Quantification of bovine and porcine heparin utilizing the Heparin Red assay. Applications in the study of pharmacokinetics and pharmacodynamics.

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INTRODUCTION

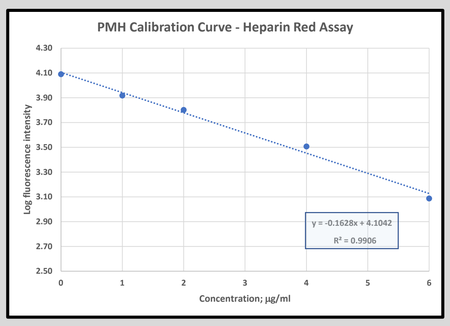
It is well known that bovine intestinal heparins (BMH) exhibit a relatively lower potency than porcine intestinal heparins (PMH). This translates to weaker anticoagulant activity when the two heparins are supplemented to plasma on a gravimetric basis and weaker antithrombotic activity when animals are administered equigravimetric dosages. Recent primate studies have suggested that when bovine and porcine heparins are dosed on the basis of equivalent anti-Xa units, equivalent anti-IIa and anti-Xa responses are observed. The pharmacokinetic behavior of heparins has traditionally been based on antithrombin-dependent anti-Xa and anti-IIa activities. Heparin Red is a polycationic substance whose intrinsic fluorescence is quenched upon binding to heparin. As such, assays utilizing Heparin Red detect all heparin oligosaccharides in a sample: those that bind to AT and produce anti-Xa and anti-IIa activities, and those that do not. These non-AT binding heparin oligosaccharides can still impact coagulation through their interaction with heparin cofactor II, TFPI and platelets.

AIM

To compare pharmacokinetic profile of bovine and porcine heparin based on heparin levels determined using a Heparin Red assay with the pharmacodynamic profile based on heparin levels determined using AT-dependent activities (anti-Xa or anti-IIa).

METHODS

Primates were administered PMH (Medefil, Glendale Heights, IL) or BMH (KinMaster, Passo Fundo, Brazil) at a dose of 100 anti-Xa U/kg IV. Blood samples were collected prior to and at 15, 30, 60 and 120 minutes post-heparin administration. Blood samples were centrifuged to produce platelet poor plasma which was aliquoted and stored frozen until analysis. Heparin levels were assessed using a chromogenic anti-Xa assay and a Heparin Red assay relative to product-specific calibration curves. Pharmacokinetic parameters were assessed using a non-compartmental model using the PKSolver® add-in software for Microsoft Excel. Statistical analysis was performed using t-test or ANOVA (SigmaPlot 12.3; Systat Software, San Jose, CA). *P*-values <0.05 were considered statistically significant.



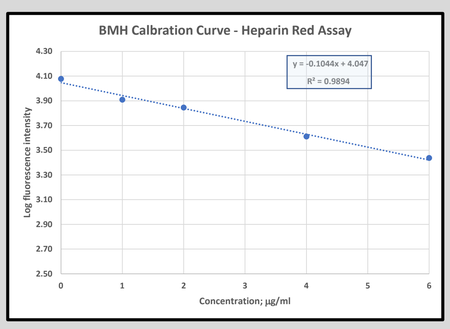


Figure 1. Product-specific calibration curves were determined for PMH and BMH. Both heparins produced linear responses for concentrations ≤ 6 µg/ml.

	BMH	PMH
t1/2; min	25.8 ± 2.6	20.6 ± 1.9
AUC ; µg*min*ml-1	728.2 ± 2.6	594.9 ± 5.4
Vol Dist	5.1 ± 0.3	5.0 ± 0.5
Clsys	0.14 ± 0.01	0.17 ± 0.01

Pharmacokinetic parameters calculated using circulating heparin levels determined by heparin red assay.

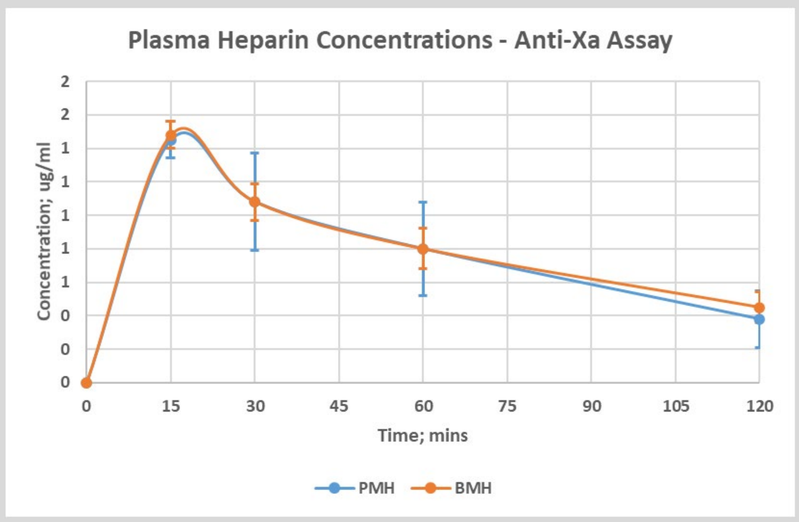


Figure 2. Heparin concentrations in plasma samples from BMH and PMH-treated primates as determined by heparin red assay. Peak circulating heparin levels were comparable following BMH and PMH administration (1.48 ± 0.08 vs. 1.45 ± 0.11 U/ml). Using drug levels determined by anti-Xa assay, AUCs for bovine and porcine heparin treated animals were calculated to be 111.5 ± 11.0 and 108.8 ± 26.7 U*min*ml-1, respectively.

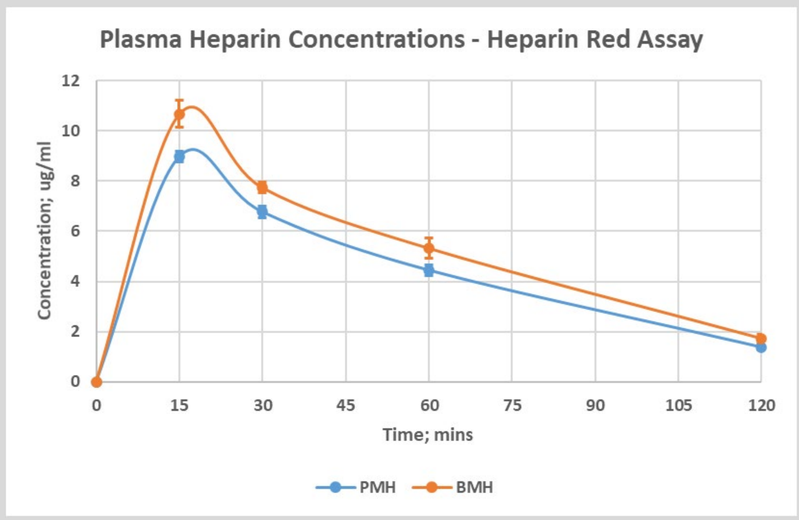


Figure 3. Heparin concentrations in plasma samples from BMH and PMH-treated primates as determined by heparin red assay. Peak circulating heparin levels were higher following BMH administration (10.7 ± 0.5 vs. 9.0 ± 0.2 µg/ml; t-test p<0.001) and the AUC for BMH-treated primates was approximately 22% larger than for PMH-treated primates (728.2 ± 35.3 vs. 594.9 ± 5.4 µg*min*ml-1; Mann-Whitney test p=0.029).

CONCLUSIONS

BMH, when administered at equivalent anti-Xa unit doses, produces comparable pharmacodynamic effects as PMH despite the presence of higher circulating GAG levels as measured by the Heparin Red assay. Measurement of BMH levels using the Heparin Red assay may be useful for identifying the appropriate dose of protamine to completely neutralize BMH. Furthermore, absolute quantitation of GAGs by Red Probe method in these studies provides a true pharmacodynamic effect of heparins accounting for the cumulative actions of both AT dependent and independent actions.