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2032 Protein Z Dependent Protease Inhibitor Inhibition of Factor XIa Is Accelerated by Unfractionated Heparin in the Presence and Absence of Protein Z: Further Evidence of the Potential Significance of XIa Inhibitory Activity

Special Interest Sessions
Poster Session: Vascular Biology and Factor VII

Tuesday, December 9, 2008, 1:00 PM-2:30 PM
310 - South (Moscone Center)
Poster Board II-126

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Protein Z dependent protease inhibitor (ZPI) is a human plasma serpin that inhibits factors Xa and XIa in the coagulation pathway. Kinetic studies have shown that ZPI is an efficient inhibitor of factor Xa. This interaction is accelerated by protein Z in the presence of calcium and phospholipid. The inhibition of factor XIa is by an alternative mechanism that does not require phospholipid but is influenced by both heparin and protein Z; heparin increases inhibition whereas protein Z /calcium delays inhibition. The latter maybe physiologically relevant to XIa inhibitory activity as ZPI and protein Z circulate in complex in plasma, with ZPI in slight excess. The kinetics of ZPI/XIa/heparin interactions have not been fully characterised. In this study we report the kinetic analysis of the interaction between factor XIa and recombinant ZPI expressed in *Escherichia coli,* in the presence of unfractionated heparin, pentasaccharide and protein Z. *Results:* Recombinant ZPI (rZPI) had characteristics similar to native protein as previously described; serpin-protease complexes were non-covalent, and reactive centre loop proteolytic cleavage of ZPI was noted during this reaction. On SDS gel rZPI had a molecular weight of 50kDa. Circular dichroism of rZPI showed an unfolding transition on thermal denaturation typical of a serpin in native conformation with a Tm of 58.8°C. In contrast loop cleaved rZPI had increased thermal stability as expected for a serpin. A discontinuous method was used to measure the rate of inhibition of target proteases by rZPI; the first order rate constant was measured over time under pseudo first order conditions, and was used to derive the second order rate constant (k_a) as shown in *Table 1*.

Table 1: Second order rate constants (k_a)

	Xa (Ms^{-1})	XIa (Ms^{-1})
ZPI	1.9×10^3	9.0×10^4
ZPI + protein Z*	6.6×10^5	1.8×10^4
ZPI + unfractionated heparin (1IU/mL)	-	1.0×10^6
ZPI + pentasaccharide (500nM)	-	1.0×10^5
ZPI + protein Z* + unfractionated heparin (1IU/mL)	-	6.0×10^5

* And calcium/phospholipid vesicles

In the presence of calcium and phospholipid vesicles the k_a for Xa in the absence and presence of protein Z was similar to previous reports. The k_a for ZPI inhibition of XIa increased 11 fold in the presence of unfractionated heparin. Heparin titration curves suggest that a template mechanism is likely; the heparin pentasaccharide had no impact on inhibition rate consistent with this observation. It has previously been reported that protein Z impedes the ZPI inhibition of XIa; our results support this with a 5-fold reduction in k_a. However the addition of heparin to ZPI in the presence of Protein Z leads to a significant 33-fold increase in inhibitory activity with a rate similar to the inhibitory activity of ZPI against Xa in the presence of protein Z. *Conclusions:* There are several physiological inhibitors of factor XIa. In previous reports ZPI inhibition only

increased 2-fold in the presence of heparin. Our results confirm that heparin increases the ZPI inhibition of XIa but with a significantly higher increase than previously reported. The increase in activity is even more marked in the presence of protein Z. Although protein Z alone inhibits ZPI activity against XIa the combination with heparin achieves a 33 fold increase in activity. Of all the serpin inhibitors of XIa, antithrombin is considered a major inhibitor in the presence of heparin. Our results show that the k_a for FXIa inhibition in the presence of ZPI, protein Z and heparin is more than 20 fold higher than the k_a reported for the antithrombin/heparin inhibition of XIa ($2\text{-}3x10^4 Ms^{-1}$). We have previously reported a higher incidence of venous thrombosis in patients with nonsense mutations in the ZPI gene suggesting the clinical significance of this protein. The suggestion that the inhibition of XIa maybe more relevant than that of Xa has been raised in recent murine work where ZPI knockouts have a more severe phenotype than protein Z knockouts. ZPI inhibition of XIa in the presence of endogenous heparins maybe an important contributor to its physiological significance.

Disclosures: No relevant conflicts of interest to declare.

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