In vivo signal transduction: mitogen activated protein kinases in inflammation
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Inhibition of coagulation, fibrinolysis and endothelial cell activation by a p38 Mitogen-Activated Protein kinase inhibitor during human endotoxemia
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Abstract

P38 mitogen-activated protein kinase (MAPK) is an important component of intracellular signaling cascades that initiate various inflammatory cellular responses. To determine the role of p38 MAPK in the procoagulant response to lipopolysaccharide (LPS), 24 healthy subjects were exposed to an intravenous dose of LPS (4 ng/kg), preceded 3 hours earlier by orally administered 600 or 50 mg BIRB 796 BS (a specific p38 MAPK inhibitor), or placebo. High dose of BIRB 796 BS strongly inhibited LPS-induced coagulation activation, as measured by the plasma concentrations of the prothrombin fragment F1+2. BIRB 796 BS also dose dependently attenuated the activation and subsequent inhibition of the fibrinolytic system (plasma tissue type plasminogen activator, plasmin-α2-antiplasmin complexes, plasminogen activator inhibitor type I), and endothelial cell activation (plasma soluble E-selectin and von Willebrand factor). Activation of p38 MAPK plays an important role in the procoagulant and endothelial cell response after in vivo exposure to LPS.
Introduction

Activation of the coagulation system is an important manifestation of the systemic inflammatory response of the host to infection\textsuperscript{160}. Several in vivo models have been used to dissect the molecular mechanisms that contribute to coagulation activation by bacteria and bacterial products, including intravenous injection of low dose lipopolysaccharide (LPS) into healthy humans\textsuperscript{4}. Tissue factor, a glycoprotein expressed on endothelial cells and monocytes upon stimulation, plays an essential role in coagulation activation induced by LPS or bacteria in vivo, as demonstrated by abolishment of this response by inhibitors of tissue factor\textsuperscript{55,161-163}.

Mitogen-activated protein kinases (MAPKs) participate in intracellular signaling cascades that mediate inflammatory responses to infectious and noninfectious stimuli\textsuperscript{6,114}. Recent in vitro studies have implicated one of these kinases, p38 MAPK, in the regulation of tissue factor expression on monocytes\textsuperscript{57}, endothelial cells\textsuperscript{164,165}, and smooth muscle cells\textsuperscript{166}. Knowledge of the role of p38 MAPK in activation of coagulation in vivo is not available. This prompted us to evaluate the effect of BIRB 796 BS, a specific and potent inhibitor of p38 MAPK\textsuperscript{118}, on the procoagulant response during human endotoxemia.

Materials and Methods

Design

This study was performed as a randomized, double-blind, placebo-controlled experiment and was performed simultaneously with a study examining the effect of BIRB 796 BS on LPS-induced cytokine release and neutrophil activation\textsuperscript{152}. Briefly, LPS (from \textit{E.coli}, lot G; United States Pharmacopeial Convention, Rockville, MD; 4 ng/kg body weight) was administered as a bolus intravenous injection to 24 healthy male volunteers (mean age 22 years, range 19-29). Three hours prior to infusion of LPS, the p38 MAPK inhibitor BIRB 796 BS (600 mg n=8, 50 mg n=8) or placebo (n=8) was administered orally. BIRB 796 BS is a highly selective and potent p38 MAPK inhibitor developed by Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT\textsuperscript{118}. Blood samples were obtained before administration of BIRB 796 BS or placebo (t = -3 h), directly before LPS administration (t = 0 h), and at several time points up to 24 hours thereafter.
Assays

Blood was collected in siliconized vacutainer tubes (Becton Dickinson, Plymouth, England) containing 0.105 M sodium citrate in a 1:9 (v/v) anticoagulant:blood ratio. ELISA’s were performed according to the instructions of the manufacturers: prothrombin fragment F1+2 (Dade Behring, Marburg GmbH, Germany), tissue-type plasminogen activator (tPA)(Asserachrom tPA, Diagnostic Stago, Asnieres-sur-Seine, France), plasminogen activator inhibitor type 1 (PAI-1) (Monozyme, Charlottelund, Denmark), Von Willebrand Factor (vWF) (Dakopatts, Älvsjö, Sweden), and soluble E-selectin (sE-selectin)(Diaclone, Besancon Cedex, France); plasmin-α2-antiplasmin complexes (PAPc) were determined by a radio immunoassay as described elsewhere\textsuperscript{167}.

Statistical analysis

All values are given as means ± SEM. Differences in results between the 3 treatment groups were tested by repeated measurements analysis of variance. A $P$-value < 0.05 was considered to represent a statistically significant difference.

Results and discussion

We recently demonstrated that oral administration of BIRB 796 BS inhibited LPS-induced p38 MAPK activation in the leukocyte fraction of blood of healthy humans \textit{in vivo}, which was associated with a profound reduction in the release of both pro- and anti-inflammatory cytokines, and in neutrophil activation\textsuperscript{152}. In the present study we sought to determine the role of p38 MAPK in the activation of the hemostatic mechanism \textit{in vivo}.

Intravenous injection of LPS was associated with activation of the coagulation system, as reflected by a rise in the plasma concentrations of the prothrombin fragment F1+2, reaching a plateau between 3 and 6 h after LPS administration (Figure 1). High dose, but not low dose, BIRB 796 BS inhibited LPS-induced coagulation activation, both delaying and diminishing the increase in plasma F1+2 concentrations ($P < 0.05$ vs placebo). The most likely
p38 MAPK mediates the procoagulant response to endotoxin

explanation for the anticoagulant effect of BIRB 796 BS is inhibition of tissue factor expression. Indeed, tissue factor is essential for activation of the coagulation system in this model of low grade endotoxemia, and p38 MAPK inhibition markedly diminished LPS-induced tissue factor expression by monocyctic cells in vitro. Moreover, p38 MAPK activation also mediates tissue factor expression by endothelial cells stimulated with thrombin or vascular endothelial growth factor, and by vascular smooth muscle cells stimulated with thrombin. Furthermore, and not mutually exclusive, the reduced IL-6 concentrations in subjects treated with BIRB 796 BS may have contributed to the attenuated coagulant response, considering that IL-6 is an important mediator of coagulation activation by LPS in vivo.

Figure 1. BIRB 796 BS dose-dependently inhibits LPS-induced coagulation and fibrinolysis. Subjects received an i.v. injection of LPS (4 ng/kg) at 0 h, preceded by oral ingestion of placebo (○), 50 mg BIRB 796 BS (●), or 600 mg BIRB 796 BS (▲) at -3 h. Data are the mean ± SEM. High dose BIRB 796 BS inhibited all parameters shown (all P < 0.05 vs placebo). Low dose BIRB 796 BS inhibited tPA and PAPc (P < 0.05 vs placebo).
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BIRB 796 BS had an even stronger inhibitory effect on activation of the fibrinolytic system (Figure 1). The LPS-induced increases in the plasma levels of tPA and PAPc were dose-dependently reduced by BIRB 796 BS (both low and high dose P < 0.05 vs placebo), and almost completely prevented by the high dose of the p38 MAPK inhibitor. High dose BIRB 796 BS also modestly inhibited the release of PAI-1 (P < 0.05 vs placebo). Knowledge of the role of p38 MAPK in the production and release of fibrinolytic mediators is highly limited. The production of PAI-1 by bovine aortic endothelial cells exposed to hypoxia was not dependent on p38 MAPK. Conceivably, the strong inhibition of tumor necrosis factor (TNF)-α release by BIRB 796 BS at least in part is responsible for the observed reduction in tPA and PAPc levels, considering that neutralization of TNF-α in this model virtually completely prevented these responses. However, other mechanisms likely are involved since anti-TNF-α strategies also strongly inhibited LPS-induced PAI-1 release.

![Figure 2. BIRB 796 BS dose-dependently inhibits LPS-induced endothelial cell activation. Subjects received an i.v. injection of LPS (4 ng/kg) at 0 h, preceded by oral ingestion of placebo (○), 50 mg BIRB 796 BS (●), or 600 mg BIRB 796 BS (▲) at -3 h. Data are the mean ± SEM. High dose BIRB 796 BS inhibited both vWF and sE-selectin (P < 0.05 vs placebo).](image)

We also were interested to establish the effect of BIRB 769 BS on endothelial cell activation, reflected by the plasma concentrations of sE-selectin and vWF. Administration of LPS elicited profound increases in the plasma levels of sE-selectin and vWF, which were attenuated by high dose BIRB 796 BS (both P < 0.05 vs placebo; Figure 2). P38 MAPK may play a direct role in signaling inflammatory effects into the interior of the vascular endothelium, including the expression of E-selectin on stimulated endothelial cells. Such direct effects, in combination with inhibition of TNF-α release, may well explain the effect of BIRB 796 BS on the secretion of sE-selectin and vWF.
We here demonstrate a role for p38 MAPK in activation of coagulation, fibrinolysis and the vascular endothelium during human endotoxemia. These findings extend earlier observations of the inhibitory effect of BIRB 796 BS and another oral p38 MAPK inhibitor on activation of the cytokine network and neutrophils\textsuperscript{152,173}, and illustrate the broad role of this kinase in inflammation \textit{in vivo}. 