Aspirin Alters the Cardioprotective Effects of the Factor XIII Val34Leu Polymorphism

Anetta Undas, MD, PhD; Wojciech J. Sydor, MD; Kathleen Brummel, PhD; Jacek Musial, MD, PhD; Kenneth G. Mann, PhD; Andrew Szczeklik, MD, PhD

Background—The mechanism underlying decreased risk for myocardial infarction in carriers of the Leu34 polymorphism of the factor (F) XIII A-subunit is unclear. Given that acetylation of fibrinogen by aspirin can alter its clotting properties and the presence of fibrin stimulates thrombin-mediated activation of FXIII, we have tested the hypothesis that treatment with aspirin differentially modulates the influence of the FXIII Val34Leu polymorphism on its activation in vivo.

Methods and Results—The rates of the disappearance of FXIIIA chain and the appearance of its activated form (FXIIIAa) in sequential 30-second blood samples collected at the site of microvascular injury were compared in 14 healthy carriers of the Leu34 allele and 23 Val34 homozygotes both before and after a 7-day aspirin ingestion (75 mg/d), with the use of quantitative Western blotting. The presence of the Leu34 allele was associated with a significant increase in the maximum rate of FXIII activation by thrombin. Although the Leu34-positive and -negative subjects were similar with respect to aspirin-related impairment of thrombin generation, aspirin led to a more pronounced inhibition of the activation of FXIII in the Leu34 carriers as compared with the Val34 homozygotes.

Conclusions—Inhibition of FXIII activation by aspirin is enhanced in the Leu34 carriers in vivo, suggesting that these subjects might benefit more than the Leu34-negative subjects from the reduction in risk for myocardial infarction with low-dose aspirin. (Circulation. 2003;107:17-20.)

Key Words: genetics ■ aspirin ■ coagulation

Recent work has highlighted importance of a common G→T mutation in the codon 34 of the factor (F) XIII A subunit gene (Val34Leu) as the only hemostatic candidate gene polymorphism, which seems to be associated with a protective effect against myocardial infarction (MI), although this issue still generates controversy.1,2 Plasma FXIII, composed of noncovalently associated 2A and 2B polypeptide subunits, in its activated form (FXIIIAa) stabilizes the hemostatic plug by cross-linking fibrin α- and γ-chains.2 Because a valine-to-leucine replacement takes place 3 amino acids away from the thrombin cleavage site at Arg37-Gly38, it has been postulated that this mutation might modulate FXIII activity. Indeed, in vitro studies demonstrated that the Leu34 allele is associated with more rapid FXIII activation and altered clot structure with enhanced fibrin cross-linking.4 It remains unclear why the Leu34 carriers have lower risk for MI despite faster FXIII activation.

Given that acetylation of fibrinogen (Fbg) by aspirin alters its clotting properties assessed in a purified system5 and that Fbg and/or fibrin stimulate thrombin-mediated FXIII activation,6 we hypothesized that treatment with aspirin might modulate the influence of the FXIII Val34Leu polymorphism on its activation, and as a consequence, alter the impact of the Leu34 allele on risk of MI. Here we report that aspirin slows FXIII activation in the Leu34 carriers to a greater extent than in the Leu34-negative subjects, providing evidence for a polymorphism selective beneficial effect.

Methods

Subjects

Participants of the study were recruited from asymptomatic, male medical students, aged 22 to 25 years, who had not taken any medication during the previous 4 weeks. The University Ethical Committee approved the study. The subjects gave informed consent. Blood samples were obtained between 8:00 AM and 10:00 AM. Platelet count was determined by standard method. Plasma Fbg levels were measured using nephelometry. All tests were performed before and after a 7-day aspirin administration (75 mg/d). Platelet aggregation in platelet-rich plasma in response to arachidonic acid (600 μmol/L) was used to exclude subjects on platelet-blocking drugs at the start of the study and to confirm platelet inhibition induced by aspirin after 7 days.

Genotyping

The Val34Leu polymorphism was determined using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique.6 Briefly, in a 114-bp fragment of exon2/intron B of FXIII, a new restriction site for CfoI in the normal sequence, was created using mismatched reverse primer (5'-GTTGACGCCCGGGCACC-3').
After digestion, a 94-bp fragment was visible in the wild genotype samples, whereas in the Leu34 homozygotes, the PCR product remained undigested. The Leu34 allele frequency was similar to those reported in other studies.\(^1,2\)

**Model of Microvascular Injury**

Evaluation of the time-course of FXIII activation was performed in blood obtained from the microvascular wounds at 30-second intervals as described.\(^7,8\) Briefly, at venous stasis of 40 mm Hg, 2 incisions were made on the lateral aspect of a forearm using a Simplate II device (Organon Teknika), and blood was collected using heparinized capillary tubes (Kabe Labortechnik) into Eppendorf tubes, containing sodium citrate, aprotinin, peptidyl chloromethyl ketone, and heparin (Diagnostica Stago). The procedure was performed by the same investigator each time, who was blinded to the results of genotyping. The supernatants were aliquoted and stored at \(-80^\circ\)C until analysis.

**Immunoblotting**

After electrophoretic separation, quantitative Western blotting was performed by technical staff blinded with respect to knowledge of genotype.\(^8\) The time-courses of FXIII activation were performed using rabbit polyclonal antibody raised against human FXIII A (D4679), a gift from Drs Paul Bishop and Gerry Lasser (ZymoGenetics, Seattle, Wash). FXIIIa levels were estimated by comparison with purified human FXIIIa standards. Prothrombin activation products were also evaluated with the use of a burro polyclonal prothrombin 1 antibody, which recognizes human \(\alpha\)-thrombin B chain (Division of Hematology Research, Mayo Clinic, Rochester, Minn). Reaction rates were analyzed using IGOR Pro Version 3.1 software (WaveMetrics Inc).

**Statistical Analysis**

Data are presented as mean±SEM. Comparisons between different genotypes were made using the Mann-Whitney U test. For intragroup differences related to aspirin, the Wilcoxon sign rank test was used. Spearman’s correlation coefficient analysis was used to quantify the relations between parameters. A probability value <0.05 was considered significant.

**Results**

Of 37 subjects, 12 were heterozygous for the Leu34 allele, 2 were homozygotes, and the remaining 23 were Val34 homozygotes. The Leu34-positive and -negative subjects did not differ with respect to the demographic characteristics. Sample volumes did not differ between the 2 groups either before \((P=0.7)\) or after aspirin ingestion \((P=0.1)\). Fbg levels, as well as platelet count, were similar in both groups before and after aspirin ingestion.

The pre-aspirin treatment evaluation of FXIIIa concentration at the wound site was similar in both groups \((84±5\text{ nmol/L})\), and decreased rapidly with time. Posttreatment concentration of FXIIIa was \(83±7\text{ nmol/L}\).

Immunoblots (Figure 1A) show the disappearance over time of an \(M_r=83\text{ 000}\) band that migrated identically with the purified FXIIIa. In the Val34 homozygotes before aspirin (upper blot), the zymogen became undetectable after 90 seconds of bleeding (median; range: 60 to 150 seconds), whereas in the Leu34 carriers before aspirin (lower blot), the
zymogen was not detected after 60 seconds (median; range: 30 to 120 seconds). This intergroup difference was significant (P = 0.01). The activated form of FXIII (FXIIIa) has an M₉₇ = 79 000 and is detectable at 120 seconds in the Val34 homozygotes and at 90 seconds in the Leu34 carriers. Aspirin administration delayed FXIII activation by approximately 60 seconds (P = 0.007) in the Val34 homozygotes and by 120 seconds in the Leu34 carriers (P = 0.002), which led to a significant intergroup difference (P = 0.01; Figure 1B).

The pre-aspirin maximum rate of FXIII removal from the fluid phase was higher by 30% in the Leu34 carriers as compared with the rate calculated for the Val34 homozygotes (1.17 ± 0.08 versus 0.90 ± 0.03 nmol·L⁻¹·s⁻¹; P = 0.03). The post-aspirin treatment velocity of FXIII activation was reduced at maximum to 0.52 ± 0.03 nmol·L⁻¹·s⁻¹ (P = 0.009) in the Leu34 carriers and to 0.70 ± 0.03 nmol·L⁻¹·s⁻¹ (P = 0.015) in the Val34 homozygotes (Figure 2). Thus, the aspirin-induced reduction in the maximum velocity of FXIIIa disappearance was greater in the Leu34 carriers (P = 0.001).

There was a nonsignificant trend toward higher maximum rates of FXIII disappearance in the Leu34 homozygotes compared with the Leu34 heterozygotes before aspirin administration and lower posttreatment rates of this process in the former group.

Analyses of the appearance of FXIIIa by quantitative immunoblotting revealed that the pre-aspirin velocity of the appearance of FXIIIa was higher in the Leu34 carriers (0.427 ± 0.027 versus 0.243 ± 0.02 nmol·L⁻¹·s⁻¹; P < 0.0001), whereas the post-aspirin maximum rate of this process was slower in these subjects than the corresponding value in the Val34 homozygotes (0.142 ± 0.014 versus 0.265 ± 0.016 nmol·L⁻¹·s⁻¹; P < 0.0001). Thus, aspirin significantly slowed the maximum rate of FXIII activation in the Leu34 carriers (P = 0.0009), but not in the remainder. Maximum levels of FXIIIa in the last minute of bleeding, however, were significantly reduced from 30.7 ± 1.52 to 10.9 ± 0.74 nmol/L (P = 0.008) in the Leu34 carriers and from 22.9 ± 1.24 to 14.8 ± 0.69 nmol/L (P = 0.03) in the Leu34-negative subjects.

Analysis of thrombin B-chain by immunoblotting indicates that the Leu34 allele had no significant influence on pro-

Discussion

This study demonstrates that the disappearance of FXIII at the site of vascular injury is significantly faster in the Leu34 carriers, which is consistent with data from in vitro models, and aspirin modulates the velocity of this process, resulting in its marked inhibition. We have also provided evidence for differential courses of FXIII activation in vivo, which depends on the Val34Leu polymorphism and on treatment with low-dose aspirin.

The molecular mechanism by which aspirin might interfere with FXIII activation is unknown. Apart from inhibiting
platelet function by blocking thromboxane A₂ synthesis, aspirin has been reported to acetylate coagulation factors and/or platelet glycoproteins, primarily via lysyl residues, which can alter charge distributions and possibly conformation. Because there is no data on acetylation of FXIII by aspirin, it might be hypothesized that fibrinogen modified by incorporation of acetyl groups may contribute to decreased rates of the disappearance of FXIIIA after aspirin. The aspirin effect is not due to differences in aspirin-induced impairment of thrombin formation related to Leu34, because aspirin inhibited thrombin generation to a similar extent in both groups studied.

In summary, in the Leu34 carriers, the amount of activated FXIII in blood collected at sites of vascular injury is decreased by aspirin to a greater extent than in the Leu34-negative subjects, which may result in the formation of clots that are less resistant to fibrinolysis. The selective effect of aspirin combined with a polymorphism reported here will be of interest with respect to pharmacogenetics, aiming to tailor the optimal therapy for an individual patient. Recently, we have shown a different thrombin-lowering effect of aspirin in relation to the PlA₂ polymorphism of β₃ integrins. Taken together, these results suggest that genotyping for both common polymorphisms may help us identify patients who are more likely to benefit from the prophylactic use of aspirin.

Acknowledgments

This work was supported by National Institutes of Health grants HL-46703 and T32 HL-07594 (to Dr Mann). Dr Undas was supported by a Fulbright Fellowship.

References

4. Trumbo TA, Maurer MC. Examining thrombin hydrolysis of the factor XIII activation peptide segment leads to a proposal for explaining the cardioprotective effects observed with the factor V34L mutation. J Biol Chem. 2000;275:20627–20631.
Aspirin Alters the Cardioprotective Effects of the Factor XIII Val34Leu Polymorphism
Anetta Undas, Wojciech J. Sydor, Kathleen Brummel, Jacek Musial, Kenneth G. Mann and
Andrew Szczeklik

_Circulation_. 2003;107:17-20; originally published online December 2, 2002;
doi: 10.1161/01.CIR.0000047062.03282.A3

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/107/1/17

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located,
click Request Permissions in the middle column of the Web page under Services. Further information about
this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/