Factor VIII expression and regulation in health and disease

Hollestelle, M.J.

Citation for published version (APA):
Chapter 7

General Discussion
GENERAL DISCUSSION

It is well established that plasma factor VIII levels are extremely prone to variation, both in physiological and pathophysiological conditions. Yet, little is known about the mechanism underlying these fluctuations. The aim of the studies presented in this thesis was to get insights into the molecular and cellular mechanisms that control plasma factor VIII levels. Factor VIII expression was examined in hepatic and extra-hepatic tissues, like kidney, spleen, lungs and bone marrow. Furthermore, the contribution of factors that play a role in regulating plasma factor VIII, including von Willebrand factor (VWF) and low-density lipoprotein receptor-related protein (LRP) were studied under various pathological conditions. Modulations of factor VIII and VWF were addressed in patients with liver disease and modulations of VWF and VWF propeptide levels in patients with malaria.

Factor VIII expression under normal physiological conditions

Hepatic factor VIII expression

The origin of factor VIII biosynthesis has been a controversial issue for many decades and is still subject of ongoing research. In many textbooks the liver is described as the primary source for factor VIII. As demonstrated by successful liver transplantation in hemophilia A patients, this view is correct (1-4). However, the idea frequently put forward that the hepatocytes synthesize factor VIII should at least be a matter of debate. The observations described in this thesis demonstrate a different picture. In mice, as studied by RT-PCR, factor VIII mRNA is clearly detectable in the liver. However, no mRNA was detected in hepatocytes by in situ hybridization (Chapter 2). Rather, factor VIII mRNA was detected in sinusoidal endothelial cells as well as factor VIII protein (Chapter 2, (5-7)). Similarly, others also detected factor VIII mRNA in sinusoidal endothelial cells, although there was detectable mRNA for factor VIII in hepatocytes as well (8). Overall, these findings indicate that factor VIII synthesis in the liver primarily occurs in sinusoidal endothelial cells, not hepatocytes.

A surprising observation is that in the various tissues analyzed factor VIII mRNA levels are extremely high compared to other protein transcripts, including VWF, urokinase-type plasminogen activator and tissue factor (Chapter 2, (9)). In contrast, factor VIII protein levels in plasma are low compared to the levels of, for example, VWF. On a molar basis the plasma VWF concentration is about 50-fold higher than the factor VIII concentration whereas the concentration of mRNA for VWF is at least 1000-fold lower than the factor VIII mRNA concentration, at least in the tissues examined (Chapter 2). The reason for the relative low plasma levels of factor VIII is probably due to inefficient transport of the primary translation product from the endoplasmic reticulum (ER) to the Golgi apparatus. A significant portion of factor VIII within the ER never transits to the Golgi compartment, but rather is degraded within the cell (10). Apparently, factor VIII is poorly transported to the outside of the cell. Indeed, although a 10-fold difference was observed in factor VIII mRNA
levels between different mouse strains (C57Bl/6 and Balb/c; M.J. Hollestelle, unpublished observations), no significant difference was observed between the factor VIII plasma levels in these strains (11). Taken together, the observation that high factor VIII mRNA levels are associated with low factor VIII protein levels suggests that the transcriptional activity of the factor VIII gene is not a critical factor in controlling factor VIII plasma levels. Rather the secretion of factor VIII is a rate-limiting step in maintaining hemostatic plasma levels of factor VIII (12).

**Extra-hepatic factor VIII expression**

Transplantation studies in hemophilic animals and our gene expression studies in anhepatic pigs (Chapter 4) showed that lung and spleen, and possibly also the kidney may contribute to the regulation of circulating factor VIII (13,14). Kidney transplantation studies in a hemophilic dog and a human individual did not show increase of plasma factor VIII (15,16). These were single observations and, to our knowledge, the results have not been confirmed. On the other hand, high levels of factor VIII mRNA were detected in the kidney, levels that are comparable with hepatic factor VIII mRNA levels (Chapter 2). *In situ* hybridization analysis indicated the presence of factor VIII mRNA in glomerular cells of the kidney (Chapter 2). Similarly, factor VIII protein was detected in the glomeruli (Chapter 4). These observations for the first time provide evidence that the kidney may produce factor VIII. Unexpectedly we observed that within the glomerulus factor VIII protein colocalized with VWF. This suggests that the factor VIII-producing cell type within the glomerulus is endothelial cell-like in nature. A remarkable feature that should await further studies on the cellular mechanism. We cannot exclude that the cellular expression of factor VIII in the glomerulus reflects an endocytotic process, rather than *de novo* synthesis, for instance mediated by megalin, a LRP-related endocytotic receptor that may bind factor VIII with high affinity (17). We feel that only refined studies with purified and well-characterized populations of glomerular and tubular cells, another candidate of factor VIII synthesis (Chapter 4), could further shed light on the significance of renal factor VIII synthesis and secretion.

More than three decades ago spleen perfusion and transplantation studies demonstrated that this organ could play a significant role in the extra-hepatic regulation of plasma factor VIII levels, either as site of synthesis (13,18,19) or as storage reservoir (15,20-22). After that it remained silent in the literature (except one report published 10 years ago which described successful transplantation of normal spleen cells into hemophilia A patients (23)). Splenic factor VIII synthesis is still a controversial issue, however (24,25). Pertinent to this view is the observation that splenectomy is not associated with a decrease of plasma factor VIII levels (26). Our studies do indicate a potential role for the spleen as a factor VIII synthetic organ, however. Both factor VIII protein and mRNA expression was detected in spleen cells, a strong indication of factor VIII synthesis, notably by reticular cells present in the red pulp (Chapter 2 and 4). To what extent factor VIII synthesized in the spleen contributes to the factor VIII plasma level can
not be inferred from our studies. As total hepatectomy results in sustained replenishment of plasma factor VIII (Chapter 4), it seems likely that the contribution of the spleen to the factor VIII plasma pool is significant, at least under these clinical conditions. This observation does not rule out the possibility that, as previously hypothesized (15,20-22), the spleen serves as a storage pool of factor VIII.

Grafting normal lungs into hemophilic dogs demonstrated a small increase of plasma factor VIII levels, indicating that also the lung is an extra-hepatic source of factor VIII synthesis (14). Consistent with this view is our observation that both factor VIII mRNA and protein are present in lung tissue (Chapter 2 and 4). Although the significance of the findings remains to be established, it lends support to our view that the factor VIII synthetic system is widely dispersed.

Whether hematopoietic cells are capable of producing factor VIII have been inconclusive (24). We demonstrated low but significant factor VIII plasma levels (about 0.04 U/mL) and factor VIII mRNA in multiple tissues after transplantation of bone marrow from wildtype mice in factor VIII knockout mice (Chapter 6). In contrast, hematopoietic grafting in hemophilic dogs showed no evidence of factor VIII synthesis (27). We have no explanation for these apparently disparate observations. It is possible that small changes of factor VIII levels in the canine study have escaped detection. As expected, we detected highest factor VIII mRNA in bone marrow. Lower levels were detected in blood cells, lung and spleen (Chapter 6). Virtually no factor VIII mRNA was detected in liver and kidney, organs which under normal physiological conditions are relatively rich in factor VIII mRNA (Chapter 2). It is possible that expression of factor VIII in lung and spleen is caused by differentiation of stem cells or fusion of stem cells with cells of the lung or spleen destined for factor VIII synthesis.

In conclusion, our studies strongly suggest that the sinusoidal endothelial cells in the liver constitute the primary site of factor VIII synthesis. Our studies also document that the liver is but one site of a widely dispersed factor VIII synthetic system. We postulate that a variety of non-hepatic cells, cells with different phenotypic characteristics, located in different organs, contributes to factor VIII synthesis. Although we were not able to quantitatively assess the contribution of these cells to the plasma factor VIII pool, we do know that the capacity of this biosynthetic system is sufficient to compensate aberrant hepatic synthesis.

**Modulations of factor VIII expression under pathophysiological conditions**

It is well established that in many clinical conditions, including liver disease, malignancies or inflammatory disorders, plasma factor VIII concentrations are frequently elevated. About the mechanism that underlies increased factor VIII levels, we can only speculate. First, increased plasma levels could be a reflection of enhanced synthesis by one or more cell types elicited by hormones or signals produced under various clinical conditions. This hypothesis is supported by the observation that administration of the pleiotropic cytokine IL-11 increases factor VIII levels in a specific (VWF-independent) manner (28,29).
Secondly, increased factor VIII levels may be secondary to rises in plasma VWF levels induced by disease-related endothelial cell agonists [review (30)] or neovascularization and subsequent enhanced VWF production (Chapter 3). Indeed, to date most evidence supports that VWF is a significant factor in the regulation of plasma factor VIII levels. Any change in VWF level is coupled with a concordant change in the plasma factor VIII level. This is most clearly demonstrated by fitting a model describing steady state factor VIII and VWF kinetics in the systemic circulation (Chapter 1) (31) to factor VIII and VWF data obtained from our study on expression of factor VIII in liver disease (Chapter 3). The model curve is in good agreement with the data obtained (fig.1), suggesting that the increase in factor VIII concentration is due more to variability in VWF concentration than to variability in the factor VIII synthetic rate (31). On the other hand, application of the model to individual factor VIII and VWF data of patients with severe Plasmodium falciparum infection (Chapter 5), showed considerable spread in factor VIII values along the model curve (M.J. Hollestelle, unpublished observations). This suggests that in these patients also an increased factor VIII synthetic rate contributes to increased plasma factor VIII levels. This view is supported by the observation that VWF propeptide and VWF levels decrease after successful treatment already after respectively one day and three day (probably because of differences in clearance rate), whereas factor VIII plasma levels remain elevated during this follow up period. This indicates that the elevation of VWF and factor VIII are independently up-regulated in these malaria patients (M.J. Hollestelle, unpublished observations).

Figure 1. Correlation between factor VIII activity and VWF antigen levels in patients with liver disease. The curve shows a model that predicts factor VIII value to their corresponding VWF plasma level (Adapted from (31)). Closed circles: patients with liver cirrhosis and a bile duct tumor, closed triangles: patients with liver cirrhosis and liver infections, open circles: patients with a bile duct tumor or a gallstone, open triangles: patients with liver infection, squares: liver metastasis.
Finally, impaired clearance of factor VIII as a factor that modulates plasma factor VIII levels, should be taken into account, notably mediated by hepatic LRP (Chapter 3). The significance of hepatic LRP as a clearance receptor is suggested by our observation (M.J. Hollestelle, unpublished) that the mean residence time of factor VIII in a hemophilia A patient suffering from liver cirrhosis is considerably longer (27.6 hours) than observed in apparently "healthy" hemophilia A patients (15.9 hours ± 7.2, mean ± SD, ref (32)). Also our observation that hepatectomy increases factor VIII mean residence time (Chapter 4) is consistent with the view that the liver plays an important role in the catabolism of factor VIII. In addition, megalin, a renal, LRP-related endocytotic receptor, could play a role in the catabolism of factor VIII (vide supra), although clinical evidence is lacking.

Future prospects

The studies presented here document features that might have impact on the management of hemophilia A by gene therapeutic approaches. Current strategies focus on organ (i.e. liver) - specific rather than cell-specific factor VIII expression. So far the success rate of these protocols is limited, both in terms of extent and duration of factor VIII expression following administration of factor VIII vectors. Although the liver is most likely the right target, it is conceivable that factor VIII is expressed in the wrong host (e.g. hepatocytes). Rather future studies should focus on the sinusoidal endothelial cell as the natural host of factor VIII synthesis. In this respect, it would also be of interest to further identify and characterize the cells that are responsible for extra-hepatic factor VIII synthesis. Reticular cells in the spleen and glomerular cells are attractive candidates. Also this knowledge could provide a rationale for a superior approach to the management of hemophilia A by gene therapy.

REFERENCES

26. Garcia VV, Coppola R, Mannucci PM. The role of the spleen in regulating the plasma levels of factor VIII--von Willebrand's factor after DDAVP. Blood 1982; 60: 1402-6