Review of Pharmacodynamics, Pharmacokinetics, and Therapeutic Properties of Sulodexide

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Abstract: Due to various side effects of heparin, such as heparin-induced thrombocytopenia type I or type II, alternative anticoagulants are in clinical development to optimize the anticoagulant regimes in patients requiring low or high anticoagulation dosages. Sulodexide is a highly purified preparation containing a fast-moving heparin fraction as well as dermatansulfate. The pharmacological effects of sulodexide differ substantially from unfractionated heparin and are mainly characterized by a prolonged half-life and reduced effect on global coagulation and bleeding parameters. The lipolytic activity of sulodexide is increased in comparison to unfractionated heparin. Clinical studies demonstrate the safety and efficacy of sulodexide. Specially, oral administration leads to fibrinolytic activities in contrast to oral administration of other glycosaminoglycans. Thus, sulodexide releases tissue plasminogen activator and decreases fibrinogen levels as well as HDL and total cholesterol levels and blood viscosity. Clinical efficacy is demonstrated in peripheral arterial disease, cardiovascular events, in postphlebitic syndrome and on albuminuria in nephropathy. *Med Res. Rev.* **18,** No. 1, 1–20, 1998.

Key words: sulodexide; anticoagulation; peripheral arterial disease; oral bioavailability; fibrinolytic effects

1. INTRODUCTION

Prophylaxis and treatment of thromboembolic diseases is mainly performed using heparins, low molecular weight heparins, and dermatan sulfates. Heparins and oral anticoagulants are also effectively adopted for prevention of recurrent thromboembolism as well as in myocardial infarction. Because for survivors of acute myocardial infarction there is a risk of recurrence and cardiac death, several clinical trials have been conducted using different antithrombotic drugs alone or in combination for secondary prevention of coronary heart diseases. It has been shown that platelet in-

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hibitors are able to reduce the cardiovascular mortality as well as subcutaneous heparin or oral anticoagulation.

Sulodexide is a highly purified glycosaminoglycan on the Italian market since 1974 as an antithrombotic drug. The simultaneous presence of a heparin fraction and dermatan sulfate provides the product with particular synergistic characteristics which increase the antithrombotic potential in comparison to heparin with less bleeding.[1,2]

2. CHEMISTRY

Sulodexide is a glycosaminoglycan obtained by a patent process (U.S. 3,936,351) from porcine mucosa. The chemical composition of sulodexide is defined as 80% of fast-moving heparin fraction (iduronylglycosaminoglycan sulfate) and 20% of dermatan sulfate.[3] The fast-moving heparin (FMH) is defined on the basis of its electrophoretic mobility in the barium-propanediamine system. This component is present in the commercial heparin together with a fraction with a lower electrophoretic mobility.[4]

The FMH is characterized by a low–medium molecular weight (7000 D), lower sulfation degree, lower anticoagulant activity than the slow-moving heparin fraction and unfractionated heparin. FMH contains the same dimeric components as heparin but with lower content of iduronic acid-2-O-sulfate and different content of glucosamine-N-acetylated-glucuronic acid dimer (Alfa Wassermann SpA—Sulodexide: 250 LRU capsules Regulatory Dossier—Part II C: Control of starting materials. data on file).

Dermatan sulfate (DS) is a polydisperse polysaccharide constituted by $\rightarrow$3)-β-D-galactosamine-N-acetylate-4-sulfate (1$\rightarrow$4)-α-L-iduronic acid as the most representative unit with a mean molecular weight of 25,000 D. The particular chemical structure of DS is responsible for its anticoagulant, specifically antithrombin activity, and for its antithrombotic activity. Due to the concomitant presence of FMH with affinity for antithrombin III (ATIII) and of DS with affinity for heparin cofactor II (HCII), sulodexide is characterized by various biological activities: lipasemic ($\geq$ 10 LRU/mg), anticoagulant ($<$ 100 IU/mg), anti-Xa (70–100 IU/mg), HCII ($\approx$ 180 U/mg), APTT ($<$ 50 U/mg) determined against the IV International Standard of Heparin and antithrombotic activity.[6]

3. PHARMACODYNAMICS

A. Anticoagulant and Venous Antithrombotic Activities

Sulodexide, which contains 80% fast-moving heparin and 20% dermatan sulfate, potentiates the antiprotease activities of both ATIII and HCII simultaneously. The prolongation by sulodexide of prothrombin time, activated partial thromboplastin time, thrombin clotting time, heptest, chromogenic antithrombin and anti-Xa assays, thrombin generation inhibition, and factor Xa inhibition in normal human plasma have been analyzed. The data are compared in Table I.[3] Sulodexide prolongs the thrombin clotting time and the aPTT; such effect in this case estimates the catalytic efficiency on the reactions of thrombin with AT III and HCII contemporarily. Moreover, in the amidolytic assays sulodexide produces strong antithrombin and anti-Xa effects, the former being also more potent than the latter. The drug's inhibitory effect shows more efficaciously by the intrinsic than by the extrinsic way.

Furthermore, several glycosaminoglycans delay the onset of prothrombin activation. Unfractionated heparin is the most effective one and it delays the onset of intrinsic activation by ca. 30 sec.

Table I. Inhibition of Various Coagulation Parameters by Sulodexide (μg/ml) (Data from Ref. 3)

A. *Test system*	*Doubling of Clotting Time (μg/ml)*	*Prolongation to 100s (μg/ml)*
Prothrombin time	25	100
aPTT	3.1	5.0
Thrombin clotting time	0.5	1.0
Heptest	0.4	3.1
B.		
	IC_{50}[a] (μg/ml)	
Chromogenic alla assay	0.10	n.a.
Chromogenic Xa assay	0.20	n.a.
	Intrinsic Activation	*Extrinsic Activation*
Inhibition of thrombin generation	30	65
Inhibition of Factor Xa generation	3	5

[a]IC_{50} = 50 % inhibition concentration.

Delay by sulodexide is less effective; furthermore, it essentially proceeds to completion within 90 s of recalcification.

Similarly, the onset of factor Xa is delayed by each glycosaminoglycan: heparin is the most effective inhibitor of intrinsic factor Xa activation, and the second most effective glycosaminoglycan is sulodexide. The co-ordinated inhibition of factor Xa and prothrombin activation suggests that sulodexide can effectively inhibit thrombin-mediated amplification reactions of coagulation.[7]

The same results obtained *in vitro* were confirmed *in vivo*, where the inhibition of thrombus formation was paralleled by a dose-related increase in antifactor Xa and antithrombin activities, but with a lower anticoagulant effect than heparin.[2,3]

The antithrombotic activity of sulodexide was analyzed after intravenous injection in the rabbit jugular vein stasis thrombosis model prior to injection of prothrombin complex (FEIBA). The ED 50 was 20 mg/kg for the 10-min stasis time. At the 10-min stasis time complete prevention of clot formation was achieved at 60 mg/kg sulodexide. In the 20-min stasis time the drug had an ED 50 of 60 mg/kg and complete prevention of clot formation was attained at 125 mg/kg. APTT and thrombin clotting time values were not prolonged up to 125 mg/kg sulodexide despite the effective inhibition of clot formation. In contrast, heptes coagulation values were prolonged up to 100 sec at 125 mg sulodexide[3]; the antithrombotic efficacy of sulodexide was comparable to that of heparin.[8]

Thrombin-induced thrombus formation in rabbits was analyzed in order to get information about this typical naturally occurring activator of fibrin formation. Preinjection of 5 antithrombin units (U)/kg of heparin inhibited clot formation to about 35%. In comparison 3 U/kg sulodexide also decreased clot formation by 35%. Increasing the dose of heparin to 10U/kg inhibited thrombin-induced clot formation by 95% and increasing the sulodexide dose to 5 U/kg resulted in a 92% inhibition of thrombus formation (Fig. 1). Thus, sulodexide when given in half the antithrombin dose of heparin, was effective as the heparin dose in preventing thrombus formation. The equivalent antithrombotic activity of sulodexide (5 U/kg) and heparin (10 U/kg) was achieved, although the circulating an-

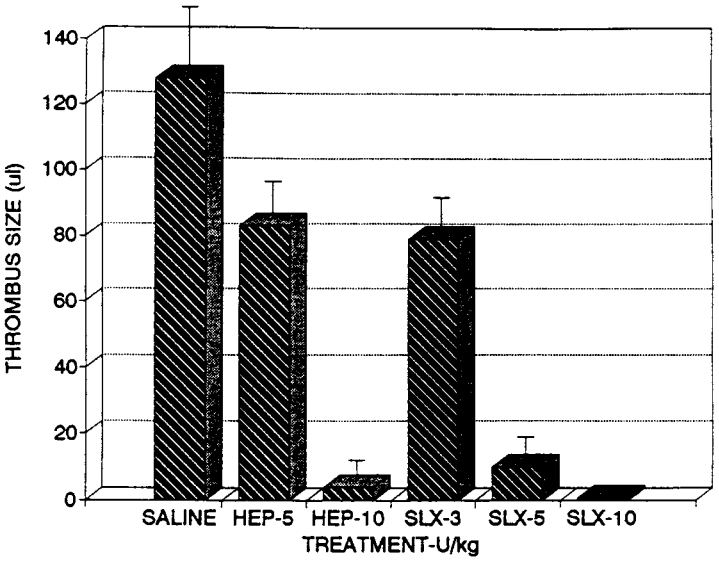

Figure 1. Inhibition of clot formation by preinjection of increasing doses of heparin or sulodexide. Five and ten units heparin correspond to 36 and 66 mg/kg. Three, five, and ten units sulodexide correspond to 50, 83, and 166 mg/kg, respectively (mean + SEM, $n \geq 10$, figure from Ref. 2).

tithrombin plasma levels of rabbits treated with sulodexide were significantly lower than those generated after heparin treatment. These results are consistent with the hypothesis that catalysis of thrombin inhibition simultaneously by HC II and AT III is synergistic; this synergism is evident by the observation that a lower dose of sulodexide achieved an equivalent antithrombotic effect as a higher dose of heparin in preventing thrombosis.[1,2]

The prevention of thrombus growth was studied in animals receiving thrombin as thrombotic stimulus and infusion of heparin or sulodexide: it was demonstrated that fibrin incorporated into the thrombus was significantly less after infusion of sulodexide. These data demonstrate a possible profibrinolytic effect of sulodexide.[2] The thrombolytic effect of sulodexide was also demonstrated after intravenous slow infusion at various doses. A complete clot lysis was obtained at an infusion rate of 2.5 mg/kg/h; this effect was comparable to that of urokinase at 10,000 U.[3,9]

After subcutaneous administration sulodexide showed complete prevention of clot formation after 10 and 20 min of stasis time at a dose of 2.5 mg/kg.[3] Oral administration of 10 mg/kg sulodexide in primates showed an increase in TPA and U-PA antigen levels over an administration time of 5 days from 5 to 10 ng/ml for TPA and from 3 to 6.5 ng/ml (U-PA).[3] The data suggest that sulodexide is a strong anticoagulant, antithrombotic, and profibrinolytic agent in pharmacological models.

The antithrombotic effect of sulodexide was demonstrated also in rats in a venous stasis model.[10] The injection of sulodexide 10 min before ligature of the *vena cava* resulted in a dose-dependent decrease of thrombus formation with an ED 50 of 0.55 mg/kg. Even at lower dosages, an effective inhibition was obtained (Table II).[11] The effect of sulodexide on 6 h-old thrombosis showed a significant reduction in thrombus with increasing doses of sulodexide.[11] To investigate the fibrinolytic effect of sulodexide on preformed thrombosis, ϵ-aminocaproic acid as an antifibrinolytic agent was given to the thrombus-bearing animals immediately before sulodexide. About 40% of the reduction of thrombus was counteracted by preinjection of the antifibrinolytic agent, indicating the fibrinolytic effect of sulodexide (Table III).[11] An increase of the plasminogen acti-

Table II. Prevention of Stasis-Induced Thrombus Formation by Different Doses of Sulodexide (Data from Ref. 11)

Groups	*n*	*Incidence of Thrombosis (%)*	*Thrombus Weight (mg) (mean ± SEM)*[a]
Saline	8	100.0	5.45 ± 0.35
Sulodexide (0.25 mg/kg)	8	100.0	2.96 ± 0.27
(0.5 mg/kg)	8	50.0	2.43 ± 0.65
(1.0 mg/kg)	8	14.3	2.34

[a]Only the animals showing thrombi are considered in the calculation of the mean weight.

vator in the euglobulin activity was demonstrated in these experiments. Administration of fluorescein-labeled sulodexide revealed diffused infiltration of the thrombus with the fluorescent compound, indicating binding as well as antithrombotic and profibrinolytic effects of sulodexide on the thrombus.[11]

Also after administration into the rat duodenum sulodexide caused 50% decrease of 6 h-old thrombi within 2 h at dosages of 50 mg/kg.[11] The experimental data gave evidence of an equivalent potency of sulodexide compared to unfractionated or low molecular weight heparins with regard to its antithrombotic effects.[8–13] The bleeding effects were comparable to dermatan sulfate and thus, lower compared to heparin.[11,14]

B. Effects on Platelet and Leukocyte Function

Leukocyte elastase is a sensitive and potent mediator of connective tissue breakdown, and degrades several of the macromolecular constituents under physiological and experimental conditions. The inefficient control of this proteinase by naturally occurring inhibitors may lead to disease states, such as emphysema and rheumatoid arthritis. Leukocyte elastase is a highly basic protein and it has been shown that several glycosaminoglycans could inhibit this proteinase via electrostatic interaction. Heparin is a potent inhibitor of leukocyte elastase as well as of catepsin G. Desulfated heparin lacks the

Table III. Reduction of 6-h-Old Thrombi by Different Doses of Sulodexide, and the Effect of ε-Aminocaproic Acid (EACA) (Data from Ref. 11)

Groups	*n*	*Thrombus Weight (mg) (mean ± SEM)*
Saline	8	15.71 ± 1.17
Sulodexide (0.5 mg/kg)	8	13.47 ± 0.91
Sulodexide (1.0 mg/kg)	8	7.87 ± 0.80
Sulodexide (2.0 mg/kg)	8	4.82 ± 0.31
Saline	8	19.26 ± 0.88
Sulodexide (2.0 mg/kg)	8	4.60 ± 0.50
EACA (1 mg/kg)	8	21.18 ± 1.44
Sulodexide + EACA	8	11.38 ± 1.20

inhibitory potential, whereas over-O-sulfated oligosaccharides are more potent inhibitors than over-N-sulfated compounds.[15]

The effect of sulodexide and other glycosaminoglycans was studied on catepsin G or thrombin-stimulated platelets and stimulated polymorphonuclear leukocytes. Sulodexide has proved to prevent platelet activation induced by cat G with an equivalent potency of heparin and it also prevented the hydrolysis of the specific substrate in the same range of concentrations. Thrombin-induced platelet aggregation was prevented by heparin and sulodexide. Sulodexide as well as heparin inhibited at equivalent potency thrombin-induced platelet aggregation in a range of concentrations from 5 to 100 mg/ml, while the effect of dermatans sulfate was much less pronounced. The effect was more pronounced in the presence of antithrombin III and heparin-cofactor II in platelet-rich plasma indicating a cofactor-mediated process.[16]

C. Antiproliferative Activity

Vascular muscle cells synthesize proteoglycans containing chondroitin sulfate with varying amounts of dermatan sulfate and heparan sulfate. Heparin is a highly negatively charged glycosaminoglycan and a potent inhibitor of smooth muscle cell proliferation *in vitro* and *in vivo.* Other glycosaminoglycans such as heparan sulfate, dermatan sulfate, and sulodexide caused a significant but lesser stimulation of proteoglycan synthesis. No dependence on the molecular weight of the heparin and sulodexide preparations was observed. In addition, the individual activity on stimulation of proteoglycan synthesis by vascular smooth muscle cells occurred with respect to the cells' state of proliferation using heparin and sulodexide.[17]

The antiproliferative effect of sulodexide on smooth muscle cell proliferation and protein synthesis of human arterial smooth muscle cells, fibroblast-like cells, and epithelial cells showed an effective reduction of proliferation at a concentration ranging from 5 to 100 mg/ml. Smooth muscle cells were less sensitive to sulodexide compared to fibroblast-like cells and epithelial cells.[18]

Fibroblast growth factors (FGFs) belong to a family of heparin-binding proteins, which display a strong affinity for heparin. They regulate cell migration, proliferation, and differentiation participating in numerous physiological processes as neovascularization or wound repair.[19] The mitogenic activity of fibroblast growth factors was potentiated by 10 mg/ml sulodexide and was comparable to 20 mg/ml heparin, as measured by the tritiated Thymidine Incorporation rate. FGFs were also stabilized by sulodexide as well as by heparin, as determined by resistance of growth factors to heat denaturation in the presence of glycosaminoglycans.[20]

D. Arterial Antithrombotic Activity

Occlusive arterial thrombosis is the principal complication of atherosclerosis. Heparins and platelet inhibitors such as aspirin or sulfinpyrazone have been demonstrated to potently inhibit arterial thrombus formation[21] as well as prostaglandin E1.[22]

In an acute experimental model where the thrombus formation was induced in the carotid arteries by electrical stimulation,[21] sulodexide was able to significantly prolong the time of maximum fall in temperature in a dose-dependent manner after intravenous bolus administration, with an efficacy similar to that of heparin or ASA.[23] The antithrombotic activity of sulodexide has been compared to heparin and dermatan sulfates in a chronic animal model[22] of arterial thrombosis with the aim to analyze the ratios of the antithrombotic/bleeding effects of the compounds. The concentrations to double the aPTT coagulation time were 1.5 mg/ml for heparin and 3.0 mg/ml for sulodexide. The heparin cofactor II-mediated thrombin inhibition was 180 U/mg for both compounds. The dose of 2 mg/kg of heparin was already effective in preventing arterial thrombosis in contrast to sulodexide. At 8 mg/kg (heparin) and at 10 mg/kg (sulodexide) both compounds were equally effective. In contrast, bleeding time was 2 times longer at the higher dosages of heparin in contrast to su-

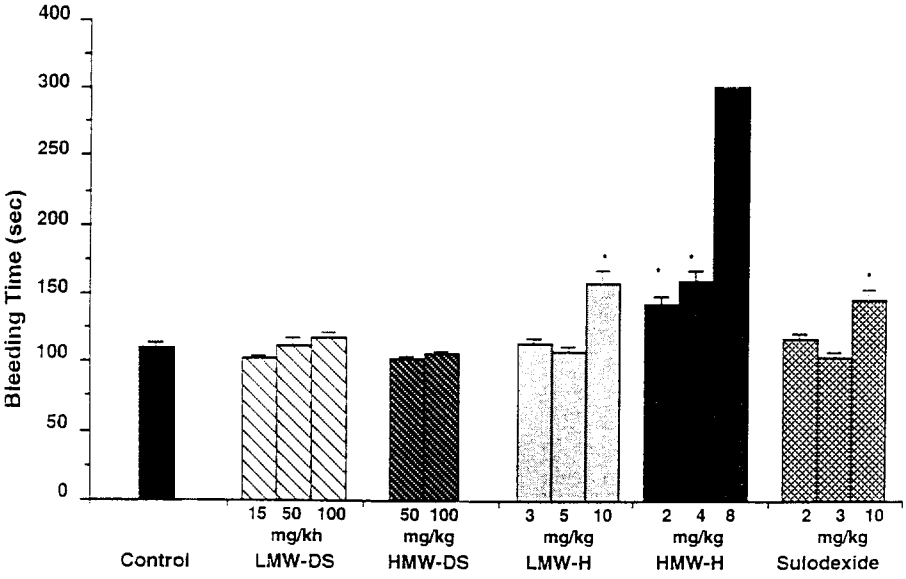

Figure 2. The antithrombotic effect (h), the effect on bleeding time (s), and the effect on aPTT coagulation time (s) of the control experiments and after administration of 2 mg, 4 mg, or 8 mg unfractionated heparin (UFH) or 2 mg, 3 mg, or 10 mg sulodexide (SDX) are shown as mean and standard deviation (data from Ref. 14).

lodexide, which produced only a 25% prolongation of bleeding time at the higher dose (14) (Fig. 2). APTT was prolonged to the same extent with heparin using a 2.5-fold lower concentration compared to sulodexide.[14]

In conclusion, sulodexide was an effective inhibitor of arterial thrombosis in rats without inducing bleeding complications and with a substantially reduced effect on bleeding time and aPTT than heparin.

E. Antiatherosclerotic and Antilipaemic Effects

Release of lipoproteinlipase activity by heparin and low molecular weight heparin is well established and is regarded as an important mechanism for prevention of arteriosclerosis due to hydrolysis of lipoproteins and triglycerides. Sulodexide has been demonstrated to be a potent liberator of lipoproteinlipase activity after intravenous, subcutaneous, and oral administration. In fact, sulodexide is often rated on its lipasemic activity, i.e., as Lipoproteinlipase Releasing Units (LRU). In cholesterol-fed rabbits sulodexide significantly reduced cholesterol plasma levels and cholesterol accumulation in the rabbit abdominal aorta compared to controls.[24,25] The disappearance of 14 C palmitate-labeled low density lipoproteins was enhanced after addition of sulodexide in rat liver perfusates of healthy, lipidemic, and hypertriglyceridemic rats. Sulodexide was shown to interact with VLDL by decreasing lipoprotein uptake into the rabbit aorta and by increasing the hepatic metabolism in normal and hypertrigylceridemic animals.[26]

F. Pharmacokinetics of Fluoresceinated Sulodexide

To study the pharmacokinetics of sulodexide, a fluoresceinated derivative has been synthesized with intact anticoagulant activity. In rats 50% of the compound was eliminated into the urine within 24 h

and 67% within 48 h after intravenous administration.[27] The calculated half-life increased with increasing doses up to 16 min after administration of 15 mg/kg.[27] The organ distribution revealed an extracellular diffusion in liver and kidney up to 4 h after administration. In particular, in the kidney cortex fluorescent material was present at the longer time intervals. Some fluorescence was also observed in the spleen, in the perivascular areas and in the media of the aortic wall, so indicating the importance of the various species for the metabolism and the biological effects.[28]

After oral administration of sulodexide in rats, anticoagulant effects were observed within 3 h and a cellular distribution of radiolabeled material was observed in kidney, liver, and the endothelium of venous and arterial system. Thus, data from fluorescent labeled compound could be reproduced by radioactive labeling of sulodexide.[29]

4. PHARMACOKINETICS AND PHARMACODYNAMICS OF THE EFFECTS IN MAN

The pharmacokinetics of sulodexide were investigated in man after a single oral administration of 60 mg ^{14}C-labeled sulodexide (8 mCi) in 3 patients.[30] The calculated clearance was 2.7 ml/min and the volume of distribution 71 $\pm$ 14 liters. Twenty-three percent of radioactivity was excreted through the bile and 55% through the kidney. Blood concentration data were submitted to pharmacokinetic analysis, fitting to a two-compartment open model. From this model, the concentration-time profile was extrapolated for two possible dosage schedules: 25 mg (equal to 250 LRU) twice daily and 50 mg (500 LRU) once daily. In both cases the steady state was reached after approximately 20 days with peak concentrations of around 1.5 mg/l. The pharmacokinetic parameters are given in Table IV. These data were confirmed using 125-iodine-sulodexide, labeled either on the fast-moving heparin fraction or on the dermatan sulfate fraction.[31] Four groups of 3 patients undergoing radiotherapy received a single dose of 50 mg, either by intravenous or oral route. This study showed that sulodexide is absorbed into the blood stream after oral dosing, the absorption rate being equivalent, either calculated from the fast-moving heparin fraction or from the dermatan fraction.

The pharmacokinetics of sulodexide was furthermore evaluated by 131-iodine labeling the fast-moving heparin fraction.[32] Three groups of 3 patients received the single administration of: 50 mg by i.v. route; 50 mg by oral route; 100 mg again by oral route. The elimination half-life was 11.7 + 2.0 h after intravenous administration, 18.7 + 4.1 h after 50 mg *per os*, and 25.8 + 1.9 h after 100 mg *per os*. The elimination occurred mainly by biliary and urinary excretion. The calculated steady state conditions after oral administration were reached after 4 to 5 days. The biological availability of sulodexide was almost equivalent after i.v. bolus and oral dosing, but oral administration yielded circulating concentrations greater than those after *i.v.* dosing at all times except the first few hours.

The pharmacokinetics of deuterium-labeled sulodexide was also studied in healthy volunteers[33] using high performance liquid chromatography and mass spectrometry. Following a cross-over design, 4 healthy volunteers received—with one month of wash-out between the two administrations—100 mg of deuterated sulodexide by single *i.v.* or oral dosing. After intravenous administration, the elimination half-life of the fast-moving heparin fraction was calculated as 1.0 h and that of the dermatan fraction as 1.6 h. Peak plasma levels were 20 and 8 mg/l, respectively. Oral administration of sulodexide resulted in peak plasma levels of both compounds ranging from 0.2 to 1.0 mg/l 1 to 10 h after dosing.[49] Also this study provided clear evidence of the availability, following oral administration, of the two sulodexide fractions.

A large number of studies have also been performed to analyze the anticoagulant/antithrombotic, fibrinolytic, and hemorrheologic effects of sulodexide in man. A dose-dependent effect was demonstrated after intravenous injection of sulodexide doses ranging from 1800 to 7200 antithrom-

Table IV. Plasma Pharmacokinetics and Excretion Profile of Sulodexide (Data from Ref. 30)

Variable	*Mean ± SD*
Plasma Pharmacokinetics	
C_{max} (ng/ml)	516.00 ± 77.54
T_{max} (h)	1.33 ± 0.58
AUC (mg.h/liter	22.83 ± 4.44
Clearance (liters/h)	2.70 ± 0.58
MRT (hours)	14.28 ± 3.29
VD (liters; β phase)	71.24 ± 14.06
Renal Excretion (% of Dose)	
Cumulated 96 h	55.3 ± 2.9
Bilary excretion (% of dose):	
Cumulated 48 h	23.5 ± 2.3
Total excretion (% of dose):	
Cumulated 96 h	78.9 ± 1.5

bin units (180–720 LRU).[34] Another study pointed out that 2 hs after intravenous injection, aPTT, and antifactor Xa activity were again normal. The calculated half-life on antifactor Xa activity was 45 min.[35] The intravenous and intramuscular administration of 300 to 1200 LRU of sulodexide were also compared to analyze the drug's bioavailability.[36] The half-life after i.m. injection ranged between 420 and 500 min and the bioavailability by this administration route was calculated to be above 90%.

The oral absorption of sulodexide has been investigated in volunteers and patients. A pilot study analyzed the influence, on fibrinolytic and viscosity parameters, of 100 mg (equal to 1000 LRU) of sulodexide, given once daily for 7 days; parameters were evaluated on day 1 and day 7.[37] Plasminogen Activator Inhibitor (PAI) activity was found decreased and the Euglobulin clot Lysis Time (ELT) increased; whole blood, plasma, and serum viscosities also decreased significantly during the treatment period. In 32 patients with chronic phlebopathies, treated for 30 days with oral sulodexide (1000 LRU/day) and placebo according to an experimental design in double-blind with cross-over of treatments,[38] fibrinogen was found decreased by 5% compared to placebo, while no changes of the coagulation parameters were detected. Another 40 patients were treated with 100 mg of sulodexide or with placebo for 3 weeks to analyze coagulation and fibrinolytic parameters.[39] During treatment PAI-1 decreased from 30 to 17 U/ml while remained unchanged during placebo. Fibrinogen decreased from 410 to 345 mg%. No effects were observed on aPTT.

The positive effects on fibrinolytic activity and on blood viscosity of oral sulodexide were again confirmed in a study of a 7-day treatment with 100 mg,[40] and in another with a treatment length of 14 days.[41] Also the administration of 1000 LRU/day for 1 month again supported the oral absorption of sulodexide as well as its effect on blood viscosity, in comparison to placebo.[42]

The effects of i.v. sulodexide, administered for 10 days at the dose of 1200 LRU/day, were investigated in 10 healthy controls and in 10 type I diabetic subjects, monitoring several coagulation and fibrinolysis parameters.[43] Sulodexide reduced the fibrinopeptide A (FPA) formation induced by intravenous glucose infusion in normal as well as in diabetic subjects. At the same time sulodexide increased tPA activity, while PAI-1 was reduced in patients and still more evidently in healthy volunteers. The increase in FPA and decrease in PAI-1 induced by intravenous glucose injection was partially counteracted by sulodexide. The circadian fibrinolytic activity is characterized by a sharp

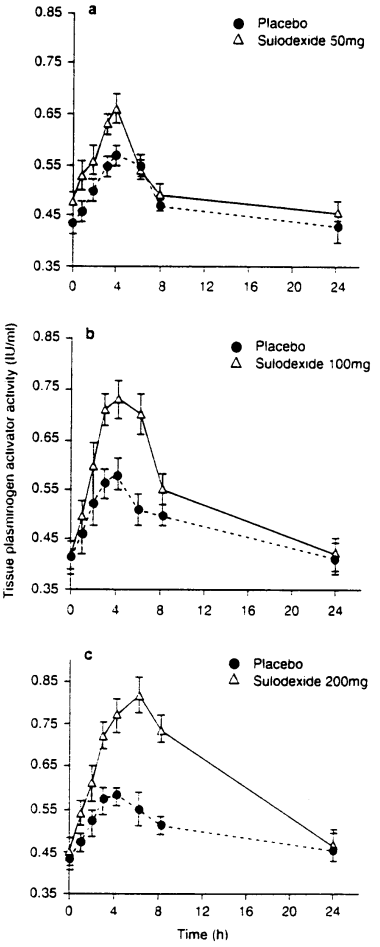

Figure 3. Activity of tissue plasminogen activator (IU/ml, mean ± SEM) during day time after oral administration of placebo, 50 mg (a), 100 mg (b), or 200 mg (c) sulodexide (data from Ref. 44).

increase in tPA during the morning; thereafter, such fibrinolytic activity decreases. A dose-dependent increase in the circadian activity of tPA by sulodexide was demonstrated in a placebo-controlled trial.[44] It is also known from the literature that the increase in tPA is mainly linked to a decrease in PAI-1. Accordingly, this study showed that PAI-1 decrease was enhanced by increasing doses (50–200 mg) of sulodexide, in comparison with placebo (see Fig. 3 and 4). Similar results were observed in patients with postphlebitic syndrome[45] and peripheral arterial occlusive disease.[46]

The effects of sulodexide on blood rheology have been studied in various trials. A dose-dependent decrease in plasma and whole blood viscosity was demonstrated after oral administration in patients with chronic venous disease[44] and in patients with peripheral vascular disease.[46–48] It is also worth mentioning a study of the effect of some glycosaminoglycans (including sulodexide) on elevated plasma fibrinogen levels in diabetic patients.[49] The parenteral administration, to type II diabetic subjects, of 12,500 IU of heparin, 7500 IU of low-molecular-weight heparin, 50 mg of der-

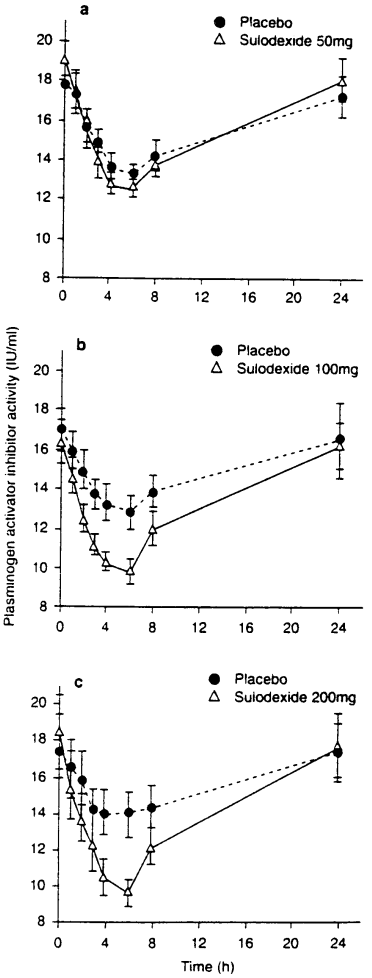

Figure 4. Plasminogen activator inhibitor (IU/ml, mean ± SEM) after oral administration of placebo, sulodexide 50 mg (a), 100 mg (b), or 200 mg (c). Data are from Ref. 44.

matan sulfate, or 40 mg (equal to 400 LRU) of sulodexide, for 10 days, resulted in a decrease in plasma fibrinogen concentrations by about 50 mg% compared to placebo, in all groups. Consequently, a decrease of 0.4 to 0.5 ng/ml in fibrinopeptide A levels was registered in all groups, compared to placebo (always $p < 0.01$).

5. CLINICAL EFFICACY

Despite the positive and well-defined effects of sulodexide on blood coagulation, fibrinolysis, and blood viscosity, its clinical efficacy is not always explained by these actions. Binding of the heparin and dermatan fractions to endothelium, erythrocytes, or leukocytes may significantly influence the

drug's clinical effectiveness.[50–53] Several clinical studies have been carried out with the aim to demonstrate sulodexide efficacy in patients affected by vascular pathologies associated with a thrombotic risk such as: peripheral occlusive arterial disease, hyperlipidemia and/or diabetes, coronary artery disease, myocardial infarction, transient cerebral ischemia, and in patients with diabetic nephropathy associated with micro- or macro-albuminuria.

A. Peripheral Occlusive Artery Diseases (POAD)

Anticoagulant and antiaggregant treatment in these patients is still debated. Compounds with additional nonanticoagulant actions such as sulodexide may offer advantages due to their capability of lowering fibrinogen levels, improving blood viscosity, and increasing fibrinolytic activity. High plasma fibrinogen levels have been shown to contribute significantly to the development of peripheral arterial diseases.[54,55]

A large number of clinical studies performed with sulodexide in this indication has been identified through literature search on Medline and M-base databases.[56–84] A great part of the investigations followed a double-blind design with or without cross-over, and compared intramuscular or oral sulodexide—given for up to 180 days—with placebo. Nineteen publications relevant to studies performed with a controlled design proved furthermore suitable, more recently, for a meta-analysis;[85] their characteristics are given in Tables V and VI. The diagnosis of POAD was made according to the criteria of Leriche-Fontaine stages: most studies included patients with stages I to III, according to this definition. The administration scheme most frequently consisted of a first parenteral phase, more often with sulodexide (600 LRU/day i.m.) for 10 to 15 days, followed by the oral administration of sulodexide (500–1000 LRU) or placebo for 2 to 6 months.

Table V. Characteristics of the Studies Considered for Meta-analysis (Data from Ref. 85)

Author	*Study Design*	*Administration Route & Length (Days)*
Bartolo *et al.*, 1984	DB	im = 20, oral = 70
Bonalumi *et al.*, 1986	DB	im = 20, oral = 70
Bonanno *et al.*, 1985	DB	im = 20, oral = 70
Borreani *et al.*, 1993	DB	im = 30, oral = 60
Calabrò *et al.*, 1985	DB-CO	im = 15
Caramelli *et al.*, 1988	DB	im = 20
Castelluccio *et al.*, 1991	DB-CO	oral = 30
Ciuffetti *et al.*, 1989	DB-CO	oral = 15
Corsi *et al.*, 1985	DB	im = 20, oral = 70
Cospite *et al.*, 1985	DB	im = 10, oral = 70
Crepaldi *et al.*, 1990	DB-CO-MC	im = 30, oral = 180
Di Stefano *et al.*, 1984	DB	im = 20, oral = 70
Lunetta *et al.*, 1992	DB-CO	oral = 30
Marzola *et al.*, 1985	DB	im = 20, oral = 70
Palmieri *et al.*, 1982	DB	im = 30
Palmieri *et al.*, 1984	DB	oral = 60
Perego *et al.*, 1981	DB	im = 30
Perego *et al.*, 1982	DB	im = 30, oral = 60
Pisano, *et al.*, 1986	DB	im = 20, oral = 70

S = Sulodexide, P = placebo, DB = double-blind, CO = cross-over, MC = multicenter

Table VI. Characteristics and Distribution by Diabetes, Hyperlipidemia, and Leriche Stage of Patients Involved in the Meta-Analysis (Data from Ref. 85)

Author	*Pts All*	*Pts S*	*Pts P*	*Mean Age (yrs)*	*Leriche Stage*	*Diabetes (n)*	*HLP IIa (n)*	*HLP IIb (n)*	*HLP IV (n)*
Bartolo *et al.*, 1984	30	16	14	63.5	I,II	13	—	10	—
Bonalumi *et al.*, 1986	30	15	15	60.1	NA	11	14	—	14
Bonanno *et al.*, 1985	30	15	15	64.5	<III	15	2	8	11
Borreani *et al.*, 1993	100	50	50	67.5	I,II	NA	—	58	—
Calabrò *et al.*, 1985	36	18	18	<40	I	NA	—	8	—
Caramelli *et al.*, 1988	60	30	30	69.1	NA	19	7	—	14
Castelluccio, 1991	30	15	15	52	I,II	8	—	—	8
Ciuffetti *et al.*, 1989	30	15	15	53	II	5	8	4	2
Corsi *et al.*, 1985	30	15	15	65, 8	I	NA	8	4	9
Cospite *et al.*, 1985	30	15	15	58	NA	15	—	—	—
Crepaldi *et al.*, 1990	164	82	82	59	I,II	NA	23	34	51
Di Stefano, *et al.*, 1984	30	15	15	67.5	NA	9	3	4	8
Lunetta *et al.*, 1992	39	20	19	—	<III	10	—	—	—
Marzola *et al.*, 1985	28	15	13	63.8	<III	8	5	9	11
Palmieri *et al.*, 1982	30	15	15	66	NA	NA	5	6	8
Palmieri *et al.*, 1984	30	15	15	42.5	I,II	NA	6	7	10
Perego *et al.*, 1981	32	16	16	58.4	I,II,III	NA	16	5	11
Perego *et al.*, 1982	60	30	30	—	NA	NA	—	—	—
Pisano *et al.*, 1986	30	15	5	—	NA	22	3	7	8
All		427	422	57.68	—	135	158	148	165

NA—not available, HLP = hyperlipoproteinemia by Fredrickson classification, S = sulodexide, P = placebo

The registration of improvement through the treadmill test was the primary endpoint of the evaluated studies: the test was performed before the start, at the end of the intramuscular phase, and at the end of the oral one. After 20 days of intramuscular administration both treatments produced on average small increases in the walking distance: + 28.7 m after 20 days with placebo (confidence interval: −131/+73) and + 70.7 m (confidence interval: −186/+44.8) after sulodexide. On the other hand, after 70 to 90 days no increase in the walking distance was observed with placebo, while patients treated with oral sulodexide showed a prolongation, on average from 180 m before treatment to 340 m ($p = 0.0066$). The overall meta-analysis of the improvement and worsening rates, performed according to several meta-analytical procedures, is summarized in Figure 5. The differences between improvement and worsening rates are due to patients with stable results, i.e., improved versus (stable + worsened) or (improved + stable) versus worsened.[85] The analysis of pooled data comparing blood fibrinogen values before the start and at the end of the mean treatment period, showed a highly significant ($p < 0.01$) decrease in fibrinogen levels in sulodexide-treated patients (from 346 + 8.3 mg% to 294 + 9.7 mg%) in comparison to those treated with placebo, in whom plasma levels remained unchanged (from 344 + 9.1 mg% to 345 + 9.5 mg%). Also the evaluation of blood viscosity performed—in the cited meta-analysis—on data from 7 studies, revealed a significant decrease in serum and whole blood viscosity, while no activity was exerted by placebo.

Only in three out of the 19 evaluated studies patients did not show hyperlipoproteinemia; in the remaining trials triglycerides values fell on average from 222 mg% to 159 mg% during treatment with sulodexide ($p = 0.015$) and from 206 mg% to 192 mg% during treatment with placebo (ns). HDL-cholesterol mean levels increased during treatment with sulodexide from 38 mg% to 47 mg%

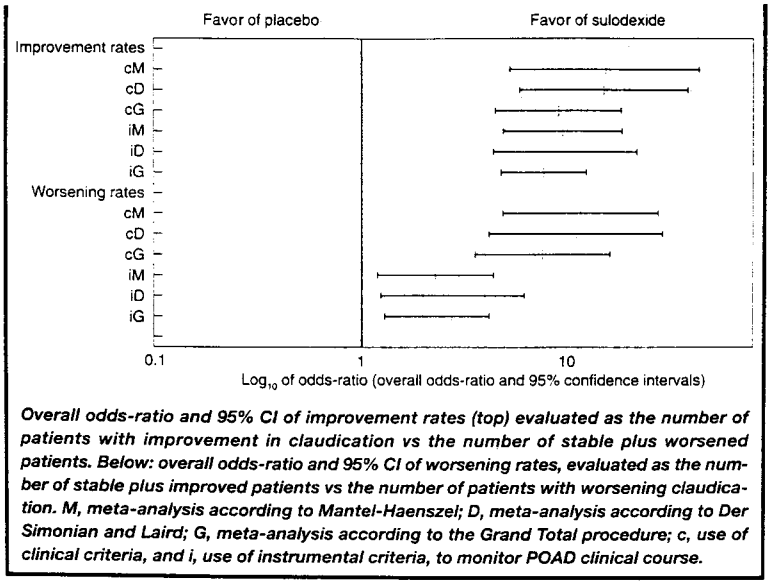

Figure 5. Overall odds ratios and 95 confidential interval of improvement rates and worsening rates of patients treated with sulodexide or placebo for POAD. The odds ratios evaluated the clinical criteria (c) as well as the instrumental criteria (i) (adapted from Ref. 85).

in contrast to placebo-treated patients, in whom HDL-cholesterol levels were on average, respectively, 38 mg% and 39.5 mg%.

B. Cardiovascular Diseases

Sulodexide effectiveness was investigated in patients with unstable angina[86] and ischemic cardiopathy[87]; its use for the prevention of left ventricular thrombosis after myocardial infarction,[88] and after aortocoronary bypass operation,[89] was also studied. Patients with atherosclerosis and hyperlipidemia and/or diabetes have also been included.[90–93] An interesting, multicenter study was also carried out with the aim of studying the prevention by sulodexide of cardiovascular events (death and thromboembolic accidents) in the first year after acute myocardial infarction;[94] the administration scheme was: 600 LRU/day i.m. for 1 month, then 1000 LRU/day by oral route for up to 12 months; a total of 3986 patients were involved. Administration of antiplatelet and other anticoagulant drugs was excluded. The recorded mortality rate was 7.1% in the control group versus 4.8% in the sulodexide group (32% risk reduction, $p = 0.002$, Chi-square test). Another 4.6% of patients in the control group had reinfarction, compared to 3.3% in sulodexide-treated patients (28% risk reduction, $p = 0.035$). Left ventricular thrombus formation was less frequent in the sulodexide group (0.6% versus 1.3% in controls; 53% risk reduction, $p = 0.026$). Sulodexide was well tolerated and devoid of significant adverse events. Figure 6 shows the results of the cumulative incidence of mortality in the two treatment groups.

C. Cerebrovascular Diseases

The benefits of sulodexide for the prevention and treatment of cerebrovascular events in patients mainly elderly have been investigated in a number of clinical studies, sometimes in comparison with

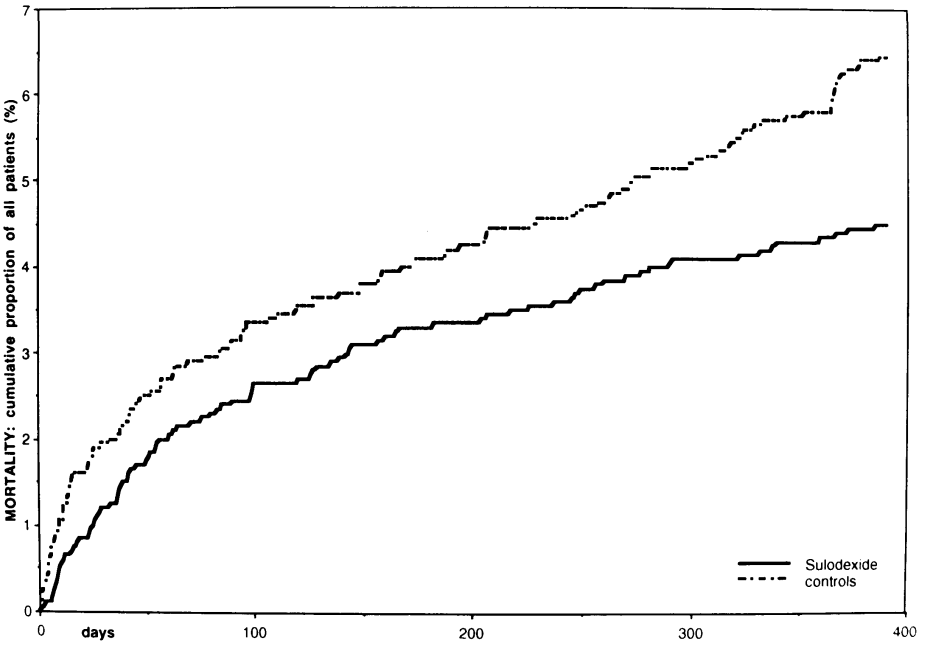

Figure 6. Cumulative proportion of mortality in the study, according to the treatment with sulodexide or in the control group (adopted from Ref. 94).

antiplatelet agents.[95–107] All of the studies evidenced the capability of such an antithrombotic treatment as that represented by sulodexide, to improve the performances of cerebrovasculopathic patients.

D. Postphlebitic Syndrome

Due to the multiple, anticoagulant and nonanticoagulant, mechanism of action of sulodexide, patients with postphlebitic syndrome may profit from long-term treatment. Several clinical studies[108–123] demonstrate the reduction of the clinical manifestations and the improvement in the instrumental findings detected in postphlebitic syndrome patients with history of deep vein thrombosis or chronic venous insufficiency. Some studies have been performed following controlled designs (see Table VII), but the most part was open. The usual treatment scheme of intramuscular administration for 2 weeks, followed by oral treatment for up to 90 days, at the dose of 500 to 1000 LRU/day, was in most studies dropped in favor of a therapy exclusively oral. About 600 patients were included into the studies.

The Maximal Venous Incremental Volume, and the indexes of Venous Capacity and of Venous Distension improved during treatment with sulodexide. Despite the high variation of the results, in some of the patients a complete restoration of the normal vein pressure has been obtained. An interesting multicenter study[119] confirmed the clear-cut improvement, during treatment, of leg edema, pain, hyperthermia, cyanosis, and impairment of function capacity. Also skin atrophy and venous stasis were shown improved by phlethysmography. Significant increases in fibrinolytic activity and decrease in fibrinogen levels and in PAI-1 were found in some of these studies.

E. Vascular Complications in Diabetes

In diabetic patients vascular lesions are the most common cause of morbidity and mortality.[55,124,125] Abnormalities in lipid metabolism and hemostasis contribute to the development of vascular

Table VII. Overview of the Studies Performed in Double-Bind and With Cross-Over in Patients With Postphlebitic Syndrome

Author	*Criteria for Evaluation*	*Dose/Day (LRU) Admin. Route*	*Admin. Length (days)*	*Reference Therapy*	*Pts No S/Ref.*	*Results*
Mauro *et al.*, 1992 DB + CO	L	1000 os	30	Placebo	14/15	S > P ($p < 0.01$)
Agrati *et al.*, 1992 DB + CO	L	1000 os	21	Placebo	40	S > P ($p < 0.01$)
Fiore *et al.*, 1991 DB	L	1000 os	15	Placebo	10/10	S > P ($p < 0.05$)
Cospite, Milio, 1992 DB	I	1000 os	45	Placebo	18/18	S > P ($p < 0.05$)
Cospite, Ferrara, 1992 DB	I	1000 os	90	Placebo	15/15	S > P ($p < 0.001$)
Saviano *et al.*, 1993 DB	I, S, L	250 × 2/500 × 2/1000 os	60	—	160/158/158	—

DB = Double Blind Design; CO = Cross-Over; I = Instrumental Tests; L = Laboratory Tests; S = Clinical Symptoms; S = Sulodexide; P = Placebo

occlusion. Several of the studies performed with sulodexide included diabetic patients;[43,49,69,72–75,78,79,83,84,119,126–129] the studies' aim, by comparing sulodexide to placebo, was frequently to demonstrate a reduction in the occurrence of vascular events. The double-blind study design was often followed, and sulodexide administration was usually by i.m. route for 20 days, and by oral route for the subsequent 70 days. The therapeutic effect of sulodexide was constantly demonstrated with statistical significance, mainly on the pain-free walking distance and on the clinical symptoms present at the start of treatment. Nocturnal pain, cramps, burning sensation, and presence of cold limbs decreased during treatment.

1. Influence of Sulodexide on Albuminuria

Microalbuminuria has been postulated to be a risk factor for diabetic nephropathy and for endstage renal disease. A loss of glycosaminoglycans has been demonstrated in this condition, and GAGs administration was shown useful in the prevention of morphological alteration and in the reduction of albuminuria in experimentally diabetic rats.[130] This behavior has been confirmed in man: patients with type II diabetes were administered orally 1000 LRU/day of sulodexide for 2 months and were observed for another 4 months.[131] During treatment, albumin excretion rate decreased, but was found again increased 4 months after cessation of treatment.

Also another investigation performed in patients with noninsulin-dependent diabetes mellitus, microalbuminuria and limb arteriopathy,[132] pointed out the effectiveness of sulodexide, which significantly reduced micro-albuminuria, (and blood fibrinogen levels and limb arteriopathy clinical signs as well) after a 6-month treatment period (1000 LRU/day).

6. CONCLUSIONS

Sulodexide pharmacological data point to a high bioavailability of this drug after intramuscular and oral administration. The biological half-lives of antithrombotic activities range from 1 to 2 h after intravenous administration and from 5 to 7 h after oral application. The antithrombotic effects and

good tolerability demonstrated in animals have constantly been confirmed in man. Sulodexide in fact releases tPA while decreasing PAI in healthy subjects and in patients. Its administration furthermore lowers blood fibrinogen levels by about 20%, while also blood viscosity is decreased. Clinical studies demonstrate the drug's efficacy and safety in patients suffering from peripheral arterial diseases, cerebrovascular events, and postphlebitic syndrome. Positive effects are observed also in patients with nephropathy, even though in this latter pathology further investigations are needed in order to better understand the real role of sulodexide during long-term treatments.

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