEM; n = 3).

Variable	FMH		DS	
	intravenous	oral	intravenous	oral
$Coefficient_{el}$ (mg/litre)	1.27 ± 0.10	1.50 ± 0.44	2.02 ± 1.30	0.78 ± 0.20
$Exponent_{el}$ (h^{-1})	0.07 ± 0.01	0.06 ± 0.01	0.10 ± 0.06	0.04 ± 0.02
$Half\text{-}life_{el}$ (h)	10.38 ± 0.45	12.21 ± 2.25	11.63 ± 5.03	21.42 ± 5.87
$Coefficient_{abs/distr}$ (mg/litre)	8.53 ± 1.43	2.60 ± 0.92	5.73 ± 0.97	1.47 ± 0.52
$Exponent_{tabs/distr}$ (h^{-1})	1.88 ± 0.22	0.29 ± 0.03	1.09 ± 0.38	0.42 ± 0.27
$Halflife_{abs/distr}$ (h)	0.38 ± 0.04	2.46 ± 0.28	0.83 ± 0.29	2.74 ± 1.74
AUC_{exp} (mg•h/litre)	24.27 ± 1.33	21.35 ± 6.69	28.48 ± 4.96	21.10 ± 2.30
Lag time (h)	–	1.91 ± 0.82	–	0.94 ± 0.94
MRT_{exp} (h)	11.50 ± 0.58	20.83 ± 3.46	12.43 ± 4.19	30.15 ± 8.28
Cl_{app} (litres/h)	2.07 ± 0.12	3.18 ± 1.36	1.89 ± 0.39	2.43 ± 0.28
V_D (litres)	31.14 ± 2.87	50.50 ± 16.27	30.59 ± 11.53	70.37 ±14.10
V_{Dss} (litres)	26.34 ± 2.52	57.56 ± 18.69	21.76 ± 6.54	76.61 ± 17.73
AUC_{model} (mg•h/litre)	23.40 ± 1.23	18.78 ± 5.91	25.63 ± 4.85	16.21 ± 5.80
MRT_{model} (h)	12.21 ± 0.59	23.07 ± 3.87	11.59 ± 4.97	31.80 ± 13.07
Concentrations (mg/litre)				
0.25 h	6.02 ± 0.41	–	6.08 ± 0.52	–
0.5 h	4.66 ± 0.59	–	5.23 ± 0.28	–
1 h	2.56 ± 0.17	–	4.03 ± 0.21	–
2 h	1.29 ± 0.04	0.19 ± 0.03	2.66 ± 0.37	0.42 ± 0.19
3 h	0.90 ± 0.08	0.20 ± 0.02	1.32 ± 0.39	0.51 ± 0.05
6 h	–	0.64 ± 0.21	0.65 ± 0.18	0.48 ± 0.05
8 h	0.76 ± 0.03	0.92 ± 0.29	0.68 ± 0.13	0.68 ± 0.24
10 h	0.69 ± 0.04	0.83 ± 0.23	0.50 ± 0.08	0.63 ± 0.14
24 h	0.25 ± 0.01	0.39 ± 0.13	0.32 ± 0.09	0.31 ± 0.10
48 h	–	0.11 ± 0.05	–	0.12 ± 0.04

The time-concentration profile of FMH after intravenous bolus injection indicates that the whole blood concentration is almost the weighed average between the higher plasma concentration and the lower particulate concentration; the overall profile appears the same in the three monitored compartments (Figure 1). The same consideration may be extended to the profile after oral dosing (Figure 2) and to the DS concentration profile after intravenous (Figure 3) and oral (Figure 4) dosing.

The statistical analysis performed to assess the influence of the administration route indicated that there are no major differences in the pharmacokinetic profile of FMH after oral or intravenous administration, except for the distribution parameters (missing after oral dosing) and the absorption parameters (missing after intravenous dosing). No statistically significant differences were observed for the elimination parameters (coefficient, constant and therefore half-life), AUC, MRT, Cl, V_D and V_{DSS}. Indeed, even the actual concentrations do not significantly

Table 3. Concentrations of sulodexide (as FMH or DMS) in whole blood after intravenous and oral administration of 50 mg and relevant pharmacokinetic parameters (mean ± SEM; n = 3).

Variable	FMH		DS	
	intravenous	oral	intravenous	oral
$Coefficient_{el}$ (mg/litre)	0.52 ± 0.06	1.06 ± 0.17	0.48 ± 0.05	0.73 ± 0.30
$Exponent_{el}$ (h^{-1})	0.04 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.04 ± 0.02
$Half\text{-}life_{el}$ (h)	23.99 ± 8.26	13.01 ± 2.74	22.17 ± 6.58	27.89 ± 11.33
$Coefficient_{abs/distr}$ (mg/litre)	4.06 ± 0.28	2.44 ± 0.82	3.52 ± 0.34	1.19 ± 0.08
$Exponen_{tabs/distr}$ (h^{-1})	1.30 ± 0.10	0.30 ± 0.04	0.53 ± 0.16	0.37 ± 0.23
$Halflife_{abs/distr}$ (h)	0.54 ± 0.05	2.36 ± 0.31	1.52 ± 0.36	3.00 ± 1.84
AUC_{exp} (mg•h/litre)	19.54 ± 4.04	15.88 ± 4.19	24.37 ± 7.00	18.88 ± 4.03
Lag time (h)	–	2.56 ± 0.88	–	0.94 ± 0.94
MRT_{exp} (h)	27.53 ± 12.17	22.20 ± 3.94	21.67 ± 7.34	39.08 ± 14.63
Cl_{app} (litres/h)	2.76 ± 0.48	3.81 ± 1.28	2.52 ± 0.85	2.89 ± 0.58
V_D (litres)	83.96 ± 11.42	64.03 ± 11.84	66.25 ± 4.10	97.42 ± 24.95
V_{Dss} (litres)	68.41 ± 12.56	71.43 ± 11.84	46.93 ± 3.13	91.11 ± 32.50
AUC_{model} (mg•h/litre)	20.19 ± 4.13	14.09 ± 3.72	23.42 ± 6.68	18.35 ± 9.58
MRT_{model} (h)	29.70 ± 11.64	24.73 ± 4.30	22.54 ± 7.64	44.94 ± 27.66
Concentrations (mg/litre)				
0.25 h	3.53 ± 0.15	–	3.80 ± 0.46	–
0.5 h	2.56 ± 0.26	–	3.23 ± 0.30	–
1 h	1.65 ± 0.16	–	2.58 ± 0.27	–
2 h	0.76 ± 0.04	0.15 ± 0.02	1.84 ± 0.30	0.34 ± 0.15
3 h	0.46 ± 0.03	0.16 ± 0.02	0.97 ± 0.29	0.35 ± 0.03
6 h	–	0.48 ± 0.14	0.46 ± 0.16	0.37 ± 0.02
8 h	0.44 ± 0.01	0.66 ± 0.18	0.50 ± 0.12	0.50 ± 0.16
10 h	0.38 ± 0.01	0.59 ± 0.14	0.40 ± 0.10	0.46 ± 0.11
24 h	0.15 ± 0.01	0.28 ± 0.07	0.22 ± 0.06	0.27 ± 0.08
48 h	–	0.09 ± 0.04	–	0.11 ± 0.04

differ between routes, starting from the eighth hour onwards. All these observations held for the three compartments tested (whole blood, plasma, particulate). The same is true also for the fraction DS. From these considerations, there is no evidence that the administration of sulodexide by oral route might entail a different pharmacokinetic profile of either or both FMH and DS, in absorbed fraction (AUC), elimination rate (Kel and relevant half-life) and persistence in the organism (MRT).

The main aim of this study was to determine possible pharmacokinetic differences of the two main components of sulodexide responsible for its specific pharmacodynamic activity, as long as they are both present in the specific native ratio. Thus an extensive analysis was performed to test the influence of the chemical entity on the pharmacokinetic profile.

The only differences that could be found between the FMH and DS profile was a greater concentration of FMH in plasma 0.25 h after intravenous bolus ($p = 0.006$), and conversely, a greater concentration of DS

Table 4. Concentrations of sulodexide (as FMH or DMS) in blood particulate after intravenous and oral administration of 50 mg and relevant pharmacokinetic parameters (mean ± SEM; n = 3).

Variable	FMH		DS	
	intravenous	oral	intravenous	oral
$Coefficient_{el}$ (mg/litre)	0.14 ± 0.01	0.37 ± 0.05	0.17 ± 0.03	0.28 ± 0.02
$Exponent_{el}$ (h–1)	0.03 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.02
$Half\text{-}life_{el}$ (h)	24.99 ± 14.74	16.96 ± 4.62	18.68 ± 4.13	38.61 ± 24.87
$Coefficient_{abs/distr}$ (mg/litre)	0.99 ± 0.17	0.64 ± 0.20	0.60 ± 0.08	0.36 ± 0.08
$Exponent_{abs/distr}$ (h–1)	1.46 ± 0.32	0.41 ± 0.21	0.57 ± 0.08	0.33 ± 0.01
$Halflife_{abs/distr}$ (h)	0.53 ± 0.12	2.66 ± 1.00	1.26 ± 0.18	2.10 ± 0.08
AUC_{exp} (mg•h/litre)	6.06 ± 1.85	7.54 ± 1.75	5.71 ± 1.32	12.87 ± 6.93
Lag time (h)	–	0.99 ± 0.34	–	0.87 ± 0.87
MRT_{exp} (h)	35.77 ± 17.53	27.32 ± 6.99	22.08 ± 5.90	52.88 ± 32.82
Cl_{app} (litres/h)	9.65 ± 2.31	7.31 ± 1.49	10.06 ± 2.86	6.34 ± 2.35
V_D (litres)	311.88 ± 45.05	159.25 ± 8.08	244.12 ± 30.40	187.92 ± 32.60
V_{Dss} (litres)	282.15 ± 49.31	175.96 ± 7.30	199.38 ± 22.75	192.59 ± 12.50
AUC_{model} (mg•h/litre)	5.86 ± 1.82	6.83 ± 1.59	5.66 ± 1.33	17.20 ± 13.76
MRT_{model} (h)	36.47 ± 17.24	29.30 ± 7.32	22.19 ± 5.74	73.58 ± 58.28
Concentrations (mg/litre)				
0.25 h	1.34 ± 0.06	–	0.81 ± 0.09	–
0.5 h	0.50 ± 0.01	–	0.55 ± 0.07	–
1 h	0.34 ± 0.03	–	0.47 ± 0.06	–
2 h	0.19 ± 0.03	0.07 ± 0.02	0.33 ± 0.05	0.17 ± 0.08
3 h	0.13 ± 0.01	0.08 ± 0.01	0.21 ± 0.04	0.19 ± 0.01
6 h	–	0.18 ± 0.02	0.16 ± 0.04	0.17 ± 0.01
8 h	0.10 ± 0.01	0.24 ± 0.02	0.14 ± 0.04	0.19 ± 0.03
10 h	0.09 ± 0.01	0.25 ± 0.02	0.10 ± 0.02	0.22 ± 0.04
24 h	0.06 ± 0.01	0.12 ± 0.02	0.07 ± 0.02	0.15 ± 0.08
48 h	–	0.05 ± 0.02	–	0.06 ± 0.04

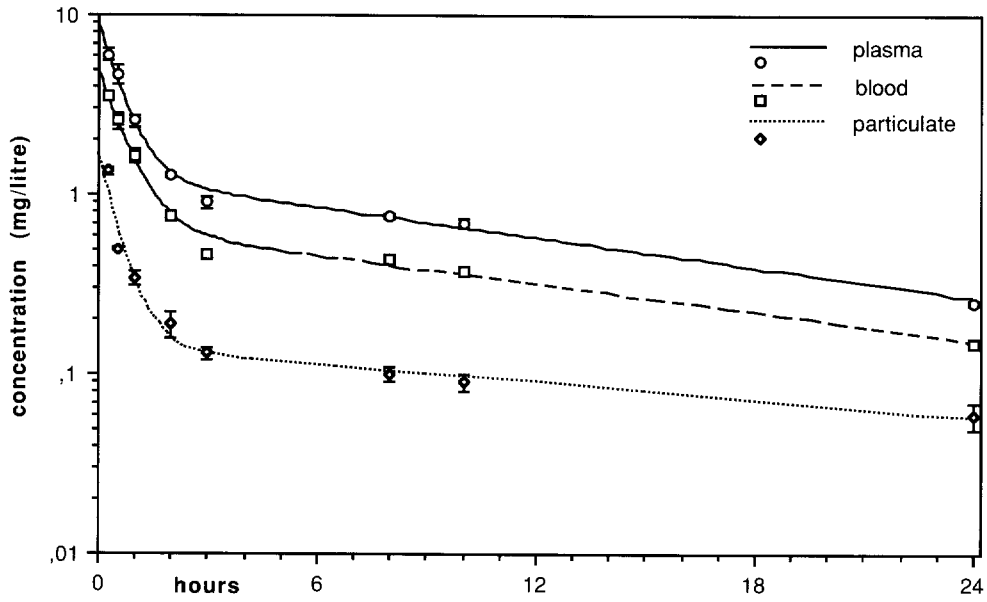
in the particulate at the same time point ($p = 0.007$) with a shorter distribution half-life; a greater distribution coefficient of FMH in whole blood after intravenous dosing was also found ($p = 0.017$) but without appreciable differences in exponent and half-life. No differences were observed after oral dosing, except for a greater concentration of DS at 4 h in blood, plasma and particulate, due to an abnormally low concentration of FMH in one subject. Lag time (approximately 1.5 h), absorption and elimination half-lives, AUC and MRT were comparable between the two components in plasma, blood and particulate. The only appreciable difference in the pharmacokinetic behaviour of FMH and DS was a slower equilibration rate of DS between plasma and particulate, in comparison with FMH. This, however, was only appreciable immediately after the intravenous injection, and did not greatly influence the overall pharmacokinetic profile.

A close examination of the profile of the fraction of FMH and DS bound to the particulate indicates that the proportion bound

Table 5. Urinary excretion of radioactivity after oral or intravenous administration of 50 mg of sulodexide labelled on FMH or DMS (mean ± SEM).

Variable		FMH		DS	
		intravenous	oral	intravenous	oral
Excreted mg	0 – 6 h	8.8 ± 0.7	0.8 ± 0.4	8.2 ± 1.9	1.3 ± 1.0
	6 – 12 h	4.2 ± 0.6	5.3 ± 1.1	3.0 ± 0.5	4.4 ± 1.4
	12 – 24 h	3.2 ± 0.3	4.7 ± 0.5	2.0 ± 0.1	4.7 ± 0.9
	24 – 48 h	1.5 ± 0.2	4.7 ± 0.4	1.4 ± 0.2	10.3 ± 4.6
	48 – 72 h	0.7 ± 0.1	1.4 ± 0.7	0.7 ± 0.1	2.6 ± 0.4
Cumulated dose %	6 h	17.6 ± 1.4	1.5 ± 0.8	16.4 ± 3.8	2.7 ± 2.0
	12 h	26.0 ± 2.5	12.2 ± 2.7	22.5 ± 2.8	11.5 ± 4.2
	24 h	32.4 ± 1.8	21.4 ± 1.9	26.4 ± 3.0	21.0 ± 5.5
	48 h	35.6 ± 2.0	30.7 ± 2.4	29.2 ± 2.6	41.5 ± 14.2
	72 h	36.9 ± 2.1	32.6 ± 2.9	30.7 ± 2.3	46.7 ± 15.1

Figure 1. Concentration – time curves of sulodexide monitored as labelled FMH in plasma (solid line), whole blood (broken line) and particulate (dotted line) computed from the experimental data, reported as mean ± SEM, after intravenous bolus injection of 50 mg.



at 0.25 h and, with less evidence, at 0.5 h after intravenous injection was appreciably greater than at later times. For each sample, the ratio of concentration in particulate to that in plasma and in whole blood was computed, obtaining an approximate index of the bound fraction. The average bound fraction of DS computed in this way ranged from 0.17 to

Figure 2. Concentration-time curves of sulodexide monitored as labelled FMH in plasma (solid line), whole blood (broken line) and particulate (dotted line) computed from the experimental data, reported as mean ± SEM, after oral administration of 50 mg.

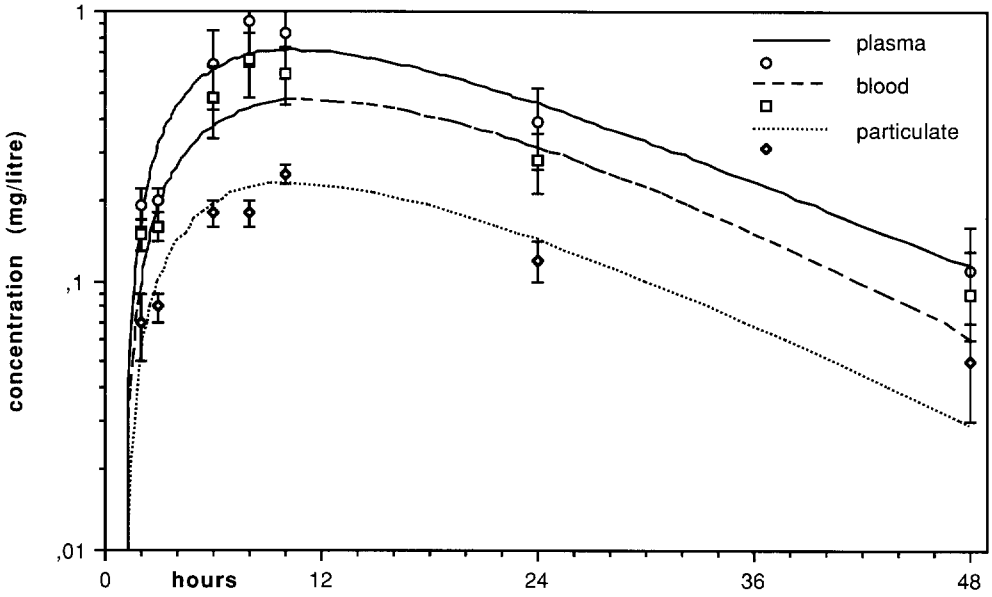


Figure 3. Concentration-time curves of sulodexide monitored as labelled DS in plasma (solid line), whole blood (broken line) and particulate (dotted line) computed from the experimental data, reported as mean ± SEM, after intravenous bolus injection of 50 mg.

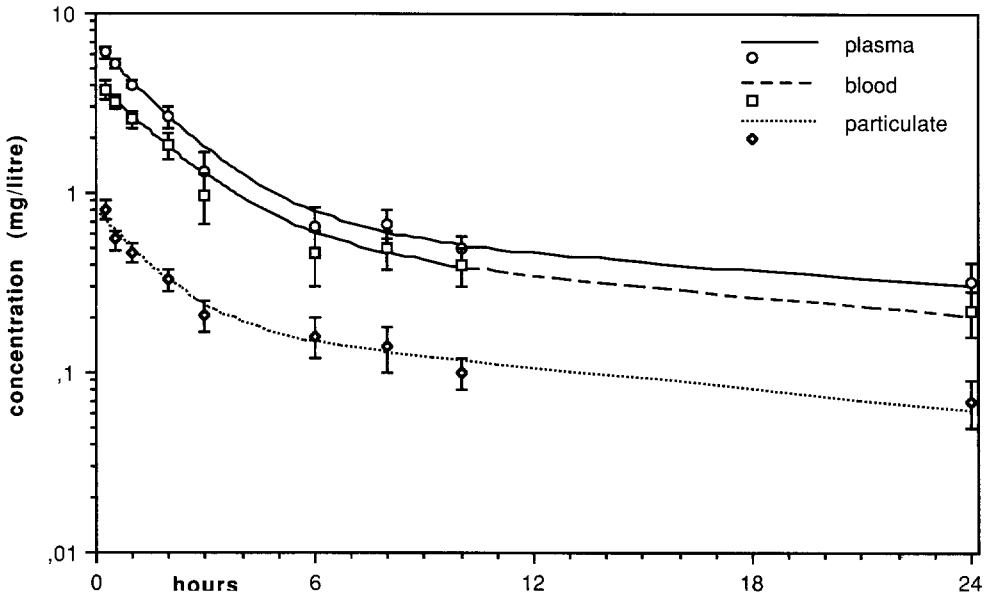
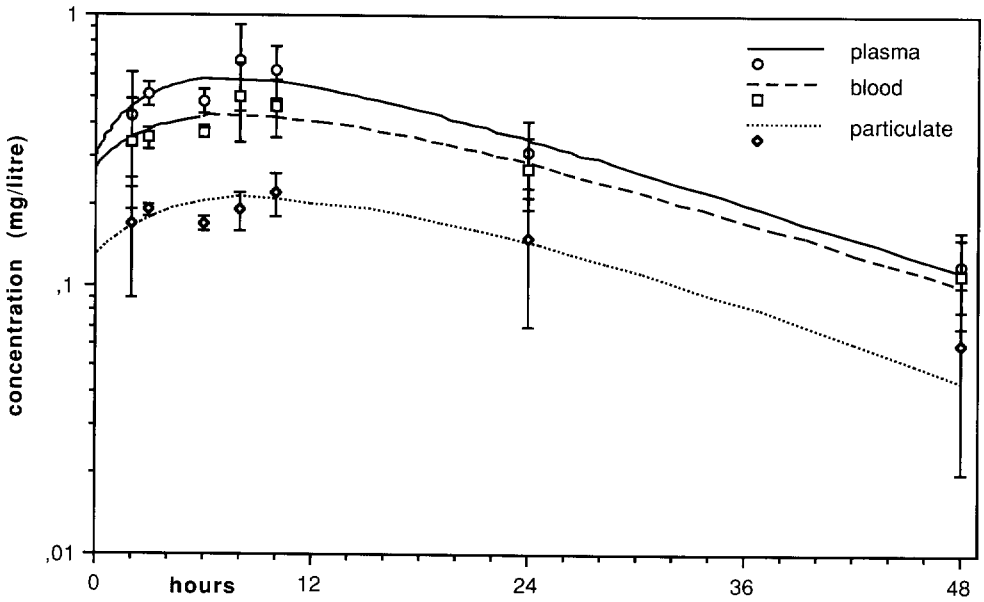


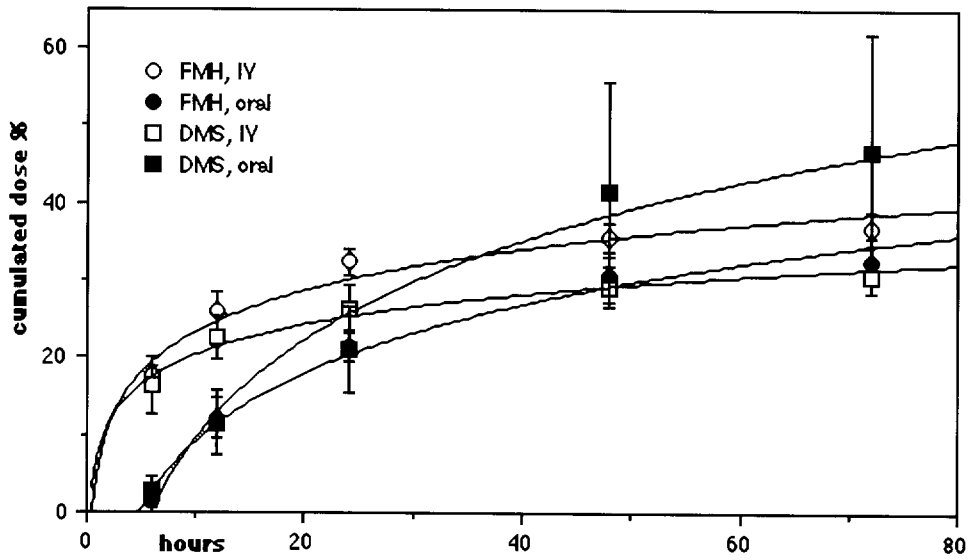
Figure 4. Concentration-time curves of sulodexide monitored as labelled DS in plasma (solid line), whole blood (broken line) and particulate (dotted line) computed from the experimental data, reported as mean ± SEM, after oral administration of 50 mg.



0.27 versus the whole blood and from 0.10 to 0.21 versus the plasma, in the samples from 0.25 to 24 h. Similarly, the bound fraction of FMH from 0.5 to 24 h ranged from 0.20 – 0.29 versus the whole blood and from 0.11 to 0.15 versus the plasma. This ratio was significantly greater at 0.25 h (0.38 versus blood and 0.23 versus plasma), and appeared to stabilise more slowly than that of DS. Thus, the distribution between fluid and particulate was a specific feature of the two components, constant in time without signs of permanent sequestration, in a dynamic, stationary equilibrium except at very early times after intravenous dosing for the component FMH, which nevertheless equilibrated later. Although the bound fraction appeared somewhat greater after oral than after intravenous dosing, the free fraction in the fluid accounted for more than 50% of the total circulating concentration at any time point.

Given the different concentration profiles observed in the three monitored compartments, all the concentration-dependent parameters were inevitably different. On the contrary, no detectable difference was observed in the concentration-independent parameters, such as elimination half-life or MRT. Comparison of the mathematical description of the three concentration curves in the different compartments failed to show appreciable differences, except for the vertical position factor, which was directly dependent on the concentration. Thus, there was no apparent kinetic difference between the three examined blood compartments, which were in a reciprocal dynamic equilibrium controlled exclusively by the mass law, in ratios determined by the partition or affinity coefficients, specific for the two monitored chemical entities.

Figure 5. Urinary excretion of sulodexide estimated from FMH or DS, after intravenous or oral administration of a single 50 mg dose.



The urinary excretion (Figure 5) showed the expected differences between intravenous and oral administration routes. At short times (0 – 6 h) after IV dosing, both examined components were excreted to a greater extent than after oral dosing. The reverse occurred at longer times after dosing. Overall, therefore, the urinary excretion of sulodexide was similar by the two monitored administration routes, both when calculated from the FMH moiety (36.9 ± 3.6% versus 32.6 ± 5.0%; p = 0.2921) and when calculated from the DS moiety (30.7 ± 4.0% versus 41.5 ± 24.7%). In this case, the large error estimate after oral dosing was due to an abnormally high excretion of one subject in the interval 48 – 72 h (39%). If this value is not considered, then the excretion of DS after oral dosing returns within the values already observed (35%).

Over the 72 hour observation period, therefore, we saw almost complete comparability between the intravenous and oral administration routes, within each monitored component. Moreover, complete comparability was found also between the two tested components, within each administration route, intravenous (36.9 ± 3.6% versus 30.7 ± 4.0%, p = 0.1172) and oral (32.6 ± 5.0% versus 41.5 ± 24.7%; p = 0.4497).

The renal excretion of sulodexide was the same after intravenous and oral dosing – although the first route favours early excretion and the second relatively late excretion. In addition, the two components of sulodexide, FMH and DS, were excreted to the same extent and with the same rate by both administration routes.

The decrease of the excreted fraction in the interval 48 – 72 hours, is in agreement with the pharmacokinetic interpretative model, that failed to show signs of a long elimination half-life or mean residence time, which might imply the existence of deep or sequestering compartments with slow equilibration rate.

These should therefore be excluded, on the ground of the considerations made.

DISCUSSION

The results of this investigation are in substantial agreement with those arising from our preliminary study with ^{14}C labelling [1].

The multivariate analysis applied to the blood concentrations observed, the urinary excretion measured and the pharmacokinetic parameters calculated, indicates a statistically significant "route" effect only for the plasma MRT, V_D and V_{DSS}, and for the clearance and experimental VD in the particulate. This last effect is in our opinion due to the absence, after oral dosing, of the rapid equilibration phase observed after intravenous bolus injection. These data suggest more a procedural difference between routes, rather than a real pharmacokinetic difference, unless this equilibration phase is dependent from the high concentrations of FMH present immediately after intravenous injection.

The differences in plasma MRT, V_D and V_{DSS} are likely to be due to the absorption factor, which is present after oral dosing with, incidentally, a measurable lag time, whilst it is obviously absent after intravenous injection. These differences are therefore structural, model-intrinsic differences.

It is appropriate to stress that in no instance substantial pharmacokinetic differences that might be attributed to the "route" effect on the basic bioavailability parameters (AUC, elimination constant, urinary excretion) were observed.

The multivariate analysis within subjects, performed to identify differences between tested compartments, confirmed the significant differences due to the differences in concentrations, already mentioned. No appreciable difference was shown for basic parameters, such as elimination constant, elimination half-life, MRT, neither as function of the monitored component nor by administration route.

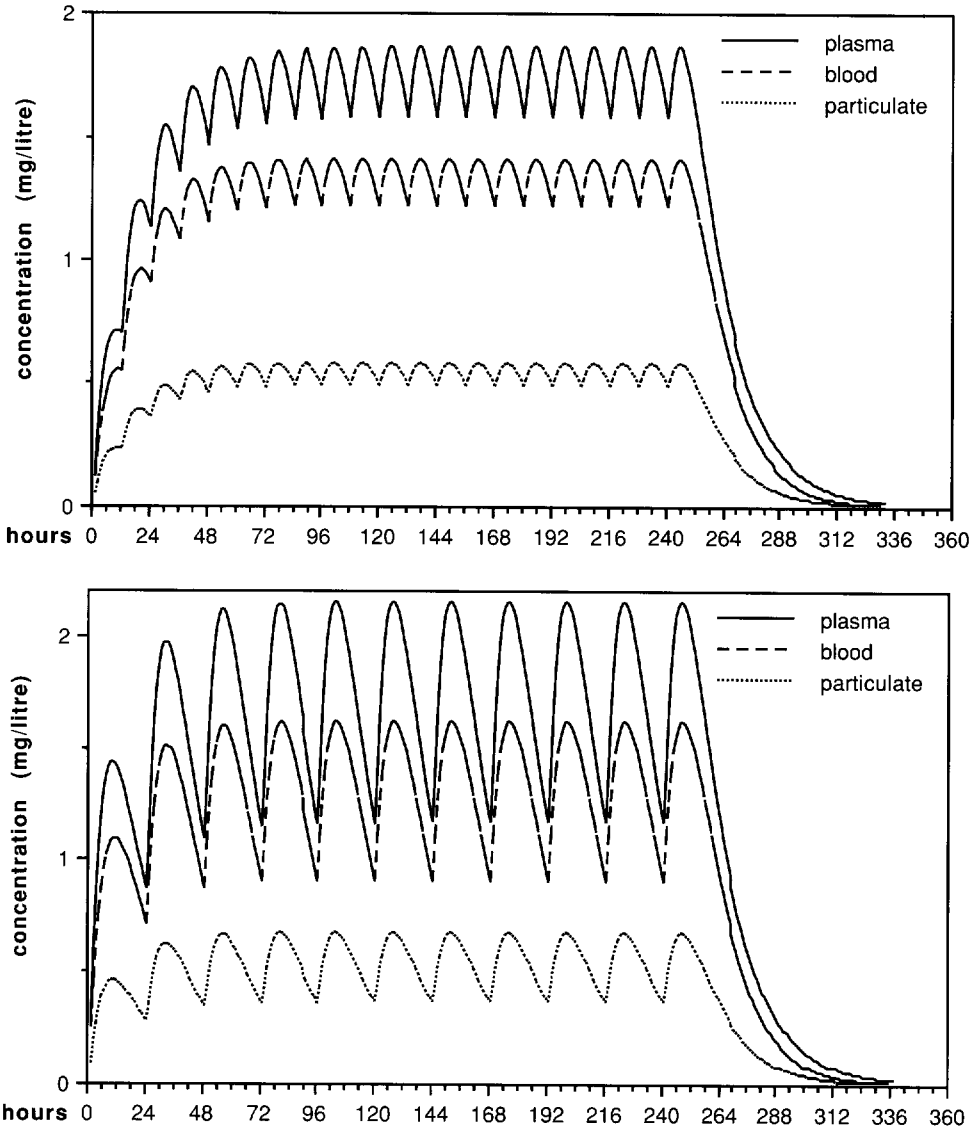
The monitored component played a significant role on the exponent – and therefore on the half life – of distribution after intravenous injection, because of the different equilibration rates between fluid and particulate seen at early observation times.

No appreciable difference of kinetic profile, that might be considered clinically relevant, in terms of pharmacodynamic effect, mode of application or safety of use (such as elimination half-life, MRT, AUC, lag-time after oral dosing, fraction bound to the particulate) was seen between the two monitored components of sulodexide and the two administration routes employed.

From the data obtained an extrapolation to the steady-state for the two most interesting oral dosage schedules, 50 mg bd and 100 mg once daily was performed. This allowed the evaluation of the risk of possible progressive accumulation with time, which is more likely to occur after oral dosing.

When numerically analysed, the stabilisation times for the sulodexide concentration profiles were relatively long, as already indicated in our previous study [1], ranging from 0 to 250 hours for the peak value, and from 160 to 280 hours for the trough

Figure 6. Concentration-time profile extrapolated at the steady-state of sulodexide monitored as FMH in plasma, whole blood or particulate, after repeated administration of 50 mg twice daily (upper graph) or 100 mg once daily (lower graph) by oral route.

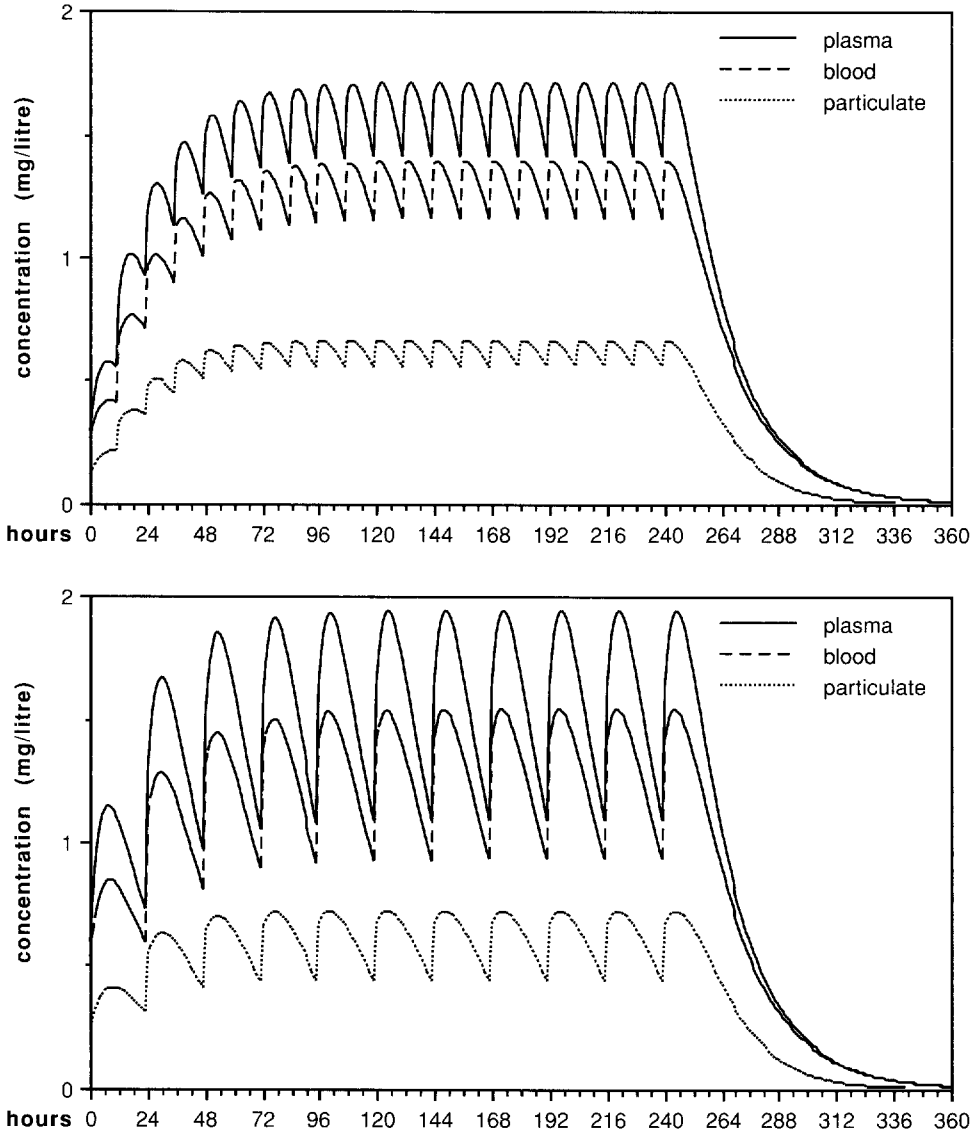


concentration, in the different compartments and with the different tested schemes.

The graphical analysis, however, indicated that, after the first few days (4 to 6) these variations were clinically irrelevant, with a magnitude of approximately 0.01 mg/litre in plasma and proportional magnitudes in the other compartments, both when calculated as FMH (Figure 6) and as DS (Figure 7).

By extrapolation from steady-state values

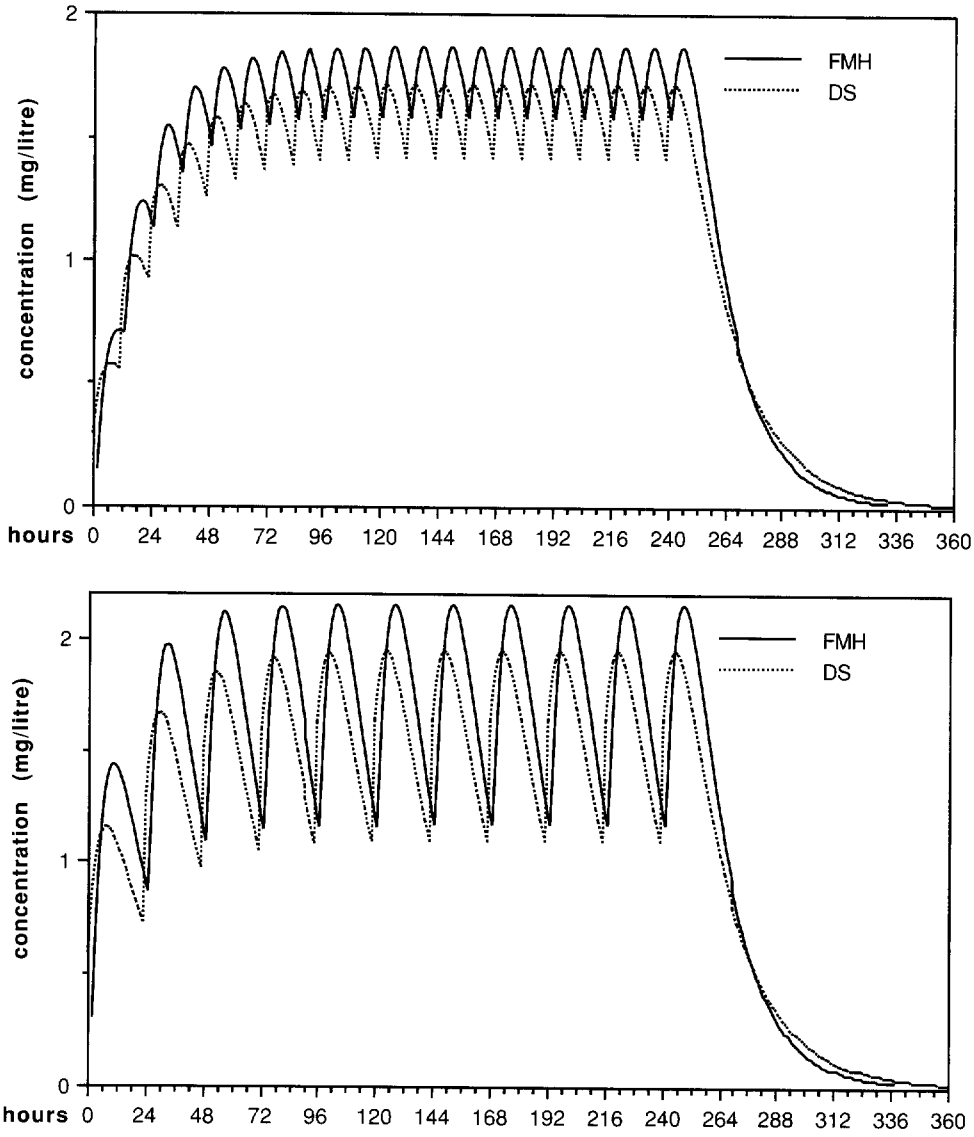
Figure 7. Concentration-time profile extrapolated at the steady-state of sulodexide monitored as DS in plasma, whole blood or particulate, after repeated administration of 50 mg twice daily (upper graph) or 100 mg once daily (lower graph) by oral route.



some clinically relevant information was derived. Steady-state was obtained in a finite, measurable time, regardless of the administration route of the measured component and of the examined compartment. Thus, accumulation and non-linear kinetics may be excluded.

The time necessary to reach steady-state in the different compartments was apparently the same, regardless of the chemical species considered and the administration route examined, for any given dosage scheme.

Figure 8. Concentration-time profile extrapolated at the steady-state of sulodexide in plasma from FMH or DS parameters, after repeated administration of 50 mg twice daily (upper graph) or 100 mg once daily (lower graph) by oral route.



The minimum concentration at steady-state was approximately the same in any given compartment and for any given dosage scheme, regardless of the administration route considered. However, the intravenous administration was associated with appreciably higher peaks than the oral route, from three to ten times higher, although these were transient. The only exception appears to be the peak concentration of DS in the particulate, which did not differ irrespective of oral or intravenous route.

There was no evidence that repeated administration may appreciably modify the equilibrium between plasma and particulate, regardless of chemical species considered, administration route or dosage scheme.

The concentration profile at steady state did not differ remarkably between the estimate from FMH and the estimate from DS (Figure 8), regardless of the administration route and the dosage scheme. The time to reach steady-state provided identical estimates when extrapolated from FMH data and DS data.

All these observations were confirmed by multivariate statistical analysis.

CONCLUSION

With all the necessary caution due to the limited sample size and the intrinsic limits of the applied methods, the reported study shows that sulodexide is absorbed into the blood stream after oral dosing. This absorption is equivalent, whether calculated from the FMH fraction or the DS fraction. The results of AUC and of urinary excretion indicate an almost equivalent biological availability after intravenous bolus injection and oral dosing. However, this statement should take into consideration that, upon the results of gel-permeation analyses, the AUC computed after IV dosing was closer to the real AUC of the parent substance, by approximately 10 – 20%, in comparison with that computed after oral dosing. The results for the elimination constant, elimination half-life, mean residence time and urinary excretion, all concur to indicate that the elimination kinetics of FMH and DS from plasma, whole blood and particulate, as well as the excretion rate, were almost identical and not influenced by the administration route. The bound fraction, or the ratio between concentration in plasma and concentration in the particulate, tended to exclude irreversible uptake or sequestration. The equilibrium between fluid and particulate appeared to be of a passive dynamic nature, for both FMH and DS. The fraction circulating unbound in the fluid was always appreciably greater than that bound to the particulate, to almost the same extent for FMH and DS.

The performed extrapolations to steady-state suggested that sulodexide reached steady-state in four to six days, in agreement with our previous report [1]; the concentration profile at steady-state was similarly well described when computed from FMH parameters as when computed from DS parameters; the two possible oral dosage schemes – 50 mg twice daily or 100 mg once daily – provided comparable concentration profiles at steady-state, with smaller fluctuations for the fractionated doses. The absence of important peaks in comparison with the intravenous route, justifies a somewhat slower onset of pharmacodynamic (anti-thrombotic) action, and the absence of detectable anticoagulant effects.

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