Inhibition of p38 Mitogen-Activated Protein Kinase Reduces Inflammation After Coronary Vascular Injury in Humans

Lea Sarov-Blat, John M. Morgan, Pedro Fernandez, Rachel James, Zixing Fang, Mark R. Hurle, Charlotte Baidoo, Robert N. Willette, John J. Lepore, Svend E. Jensen, Dennis L. Sprecher

Objective — To evaluate whether a p38α/β mitogen-activated protein kinase inhibitor, SB-681323, would limit the elevation of an inflammatory marker, high-sensitivity C-reactive protein (hsCRP), after a percutaneous coronary intervention (PCI).

Methods and Results — Coronary artery stents provide benefit by maintaining lumen patency but may incur vascular trauma and inflammation, leading to myocardial damage. A key mediator for such stress signaling is p38 mitogen-activated protein kinase. Patients with angiographically documented coronary artery disease receiving stable statin therapy and about to undergo PCI were randomly selected to receive SB-681323, 7.5 mg (n = 46), or placebo (n = 46) daily for 28 days, starting 3 days before PCI. On day 3, before PCI, hsCRP was decreased in the SB-681323 group relative to the placebo group (29% lower; \( P = 0.02 \)). After PCI, there was a statistically significant attenuation in the increase in hsCRP in the SB-681323 group relative to the placebo group (37% lower on day 5 \( P = 0.04 \); and 40% lower on day 28 \( P = 0.003 \)). There were no adverse safety signals after 28 days of treatment with SB-681323.

Conclusion — In the setting of statin therapy, SB-681323 significantly attenuated the post-PCI inflammatory response, as measured by hsCRP. This inflammatory dampening implicates p38 mitogen-activated protein kinase in the poststenotic response, potentially defining an avenue to limit poststenotic restenosis. (Arterioscler Thromb Vasc Biol. 2010;30:00-00.)

Key Words: angina pectoris ■ atherosclerosis ■ ischemia ■ stent ■ vascular biology ■ inflammation

Percutaneous coronary intervention (PCI) has become the predominant form of coronary artery revascularization. The benefits for resolution of chronic angina and/or salvage of ischemic myocardium during acute coronary syndromes are well documented. However, PCI procedures can also injure the vessel wall, generating a considerable inflammatory response and smooth muscle cell proliferation, culminating in restenosis at the lesion site (observed in 5% to 10% of subjects during the first year after PCI).

The implantation of a stent drives cytokine production and amplification at the site of the vascular injury, with subsequent signaling to the liver, leading to C-reactive protein (CRP) production. This is the presumed basis for elevations in serum CRP after PCI procedures. CRP is relatively stable in the plasma (half-life of approximately 19 hours) and peaks approximately 48 hours after PCI regardless of underlying disease or circulating concentration of CRP; thus, levels can be evaluated as a measure of the PCI-induced response because they constitute de novo synthesis. After PCI, CRP levels are predictive of restenosis.

The initiation and progression of inflammatory signals as a result of vascular stressors is mediated in part by the p38 mitogen-activated protein kinase (MAPK) cascade. This intracellular signal transduction pathway is a key sensor for traumatic, oxidative, or inflammatory stress within the vasculature. Selective inhibitors of p38 MAPK have inhibited lipopolysaccharide-stimulated interleukin (IL) 1 and tumor necrosis factor α production in human monocytes and the production of several other cytokines, including IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor.

Because of the key role of p38 MAPK cellular signaling in the initiation and amplification of the inflammatory response, we hypothesized that a potent p38α/β MAPK inhibitor, SB-681323, should mitigate the anticipated increase in serum high-sensitivity CRP (hsCRP) after stent placement.

Methods

This phase 2, randomized, double-blind, placebo-controlled study took place at 10 sites in Denmark, Poland, and Spain. The study was conducted between May 2006 and August 2007. The protocol and consent forms were approved by each center’s ethics committee. Written informed consent was obtained from each patient before the performance of any study-specific procedures. The protocol was registered on clinicaltrials.gov as NCT00291902.
Study Population
Men and women aged 18 years or older who were about to undergo elective single-vessel PCI within 12 weeks of diagnostic coronary angiography confirming obstructive coronary heart disease, who were receiving a stable dose of a statin, and who had an hsCRP concentration of less than 10 mg/L and a low-density lipoprotein concentration of less than 5.0 mmol/L were eligible. Patients were not eligible if the planned PCI involved the left main coronary artery, a bypass graft, or a procedure other than stenting; or if additional revascularization procedures staged on different days during the study, cardiac surgery, or another major noncardiac surgery was planned. Patients with severe noncardiac underlying disease were excluded.

Interventions
SB-681323 is a specific and potent p38α/β MAPK inhibitor whose terminal half-life is approximately 10 hours, with steady state achieved within 3 days of repeat dosing. Bioavailability (percentage of oral dose achieving systemic exposure) is approximately 50%. Eligible patients were randomly selected (1:1) to receive either SB-681323, 7.5 mg (administered as a 5-mg tablet each morning and a 2.5-mg tablet each evening), or matching placebo, orally, for 28 days. SB-681323 and placebo tablets were manufactured by GlaxoSmithKline, Harlow, England.

Procedures
Elective PCI was planned for the third day of the 28-day treatment period (day 3). The number and type of stents (drug-eluting stent [DES] or bare metal stent [BMS]) were at the discretion of the investigator. Patients received concomitant medications according to the local community standard. Post-PCI assessments were scheduled for days 4, 5, 10 or 11, 16 or 17, 21 or 22, and 28. After the last dose of study drug, patients were observed for 14 days. Blood samples were collected for the measurement of serum hsCRP and troponin I, and routine safety laboratory tests were performed at each scheduled assessment. Blood samples were collected for the measurement of other serum-based inflammatory biomarkers (IL-6, IL-8, lipoprotein-associated phospholipase A2, activity, paraoxonase 1, and myeloperoxidase) at baseline and days 3, 5, and 28. Urine samples were collected for urinalysis. Vital signs and 12-lead electrocardiograms were recorded at each scheduled assessment. All assays were performed at a central laboratory using established methods (enzyme-linked immunosorbent assay for hsCRP and multiplex protein microarray assays for other biomarkers).

Details of adverse events, serious adverse events, major adverse cardiovascular events (MACEs), and deaths were collected from receipt of the first dose of study drug to study completion at the follow-up visit. MACEs were defined as cardiovascular death, nonfatal myocardial infarction, stroke, coronary revascularization (in a nonintervened or previously intervened coronary artery), or hospitalizations for recurrent myocardial ischemia (defined as overnight admission for a myocardial ischemic event, including cardiac chest pain or worsening/recurrent angina without evidence of myocardial infarction).

A longer-term post hoc safety follow-up was conducted for patients enrolled in Denmark (approximately 75% of the total cohort). From examination of the Danish National Patient Registry, hospital/physician notes, and patient interviews, details of any deaths or MACEs that occurred since study completion were recorded. In addition, details of general infections were collected because these could theoretically be increased when limiting an inflammatory response.

Statistical Analysis
The sample size for this study was based on feasibility. It was anticipated that the planned sample size of 90 patients would provide 80 evaluable patients (40 per group). The prospectively defined coprimary end points were change from baseline to day 5 (2 days after PCI) in serum hsCRP in the SB-681323 group compared with the placebo group, safety, and tolerability. The secondary end points included change from baseline in serum hsCRP at other time points and change from baseline in serum concentrations of other biomarkers on days 3, 5, and 28.

Statistical analyses were performed using computer software (SAS, version 9.1.3; SAS Institute Inc, Cary, NC). The safety population was defined as all randomly selected patients who received at least 1 dose of study drug. The intent-to-treat (ITT) population was defined as all randomly selected patients who received at least 1 dose of study drug and who had hsCRP values at baseline and day 5 (the primary point of interest).

After log transformation, biomarker data for patients in the ITT population were analyzed using analysis of covariance, fitting terms for treatment, visit, and interaction of treatment and visit; country and baseline value were included as covariates. Comparisons of SB-681323 with placebo were obtained at each point of interest.

A categorical summary of the proportion of patients with elevated troponin I after PCI on either day 4 or day 5 was produced for patients in the ITT population, using a cut point of greater than 0.04 μg/L (upper limit of the normal range of the central laboratory). We also calculated geometric mean (95% CI) values for troponin I on day 4 or 5.

The area under the hsCRP concentration curve (AUC) from baseline to day 28 was derived for each patient using linear trapezoidal rules. After log transformation, AUC data were analyzed with an ANOVA model with fixed-effect terms as treatment group in the model; this analysis was defined retrospectively.

The Fisher exact test was used to test for statistically significant differences for categorical data (eg, stent number and type at baseline, adverse events, MACE, and infections), and the 2-sample t test was used to test for statistically significant differences in continuous variables (eg, stent length); these significance tests were defined retrospectively.

Results

Study Population
Ninety-three patients were randomly assigned to the study, and 92 received at least 1 dose of study drug and were included in the safety population (Figure 1). The ITT population, for analysis of biomarker end points, comprised 78 patients. Sixty-eight patients from Denmark (34 in each treatment group) were included in the post hoc analysis of long-term safety, in which patients were observed for up to 30 months after the last dose. Seventy-four patients completed the study (and received 28 days of study drug), and 19 withdrew from the study.

Demographic characteristics were similar between treatment groups for the ITT population (Table 1) and for all randomly selected patients (data not shown). Baseline concomitant medications were similar in each group (Table 1). More patients in the placebo group than the SB-681323 group had a history of myocardial infarction (42% versus 25%) or had a previous PCI (50% versus 32%) (Table 1). Of the 78 patients in the ITT population, 73 underwent stent placement (SB-681323, n=38; and placebo, n=35); PCI was not performed in the remaining 5 patients for clinical or technical reasons. More patients in the SB-681323 group than the placebo group underwent single stent placements (84% versus 66%), although the difference was not statistically significant (Table 1). In both treatment groups, more patients had DES than BMS.

High-Sensitivity CRP
In the placebo group, hsCRP increased on day 4, 24 hours after stent placement (74% increase from baseline); hsCRP
peaked on day 5 (177% increase from baseline: from a geometric mean of 1.41 to 3.86 mg/L), and levels did not return to baseline until day 28 (25 days after PCI) (Figure 2 and Table 2).

In the SB-681323 group, hsCRP levels were reduced from baseline before the PCI procedure on day 3 (29% reduction relative to placebo: 95% CI, 46.6% to 5.2%) (Table 2). After stent placement, the increase from baseline in hsCRP levels was statistically significantly attenuated by SB-681323 on day 4 (36% reduction relative to placebo: 95% CI, 56.0% to 7.4%), at the primary point of interest on day 5 (37% reduction: 95% CI, 59.5% to −3.3%), and at every post-PCI point thereafter to the end of treatment on day 28. In the SB-681323 group, hsCRP levels were observed to return to baseline or less by day 10 or 11 (7 or 8 days after PCI). At follow-up (14 days after the last dose of study drug), concentrations of hsCRP were generally similar to the levels observed at baseline in both treatment groups.

During the period from baseline to day 28, there was a 40% reduction in hsCRP AUC in the SB-681323 group compared with placebo (P=0.02); the geometric mean hsCRP AUC was 43.90 (95% CI, 33.76 to 57.10) mg·d⁻¹·L⁻¹ in the SB-681323 group and 73.00 (95% CI, 52.66 to 101.08) mg·d⁻¹·L⁻¹ in the placebo group.

To explore the relevance of differences between groups for atherosclerosis burden, data from patients with past myocardial infarction (n=26) were removed from the analysis of hsCRP on day 5. The reduction in hsCRP in the SB-681323 group, compared with the placebo group (−42.9%; 95% CI, −63.9% to −9.5%), was similar to that observed in the total population.

To elucidate whether the type of stent affected post-PCI hsCRP levels, exploratory subgroup analyses were performed in those patients who underwent single stent placement (n=55). In the placebo group, the day 5 peak in hsCRP was higher in patients with BMS (5.7 mg/L) than with DES (3.7 mg/L), despite equivalent baseline values (1.51 and 1.54 mg/L, respectively). SB-681323 attenuated the day 5 increase in hsCRP relative to placebo by 58% (95% CI, −79% to −16%) in the BMS subgroup and by 44% (95% CI, −68% to −2%) in the DES subgroup. The multiple stent cohort was usually of mixed type and of small number, not lending itself to further analyses. The number of stents did not correlate with the levels of hsCRP (P=0.32).

Other Biomarkers
Corrected concentrations of several other inflammatory biomarkers were reduced from baseline in SB-681323–treated patients on day 3 before the PCI intervention (Table 3). On average, the following values were reduced from baseline, relative to placebo: IL-6, 14% (95% CI, −29% to 4%); IL-8, 28% (95% CI, −42% to −11%); lipoprotein-associated phospholipase A2 activity, 5% (95% CI, −9.6% to −0.1%); paraoxonase 1, 9% (95% CI, −21.2% to 5.0%); and myeloperoxidase, 30% (95% CI, −50% to −3%).

Figure 1. Flow diagram of participants through the study. Other reasons for withdrawal were percutaneous coronary intervention not possible (SB-681323, 2 patients; and placebo, 3 patients) and receipt of the prohibited oral corticosteroid (SB-681323, 1 patient). The asterisk indicates.
no other clinically relevant differences were observed between the SB-681323 and placebo groups after PCI (Table 3).

The similarity in temporal profiles for the change from baseline in hsCRP and IL-6 was further corroborated by a correlation between these 2 variables. The overall Spearman rank correlation coefficient for percentage change from baseline in hsCRP versus percentage change from baseline in IL-6 on day 5 (log scale) was 0.63. Moreover, the results of regression analysis indicated a similar slope of the regression line for the percentage change from baseline in hsCRP versus the percentage change from baseline in IL-6 on day 5 for SB-681323–treated and placebo subjects (Figure 3). Despite the lack of apparent IL-6 change on days 3 and 28, similar correlations were observed on these days (data not shown).

Safety and Tolerability
SB-681323 was generally well tolerated. Cardiac and vascular disorders were the most commonly reported categories of
adverse events; these disorders were reported for more patients in the placebo group than in the SB-681323 group (16 [35%] versus 7 [15%] of 46 for cardiac disorders, and 13 [28%] versus 8 [17%] of 46 for vascular disorders, respectively). Angina was the most frequently reported adverse event and was identified by more patients in the placebo group than in the SB-681323 group (11 [24%] versus 3 [7%] of 46). To the end of the 14-day follow-up, 2 (4%) of the 46 patients in the SB-681323 group reported 3 MACEs and 6 (13%) of the 46 patients in the placebo group reported 10 MACEs. The corresponding values were 3 (9%) of 34 patients and 9 (26%) of 34 patients for the long-term follow-up (up to 30 months after the last dose) (P=0.04), with these events determined to be exclusively coronary revascularizations. Infections were reported for 6 (18%) of 34 patients and 3 (9%) of 34 patients, respectively, during this longer follow-up (P=0.48).

For patients in the ITT population, troponin I levels were elevated to greater than 0.04 μg/L on day 4 or 5 (1 to 2 days after PCI) for 16 (40%) of 40 patients in the SB-681323 group and 20 (53%) of 38 patients in the placebo group. On day 4, geometric mean troponin I levels were minimally higher in the SB-681323 group (0.54 [95% CI, 0.175 to 1.693] μg/L) than in the placebo group (0.39 [95% CI, 0.207 to 0.798] μg/L); more patients had higher troponin I values (>1 μg/L) in the SB-681323 group (7 [18%] of 39 patients versus 4 [11%] of 36 patients). On day 5, geometric mean troponin I levels were similar in the SB-681323 and placebo groups (0.34 [95% CI, 0.103 to 1.111] μg/L versus 0.39 [95% CI, 0.125 to 1.211] μg/L, respectively).

**Discussion**

We demonstrated that after vascular trauma by coronary artery stent placement, p38 MAPK inhibition attenuated the
expected elevation in hsCRP 48 hours after PCI and produced a trend to attenuate the increase in serum IL-6. Furthermore, treatment with SB-681323 for 3 days, before stent placement, reduced baseline levels of hsCRP and other inflammatory markers (ie, IL-8, lipoprotein-associated phospholipase A2 activity, paraoxonase 1, and myeloperoxidase). Overall, these results indicate that p38 MAPK inhibition can suppress the chronic systemic inflammatory state associated with atherosclerosis and can also suppress the acute systemic inflammatory signaling cascade associated with acute vascular injury.

We corroborate that vascular injury caused by PCI leads to hsCRP elevation, which peaks 48 hours after the procedure, and that DES decrease the poststent inflammatory response compared with BMS. However, p38 MAPK inhibition effectively and significantly limited the increase in hsCRP overall and for both stent types. Furthermore, in the SB-681323 group, hsCRP levels returned to baseline within 8 days of stent placement, whereas in the placebo group, hsCRP levels did not return to baseline until 25 days after the procedure. This suggests that the overall inflammatory burden originating from the vasculature was reduced after treatment with SB-681323. Our results are consistent with a series of preclinical studies demonstrating that p38 MAPK inhibitors suppress cytokines and vascular inflammation in cellular and in vivo models of cardiovascular disease.

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<th>Table 3. Concentration of Other Inflammatory Biomarkers During the Study for the ITT Population*</th>
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<td><strong>Biomarker and Visit</strong></td>
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<td>LpPLA2, μmol/min/L</td>
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<td>PON-1, U/L</td>
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<td>MPO, μg/L</td>
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LpPLA2 indicates lipoprotein-associated phospholipase A2; MPO, myeloperoxidase; PON, paraoxonase.

*Data are included for patients who had a value at baseline and at the specified point of interest.

There was a modest trend to attenuate IL-6 levels 2 days after stent placement in the SB-681323 group; however, the effects were variable and not statistically significant. This is consistent with a modest, but attenuated, synthesis of a short half-life cytokine, in concert with other cytokines, signaling a longer half-life liver-derived molecule, such as CRP. Correlation of the changes in IL-6 and hsCRP from baseline, in both placebo and drug-treated groups, supports the notion that the 2 biomarkers are linked, as might be expected if 1 marker is driving the other. Thus, we propose that p38 MAPK inhibition attenuates hsCRP by limiting stent-induced vascular inflammation and the attendant generation of IL-6, a known stimulus for CRP production. However, more direct hepatic actions to limit CRP production cannot be excluded.

Necropsy studies reveal mural thrombus and enhanced content of inflammatory cells during the 4 weeks after stent implantation. Such changes, and concurrent elevations in hsCRP, have been associated with restenosis. Although the inflammatory response is both expected and purposeful...
for a healing phase, there is growing attention to the fact that these inflammatory consequences after PCI might cause some degree of damage to the myocardium distal to the stent. This has been inferred from data derived from both prednisone and statin use peri–acute coronary syndromes, revealing improved short-term clinical outcomes.30–32 Our data suggest that inhibiting the p38 MAPK pathway would limit the amplification of inflammatory pathways during stent-induced vascular trauma and, thus, provide additional cardiac protection in the setting of stents and DES.

SB-681323 was well tolerated, with a safety profile similar to that observed in the placebo group. No unfavorable signals were observed, and it was encouraging that there were fewer patients reporting anginal symptoms and a lower incidence of cardiac events in the drug-treated group when compared with placebo. Finally, there were no emergent issues identified in a 30-month follow-up of a major subset of participants.

There are several important limitations of this study. The study was small and not designed, nor powered, to compare highly variable biomarkers (eg, IL-6) or clinical outcomes between treatment groups. Poststent care (including pharmaceticals) was left to the community standard at each site. In addition, the moderate differences in history of myocardial infarction between groups (ostensibly suggesting a placebo population with a greater atherosclerosis burden) could have biased our results, including poststent hsCRP level or reporting of events. Furthermore, stent type and number add variability to our results. Yet, these features do not undermine the central conclusion of an anti-inflammatory effect because removal of patients with a history of myocardial infarction and stratification according to both stent types, with a 30-month follow-up of a major subset of participants.

In summary, our observations indicate that treatment with SB-681832 is a safe and effective means of reducing hsCRP in patients undergoing elective PCI. The drug effects implicate p38 MAPK in the poststent hsCRP increase and most likely represent suppression of the systemic inflammatory response induced by vascular injury. These data suggest the potential value of p38 MAPK inhibition in limiting poststen restenosis and related clinical sequelae.

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Disclosures

Drs Sarov-Blat, James, Fang, Hurle, Baidoo, Willette, Lepore, and Sprecher are employees of GlaxoSmithKline; Drs Sarov-Blat, Fernandez, James, Hurle, Baidoo, Willette, Lepore, and Sprecher own stock in GlaxoSmithKline; and Drs Morgan and Fernandez are former employees of GlaxoSmithKline (they were employed by GlaxoSmithKline during the operation of the present study).

References


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