Decreased Factor VIII Levels During Acetaminophen-Induced Murine Fulminant Hepatic Failure

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Abstract

During human fulminant hepatic failure (FHF) circulating levels of most hemostatic proteins fall dramatically. Concurrently factor VIII (fVIII) procoagulant activity rises despite destruction of the hepatocytes considered responsible for fVIII synthesis. This observation suggests a role for cells other than hepatocytes in fVIII biosynthesis during FHF. We have attempted to identify non-hepatocytic sites of fVIII biosynthesis by inducing FHF in mice using acetaminophen overdose, a common cause of human FHF. Acetaminophen treated mice consistently displayed signs characteristic of FHF including elevated plasma aminotransferase activity. However, acetaminophen treated mice demonstrated markedly reduced fVIII activity, contrary to the observation in human FHF. von Willebrand factor antigen levels were only mildly reduced, suggesting that the decrease in fVIII is not secondary to loss of von Willebrand factor. These results imply that there are fundamental differences in the regulation of plasma fVIII levels between humans and mice. jlollar@emory.edu
Introduction

Much of the knowledge concerning factor VIII (fVIII) biosynthesis has been gained through the use of heterologous expression of fVIII transgenes in cultured cells (for review see Kaufman et al.). Unfortunately in vitro experiments cannot identify all of the factors involved in fVIII biosynthesis in vivo. Thus, a substantial gap in the understanding of fVIII gene regulation in vivo remains, including the identification of endogenous sites of fVIII production. Beyond hepatocytes, several cell types in mice have been shown to contain significant levels of fVIII mRNA, but only murine liver sinusoidal endothelial cells have been demonstrated to secrete fVIII in vitro. Additionally, liver is the only tissue that has been shown conclusively to produce fVIII when transplanted into fVIII deficient (hemophilia A) canines or humans, as evident by alleviation of the bleeding phenotype. However, during the course of fulminant hepatic failure (FHF) induced by acetaminophen or viral hepatitis, plasma fVIII activity can increase to greater than 10 times normal levels. The cellular source of this super-physiologic fVIII activity is currently unknown. We previously reported that fVIII levels were decreased in a murine model of FHF induced by the hepatotoxin, azoxymethane. Since azoxymethane has not been proven to cause FHF in humans, we have continued to identify other models that may be more representative of human FHF pathophysiology. In the current study we sought to determine whether fVIII levels are elevated in a recently described murine model of acetaminophen–induced FHF and possibly identify alternative sites of fVIII biosynthesis in vivo.
Study design

Twelve week-old C57BL/6J male mice (21-26 g) were purchased from Charles River Laboratory (Wilmington, MA). Mice were fed standard rodent chow and were kept on a 12 hr/12 hr - light/dark cycle. Murine fulminant hepatic failure was induced as described previously. Briefly, mice were given an intraperitoneal injection of 100 mg/kg phenobarbital (Sigma, St. Louis) suspended in corn oil, for three consecutive days followed by an intraperitoneal injection of 250 mg/kg acetaminophen (Sigma), dissolved in basic phosphate-buffer saline (10 mM Na₂HPO₄, 150 mM NaCl, pH 11), on day 4. Twenty-four hours after acetaminophen administration, the mice were sacrificed and blood was collected by cardiac puncture into one-tenth volume 3.8% sterile trisodium citrate. Plasma was isolated from whole blood by centrifugation at 1,800 g for 15 min at 4°C and stored at -70°C. The control plasma used in this study was pooled plasma from six untreated C57BL/6J male mice.

FVIII activity was determined using a chromogenic substrate assay in which the rate of activation of factor X is linearly related to the concentration of fVIII as follows. Plasma samples were diluted 1/81 in 20 mM HEPES, 0.15 M NaCl, 1% BSA, pH 7.4. Thirty microliters of a solution containing 3.6 nM factor IXa, 150 µM phosphatidylcholine/phosphatidylserine vesicles (75%/25%) and 15 mM CaCl₂ was added to 30 µl of diluted sample plasma. Next, 30 µl of a solution containing 48 nM factor IIa and 900 nM factor X was added. After the reaction mixture was incubated for 5 min at room temperature, 30 µl of 1 mM Spectrozyme factor Xa (American Diagnostica), 400 nM desulfatohirudin, 100 mM EDTA, 20 mM HEPES, 0.15 M NaCl, 0.1% polyethylene glycol, pH 7.4 was added and the absorbance at 405 nm was measured.
over a 10 min period. Murine plasma fVIII levels were obtained by comparison to standard curve using pooled normal human plasma (FACT, George King Biomedical, Overland Park, KA). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured using the Sigma Diagnostic Transaminases Reagent kit (Sigma) following the manufacturer’s instructions. vWF levels were measured by enzyme-linked immunosorbent assay (ELISA) using a previously published method.\textsuperscript{11} The ELISA titer was defined as the absorbance at 490 nm obtained for a 1/20 dilution of plasma. Data acquired at this dilution were within a linear range. All numerical data are presented as mean ± 1 standard deviation with the data range presented in parentheses.
Results and discussion

Twenty-four hours after acetaminophen treatment, all mice displayed signs of stage II hepatic encephalopathy, including reduced locomotive activity. This treatment regimen previously has been shown to induce extensive hepatic centrilobular necrosis within 24 hr.\textsuperscript{10} One indicator of liver necrosis is the elevation of serum ALT activity and a change in the ratio of AST:ALT from greater than one to less than one. Plasma from control and acetaminophen treated mice was assayed for AST and ALT activity (Figure 1). Pooled plasma from untreated mice had 30 Units/ml AST activity and 19 Units/ml ALT activity for an AST:ALT ratio of 1.58, whereas the plasmas from all acetaminophen treated mice demonstrated elevated AST and ALT levels and decreased AST/ALT ratios. The mean values for AST and ALT activity were $188 \pm 10$ (175 – 209) Units/ml and $28,200 \pm 6,000$ (20,300 – 37,300) Units/ml, respectively, corresponding to an AST/ALT ratio of 0.007. These findings indicate that FHF was successfully induced in all of the mice.

Plasma fVIII activity was measured using a chromogenic substrate assay. The concentration of fVIII activity measured in control mouse plasma was 2.9 units/ml, which is consistent with previously published results.\textsuperscript{12,13} Average fVIII activity was decreased by 91\% to $0.27 \pm 0.07$ (0.16 – 0.37) units/ml in the acetaminophen treated murine plasmas (Figure 2). Therefore, we were unable to replicate the increase in plasma fVIII activity observed in human acetaminophen-induced FHF in a murine model. The decrease in fVIII activity observed in the present study corresponds well with the results previously reported in azoxymethane treated mice where, at the highest dose of azoxymethane tested (50 µg/g body weight), fVIII activity dropped to 10\% of control levels at 24 hr post-administration.\textsuperscript{9}
Several factors have been shown to influence circulating fVIII levels, including von Willebrand factor (vWF) whose levels display a strong positive correlation with fVIII levels (for review see Kamphuisen et al.\textsuperscript{14}) Accordingly, in the complete absence of vWF, fVIII levels are reduced by approximately 80% in mice.\textsuperscript{11} We measured vWF antigen levels in the acetaminophen treated and control murine plasmas and found that the levels in acetaminophen treated mice were mildly reduced to 62 ± 18% (29 – 97%) of the control murine plasma level (Figure 2). However, the extent of decline in vWF was not equivalent to the decrease in fVIII, and there was not a significant correlation between vWF antigen and fVIII activity within the acetaminophen treated mice. Therefore, the decrease in fVIII was not secondary to a reduction in vWF. The current data support our previous findings of reduced fVIII activity following azoxymethane-induced murine fulminant hepatic failure. Combined these studies argue that there are fundamental differences in the regulation of circulating fVIII levels between mice and humans and that these differences should not be ignored when using the mouse as an experimental model system to study fVIII biology.
References


Figures and legends

Figure 1. Aminotransferase levels in acetaminophen treated mice. Pooled control plasma from six normal mice and individual phenobarbital/acetaminophen (PB+APAP) treated murine plasmas (n = 12) were assayed for AST (grey bars) and ALT (black bars) activity as described in Study design.
Figure 2. FVIII activity vs. vWf antigen levels in acetaminophen treated mice.

Plasma from acetaminophen treated mice (n = 12) was assayed for fVIII activity by chromogenic assay and vWf antigen level by ELISA. Results expressed relative to pooled normal murine plasma.
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