Bosentan Enhances Viral Load via Endothelin-1 Receptor Type-A–Mediated p38 Mitogen-Activated Protein Kinase Activation While Improving Cardiac Function During Coxsackievirus-Induced Myocarditis

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Abstract—Reduced cardiac output is one of the consequences of myocarditis. Bosentan, an endothelin-1 receptor (ET1R) antagonist, could be useful to reduce cardiac afterload, preserving cardiac output. In this study, we investigated the potential therapeutic use of bosentan in an animal model of viral myocarditis. Using a mouse model of coxsackievirus B3 (CVB3)-induced myocarditis, we demonstrated preserved ejection fraction (EF) and fractional shortening (FS) by treatment with bosentan (68±5.8% EF and 40±3.7% FS for treated versus 48±2.2% EF and 25±2.6% FS for controls; \( P = 0.028 \)). However, bosentan enhanced cardiac viral load (10.4±6.7% in the bosentan group versus 5.0±5.5% in control group; \( P = 0.02 \)), likely through enhancement of p38 mitogen-activated protein kinase (MAPK) phosphorylation (0.77±0.40% ATF2 activation in the bosentan group versus 0.03±0.02% in controls; \( P = 0.0002 \)), mediated by endothelin receptor type-A. We further demonstrate that a water soluble inhibitor of p38 MAPK, SB203580 HCl, is a potent inhibitor of virus replication in the heart (0.28% antisense viral genome stained area for 3 mg/kg dose versus 2.9% stained area for controls; \( P = 0.01 \)), attenuates CVB3-induced myocardial damage (blinded cardiac histopathologic scores of 1.8±1.6 and 2.05±1.2 for the 3 mg/kg and 10 mg/kg doses, respectively, versus 3.25±1.2 for the controls), and preserves cardiac function (69±3.5% EF for 3 mg/kg dose and 71±6.7% EF for 10 mg/kg dose versus 60±1.5% EF control; \( P = 0.038 \) and \( P = 0.045 \), as compared to control, respectively). Bosentan, a prescribed vasodilator, improves cardiac function but enhances viral load and myocarditis severity through ETRA mediated p38 MAPK activation; p38 MAPK is a desirable antiviral target. Caution must be exercised during treatment of suspected infectious myocarditis with supportive vasoactive remedies. (Circ Res. 2009;104:00-00.)

Key Words: echocardiography ■ endothelin-1 ■ p38 ■ receptors ■ signal transduction ■ signaling pathways

Myocarditis is a common inflammatory heart disease in children and young adults that is associated with cardiac dysfunction. A diminished ejection fraction (EF) in these patients translates into increased mortality.1

The most common etiologic agents for this disease are enteroviruses; however, many other viruses are also known to cause myocarditis.2 Because of the insidious presentation of symptoms during viral myocarditis the disease is often misdiagnosed. Indeed, a major problem relates to the lack of diagnostic procedures to properly identify the pathogen in a timely manner. This is why a broadly effective and safe antiviral approach would be desirable from a clinical viewpoint. If a patient presents with suspected infectious myocarditis, with early diagnosis, antiviral therapy could minimize replication and direct virus-induced damage.

Experimental and clinical studies have demonstrated activation of the endothelin (ET) system in many diseases characterized by inflammation or fibrotic remodeling such as atherosclerosis3 and myocardial ischemia.4 The result of ET1 signaling is vasoconstriction, an undesirable response during myocarditis. Bosentan,5 an ET1 antagonist has been indicated for the treatment of pulmonary hypertension in the clinic. In mice with experimental myocarditis, treatment with this antagonist has been shown to reduce cellular infiltration and...
myocardial necrosis. In addition, acute and chronic ET1 receptor type-A (ETRA) blockade was found to improve cardiac function and reduce the incidence of arrhythmia in rats with myocardial infarction. Thus, antagonism of ET1 signaling is potentially a desirable therapeutic strategy. As such, we hypothesized that blockade of the ET1R may have a cardioprotective effect in myocarditis, and we investigated the therapeutic use of ET1 antagonist, bosentan, in a mouse model of viral myocarditis.

We report here that bosentan preserves cardiac function but also increases cardiac viral load, the latter likely through enhancement of p38 mitogen-activated protein kinase (MAPK) phosphorylation. Indeed, bosentan enhanced p38 MAPK phosphorylation both in vitro and in vivo, elevating viral load in cells and tissues studied, and resulted in increased global histopathology scores in vivo. We also show here that inhibition of p38 MAPK in CVB3-infected A/J mice preserves physiological parameters of heart function, while reducing viral load. We propose a new approach to treatment of enterovirus-induced myocarditis through pharmacological inhibition of p38 MAPK and also provide a warning about the use of the ET1R antagonist, bosentan, during infectious myocarditis.

**Materials and Methods**

### Mice and Viruses

Sixty (24 Bosentan treatment mice and 36 in SB203580 HCl treatment groups) male 5-week-old A/J mice (The Jackson Laboratory) were used for this study. All mice were observed for appearance and behavior and weighed daily for 5 days, a timeframe that brackets acute virus infection in this model. The protocol was approved by the Committee on Animal Care at the University of British Columbia.

CVB3-Gauntt was used for mouse infection. A molecular clone of CVB3 RNA genome, respectively, was hybridized, in situ, to detect the presence of active virus in cardiac tissue was assessed by in situ hybridization. Briefly, digoxigenin-conjugated positive and negative-sense RNA probes, complementary to negative and positive-sense regions of the CVB3 RNA genome, respectively, were hybridized, in situ, and detected by X-gal substrate cleavage by β-galactosidase conjugated anti-digoxigenin secondary antibody (Santa Cruz Biotechnology).

### Echocardiography

Cardiac structure and function were examined via transthoracic echocardiography using a Vevo 770 (Visual Sonics, Toronto, Canada) equipped with a 35-MHz transducer with mice under 1.2% to 1.5% isoflurane anesthesia on a warm pad (37°C). Measurements were obtained before infection and day 5 pi. Measurements of interventricular septum, left ventricular posterior wall thickness, and the diameter of the left ventricle at the end-diastole and end-systole were obtained. Left ventricular EF and fractional shortening (FS) were calculated using Vevo 770 (version 2.30) software.

### Determination of p38 MAPK Phosphorylation in HL1 Cardiomyocytes

HL1 murine cardiomycocytes (a gift from William Claycomb, Louisiana State University Health Sciences Center, New Orleans) were plated on fibronectin coated plates in Claycomb medium with 10% FBS, and the experiment was conducted at confluence. HL1 cells beat when confluence was obtained. Bosentan was added 30 minutes before addition of CVB3 or ET1. Cells were harvested after 0, 5, 15, and 30 minutes of exposure using lysis buffer (10 mmol/L HEPES [pH 7.4], 50 mmol/L Na₃P₂O₇, 50 mmol/L NaF, 50 mmol/L NaCl, 5 mmol/L EDTA, 5 mmol/L EGTA, 1 mmol/L NaᵥVO₄, 0.5% Triton X-100, 10 μg/mL leupeptin, and 1 mmol/L phenylmethylsulfonyl fluoride). Protein concentration was determined by Bradford protein determination assay (Bio-Rad) and equal concentration (10 μg total) of each sample was loaded onto phospho–p38 MAPK (pThr180/pTyr182) ELISA plates (Biosource, Invitrogen) and performed according to the instructions of the manufacturer.

### In Vitro Determination of Virus Progeny Release by HL1 Cardiomyocytes

HL1 cardiomycocytes pretreated with SB203580 (5 μmol/L) and bosentan (10 μmol/L) for 1 hour were infected with CVB3 or ET1 (10 μmol/L) for 2 hours. Cells were washed 3 times with PBS, and time 0 (t=0) was obtained after replacement of fresh growth media. Supernatants were collected at the peak of CVB3 progeny release from HL1 cells (16 hours pi). Supernatants were titrated on indicator HeLa cells and infection of CBV3-GFP was enumerated by flow cytometry 8 to 10 hours after initial infection (Beckman Coulter EPICS XL-MCS flow cytometer). Infected cells were differentiated from the main cell population by gating for the main population from a sample of uninfected HeLa cells.

### In Vitro Determination of p38 MAPK Activation From ET Receptors Types A and B in HL1 Cardiomyocytes

HL1s were pretreated with 10 μmol/L ETR signaling inhibitors (BQ123 [ETRA; Sigma] or BQ788 [ETRB; Sigma]) 1 hour before 10 μmol/L concentrations of ET1 or bosentan. Signal activation proceeded for 30 minutes before lysis of cells on ice using protein lysis buffer (as described in detail above). ELISA for p38 MAPK was conducted on a total of 10 μg of protein, as determined by Bradford protein determination assay (described above). The activation of p38 MAPK resulting from ET1 and bosentan treatment was divided by the basal p38 MAPK activation in the control DMSO treatment, in the absence and presence of BQ123, BQ788, or both BQ123 and 788 together (10 μmol/L each).

### Immunohistochemical Staining for Phospho-ATF2 and ET1

Five-micron tissue sections cut from paraffin-embedded tissue were stained with an anti–phospho-ATF2 antibody (pThr71) or anti-ET1 antibody per the instructions of the manufacturer for immunohistochemistry (no. 9221; Cell Signaling Technology).
Images captured using the Nikon Coolscope (Nikon) were analyzed using ImagePro Plus software (Media Cybernetics, Silver Spring, Md). The fraction of positively stained tissue brown for ATF2 staining and blue for in situ hybridization to total tissue area was given as a percentage. A color cube algorithm was used in Image Pro Plus with manual selection of positive (stained) colors to define the colors used for thresholding between positive and negative staining and calculation of volume fraction. Volume fraction is defined as the area of positive staining divided by total tissue area.

**Histopathologic Analysis and Grading**

Ventricular samples were fixed in 10% neutral-buffered formalin and processed for standard histological staining. Hematoxylin/eosin (H&E)-stained sections were coded and graded by an experimental cardiovascular pathologist, who was unaware of animal group assignment, on the following scale: 0, no or questionable presence; 1 to 2, limited focal distribution; 3 to 4, intermediate severity; and 5, coalescent and extensive foci over the entirety of the transversely sectioned ventricular, pancreatic, or liver tissues. Images were all captured using a Nikon Coolscope.

**Statistical Analysis**

Post hoc pairwise comparisons between the vehicle and drug-treated mice were conducted with the Student’s t test. A probability value <0.05 was considered to be statistically significant.

**Results**

**Bosentan Attenuates the Functional Cardiac Abnormalities Induced by CVB3 Infection**

We previously reported the presence of ET1 in the myocardium during CVB3 infection.11 There was no difference in ET1 expression between sham and CVB3-infected mouse hearts at day 42 pi, long after virus infection and replication had been cleared. We investigated the presence of ET1 in myocardium during acute infection, because ET1 has been shown to cause vasoconstriction, focusing on the status of ET1 within the myocardium during active virus replication. We stained for the presence of ET1 in mouse myocardium 5 days pi, the time after inoculation when viral load and viremia are maximal. By immunohistochemical staining, we noted that ET1 was dispersed throughout the ventricular myocardium (Figure 1), as compared to the uninfected control.

Bosentan, an ET1R antagonist that prevents ET1-mediated vasoconstriction, is currently used to treat pulmonary hypertension and has been proposed for the treatment of myocarditis.12,13 We treated 5-week-old A/J mice with oral doses of bosentan at 100 mg/kg each day, and we conducted echocardiography at day 5 pi. Figure 2 shows the benefit of treatment with bosentan during CVB3 infection. EF was better maintained with treatment as compared to the vehicle control (69 ± 5.8% versus 49 ± 2.2%; P = 0.028; Figure 2A). Similarly, FS was preserved in the treated mice (40 ± 3.7% versus 25 ± 2.6%; P = 0.04; Figure 2B). However, there was no apparent improvement in survival after bosentan treatment (Figure 2C).

**Bosentan Enhances Viral Load and Global Histopathologic Scores Despite Preservation of Cardiac Function During CVB3 Infection**

Physiological parameters obtained by echocardiography in Figure 2 suggested that viral load may have been decreased by bosentan treatment, so we sought to determine the viral load in the myocardium of CVB3-infected mice. We used in
Slides from sectioned hearts were also stained with H&E and blindly graded on a global histopathology scale from 0 to 5. Five indicates the most severe destruction. The bosentan treatment group demonstrated severely exacerbated cardiac damage that was statistically significant ($P=9.0 \times 10^{-6}$; Figure 3B).

**Bosentan and ET1 Activate p38 MAPK via ETRA and not ETRB**
We wanted to determine whether there were any ET1- and bosentan-associated p38 MAPK signaling effects in cardiomyocytes attributable to the widespread exposure of myocardium to this cytokine (Figure 1). ET1 has been reported to activate p38 MAPK in HeLa cells, and p38 MAPK activation has been shown to drive CVB3 replication in HeLa cells, in vitro. We were also curious as to whether ET1 or Bosentan could activate p38 MAPK via ETRA or ETRB.

We used a murine HL-1 cardiomyocyte cell line to determine whether the activation of p38 MAPK was from either ETRA and/or -B. Figure 4A shows that ET1 and bosentan cause activation of p38 MAPK from ETRA but not ETRB. p38 MAPK activation by ET1 and bosentan was not inhibited by treatment with ETRB antagonist BQ788 (ET1: 2.4-fold \(P=0.04\); bosentan: 2.0-fold \(P=0.02\) over BQ788-treated control) or control DMSO (ET1: 1.5-fold \(P=0.04\) and bosentan: 1.6-fold \(P=0.007\) over control, respectively). Treatment of HL-1s with either BQ123 (ETRA) alone or both BQ123 and BQ788 (ETRB) simultaneously resulted in no significant difference between the p38 MAPK activation caused by the control or ET1 and bosentan treatments, indicating inhibition of p38 signal propagation.

**Bosentan Increases and Prolongs p38 Phosphorylation in Response to ET1 Treatment or CVB3 Infection**
We determined the effect of ET1, bosentan, and CVB3 on p38 MAPK signaling. Bosentan competitively binds ET1A and ET1B receptors to antagonize ET1 vasoconstriction. HL1 cells were preincubated with bosentan then treated with ET1 or infected with CVB3. Bosentan increased and prolonged the amount of p38 MAPK phosphorylation attributable to CVB3 and ET1. Bosentan with CVB3 enhanced p38 MAPK phosphorylation at 5 and 30 minutes, compared to CVB3 alone ($P=0.02$ and $P=0.01$, respectively; Figure 4B).

**Bosentan Enhancement of Viral Progeny Release Is Reduced by Treatment With a p38 MAPK Inhibitor**
We have reported the requirement of CVB3 on p38 MAPK signaling for optimal virus replication in vitro. Inhibition of p38 MAPK activation results in decreased release of progeny virion from infected cells but does not affect viral protein production. Consistent with this hypothesis, we observed no alteration of primary infection in target HL1 cells with SB203580 treatment (data not shown). However, we showed a significant enhancement of viral progeny release with bosentan treatment ($37 \pm 0.5\%$ of indicator HeLa cells infected with bosentan treatment as compared to $17 \pm 0.4\%$ of control cells; $P=0.0006$; Figure 4C). This enhancement

**Figure 3.** Myocarditis is exacerbated by bosentan treatment as indicated by increased viral load and global histopathologic scores. A, Mouse heart was harvested 5 days pi, and CVB3 sense and antisense genome was detected by in situ hybridization using antisense and sense RNA probes, respectively. Bosentan treatment significantly enhances cardiac viral load, presented as percentage of infected area (means $\pm$ SD). *$P<0.05$ as compared to vehicle-treated group. B, Histopathologic scores (means $\pm$ SD) by H&E staining were significantly presented as percentage of infected area (means $\pm$ SD). **$P<0.001$ as compared to vehicle-treated group.

**Figure 4.** Bosentan enhances viral progeny release from infected cells. A, Mouse heart was harvested 5 days pi, and CVB3 sense and antisense genome was detected by in situ hybridization using antisense and sense RNA probes, respectively. Bosentan treatment significantly enhances cardiac viral load, presented as percentage of infected area (means $\pm$ SD). *$P<0.05$ as compared to vehicle-treated group. B, Histopathologic scores (means $\pm$ SD) by H&E staining were significantly presented as percentage of infected area (means $\pm$ SD). **$P<0.001$ as compared to vehicle-treated group.
Effect by bosentan was eliminated by treatment with P38 MAPK inhibitor SB203580.

The Phosphorylation of ATF2, a Downstream Target of p38 MAPK, Is Enhanced in Bosentan-Treated Mice

ATF2 phosphorylation at Thr71 and subsequent activation is a product of p38 MAPK activation. Therefore, we detected ATF2 phosphorylation at Thr71 in bosentan-treated mice. We stained heart sections from bosentan-treated mice for ATF2 phosphorylation by immunohistochemistry. Figure 5 shows bosentan enhanced cardiac phospho-ATF2 expression in CVB3-infected A/J mice (0.77 ± 0.40% of anti–phospho-ATF2–stained tissue area in bosentan-treated mice versus 0.03 ± 0.02% in the control mice; \( P < 0.0002 \)).

SB203580 Preserves Parameters of Cardiac Function During CVB3-Induced Myocarditis

We next determined the effect of p38 inhibition during CVB3-myocarditis. SB203580 HCl is a water soluble hydrochloride that inhibits p38 MAPK activation. Because of its water soluble properties this inhibitor is a desirable drug for in vivo studies.

Mice were infected with CVB3 and treated once every 2 days by intraperitoneal injection with SB203580 HCl at 3 and 10 mg/kg doses. There was no difference between the control and SB203580 treatment groups, with regard to ATF2 phosphorylation in the heart (data not shown). However, the pancreas, an organ with the greatest virus replication and virus-induced destruction, demonstrated significant decreases (Figure 6A) (2.6 ± 2.05% in the 3 mg/kg group \( P = 0.04 \), 1.7 ± 1.88% in the 10 mg/kg group \( P = 0.03 \) as compared to 7.3 ± 8.7% in the control group) in phospho-ATF2 staining, as compared to the control, and a trend toward decreased activation effect by bosentan was eliminated by treatment with P38 MAPK inhibitor SB203580.
in the 10 mg/kg SB203580 treatment group as compared to the 3 mg/kg group.

Murine echocardiography in Figure 6B shows that EF and FS are significantly preserved, as is survival (Figure 6C), with SB203580 treatment as compared to the vehicle control. The EF mean of mice treated with 3 mg/kg SB203580 was 69% ± 3.5% (P = 0.038) and was 71% ± 6.7% EF with the 10 mg/kg SB203580 dose (P = 0.045), as compared to 60% ± 1.5% in the mice treated with vehicle only (P = 0.038). The FS was improved to a mean of 37% ± 4.4% and 39% ± 5.6% with 3 mg/kg and 10 mg/kg treatments, respectively, as compared to 31% ± 7.2% in the vehicle control (P = 0.008 and P = 0.041, respectively).

SB203580 Reduces Pancreas and Cardiac Viral Loads During CVB3 Infection

We have reported that p38 inhibitors reduce CVB3 replication in HeLa cells. Figure 7 shows the inhibition of virus infection with SB203580 treatment in both heart (Figure 7A) and pancreas (Figure 7B). Therefore, inhibition of virus replication by the SB hydrochloride was system-wide.

The myocardium in Figure 7A is "pock-marked" blue, with more discrete regions of antisense staining, but, most importantly, sense probe staining showing the presence of negative-sense CVB3 RNA indicates very active virus replication. The 10 mg/kg dose of SB203580 was most effective at reducing the presence of CVB3 positive-sense genome (antisense staining), which was significantly decreased in the heart (AS: 4.0% ± 1.8% of control as compared to 0.9% ± 0.4% in 10 mg/kg treatment; S: 3.0% ± 1.9% of control as compared to 0.31% ± 0.29% of 10 mg/kg SB203580 treatment; P = 0.05). The 3 mg/kg dose was less effective than the 10 mg/kg dose at reducing AS probe–stained cardiac infection by CVB3. There was a trend toward less AS staining (AS: 3.9% ± 1.8% in control as compared to 3.0% ± 1.9% in 3 mg/kg treated group; P = 0.43). The S probe results were significant: S: 3.0% ± 1.9% in the control group as compared to 0.28% ± 0.1% in the 3 mg/kg treated group (P = 0.02).

Global Histopathologic Scores Were Lower in the SB203580 Treatment Group

We conducted blinded global histological assessment of H&E-stained sections from 3 mg/kg SB203580-treated, 10 mg/kg SB203580-treated, and control mice. As shown in Figure 7C, the 3 mg/kg (1.8 ± 1.5; P = 0.05) and 10 mg/kg...
(2.1±1.2; P=0.04) doses both significantly decreased scores in the heart as compared to the control mice (3.25±1.2).

There were trends toward decreased histological scores in the liver (1.8±1.6; P=0.07) and pancreas (3.1±2.4; P=0.06) with the 3 mg/kg dose, but only the 10 mg/kg dose decreased scores by significant levels in the liver (1.55±1.4; P=0.04) and pancreas (2.85±1.5; P=0.006), as compared to the control (3.1±1.1 for the liver and 4.9±1.8 for the pancreas).

**Discussion**

This is the first report of the use of MAPK inhibitors to control virus infection in an in vivo model. Bosentan increased viral load and this drug also augmented p38 MAPK activation via ETAR in the presence of ET1 in HL1 cardiomyocytes, suggesting that this may provide a boost to virus replication by increased p38 activation. However, bosentan prevents the vasoconstriction that is induced by ET1 ligation of ET1 receptors, suggesting that increased circulation and lower cardiac afterload are therapeutic during CVB3 induced myocarditis. We also describe here, for the first time, the use of a well-characterized murine model to study the effect of p38 activation on virus replication in vivo, because any reliable model of signaling should include a means of constitutive activation as well as inhibition. This study characterizes the use of SB203580 to inhibit virus replication in vivo while also identifying a contraindication of an antipressor drug that paradoxically increases viral replication while preserving cardiac output.

We noted that the level of ATF2 phosphorylation resembled the SB203580 inhibition of virus replication and the enhancement of replication by bosentan treatment. However, we were unable to detect variation of ATF2 activation in the hearts of SB203580 HCl-treated mice. Although we could detect enhancement of ATF2 activation in the bosentan-treated mice, the amount of ATF2 activation in the heart during control infection and SB203580 inhibition may have been too subtle for detection. In light of these results, the ability to detect ATF2 activation by bosentan may at test to the profound enhancement of p38 MAPK by this drug.

Our laboratory has shown the requirement of CVB3, the most common cause of viral myocarditis, for activation of extracellular signal-regulated kinase and p38 MAPKs for productive virus replication in vitro. Pharmacological inhibition of these MAPKs greatly reduces CVB3 replication in tissue culture cells, but the effect of this inhibition has not been examined in an animal model. The benefit of altering the host cell’s environment to reduce the suitability of the cell to virus replication is a recently proposed antiviral therapeutic strategy. Moreover, the control of virus replication early, at the onset of symptoms, is desirable because the majority of damage is mediated by virus replication, directly (reviewed elsewhere). We have shown here that MAPK
Inhibition can reduce acute viral replication in vivo. However, we also recognize the need to investigate the long-term effects of this pharmacological intervention.

We noted that the bosentan was more effective in maintaining the integrity of key cardiac parameters, such as EF and FS, than the hydrochloride of SB203580. This is paradoxical because bosentan increased the replication of CVB3 in cardiac tissue. However, the vasodilatory properties of bosentan may increase cardiac circulation, while enhancing virus infection and replication through p38 MAPK activation enhancement. However, any therapeutic strategy would optimally minimize further infection and not make the organ a more suitable site for virus replication. The use of bosentan to treat CVB3 myocarditis may be palliative in the short term; however, there is the possibility of exacerbating disease by making the organ a more suitable locale for virus replication in the long term. One of the reasons we conducted our study over 5 days was to determine whether short-term bosentan treatment could enhance acute viral load. At the same time, this study identifies a need to further investigate the longer-term effects of pharmacological virus load enhancement. It is because of this observation that we would suggest that bosentan be used sparingly for the treatment of viral myocarditis. This also underlines the need to accurately establish the etiology of the myocarditis to prevent the possibility of treatment-induced exacerbation or relapse.

Our results show that p38 MAPK activation occurs via ETRA and not ETRB, which is consistent with what has been shown previously for ET1 activation of p38 MAPK in rat myocytes and consistent with the observation that ETRB is involved primarily in the clearance of ET1 from the circulation. However, we also show that bosentan, an ET1 pressor antagonist, can activate p38 MAPK on its own. Furthermore, it can enhance ET1-mediated p38 activation when administered in combination. This behavior suggests that there may be separate bosentan and ET1-binding sites on the ETRA receptor, a proposition supported by a previous molecular modeling study showing dual and separate binding sites for ET1 and bosentan on ETRA.23 We did not detect any background p38 signaling activity during ET1A inhibition, suggesting that ETRA signaling is the only source of p38 activation during ET1 and bosentan stimulation. Therefore, our results suggest that the p38 MAPK activation and viral load enhancement mediated by bosentan is independent of its ability to antagonize ET1 pressor activity.

With the possibility that present treatments may be exacerbating the causative mechanisms of infectious myocarditis, new therapies are crucial to shut down viral replication as easily as possible such that exacerbating therapies may be avoided in the long term. Altering the signaling profile of the host cell is a desirable strategy to treat virus infection because this makes the host a less favorable environment for virus infection; “the door is shut.”78 Small molecule entry inhibitors for enteroviruses such as Pleconaril have demonstrated resistance by amino acid changes in the structural proteins, but altering the signaling profile alters the fundamental requirements of the virus.24

In conclusion, the ET1 receptor antagonist bosentan in virus-infected mice had beneficial effects on indices of cardiac function but made organs that were normally resistant to CVB3 infection, highly susceptible to CVB3 infection and replication. By contrast, administration of the p38 MAPK antagonist SB203580 in virus-infected mice was associated with benefits to cardiac function, while decreasing viral replication and improving histopathologic changes of myocarditis. These findings suggest that p38 MAPK inhibition is a promising regimen for the therapy of viral myocarditis and warrants further investigation.

Sources of Funding
Supported by Canadian Institutes of Health Research, Michael Smith foundation for health research, and the Heart and Stroke Foundation of Canada.

Disclosures
None.

References


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Circ Res. published online February 12, 2009; Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231 Copyright © 2009 American Heart Association, Inc. All rights reserved. Print ISSN: 0009-7330. Online ISSN: 1524-4571

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