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Does Dietary Fatty Acid Fluctuation Influence Factor XII?

Erratum
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Does Dietary Fatty Acid Fluctuation Influence Factor XII?

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ABSTRACT

FXII is considered a potential prothrombotic, proinflammatory and profibrinolytic protease. Evidence supports the concept that women with reduced plasma FXII level may be at high risk of developing premature delivery. FXII C46T polymorphism influences venous thrombosis and puerperium in asymptomatic women with no history of miscarriages. The prevalence of preeclampsia in obese women is high. The aim of this study was to examine whether there would be a correlation between the factor XII and dietary fatty acid. Immunochemical methods and functional techniques using a chromogenic peptide substrate assay were used. Although our findings were inconclusive, further research is needed.

INTRODUCTION & BACKGROUND

FXII (Hageman Factor) activates the plasma Kallikrein-Kinin System (KKS). This plasma protein is involved in the contact activation (intrinsic) pathway of blood coagulation. Activation of FXII to FXIIa begins the coagulation cascade, with the eventual formation of a fibrin clot. Circulating fatty acids have been proposed to provide a substrate for FXII activation¹. Once activated, FXIIa, can catalyze Prekallikrein (PK) to kallikrein. Kallikrein then acts on High molecular weight Kininogen (HK) to generate Bradykinin (BK). Elevated BK has been shown to play a significant role in the pathogenesis of Hereditary Angioedema (HAE), and may be implicated in preeclamptic swelling. In the absence of the C1-inhibitor protein, there is uncontrolled generation of kallikrein and BK, which leads to severe edema in HAE patients.

FXII is converted to FXIIa in an excess manner under pathophysiological conditions. FXIIa is significantly elevated in diseases such as non-alcoholic fatty liver disease², diabetes mellitus, and diabetic retinopathy³. As these are diseases where fatty acids have been shown to be elevated⁴,⁵, our lab investigated the relationship between saturated, monounsaturated, and polyunsaturated fatty acids and FXII activation. Following experiments on fatty acids, we begun characterizing a new FXIIa inhibitor. Several hundred compounds from the National Center for Natural Products Research were screened in a double blind manner. Five compounds were found to inhibit FXIIa to various extents. Among these lead compounds, G1 avidly inhibited FXIIa.
The goal of the current study is to determine the effects of fatty acids on plasma proteins, specifically FXII. Subsequently, the inhibitory characteristics of G1 were determined in order to understand how this compound interacts with different proteins. It is our hope that G1 will prove to be a selective inhibitor of FXIIa, and thus be a novel therapeutic for treating diseases such as Hereditary Angioedema.

MATERIAL & METHODS

Chromogenic FXIIa Activity Assay: The effect of fatty acids on the activity of FXIIa was quantified using a chromogenic assay. Effect of G1, a new inhibitor of plasma enzymes, was also characterized with the use of a chromogenic assay in the presence of kallikrein, FXIa, and FXIIa FXIIa (Enzyme Research Laboratories, South Bend, IN, USA) in fluid phase. The effect of fatty acids on FXIIa activity was determined using the chromogenic substrate CS-31(02) (Aniara Diagnostica, West Chester, OH, USA). In this experiment, 9 nM FXIIa was incubated with the chromogenic substrate CS-31(02). Subsequently, free para-nitroaniline was measured. Kallikrein (1 nM) and FXIa (18 nM) were measured in a similar manner, utilizing 0.23 mM CS-31(02) and 0.43 mM S2366 respectively. Plasma proteins were incubated with their respective enzyme in the absence or presence of increasing concentrations of fatty acids or potential inhibitory compound G1 with a final volume of 100 µL HEPES-NaHCO3 buffer (137 mM NaCl; 3 mM KCl; 14.7 mM HEPES; 1 mM MgCl2; 2 mM CaCl2; 5.5 mM glucose; and 0.1% gelatin, pH 7.1). For FXIa activity, (18 nM) FXIa (Enzyme Research Laboratories) was incubated with 0.43 mM S2366 (Glu-Pro-Arg-p-nitroanilide) in the absence or presence of the inhibitor. After 1 h of incubation at 37°C, the activity of kallikrein, FXIIa or FXIa was measured as a change in absorbance at OD 405 nm using BioTek ELx800 Absorbance Microplate Reader (Winooski, VT, USA). Data were analyzed using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA).
RESULTS

Enzyme Kinetic Determination: Optimum concentrations of enzymes and substrates were determined. Once proper concentrations were specified, experimental time length was established through a time course.

FIGURE 2. A) FXIIa activity was assayed to determine the optimal concentration of FXIIa in the presence of CS-31(02). Due to this experiment, 9 nM FXIIa was used in subsequent experiments to measure changes in FXIIa activity. B) The Km range obtained for CS-31(02) in the presence of FXIIa was 0.4375 to 0.5899 mM. For subsequent measurements of FXIIa activity, the final concentration of CS-31(02) equaled 0.43 mM. C) A time course using 9 nM FXIIa and 0.43 mM CS-31(02) was used to determine the ideal incubation time for the following experiments. Following this result, an incubation time of 1 hour was used for all experiments utilizing FXIIa, FXIa, and Kallikrein.

FIGURE 3. Effect of fatty acid on FXIIa activity. Fatty acids were tested (replicates=3) to determine their effects on the catalytic activity of FXIIa. No fatty acids were found to show inhibition or stimulation of FXIIa. Data are presented as Mean ± SEM.
FIGURE 4. A) Determination of enzyme inhibition by compound G1. FXIIa and FXIa activity were reduced significantly by compound G1. However, G1 did not inhibit Kallikrein, which indicates its specificity for FXIIa and FXIa. B) G1 does not inhibit Kallikrein activity. Kallistop, a synthetic Kallikrein inhibitor was used to show that Kallikrein was not inhibited by G1. Data are presented as Mean ± SEM.

DISCUSSION

Substrate hydrolysis occurred in a time and concentration dependent manner. Fatty acids were not found to modulate the activity of FXIIa. G1 was found to inhibit coagulation factors XIIa and XIa, but not kallikrein. Further experiments are needed to determine fatty acid modulation of FXIIa, along with the effects of G1 on downstream components of the blood coagulation pathway and the Kallikrein-Kinin System.

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REFERENCES

2) Kotronen, Anna, et al. "Increased coagulation factor VIII, IX, XI and XII