Abstract: **P185**

**Regulation of coagulation by zinc: characterisation of zinc-dependent heparin neutralisation by fibrinogen and histidine-rich-glycoprotein**

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Background: Zinc ions (Zn2+) are released from activated platelets and are important regulators of coagulation. The majority of plasma Zn2+ are bound to human serum albumin (HSA), yet binding of free fatty acids (FFAs) to HSA alters the protein conformation disrupting Zn2+ binding. Elevated concentrations of plasma FFAs are associated with certain disease states characterised by increased incidence of thrombotic complications. It is therefore important to understand this dynamic and the roles zinc plays in coagulation. Among these roles is modulation of histidine-rich-glycoprotein (HRG) and fibrinogen interactions with heparins, important natural anticoagulants which are "neutralised" by these interactions.

Aims: The primary aims of this project were to: 1) Investigate the interplay between binding of FFAs and Zn2+ to HSA. 2) Determine the effect of this dynamic upon Zn2+ -dependent neutralisation of heparin by fibrinogen and HRG and on fibrin clot formation/lysis. These constitute two potential mechanisms by which Zn2+ can influence clotting.

Methods: Isothermal titration calorimetry (ITC) was carried out to study the effect of FFAs of different length on the binding of Zn2+ to HSA. ITC and ELISA-based binding assays were then used to study the effect of Zn2+ on those interactions. Finally the effect of Zn2+ and FFAs on fibrin clot parameters was investigated in both a purified fibrinogen system and in citrated plasma by turbidity assays.

Results: ITC data showed that when FFA concentration (0 to 5 mol.eq.) or chain length (C8:0 to C18:0) increase, the availability of the major Zn2+ binding site on albumin decreases up to being completely abolished. ITC and ELISA-based binding assays were then used to study the effect of this freed Zn2+ on heparin neutralisation. It was found that the affinity of HRG for unfractionated heparins (UFHs) (but not low-molecular-weight heparins (LMWHs)) was increased in the presence of 1 µM ZnCl2 (Kd[with zinc] = 5 nM compared to Kd[no zinc] = 33 nM) and that of fibrinogen for UFHs and LMWHs was increased by the presence of 10 µM ZnCl2 (Kd[with zinc] = 24 nM compared to Kd[no zinc] = 56 nM). The effect of Zn2+ and FFAs on fibrin clot parameters was then investigated. In the purified fibrinogen system, Zn2+ caused an enhanced clot formation and an increase in clot maximum absorbance associated with prolongation in clot lysis time and reduced clot lysis rate, effects that were accentuated in presence of FFAs. Similar results were obtained using plasma samples where clot maximum absorbance increased in presence of Zn2+ and FFAs.

Conclusion: These results confirm that FFAs directly affect Zn2+ levels and that Zn2+ is likely to influence coagulation through modulation of HRG and fibrinogen interactions with heparins. This suggests that the increased thrombotic risk in patient with high level of plasma fatty acids is acting through serum albumin and
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