Inhibition of Soluble Epoxide Hydrolase Attenuates Bosutinib-Induced Blood Pressure Elevation

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ABSTRACT: Endothelial cells play a critical role in maintaining homeostasis of vascular function, and endothelial activation is involved in the initial step of atherogenesis. Previously, we reported that Abl kinase mediates shear stress–induced endothelial activation. Bosutinib, a dual inhibitor of Src and Abl kinases, exerts an atheroprotective effect; however, recent studies have demonstrated an increase in the incidence of side effects associated with bosutinib, including increased arterial blood pressure (BP). To understand the effects of bosutinib on BP regulation and the mechanistic basis for novel treatment strategies against vascular dysfunction, we generated a line of mice conditionally lacking c-Abl in endothelial cells (endothelial cell-AblKO). Knockout mice and their wild-type littermates (Abf/f) were orally administered a clinical dose of bosutinib, and their BP was monitored. Bosutinib treatment increased BP in both endothelial cell-Abf/f and Abf/f mice. Furthermore, acetylcholine-evoked endothelium-dependent relaxation of the mesenteric arteries was impaired by bosutinib treatment. RNA sequencing of mesenteric arteries revealed that the CYP (cytochrome P450)-dependent metabolic pathway was involved in regulating BP after bosutinib treatment. Treatment with 1-Trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea, a soluble epoxide hydrolase inhibitor, reversed the bosutinib-induced changes to the eicosanoid metabolite profile, endothelium-dependent vasorelaxation, and BP. Thus, the present study demonstrates that upregulation of soluble epoxide hydrolase mediates bosutinib-induced elevation of BP, independent of c-Abl. The addition of soluble epoxide hydrolase inhibitor in patients treated with bosutinib may aid in preventing vascular side effects. (Hypertension. 2021;78:1527–1540. DOI: 10.1161/HYPERTENSIONAHA.121.17548.) • Data Supplement

Key Words: blood pressure • bosutinib • endothelium • incidence • mice, knockout

Endothelial cells (ECs) line the lumen of vascular beds and release vasoactive factors, which are critical for the maintenance of vascular tone and normal blood pressure (BP). Nitric oxide (NO) and arachidonic acid (AA)-derived epoxyeicosatrienoic acid (EETs) are well-described endothelium-derived relaxing factors, whereas ET-1 (endothelin-1) is a strong factor contributing to vessel contraction; the balance of these vasoactive factors maintains endothelial integrity and normal BP. Endothelial dysfunction disrupts this balance, leading to vascular disorders such as hypertension and atherosclerosis.

PTK (protein tyrosine kinase) is one of the major signaling enzymes implicated in tumorigenesis and tumor progression and has emerged as a crucial target for drug discovery. At least 90 PTKs (58 RTKs [receptor tyrosine kinases] and 32 NRTKs [nonreceptor tyrosine kinases]) have been identified. Tyrosine kinase...
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nonstandard Abbreviations and Acronyms

| Acronym | Definition |
|---------|------------|
| AA      | arachidonic acid |
| ACE     | angiotensin-converting enzyme |
| AGT     | angiotensigenon |
| ALT     | alanine aminotransferase |
| AST     | aspartate aminotransferase |
| BP      | blood pressure |
| CML     | chronic myeloid leukemia |
| COX     | cyclooxygenase |
| CYP     | cytochrome P450 |
| EC      | endothelial cell |
| EDHF    | endothelium-derived hyperpolarizing factor |
| EET     | epoxyeicosatrienoic acid |
| ET-1    | endothelin-1 |
| LOX     | lipoxigenase |
| NO      | nitric oxide |
| NRTK    | nonreceptor tyrosine kinase |
| PTK     | protein tyrosine kinase |
| RAAS    | rennin-angiotensin-aldosterone system |
| RTK     | receptor tyrosine kinase |
| SBP     | systolic BP |
| sEH     | soluble epoxide hydrolase |
| SNP     | sodium nitroprusside |
| TKI     | tyrosine kinase inhibitor |
| TPPU    | 1-Trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea |

Novelty and Significance

What Is New?
• Bosutinib dose-dependently increases systolic blood pressure and impairs endothelium-dependent vasorelaxation.
• Inhibition of the CYP-soluble epoxide hydrolase (sEH) pathway by a sEH inhibitor (1-Trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea) attenuates endothelial-dependent vascular dysfunction and elevates blood pressure induced by bosutinib.

What Is Relevant?
• The sEH inhibitor, 1-Trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea, protects against endothelial-relative vasodilation dysfunction and abnormal blood pressure induced by bosutinib, which provides great potential for the application of sEH inhibitors in bosutinib-treated patients with chronic myeloid leukemia with elevated blood pressure.

Summary
Our results demonstrate that endothelial c-Abl is not involved in blood pressure elevation caused by bosutinib, while the CYP-sEH pathway contributes to abnormal blood pressure, which is reversed by a sEH inhibitor, 1-Trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea. These results indicate that 1-Trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea exerts beneficial effects in attenuating vasodilation dysfunction resulting from bosutinib treatment and may represent a novel therapeutic strategy for patients with chronic myeloid leukemia undergoing bosutinib treatment.
endothelial-derived EETs are endothelium-derived hyperpolarizing factors and have strong antihypertensive and antihypertrophic effects in the cardiovascular system.\(^\text{16}\) Different isozymes of EETs have been reported to mediate vasodilative effects.\(^\text{17-20}\) EETs can be further hydrolyzed to less active diols by soluble epoxide hydrolase (sEH); therefore, low EETs expression and high sEH activity or expression may contribute to endothelial dysfunction and cardiovascular diseases such as hypertension and atherosclerosis. Consistently, inhibition of sEH seems to confer cardiovascular protection. Our previous studies demonstrated that sEH inhibition increases EETs levels and markedly reduces arterial blood pressure in the angiotensin II–induced hypertension mouse model.\(^\text{21,22}\) Recent exploration of sEH inhibitors in the treatment of sepsis,\(^\text{23}\) myocardial infarction,\(^\text{24}\) and hypoxic pulmonary vasorestriction\(^\text{25}\) illustrate the novel therapeutic potency of sEH inhibitors. However, it is unclear whether EETs or sEH plays a role in bosutinib-altered BP.

In the current study, we used EC-specific c-Abl knockout mice to demonstrate that high doses of bosutinib increase systolic BP (SBP) and vasodilative dysfunction in an endothelial c-Abl-independent manner. We found that the expression of sEH in the arteries of mice was upregulated by bosutinib. Metabolic profiling revealed that the plasma content of eicosanoid metabolites in the CYP pathway, including EETs, was significantly depleted in bosutinib-treated mice. Treatment with the sEH inhibitor, 1-Trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU), not only improved the bosutinib-induced depletion of eicosanoid metabolites and endothelium-dependent vasorelaxation, but also attenuated bosutinib-induced BP elevation. Altogether, our results suggest that sEH upregulation plays a crucial role in the adverse vascular events induced by bosutinib. The use of sEH inhibitor in patients undergoing bosutinib treatment may help to reduce the vascular side effects.

**MATERIALS AND METHODS**

The authors declare that all supporting data are available within the article (and its Data Supplement).

**Data Availability**

RNA-seq data generated in this study have been deposited in the Genome Sequence Archive in the BIG Data Center under accession number PRJCA004741.

**Cell Culture and Transfection**

Human umbilical vein ECs were isolated and cultured in EC basal medium supplemented with EGM SingleQuots from LONZA (Walkersville, MD), 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL), as previously described.\(^\text{11}\) Cells between passages 5 and 7 were used for the experiments. Human umbilical vein ECs were plated on 6-well plates at 85% confluence and transfected with 10 nmol/L siRNA using Lipofectamine RNAiMAX transfection reagents (MA, Thermo Fisher Scientific). Transfected cells were treated with bosutinib (0.5 μmol/L)\(^\text{26}\) or vehicle for 3 hours, and protein was harvested 24 hours after initial transfection.

**Animals**

Floxed Abl (stock no. 013224), and Cdhl5-cre (stock no. 006137) mice were obtained from the Jackson Laboratory. We established tamoxifen-inducible EC-specific Abl-deficient (EC-Abl\(^\text{-}\)) and littermate control (Abl\(^\text{+}\)) mice. Mice carrying the floxed Abl allele were crossed with mice harboring Cre recombinase under the control of the Cdh5 promoter, which contained a tamoxifen-inducible EC-specific Cre. Tamoxifen (60 mg/kg per day, subcutaneously) was administered once every 24 hours for 5 consecutive days. Male mice on a C57BL/6J background in 8 to 10 weeks old were maintained under a 12:12 hour light/dark cycle (lights on at 7:00 and lights off at 19:00). The investigation conformed to the Guide for the Care and Use of Laboratory Animals by the US National Institutes of Health (NIH Publication No. 85–23, revised in 2011). All study protocols and the use of animals were approved by the Institutional Animal Care and Use Committee of Tianjin Medical University.

**Bosutinib/TPPU Treatment and BP Measurements**

BP was measured using a tail-cuff system (Visitech Systems, Inc). Mice were trained for 3 to 5 days before the experiments to acquaint them with the procedure. Mice were randomly assigned, and intragastrically administered bosutinib or control in a carboxymethylcellulose sodium (CMC Na) vehicle every day for 4 to 7 days. BP values were averaged from at least 10 consecutive measurements for each mouse. For experiments involving TPPU treatment, water was provided ad libitum containing 0.2% polyethylene glycerol (PEG400) with and without TPPU at a concentration of 17.5 mg/L during the treatment period. The mice were administered TPPU at 3 mg/kg per day (drinking) 5 days before the experiment, then initiating bosutinib at 100 mg/kg (by oral gavage) with or without TPPU treatment. Animals that served as controls were given the corresponding vehicle solutions.

**Telemetry for BP Monitor**

BP was measured using implanted radio-telemetry probes (Data Sciences International, Cat No. HD-X11, New Brighton, MN) as described previously.\(^\text{26}\) Eight-week-old male mice were anesthetized with 1% to 2% isoflurane. The left carotid artery was isolated and the probe catheter inserted to a depth of ≈1 cm and secured with sutures. The probe body was placed subcutaneously on the left side of the abdomen. Animals were allowed to recover for 7 days, then were intragastrically administered bosutinib for 4 days. BP was measured before administered (first day), at fourth day after bosutinib treatment and after stopping bosutinib at fifth day, as indicated.

**Isometric Force Measurement in Wire Myograph**

Second-order mesenteric arteries were isolated from 8-week-old male mice and stored in an oxygenated organ chamber
containing physiological salt solution with the following composition (mmol/L): 130 NaCl, 4.7 KCl, 1.18 KH₂PO₄, 1.17 MgSO₄, 0.026 EDTA, 1.6 CaCl₂, 14.9 NaHCO₃, and 5.5 glucose (Danish Myo Technology, Denmark), as previously described.²⁷ Vasoconstriction of mesenteric arteries was evaluated by adding phenylephrine (0.1 nmol/L to 10 μmol/L). Endothelium-dependent relaxation (EDR) was induced by acetylcholine (ACH) (0.01–10 μmol/L) in phenylephrine (3 μmol/L) precontracted segments with or without L-NAME (100 μmol/L) and bosutinib. In some experiments, the mixed 4 kind of EET isoforms (5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET, 0.312 μmol/L) and the sEH inhibitor TPPU (3 μmol/L) were co-incubated with bosutinib (0.5 μmol/L). Cumulative concentration-response curves in response to sodium nitroprusside (SNP) (0.1 nmol/L to 1 μmol/L) were used to assess the endothelial-independent relaxation of vessels.

Metabolomics Profiling

Mouse plasma samples were extracted using solid-phase extraction. First, the samples were spiked with an internal standard mixture (5 ng) and then the extraction column was simultaneously activated by washing with 2 mL of 5% methanol. The analytes were eluted using methanol and evaporated to dryness. The dried remnants extracted using solid-phase extraction and liquid-liquid extraction were dissolved in 30% acetonitrile. To perform liquid chromatography, a 5500 QTRAP hybrid triple quadruple linear ion-trap mass spectrometer (AB Sciex, Foster City, CA) equipped with a turbo ion spray electrospray ionization source was used (ultra-high-performance liquid chromatography/tandem mass spectrometry, UPLC-MS/MS). Metaboanalyst 5.0 (http://www.metaboanalyst.ca) was used for metabolomic data analysis, interpretation, and visualization. In the current study, Partial Least Squares Discrimination Analysis produced a 2-dimensional visual summary of the observed variation in plasma eicosanoid profiles from each group. The calibration curves (linear range, 0.5–2000 ng/mL) were established for each EET molecule relying on their respective standard chemicals. During sample pretreatment, we added 8,9-EET-d₈ (the isotopic substitution of 8,9-EET) as a common internal reference for the 4 kinds of EETs, which was used to correct for errors during sample extraction. Since our method was an absolute quantification, the level of EET was presented as the raw value of the measurement.

Statistical Analysis

Data are represented as the mean±SEM. GraphPad Prism v8.0 (GraphPad Software, San Diego, CA) was used for all statistical analyses. Unpaired Student t test (2-tailed), 1-way ANOVA, or 2-way ANOVA with Bonferroni multiple comparison post-test were used for analyses as appropriate. In all experiments, statistical significance was set at P<0.05.

RESULTS

Bosutinib Dose-Dependently Increases Systolic BP and Impairs Endothelium-Dependent Vasorelaxation

We previously reported that low-dose bosutinib treatment (30 mg/kg every 3 days, IP) for 5 weeks in Apoe−/− mice had an atheroprotective effect and did not affect BP.¹¹ To investigate the BP change associated with a clinically relevant dose of bosutinib, wild-type mice were treated with bosutinib (25, 50, 100, or 150 mg/kg per day) by oral gavage for 5 days. SBP increased by 14.1±2.5 and 12.5±4.0 mm Hg in a time-dependent manner in mice treated with 100 and 150 mg/kg bosutinib but was not affected in mice treated with 25 or 50 mg/kg (Figure 1A). The body weight, heart rates, and cardiac function were unaffected in all mice (Figure S1A through S1D in the Data Supplement). As 100 mg/kg of bosutinib is similar to that through drug exposure in patients with CML,²⁸ we chose this dose for subsequent experiments. Radiotelemetry was conducted to monitor daily changes in SBP at fourth day after 100 mg/kg of bosutinib treatment. The results showed a typical circadian variation in SBP with higher BP at night when mice are active, and a similar result of BP elevation (delta SBP 10.7±1.2 mm Hg) in mice with bosutinib was detected (Figure 1B).

Treatment with bosutinib markedly impaired acetylcholine (ACh)-induced EDR in second-order mesenteric arteries when compared with that in control mice (Figure 1C and 1D), without affecting vasoconstrictive responses to phenylephrine (Figure 1E). Endothelium-independent vasodilation with SNP was similar between the 2 groups (Figure 1C and 1E). These results suggest that bosutinib-impaired EDR results from EC dysfunction.

Bosutinib-Induced Impairments Are Independent of c-Abl in ECs

Our previous study revealed that bosutinib inhibits the endothelial activity of c-Abl and exerts an atheroprotective effect.¹¹ To further explore whether endothelial c-Abl contributes to bosutinib-induced BP elevation, tamoxifen-inducible, EC-specific c-Abl-deficient (EC-cAblKO) mice were generated by crossing mice homozygous for IfoxP-flanked Abl (Abfl/KO) with mice expressing Cre recombinase under the control of the Cdh5 promoter (Figure S2A and S2B). En face staining confirmed a knockdown efficiency of c-Abl in ECs of ≈75% (Figure S2C and S2D). The results of the telemetry of BP monitor showed a similar pattern as Figure 1B, the SBP was higher in mice with bosutinib treatment regardless the presence or absence of endothelial c-Abl. Furthermore, the increased SBP was returned to normal at the fifth day after stopping bosutinib (Figure 2A). Similarly, the increased SBP caused by bosutinib was still present in mice with c-Abl deficiency for 7 days, measured with tail-cuff method (Figure S2E), whereas diastolic BP, mean arterial pressure, heart rates, and cardiac function were not different between groups (Figure S3A through S3F). Furthermore, EDR of mesenteric arteries was blocked in both EC-cAblKO and control mice treated with bosutinib (Figure 2B), whereas the responses to SNP and phenylephrine were
similar in all groups (Figure 2C and 2D). It was reported that bosutinib administration might be associated with a reversible decline in renal function, renal function was evaluated as the ratio of kidney weight to body weight and was unanimous in all mice, which was proportional to creatinine, blood urea nitrogen, and cystatin C.
Figure 2. Bosutinib-impaired acetylcholine (ACh)-induced relaxation of mesenteric arteries is independent of endothelial c-Abl.

A. Mean SBP measurements during 12 h light/dark cycles over 72 h (before bosutinib at first day, bosutinib treatment at fourth day, and stopping bosutinib at fifth day). Data represent mean±SEM, *P<0.05 (2-way ANOVA with Bonferroni multiple comparison post-test), n=5. B. Abf/f and EC-AbfKO mice were administrated with bosutinib or control, mesenteric arteries were isolated from mice pretreatment with L-NAME (100 μmol/L) in response to ACh. Data represent mean±SEM, *P<0.05 (2-way ANOVA with Bonferroni multiple comparison post-test), n=7. C. Endothelium-independent relaxation in response to SNP in Abf/f and EC-AbfKO mice intragastrically administrated with bosutinib. Data represent mean±SEM, n=7. D. The vasoconstrictive response to phenylephrine (PE). Data represent mean±SEM, n=7.
concentrations in the plasma (Figure S4A through S4D). These results suggest that short term of bosutinib (within 7 days) has no effect on renal function in mice. Furthermore, as bosutinib is associated with acute liver injury, we measured liver function and found that ALT (alanine aminotransferase) and AST (aspartate aminotransferase) levels were unchanged between genotypes (Figure S4E and S4F). Overall, these results suggest that vascular complications resulting from bosutinib treatment are independent of c-Abl in ECs.

To explore the potential mechanism by which bosutinib increases BP, we treated the mesenteric arteries of the mice with the NOS inhibitor, L-NAME, and assessed ACh-induced EDR. EDR was reduced in arteries treated with L-NAME, but not abolished the differences in EDR between the 2 genotypes (Figure 2B). Additionally, we measured the total levels of NO in plasma and the expression and phosphorylation of eNOS in vivo and in vitro. No differences were observed among groups in total NO in the plasma or immunofluorescent staining of eNOS (Figure S5A through S5C). Moreover, neither c-Abl knockdown by siRNA nor bosutinib treatment in vitro affected the phosphorylation of eNOS in human umbilical vein ECs (Figure S5D through S5F). The rennin-angiotensin-aldosterone system (RAAS) is a master regulator of BP. To determine the potential role of RAAS in mediating bosutinib-induced BP changes, we measured mRNA expression of angiotensinogen (Agt), renin, and angiotensin-converting enzyme (Ace) in the liver, kidney, and lung. We observed no differences among all groups (Figure S6A through S6C). Moreover, there were no significant differences in the protein levels of AGT (angiotensinogen), renin, angiotensin II, aldosterone, or ACE (angiotensin-converting enzyme) in the plasma among all groups (Figure S6D through S6I). These results revealed that NO production and RAAS were not involved in bosutinib-induced BP elevation.

**Bosutinib Upregulates the Expression of sEH in the Arteries and Reduces Epoxy-Eicosanoids in the Plasma of Mice**

To further explore the mechanisms involved in bosutinib-elevated BP, we performed RNA sequencing (RNA-seq) on the mesenteric arteries of mice treated with bosutinib. We observed several differentially expressed genes, including 646 upregulated and 770 downregulated genes (Figure 3A). Gene enrichment analysis using kyoto encyclopedia of genes and genomes and gene-set enrichment analysis revealed high enrichment in drug and xenobiotic metabolism by the CYP pathway. Additionally, RNAseq analysis indicated upregulation of some CYP pathway genes, including Cyp1b1, Cyp2d22, Cyp4f10, and Cyp4f14, following bosutinib treatment (Figure 3B and Figure S7A and S7B). These observations were verified using qPCR (Figure 3C). Interestingly, the expression of sEH, hydrolytic enzyme of EETs, was upregulated in the arteries of the bosutinib-treated mice, suggesting the involvement of AA metabolism. We further assessed the expression of all enzymes involved in AA metabolism through the CYP pathway in mice, only sEH, but not those to generate epoxy-eicosanoid, was regulated by bosutinib (Figure 3D). We also evaluated the protein levels of sEH in mice treated with different doses of bosutinib, which confirmed the upregulation of sEH at the protein level (Figure 3E and 3F).

Further lipid metabolomic studies were performed on the plasma of the mice. The eicosanoids profile revealed that bosutinib treatment decreased the levels of all 4 isoforms of EET and increased the levels of DHETs and their hydrolyzed diols by sEH, compared with those in the control group (Figure 4A through 4C). The ratio of DHETs/EETs in the bosutinib treatment group increased, suggesting an increase in the activity of sEH (Figure 4D). Furthermore, the plasma levels of epoxyoctadecanoic acids, CYP pathway metabolites from LA, and their hydrolyzed diols dihydroxyoctadecanoic acids, showed a pattern similar to that of EETs and DHETs (Figure 4A, 4B, 4E, 4F). Thus, our data suggest that polyunsaturated fatty acid metabolites from the CYP-sEH pathway may be involved in bosutinib-induced BP elevation.

**Inhibition of sEH Ameliorates Vasodilation Dysfunction Induced by Bosutinib**

To examine the role of EETs and sEH in BP regulation, we investigated the effects of the EET complex and TPPU, the sEH inhibitor, on vascular reactivity. Mesenteric arteries of wild-type mice were preincubated with bosutinib (0.5 μM) for 30 minutes, followed by co-incubation with EETs and TPPU. As shown in Figure 5A, preincubation with bosutinib caused dysfunction of ACh-mediated vasodilation and was reversed by EETs and TPPU; the SNP-mediated vasodilation did not change (Figure 5B). In vivo, bosutinib-induced high SBP gradually decreased to the baseline level with TPPU treatment, while diastolic BP remained unaffected (Figure 5C and 5D). Moreover, TPPU also abolished the dysfunction of vasodilation in mesenteric arteries, isolated from the bosutinib-treated mice, without affecting phenylephrine-induced contraction and SNP-induced endothelium-independent relaxation (Figure 5E and 5F; Figure S8A).

**TPPU Modulates sEH Inhibition and EETs Production**

We identified the content of sEH substrates in the plasma of TPPU- and bosutinib-treated mice. Metabolomic profiling revealed that TPPU significantly reversed the reduction of EETs mediated by bosutinib. Both TPPU alone and together with bosutinib, decreased DHET levels, as expected (Figure 6A through 6C). TPPU decreased the
Figure 3. Epoxigenases may be involved in bosutinib-induced blood pressure (BP) elevation. 

A–D, RNA sequencing was performed from mesenteric arteries from Abf1−/− mice given bosutinib or vehicle, n=3. A, Volcano plot of each group. Blue indicates downregulated genes; red indicates upregulated genes. B, Bubble chart of enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. C, qPCR analysis of Cyp1b1, Cyp2e1, Cyp2d22, Cyp4a10, and Cyp4f14 in mesenteric arteries after bosutinib treatment. Data represent mean±SEM, *P<0.05 (Student t test), n=5. D, The mRNA levels of Cyp2j5, Cyp2j6, Cyp2j28, Cyp2j29, Cyp2j44, and Ephx2 in mesenteric arteries after bosutinib treatment. Data represent mean±SEM, *P<0.05 (Student t test), n=5. E and F, Protein was extracted from the mesenteric arteries of mice treated with different doses of bosutinib. E, Western blot analysis of expression of Ephx2 and GAPDH. F, Quantification of protein expression in (E). Data represent mean±SEM, *P<0.05 (1-way ANOVA with Bonferroni multiple comparison post-test). Protein extracts of mesenteric arteries from 2 mice were pooled as 1 sample, n=3.

The ratio of DHETs/EETs, confirming the decreased activity of sEH (Figure 6D). Furthermore, the plasma levels of epoxyoctadecanoic acids and dihydroxyoctadecanoic acids showed a pattern similar to that of EETs and DHETs (Figure 6A, 6B, 6E, 6F). These results indicate that the utilization of TPPU effectively restores EET levels and reduces...
Figure 4. Bosutinib elevates soluble epoxide hydrolase (sEH) activity and decreases epoxyeicosatrienoic acids (EETs) production.

A. Heat map showing the eicosanoid profile of EETs/DHETs and epoxyoctadecanoic acids (EpOMEs)/DiHOMEs in plasma samples from bosutinib treated Abl-/- mice using LC-MS/MS. B. The VIP scores of metabolites between control and bosutinib groups. Concentration of EETs and DHETs (C) and relative ratio of total DHETs/EETs (D) in plasma of control and bosutinib-treated mice were determined using LC-MS/MS. Concentration of EpOMEs and DiHOMEs (E) and relative ratio of DiHOME/EpOME (F) in plasma of control and bosutinib-treated mice were determined using LC-MS/MS. D and F represent sEH activity. Data represent mean±SEM, *P<0.05 (Student t test), n=6.
Figure 5. Inhibition of soluble epoxide hydrolase (sEH) attenuates vasodilation dysfunction induced by bosutinib. A and B, Mesenteric arteries from wild-type mice were pretreated with bosutinib (0.5 μmol/L) with or without epoxyeicosatrienoic acids (EETs) (0.312 μM) or 1-trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU; 3 μmol/L) for 30 min. Endothelial-dependent relaxation to acetylcholine (ACh) (A) and endothelium-independent relaxation to sodium nitroprusside (SNP) (B) were assessed. Data represent mean±SEM, *P<0.05 (2-way ANOVA with Bonferroni multiple comparison post-test), n=8. C and D, Noninvasive tail-cuff monitoring of SBP (C) and DBP (D) of 8-wk-old Abf−/− mice intragastrically administrated bosutinib with or without 1-Trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU). Data represent mean±SEM, *P<0.05 (Student t test), n=8. E and F, Abf−/− mice were pretreated with bosutinib (100 mg/kg per d) with or without TPPU (3 mg/kg per d) for 4 d, mesenteric arteries were isolated from the mice for endothelial-dependent relaxation in response to ACh (E), and endothelium-independent relaxation in response to SNP (F). Data represent mean±SEM, *P<0.05 (2-way ANOVA with Bonferroni multiple comparison post-test), n=12.
Figure 6. 1-Trifluoromethoxyphenyl-3-(1-propionylpiperidin-4-yl) urea (TPPU) modulates soluble epoxide hydrolase (sEH) inhibition and epoxyeicosatrienoic acids (EETs) production.

A, Heat map showing eicosanoids profile of EETs/DHETs and epoxyoctadecanoic acids (EpOMEs)/dihydroxyoctadecanoic acids (DiHOMEs) in plasma samples from bosutinib (100 mg/kg per d) treated Ablf/f mice with or without TPPU (3 mg/kg per d) using LC-MS/MS. B, The VIP scores of metabolites in each group. Concentration of EETs and DHETs (C) and relative ratio of total DHETs/EETs (D) in plasma of vehicle and bosutinib-treated mice with or without TPPU were determined using LC-MS/MS. Concentration of EpOMEs and DiHOMEs (E) and relative ratio of DiHOME/EpOME (F) in plasma of vehicle and bosutinib with or without TPPU were determined using LC-MS/MS. D and F represent sEH activity. Data represent mean±SEM, *P<0.05 (2-way ANOVA with Bonferroni multiple comparison post-test), n=5.
BP in patients with CML treated with bosutinib. Overall, our results demonstrated that inhibition of sEH could reverse the deceased in plasma levels of EETs, attenuate the vasodilation dysfunction and BP elevation induced by bosutinib. These findings are summarized in the Graphical Abstract.

DISCUSSION

Bosutinib is a second-generation dual Src/Abl inhibitor that exerts an atheroprotective effect at a low dose.11 However, high doses of bosutinib are associated with vascular toxicity including increased arterial BP and cardiovascular effects.33,34 Our results reveal that the CYP-sEH pathway plays a critical role in regulating bosutinib-impaired vasodilation and increased BP. First, a clinical dose of bosutinib (100 mg/kg) increased SBP and impaired endothelium-dependent vasodilation independently of c-Abl. Additionally, RNA sequencing and metabolomic profiling revealed that EETs and sEH are involved in bosutinib-increased BP. Finally, the sEH inhibitor (TPPU) reversed the effects of bosutinib, demonstrating a novel therapeutic approach for patients with CML treated with bosutinib leading to hypertension.

As with most other kinase molecules, some of the adverse effects of TKIs are unrelated to their primary pharmacodynamic activity and are considered off-target effects resulting from unexpected secondary pharmacological properties. Src and c-Abl are key targets of bosutinib and have been verified in numerous studies. Our data showed that bosutinib-induced BP elevation was independent of endothelial c-Abl. It has been previously reported that SBP was lower in Abl knockout mice than that in wild-type mice, likely due to the effects of c-Abl in vascular smooth muscle.36 In our study, we used endothelium-specific c-Abl knockout mice and did not observe differences in BP between genotypes. Consistently, a study by Elizabeth et al did not find changes in BP with endothelial Abl deletion in Arg-null mice.36,37 EDR of mesenteric arteries evoked by ACh was also similar between genotypes, further establishing that c-Abl is not involved in bosutinib-induced BP elevation (Figure 2A and 2B and Figure S2E).

Three generations of TKIs have been developed for the treatment of CML. Although typically well tolerated, TKIs can have unanticipated adverse events, particularly related to BP dysregulation.38 Imatinib was the first TKI to be developed for clinical use, followed by drugs such as nilotinib, dasatinib, bosutinib, and ponatinib. Nilotinib and dasatinib lead to stronger and faster biological responses, than those with imatinib, but result in serious cardiovascular events after long-term treatment.39 Bosutinib and ponatinib share similar target genes and inhibit parallel pathways.40 It has been reported that the administration of ponatinib (5 mg/kg) did not affect SBP or heart rates for 2 to 15 weeks.41 Bosutinib has a 100-fold increased potency toward BCR-ABL1 compared with that with imatinib and has been approved for use in Ph+ CML patients with resistance or intolerance to prior therapy.42 In our previous study, injection of bosutinib (30 mg/kg, IP every 3 days) for 5 weeks into ApoE−/− mice did not affect BP.11 Here, we administered bosutinib (100 mg/kg per day, IG) for 7 days, consistent with clinical practice. These data suggest that different doses of TKIs, especially bosutinib, may play opposing roles in cardiovascular regulation, including BP regulation.

The maintenance of physiological BP levels involves an integrated neurohumoral system that includes the endothelium, RAAS, natriuretic peptides, and circulating metabolites.43 The RAAS is involved in a wide range of processes, including mediation of vasoconstriction, endothelial dysfunction, and pathogenesis of hypertension.44 In our study, we observed no significant changes in RAAS, and kidney and liver function were normal in control mice. The endothelium is a major regulator of vascular tone and secretes a variety of vasoregulatory substances, including endothelium-derived relaxing factors such as NO, EDHFs (endothelium-derived hyperpolarizing factors), and vasoconstrictors such as ET-1.43 However, interruption of NO production via L-NAME had no effect on bosutinib-impaired vasodilatation, suggesting that NO is not involved in bosutinib-induced changes to BP.

RNA-seq of mesenteric arteries helped in identifying gene expression changes related to bosutinib treatment. Our results suggest that bosutinib induced an expression of several genes involved in drug metabolism and the cytochrome P450 signaling pathway (Figure 3A through 3D). Ingenuity pathway analysis revealed that bosutinib has an indirect link with EPHX2 through PTGS2,24,45 JUN,46 cyp2d22,47 and cyp1b148 (data not shown); all of which contribute to hypertension. Furthermore, Cyp2d22 and Cyp1b1 were upregulated with bosutinib treatment in our RNA-seq data (Figure 3E). As an inhibitor of Src/c-Abl kinases, bosutinib can inhibit not only c-Abl but also Src and other tyrosine kinase activity. The signaling pathways other than c-Abl mediated are worth to be explored in the future study.

Numerous studies have demonstrated the involvement of sEH in various physiological processes, which is due to its ability to efficiently hydrolyze AA-derived EETs49 and LA-derived epoxyoctadecenoic acids.50 The active compounds of lipids mediated signaling may alter the metabolic process in the body and obesity plays an important role in endothelial function and cardiovascular disease. We previously reported that sEH was involved in high-fat diet-induced adipose inflammation and fatty liver disease.51 However, no association between bosutinib and obesity has been reported, and the body size was not to be predictive of bosutinib exposure.52 In current study, we focused on the mechanism of the side effect of bosutinib on BP elevation, and discovered the role of sEH upregulation in vascular ECs.

EETs are products of CYP450 epoxygenase that have vasodilatory properties similar to those of endothelium-derived hyperpolarizing factors, especially in the
vascular system; their diols, DHETs, are generally regarded as being less active. In contrast, diols derived from eicosapentaenoic acids, termed dihydroxyoctadecanoic acids, are more potent than their parental epoxides, and display pro-inflammatory properties. In our study, bosutinib treatment was positively correlated with the DHET/EET ratios, which was reversed by TPPU. Supplementation with EETs complex and TPPU alleviated the impairment of EDR induced by bosutinib.

In conclusion, the present study demonstrates that the clinically relevant dose of bosutinib impairs vasodilation and elevates BP by increasing sEH levels and subsequently depleting EETs, which is reversed by TPPU. Using endothelial c-Abl knockout mice treated with bosutinib, we demonstrated that endothelial c-Abl is not involved in the BP elevation induced by bosutinib, indicating that more specific and effective Abl-target inhibitors are urgently needed for patients with CML for long-term TKI treatment. Overall, our results suggest a great potential for the application of sEH inhibitors in bosutinib-treated patients with CML. These results also highlight the need for the development of more specific inhibitors for patients with CML.

Perspectives
This study elucidates the mechanism of elevated BP after bosutinib administration and confirms that sEH inhibitors have the potential to reduce this effect in patients. Overall, we demonstrate that the CYP-sEH pathway may be involved in the off-target side effects of bosutinib. The highlight of this study is that bosutinib causes BP elevation in a c-Abl-independent manner, reminding us to find more specific c-Abl-target drugs. With RNA-seq and metabolomic analysis, we discovered the role of upregulation of sEH by bosutinib, which should have a great potential for the application of sEH inhibitors, and illuminate a new strategy for controlling BP in patients treated with bosutinib.

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