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SB203580, a p38 Inhibitor, Improved Cardiac Function but Worsened Lung Injury and Survival During *Escherichia coli* Pneumonia in Mice

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Abstract

Background—Supporting its therapeutic application in sepsis, p38 mitogen-activated protein kinase (MAPK) inhibition decreases cardiopulmonary injury and lethality with lipopolysaccharide challenge. However, only one preclinical study has reported the survival effects of a p38 inhibitor (SB203580, 100 mg/kg) during infection. We therefore tested SB203580 in mice (n = 763) challenged with intratracheal *Escherichia coli* and treated with antibiotics and fluids.

Methods and Results—Compared with placebo, high dose SB203580 (100 mg/kg) pretreatment increased the hazards ratio of death (95% confidence interval) (3.6 [2.1, 6.1], $p < 0.0001$). Decreasing doses (10, 1, or 0.1 mg/kg) went from being harmful to having no significant effect ($p < 0.0001$ for the effect of decreasing dose). At 48 hours, but not 24 hours after *E. coli*, high and low dose SB203580 pretreatment decreased cardiac phosphorylated p38 MAPK levels and improved cardiac output either ($p = 0.07$). Low dose SB203580 did not alter lung neutrophils significantly but increased lung injury at 48 hours ($p = 0.05$). High dose decreased lung neutrophils and injury at 24 hours ($p = 0.09$ and 0.01 , respectively) but then increased them at 48 hours (both $p = 0.01$). Lung injury was greater with high versus low dose at 48 hours ($p = 0.002$).

Conclusion—Thus, SB203580 had divergent effects on cardiac and lung function in *E. coli* challenged mice. Furthermore, high dose worsened survival and low dose did not improve it. Altogether, these findings suggest that clearly defining the risks and benefits of p38 MAPK inhibition is important before such treatment is applied in patients with or at risk of serious infection.

Keywords

p38 MAP kinase inhibitor; Treatment; Sepsis; Pneumonia; Cardiopulmonary dysfunction

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Although host inflammation is important for microbial clearance, if excessive, it contributes to cardiopulmonary injury and mortality during septic shock.¹ Mitogen-activated protein kinase (MAPK) p38 is an intracellular signaling protein central to this inflammatory response.^{2–4} Activation of p38 has been implicated in lipopolysaccharide (LPS) associated heart and lung injury.^{5,6} Such data have increased interest in therapeutic inhibition of p38 during sepsis.⁷ Pre-clinical in vivo studies have demonstrated that p38 inhibitors reduce inflammatory lung injury and cardiac dysfunction with LPS and other challenges.^{8–14} Such inhibitors also reduced circulating cytokines and symptoms related to LPS administration in normal humans.^{15,16} Importantly, p38 inhibition improved survival in LPS challenged animals.^{12,17,18}

Although such studies support therapeutic p38 MAPK inhibition during sepsis, this mediator's role in host defense suggests its inhibition could also be harmful during infection.^{3,19–24} However, a literature search found only one investigation of the survival effect of p38 inhibition with bacteria challenge.²⁵ In this study, SB203580 (100 mg/kg), a pyridinyl imidazole that selectively inhibits p38 MAPK phosphorylation, improved survival in mice undergoing cecal ligation and puncture (CLP).^{17,25–27} No study assessed p38 inhibition in bacterial pneumonia, a common cause of sepsis.²⁸ We therefore tested the effects of SB203580 on survival and lung and cardiac dysfunction in mice challenged with intratracheal (IT) *Escherichia coli*.

MATERIALS AND METHODS

Animal Care

The protocol used in this study was approved by the Animal Care and Use Committee of the Clinical Center of the National Institutes of Health.

Study Design

In survival studies, C57BL/6J mice (Jackson Labs., MA) weighing 20 g to 30 g were briefly anesthetized with isoflurane and challenged with 0.05 mL of IT normal saline (NS, noninfected controls) or *E. coli* (15×10^9 CFU/kg) as previously described.²⁹ One hour before NS challenge, mice (n = 24) received either intraperitoneal SB203580 (100 mg/kg in 0.25 mL) or diluent only (placebo). Infected animals received SB203580 in doses of 100, 10, 1, or 0.1 mg/kg or placebo 1 hour before IT *E. coli* (n = 241); SB203580 100 or 0.1 mg/kg or placebo 1 hour after *E. coli* (n = 121); or SB203580 100 mg/kg or placebo 12 hours after *E. coli* (n = 72). All animals received ceftriaxone (100 mg/kg in 0.1 mL, subcutaneously) for 4 days and NS (0.5 mL, subcutaneously) for 1 day beginning 4 hours after challenge. Animals were observed every 2 hours for the initial 48 hours, every 4 hours from 48 hours to 72 hours, every 8 hours from 72 hours to 96 hours, and then twice daily until study completion (168 hours). Sequential weekly experiments with 24 animals each compared either two to three doses of SB203580 versus placebo administered at similar times or similar doses of SB203580 versus placebo at differing treatment times. Study groups in each experiment were of equivalent sample size (i.e., 6 – 8 per group).

Experiments in additional animals (n = 305) investigated the effects of *E. coli* alone compared with NS challenge and of pretreatment with SB203580 100 or 0.1 mg/kg 1 hour before *E. coli* on laboratory measures. Animals were supported as above and then randomly selected at 24 or 48 hours to have either (1) quantitative blood bacteria counts, complete blood counts and lung lavage cell counts, protein levels, and quantitative bacteria counts or (2) plasma cytokine levels, lung wet to dry weight (*W/D*) ratios, lung and heart histology, and phosphorylated and nonphosphorylated p38 MAPK levels. Randomly selected animals had echocardiography before other measures.

Lung and Blood Measures

Animals were anesthetized with isoflurane and blood was drawn for complete blood counts and bacteria counts as previously described.²⁹ Remaining blood was prepared for analysis of tumor necrosis factor (TNF) α , interleukin (IL)-1 α and β , IL-2, IL-4, IL-6, IL-10, interferon- γ , granulocyte macrophage-colony stimulating factor (GM-CSF), two migratory inhibitory proteins (JE) (MIP-1 α and MIP-2), monocyte chemoattractant protein-1, and regulated on activation, normal T-cell expressed and secreted (RANTES) using a multiplexed sandwich enzyme-linked immunosorbent assay (Search Light Cytokine Array, Pierce, Rockford, IL). Animals were then sacrificed and lungs harvested for lavage with cell, protein and bacteria analysis, *W/D* ratios, and histology measures.³⁰

Echocardiography

M-mode echocardiography was performed using a Vevo 770 (Visualsonics Inc, Toronto, Canada) and frame rate of 300 to 500 frames per second in anesthetized (2% isoflurane) mice. Two-dimensional parasternal short-axis imaging was performed at the papillary muscle level. Data represent the average of nine selected cardiac cycles from at least two separate scans. After tracing end-diastolic and end-systolic dimensions, manufacturer software computed end-diastolic and end-systolic volumes (EDV and ESV respectively, μ L), stroke volume (SV, μ L), cardiac output (CO, mL/min), and percent fractional shortening (%) and ejection fraction (%). Studies were interpreted without knowledge of experimental group.

Histology

Lung and heart samples were prepared as previously described and analyzed without knowledge of experimental group.^{30–32} Lung injury parameters scored included interstitial capillary congestion, interstitial edema, alveolar edema, alveolar hemorrhage, alveolar fibrin, and pneumocyte hyperplasia.^{30,31} Scoring based on degree of lung involvement: 0 (normal histology), 1 (<5% surface area), 2 (5–25%), 3 (25–50%), 4 (50–75%), and 5 (>75%). Alveolar neutrophil infiltration was quantified and averaged from 10 high power fields (400 $\times$). Histologic sections of the heart were evaluated for features of myocardial injury and inflammation, as described.³³ Myocardial injury was scored on a scale of 0 to 4 based on the extent of cellular swelling, contraction bands, and/or myocyte necrosis. Inflammation was scored on a scale of 0 to 4 based on the extent of endothelial swelling in interstitial blood vessels, neutrophil margination, and tissue inflammatory exudate.

Tissue p38 MAPK Measures

After sacrifice, lung and heart tissues were immediately collected, weighed, and frozen in liquid nitrogen. Tissue was cut into 3 $\times$ 3 mm pieces, washed, lysed, homogenized, and sonicated in an ice bath. After centrifugation, supernatants were stored at -70°C . Samples were later thawed, centrifuged, and the supernatants were collected. Protein concentrations were determined and adjusted for total and phosphorylated p38 MAPK assays (Bio-Rad Laboratories, Hercules, CA). Beads were vortexed and added to the wells of a 96-well filter plate. Fifty microliters of either tissue lysates or positive control samples were added to wells in duplicate and incubated for 18 hours with agitation at 4°C . After vacuum filtration and washing, 25 μ L of detection antibodies were added to wells and the plate was incubated for 30 minutes at room temperature. The plate was again vacuum filtered and washed. Streptavidin-PE was added to each well for 10 minutes, after which the plate was vacuum filtered, rinsed, and 125 μ L of resuspension buffer added. The activity of total and phosphorylated p38 MAPK was measured with a Bioplex reader and expressed as fluorescence intensity.

Treatments

A stock solution of SB203580, 50 mg (Tocris Cookson Ltd, Ellisville, MD) in 0.5 mL dimethyl sulfoxide was mixed and then further diluted using 0.03N HCl 0.9% NS to prepare treatment doses. Dimethyl sulfoxide concentrations were maintained constant for each SB203580 dose. Placebo consisted of diluent only.

Statistics

SAS version 9.13 was used for analysis. The Cox proportional hazard model was used to evaluate the effect of SB203580 on survival (hazard ratio of death) across time accounting for treatment (placebo vs. SB203580), treatment doses (the effects of 100 vs. 10 vs. 1 vs. 0.1 mg/kg) and treatment time (–1 hour vs. 1 hour vs. 12 hours) in each experiment. Mean ($\pm$ SEM) of other data are shown in the tables (Tables 1, 2 and 3). Three-way analysis of variance (ANOVA) was used to test the effects of challenge (*E. coli* vs. NS), treatment (SB203580 vs. placebo with *E. coli*), and time on all lung, heart, and blood measures. To facilitate data presentation, figures show the mean ($\pm$ SEM) effects of *E. coli* (placebo-treated NS challenged group subtracted from the placebo-treated *E. coli* challenged group) or treatment (placebo-treated *E. coli* challenged group subtracted from the SB203580 treated *E. coli* challenged group). Tables 1, 2 and 3 are provided so that the reader can determine the range of values within which a particular parameter was changing with either infection or treatment. In addition, as previously described, eight parameters of lung injury including alveolar edema, fibrin and hemorrhage, interstitial congestion and hyperplasia and perivascular edema from histology, lung lavage protein concentration, and wet to dry weight (*W/D*) ratio were used to calculate an overall lung injury score.³⁰ For each parameter the mean value in the control group was subtracted from the mean value in the group of interest. To ensure that the scale was similar across the eight parameters, the difference for each parameter was divided by a measure of variability (i.e., the root mean square error from ANOVA). These standardized lung injury values were then analyzed with an ANOVA partitioned by challenge and treatment, which showed that data could be summarized across the eight parameters to produce a single lung injury score. Data were log-transformed where applicable and $p < 0.05$ was considered significant and p values >0.05 and >0.10 considered a trend toward significance.

RESULTS

Survival

All animals challenged with NS (noninfected controls) and treated with either SB203580 or placebo survived. Compared with placebo, pretreatment with the highest dose of SB203580 (100 mg/kg) 1 hour before *E. coli* increased the hazards ratio of death (confidence interval 3.55 [2.05, 6.14], $p < 0.0001$) (Fig. 1). Decreasing the pretreatment dose (10, 1, and 0.1 mg/kg) changed its effects from being on the side of harm to the side of benefit (1.10 [0.58, 2.09], 0.81 [0.47, 1.39], and 0.73 [0.45, 1.18], respectively, $p < 0.0001$ for the effect of decreasing dose on the efficacy of SB203580), although no dose was significantly beneficial. The effect of the lowest and highest doses differed significantly ($p = 0.02$). Finally, compared with pretreatment, delaying treatment with the highest dose until 1 or 12 hours after *E. coli* decreased its harmful effect (1.66 [0.94, 2.960] and 1.40 [0.83, 2.35], respectively, $p = 0.075$ for the effect of time). Treatment with the lowest dose 1 hour after *E. coli* was not beneficial (1.65 [0.92, 2.96], $p = ns$).

Cardiac Effects of *E. coli* and SB203580

To determine why decreasing SB203580 pretreatment doses influenced the agent's efficacy, experiments compared the effects of the highest and lowest doses on *E. coli* induced heart,

lung, and blood changes. *E. coli* did not significantly alter total p38 MAPK in heart tissue at 24 or 48 hours ($p = \text{ns}$) (Table 1). At 48 hours, but not 24 hours, *E. coli* increased phosphorylated (activated) p38 MAPK and the phosphorylated to total p38 ratio ($p = 0.001$ for both) (Fig. 2, Panel A) (Table 1). With *E. coli*, compared with placebo neither low or high dose SB203580 significantly altered total p38 MAPK at either time point ($p = \text{ns}$). However, at 48 hours, but not 24 hours, low and high dose SB203580 decreased phosphorylated p38 MAPK and the ratio of phosphorylated to total p38 (Fig. 2 panel B, $p = 0.01$ for each).

Compared with noninfected controls, at 48 hours, but not 24 hours, *E. coli* significantly decreased left ventricular ESV and EDV ($p = 0.01$ for each) (Table 1). Although these changes resulted in increases in fractional shortening and ejection fraction ($p = 0.01$ for both, data not shown), SV and CO were significantly decreased ($p = 0.01$ for each) (Fig. 2, Panel C and D) (Table 1). With *E. coli*, compared with placebo at 48 hours, but not 24 hours, low and high SB203580 doses increased EDV, SV, and CO either significantly ($p = 0.05$) or in a trend approaching significance ($p = 0.07$ for CO with high dose) (Fig. 2, Panel C and D) (Table 1). Other cardiac parameters were not altered significantly by SB203580 ($p = \text{ns}$ for all).

E. coli increased heart histologic measures of hypoxia at 24 hours and 48 hours and inflammation at 24 hours, but not significantly ($p = \text{ns}$ for all) (Table 1). Neither dose of SB203580 altered these measures throughout ($p = \text{ns}$ for all).

Lung Effects of *E. coli* and SB203580

Compared with noninfected controls, *E. coli* increased lung neutrophils on histology and in lavage at 24 hours and 48 hours ($p = 0.05$ for each) (Fig. 3, Panel A; Fig. 4) (Table 2). With *E. coli*, low dose SB203580 did not alter lung neutrophils at either time point compared with placebo ($p = \text{ns}$). High dose SB203580, however, decreased lung neutrophils on histology at 24 hours in a trend approaching significance ($p = 0.09$) and increased them significantly at 48 hours ($p = 0.01$) in patterns different over time ($p = 0.05$ comparing 24 vs. 48 hours). High dose SB203580 also first decreased and then increased lavage neutrophils at 24 hours and 48 hours but not significantly ($p = \text{ns}$ for all).

Eight parameters of lung injury were analyzed and presented (Table 2). These eight were standardized (see methods) to compute an overall lung injury score (Fig. 3, Panels B and C). *E. coli* increased all measures at 24 hours and 48 hours ($p = 0.05$ for all except lavage protein at 48 hours) as well as the overall score ($p < 0.0001$ at each time point) (Figs. 3 and 4). With *E. coli*, low dose SB203580 did not alter any individual lung injury parameter significantly at either 24 or 48 hours ($p = \text{ns}$ for all) but did increase the lung injury score at 48 hours ($p = 0.05$). By contrast, high dose SB203580 decreased seven lung injury parameters at 24 hours, one significantly (alveolar fibrin, $p = 0.03$) and increased seven at 48 hours, five significantly ($p = 0.05$) and two approaching significance ($p = 0.07$ and 0.09). High dose decreased the lung injury score at 24 hours ($p = 0.01$) and increased it at 48 hours ($p < 0.0001$) in very different patterns ($p = 0.0002$ comparing 24 vs. 48 hours). Finally, at 48 hours, the lung injury score was higher with high compared with low dose SB203580 ($p = 0.002$).

At both 24 hours and 48 hours, compared with noninfected controls, *E. coli* increased total lung p38 MAPK levels ($p < 0.05$ for each) (Table 2). Phosphorylated p38 MAPK levels and the ratios of phosphorylated to total p38 levels were low and not significantly different comparing noninfected and infected animals ($p = \text{ns}$ for all). In *E. coli* animals, although SB203580 treatment altered both lung neutrophils and injury, compared with placebo, no changes in p38 levels could be detected with either dose of treatment ($p = \text{ns}$ for all).

Bacteria were undetectable in lung lavage from uninfected animals while *E. coli* produced positive bacteria counts (mean $\pm$ SEM CFU $\times 10^4$ /mL) that were greater at 24 hours (12.7 ± 0.3) than 48 hours (9.9 ± 0.7) ($p < 0.0001$ for 24 vs. 48 hours). With *E. coli*, lavage bacteria counts at 24 hours and 48 hours were not different ($p = \text{ns}$) compared with placebo with either low dose SB203580 (12.3 ± 0.4 and 9.6 ± 0.7 , respectively) or high dose (12.4 ± 0.4 and 8.6 ± 0.9 , respectively).

Plasma Cytokine Levels and Other Blood Measures

Compared with noninfected controls, *E. coli* increased all cytokines at both 24 hours (11 significantly, each $p \leq 0.05$) and 48 hours (10 significantly, each $p \leq 0.05$) (Fig. 5) (Table 3). With *E. coli*, compared with placebo neither dose of SB203580 significantly altered any individual cytokine at either time point (except high dose decreased IL-4 and MIP-2 at 24 hours, $p \leq 0.05$ for each). However, the overall patterns of cytokine change were different comparing the two doses. With low dose, 11 cytokines were increased at 24 hours while 12 were decreased at 48 hours ($p < 0.0001$ for low dose at 24 vs. 48 hours, χ^2). In contrast, with high dose, 11 cytokines were decreased at 24 hours ($p = 0.0003$ for low vs. high dose at 24 hours, χ^2) and 10 were decreased at 48 hours.

With placebo, *E. coli* compared with noninfected animals decreased circulating neutrophils, lymphocytes, and platelets at 24 hours and 48 hours (all $p \leq 0.01$) and produced positive blood cultures at 24 hours (Table 3). Other measures were not significantly altered by *E. coli* or SB203580 throughout study ($p = \text{ns}$ for all).

DISCUSSION

In this *E. coli* pneumonia model, high dose SB203580 worsened survival whether administered before or after inoculation, although its deleterious effect decreased with later treatment. Reducing the pretreatment dose of SB203580 caused its effects to change from harm to the side of benefit in a dose-dependent pattern, although even the lowest dose did not improve survival significantly. The highest and lowest doses had significantly different effects on survival.

One potential explanation for the differing effect high and low SB203580 doses had on survival with *E. coli* challenge relates to their effects on cardiac and lung function. Consistent with previous studies testing selective p38 MAPK inhibitors, both high and low SB203580 reduced cardiac phosphorylated p38 levels and improved cardiac function (i.e., increases in SV and CO), while the high dose also reduced early (24 hours) lung neutrophil recruitment and injury.⁸⁻¹⁴ However, not reported previously, after first reducing lung neutrophils and injury at 24 hours, high dose SB203580 then increased these at 48 hours in patterns that were very different over time. Although low dose SB203580 also increased the lung injury score later, this change was significantly less compared with high dose. Thus, substantially greater lung injury later with high compared with low dose SB203580 may have outweighed the agent's beneficial hemodynamic effects. The present findings add to other studies suggesting that treatment dose is one of several important factors influencing the benefits and risks of anti-inflammatory agents. We showed previously in rats that higher doses of E5564, a competitive inhibitor of LPS, while highly beneficial with intravenous *E. coli*, had little effect with IT challenge.³⁴ However, reducing the dose of E5564 in animals challenged with IT *E. coli* resulted in benefit.

Improved cardiac function with SB203580 may be related to the positive inotropic actions reported with this agent in LPS perfused heart models and with SB202190, another selective p38 inhibitor, in LPS challenged mice.^{12,14} However, increases in left ventricular volumes with SB203580 with *E. coli* suggest that treatment may have also reduced systemic

vasodilation or extravasation of fluid and improved cardiac filling rather than directly altering myocardial function. Treatment with SB203580 reduced systemic intravascular NO levels for up to 72 hour after LPS challenge in mice.³⁵ Previous studies have also shown that p38 MAPK activation mediates LPS-induced endothelial cell dysfunction and increased permeability, effects blocked by SB203580.^{36,37} It is important to note that improved organ injury with treatment may not correlate with outcome. For example, in septic patients, the nitric oxide inhibitor 546C88 increased blood pressure but worsened survival.³⁸

There are several potential explanations for the changing effects high dose SB203580 had on lung neutrophil recruitment and injury. Greater or more rapid anti-inflammatory effects with high compared with low dose SB203580, while potentially limiting early lung injury from pneumonia may ultimately have been deleterious by limiting the innate immune response and subsequent reparative processes. High dose SB203580 significantly reduced MIP-2 levels at 24 hours and this chemokine has been shown to be important in microbial clearance in different models of infection.^{39,40} Bacteria counts did not differ between groups but concurrent antibiotic administration may have confounded these measures. Activation of p38 MAPK plays an important role in extravascular leukocyte recruitment via cytokine and chemokine dependent and independent mechanisms.⁴¹ Consistent with high dose SB203580 in this study, in an *E. coli* rat pneumonia model, leukocyte adhesion molecule directed monoclonal antibodies (mAbs), decreased early lung neutrophil recruitment and lung injury but increased both measures later and worsened survival.^{30,32} Such studies emphasize the importance of early extravascular neutrophil recruitment during bacterial infection and sepsis and the risks of therapies that alter it. However, just as p38 stimulates proinflammatory responses, it also contributes to compensatory anti-inflammatory responses. Thus, in this study, early and persistent reductions in mediators such as IL-10 with high dose SB203580 may have interfered with the control of excessive intrapulmonary inflammation and contributed to later neutrophil recruitment and injury.^{42,43}

Results from this *E. coli* pneumonia model are the first to directly support concerns that inhibition of p38 MAPK may have a detrimental effect during bacterial infection and sepsis.²³ Despite the beneficial trend when SB203580 dose was decreased, no individual dose improved survival significantly, even with delayed treatment. These findings contrast with previous studies of SB203580 with LPS or CLP challenge.^{12,17,18,25} Thus, the type or site of infectious challenge may also influence the effect p38 MAPK inhibition just as dose did in this study. Additionally, while this investigation provided standard therapies including fluid and antibiotic administration, previous studies with CLP did not.^{25,29} Thus concurrent treatment regimens may also influence the effects of p38 MAPK inhibition.

The array used in this study measured cytokines associated with TH1 cells (IL-2, interferon- γ , GM-CSF, and TNF- α), TH2 cells (IL-4, 6 and 10), and antigen-presenting cells (IL-1, IL-6, and TNF α) as well as chemokines such as MIP-1 α and MIP-2.⁴⁴ In addition, while some of these cytokines and chemokines are recognized to be inflammatory in nature (e.g., IL-1 β , TNF α , MIP-1 α , and MIP-2), others are either strongly associated with anti-inflammatory effects (e.g., IL-10) or may have both inflammatory and anti-inflammatory effects (e.g., G-CSF). Despite this broad range of functions and effects, *E. coli* challenge itself resulted in generalized increases in all of these cytokines and chemokines at both 24 hours and 48 hours. Furthermore, when one considers those cytokines that were significantly altered with SB203580 treatment (IL-4 and MIP-2 decreased with high dose at 24 hours and GM-CSF decreased with high dose at 48 hours), these also represent a range of functions and effects. Although activation of p38 has been associated with stimulation of all three of these, MIP-2 and GM-CSF but not IL-4 have been implicated in the pulmonary and cardiac injury occurring during sepsis.^{2,39,45-47} Although it is possible that higher or repetitive doses of SB203580 treatment might have resulted in significant decreases in a greater

number of the cytokines and chemokines measured, worsened survival with the highest dose tested precluded such studies. Overall, however, in contrast to studies of specific lymphocyte responses during sepsis in vitro, neither challenge nor treatment appeared to significantly alter circulating cytokines or chemokines selectively with respect to particular functions or effects.⁴⁸

Of note though were the differing effects low and high doses of SB203580 had on overall patterns of circulating cytokines. Although low dose was associated with small but consistent early (i.e., 24 hours) increases in 12 of the 13 cytokines measured, a similar number were decreased later (i.e., 48 hours) in patterns that differed comparing early and late time points. In contrast, high dose treatment decreased 11 of 13 cytokines early in a pattern different from low dose and 10 of 13 later. Thus, early and persistent depression in the overall cytokine and chemokine response with high dose SB203580, while potentially contributing to early reductions in lung injury, may have ultimately put infected animals at a disadvantage either with regard to bacterial clearance or reparative processes.

One limitation of this study is that in contrast to myocardial tissue in which SB203580 was documented to reduce phosphorylated p38 MAPK levels, this was not the case in lung tissue. This inability may have been because levels of total p38 protein were substantially higher in the lung with infection, and our techniques were insufficient to detect differences in the phosphorylated fraction with treatment. On the one hand, this inability prevents firm conclusions regarding the role of p38 activation in lung injury in the model and its reduction with SB203580. On the other hand, the reductions in lung neutrophils and inflammatory injury noted with SB203580 are consistent with the reported effects of this agent on inhibiting phosphorylated p38.^{8–11,13} These lung findings, in combination with the documented reductions in myocardial phosphorylated p38 levels and improved cardiac function with SB203580 in infected animals, support a direct inhibitory effect of treatment on p38 in the model.

The p38 MAPK family is composed of four members (i.e., α , β , δ , and γ). Although p38 δ and γ are relatively tissue specific (testes, prostate, pancreas, small intestine, and salivary, pituitary and adrenal glands vs. skeletal muscles, respectively) p38 α and β are ubiquitous.^{49,50} Studies suggest that p38 α may be most important for the proinflammatory effects associated with p38 activation.⁵¹ There are now several different classes of p38 inhibitors: (1) pyridinyl- and pyrimidyl-imidazoles, (2) bicyclic 6,6-heterocycles, (3) *N,N'*-diarylureas, (4) substituted benzamides, (5) diaryl ketones, and (6) indole amides.⁵² Members from each of these classes of agents are designed to inhibit phosphorylation and activation of p38 MAPK, although by differing mechanisms. While SB-203580, a pyridinyl-imidazole, was one of the first agents to demonstrate the potential anti-inflammatory effects of such inhibition, it is known to inhibit both p38 α and β . In fact to date, besides SB203580, the only other two p38 inhibitors investigated regarding their effects on survival in animal sepsis models (i.e., FR167653 and SB202190) are also both of the pyridinyl-imidazole class.^{12,18} However, compounds from subsequently developed classes of inhibitor have demonstrated increased selectivity for p38 α . For example, BIRB 796, a diaryl urea, is highly selective for p38 α and has been shown to inhibit the proinflammatory effect of LPS in vitro and in normal human volunteers.⁴⁹ BIRB 796 has also been studied clinically in rheumatoid arthritis, Crohns disease, and psoriasis and may be a better candidate for study in sepsis than SB203580.

In conclusion, in this study, SB203580 had divergent effects on cardiac and pulmonary function in *E. coli* challenged rats. Furthermore, high dose treatment worsened survival and low dose treatment did not improve it. These findings in combination with previous ones suggest that agents designed to inhibit p38 MAPK may have unpredictable effects during

sepsis or infection. In light of the complex role p38 MAPK has in regulating protective as well as maladaptive host responses, these findings emphasize the need to fully define the risks and benefits of agents inhibiting this intracellular pathway before application in patients with or at risk of infection.

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* p value for the change in effect of SB203580 with decreasing dose

Figure 1.

Effects of increasing doses of SB203580 or placebo administered 1 hour before IT challenge with *E. coli* on the proportion of animals surviving (*Panel A*) and on the hazards ratio of death (hazard ratio of death, 95% confidence interval) (*Panel B*). Apparent differences in animal number between Figure 1 and the methods relate to the fact that one control group was compared with several doses of SB203580 in some cycles.

* $p \leq 0.05$ for the effect of *E. coli*, + $p \leq 0.05$ for the effect of SB203580, # $p=0.07$ for the effect of SB203580

Figure 2.

Mean effect ($\pm$ SEM) of *E. coli* alone and low or high dose SB203580 with *E. coli* on heart phosphorylated p38 (Panel A) and the ratio of phosphorylated to total p38 (Panel B) and on SV (Panel C) and CO (Panel D) at 24 hours or 48 hours after challenge.

Figure 3.

Mean effect ($\pm$ SEM) of *E. coli* alone and low or high dose SB203580 with *E. coli* on lung neutrophils (*Panel A*) and eight parameters of lung injury (*Panel B*) and the lung injury score (*Panel C*) based on histology or lavage measures at 24 hours or 48 hours after challenge. High dose SB203580 caused an early decrease and then increase in lung neutrophils on histology and in lung injury score that occurred in significantly different patterns comparing the two time points ($p < 0.0001$ as shown by the lower bracket). Note that the effect of *E. coli* and SB203580 on lung neutrophils and lung injury score employ differing scales.

Figure 4.

Representative photomicrographs of lung histology at 24 hours or 48 hours after intratracheal challenge with NS and treatment with placebo (*Panels A and E*, respectively) or with *E. coli* challenge and treatment with placebo (*Panels B and F*), low dose SB203580 (*Panels C and G*), or high dose SB203580 (*Panels D and H*) (H&E, 400×).

* $p \leq 0.05$ for the effect of *E. coli*, ** $p < 0.0001$ for the effect of *E. coli*, + $p \leq 0.05$ for the effect of SB203580

Figure 5.

Mean effect (±SEM) of *E. coli* alone and low or high dose SB203580 with *E. coli* on 13 plasma cytokine levels at 24 hours or 48 hours after challenge.

TABLE 1

Mean ($\pm$ SEM) Heart Tissue Total p38 (FI), Phosphorylated p38, and Phosphorylated to Total p38 Ratio (P/T), and Echocardiographic Left Ventricular ESV and EDV Respectively, SV, and CO, and Heart Tissue Histology Measures at 24 or 48 h After Challenge With NS Alone, or *E. coli* Followed by Treatment With SB203580 in Low (0.1 mg/kg) or High (100 mg/kg) Doses or Placebo (0 Dose)

	Hours After NS or <i>E. coli</i> Challenge									
	24					48				
	NS		<i>E. coli</i>			NS		<i>E. coli</i>		
Dose of SB203580 (mg/kg)	0 (n = 7-9)	0 (n = 9-13)	0.1 (n = 10-13)	100 (n = 10-12)	0 (n = 8-9)	0 (n = 9-10)	0.1 (n = 9-13)	100 (n = 9-10)	0.1 (n = 9-13)	100 (n = 9-10)
Heart tissue p38										
Total p38 (FI, $\times 10^3$)	2.8 $\pm$ 0.3	4.2 $\pm$ 0.8	3.9 $\pm$ 0.5	5.1 $\pm$ 0.9 [*]	2.1 $\pm$ 0.3	2.8 $\pm$ 0.7	2.7 $\pm$ 0.5	3.4 $\pm$ 0.6		
Phosphorylated p38 (FI)	95.0 $\pm$ 28.1	289.9 $\pm$ 60.2	564.6 $\pm$ 156.2 [*]	185.0 $\pm$ 238.2	68.9 $\pm$ 19.0	475.0 $\pm$ 60.2 [*]	162.0 $\pm$ 42.7 [†]	196.4 $\pm$ 55.1 [†]		
P/T p38 ratio	0.03 $\pm$ 0.01	0.07 $\pm$ 0.01	0.20 $\pm$ 0.11	0.04 $\pm$ 0.01	0.03 $\pm$ 0.01	0.16 $\pm$ 0.02 [*]	0.06 $\pm$ 0.01 [†]	0.06 $\pm$ 0.02 [†]		
Echocardiography										
ESV (μ L)	8.0 $\pm$ 1.6	7.4 $\pm$ 1.1	7.7 $\pm$ 1.0	6.0 $\pm$ 0.6	11.7 $\pm$ 1.4	3.9 $\pm$ 0.9 [*]	6.8 $\pm$ 1.3 [*]	6.6 $\pm$ 1.4 [*]		
EDV (μ L)	32.6 $\pm$ 3.1	34.0 $\pm$ 3.3	35.3 $\pm$ 2.3	33.9 $\pm$ 2.2	40.6 $\pm$ 2.0	23.6 $\pm$ 2.4 [*]	32.1 $\pm$ 2.1 [†]	31.6 $\pm$ 2.8 [†]		
SV (μ L)	24.7 $\pm$ 1.8	26.6 $\pm$ 2.4	27.7 $\pm$ 1.7	27.9 $\pm$ 1.8	29.2 $\pm$ 1.3	19.6 $\pm$ 2.1 [‡]	25.4 $\pm$ 1.3 [†]	25.1 $\pm$ 2.0 [†]		
CO (mL/min)	9.7 $\pm$ 0.8	10.9 $\pm$ 1.0	10.6 $\pm$ 0.6	11.2 $\pm$ 0.8	11.9 $\pm$ 0.4	7.8 $\pm$ 0.9 [‡]	10.1 $\pm$ 0.5 [†]	9.8 $\pm$ 0.8 [§]		
Heart histology (scored 0-4)										
Hypoxic changes	1.5 $\pm$ 0.3	1.8 $\pm$ 0.4	2.4 $\pm$ 0.5	1.9 $\pm$ 0.4	1.0 $\pm$ 0.2	1.2 $\pm$ 0.4	1.3 $\pm$ 0.3	2.0 $\pm$ 0.4		
Inflammation	1.8 $\pm$ 0.3	2.3 $\pm$ 0.2	2.5 $\pm$ 0.2	2.5 $\pm$ 0.2	2.4 $\pm$ 0.3	2.3 $\pm$ 0.3	2.6 $\pm$ 0.2	2.6 $\pm$ 0.2		

FI, fluorescence intensity.

^{*} *p* 0.05 compared with NS.

[†] *p* 0.05 compared with placebo-treated *E. coli*.

[‡] *p* 0.0001 compared with NS.

[§] *p* 0.07 compared with placebo-treated *E. coli*.

Mean ($\pm$ SEM) Lung Tissue Total p38 (FI), Phosphorylated p38, and Phosphorylated to Total p38 Ratio (P/T), Lung Neutrophils in Histology and Lavage, and Lung Injury Parameters (Histological, LL-Pro, and Lung W/D Ratios) at 24 or 48 h After Challenge With NS Alone, or *E. coli* Followed by Treatment With SB203580 in Low (0.1 mg/kg) or High (100 mg/kg) Doses or Placebo (0 Dose)

TABLE 2

	Hours After NS or <i>E. coli</i> Challenge									
	24					48				
	NS		<i>E. coli</i>			NS		<i>E. coli</i>		
Dose of SB203580 (mg/kg)	0 (n = 7-9)	0 (n = 9-12)	0.1 (n = 9-12)	100 (n = 9-12)	100 (n = 10-12)	0 (n = 8-9)	0 (n = 8-12)	0.1 (n = 9-11)	100 (n = 8-10)	100 (n = 8-10)
Lung tissue p38										
Total p38 (FI $\times 10^3$)	2.3 $\pm$ 0.9	8.2 $\pm$ 2.4*	8.1 $\pm$ 2.1*	6.9 $\pm$ 2.5*	2.5 $\pm$ 1.6	2.5 $\pm$ 1.6	6.6 $\pm$ 1.7*	5.0 $\pm$ 2.2	11.3 $\pm$ 2.5*	11.3 $\pm$ 2.5*
Phosphorylated p38 (FI)	44.2 $\pm$ 27.8	34.3 $\pm$ 11.0	40.4 $\pm$ 8.5	23.9 $\pm$ 13.7	12.9 $\pm$ 9.1	12.9 $\pm$ 9.1	26.9 $\pm$ 12.8	22.7 $\pm$ 11.5	30.7 $\pm$ 14.5	30.7 $\pm$ 14.5
P/T p38 ratio	0.02 $\pm$ 0.01	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01
Lung neutrophils										
Histology (cells $\times 10^3$ /HPF)	0.1 $\pm$ 0.1	5.6 $\pm$ 2.0 [†]	5.2 $\pm$ 0.6*	3.5 $\pm$ 0.6*	0.1 $\pm$ 0	0.1 $\pm$ 0	4.1 $\pm$ 1.1*	5.2 $\pm$ 1.2*	6.9 $\pm$ 0.9 ^{†§}	6.9 $\pm$ 0.9 ^{†§}
Lavage (cells $\times 10^4$ /mL)	0	13.9 $\pm$ 3.8 [†]	12.1 $\pm$ 2.6 [†]	10.1 $\pm$ 2.2 [†]	0.1 $\pm$ 0.1	0.1 $\pm$ 0.1	13.6 $\pm$ 3.9*	14.7 $\pm$ 4.3*	15.2 $\pm$ 7.4*	15.2 $\pm$ 7.4*
Lung injury										
Histology (scored 0-4)										
Alveolar edema	0	1.4 $\pm$ 0.4 [†]	1.6 $\pm$ 0.3 [†]	1.0 $\pm$ 0.3*	0	0	1.1 $\pm$ 0.4*	1.2 $\pm$ 0.3 [†]	2.0 $\pm$ 0.3 ^{†§}	2.0 $\pm$ 0.3 ^{†§}
Alveolar fibrin	0	1.2 $\pm$ 0.5*	1.6 $\pm$ 0.3 [†]	0.4 $\pm$ 0.2 [§]	0	0	1.2 $\pm$ 0.4*	1.7 $\pm$ 0.4 [†]	2.5 $\pm$ 0.5 ^{†§}	2.5 $\pm$ 0.5 ^{†§}
Alveolar hemorrhage	0	2.1 $\pm$ 0.5 [†]	2.6 $\pm$ 0.2 [†]	1.9 $\pm$ 0.3 [†]	0	0	1.7 $\pm$ 0.5 [†]	2.3 $\pm$ 0.3 [†]	2.8 $\pm$ 0.4 ^{†§}	2.8 $\pm$ 0.4 ^{†§}
Interstitial congestion	0.4 $\pm$ 0.2	2.4 $\pm$ 0.4 [†]	3.0 $\pm$ 0.3 [†]	2.9 $\pm$ 0.2 [†]	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	2.1 $\pm$ 0.5 [†]	2.8 $\pm$ 0.3 ^{††}	3.1 $\pm$ 0.3 ^{†§}	3.1 $\pm$ 0.3 ^{†§}
Interstitial hyperplasia	0.3 $\pm$ 0.2	1.9 $\pm$ 0.5*	2.1 $\pm$ 0.3 [†]	1.6 $\pm$ 0.4*	0	0	1.8 $\pm$ 0.3 [†]	2.0 $\pm$ 0.3 [†]	2.5 $\pm$ 0.4 ^{††}	2.5 $\pm$ 0.4 ^{††}
Perivascular edema	0.3 $\pm$ 0.2	2.9 $\pm$ 0.4 [†]	2.7 $\pm$ 0.2 [†]	2.5 $\pm$ 0.2 [†]	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	2.2 $\pm$ 0.4 [†]	2.8 $\pm$ 0.3 [†]	3.2 $\pm$ 0.3 ^{†§}	3.2 $\pm$ 0.3 ^{†§}
LL-Pro (100 mg/dL)	0.2 $\pm$ 0.0	3.2 $\pm$ 1.7 [†]	1.7 $\pm$ 0.3 [†]	1.1 $\pm$ 0.1 [†]	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	1.4 $\pm$ 0.3 [†]	1.7 $\pm$ 0.2 [†]	5.1 $\pm$ 3.4 ^{†§}	5.1 $\pm$ 3.4 ^{†§}
W/D	3.8 $\pm$ 0.1	4.3 $\pm$ 0.2*	4.6 $\pm$ 0.2*	4.2 $\pm$ 0.1*	3.9 $\pm$ 0.1	3.9 $\pm$ 0.1	5.1 $\pm$ 0.3*	5.0 $\pm$ 0.3*	5.1 $\pm$ 0.2*	5.1 $\pm$ 0.2*

FI, fluorescence intensity; LL-Pro, lung lavage protein.

* *p* 0.05 compared with NS.

[†]*p* 0.0001 compared with NS.

^{††}*p* 0.09 compared with placebo-treated *E. coli*.

δp 0.05 compared with placebo-treated *E. coli*.

TABLE 3

Mean ($\pm$ SEM) Cytokines, Circulating Neutrophils, Lymphocytes and Platelets, and Quantitative Blood Bacterial Counts at 24 or 48 h After Challenge With NS Alone, or *E. coli* Followed by Treatment With SB203580 in Low (0.1 mg/kg) or High (100 mg/kg) Doses or Placebo (0 Dose)

Dose of SB203580 (mg/kg)	Hours After NS or <i>E. coli</i> Challenge							
	24				48			
	NS		<i>E. coli</i>		NS		<i>E. coli</i>	
	0 (n = 8)	0 (n = 9-13)	0.1 (n = 11-12)	100 (n = 10-12)	0 (n = 9-11)	0 (n = 8-9)	0.1 (n = 9-12)	100 (n = 8-12)
Cytokines (log [pg/mL])								
IL-1 α	1.6 $\pm$ 0.3	2.5 $\pm$ 0.4	2.5 $\pm$ 0.4	1.9 $\pm$ 0.3	1.7 $\pm$ 0.4	1.9 $\pm$ 0.6	2.8 $\pm$ 0.3 *	2.6 $\pm$ 0.2
IL-1 β	0	1.9 $\pm$ 0.5 *	2.3 $\pm$ 0.6 *	1.6 $\pm$ 0.4 *	0.5 $\pm$ 0.5	2.2 $\pm$ 0.7 *	1.7 $\pm$ 0.5 *	1.2 $\pm$ 0.4
IL-2	4.2 $\pm$ 0.6	4.9 $\pm$ 0.2	5.1 $\pm$ 0.1	4.5 $\pm$ 0.5	4.4 $\pm$ 0.5	4.9 $\pm$ 0.1	4.8 $\pm$ 0.1	4.7 $\pm$ 0.1
IL-4	0 $\pm$ 0	2.1 $\pm$ 0.4 $\ddagger$	2.4 $\pm$ 0.5 $\ddagger$	1.1 $\pm$ 0.4 **	0.4 $\pm$ 0.4	1.4 $\pm$ 0.7	0.9 $\pm$ 0.4	0.6 $\pm$ 0.3
IL-6	3.4 $\pm$ 0.7	7.6 $\pm$ 0.3 $\ddagger$	8.2 $\pm$ 0.4 $\ddagger$	6.9 $\pm$ 0.3 $\ddagger$	3.3 $\pm$ 0.5	7.9 $\pm$ 1.0 $\ddagger$	7.3 $\pm$ 0.5 $\ddagger$	6.5 $\pm$ 0.5 $\ddagger$
IL-10	1.7 $\pm$ 0.5	6.0 $\pm$ 0.2 $\ddagger$	6.4 $\pm$ 0.3 $\ddagger$	5.5 $\pm$ 0.2 $\ddagger$	1.8 $\pm$ 0.6	5.2 $\pm$ 0.7 $\ddagger$	4.8 $\pm$ 0.5 $\ddagger$	4.3 $\pm$ 0.5 $\ddagger$
Interferon- γ	0 $\pm$ 0	2.0 $\pm$ 0.8 *	2.7 $\pm$ 0.8 *	2.3 $\pm$ 0.8 *	0.5 $\pm$ 0.5	3.2 $\pm$ 1.0 *	2.6 $\pm$ 0.9 *	1.6 $\pm$ 0.7
TNF α	0.4 $\pm$ 0.4	4.7 $\pm$ 0.3 $\ddagger$	4.9 $\pm$ 0.3 $\ddagger$	4.5 $\pm$ 0.2 $\ddagger$	0.1 $\pm$ 0.1	4.4 $\pm$ 0.5 $\ddagger$	4.1 $\pm$ 0.3 $\ddagger$	4.0 $\pm$ 0.6 $\ddagger$
GM-CSF	0.1 $\pm$ 0.1	2.5 $\pm$ 0.3 $\ddagger$	2.9 $\pm$ 0.3 $\ddagger$	2.1 $\pm$ 0.2 $\ddagger$	0.6 $\pm$ 0.3	2.4 $\pm$ 0.8 *	1.9 $\pm$ 0.4 *	1.7 $\pm$ 0.3 *
MIP1 α	0.2 $\pm$ 0.2	3.6 $\pm$ 0.2 $\ddagger$	4.0 $\pm$ 0.2 $\ddagger$	3.7 $\pm$ 0.2 $\ddagger$	0.3 $\pm$ 0.3	3.5 $\pm$ 0.8 $\ddagger$	2.6 $\pm$ 0.4 $\ddagger$	2.6 $\pm$ 0.4 $\ddagger$
MIP2	0.5 $\pm$ 0.5	6.9 $\pm$ 0.3 $\ddagger$	7.0 $\pm$ 0.4 $\ddagger$	5.5 $\pm$ 0.7 $\ddagger$	0.6 $\pm$ 0.6	6.2 $\pm$ 1.2 $\ddagger$	5.0 $\pm$ 0.7 $\ddagger$	4.9 $\pm$ 0.7 $\ddagger$
JE	4.2 $\pm$ 0.2	7.1 $\pm$ 0.3 $\ddagger$	7.4 $\pm$ 0.5 $\ddagger$	6.7 $\pm$ 0.3 $\ddagger$	3.9 $\pm$ 0.1	7.1 $\pm$ 0.9 $\ddagger$	6.4 $\pm$ 0.4 $\ddagger$	5.9 $\pm$ 0.4 $\ddagger$
RANTES	2.8 $\pm$ 0.1	7.0 $\pm$ 0.3 $\ddagger$	6.9 $\pm$ 0.2 $\ddagger$	7.0 $\pm$ 0.2 $\ddagger$	2.7 $\pm$ 0.1	5.9 $\pm$ 0.7 $\ddagger$	5.4 $\pm$ 0.4 $\ddagger$	5.1 $\pm$ 0.4 $\ddagger$
Circulating cells (cells $\times 10^3/\mu$ L)								
Neutrophils	1.6 $\pm$ 0.3	0.6 $\pm$ 0.1 $\ddagger$	0.6 $\pm$ 0.1 $\ddagger$	0.9 $\pm$ 0.1 *	1.8 $\pm$ 0.4	0.8 $\pm$ 0.1 *	1.2 $\pm$ 0.1 *	0.9 $\pm$ 0.1 *
Lymphocytes	6.8 $\pm$ 0.2	1.2 $\pm$ 0.1 $\ddagger$	1.2 $\pm$ 0.1 $\ddagger$	1.3 $\pm$ 0.2 $\ddagger$	7.7 $\pm$ 0.8	1.1 $\pm$ 0.2 $\ddagger$	1.3 $\pm$ 0.2 $\ddagger$	1.2 $\pm$ 0.2 $\ddagger$
Platelets	802.6 $\pm$ 69.3	561.0 $\pm$ 21.5 $\ddagger$	533.6 $\pm$ 36.9 $\ddagger$	594.6 $\pm$ 28.9 $\ddagger$	800.8 $\pm$ 76.9	552.5 $\pm$ 38.5 *	608.4 $\pm$ 28.4 *	560.5 $\pm$ 38.6 *
Quantified blood bacterial counts (CFU $\times 10^4/\mu$ L)								
Blood culture	NA	1.7 $\pm$ 0.5	1.3 $\pm$ 0.5	0.8 $\pm$ 0.4	NA	0	0	0

RANTES, regulated on activation, normal T-cell expressed and secreted.

**p* 0.05 compared with NS.
 †*p* 0.0001 compared with NS.
 ‡*p* 0.05 compared with placebo-treated *E. coli*.