Endotoxin-Induced Activation of the Coagulation Cascade in Humans
Effect of Acetylsalicylic Acid and Acetaminophen


Abstract—During Gram-negative septic shock, lipopolysaccharide (LPS, endotoxin) induces tissue factor (TF) expression. TF expression is mediated by nuclear factor \( \kappa \)B and amplified by activated platelets. TF forms a highly procoagulant complex with activated coagulation factor VII (FVIIa). Hence, we hypothesized that aspirin, which inhibits LPS-induced, nuclear factor \( \kappa \)B–dependent TF expression in vitro and platelet activation in vivo, may suppress LPS-induced coagulation in humans. Therefore, we studied the effects of aspirin on systemic coagulation activation in the established and controlled setting of the human LPS model. Thirty healthy volunteers were challenged with LPS (4 ng/kg IV) after intake of either placebo or aspirin (1000 mg). Acetaminophen (1000 mg) was given to a third group to control for potential effects of antipyresis. Neither aspirin nor acetaminophen inhibited LPS-induced coagulation. However, LPS increased the percentage of circulating TF

monocytes by 2-fold. This increase was associated with a decrease in FVIIa levels, which reached a minimum of 50% 24 hours after LPS infusion. Furthermore, LPS-induced thrombin generation increased plasma levels of circulating polymerized, but not cross-linked, fibrin (ie, thrombus precursor protein), whereas levels of soluble fibrin were unaffected. In summary, a single 1000-mg dose of aspirin did not decrease LPS-induced coagulation. However, our study showed, for the first time, that LPS increases TF

monocytes, substantially decreases FVIIa levels, and enhances plasma levels of thrombus precursor protein, which may be a useful marker of fibrin formation in humans. (Arterioscler Thromb Vasc Biol. 1999;19:2517-2523.)

Key Words: acetylsalicylic acid ■ acetaminophen ■ lipopolysaccharide ■ tissue factor ■ factor VIIa ■ coagulation

Disseminated intravascular coagulation (DIC) is a frequently encountered complication of sepsis and is triggered in part by endotoxin (lipopolysaccharide [LPS]). DIC is defined by enhanced activity of coagulation factors, exhaustion of endogenous coagulation inhibitors, and activation and depletion of platelets by formation of thrombi in the vasculature. Results of several studies suggest that initiation of coagulation in sepsis is driven primarily by the tissue factor (TF)–dependent pathway of coagulation.

Inhibition of the TF-dependent activation of coagulation using antibodies against TF or activated factor VII (FVIIa) completely prevents LPS-induced activation of coagulation in animals. Thus, inhibition of TF expression on monocytes during endotoxia may present a promising therapeutic option for dampening the coagulation cascade. A recent in vitro study showed that acetylsalicylic acid (ASA) reduces LPS-induced TF expression on isolated monocytes. Conversely, whole-blood experiments showed that ASA enhanced TF activity on monocytes. Furthermore, TF expression on human monocytes is enhanced by the presence of granulocytes and activated platelets, suggesting an even more complex regulation of TF in vivo. In addition, results of animal studies suggest that inhibition of thromboxane A\(_2\)–mediated platelet activation may improve DIC induced by LPS.

Besides in vitro studies and experiments in animals, infusion of small doses of LPS into humans has emerged as a valuable tool to explore the pathogenesis and new treatment options of LPS-induced coagulation activation. Thus, we used, for the first time, a human LPS model to clarify, in a properly controlled setting, the influence of ASA on LPS-induced TF expression and coagulation activation in vivo. We hypothesized that ASA could block TF-mediated activation of coagulation by 2 mechanisms in vivo, direct inhibition of TF expression on monocytes and its well-known inhibition of platelets.

Anticipating that the maximal TF expression on monocytes might coincide with the well-known LPS-induced monocytopenia and that monocytes may not be eligible for flow cytometric analysis, we focused the study on established

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markers of coagulation events downstream from TF. Thus, we defined a priori the increase of prothrombin fragment F1+2 after LPS and its inhibition by ASA as the primary end point of our investigation. Furthermore, we used this model, for the first time, to characterize the effects of endotoxin on the changes of plasma levels of FVIIa and new markers of fibrin generation, namely soluble fibrin and thrombus precursor protein (Ttp), which represents polymerized fibrin oligomers.

Methods

Study Design

The study was designed as a randomized, double-blind, placebo-controlled trial in 3 parallel groups of 30 healthy men (n=10 per group). The study was approved by the Institutional Ethics Committee of Vienna University Medical School. The effects of aspirin and acetylsalicylic acid on activation of the coagulation cascade (reported earlier19) were assessed concomitantly. Written informed consent was obtained from all subjects before enrollment in the study.

Subjects

Mean age of subjects was 27.1±5 years (±SD), and mean body mass index was 23.7±2.2 kg/m². Health status was determined by using a battery of laboratory and clinical tests, including medical history; physical examination; hematological, biochemical, virological, and drug screening; and a detailed family history of thrombotic disorders, as previously described.20 Exclusion criteria were hypersensitivity to aspirin or acetylsalicylic acid and regular or recent intake of medication, including over-the-counter medication.

Study Protocol

Subjects were admitted to the study ward at 8:00 AM after an overnight fast. Throughout the entire study period, they were confined to bed rest and fasted for 8.5 hours after endotoxin infusion. A 5% glucose infusion (Leopold Pharma) was started at 8:15 AM and continued for 8.5 hours at a rate of 3 mL/kg·h to ensure adequate blood glucose levels and urinary output. After baseline blood samples were drawn, subjects were randomly assigned to receive placebo, 1000 mg of acetylsalicylic acid (Paracetamol Genericon Pharma), or 1000 mg of ASA (ASS Genericon) at 8:15 AM. Thirty minutes later, they received 4 ng/kg LPS (national reference endotoxin, Escherichia coli, US Pharmacopeial Convention Inc) as an intravenous infusion over 1 to 2 minutes.

Vital parameters (ECG, heart rate, oxygen saturation, and blood pressure) were recorded before and 1.5, 6, and 24 hours after administration of aspirin.

Sampling and Analysis

Blood samples for coagulation and fibrinolysis parameters were collected by venipuncture before drug administration (baseline) and 1, 1.5, 2, 3, 4, 8, and 24 hours after administration of endotoxin, applying minimal venostasis, into Vacutainer tubes (Becton Dickenson) containing 0.129 mol/L sodium citrate (1 vol anticoagulant and 9 vol whole blood). Citrated plasma samples were processed immediately by centrifugation at 2000g at 4°C for 15 minutes and stored at −30°C for <8 weeks before analysis.

The following commercially available assays were used: whole blood factor assay (American Diagnostica); FVIIa (Staclot VII-rTF 1-step clotting assay, Diagnostica Stago; normal range, 28 to 113 mU/mL); factor VIlc (FVIIc; Diagnostica Stago; normal range, 60% to 180%); factor VII a (FVIIa; Asserachrom VII:Ag, Diagnostica Stago; normal range, 76% to 123%); prothrombin fragment F1+2 (Behring; normal value, <1.9 µmol/mL); polymers of soluble fibrin, ie, Ttp (American Biogenetic Sciences), which shows no cross-reactivity with D-dimer in vitro (data not shown); soluble fibrin (Chromogenix AB; normal value, <6 µg/mL); and the fibrin-split product D-dimer (Boehringer Mannheim; normal value, <400 ng/mL). Data for soluble fibrin are expressed in soluble fibrin units (SF; normal range, 25 to 75 SF).23 Fibrinogen (fibrinogen reagent, Immuno AG; normal range, 180 to 350 mg/dL), antithrombin activity (STA Antithrombin, Diagnostica Stago; normal range, 75% to 125%), and protein C activity (Chromogenix; normal range, 65% to 130%) were determined by using the STA analyzer (Diagnostica Stago).

Blood Cell Counts, Flow Cytometry, and Albumin Levels

Differential blood counts and hematocrit values were determined with a cell counter (Sysmex, Toa-Medical Electronics). However, blood smears revealed that monocyte counts determined with this counter 1.5 and 6 hours after endotoxin administration were spuriously high. Hence, monocyte counts were calculated from scatter histograms obtained with a flow cytometer (Becton Dickinson), and only flow cytometry results are presented for monocytes. Flow cytometry was performed by analyzing 30 000 gated events as previously described.24 Because all samples required immediate processing to avoid artificial activation of leukocytes or platelets, leukocytes were stained only before and 1.5, 6, and 24 hours after endotoxin administration. Staining of TF on monocytes and neutrophils was assessed using FITC-coupled antibodies (American Diagnostics) as previously described.24

In Vitro Whole-Blood Experiments

Citrated whole-blood samples (n=10) were assayed for TF⁺ monocytes and TF⁺ granulocytes before and after 2 hours of incubation as described above for the in vivo experiments. Incubated samples (500 µL/well) were kept at 37°C in 5% CO₂ in 12-well flasks (Costar) with or without 50 µg/mL LPS or LPS together with sodium salicylate (Sigma Chemical Co) adjusted to final concentrations of 0.2, 2, and 10 mmol/L·L⁻¹.

The concentration of LPS was chosen because a similar peak concentration of LPS could be expected in our in vivo experiments, assuming that the initial volume of distribution of LPS IV equals blood volume in circulation. Similarly, the lowest dose in vitro concentration of sodium salicylate was selected to parallel plasma levels of salicylates measured in our in vivo trial after administration of 1000 mg of ASA. The higher concentrations of sodium salicylate were chosen to allow comparisons with results from a previous in vitro trial.9

Data Analysis

Data are expressed as the mean and 95% confidence interval (CI). Because data were nonnormally distributed, all comparisons were made by using nonparametric statistics. For statistical comparisons within groups, Friedman ANOVA and the Wilcoxon signed ranks test for post hoc comparisons were used. For comparisons between groups, Kruskal-Wallis ANOVA and the Mann-Whitney U test were applied.

Because hematocrit and albumin levels changed by <6.5% during the entire study period in all subjects, impairment of vascular permeability was considered unlikely under our experimental conditions (data not shown). Hemodilution of this order of magnitude has been observed in healthy subjects without LPS challenge.25 Hence, hemostatic parameters were not corrected for hemodilution.

Results

Baseline data for the 3 treatment groups are presented in the Table. Coagulation parameters of all study subjects were similar in the 3 groups at baseline with the exception of 25% lower values for FVIIa in the aspirin group (P=0.045, placebo group versus aspirin group). Salicylate concentrations in plasma averaged 0.21 mmol/L·L⁻¹ (95% CI, 0.13 to 0.28) at 1 hour and were maintained at that level until 3 hours (mean, 0.22 mmol/L·L⁻¹; 95% CI, 0.16 to 0.27) after administration of aspirin.
Changes in Differential White Blood Cell Counts and Platelet Counts

The time course of leukocytes and monocytes is shown in Figure 1. Neither ASA nor acetaminophen influenced LPS-induced effects on white blood cells, as shown by the nearly identical curves in the treatment groups (Figure 1). After an initial drop, leukocyte counts increased 3-fold over baseline values by 6 hours (10.6 G/L; 95% CI, 9.6 to 11.6; P<0.05, first significant change versus baseline). After 24 hours, platelet counts were, on average, 12% lower than at baseline (P<0.05 versus baseline; data not shown).

Tissue Factor Positivity on Monocytes and Neutrophils and Soluble TF

Mean percentage of TF expression on monocytes and mean fluorescence intensity (MFI) at baseline are presented in the Table. Because of monocytopenia, TF expression on monocytes could not be evaluated at 1.5 hours, and because of the low numbers of circulating monocytes in half of the subjects (n=16) at 6 hours after LPS infusion, data for this time point are pooled. Whereas MFI in monocytes did not change (data not shown), TF⁺ monocytes in subjects eligible for flow cytometry (n=15) increased 2-fold to 7.8% (95% CI, 5.6 to 10.0; P<0.01; Figure 1) at 6 hours. After 24 hours, monocyte counts returned to baseline levels (Figure 1). TF⁺ neutrophils increased in a trendwise manner by ≈1% (P=0.056, Friedman ANOVA; Figure 1). Levels of circulating TF exhibited greater variability after LPS administration in all groups without differences versus baseline values or between groups (P>0.05; data not shown).

In Vitro Effects of Sodium Salicylate and LPS on TF⁺ Leukocytes in Whole-Blood Experiments

Results from in vitro studies on TF positivity of monocytes and neutrophils are presented in Figure 2. TF⁺ monocytes averaged 11% (95% CI, 4% to 18%) at baseline and increased more than 3-fold after 2 hours in culture (Figure 2). Addition of 50 pg/mL LPS further enhanced TF⁺ monocytes (P=0.005 versus 2 hours of culture without LPS). Incubation of whole-blood specimens with LPS and sodium salicylate resulted in a dose-dependent increase in the percentage of TF⁺ monocytes, but this increase did not reach statistical significance (P=0.13 versus LPS alone; Figure 2). MFI for TF at baseline averaged 34 (95% CI, 30 to 39) and increased to 50 (95% CI, 38 to 63; P=0.028 versus baseline) after 2 hours of culture with LPS. Addition of sodium salicylate did not alter MFI for baseline; P>0.05 between groups). After 24 hours, platelet counts were, on average, 12% lower than at baseline (P<0.05 versus baseline; data not shown).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo (n=10)</th>
<th>Acetaminophen (n=10)</th>
<th>Aspirin (n=10)</th>
</tr>
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<tbody>
<tr>
<td>Neutrophils, 10⁷/L</td>
<td>3.3 (2.4–4.3)</td>
<td>3.1 (2.5–3.6)</td>
<td>3.5 (2.7–4.3)</td>
</tr>
<tr>
<td>Monocyte counts, 10⁷/L</td>
<td>0.46 (0.39–0.62)</td>
<td>0.39 (0.26–0.54)</td>
<td>0.44 (0.36–0.48)</td>
</tr>
<tr>
<td>Platelet counts, 10⁹/L</td>
<td>215 (187–243)</td>
<td>242 (212–272)</td>
<td>244 (201–286)</td>
</tr>
<tr>
<td>TF⁺ monocytes, %</td>
<td>3.2 (1.9–4.5)</td>
<td>3.3 (1.7–4.9)</td>
<td>2.7 (1.5–4.0)</td>
</tr>
<tr>
<td>MFI of TF⁺ monocytes</td>
<td>36 (22–50)</td>
<td>34 (30–39)</td>
<td>35 (31–40)</td>
</tr>
<tr>
<td>Soluble TF, pg/mL</td>
<td>36 (28–44)</td>
<td>35 (23–48)</td>
<td>48 (33–63)</td>
</tr>
<tr>
<td>FVIIa, nM</td>
<td>50 (36–64)</td>
<td>53 (38–66)</td>
<td>33 (22–43)</td>
</tr>
<tr>
<td>FVIIc, %</td>
<td>87 (71–114)</td>
<td>88 (71–104)</td>
<td>71 (61–104)</td>
</tr>
<tr>
<td>FVIIa, mU/mL</td>
<td>0.55 (0.45–0.85)</td>
<td>0.45 (0.3–0.6)</td>
<td>0.53 (0.43–0.63)</td>
</tr>
<tr>
<td>TpP, µg/mL</td>
<td>2.3 (1.1–3.5)</td>
<td>3.6 (0.6–6.5)</td>
<td>1.7 (0.6–2.8)</td>
</tr>
<tr>
<td>Soluble fibrin, arbitrary units</td>
<td>60 (45–75)</td>
<td>70 (49–90)</td>
<td>62 (37–87)</td>
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<tr>
<td>D-dimer, ng/mL</td>
<td>285 (242–329)</td>
<td>361 (236–486)</td>
<td>247 (201–293)</td>
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<tr>
<td>Fibrinogen, mg/dL</td>
<td>209 (184–233)</td>
<td>228 (200–255)</td>
<td>189 (174–204)</td>
</tr>
<tr>
<td>Protein C, %</td>
<td>86 (78–96)</td>
<td>90 (81–100)</td>
<td>101 (78–124)</td>
</tr>
<tr>
<td>Anti-thrombin III, %</td>
<td>91 (87–96)</td>
<td>89 (83–95)</td>
<td>89 (83–95)</td>
</tr>
</tbody>
</table>

Figure 1. Monocyte counts (mean ± SEM, below y axis break, top) and granulocyte counts (above y axis break, top) before and after infusion of LPS (4 mg/kg of body weight) in humans after oral premedication with placebo (●), 1000 mg of acetaminophen (○), and 1000 mg of aspirin (■) in 3 groups (n=10 per group). Percentages of monocytes (●) and granulocytes (■) staining for anti-TF antibody at baseline and 1.5, 6, and 24 hours after LPS infusion (bottom; pooled data from all 3 groups, n=16; *P<0.05, first significant change versus baseline). Because of profound monocytopenia at 1.5 hours, no monocytes were detectable.
TF of monocytes at any concentration tested ($P>0.05$; data not shown).

Furthermore, the number of TF$^+$ neutrophils was consistent with that observed in our in vivo experiments at baseline (1.6%; 95% CI, 1.1% to 2.2%; Figure 2) and increased to 18% after 2 hours of culture (95% CI, 10 to 26; $P=0.005$ versus baseline). Addition of LPS increased TF$^+$ neutrophils by $\approx 30\%$ ($P=0.005$ versus baseline; $P=0.06$ versus 2 hours of culture without LPS), and this LPS-mediated increase was not affected by sodium salicylate at any concentration (Figure 2). MFI for TF on neutrophils at baseline was 34 (95% CI, 30 to 39) and did not change throughout the entire experiment (data not shown).

**FVIIa, FVIIc, and FVII:Ag**

FVIIa levels decreased in all groups at 3 hours after LPS administration and reached minimum levels of 50% at 24 hours ($P<0.046$ for all groups; Figure 3). Interpretation of these data is compromised by the fact that baseline values for FVIIa were $\approx 20\%$ lower in the aspirin group ($P=0.045$ versus placebo; $P=0.08$ versus acetaminophen). Decreases of FVIIa were somewhat less pronounced in this group. Changes of FVIIa were mirrored by reductions of FVIIc levels of the same order of magnitude at 8 and 24 hours after LPS administration ($P<0.018$ in all groups versus baseline; $P>0.05$ between groups). FVII:Ag levels remained unchanged after LPS infusion in all groups until 8 hours but were decreased by $\approx 25\%$ at 24 hours ($P<0.012$ versus baseline in all groups; $P>0.05$ between groups; Figure 3).

**Prothrombin Fragment F$_{1+2}$**

Levels of F$_{1+2}$ increased almost 20-fold within 2 to 3 hours after LPS injection with no differences between groups. F$_{1+2}$ values remained elevated over baseline until 8 hours ($P<0.02$ for comparisons versus baseline; Figure 4) but returned to baseline at 24 hours after LPS administration ($P>0.05$ versus baseline).

**Fibrinogen, TpP, Soluble Fibrin, and D-Dimer**

Fibrinogen levels increased by $\approx 30\%$ in all groups at 24 hours ($P<0.017$ versus baseline in all groups; data not shown). Plasma levels of TpP increased significantly (at least 6-fold) in all groups at 3 hours and remained elevated for 24 hours after LPS infusion (Figure 4). In contrast, plasma levels of soluble fibrin increased in a trendwise manner by only $\approx 30\%$ in all groups, reaching levels of significance only at 4 hours in the placebo group and at 8 hours in the aspirin group (data not shown). D-dimer plasma levels increased 5- to 10-fold in all groups (Figure 4).

**Protein C and Antithrombin III**

Plasma levels of protein C decreased by $\approx 15\%$ at 8 and 24 hours after LPS infusion ($P<0.027$ at 8 and 24 hours in all groups; data not shown). Variations of antithrombin III levels did not exceed 5% in all groups ($P>0.05$ versus baseline and for comparisons between groups; data not shown).

**Discussion**

LPS is one trigger of disseminated intravascular coagulation during sepsis, inducing TF expression on monocytes and endothelial cells. This LPS-induced TF expression is amplified by the presence of activated platelets in vitro, emphasi-
ing the close interaction between coagulation factors and platelets.\textsuperscript{13,14,26}

Results of animal studies suggest that inhibition of TF activity abolishes thrombin formation in endotoxemia.\textsuperscript{3} Accordingly, inhibition of TF expression might represent the most upstream and hence most specific anticoagulatory intervention during endotoxemia.

Recent studies have produced conflicting results, ie, that ASA either inhibited or enhanced LPS-induced TF expression on monocytes in vitro.\textsuperscript{9–11} Although toxic concentrations of ASA and salicylate have been shown to inhibit LPS-stimulated TF expression on isolated monocytes,\textsuperscript{9} experiments in whole blood have demonstrated that ASA at substantially lower doses stimulates TF expression.\textsuperscript{10}

Attempting to address these controversial findings and to put them into a clinically relevant context, we studied whether 1000 mg of ASA would inhibit TF expression and subsequent generation of thrombin in vivo. The human LPS model, which resembles the early phase of sepsis-induced DIC, was chosen because it allows investigation of the complex interplay between coagulation, platelets, leukocytes, and endothelium within human vasculature. We hypothesized that inhibition of TF expression by ASA may occur by a dual mode of action in vivo, first, by inhibiting NF-kB–mediated TF expression\textsuperscript{9} and, second, by inhibiting platelet activation.

However, in contrast to our hypothesis, aspirin did not inhibit thrombin generation in this model. The lack of an effect of ASA on thrombin generation was evidenced by a 10-fold increase of $F_{1,2}$, an established indicator of the conversion of prothrombin to thrombin (Figure 4). The lack of an effect of ASA on the increase in thrombin formation was consistent with the lack of effect of ASA on the upstream coagulation factor $FVII_{II}$ (Figure 3).

Interest in $FVII_{II}$ has risen recently because elevated $FVII_{II}$ levels are associated with chronic activation of coagulation.\textsuperscript{27–29} It therefore seemed reasonable to expect an increase in plasma levels of $FVII_{II}$ during the initial phase of LPS-induced coagulation. Interestingly, activation of coagulation by LPS decreased $FVII_{II}$ as early as 3 hours in the placebo group, and $FVII_{II}$ further declined to 50% of baseline levels at 24 hours (Figure 3). However, interpretation of this observation is compromised by the fact that baseline $FVII_{II}$ levels were 20% lower in the aspirin group than in the other groups. Furthermore, this decline of $FVII_{II}$ was attenuated in subjects treated with aspirin, whereas $FVII_{II}$ levels were similar in all groups at later times. In the absence of an effect on thrombin generation, this intergroup difference cannot be regarded as an effect of aspirin on $FVII_{II}$.

Although it is puzzling that TF-induced coagulation during endotoxemia is not reflected by an increase in $FVII_{II}$ levels, our data are consistent with the decreased $FVII_{II}$ levels found in a study of septic patients.\textsuperscript{30} Of note is that low $FVII_{II}$ levels were predictive of mortality in this trial, but interpretation of these data is difficult because many of the patients who died had liver failure. Our data from the LPS model extend these findings to healthy subjects with normal liver function and hence normal hepatic synthesis of $FVII$. It also seems that the 50% decrease in $FVII_{II}$ is disproportionately high when compared with the 25% decline in $FVII_{II}$:Ag. This finding could be explained by firm binding of $FVII_{II}$ to TF or increased turnover of $FVII_{II}$. This notion is supported by the in vitro observation that even physiological antithrombin concentrations inactivate $FVII_{II}$, provided that $FVII_{II}$ is complexed to membrane-bound TF.\textsuperscript{31,32} Thus, it could be speculated that the decline of $FVII_{II}$ indirectly reflects increased in vivo TF expression on monocytes and/or endothelial cells.

In addition, we showed directly an increase in TF expression on monocytes by flow cytometry in subjects who had recovered from profound LPS-induced monocytopenia at 6 hours. This is in line with the observation that TF expression on monocytes was increased in septic patients, particularly those who later died.\textsuperscript{33} However, the anticipated monocytopenia at 6 hours coincided with the expected maximum of TF expression, so the number of eligible subjects (ie, those with monocyte counts >3% of white blood cells; $n=16$) did not provide sufficient power to determine whether aspirin had an effect on LPS-induced TF expression. Although we were forced to pool data from all 3 groups because 50% of our study subjects exhibited monocytopenia, this is the first in vivo demonstration of an increase of TF$^+$ monocytes in humans exposed to LPS.

Could the lack of an effect of ASA on TF-mediated coagulation in vivo be caused by an inadequately low dose of ASA? To address this question, we performed a series of whole-blood experiments ($n=10$) using approximately the same concentration of LPS and salicylate as in our in vivo trial. In addition, 2 higher doses of salicylate were used to allow comparisons with the conflicting in vitro data from Osnes et al\textsuperscript{9} and Osterud et al.\textsuperscript{10} Our findings show that 0.2 mmol/L sodium salicylate, which resembles the plasma levels of salicylate attained in our subjects, does not affect LPS-induced TF expression on monocytes in humans. On the contrary, the higher concentrations of salicylate used in our in vitro experiments (ie, 2 and 10 mmol/L) resulted in a dose-dependent increase in TF$^+$ monocytes compared with incubation with LPS alone, although this increase did not reach statistical significance ($P=0.13$; Figure 2). Still, the 25% higher TF expression found after incubation with 10 mmol/L salicylate is in line with results of in vitro experiments conducted by Osterud et al\textsuperscript{10} and shows that only toxic ASA concentrations would be expected to cause this effect in humans. When our in vivo findings are compared with those from the in vitro study of Osnes et al,\textsuperscript{9} it is noteworthy that Osnes et al used a 30-fold higher LPS stimulus and that the 50% reduction of TF expression on monocytes was demonstrated with 1.5 mmol/L sodium salicylate (ie, 8 times higher concentration than achieved in our trial). However, similar concentrations of salicylate in plasma are considered toxic in humans.\textsuperscript{34} Our results suggest that the in vitro findings of an effect of ASA on TF expression may have only limited relevance in the in vivo situation of our human LPS model.

Surprisingly, a small percentage of neutrophils exhibited binding of TF antibodies, and this percentage of TF$^+$ neutrophils increased in a trendwise manner after LPS infusion (Figure 1). It is currently assumed that neutrophils do not express TF in relevant amounts.\textsuperscript{35} Hence, this finding could be explained by the binding of soluble TF to neutrophils by an as-yet unidentified receptor. This increase in TF$^+$ neutrophils could also be reproduced by our in vitro experiments when whole blood was incubated with LPS concentrations that could also be reached after administration of the LPS bolus in our in vivo study (Figure 2). Still, the biological...
relevance of a small increase in the percentage of TF\textsuperscript{+} neutrophils is far from resolved.

Like the upstream markers of coagulation activation (ie, TF and FVIIa), coagulation factors downstream from thrombin, particularly the circulating polymerized fibrin molecules called TpP,\textsuperscript{36} were also unaffected by ASA. TpP increased in parallel with F\textsubscript{1+2} and peaked \( \approx 10 \text{-fold} \) over baseline levels at 8 hours (Figure 4). Soluble fibrin, which was determined for the first time in this model, did not increase after infusion of LPS. Differences in the sensitivity of both assays may account for this discrepancy. Alternatively, the lack of increased levels of soluble fibrin despite increased levels of TpP could be explained by the higher reactivity of soluble fibrin and its spontaneous tendency to precipitate in the vessels (ie, its low solubility). Interestingly, elevated D-dimer levels were detected concomitantly with the increase in F\textsubscript{1+2} and TpP, indicating that onset of fibrin generation and fibrin dissolution occur almost in parallel. Whereas thrombin generation returned to baseline levels at 24 hours (Figure 4), the persistent elevation of TpP, similar to that of D-dimer, may indicate the stability of this intermediate of fibrin generation (Figure 4).

We compared aspirin with acetaminophen given to a third group of subjects because we could not exclude that hyperthermia per se has an activating effect on coagulation. Hence, we studied whether acetaminophen, a drug with documented antipyretic activity but no effects on peripheral cyclooxygenase activity or platelet function, might have a dampening effect on coagulation during endotoxemia. Our findings indicate that decreasing hyperthermia by acetaminophen had no impact on activation coagulation (Figures 1 to 4). This is of interest in view of the widespread use of acetaminophen and aspirin to treat fever in the clinical setting.

In summary, our study showed that a single 1000-mg dose of ASA has no influence on LPS-induced activation of the coagulation cascade, fibrin formation, or fibrinolysis. Similarly, amelioration of fever by acetaminophen had no effect. The results also indicate that LPS increases TF\textsuperscript{+} expression on circulating monocytes in vivo but decreases FVIIa levels. The resultant thrombin formation increased TpP levels log-fold, similar to the increase in D-dimer levels. Thus, TpP may become a useful coagulation marker during endotoxemia.

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References


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