Tissue factor pathway inhibitor release and depletion by sulodexide in humans

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ABSTRACT

Purpose: Anticoagulant tissue factor pathway inhibitor (TFPI) is released from its endothelial stores by heparin, which may lead to its untoward depletion. We investigated the effects of sulodexide – a commercially available mixture of heparan and dermatan sulfate, on plasma TFPI release and depletion.

Material and Methods: An open-label pilot trial of intravenous and/or oral sulodexide effects on plasma immunoreactive total TFPI antigen level was performed in 11 healthy men. The drug was initially administered i.v. at a single dose of 120 mg, then-orally for 12 days (50 mg b.i.d), and again by i.v route after 2 weeks.

Results: Sulodexide injections induced marked increases in plasma TFPI; they were more pronounced on day 14 than on study initiation (3-fold vs. 2-fold after 10 min) and still evident after 120 min. TFPI levels did not change when measured at 120 min after oral sulodexide administration. The percentage increment in plasma TFPI after 10 min from initial sulodexide injection inversely correlated with baseline TFPI levels ($r = -0.664$, $P = 0.026$). On day 14, the association became strong ($r = -0.970$, $P < 0.0001$) and evident also after 120 min ($r = -0.810$, $P < 0.002$). Baseline TFPI levels decreased over the trial; on day 14 they were lower by 34% than on study initiation ($P = 0.001$).

Conclusions: TFPI release by i.v. sulodexide and its depletion during oral administration of this heparinoid compound constitute novel and likely important hemostatic effects of the drug.

Key words: glycosaminoglycan, human study, sulodexide; tissue factor pathway inhibitor

INTRODUCTION

Sulodexide is a purified glycosaminoglycan medication consisting of 80% fast moving heparin (containing heparan sulfate) and 20% dermatan sulfate [1]. Because of low molecular weight of both fractions, intestinal absorption of sulodexide is feasible and the compound is commercially available in oral and parenteral form [2,3]. The drug shows benefit in the prevention/treatment of diabetic nephropathy [4], cardiovascular diseases, deep vein thrombosis, cerebrovascular dementia [1], complications of peritoneal dialysis [5] and others. It also has endothelium-protective properties, diminishes restenosis of injured arteries and blunts systemic and local inflammation [1,4]. Noteworthy, in contrast to heparin, sulodexide is also active against fibrin-bound thrombin due to marked contents of dermatan sulfate [6,7].

Anticoagulant effect of unfractionated heparin (UFH) is, approximately in 30%, mediated by tissue factor pathway inhibitor (TFPI) release from its endothelial membrane-bound complexes with glycosaminoglycans and mobilization of the inhibitor from intracellular stores into circulating blood [8,9]. However, prolonged UFH treatment leads to depletion of vascular stores of TFPI, which results in a partial loss of heparin's anticoagulant activity [10-12].

It is not known if the phenomenon of TFPI "release and depletion" is also applicable to sulodexide. To address the issue, we studied the effects of intravenous (i.v.) injections of sulodexide and its 2 week oral (p.o.) administration on plasma levels of total TFPI antigen in healthy volunteers.

MATERIALS AND METHODS

Study subjects

Eleven healthy males aged 30.4 ± 1.16 years, of a mean body mass index of 25.7 ± 2.44 kg/m^2, non-smokers, without a history of substance abuse, drug hypersensitivity, bleeding or thrombotic events and gastroduodenal disease were enrolled. All volunteers had undergone a physical examination and laboratory work-up with special attention given to serum C-reactive protein, platelet counts and routine hemostatic tests such as plasma fibrinogen, prothrombin time, whole blood activated partial thromboplastin time, and euglobulin clot lysis time. None of the participants experienced major trauma, surgery or infection in the preceding month and was not receiving any medications within the past 2 weeks.

Study protocol

The study was designed as an open-label pilot trial. It conformed to the Declaration of Helsinki, was approved by the Ethics Committee of Medical University of Bialystok, and written informed consent was obtained from each participant.

On day 1, fasted blood samples (T0) were obtained from the antecubital vein by atraumatic puncture with an 18-gauge needle without the use of tourniquet. Then, sulodexide (Vessel Due F, Alfa Wassermann SpA, Bologna, Italy) was injected i.v. at a dose of 1200 Lipoprotein Lipase Releasing Units (LRU, a single 2 ml ampoule contains 600 LRU = 60 mg), prompted by a 10 ml normal saline flush. Afterwards, the subjects remained ambulatory and allowed habitual water intake; the consecutive blood samples were drawn after 10 min (T10) and after 120 min (T120). Next, the volunteers were asked to take oral sulodexide at a dose of 500 LRU b.i.d. for 12 consecutive days. On day 7, fasted blood samples were obtained at T0; then the participants ingested 500 LRU of sulodexide and the sampling was repeated at T120. On day 14, no usual morning oral dose was given and 1200 LRU of the drug was again injected i.v.; further procedures and timing of the blood draw strictly followed those employed on day 1. After that, the study was terminated.

The daily oral sulodexide dose of 1000 LRU was chosen based on that used in a majority of previous clinical trials [1,4]. The i.v. dose of 1200 LRU was selected as a part of a phase I study. The actual oral dose employed in this trial was 12.0 ± 1.57 LRU/kg/day and the i.v. dose was 14.4 ± 1.88 LRU/kg. Drug compliance was evaluated by questioning the participants and counting the capsules. The study period was uneventful; no delayed adverse effects of sulodexide were revealed on medical history and examination performed 1 month after study completion.

Plasma TFPI determination

Blood was drawn into Monovette vacutainers (Sarstedt, Nümbrecht, Germany) containing 0.129 M trisodium citrate (1 vol. anticoagulant and 9 vol. whole blood). Plasma was prepared by immediate centrifugation at 3000 g for 20 min at ambient temperature, aliquoted, and stored at $-70\,^{0}$C until assay.

Plasma concentrations of total TFPI antigen were quantified with an enzyme-linked immunosorbent assay kit from American Diagnostica Inc., Stamford, CT, USA (Imubind® Total TFPI Elisa Kit; product no. 849). The measurements were performed in duplicate using 400SFC microplate reader (SLT-Labinstruments, Gröding/Salzburg, Austria), and calibrated using provided recombinant human reference samples and standards. For calculation of the results, a computer and a curve fitting program were used. The intra- and inter-plate coefficients of variations were < 8%.

Statistical analysis

All data were normally distributed as provided by Shapiro-Wilk W test, and expressed as means ± 1 SD. Differences in plasma TFPI following i.v. sulodexide injection on days 1 and 14 as well as those between baseline (T0) TFPI levels on days 1, 7 and 14 were assessed using the analysis of variance for repeated measures and the post-hoc Newman-Keuls test. The values measured at two time points (day 7) were compared with paired Student t-test. Bivariate correlations were assessed with Pearson regression analysis. The tests were two-sided, and P values < 0.05 were considered significant. Statistica 6.0 PL for Windows (StatSoft, Tulsa, OK, USA) data analysis package was used.

RESULTS

Plasma TFPI under intravenous and oral sulodexide

As shown in *Tab. 1*, plasma TFPI antigen levels significantly changed following i.v. sulodexide administration on day 1 (F = 33.2, P < 0.0001). They increased by a mean of 111 % at T10 vs.T0 (P < 0.0001) and the increase was observed in all participants (*Fig. 1a*). At T120 TFPI levels were lower than those at T10 (P = 0.001) but still elevated by a median of 62 % compared with baseline values (P = 0.004).

Following oral sulodexide administration on day 7, plasma TFPI antigen concentrations remained unchanged when measured after 120 min from ingestion (*Tab. 1*).
On day 14, i.v. sulodexide injection resulted in another prominent increase in TFPI antigen concentration (F = 133, P < 0.0001; *Tab. 1*). The change was even more consistent than that observed on day 1 (F = 133 on day 14 vs. F = 33.2 on day 1; *Fig. 1b* vs. *Fig. 1a*). The percentage increments in TFPI were T10 vs. T0 $220 \pm 82.9\%$ and T120 vs. T0 $114 \pm 50.0\%$ (*Tab. 1*).

Baseline plasma TFPI levels over sulodexide trial

Regarding baseline (T0) total TFPI levels (*Tab. 1; Fig. 1c*), they changed significantly during the 2-week study (F = 29.8, P < 0.0001) Compared to day 1, the T0 TFPI values on day 7 were numerically lower but the decrease was not statistically significant (P = 0.137) On study termination (day 14), plasma

Table 1. **Plasma total tissue factor pathway inhibitor (TFPI) antigen levels following sulodexide administration in healthy humans.**

	TFPI at T0(ng/ml)	TFPI at T10(ng/ml)	TFPI at T120(ng/ml)	*P* value [a]
Day 1 - sulodexide i.v.	152 ± 24.9 [b, c]	316 ± 63.2	233 ± 30.6 [d]	< 0.0001
Day 7 - sulodexide p.o.	138 ± 21.8	ND	130 ± 22.4	0.748
Day 14 - sulodexide i.v.	101 ± 27.5 [b, e, f]	304 ± 13.9	216 ± 35.2 [b]	< 0.0001

Values expressed as means ± 1 SD.
[a] By ANOVA for repeated measures (days 1 and 14) or paired Student t-test (day 7).
[b] $P < 0.0001$ vs. TFPI at T10; [c] $P = 0.004$ vs. TFPI at T120; [d] $P = 0.002$ vs. TFPI at T10;
[e] $P < 0.0001$ vs. TFPI at T120; [f] $P < 0.008$ vs. baseline (To) TFPI levels on days 1 and 7
Abbreviations: T0, before sulodexide administration; T10 and T120, 10 min and 120 min after sulodexide administration; i.v., intravenously; p.o., orally; ND, not determined.

Figure 1. **Case profiles of total tissue factor pathway inhibitor (TFPI) levels following sulodexide administration in healthy humans. Panel A – effects of initial intravenous injection; panel B – effects of intravenous injection after 12-day oral treatment; panel C – baseline (T0) TFPI levels during the 2-week study.**
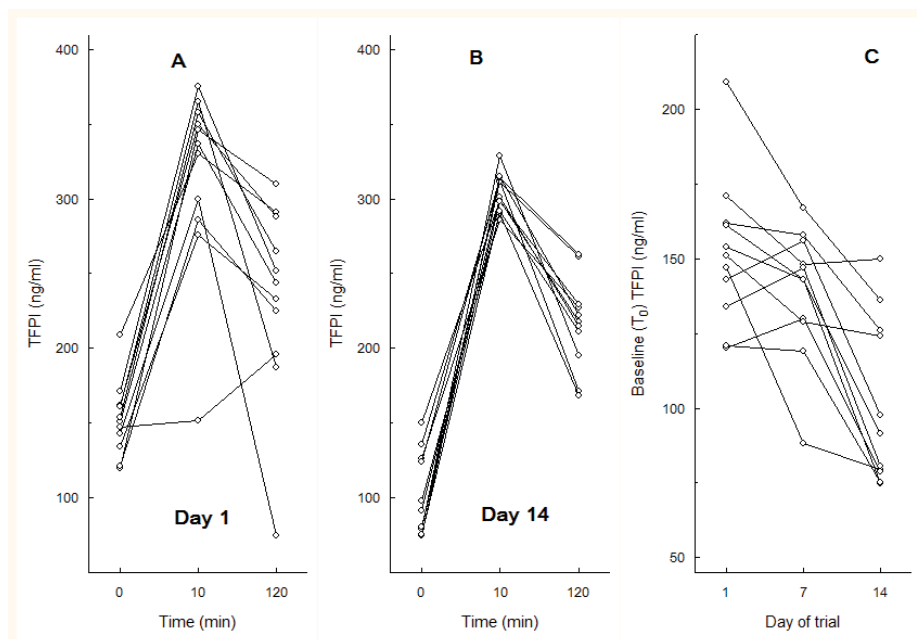
TFPI levels were lower by a median of 34 % compared to those on study initiation ($P = 0.001$) and by a median of 27 % compared to those on day 7 ($P = 0.007$).

Variables affecting sulodexide-induced TFPI release
On day 1, significant negative correlation between baseline TFPI concentration and its subsequent percentage increment at T10 was found ($r = - 0.664$, $P = 0.026$; *Fig. 2a*). On day 14, the inverse associations between the T0 TFPI level and its increase at T10 became very strong ($r = - 0.970$, $P < 0.0001$; *Fig. 2b*) and the correlation between baseline TFPI and the percentage change at T120 also became remarkable ($r = - 0.810$, $P = 0.002$; *Fig. 2c*).

No significant associations between the i.v. sulodexide dose per kg of body weight and plasma TFPI antigen levels at each of the subsequent time points were found (either on day 1 or day 14).

DISCUSSION

The main findings of this pilot study performed for the first time in healthy men are: (i) i.v. injection of sulodexide induces a marked increase in plasma TFPI, (ii) the increase is inversely related to the baseline level of the inhibitor, and (iii) a 2-week oral administration of the drug results in a fall in the circulating TFPI level. The phenomena indirectly indicate that sulodexide profoundly affect the balance between circulating and vascular wall-bound forms of TFPI, and that the prolonged exposure to the enteral form of this heparinoid compound may lead to TFPI depletion.

The effect of TFPI release by i.v. and subcutaneous UFH has been known for 20 years and thoroughly studied [13,14]. After invention of low-molecular-weight heparins (LMWHs), the action was confirmed and found to be less pronounced for both routes of LMWH administration compared with UFH [10,11]. Recent studies clearly indicate that the ability

Figure 2. Associations between baseline (To) levels of plasma total tissue factor pathway inhibitor (TFPI) and its increments due to intravenous sulodexide injection at study initiation (panel A) and termination (panel B and C).
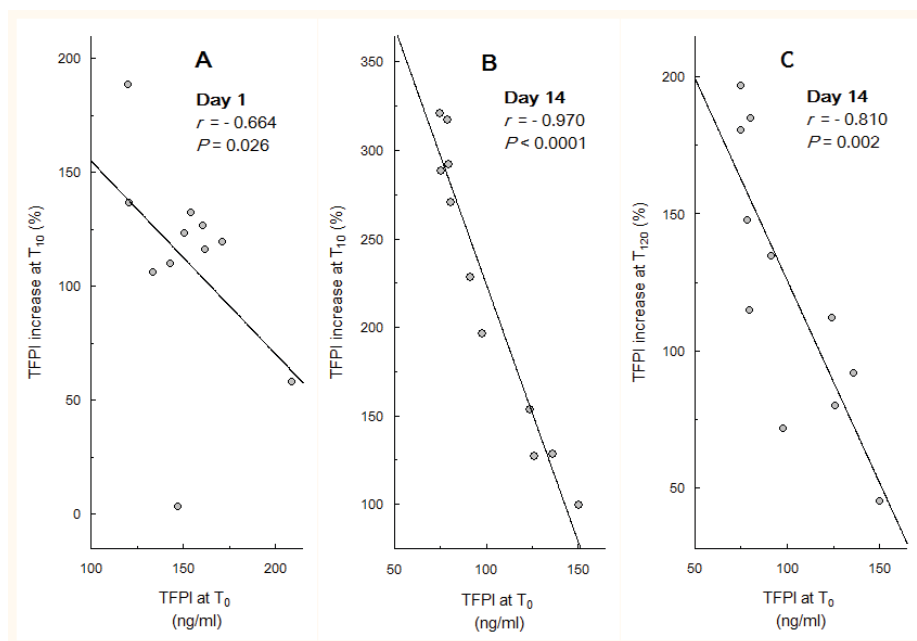
of heparins to release TFPI from its endothelial stores is strictly and inversely related to the molecular weight of the anticoagulant [15,16]. The degree of ongoing vascular depletion of TFPI during prolonged heparin administration/ treatment also seems to be less significant with LMWHs than with UFH [10,11,17] This may account for therapeutic superiority of LMWHs over regular heparin in many clinical settings, and is a consequence of the fact that the endothelium-bound TFPI is more hemostaticaly active than the circulating lipid-bound form of the inhibitor [13]. Notably, the above effect of TFPI exhaustion seems to overwhelm the ability of heparin to augment TFPI synthesis in endothelial cells [9,13]. To our knowledge, the effects of sulodexide on TFPI release have not been reported before. Furthermore, PubMed and Scopus database searches with the MeSH headings "tissue factor pathway inhibitor", "TFPI", and "sulodexide" as key words revealed no relevant studies. This is somewhat surprising because the drug has been under investigation for almost 30 years, is widely distributed on the European market and has been registered by the US Food and Drug Administration in 2004 [1,4]. Our present results imply that besides the prominent TFPI release into plasma, sulodexide also influence the equilibrium between circulating and endothelium-bound inhibitor. This assumption is based on strong inverse associations between the post-sulodexide TFPI increments and the baseline levels of the inhibitor, similarly to those reported previously for heparin [12,17] Moreover, our data show that the increase in plasma TFPI induced by i.v. sulodexide is more prominent and regular after pretreatment with oral drug than on initial exposure (3-fold rise vs. 2-fold). Hypothetically, this may reflect a kind of ongoing, specific and vague adaptation and/or sensitization to the drug. Another

unexpected finding of this study is that even parenteral exposure of only 2-week duration may lead to a substantial decrease in plasma total TFPI antigen concentration and thus a partial loss of the anticoagulant efficacy of sulodexide. The results should be, however, viewed with caution because our pilot trial was open-labeled with its resulting limitations.

It should also be mentioned that from a pharmacological point o view, it is plausible that the rise in plasma TFPI may be due to its liberation from circulating complexes with lipoproteins under influence of lipolytic sulodexide, as it was reported for heparin [18].The effect is, however, understudied, and of negligible significance.

It is premature to discuss the clinical relevance of our present findings in healthy men, albeit the design of the present study imitates clinical practice – sulodexide treatment is often intensive at the beginning (usually i.v. for several days) and then prolonged for months (in parenteral form). Foremost, the premise needs confirmation and additional investigations in larger groups and various disease states. The results also need to be viewed in light of the pleiotropic effects of sulodexide in many (mostly cardiovascular) human diseases – particularly those which cannot be ascribed only to the simple anticoagulant action of this polytherapeutic heparinoid compound [1,4,5]. The encouragement for further research on this somewhat neglected drug are the new findings that, similarly to regular heparin [19], it also induces release of hepatocyte growth factor – a powerful regenerative and healing cytokine [20] and downregulates plasma transforming growth factor-β1 - a prototypical profibrotic cytokine [21]. In addition, sulodexide could be a promising replacement in patients with heparin-induced thrombocytopenia type II [22].

CONCLUSIONS

Tissue factor pathway inhibitor release by intravenous sulodexide, its pharmacological interference with the equilibrium between circulating and tissue-bound form of the coagulation inhibitor and TFPI depletion during oral administration of sulodexide may constitute novel and likely important hemostatic actions of this heparinoid drug.

ACKNOWLEDGEMENTS

REFERENCES

1. Lauver DA, Lucchesi BR. Sulodexide: a renewed interest in this glycosaminoglycan. Cardiovasc Drug Rev. 2006 Fall-Winter;24(3-4):214-26.

2. Harenberg J. Review of pharmacodynamics, pharmacokinetics, and therapeutic properties of sulodexide. Med Res Rev. 1998 Jan;18(1):1-20.

3. Ofosu FA. Pharmacological actions of sulodexide. Semin Thromb Hemost. 1998; Feb;24(2):127-38.

4. Abaterusso C, Gambaro G. The role of glycosaminoglycans and sulodexide in the treatment of diabetic nephropathy. Treat Endocrinol. 2006 Apr;5(4):211-22.

5. Gozdzikiewicz J, Borawski J, Mysliwiec M. Pleiotropic effects of heparin and heparinoids in peritoneal dialysis. Clin Appl Thromb Hemost. 2009 Feb;15(1):92-7.

6. Liaw PC, Becker DL, Stafford AR, Fredenburgh JC, Weitz JI. Molecular basis for the susceptibility of fibrin-bound thrombin to inactivation by heparin cofactor II in the presence of dermatan sulfate but not heparin. J Biol Chem. 2001 Jun;276(24):20959-65.

7. Linhardt RJ, al-Hakim A, Liu JA, Hoppensteadt D, Mascellani G, Bianchini P, Fareed J. Structural features of dermatan sulfates and their relationship to anticoagulant and antithrombotic activities. Biochem Pharmacol. 1991 Sep 27;42(8): 1609-19.

8. Hoppensteadt DA, Walenga JM, Fasanella A, Jeske W, Fareed J. TFPI antigen levels in normal human volunteers after intravenous and subcutaneous administration of unfractionated heparin and a low molecular weight heparin. Thromb Res. 1995 Jan 15;77(2):175-85.

9. Lupu C, Poulsen E, Roquefeuil S, Westmuckett AD, Kakkar VV, Lupu F. Cellular effects of heparin on the production and release of tissue factor pathway inhibitor in human endothelial cells in culture. Arterioscler Thromb Vasc Biol. 1999 Sep;19(9):2251-62.

10. Bendz B, Hansen JB, Andersen TO, Ostergaard P, Sandset PM. Partial depletion of tissue factor pathway inhibitor during subcutaneous administration of unfractionated heparin, but not with two low molecular weight heparins. Br J Haematol. 1999 Dec;107(4):756-62.

11. Hansen JB, Sandset PM. Differential effects of low molecular weight heparin and unfractionated heparin on circulating levels of antithrombin and tissue factor pathway inhibitor (TFPI): a possible mechanism for difference in therapeutic efficacy. Thromb Res. 1998 Aug 15;91(4):177-81.

12. Hansen JB, Sandset PM, Huseby KR, Huseby NE, Nordøy A. Depletion of intravascular pools of tissue factor pathway inhibitor (TFPI) during repeated or continuous intravenous infusion of heparin in man. Thromb Haemost. 1996 Nov;76(5):703-9.

13. Crawley JT, Lane DA. The haemostatic role of tissue factor pathway inhibitor. Arterioscler Thromb Vasc Biol. 2008 Feb;28(2):233-42.

14. Sandset PM, Abildgaard U, Larsen ML. Heparin induces release of extrinsic coagulation pathway inhibitor (EPI). Thromb Res. 1988 Jun 15;50(6):803-13.

15. Alban S. Molecular weight-dependent influence of heparin on the form of tissue factor pathway inhibitor circulating in plasma. Semin Thromb Hemost. 2001 Oct;27(5):503-11.

16. Ma Q, Tobu M, Schultz C, Jeske W, Hoppensteadt D, Walenga J, Cornelli U, Lee J, Linhardt R, Hanin I, Fareed J. Molecular weight dependent tissue factor pathway inhibitor release by heparin and heparin oligosaccharides. Thromb Res. 2007 Feb;119(5):653-61.

17. Naumnik B, Borawski J, Mysliwiec M. Different effects of enoxaparin and unfractionated heparin on extrinsic blood coagulation during haemodialysis. Nephrol Dial Transplant. 2003 Jul;18(7):1376-82.

18. Novotny WF, Brown SG, Miletich JP, Rader DJ, Broze GJ. Jr. Plasma antigen levels of the lipoprotein-associated coagulation inhibitor in patient samples. Blood. 1991 Jul 15;78(2):387-93.

19. Salbach PB, Brückmann M, Turovets O, Kreuzer J, Kübler W, Walter-Sack I. Heparin-mediated selective release of hepatocyte growth factor in humans. Br J Clin Pharmacol. 2000 Sep;50(3):221-6.

20. Borawski J, Dubowski M, Pawlak K, Mysliwiec M. Sulodexide induces hepatocyte growth factor release in humans. Eur J Pharmacol. 2007 Mar;558(1-3):167-71.

21. Borawski J, Dubowski M, Pawlak K, Mysliwiec M. Effect of sulodexide on plasma transforming growth factor-beta1 in healthy volunteers. Clin Appl Thromb Hemost. 2008 doi: 10.1177/1076029608326170.

22. Borawski J, Zbroch E, Rydzewska-Rosolowska A, Pawlak K, Mysliwiec M. Sulodexide for hemodialysis anticoagulation in heparin-induced thrombocytopenia type II. J Nephrol. 2007 May-Jun;20(3):370-2.