

Scavenger Receptor Stabilin-2 is a Major Regulator of VWF Propeptide Clearance

Background

The VWF propeptide (VWFpp) to VWF antigen (VWF:Ag) ratio is widely used as a marker for increased VWF clearance and to determine VWD subtype. The major caveat in interpreting the VWFpp/VWF:Ag ratio is the assumption that VWFpp clearance is relatively constant. Elevated VWFpp/VWF:Ag ratios are assumed to arise from altered VWF clearance and variations in VWFpp clearance are not considered. This is mainly due to the paucity of data on the mechanisms underlying VWFpp clearance.

Aims

To investigate the role of scavenger receptor stabilin-2 in mediating VWFpp clearance.

Materials & Methods

VWFpp was expressed in HEK293T cells and was subsequently infused (200U mVWFpp/kg) into VWF^{-/-} or Stab2^{-/-}/VWF^{-/-} mice. Mice were then retro-orbitally sampled and VWFpp was quantified by ELISA. Solid-phase binding assays were carried out utilizing the secreted ectodomain of the short stabilin-2 isoform. Age and sex-matched wild-type C57Bl6 mice and STAB2^{-/-} mice were retro-orbitally sampled and endogenous VWF and VWFpp were quantified by ELISA.

Results

Clearance studies involving VWF^{-/-} and Stab2^{-/-}/VWF^{-/-} mice revealed that stabilin-2 deficiency significantly prolonged VWFpp survival (7.8 mins versus 18.2 mins, $p < 0.0001$). Subsequent immunohistochemical analysis of VWF^{-/-} mouse liver sections harvested following VWFpp infusion, demonstrated co-localization of both VWFpp and stabilin-2 with the liver sinusoidal endothelial cell markers LYVE1 and FcYRIIB. To further investigate this interaction, solid-phase binding assays were carried out. Binding of VWFpp to stabilin-2 was found to be dose-dependent and saturable. Furthermore, the interaction was inhibited by the presence of propeptide-deleted full-length VWF ($31.5 \pm 8.1\%$, $p < 0.0001$) and was enhanced by removal of the VWFpp N-linked glycans ($180.0 \pm 0.6\%$, $p = 0.0079$). Notably, although STAB2^{-/-} mice were found to have normal VWF plasma levels (STAB2^{-/-} versus wild-type C57BL/6 mice, 0.9 ± 0.1 versus 1.0 ± 0.9 , $p > 0.05$), significantly elevated endogenous VWFpp levels were observed (3.1 ± 0.2 versus 1.0 ± 0.1 , $p < 0.0001$). Consequently, STAB2^{-/-} mice had elevated VWFpp/VWF:Ag ratios (2.7 ± 0.4 versus 0.7 ± 0.1 , $p < 0.0001$) that result from altered VWFpp clearance rather than altered VWF clearance.

Conclusion

Stabilin-2 is a major regulator of VWFpp clearance. Altered VWFpp clearance significantly effects the VWFpp/VWF:Ag ratio in stabilin-2 deficient mice.