Genotype-specific increase in plasma concentrations of activated coagulation factor VII in response to experimental inflammation

A link between infection and acute myocardial infarction?

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Summary
There is evidence that infection and inflammation might trigger an acute coronary event, but the mechanisms are unclear. Activated factor VII (FVIIa) is a potent coagulant that is under genetic control and a potential determinant of the outcome of acute myocardial infarction. This study investigated the acute FVIIa response to experimental inflammation. Forty healthy men and women were vaccinated with 1 ml of Salmonella Typhii vaccine. Plasma levels of FVIIa, FVII antigen (FVIIag), tissue factor (TF) activity and thrombin-antithrombin complex (TAT) were measured at baseline and up to 24 hours after inoculation. All subjects were genotyped for the FVII gene Arg353Gln polymorphism. Plasma concentrations of FVIIa, but not FVIIag, increased significantly with a peak at 10 hrs after vaccination. At 24 hrs FVIIa levels had returned to baseline. The FVIIa response to vaccination was significantly greater in subjects with the ArgArg genotype compared with ArgGln subjects. TAT increased, but TF activity was unchanged after vaccination. The results are of interest from a mechanistic viewpoint, since one explanation for the link between infection and acute myocardial infarction might be activation of coagulation. However, there is a need for further studies of the role of infection and inflammation in haemostasis.

Keywords
Gene regulation, inflammatory mediators, tissue factor/factor VII

Introduction
Thrombosis underlies most of the acute manifestations of coronary heart disease (CHD) including myocardial infarction (1). Plaque disruption followed by exposure of tissue factor (TF) to blood and further binding of TF to circulating coagulation factor VII (FVII) resulting in activated FVII (FVIIa) and subsequent initiation of the coagulation cascade (1, 2), is considered to be the major cause of thrombosis in acute myocardial infarction. An association between an elevated plasma concentration of FVII and an increased risk of CHD or cardiovascular death has been reported in some studies (3, 4), but not in others (5–7). Data suggesting an association between the plasma concentration of FVIIa and CHD are sparse and positive associations seem to be limited to women (8). The plasma concentration of FVII is under genetic and environmental control, the plasma triglyceride concentration being a major determinant of FVII activity in blood (9, 10). Furthermore, age, body-mass-index, oral contraceptive use, menopausal status and hormone replacement therapy are associated with variation of mass and activity of FVII (11, 12). Several common polymorphisms in the FVII gene that strongly influence plasma concentrations of FVII have been described. In particular, the Arg353Gln polymorphism in exon 8 of the FVII gene is associated with mass and activity of FVII in plasma (13–15). The Arg353Gln is a single nucleotide polymorphism characterised by a guanine-to-adenine substitution resulting in replacement of arginine (Arg) by glutamine (Gln) in codon 353 of the protein (13). Presence of the rare Gln-allele is associated with a 20–30% decrease in FVII activity compared with the more common Arg-allele (13–16). These findings suggest that the Gln-allele may be protective against acute thrombotic events. Accordingly, two case-control studies reported that the Gln-allele was associated with a reduced risk of myocardial infarction (17, 18). However, other studies failed to show any such association (19–21).

It has been proposed that infection and inflammation might trigger myocardial infarction (22, 23), but the mechanism remains unclear. It has been shown that in a chronic inflammatory
state, such as obesity with insulin resistance, markers of coagulation activation, including FVIIa, and inflammation are concomitantly increased (24). Furthermore, FVIIa was observed to increase during the inflammatory response following endotoxin-induced sepsis (25).

Recently, vaccination with Salmonella Typhii, an experimental model of infection and inflammation, has been shown to cause endothelial dysfunction, an established risk factor for CHD (26). The aim of the present study was to determine the response of activated FVII to experimental inflammation and to evaluate whether this response is genotype-specific with respect to a FVII gene polymorphism known to influence FVIIa concentrations.

Material and methods

Forty healthy subjects from a cohort of 387 healthy men and women recruited from the general population were invited. These individuals were controls to patients with a first myocardial infarction before the age of 60 years who participated in a study aiming at identification of novel risk factors for atherosclerosis and CHD (27). Exclusion criteria were treatment for hyperlipidemia and hypertension, on-going postmenopausal substitution therapy, use of acetyl salicylic acid and on-going infection with symptoms or fever at the time of investigation. Smokers were asked to refrain from smoking 24 hrs before and during the study. Subjects were originally selected for another study according to their genotype for the –174G >C and –572 G>C polymorphisms of the interleukin-6 (IL-6) gene located on chromosome 7. Eighteen were homozygous for the common –174 G-allele, 20 were homozygous for the rare –174 C-allele and two were heterozygotes. All subjects were homozygous for the common –572 G-allele (28).

Study protocol

Subjects arrived at the Clinical Research Unit at Danderyd University Hospital in the morning after 10 hrs of fasting. Venous blood samples were taken before and at 2, 4, 6, 8, 10 and 24 hrs after vaccination. An intramuscular injection of 1 ml of Salmonella Typhii vaccine (Merieux) was administered after the initial blood sampling. Directly after the vaccination, the participants consumed a light breakfast (a sandwich with cheese or ham and coffee or tea with sugar and milk as preferred). After blood sampling at 4 hrs, they were offered the same type of sandwich as in the morning together with water. In the afternoon, after blood sampling at 8 hrs, they had coffee or tea with a sweet-roll.

All subjects gave informed consent to their participation in the study which had been approved by the Ethics Committee of the Danderyd and Karolinska University Hospitals.

Laboratory analyses

Plasma concentrations of FVIIa, FVII antigen (FVIIag), free fatty acids (FFA), IL-6, thrombin-antithrombin complex (TAT), TF and triglycerides were analysed at all time-points. The FVIIa concentration in citrated plasma was determined with a clotting assay using soluble recombinant truncated TF (29), kindly supplied by Professor James H Morrissey, University of Illinois at Urbana-Champaign, Urbana, IL, in an ACL automated coagulation instrument (Instrumentation Laboratory Spa). Samples were diluted in FVII deficient plasma (Instrumentation Laboratory Spa) and activated with rabbit brain cephalin (Haemochem Inc) before addition of TF and calcium. A standard curve was prepared with the First International Standard VIIa Concentrate (National Institute for Biological Standards and Control, United Kingdom). Total FVII mass in citrated plasma was determined by an ELISA from Affinity Biological Inc. Genotyping for the Arg353Gln polymorphism of the FVII gene, located on chromosome 13, was performed as described (13). TAT was determined with the Enzygnost TAT micro ELISA from Dade Behring. TF procoagulant activity was quantified with a chromogenic assay using the Actichrome TF kit from American Diagnostica Inc. Determinations of plasma IL-6 concentration and genotype have been described previously (28). Plasma concentrations of FFAs and triglycerides were analysed by enzymatic methods. Baseline plasma, low and high density lipoprotein cholesterol and plasma glucose concentrations were analysed by clinical routine instrumental analyses.

Statistics

Values are presented as numbers or mean (± SD or 95% confidence interval). All skewed parameters were logarithmically transformed before statistical testing. Differences in basic characteristics between genotypes were tested by chi-square or unpaired t-test. Differences in the FVIIa, FVIIag and TAT response between genotypes were tested by a mixed-effects model including time as a covariate. Associations between different parameters were tested by Spearman rank.

Results

Basic characteristics of the study subjects, grouped according to FVII Arg353Gln genotype, are shown in Table 1. Twenty-seven subjects were homozygous for the Arg-allele (ArgArg) whereas 13 individuals were heterozygous (ArgGln). None were homozygous for the Gln-allele. There were no statistically sig-

<table>
<thead>
<tr>
<th>Sex (M/F)</th>
<th>23/4</th>
<th>9/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smokers</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Interleukin-6 –174 genotype (CC/CG/GG)</td>
<td>1/1/1</td>
<td>5/8/0</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>132 ± 11</td>
<td>133 ± 9</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>82 ± 6</td>
<td>82 ± 7</td>
</tr>
<tr>
<td>Plasma cholesterol, mmol/l</td>
<td>5.5 ± 0.8</td>
<td>5.4 ± 0.7</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>1.5 ± 0.3</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>3.7 ± 0.4</td>
<td>3.2 ± 0.6</td>
</tr>
<tr>
<td>Plasma triglycerides, mmol/l</td>
<td>1.3 ± 0.5</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>5.4 ± 0.5</td>
<td>5.4 ± 0.3</td>
</tr>
</tbody>
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LDL: low-density-lipoprotein, HDL: high-density-lipoprotein. There are no significant differences between the two groups.
significant differences between the two groups regarding basic characteristics. In all subjects, plasma concentrations of FVIIa started to increase 6 hrs after vaccination and reached a maximum at 10 hrs after vaccination and had returned to baseline after 24 hrs (p<0.001), whereas FVIIag did not show any major changes (Fig. 1). Of note, there were no statistical significant differences in baseline FVIIa or FVIIag concentrations between the Arg353Gln genotype groups. After vaccination, subjects with the ArgArg genotype had higher plasma concentrations of FVIIa compared with individuals with the ArgGln genotype (p=0.02) (Fig. 2A). After the baseline determination, differences in plasma concentrations of FVIIa between the two groups were significant at all time points (p<0.05). Plasma concentrations of FVIIag tended to be lower in subjects with the ArgGln genotype compared with the ArgArg genotype, however, this difference did not reach the level of statistical significance (Fig. 2B). Plasma concentrations of TAT increased after vaccination (p<0.005) without differences between Arg353Gln genotypes (Fig. 3). TF activity was unchanged after vaccination (data not shown).

Plasma concentrations of IL-6 increased from 2 hrs to a maximum at 10 hrs after vaccination and had returned to baseline after 24 hrs (p<0.001) (data previously shown in reference 28). Plasma concentrations of FVIIa were higher at baseline in subjects homozygous for the G-allele compared with the C-allele of the −174 IL-6 polymorphism (p=0.02) (Fig. 4). There were no differences in the response to vaccination between the −174 G>C genotypes. Plasma levels of triglycerides increased significantly over the day and reached a maximum at 8 hrs after vaccination and had returned to baseline at 24 hrs (p<0.0001) (data not shown). The plasma concentrations of FFAs decreased significantly between 0 and 2 hrs with no change thereafter (p<0.01) (data not shown). There were no significant correlations between the responses to vaccination expressed as area under-the-curve of IL-6, triglycerides and FVIIa (data not shown).

**Discussion**

The main finding of the present study was an increase in plasma FVIIa concentration after experimental inflammation caused by vaccination. Furthermore, healthy subjects homozygous for the Arg-allele of codon 353 of the FVII gene had a stronger FVIIa response compared with subjects who were heterozygous. These findings are of special interest since it has recently been shown that infection might be a trigger of myocardial infarction (23). One possible link between infection and myocardial infarction might be activation of the coagulation cascade. It can be specu-
lated that an acute infection or inflammation increases the propensity for thrombus formation in subjects who are homozygous carriers of the Arg-allele in the event of plaque rupture with release of TF. Together with endothelial dysfunction this response could result in an acute myocardial infarction.

There is inconsistent evidence regarding the association between FVII and the risk of CHD or cardiovascular death (3–8). One reason for this discrepancy might be that FVII levels are well-balanced when blood sampling is performed at rest in subjects who are not infected or otherwise affected by an acute phase reaction. An acute infection or inflammatory reaction might provoke the haemostatic system resulting in activation of FVII, particularly in individuals with a certain genotype such as homozygotes for the Arg-allele. Accordingly, Girelli and coworkers (17) found that patients with CHD more often had a previous diagnosis of myocardial infarction if they were homozygous for the Arg-allele. Similarly, carriers of the Arg-allele undergoing percutaneous coronary intervention, a strong stimulus to inflammation, had an increased risk of periprocedural thrombotic complications (30). Further support for the relevance of infection and inflammation as a potential trigger of acute myocardial infarction comes from the fact that vaccination against Salmonella Typhii has been shown to cause endothelial dysfunction (26).

Several factors have been described that promote FVII activation, dependent or independent of TF (8–12, 31–33). The most likely factors to be involved in the present study are TF and triglycerides. Several studies have shown an increase in FVIIa after a fat-rich meal (31, 33, 34). However, the participants in the present study did not receive a fat-rich meal and TF activity was unchanged. The most probable mechanism for the rise in plasma triglyceride concentrations in our study is that inflammation caused by the vaccination, as indicated by the increased IL-6 levels, triggered the increase in plasma triglycerides. This interpretation is supported by previous studies showing that plasma triglycerides increase after lipopolysaccharide administration in mice and that patient with bacterial or viral infections have increased serum triglycerides levels (6, 35). Another possibility for activation of FVII, in the absence of increased plasma TF activity, that needs to be further examined is that TF activity might be expressed on the surface of endothelial cells or monocytes after vaccination.

The mechanism behind the effect of the Arg353Gln polymorphism on plasma FVII concentrations is not fully elucidated. A decreased FVII mass associated with the Gln-allele might be due to a conformational change produced by the Arg to Gln substitution that affects the processing of FVII in hepatocytes resulting in reduced secretion of the protein as shown in in vitro studies (16). The reason for the decreased FVII activation associated with the Gln-allele is not known but similarly the amino acid substitution might affect the interaction between FVII and activating factors. Accordingly, the Arg353Gln polymorphism has been observed to account for a large proportion of the variation of FVIIa concentration in plasma of women without (30 %) and with (24 %) CHD, whereas promoter polymorphisms mainly affect FVII mass (37).

One limitation of the study is the lack of control subjects. It could be speculated that the fluctuation in the ArgGln genotype group over the day might be caused by the diurnal variation which has been described (38). However, the increase in the ArgArg genotype group is unlikely to be explained by diurnal variation. Another limitation is the lack of consistency regarding thrombin generation measured as TAT. The vaccination activated TAT but the time course and lack of significant differences between Arg353Gln genotypes could not confirm that genotype-specific activation of FVII resulted in differences in thrombin generation. This needs to be addressed more closely in future studies. A third limitation is the size of the study and the fact that study subjects were selected according to –174 G>C IL-6 genotype. Although, the FVII and IL-6 polymorphisms are in linkage equilibrium, on separate chromosomes, some bias due to selection for IL-6 genotype could not be excluded. Therefore, the results need to be confirmed in a larger study of unselected healthy subjects.

Figure 3: Plasma concentrations of thrombin-antithrombin complex before and up to 24 hrs after vaccination in subjects grouped according to genotype of the Arg353Gln polymorphism. Values are means with 95 % confidence interval.

Figure 4: Plasma concentrations of activated coagulation factor VII before and up to 24 hrs after vaccination in subjects grouped according to genotype of the –174 G>C interleukin-6 polymorphism. Values are means with 95 % confidence interval.
Conclusion

The plasma FVIIa concentration increases after experimental inflammation in a genotype-specific manner. This is of interest from a mechanistic viewpoint, since one explanation for the link between infection and acute myocardial infarction might be activation of coagulation. However, there is a need for further studies of the role of infection and inflammation in haemostasis.

References