Comparative Anticoagulant Effects of Parenteral Sulodexide as Measured by Activated Clotting Time. Relevance to Dosing in Various Indications

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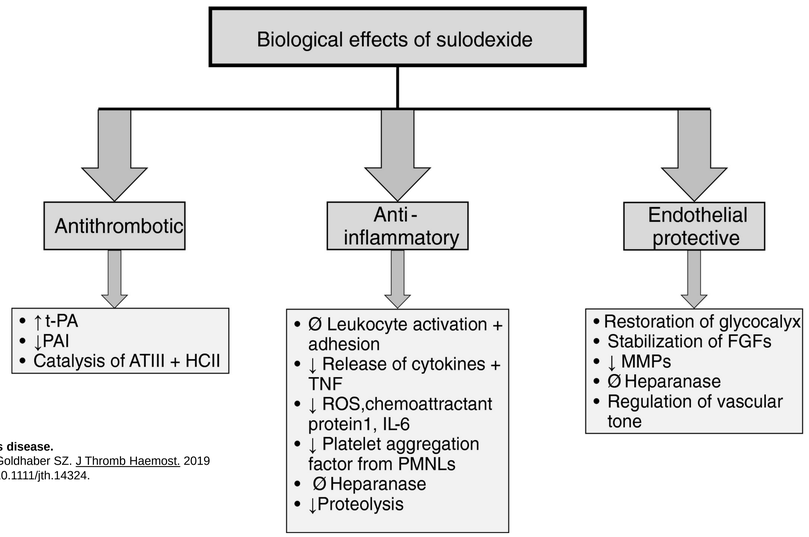
Introduction

Sulodexide is a mucosal tissue derived glycosaminoglycan of mammalian origin which is clinically used in oral (capsule) and parenteral (ampules) forms for various clinical indications. The biologic potency of sulodexide is expressed in lipoprotein lipase releasing units (LSU). As a parenteral drug sulodexide produces sizable anticoagulant effects in various laboratory assays including the activated clotting time which is routinely used during surgical procedures. In comparison to heparin the USP referenced potency of sulodexide is reported to be 95 – 105 U/mg by anti-Xa assay and 30 – 40 U/mg by anti-IIa assay. The purpose of this study is to determine the whole blood anticoagulant effect of parenteral preparations of sulodexide and its neutralization by protamine sulfate, using Hemochron Activated Clotting Time (ACT) assay.

Effects of Sulodexide



Sulodexide in venous disease.
Carroll BJ, Piazza G, Goldhaber SZ. J Thromb Haemost. 2019 Jan;17(1):31-38. doi: 10.1111/jth.14324.

Methods

Parenteral formulations (ampules) containing 600 LSU/2 ml were diluted with saline to make 10 and 100 LSU/ml working solutions. Protamine sulfate was obtained from the Loyola hospital pharmacy and diluted to 1.0 – 0.12 mg/ml. Anticoagulant effects of sulodexide over the concentration range of 1.0 – 0.25 LSU/ml were measured on the Hemochron ACT instrument in freshly drawn normal human blood (n=5-10). Protamine neutralization studies were carried out at a fixed sulodexide level (1.0 LSU/ml) and variable protamine sulfate levels (12.5-50 µg/ml). Under an IRB approved protocol and upon written informed consent, and following a double-syringe technique, blood was drawn up to 2ml mark in labeled syringes containing 200ul of the drug at respective concentrations including a saline control. The contents of the syringes are gently mixed by inverting the syringes and the blood transferred to Celite ACT tubes, and immediately placed in the Hemochron instrument to determine the clotting times. The clotting times in seconds are recorded. For studying the protamine neutralization of sulodexide at different concentrations, in addition to 200ul of the drugs, 200ul of protamine at 500ug/ml and 250ug/ml were placed and the blood drawn to obtain a final concentration of 50 ug/ml and 25 ug/ml and similarly processed to determine the clotting times. A saline control is also performed.

Results

Effect of Sulodexide on ACT

Drugs	Mean ± SD
Sulodexide (1.0 U/ml)	401 ± 20
Sulodexide (0.5 U/ml)	278 ± 29
Sulodexide (0.25 U/ml)	238 ± 24
Control	136 ± 12

Neutralization of Sulodexide on ACT

Drugs	Mean ± SD
Sulodexide (1.0 U/ml) + Protamine Sulfate (50 µg/ml)	158 ± 14
Sulodexide (1.0 U/ml) + Protamine Sulfate(25 µg/ml)	192 ± 10
Sulodexide (1.0 U/ml) + Protamine Sulfate (12.5µg/ml)	263 ± 6
Control + Protamine Sulfate (25µg/ml)	173 ± 3

Figure 1: Comparative effects of sulodexide in whole blood activated clotting time

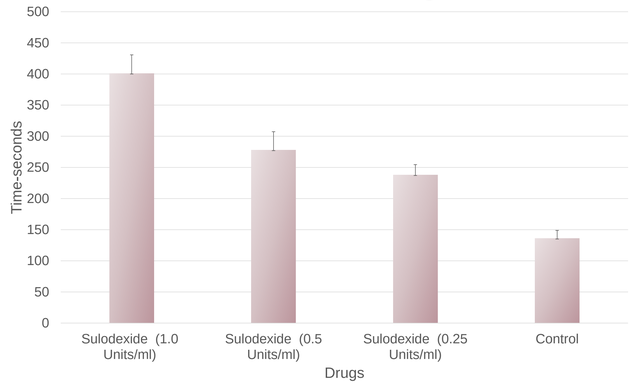
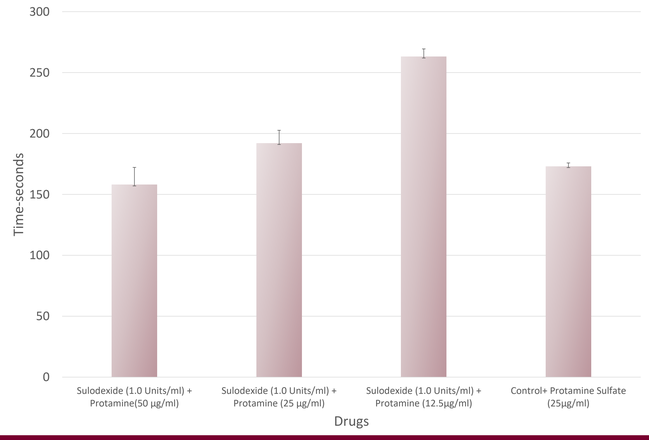


Figure 2: Relative neutralization of sulodexide by protamine sulfate in whole blood activated clotting time



Summary

Sulodexide produced a concentration-dependent anticoagulant effect in normal human whole blood over a concentration range of 0.25 – 1.0 U/ml. At the highest concentration sulodexide (1.0 U/ml), the clotting time was found to be 401 ± 30 seconds. At the lowest concentration of sulodexide (0.25 U/ml), the mean clotting time was observed to be 238 ± 24 seconds. All concentrations of sulodexide demonstrated an elongation in the clotting time when compared to saline control (136 ± 12 seconds). Protamine sulfate supplementation at a concentration range of 12.5 – 50 µg/ml produced varying levels of neutralization of the anticoagulant effects of sulodexide (FC= 1.0 U/ml). With the supplementation of protamine sulfate at the highest concentration of (50 µg/ml) with sulodexide at a final concentration of 1.0 U/ml, protamine sulfate completely neutralized sulodexide as seen in the resolution of clotting time (158 ± 14 seconds, control 173 ± 3 seconds). However, in the lower concentrations of protamine sulfate (1.25 and 2.5 µg/ml) only partial neutralization of the anticoagulant effect was observed, as seen in the variable decrease in clotting time.

Conclusion

Parenteral (ampules) sulodexide can be used for anticoagulation in dose dependent fashion. As observed in this study, protamine sulfate at higher concentrations can be used as an antidote for the anticoagulant effect of sulodexide. Thus in the event of a shortage of unfractionated heparin, sulodexide can possibly be used as a substitute anticoagulant drug for the indications in which heparin is considered.

Acknowledgments

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References

Carroll BJ, Piazza G, Goldhaber SZ. Sulodexide in venous disease. J Thromb Haemost 2019: 17: 31-8.

Jiang Q-J, Bai J, Jin J, Shi J and Qu L (2018) Sulodexide for Secondary Prevention of Recurrent Venous Thromboembolism: A Systemic Review and Meta-Analysis. Front. Pharmacol. 9:876.

Masola V, Zaza G, Onisto M, Lupo A, Gambaro G. Glycosaminoglycans, Proteoglycans and sulodexide and the endothelium: biological roles and pharmacological effects. Int Angiol 2014; 33: 243-54.