buffer, human rFVIII (Baxter Hyland Immuno, Thousand Oaks, CA), and RAP (made as a recombinant fusion protein with glutathione S-transferase), as described in our original study. Briefly, 3 groups were tested: (1) a control group received 40 mL/kg buffer followed 15 minutes later by a second injection of 20 mL/kg buffer (n = 11); (2) the rFVIII group received 40 mL/kg buffer followed by 200 U/kg human rFVIII in a volume of 20 mL/kg (n = 15); (3) the RAP preadministration group received 40 mg/kg RAP in a volume of 40 mL/kg followed by 200 U/kg rFVIII in a volume of 20 mL/kg (n = 18). In vivo recovery was determined at 15 minutes as described, and FVIII levels were measured with an ELISA that is specific for human FVIII (Immunozym FVIII:Ag, Baxter, Vienna, Austria). Statistical comparisons were based on repeated measure analysis of variance. Because of the difficulty in drawing blood at frequent intervals from vWf knockout mice, sufficient material for analysis was not available from each animal at each data point and the number of data points was limited, which allowed only fitting of a 1-compartment model for calculation of the half-life of rFVIII.

As expected for detection of a human protein in mice, FVIII levels were below the limit of detection in all animals before infusion of rFVIII and in the control group at all time points. Mean recovery (±SD) was only 5.8% (±3.7%) in the rFVIII group (6 males, 7 females), but 12.6% (±5.9%) in the group with preadministration of RAP (8 males, 8 females). P = .0023. There was neither an effect of sex (P > .1) nor an interaction between sex and group.

FVIII was maintained at higher levels and was detectable in plasma for a longer period of time with preadministration of RAP (6 hours) than without preadministration of RAP (3 hours) (Figure). Blocking LRP by preadministration of RAP prolonged the half-life of the infused rFVIII from only 42 minutes with rFVIII alone to 67 minutes with preadministration of RAP.

Saenko et al. reported that RAP slows the clearance of infused plasma-derived FVIII/vWf in normal mice. Our extended study now demonstrates that inhibition of LRP by a single bolus administration of RAP has a significant inhibitory effect on the clearance of infused FVIII in the absence of vWf, thus further supporting the involvement of LRP in the clearance mechanisms of FVIII.

References


To the editor:

A novel missense mutation (C329Q) in factor VII gene

FVII is a vitamin-K-dependent plasma glycoprotein. It is synthesized in the liver and circulates in the blood as an inactive zymogen. Upon vascular injury and the presence of tissue factor (TF), FVII is complexed to TF and is cleaved to its active form, FVIIa. The VIIa/TF complex then cleaves and activates both factors X and IX to initiate the coagulation process. Deficiency of factor VII results in a defect in the initiation of coagulation by the extrinsic pathway. Hereditary factor VII deficiency is a rare autosomal recessive bleeding disorder with variable clinical expressions. It is estimated to occur in 1 out of 500,000 persons. The severity of bleeding is variable. The patients may have symptoms such as easy bruising, epistaxis, gingival hemorrhage, increased menstrual blood loss, and cerebral hemorrhage. The clinical symptoms of patients can be improved by transfusion of fresh plasma or blood but not by the administration of vitamin K or hemostat. The FVII gene is located on 13q34-qter and contains 9 exons and 8 introns. Characterization of FVII gene mutations will provide insight into the understanding of function/structure correlation of FVII and the heterogeneity of the deficiency. More than 50 mutations responsible for FVII deficiency have been identified so far. Those include missense mutation, base deletion, splicing site mutation, and promotor mutation. In this short report, we describe a novel missense mutation from a Chinese patient of FVII deficiency with mild symptoms.

The female patient, a 53-year-old, was born of parents with a known consanguinity in Zhousan, Zhenjian, China. She has been easy to bruise and has had gingival hemorrhaging since her childhood, with no liver disease but a history of increased
A cysteine residue substituted by glycine in FVII protein. This base substitution caused a G mutation at codon 329 (location from 10813 to 10849) and antisense primer (location from 11185 to 11203), were digested with endonuclease Hgi C I, under the manufacturer’s (MBI, Fermentas, Italy) suggested condition. Digest DNA was electrophoresed on a 12% polyacrylamide gel. Since the nucleotide change generates another Hgi C I recognition site in the 358 bp PCR product, 82 and 69 bp fragments would be observed in a mutant digestion pattern instead of 151 bp in the normal pattern. The digestion result shows 207, 82, and 69 bp in the patient, while 207 and 151 bp in normal controls (n = 12), and a heterozygous digestion pattern (207, 151, 82, and 69 bp) in the patient’s son (Figure 2). A heterozygous digestion pattern for the mutation in the patient’s 2 daughters, and healthy digestion patterns in the patient’s husband and her 3 grandchildren were also observed (data not shown).

Previously, Bernardi et al 6 found a missense mutation C310F in 2 unrelated pedigrees. It was proposed that such a mutation would destroy the formation of a disulphide bond between Cys310 and Cys329, which is highly conserved in serine proteases and is critical to maintain the structure and function of FVII. Although C329Q mutant factor VII was not expressed and characterized in vitro, we predict it to be the causative mutation for the phenotype of the patient. More than one-half the mutations found so far in FVII are located in exon 8. 5 The mutant allele identified herein provided more evidence of exon 8 being the hot region of mutation and further demonstrates the heterogeneity of the FVII deficiency.

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To the editor:

Hepatitis C virus infection in patients with non-Hodgkin lymphoma in Thailand

The role of hepatitis C virus (HCV) in the pathogenesis of non-Hodgkin lymphoma (NHL) is controversial. Recently, Germanidis et al. recently reported in Blood the lack of association of HCV infection and NHL in France, where HCV infection is not common. This is in contrast to the reported high prevalence of HCV infection in patients with NHL (9% to 35%) in Italy and North America, where HCV infection in normal population is quite frequent (1% to 5%). These results lead to speculation that HCV infection may be associated with NHL only in areas where HCV is highly prevalent.

Thailand has had an increasing incidence of NHL in recent years. It also has a high prevalence of HCV infection, averaging 1% to 5% in the general population. The aim of our study was therefore to determine whether high prevalence of HCV infection exists in our Thai patients with NHL. Ninety-eight patients with intermediate- to high-grade NHL and 32 patients with low-grade NHL seen at Siriraj Hospital were screened for HCV using Cobas Core anti-HCV indirect EIA assay (Roche, Basel, Switzerland) after informed consent. NHL was classified according to working formulation. The Table shows the prevalence of anti-HCV antibody according to NHL subtype. The overall prevalence of HCV antibody in Thai NHL patients was 2.3%. All patients were HIV-negative and not previously transfused. Only 3 out of 130 cases were HCV-positive including 2 patients with diffuse large-cell lymphoma and 1 patient with follicular mixed small- and large-cell lymphoma. The route of HCV infection in the first 2 patients with intermediate-grade NHL was not clear because no history of blood transfusion or drug abuse could be elicited. The route of viral acquisition in the third patient with low-grade NHL, however, is quite unique because he developed hepatitis after a cut injury occurred while he was performing a surgical procedure in North America. He received interferon treatment for hepatitis and subsequently cleared the virus several years prior to the diagnosis of NHL. PCR for 8 HCV genotypes did not reveal HCV RNA at the time of diagnosis of NHL in Thailand. Whether HCV infection led to the development of NHL in this third patient is unknown. Our overall results, however, do not support the existence of a significant relationship between HCV infection and NHL in Thailand.

HNL in Thailand has a different distribution of histologic subtypes than does the West, with a lower prevalence of low-grade B-cell NHL (averaging 10%). Whether this may account for the overall low prevalence of HCV infection in NHL in our country is not known. Source and genotype of HCV may play an important role. The predominant HCV genotypes in Thailand appeared to be different from those found in the West.

In conclusion, although HCV infection is common in Thailand, the majority of Thai NHL patients do not carry the HCV antibody. HCV infection is unlikely to play a major role in the pathogenesis of NHL in Thailand, where HCV infection is highly prevalent.

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