

# Small-Molecule Inhibitors of Signal Transducer and Activator of Transcription 3 Protect Against Angiotensin II–Induced Vascular Dysfunction and Hypertension

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**Abstract**—Angiotensin II (Ang II) is known to promote vascular disease and hypertension in part by formation of cytokines, such as interleukin-6. However, the role of signal transducer and activator of transcription 3 (STAT3) in these processes and Ang II/interleukin-6 signaling is unclear. Using 2 models, we tested the hypothesis that STAT3 is essential for Ang II–induced vascular dysfunction and hypertension. Incubation of isolated carotid arteries from C57BL/6J mice with Ang II overnight increased superoxide  $\approx$ 2-fold and reduced vasodilator responses to the endothelium-dependent agonist acetylcholine by  $\approx$ 50% versus controls ( $P<0.05$ ). These effects were prevented by the addition of small-molecular inhibitors of STAT3 activation (S3I-201 or STATTIC). In vivo, administration of Ang II ( $1.4 \text{ mg kg}^{-1} \text{ day}^{-1}$ ) using osmotic minipumps increased arterial pressure by  $\approx$ 40 mmHg at day 14 compared with vehicle-treated mice, and this effect was prevented by S3I-201 treatment ( $5 \text{ mg/kg IP, QOD}$ ). After systemic treatment with Ang II, dilator responses to acetylcholine were reduced by  $\approx$ 30% to 50% in carotid artery and basilar arteries, whereas S3I-201 treatment prevented most of this impairment ( $P<0.05$ ). In contrast to effects on vascular function and blood pressure, S3I-201 did not prevent Ang II–induced hypertrophy in the carotid artery. These findings provide the first evidence that inhibitors of STAT3 activation protect against Ang II–induced oxidative stress, endothelial dysfunction, and hypertension. Because Ang II promotes vascular disease in the presence of multiple cardiovascular risk factors, these results suggest that selective targeting of STAT3 may have substantial therapeutic potential. (*Hypertension*. 2013;61:437-442.) • [Online Data Supplement](#)

**Key Words:** carotid artery disease ■ cerebral circulation ■ endothelium ■ hypertension ■ NO

The renin–angiotensin system plays a key role in many animal models of hypertension and is a major therapeutic target in patients with hypertension.<sup>1</sup> Most of the detrimental effects of this system are mediated by angiotensin II (Ang II) acting via type 1 ( $AT_1$ ) receptors.<sup>1</sup> In addition to a fundamental role in hypertension, Ang II promotes atherosclerosis and vascular disease in the presence of multiple cardiovascular risk factors, including diabetes mellitus and aging.<sup>2–5</sup>

Inflammatory- and oxidant-related effects of Ang II promote vascular disease and are propagated via intermediate molecules, including interleukin (IL) 6.<sup>6,7</sup> Genetic and pharmacological studies in patients highlight the importance of IL-6 in vascular disease, although details regarding the signaling mechanisms involved are lacking.<sup>8</sup> The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway can be driven by  $AT_1$  or IL-6 receptors, and at least some key vascular effects of Ang II are dependent on the expression of IL-6.<sup>6,9</sup> Multiple STAT family members are targets of JAK, of which STAT3 represents one potential effector.<sup>10,11</sup> Once phosphorylated, STAT3 hetero- or homodimers translocate to the nucleus and affect transcription.<sup>11</sup> Progress

in defining the importance of STAT3 in vascular disease has been hampered by a lack of genetic models or specific pharmacological inhibitors.<sup>12–14</sup>

Using virtual screening, S3I-201 was discovered to be an inhibitor of the Src homology 2 (SH2) domain of STAT3 needed for dimerization.<sup>13</sup> Unlike agents such as AG490 that inhibit JAK (thus acting upstream of STATs), S3I-201 selectively inhibits STAT3 SH2–dependent complex formation, STAT3 dimerization, and STAT3–dependent gene transcription.<sup>13–15</sup> STATTIC is a second, structurally distinct molecule that inhibits STAT3 activation, regardless of its phosphorylation state.<sup>14</sup>

Our limited understanding of mechanisms that regulate oxidative stress and inflammation in vascular cells hampers the development of new approaches to inhibit the progression of vascular disease and subsequent clinical events. Because Ang II plays a fundamental role in promoting vascular disease, we examined the hypothesis that inhibitors of STAT3 would protect against Ang II–induced oxidative stress, vascular dysfunction, and hypertension. In relation to end-organ damage, we studied carotid arteries and cerebral arteries because disease in these vascular segments is a

Received October 11, 2012; first decision November 16, 2012; revision accepted November 28, 2012.

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The online-only Data Supplement is available with this article at <http://hyper.ahajournals.org/lookup/suppl/doi:10.1161/HYPERTENSIONAHA.111.00299/-/DC1>.

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*Hypertension* is available at <http://hyper.ahajournals.org>

DOI:10.1161/HYPERTENSIONAHA.111.00299

major cause of stroke and a potential contributor to cognitive impairment. Our findings provide the first evidence that small-molecule inhibitors of STAT3 protect against key elements of Ang II–induced vascular disease, as well as hypertension.

## Methods

### Animals

Adult male C57BL/6J mice (4–6 months old, 25–33 g body weight) were used. Protocols were in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health) and approved by the Institutional Animal Care and Use Committee at the University of Iowa.

### Direct Effects of Ang II on the Vasculature

After euthanasia with pentobarbital (100 mg/kg IP), carotid arteries were removed, cleaned, cut into rings, and placed in culture wells at 37°C for 22 hours.<sup>6,16,17</sup> Details regarding the culture media are described elsewhere.<sup>17</sup> Individual wells were treated with vehicle (saline or <0.1% dimethyl sulfoxide), S3I-201 (10 μmol/L; Calbiochem), Ang II (10 nmol/L; Sigma), or a combination of S3I-201 and Ang II. In some studies, STAT3C (1 μmol/L; Sigma) was used instead of S3I-201. In other experiments, vessels were incubated with lipopolysaccharide (LPS, *Escherichia coli*, serotype 026:B6; Sigma [0.5 μg/mL]) instead of Ang II.<sup>16</sup>

After incubation, arteries were suspended in organ baths.<sup>6,16–19</sup> To evaluate endothelial function, responses to acetylcholine were measured after precontraction (≈50%–60% of maximum) using U46619. Nitroprusside was used to assess endothelium-independent relaxation. Tempol (1 mmol/L; Sigma), a superoxide scavenger, was used to determine whether vascular dysfunction was mediated by superoxide.

### Superoxide Measurements

Superoxide levels were measured in aorta after incubation (described above) using lucigenin-enhanced (5 μmol/L) chemiluminescence.<sup>6,17,20</sup> Vessels were then homogenized, and total protein was quantified using a Bio-Rad DC assay.

### Immunoblotting

To extract protein, vessels were pulverized in liquid N<sub>2</sub>, and then immersed in cell lysis buffer (1% [vol/vol] NP-40, 5% Na deoxycholate, 0.1% sodium dodecyl sulfate in PBS) with protease (Complete Mini; Roche Diagnostics) and phosphatase inhibitors (PhosSTOP; Roche Diagnostics). Protein was electrophoresed using standard protocols. Primary antibodies used were rabbit anti-STAT3 (1:1000; Cell Signaling), rabbit anti-phospho-STAT3 Y705 (1:1000; Cell Signaling), rabbit anti-phospho-STAT3 S727 (1:1000; Santa Cruz Biotech), or rabbit anti-α-actin (1:2000; Sigma). Protein abundance was calculated using Image J software (National Institutes of Health).

### Ang II–Dependent Hypertension

After anesthesia with ketamine/xylazine (87.5 and 12.5 mg/kg IP, respectively), osmotic minipumps (Alzet) containing saline or apressor dose of Ang II (1.4 mg kg<sup>-1</sup> day<sup>-1</sup> for 14 days) were implanted subcutaneously. In addition, animals were administered vehicle or S3I-201 (5 mg/kg IP) every other day for 14 days. In previous studies, this dose of S3I-201 was well tolerated and induced regression of tumor xenografts with constitutively active STAT3.<sup>13</sup> Blood pressure was measured using tail-cuff plethysmography.<sup>6,20,21</sup> After these treatments and anesthesia, carotid arteries were removed and placed in organ baths as described above. Basilar arteries were isolated, cannulated, and pressurized to 60 mm Hg so that the lumen diameter could be measured. To examine dilator responses to acetylcholine, A23187, and papaverine, arteries were first constricted by ≈30% (≈60% of the response to 50 mmol/L KCl) with U46619.<sup>4,19</sup>

### Vessel Cross-Sectional Area

Carotid arteries were fixed in situ in anesthetized mice using topical 4% buffered paraformaldehyde as described.<sup>6</sup> Vessels were then embedded in paraffin, sectioned, stained with hematoxylin and eosin, and images were stored for analysis using Image J. The total area measured included vascular muscle, endothelium, and the lumen, excluding adventitia. The cross-sectional area of the vessel wall was then determined by subtracting the luminal (inner) area from total arterial (outer) area.

### Quantitative Real-Time Reverse Transcriptase Polymerase Chain Reaction

RNA from carotid arteries was prepared using the RNeasy (Qiagen) method after extraction with TRIzol reagent (Invitrogen). Reverse transcription of RNA was performed using random hexamers as primers. Expression levels of select mRNAs (Table S1 in the online-only Data Supplement) were determined by quantitative real-time reverse transcriptase polymerase chain reaction using the TaqMan method (Applied Biosystems). A housekeeping gene, β-actin was used in dual-plex (VIC-MGB 4352341E) as an internal control within each sample, and C<sub>t</sub> values were found to be consistent among all groups. Relative fold expression was calculated using the ΔΔC<sub>t</sub> method.

### Statistics

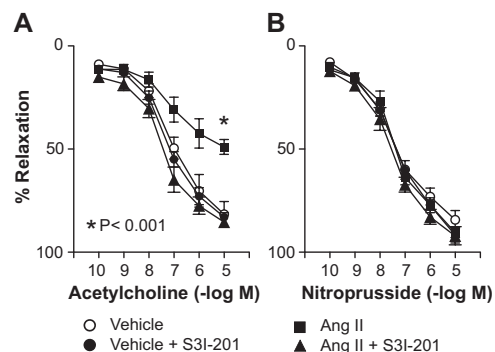
All values are expressed as mean±SE. Data analysis was performed using repeated-measures ANOVA followed by the Tukey or Student-Newman-Keuls post hoc test. When only 2 comparisons were made, a 2-tailed *t* test was used. *P*<0.05 was considered significant.

## Results

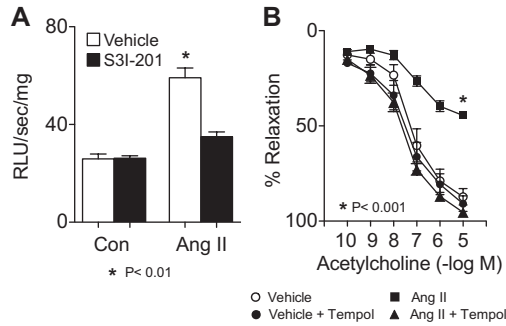
### Ang II–Induced Endothelial Dysfunction Is Prevented by Inhibitors of STAT3 Activation

To first test our hypothesis, we used an in vitro model of Ang II–induced vascular dysfunction. Relaxation of carotid arteries to acetylcholine was not altered by S3I-201 alone but was substantially reduced by Ang II (Figure 1A). S3I-201 prevented Ang II–induced vascular dysfunction. Responses to nitroprusside and U46619 were similar in these groups (Figure 1B and Figure S1), indicating that effects of Ang II were endothelium-specific.

To further evaluate the importance of STAT3, a second inhibitor was used.<sup>14</sup> Treatment with STAT3C alone did not affect responses to acetylcholine, but STAT3C prevented effects of Ang II on endothelial function (Figure S2). STAT3C did not alter responses to nitroprusside or U46619 (Figure S2).



**Figure 1.** Responses of carotid arteries (n=7) to acetylcholine (A) and nitroprusside (B) after overnight incubation with vehicle or angiotensin (Ang II) in the presence or absence of S3I-201. \**P*<0.001 vs vehicle at the highest concentration of acetylcholine.



**Figure 2.** Superoxide levels (A) in aorta treated with vehicle or angiotensin (Ang) II in the presence or absence of S3I-201 ( $P < 0.01$ ;  $n = 5$ ). Effects of tempol (B) on responses of carotid arteries to acetylcholine after overnight treatment with vehicle or Ang II ( $n = 5$ ).  $*P < 0.001$  vs vehicle.

### S3I-201 Did Not Alter Vascular Effects of LPS

Incubation with LPS impaired acetylcholine-induced vasodilation (Figure S3). In contrast to effects in Ang II-treated vessels, S3I-201 failed to protect against LPS-induced endothelial dysfunction (Figure S3). Responses to nitroprusside and U46619 were similar in each of these groups (Figure S3).

### STAT3 Contributes to Ang II-Induced Oxidative Stress

Effects of Ang II on endothelial function were prevented by tempol (Figure 2). In contrast, tempol had no effect on responses to nitroprusside or U46619 in any group (data not shown).

Vascular superoxide was increased  $\approx 2$ -fold by Ang II compared with treatment with vehicle (Figure 2). S3I-201 had no effect on baseline levels but prevented Ang II-induced increases in superoxide (Figure 2). Increases in superoxide in response to Ang II are mediated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase.<sup>6</sup> To evaluate whether S3I-201 could act directly as an antioxidant or affect the activity of NADPH oxidase, aorta was incubated with Ang II and analyzed for superoxide the following day. Sequential addition of S3I-201 (1–100  $\mu\text{mol/L}$ ) to vessels in the presence of NADPH (100  $\mu\text{mol/L}$ ), to stimulate superoxide formation by NADPH oxidase, produced no significant change in the superoxide signal (data not shown). In contrast, tiron (1 mmol/L) immediately reduced the signal by  $\approx 50\%$  ( $P < 0.05$ ).

### S3I-201 Alters STAT3 Phosphorylation and Expression

Protein was extracted from cytoplasmic fractions of aorta incubated with vehicle or Ang II. Subsets of Ang II-treated aorta were also treated with S3I-201 for 1 hour at the end of the incubation period. Phosphorylated STAT3 was assessed by the ratio of phosphorylated STAT3/total STAT3. Ang II increased phosphorylated STAT3, and this effect was inhibited by S3I-201 (Figure S4). In other experiments, total protein was extracted from aorta incubated overnight with vehicle or Ang II, with or without S3I-201. Total aortic STAT3 protein increased by  $\approx 55\%$  ( $P < 0.01$ ) after treatment with Ang II, and this effect was prevented by S3I-201 (data not shown).

### S3I-201 Treatment Prevents Ang II-Induced Hypertension

Baseline arterial pressure was similar in all groups and averaged  $\approx 110$  mm Hg (Figure 3A). Infusion of Ang II for 14 days increased systolic blood pressure by  $\approx 40$  mm Hg compared with vehicle-infused mice (Figure 3A). S3I-201 caused a small reduction in blood pressure in saline-infused mice but prevented increases in arterial pressure in response to Ang II (Figure 3A).

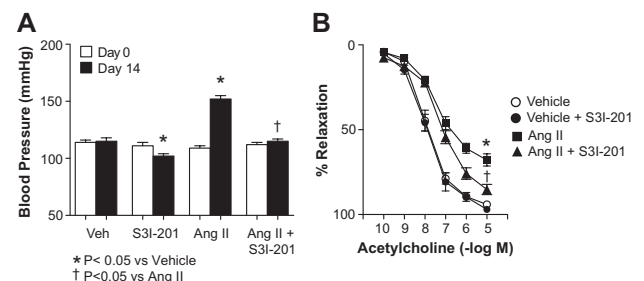
### S3I-201 Protects Against Ang II-Induced Vasomotor Dysfunction Ex Vivo

Acetylcholine produced relaxation of carotid arteries, and this effect was not altered in mice treated with S3I-201 (Figure 3B). Endothelial function was impaired in carotid arteries from Ang II-infused mice, and administration of S3I-201 prevented most of this dysfunction (Figure 3B). Responses to nitroprusside and U46619 were similar in these groups and were not affected by S3I-201 (data not shown).

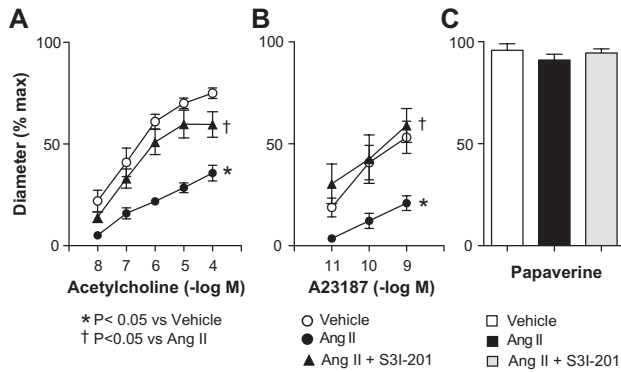
We studied basilar arteries to determine whether STAT3 was a mediator of dysfunction in cerebral arteries, important resistance vessels in brain. Acetylcholine and A23187 caused dilation in basilar arteries from vehicle-treated mice. Both responses were substantially impaired in Ang II-infused mice but were restored largely to normal in mice that received Ang II and S3I-201 (Figure 4A and 4B). Vasodilation to papaverine was similar in arteries from each group (Figure 4C). These data indicate that the protective effects of S3I-201 extend to cerebral arteries (resistance vessels) and involve postreceptor mechanisms (not simply responses mediated by muscarinic receptors).

### S3I-201 Does Not Protect Against Ang II-Induced Vascular Hypertrophy

Treatment with Ang II increased the cross-sectional area of the carotid artery by  $\approx 65\%$  relative to vehicle-infused mice ( $2.66 \pm 0.25$  versus  $4.42 \pm 0.33 \times 10^3 \mu\text{m}^2$ ;  $P < 0.05$ ). In contrast to effects on vascular function, vascular hypertrophy was not reduced by S3I-201 and tended to increase further in mice receiving both Ang II and S3I-201 ( $6.20 \pm 0.52 \times 10^3 \mu\text{m}^2$ ). These data suggest that S3I-201 does not inhibit and may potentiate hypertrophic effects of Ang II, possibly by inhibiting STAT3-dependent negative feedback mechanisms that affect vascular growth.



**Figure 3.** Effects of angiotensin (Ang) II on arterial blood pressure ( $n = 9$ ; A) and responses of the carotid artery to acetylcholine (B) after chronic infusion of Ang ( $1.4 \text{ mg kg}^{-1} \text{ day}^{-1}$ ) in mice treated with S3I-201 or vehicle.  $*P < 0.05$  vs vehicle.



**Figure 4.** Effects of angiotensin (Ang) II on responses of basilar arteries ( $n=5$ ) to acetylcholine (A), A23187 (B), and papaverine (C) after chronic infusion of Ang II ( $1.4 \text{ mg kg}^{-1} \text{ day}^{-1}$ ) treated with S3I-201 or vehicle. \* $P<0.05$  vs vehicle. † $P<0.05$  vs Ang II.

### Vascular Gene Expression

To gain additional insight into mechanisms, we measured the expression of several oxidant- and immune-related genes thought to be important in vascular disease and hypertension (Table S2). Consistent with previous studies, treatment with Ang II increased the expression of IL-6, IL-10, JAK3, suppressor of cytokine signaling-3, inducible NO synthase, p91<sup>phox</sup>, and p47<sup>phox</sup>. These changes were selective because expression of Nox4 and MyD88 was not significantly altered. The expression of anti-inflammatory and JAK/STAT-related genes (IL-10 and suppressor of cytokine signaling-3) was increased further in mice treated with S3I-201 and Ang II. IL-10 is an anti-inflammatory cytokine known to protect against Ang II-induced vascular dysfunction.<sup>20</sup> Suppressor of cytokine signaling-3 can inhibit JAK- and IL-6-mediated signaling. Little is known regarding its potential role in hypertension, although expression of suppressor of cytokine signaling-3 is reduced in pre-eclampsia.<sup>22</sup> A decrease in endothelial NO synthase expression occurred with Ang II treatment, and this effect was prevented by S3I-201. Thus, another protective effect of S3I-201 may involve maintenance of endothelial NO synthase expression.

### Discussion

There are several major new findings in this study. Small-molecule inhibitors of STAT3 dimerization did not affect vascular function or superoxide levels under baseline conditions but protected against Ang II-induced STAT3 phosphorylation, increases in superoxide, endothelial dysfunction, and increases in arterial pressure. Protective effects were seen in 2 different models and using 2 inhibitors of STAT3 activation. S3I-201 has high affinity for the STAT3 SH2 domain, having minimal effects on the most closely homologous SH2 domains in STAT1, STAT5, or the Src family of kinases.<sup>13</sup> STATTIC, a structurally distinct inhibitor of dimerization,<sup>14</sup> had similar effects. In contrast to effects of Ang II on endothelial function, S3I-201 did not prevent Ang II-induced hypertrophy in a large muscular artery. Overall, this work provides the first evidence that STAT3 plays a major role in mechanisms that underlie Ang II-induced endothelial dysfunction and hypertension. The findings support the concept that STAT3 is an essential mediator of Ang II-induced hypertension and some key features of Ang II-induced vascular disease.

We focused on Ang II in this study for several reasons. Ang II plays a key role in many models of hypertension and is a major therapeutic target in patients with hypertension.<sup>1</sup> Endothelial dysfunction produced by Ang II is mediated by AT<sub>1</sub> receptors.<sup>2,21</sup> Ang II has a primary role in promoting atherosclerosis, as well as vascular disease during aging, diabetes mellitus, and other conditions.<sup>2-5</sup> Ang II increases vascular expression of IL-6 (present study),<sup>6</sup> which is essential for Ang II-mediated oxidative stress and endothelial dysfunction.<sup>6</sup> In addition to inhibiting formation of Ang II and AT<sub>1</sub>-mediated effects, recent studies suggest that inhibition of IL-6 and IL-6-related signaling may be an attractive approach to limit vascular disease in people.<sup>8</sup> Many effects of Ang II on the vasculature are independent of hypertension.<sup>2,6,17,21,23</sup> For these reasons, we studied direct effects of Ang II on the vessel wall in these experiments.

We studied endothelial function because abnormalities in this cell type are key contributors to both the onset and the progression of vascular disease.<sup>2</sup> Disease of the carotid artery and cerebral circulation greatly increases the risk for ischemic stroke and contributes to cognitive impairment.<sup>2,24,25</sup> Carotid arteries are a primary site for development of atherosclerosis, a process in which endothelial dysfunction plays a fundamental role. To determine whether the impact of STAT3 extended into resistance vessels supplying a vital organ, we also studied basilar arteries. Previous work suggested that dilator responses of resistance vessels to acetylcholine in people are a strong predictor of future cardiovascular events.<sup>26</sup>

Consistent with previous work,<sup>6,17,20,27,28</sup> we found that Ang II produced superoxide-mediated oxidative stress and endothelial dysfunction. These effects require production of IL-6 and activation of NADPH oxidase.<sup>6,27,28</sup> Both tempol and structurally distinct inhibitors of STAT3 prevented Ang II-induced superoxide formation and endothelial dysfunction. In addition, Ang II increased the phosphorylation of STAT3 at tyrosine 705, an effect that was prevented by S3I-201. These observations support the concept that Ang II stimulates STAT3-dependent processes. Ang II also increased the expression of total STAT3, another effect that was normalized by S3I-201. This result is consistent with the concept that STAT3 auto-activates the *Stat3* gene via effects on its promoter.<sup>29</sup> Together, these observations suggest that S3I-201 acts by inhibiting STAT3 activation and transcriptional activity in response to Ang II.

Activation of JAK2 by Ang II is well documented; however, the extent that STAT3 contributes to effects of Ang II has been unclear.<sup>30,31</sup> There has been a lack of useful genetic models given that complete STAT3 deficiency produces embryonic lethality.<sup>12</sup> Previously used inhibitors of JAK/STAT-related signaling have off-target effects. For instance, AG490 has been used to define the role of JAK2 in AT<sub>1</sub>-receptor signaling,<sup>9,32,33</sup> oxidative stress,<sup>34</sup> and hypertension.<sup>35</sup> Although AG490 inhibits JAK2, it also affects many other tyrosine kinases.<sup>36-38</sup> Once activated, JAKs have multiple potential targets, including members of the STAT family. Thus, approaches that inhibit JAK2, even if selective, do not provide insight into the importance of STAT3 itself. To avoid these limitations and more directly examine the role of STAT3, we used S3I-201 and STATTIC. An attractive feature

of S3I-201 in this regard is its high selectivity for the SH2 domain of STAT3.

The protection produced by inhibition of STAT3 was specific for Ang II–induced endothelial dysfunction. Like Ang II, LPS increases vascular superoxide and induces endothelial dysfunction.<sup>16</sup> Consistent with this concept, LPS impaired endothelium-dependent vasodilation. However, in contrast to effects on Ang II–induced vascular dysfunction, S3I-201 did not protect against effects of LPS. Unlike Ang II, LPS binds to toll-like receptor 4, activating immune-related responses including expression of inducible NO synthase. Because S3I-201 failed to prevent effects of LPS, STAT3 may not play an essential role in LPS-induced endothelial dysfunction, a result that was not easily predicted. Although inducible NO synthase is an important mediator of endothelial dysfunction in response to LPS,<sup>39</sup> LPS-induced expression of inducible NO synthase is independent of STAT3.<sup>40</sup>

S3I-201 is highly selective in its effects, binding to the SH2 domain of STAT3 and inhibiting its signaling.<sup>13</sup> Despite these findings, we considered the possibility that the compound might also have antioxidant effects. Several lines of evidence suggest this was not the case. First, endothelial dysfunction in response to both LPS and Ang II is mediated by superoxide.<sup>6,16,17,20,28</sup> The observation that S3I-201 did not prevent effects of LPS suggests that its mechanism of action must be independent of direct scavenging of superoxide. Second, S3I-201 did not directly reduce superoxide produced by NADPH oxidase in vessels treated with Ang II. These observations suggest that S3I-201 has no direct oxidant scavenging capability. Whether protective effects of S3I-201 and STAT3 are caused by inhibition of transcriptionally dependent or independent effects of STAT3 is unclear at present. One possible mechanism involves the interaction of monomeric STAT3 with the Rac1 guanine nucleotide exchange factor ARHGEF7 (or  $\beta$ PIX).<sup>41</sup> Thus, pharmacological inhibition of STAT3 dimerization may suppress  $\beta$ PIX/Rac1 activation, resulting in decreased NADPH oxidase activity.<sup>42</sup> Several studies have shown that vascular effects of Ang II require activation of NADPH oxidase.<sup>6,27,28</sup>

Administration of Ang II for 2 weeks increased arterial blood pressure by  $\approx$ 40 mm Hg compared with vehicle-infused mice. These findings are similar to previous work, including studies that measured blood pressure using radiotelemetry.<sup>6,19,21,43</sup> A key new finding was that S3I-201 prevented Ang II–induced hypertension. Thus, findings for both the vasculature and arterial blood pressure support the concept that STAT3 is a key mediator of some Ang II–induced effects.

In conclusion, this study provides molecular, biochemical, and pharmacological data supporting a key role for STAT3 in Ang II–induced vascular disease through its impact on oxidative stress and endothelial function. The data also suggest that STAT3 plays a major role in mechanisms that produce Ang II–dependent hypertension. In contrast to effects related to vasomotor function, S3I-201 did not prevent Ang II–induced hypertrophy of the carotid artery.

## Perspective

Ang II promotes vascular disease as a result of the presence of multiple cardiovascular risk factors. Endothelial dysfunction is a key element in the pathogenesis and clinical impact of

vascular disease. The current findings provide the first evidence that targeted inhibition of STAT3 activation protects against hypertension and key elements of Ang II–induced vascular effects. Overall, the findings suggest that pharmacological or genetic approaches that target STAT3 in vascular cells may have beneficial therapeutic effects to suppress the onset or progression of vascular disease where Ang II is known to play key roles.

## Sources of Funding

This work was supported by grants from the National Institutes of Health (HL-38901, NS-24621, HL-62984, and HL-113863) and the American Heart Association (0575092N and 0910131G).

## Disclosures

None.

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## Novelty and Significance

### What Is New?

- Selectively targeting signal transducer and activator of transcription 3 using small-molecule inhibitors protects against angiotensin (Ang) II-induced oxidative stress, endothelial dysfunction, and hypertension. Protective effects were seen in both carotid arteries and resistance vessels in brain.
- Lipopolysaccharide-induced endothelial dysfunction and Ang II-induced vascular hypertrophy were not dependent on signal transducer and activator of transcription 3 signaling.

### What Is Relevant?

- Ang II promotes vascular disease during hypertension and in the presence of other cardiovascular risk factors.
- Endothelial dysfunction plays a fundamental role in carotid and cerebrovascular disease, major causes of stroke and cognitive impairment.

- Ang II-induced vascular dysfunction requires interleukin-6. Recent studies highlight the importance of these signaling pathways for vascular disease in people.

### Summary

This study provides molecular, biochemical, and pharmacological data supporting a key role for signal transducer and activator of transcription 3 in Ang II-induced oxidative stress and endothelial function. Pharmacological or genetic approaches that target signal transducer and activator of transcription 3 in vascular cells may have beneficial therapeutic effects to suppress the onset or progression of vascular diseases due to cardiovascular risk factors where Ang II is known to play a key role.