Effective Treatment of Edema and Endothelial Barrier Dysfunction With Imatinib

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Background—Tissue edema and endothelial barrier dysfunction as observed in sepsis and acute lung injury carry high morbidity and mortality, but currently lack specific therapy. In a recent case report, we described fast resolution of pulmonary edema on treatment with the tyrosine kinase inhibitor imatinib through an unknown mechanism. Here, we explored the effect of imatinib on endothelial barrier dysfunction and edema formation.

Methods and Results—We evaluated the effect of imatinib on endothelial barrier function in vitro and in vivo. In human macro- and microvascular endothelial monolayers, imatinib attenuated endothelial barrier dysfunction induced by thrombin and histamine. Small interfering RNA knock-downs of the imatinib-sensitive kinases revealed that imatinib attenuates endothelial barrier dysfunction via inhibition of Abl-related gene kinase (Arg/Abl2), a previously unknown mediator of endothelial barrier dysfunction. Indeed, Arg was activated by endothelial stimulation with thrombin, histamine, and vascular endothelial growth factor. Imatinib limited Arg-mediated endothelial barrier dysfunction by enhancing Rac1 activity and enforcing adhesion of endothelial cells to the extracellular matrix. Using mouse models of vascular leakage as proof-of-concept, we found that pretreatment with imatinib protected against vascular endothelial growth factor–induced vascular leakage in the skin, and effectively prevented edema formation in the lungs. In a murine model of sepsis, imatinib treatment (6 hours and 18 hours after induction of sepsis) attenuated vascular leakage in the kidneys and the lungs (24 hours after induction of sepsis).

Conclusions—Thus, imatinib prevents endothelial barrier dysfunction and edema formation via inhibition of Arg. These findings identify imatinib as a promising approach to permeability edema and indicate Arg as novel target for edema treatment. **(*Circulation*. 2012;126:2728-2738.)**

Key Words: Abl-related gene tyrosine kinase ■ edema ■ endothelium ■ imatinib ■ sepsis

The endothelium tightly controls the exchange of fluid from the circulation to the surrounding tissues. Dysfunction of this barrier leads to uncontrolled fluid extravasation and edema,[1–3] and characterizes life-threatening conditions like sepsis[1] and acute lung injury.[4] Despite high mortality rates—up to 50% in sepsis—no treatment is currently available for endothelial barrier dysfunction and edema.[1] However, in a recent case report we described fast resolution of pulmonary edema on treatment with imatinib.[5]

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Imatinib is a small molecule inhibitor, blocking the ATP-ase activity of the kinases c-Abl, Abl-related gene (Arg/Abl2), platelet-derived growth factor receptor (PDGFR), c-KIT, and discoid domain receptor-1.[6] Thus far, imatinib has found its major application in the treatment of Brc-Abl positive chronic myeloid leukemia and gastro-intestinal stromal tumors,[6] whereas nonmalignant proliferative disorders like lung fibrosis[7] and pulmonary hypertension[8] may form future applications of imatinib. Although designed as a smart drug specifically targeting overactive kinases, imatinib is associated with several side effects. Long-term treatment with imatinib may lead to cardiac failure by inducing cardiomyocyte apoptosis,[9] and, of note, long-term treatment with imatinib was associated with subcutaneous edema.[10]

In the light of these studies the association of imatinib treatment with resolution of edema is surprising. Yet, increas-

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ing evidence indicates that imatinib may protect against edema.[11–13] A second case report revealed clinical improvement of acute lung injury on initiation of imatinib,[11] whereas two experimental studies demonstrated that imatinib protects against brain edema after stroke.[12,13] The mechanism by which imatinib may protect against edema remains largely unclear. The protective effect of imatinib on brain edema was mainly attributed to PDGFR-α inhibition on perivascular astrocytes,[12,13] which is unlikely to explain the protective effects of imatinib observed in pulmonary edema. Otherwise, the descriptive character of mentioned case reports[5,11] limited mechanistic interpretation, although an effect of imatinib on endothelial barrier function was suggested.[5]

Little is known about the direct effects of imatinib on the endothelial barrier as main regulator of fluid exchange. In the current study we hypothesized that imatinib reduces edema formation via direct preservation of endothelial barrier integrity. Using in vitro and in vivo models of endothelial barrier dysfunction, we show that imatinib effectively protects against endothelial barrier dysfunction and edema formation.

Methods

Endothelial Barrier Function Assays

Endothelial barrier function was evaluated with horseradish peroxidase (HRP) passage and electric cell-substrate impedance sensing. For measurement of HRP passage, confluent cells were seeded in 1:1 density on 0.33 cm^2 Costar polycarbonate filters, pore-size 3.0 μm (Corning, Lowell, MA), and grown to confluence in 5 days. For pretreatment, pharmacological inhibitors or vector were dissolved in M199 (Biowhittaker/Lonza, Verviers, Belgium) supplemented with 1% human serum albumin (HSA; Sanquin Blood Supply, Amsterdam, The Netherlands), and added to the upper compartment of the filters during 60 minutes. For stimulation, pretreatment medium was changed for 1%HSA/M199 containing designated inhibitors, HRP 5 μg/mL (Sigma Aldrich, Zwijndrecht, the Netherlands) and thrombin 1 U/mL (Sigma Aldrich). 1%HSA/M199 was added to the lower compartment. At indicated time points, samples were taken from the lower compartment. The HRP concentration was detected by measuring chemoluminiscence after addition of TMB/E (Upstate/Millipore, Temecula, CA).

For electric cell-substrate impedance sensing measurements, cells were seeded in 1:1 density on gelatin-coated electric cell-substrate impedance sensing arrays, each containing 8 wells with 10 gold electrodes per well (Applied Biophysics, Troy, NY). Culture medium was renewed 24 hours after seeding, and experiments were performed 48 hours after seeding. For pretreatment, pharmacological inhibitors or vector were dissolved in 1%HSA/M199. After 90 minutes of pretreatment, thrombin or histamine were added directly to the wells for final concentrations of 1 U/mL or 10^{-5} mol/L, respectively. During stimulation, resistance was measured at multiple frequencies to allow for calculation of resistance attributable to cell–cell adhesion (Rb) and to cell–matrix interaction (Alpha).[14,15]

Evans Blue/Albumin Extravasation in Mouse Skin

Extravasation of albumin was visualized in the Miles assay by extravasation of Evans blue.[3] Male Balb/cByJ mice (Charles River, 25–30 g) were anesthetized with fentanyl, midazolam, and acepromazine. Anesthetized mice were treated with imatinib mesylate (20 mg/kg in PBS, intraperitoneally) or vector. Five minutes later 150 μL Evans Blue (Merck, Darmstadt, Germany, 0.5% in PBS) was administered via the tail vein and left circulating for 30 minutes. Subsequently, vascular endothelial growth factor (VEGF) was injected intradermally in the back skin. Mice were euthanized after 30 minutes of VEGF stimulation, and circular skin patches (ø 8 mm) from the injection sites were incubated in formamide for 24 hours. Extracted Evans Blue and hemoglobin was measured spectrophotometrically at 610 and 450 nm, respectively. The Evans Blue/hemoglobin ratio is given.

Edema Formation in the Isolated Perfused Mouse Lung

Pulmonary vascular permeability for fluid was analyzed as described earlier.[16] In brief, mixed C57/Bl6 mice (Jackson Laboratories, 25–30 g) were anesthetized with ketamine and xylazine. Anesthetized mice were treated with imatinib mesylate (50 mg/kg in PBS, intraperitoneally) or vector. Thirty minutes after imatinib administration, thrombin receptor-1 activating peptide (TRAP, TFLLRN, 5 mg/kg) was administered via the jugular vein and left circulating for 30 minutes. After 30 minutes, mice were intubated by tracheostomy, and heart and lungs were removed en bloc. The pulmonary artery and left atrium were cannulated, and the heart and lungs were positioned on a weighing scale. The lungs were ventilated and perfused with RPMI1640/HEPES buffer (2 mL/min). After 20 minutes of equilibration the lung weight was zeroed, and the outflow pressure was increased with 8 cm H_2O for 20 minutes while lung weight was monitored. The weight increase of the lungs over time yields the K_{fc} (mL/min/cmH_2O/g) reflecting pulmonary vascular permeability for fluid.

Cecal Ligation and Puncture

Male C57/BL6J mice (Harlan, 25–30 g) were anesthetized with isoflurane (4% vol/vol in air during induction and 1.5%–2% maintenance) and oxygen 0.5 L/min. The abdominal cavity was opened with a 1-cm cut over the medial line, and the cecum was positioned outside the abdominal cavity. For cecal ligation and puncture (CLP), 75% of the cecum was ligated with 5-0 vicryl (Johnson-Johnson Intl, New Brunswick, NJ) and perforated through-and-through with a 21-G needle.[17] After extrusion of a column of 1 mm feces, the cecum was repositioned in the abdominal cavity. For sham surgery, the cecum was only positioned outside the abdominal cavity and repositioned. The abdominal cavity was closed with a continuous ligature through the abdominal muscle wall and single ligatures in the skin. After surgery, mice received fluid resuscitation and analgesia. At t=6 hours and t=18 hours after surgery, mice were treated with imatinib mesylate (50 mg/kg in PBS) or vector by subcutaneous injection in the neck. At t=23 hours, 100 μL Evans Blue (1% in PBS) was administered via the tail vein and left circulating for 1 hour. At 24 hours after surgery, mice were anesthetized with fentanyl, midazolam, and acepromazine, and euthanized by withdrawal of 0.5 to 1 mL blood from the heart. Whole blood was collected in heparinized tubes, centrifuged for 10 minutes at 1800*g* and 4°C. Plasma (75 μL) was added to 150 μL formamide for determination of the Evans Blue concentration in the plasma. The kidneys, liver, and lungs were collected and thoroughly washed in saline. Evans Blue was extracted from organ tissue by incubating organs in 300 μL (kidneys and lungs) or 500 μL (liver lobe) formamide at 55°C. After 48 hours the organs were removed; the remaining formamide was centrifuged (13 500 rpm for 5 minutes) and analyzed spectrophotometrically at 610 nm (Evans Blue) and 740 nm (overlap of hemoglobin in the Evans Blue range). The corrected Evans Blue absorbance was calculated by the following formula: OD610−[1.426×OD740+0.03].[18] After Evans Blue measurement, organs were washed to remove the formamide and air dried at 90°C to determine dry weight. Vascular leakage is presented as the amount of organ Evans Blue absorbance, corrected for organ dry weight and plasma Evans Blue absorbance.

All animal experiments were performed with approval of the Animal Ethical Committees of the VU University Medical Center or the University of Illinois at Chicago.

Statistical Analyses

Data are reported as mean±standard error of the mean (SEM). n refers to the number of independent experiments with cells from different donors, unless stated otherwise. With the hypothesis that imatinib decreases endothelial hyperpermeability via an effect on Arg activation, the effect of interventions (imatinib, siRNAs) on

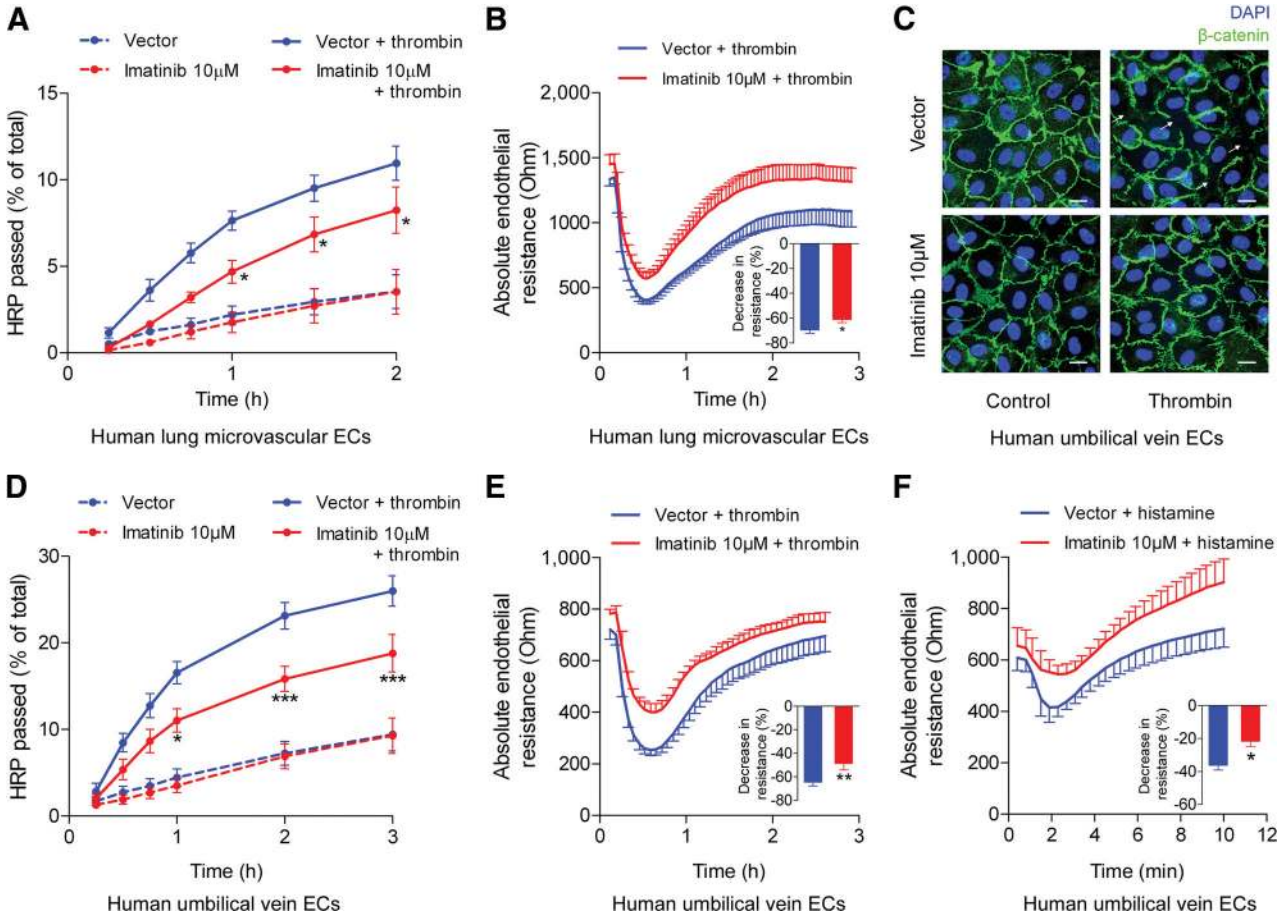

Figure 1. Imatinib protects against endothelial barrier dysfunction. **A**, Macromolecule passage (horseradish peroxidase, HRP) over human pulmonary microvascular endothelial cell (HPMVEC) monolayers during thrombin (1U/mL) stimulation. *$P<0.05$ compared with Vector + thrombin in Bonferroni post hoc test of repeated measures ANOVA (n=3 donors). **B**, Absolute endothelial electric resistance of HPMVEC monolayers during thrombin stimulation. Inset, Quantification of the thrombin response by calculating the maximal drop in resistance (%). *$P<0.05$ (n=9 experiments with cells from 3 donors). **C**, Immunofluorescence imaging of the adherens junction protein β-catenin (green) and the nuclei (blue) in human umbilical vein endothelial cells (HUVECs). **Arrows** indicate presence of intercellular gaps. Scale bars, 10 μm. Representative images of n=3 experiments. **D**, Effects of imatinib on endothelial barrier function under basal and thrombin stimulated conditions as measured by macromolecule passage over HUVEC monolayers. *$P<0.05$, ***$P<0.001$ compared with Vector + thrombin in Bonferroni post hoc test of repeated measures ANOVA (n=4 donors). **E**, Absolute endothelial resistance of confluent HUVEC monolayers during thrombin stimulation. Inset, The thrombin response (% decrease in resistance) in imatinib- versus vector-pretreated cells. **$P<0.01$ (n=5 donors). **F**, Absolute endothelial resistance of confluent HUVECs during stimulation with histamine (10^{-5} mol/L). Inset, Effects of imatinib on the histamine response (% decrease in resistance). *$P<0.05$ (n=4 donors).

endothelial barrier function and activation of specific signaling molecules was tested for statistical significance. For comparison of 2 conditions a Student *t* test was used, for comparison of >2 conditions a 1-way ANOVA with Tukey post hoc test or a repeated measures ANOVA with Bonferroni post hoc test was used when appropriate, as indicated in the figure legends. *P* values <0.05 were considered statistically significant.

Additional methods and materials used for this study can be found in the online-only Data Supplement.

Results

Imatinib Attenuates Disruption of the Endothelial Barrier by Thrombin and Histamine

The direct effect of imatinib on endothelial barrier function was evaluated in isolated human endothelial cell monolayers under basal and stimulated conditions. Short-term treatment of human lung microvascular endothelial cells and human umbilical vein endothelial cells (HUVECs) with imatinib did not affect endothelial barrier function under basal conditions (Figure 1A and 1D). However, imatinib dose-dependently attenuated endothelial barrier disruption by thrombin with an optimal dose at 10 μmol/L in HUVECs (Figure I in the online-only Data Supplement). Imatinib 10 μmol/L effectively protected against endothelial barrier dysfunction, shown by a 46% and 44% reduction in thrombin-induced macromolecule passage (Figure 1A and 1D) and a 9% and 28% attenuation of the thrombin-induced decrease in endothelial electric resistance (Figure 1B and 1E). Immunostaining of the cell–cell junctional proteins β-catenin and VE-cadherin revealed that imatinib prevents the formation of intercellular gaps after thrombin stimulation. (Figure 1C and Figure II in the online-only Data Supplement). Imatinib also attenuated endothelial barrier dysfunction in microvascular endothelial cells isolated from human skin (Figure III in the online-only Data Supplement) and endothelial barrier dysfunction induced by histamine (Figure 1F). Together these data show that imatinib effectively protects against endothe-

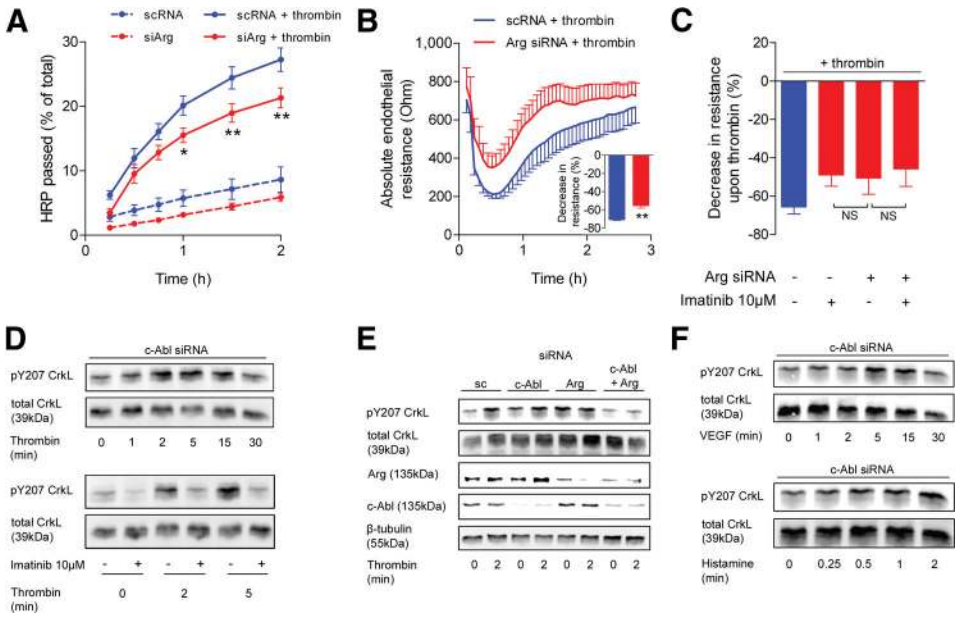

Figure 2. Imatinib protects against endothelial barrier dysfunction through inhibition of Abl-related gene (Arg). **A**, Arg knockdown attenuates thrombin-induced macromolecule passage. Macromolecule (horseradish peroxidase, HRP) passage over wild-type vs Arg-depleted human umbilical vein endothelial cells (HUVECs) under basal and stimulated (thrombin 1 U/mL) conditions. $^{*}P<0.05$, $^{**}P<0.01$ compared with scRNA + thrombin in Bonferroni post hoc test of repeated measures ANOVA (n=4). **B**, Absolute endothelial electric resistance of wild-type vs Arg-depleted HUVECs during thrombin (1 U/mL) stimulation. Inset, The thrombin response (% decrease in resistance) in wild-type vs Arg-depleted cells. $^{**}P<0.01$ (n=4). **C**, The thrombin response (% decrease in resistance) in wild-type or Arg-depleted HUVECs, pretreated with imatinib or vector. NS=non significant in Tukey post hoc test of 1-way ANOVA (n=5). **D**, Western Blot analysis of CrkL phosphorylation at Tyr207 during thrombin stimulation. Upper, Thrombin-induced Tyr207 CrkL phosphorylation in c-Abl depleted HUVECs. Lower, Effects of imatinib on thrombin-induced Tyr207 CrkL phosphorylation. **E**, Thrombin-induced Tyr207 CrkL phosphorylation in HUVECs transfected with scRNA or siRNA against c-Abl, Arg or c-Abl, and Arg. **F**, Western Blot analysis of Tyr207CrkL phosphorylation in c-Abl–depleted endothelial cells. Upper, Tyr207 CrkL phosphorylation in c-Abl depleted HUVECs stimulated with VEGF 10 ng/mL. Lower, Tyr207 CrkL phosphorylation in c-Abl–depleted HUVECs stimulated with histamine 10^{-5} mol/L. scRNA indicates scrambled RNA; siRNA, small interfering RNA.

lial barrier dysfunction, independent of endothelial cell type or barrier-disruptive agent.

Imatinib Exerts its Protective Effect via Inhibition of the Tyrosine Kinase Abl-Related Gene (Arg)

To elucidate the kinase through which imatinib exerts its protective effect on endothelial barrier function, we performed siRNA knock-downs of the known imatinib-sensitive tyrosine kinases (c-Abl, Arg, PDGFR, c-KIT, discoid domain receptor-1) and evaluated the effects on thrombin-induced endothelial barrier dysfunction. Knock-down of PDGFR-α/β, c-Abl, c-KIT, or discoid domain receptor-1 did not affect the thrombin response (Figure IVA–IVH in the online-only Data Supplement). In contrast, knock-down of Arg attenuated the thrombin-induced increase in macromolecule passage (Figure 2A) and decrease in endothelial resistance (Figure 2B). Knock-down of Arg and treatment with imatinib similarly attenuated the thrombin response, whereas imatinib had no additive protective effect in Arg-depleted cells (Figure 2C), indicating that imatinib exerts its protective effects predominantly via inhibition of Arg. To establish whether Arg is activated during endothelial barrier dysfunction, we measured CrkL phosphorylation at Tyr207 (an exclusive target for c-Abl and Arg[19]) in c-Abl–depleted cells. Thrombin induced robust CrkL phosphorylation in c-Abl–depleted cells, which could be prevented by imatinib or combined c-Abl/Arg knock-down (Figure 2D and 2E). Arg was also activated on stimulation with the barrier-disruptive agents VEGF and histamine (Figure 2F). These findings identify Arg as a novel mediator of endothelial barrier dysfunction. Arg-mediated endothelial barrier dysfunction can be effectively inhibited with imatinib.

Arg Inhibition Prevents Loss of Cell–Matrix Interaction During Endothelial Stimulation

Our next step was to analyze the effect of imatinib on processes regulating endothelial barrier function. A functional endothelial barrier is characterized by low actomyosin tension and stable cell–cell junctions. During endothelial barrier dysfunction increased actomyosin contraction and disruption of cell–cell junctions result in gap formation.[1,2] Tight adhesion of endothelial cells to the subcellular matrix counteracts cell retraction, and as such limits junction disruption and gap formation.[2,20,21] Imatinib did not affect RhoA/Rho kinase activity or calcium-dependent signaling (Figure 3), as main determinants of actomyosin contraction. Moreover, imatinib did not visibly change the morphology of actin fibers (data not shown). Resolving the endothelial resistance measurements into separate components reflecting cell–cell contact and cell–matrix interaction[14,15] displayed that Arg inhibition with siRNA or imatinib predominantly attenuated the loss of cell–matrix interaction during thrombin stimulation (Figure 4A and 4B and Figure V in the online-only Data Supplement). Using immunofluorescence and live-cell imaging of the focal adhesion marker paxillin we indeed observed

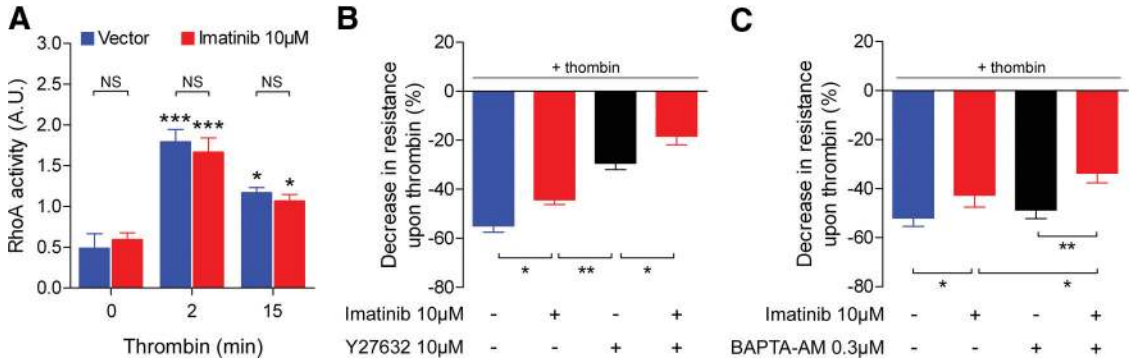

Figure 3. The protective effect of imatinib does not involve the RhoA/Rho kinase pathway and calcium-dependent signaling. **A**, RhoA activity on thrombin (1 U/mL) stimulation in human umbilical vein endothelial cells (HUVECs). Imatinib did not affect thrombin-induced RhoA activation. *$P<0.05$, ***$P<0.001$ compared with 0 minutes, NS=non significant in Bonferroni post hoc test of repeated measures ANOVA (n=3–4). **B**, The thrombin response (% decrease in resistance) in HUVECs treated with imatinib, the Rho kinase inhibitor Y27632, or the combination. Imatinib had an additive effect to Y27632, indicating that imatinib exerts its protective effect independent of Rho kinase activity. *$P<0.05$, **$P<0.01$ in Tukey post hoc test of 1-way ANOVA (n=3). **C**, The thrombin response (% decrease in resistance) in HUVECs treated with imatinib, the intracellular calcium chelator BAPTA-AM, or the combination. Imatinib had an additive effect to BAPTA-AM, indicating that imatinib exerts its protective effect independent of calcium-dependent pathways. *$P<0.05$, **$P<0.01$ in Tukey post hoc test of 1-way ANOVA (n=3). RhoA indicates Ras homolog family member A; and BAPTA-AM, 1,2-bis-(o-Aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid, tetraacetoxymethyl ester.

that imatinib enhanced the formation of focal adhesions, in particular at the cell periphery (Figure 4C and 4D and Figure VI and Movie I in the online-only Data Supplement). Furthermore, the activity of Rac1—a GTPase known to reinforce both cell–matrix interaction[21] and cell–cell junctions[1,2]—was enhanced by imatinib during thrombin stimulation (Figure 4E). Together, these data indicate that imatinib limits Arg-mediated endothelial barrier dysfunction by enhancing Rac1 activity and by enforcing adhesion of endothelial margin areas to the extracellular matrix.

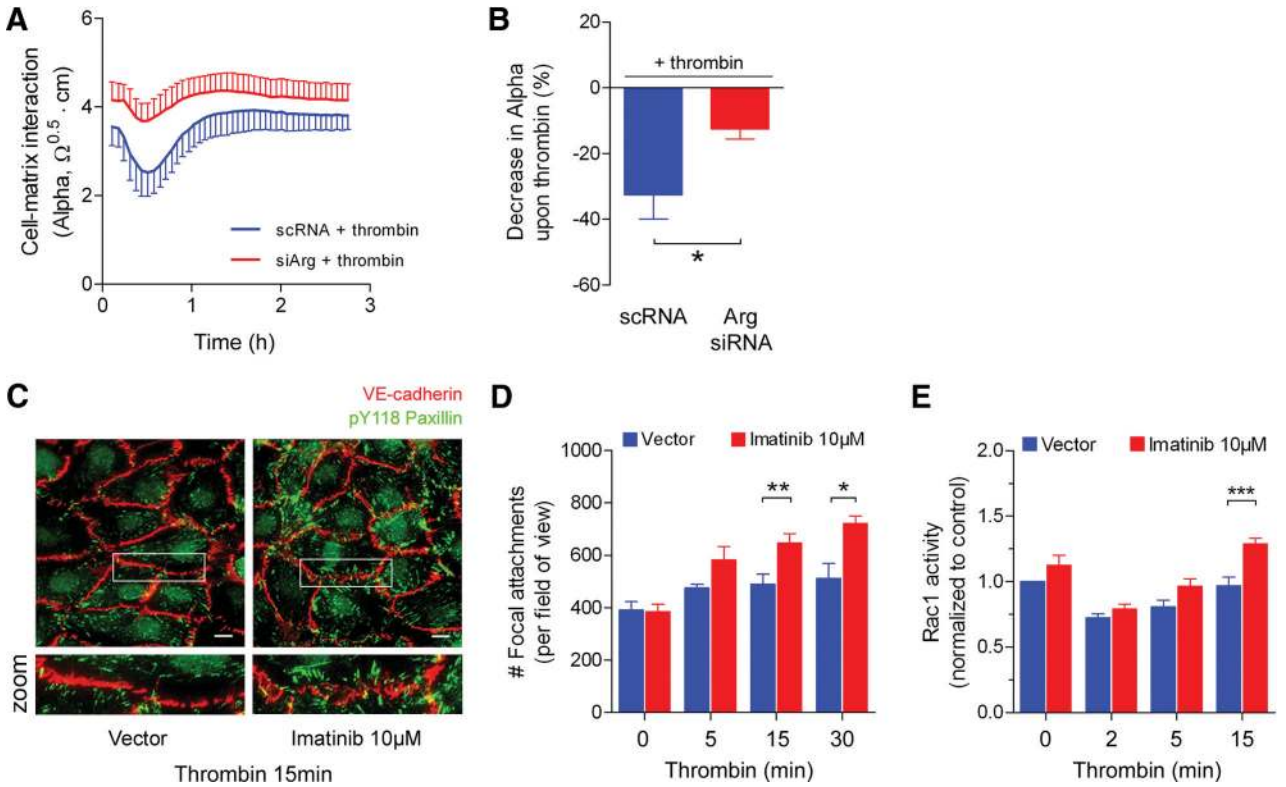

Figure 4. Abl-related gene (Arg) inhibition improves cell–matrix interaction during thrombin stimulation. **A**, Absolute endothelial electric resistance attributable to cell–matrix interaction (Alpha) of wild-type vs Arg-depleted human umbilical vein endothelial cells (HUVECs) during thrombin (1 U/mL) stimulation (n=4). **B**, The effects of thrombin on cell–matrix interaction (%) of wild-type vs Arg-depleted HUVECs. *$P<0.05$ (n=4). **C**, Immunofluorescence staining of pY118 paxillin (green) and VE-cadherin (red) for visualization of focal adhesion (FA) formation. Scale bars, 10 μm. Representative images of n=3 to 4 experiments. **D**, Quantification of the number of FAs during thrombin stimulation as observed during pY118 paxillin staining. *$P<0.05$, **$P<0.01$ in Bonferroni post hoc test of repeated measures ANOVA (n=3–4). **E**, Normalized Rac1 activity in HUVECs during thrombin stimulation. ***$P<0.001$ in Bonferroni post hoc test of repeated measures ANOVA (n=4). scRNA indicates scrambled RNA; siRNA, small interfering RNA.

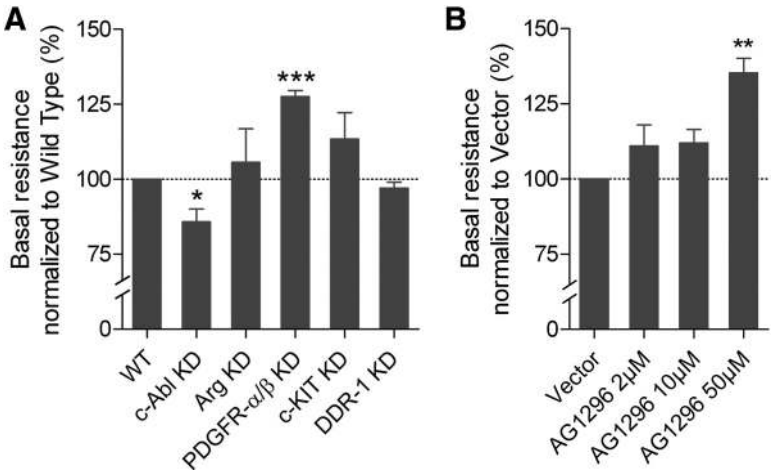

Figure 5. The effect of individual inhibition of the imatinib-sensitive kinases on basal endothelial barrier function. **A**, Basal endothelial electric resistance in human umbilical vein endothelial cells (HUVECs; % of wild type, WT) treated with siRNA against the imatinib-sensitive kinases. KD indicates knock-down. $^{*}P<0.05$, $^{***}P<0.001$ compared with WT in paired *t* test (n=3–5). **B**, The effects of combined platelet-derived growth factor receptor (PDGFR) and c-KIT inhibition on endothelial barrier function. Basal endothelial resistance (% of Vector) of HUVECs treated with the PDGFR/c-KIT inhibitor Tyrphostin AG1296. $^{**}P<0.01$ in 1-way ANOVA with Tukey post hoc test (n=3).

Effect of Imatinib-Sensitive Tyrosine Kinases on Basal Barrier Function

Although imatinib had no effect on basal barrier function (Figure 1A and 1D), we observed that inhibition of individual imatinib-sensitive kinases did change basal endothelial barrier function. Under nonstimulated conditions PDGFR- and c-KIT–depleted endothelial monolayers displayed improved barrier function, whereas c-Abl depletion reduced barrier function (Figure 5A). The finding that simultaneous inhibition of all these kinases (cq, by imatinib) has no effect on basal barrier function, suggests that the barrier-impairing effect of c-Abl inhibition is balanced by the beneficial effect of PDGFR and c-KIT inhibition, rendering a net zero effect on basal endothelial barrier function. To test this, we treated endothelial cell monolayers with Tyrphostin AG1296, a selective inhibitor of PDGFR and c-KIT. Tyrphostin AG1296 enhanced endothelial resistance up to 35% (Figure 5B). These data indicate that under basal conditions c-Abl inhibition opposes the barrier enforcing effects of Arg/PDGFR/c-KIT inhibition, whereas thrombin-induced endothelial barrier dysfunction was only influenced by Arg.

Imatinib Protects Against Vascular Leakage and Pulmonary Edema Formation In Vivo

To establish the protective effect of imatinib on endothelial barrier function in vivo, we tested imatinib in mouse models of vascular leakage and pulmonary edema. VEGF-induced vascular leakage of albumin was measured by intravenous injection of Evans Blue, followed by injection of VEGF in the skin.[3] Vascular leakage was compared between mice pretreated with imatinib and mice pretreated with vector. Imatinib treatment (20 mg/kg) attenuated VEGF-induced extravasation of Evans Blue in the skin by 39% to 55% (Figure 6A and 6B). Next, we measured the effect of imatinib on pulmonary edema formation. Acute pulmonary edema was induced in vivo by intravenous injection of thrombin-receptor activating peptide in mice pretreated with imatinib or vector. Ex vivo, the weight gain of isolated perfused lungs was measured, reflecting the pulmonary vascular permeability for fluid (K_{fc}).[16] Imatinib treatment (50 mg/kg) reduced pulmonary edema formation, shown by 66% reduction of K_{fc} (Figure 6C). Thus, imatinib effectively prevents vascular leakage and edema formation in vivo.

To exclude the possibility that the attenuation of vascular leakage resulted from a smaller hydrostatic pressure difference or a decrease in microvascular perfusion, we measured the effect of imatinib on these parameters in an experimental set-up similar to the Miles assay and the K_{fc} measurements. First, systemic blood pressure was measured using radio telemetry. Blood pressure was monitored before and after administration of imatinib (Figure VIIA in the online-only Data Supplement). Comparing mean arterial pressure 5 minutes before and 30 minutes after administration of imatinib, no effect of imatinib on mean arterial pressure was observed (Figure 6D; Figure VIIA in the online-only Data Supplement). Subsequently, the effect of imatinib on microvascular perfusion was evaluated in skin and muscle by contrast-enhanced ultrasonography. Comparing microvascular blood volume (as measure of microvascular perfusion) before and after administration of imatinib, we found that imatinib did not decrease microvascular perfusion in skin (Figure 6E and 6F; Figure VIIB in the online-only Data Supplement) or muscle (Figure VIIC–VIIE in the online-only Data Supplement), but rather caused a nonsignificant increase.

Together, these experiments indicate that the protective effect of imatinib on vascular leakage cannot be explained by smaller hydrostatic pressure differences or decreased microvascular perfusion. As proof-of-concept these data therefore support the hypothesis that imatinib prevents vascular leakage through a direct protective effect on the endothelial barrier.

Imatinib Treatment Attenuates Vascular Leakage During Sepsis

To evaluate the effect of imatinib in a clinically relevant disease model, sepsis was induced by CLP,[17] and mice were treated with imatinib or vector 6 hours and 18 hours after induction of sepsis (Figure 7A). For evaluation of vascular leakage, Evans Blue was administered intravenously 23 hours after induction of sepsis, and organs were harvested 1 hour after Evans Blue administration. Septic mice treated with vector showed a 2- to 3-fold increase in Evans Blue in the kidneys, which was attenuated by 50% in septic mice treated with imatinib 50 mg/kg (Figure 7B). A similar trend was

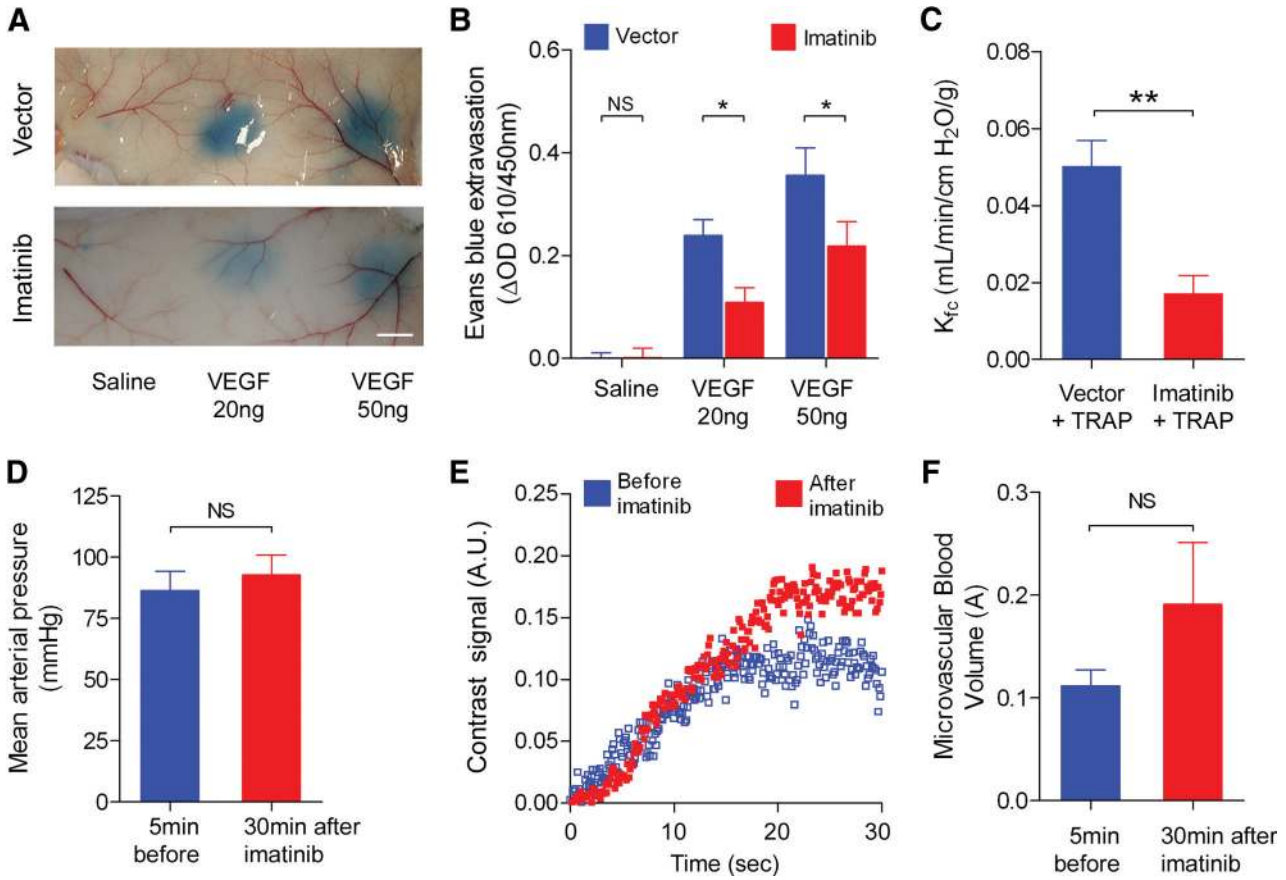

Figure 6. Effects of imatinib on vascular leakage and edema formation in vivo. **A**, Vascular endothelial growth factor (VEGF)–induced vascular leakage of albumin-bound Evans Blue in the back skin of Balb/cJ mice pretreated with saline or imatinib (20 mg/kg). Scale bar, 5 mm. **B**, Quantification of formamide-extracted Evans Blue by spectrophotometrically (610 nm), corrected for hemoglobin (450 nm). NS indicates nonsignificant; $^{*}P<0.05$ (n=6 or 7 mice per group). **C**, Permeability of the lung vasculature (K_{fc}) on thrombin receptor activation in C57/Bl6J mice pretreated with saline or imatinib (50 mg/kg). TRAP indicates thrombin receptor activating peptide. $^{**}P<0.01$ (n=3 or 4 mice per group). **D**, Effect of imatinib administration on systemic arterial blood pressure of mice, measured by radio telemetry. Systemic mean arterial blood pressure was measured before (5 minutes) and after (30 minutes) treatment with imatinib 20 mg/kg. NS indicates nonsignificant in a paired *t* test (n=3 mice). **E**, Analysis of microvascular perfusion of the skin by contrast-enhanced ultrasonography. The graph shows representative contrast replenishment curves in a single mouse before and after treatment with imatinib 50 mg/kg. The plateau of the replenishment curve represents the microvascular blood volume [A] of the skin. Further explanation and details of this analysis are provided in the online-only Data Supplement. AU indicates arbitrary units. **F**, The effect of imatinib 50 mg/kg on microvascular tissue perfusion of the skin, shown by the microvascular blood volume [A] before (5 minutes) and after (30 minutes) treatment with imatinib. NS indicates nonsignificant in paired *t* test (n=3 mice).

observed for the liver, although post hoc analyses did not show a statistical difference between vector- and imatinib-treated mice (Figure 7C). In the lungs, a slight increase of Evans Blue was observed in septic mice treated with vector. This increase was not significant, mainly because not all mice developed vascular leakage in the lungs (Figure 7D). Yet, the number of mice that developed pulmonary vascular leakage on sepsis was significantly higher in vector-treated mice than in imatinib-treated mice (0/5 in the sham group versus 3/6 in the CLP+vector group versus 0/5 in the CLP+imatinib group; $P<0.05$ in a χ^2 test). This animal study demonstrates that imatinib attenuates vascular leakage in a clinically relevant disease model and indicates that imatinib is also effective when imatinib treatment is initiated after induction of disease.

Discussion

Here we show that treatment with imatinib is an effective therapeutic approach to endothelial barrier dysfunction and vascular leakage. Imatinib attenuated endothelial barrier dysfunction in human endothelial cells isolated from multiple origins and stimulated with a variety of barrier-disruptive agents. Specifically, we found that imatinib exerts its protective effects via inhibition of Arg, a thus far unknown mediator of endothelial barrier dysfunction. Imatinib limited Arg-mediated endothelial barrier dysfunction by enhancing Rac1 activity and enforcing adhesion of endothelial cells to the extracellular matrix. The barrier-protective effect of imatinib was established in in vivo models of vascular leakage and pulmonary edema.

Effect of Imatinib on Endothelial Barrier Function

Our finding that short-term treatment with imatinib protects against endothelial barrier dysfunction and edema formation provides first mechanistic insight regarding previous case reports on patients in whom initiation of imatinib treatment was followed by fast resolution of pulmonary edema.[5,11]

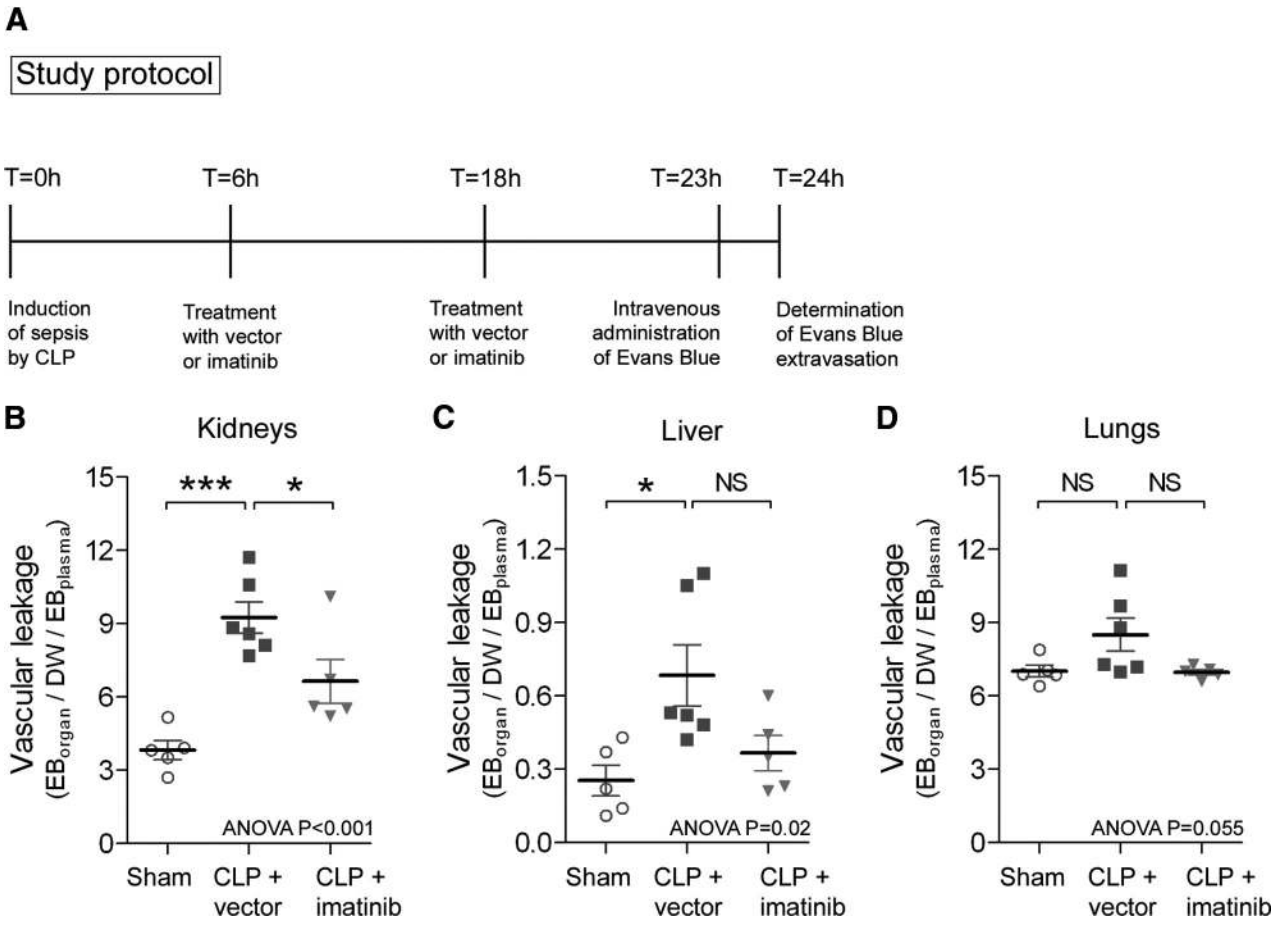

Figure 7. Imatinib attenuates vascular leakage in a murine model of sepsis. **A**, Study protocol to assess the effect of imatinib on sepsis-induced vascular leakage of Evans Blue (EB). Sepsis was induced in male C57/BL6J mice by cecal ligation and puncture (CLP). Vascular leakage of EB was compared between vector- and imatinib-treated mice. Imatinib (50 mg/kg subcutaneous) or vector were administered 6 hours and 18 hours after induction of sepsis. EB was administered after 23 hours (100 μL of 1% EB solution, intravenous), followed by euthanization of the mice at t=24 hours. Vascular leakage of EB was measured in the kidneys, the liver, and the lungs. **B**, Vascular leakage of EB in the kidneys, corrected for organ dry weight (DW) and the EB plasma concentration. *$P<0.05$, ***$P<0.001$ in Tukey post hoc test of 1-way ANOVA (n=5–6 mice per group). **C**, Vascular leakage of EB in the liver, corrected for organ dry weight (DW) and the EB plasma concentration. NS indicates nonsignificant, *$P<0.05$ in Tukey post hoc test of 1-way ANOVA (n=5–6 mice per group). **D**, Vascular leakage of EB in the lungs, corrected for organ dry weight (DW) and the EB plasma concentration. NS indicates nonsignificant in Tukey post hoc test of 1-way ANOVA (n=5–6 mice per group).

Combining in vitro and in vivo measurements of endothelial barrier dysfunction and vascular leakage, we found that imatinib protects against edema formation by enforcing the endothelial barrier. Although edema formation and vascular leakage may also be affected by changes in blood pressure, microvascular perfusion, or vascular remodeling, these factors are less likely to underlie the protective effect of imatinib. Alteration of blood pressure and microvascular perfusion as explanation for edema resolution was excluded in this study, because (1) imatinib did not affect systemic blood pressure in an experimental set-up similar to the Miles assay or K_{fc} measurements, (2) the pressure and the flow in the pulmonary circulation was kept constant in the K_{fc} measurements, and (3) no effects of imatinib on microvascular perfusion were observed. The acute character of the in vivo experiments further excludes chronic vascular remodeling as explanation for the protective effects of imatinib on edema formation and vascular leakage. Therefore, we conclude that imatinib protects against edema formation by preservation of endothelial barrier integrity.

Whereas the Miles assay and the K_{fc} measurements serve as proof-of-concept experiments in which imatinib was given as pretreatment and possible confounders were excluded, the clinical relevance of the protective effect of imatinib on endothelial barrier function was evaluated in a murine model of sepsis (CLP). This experiment mimics the clinical setting, because CLP is considered the most reliable disease model available for sepsis,[17] and because the treatment sequence in this experiment mimicked the clinical sequence of development of disease and subsequent initiation of treatment. In septic mice we found that imatinib reduced vascular leakage of Evans Blue in the kidneys by 50%, resembling the attenuating effect found in the Miles assay. In addition, the number of septic mice developing vascular leakage in the lungs was significantly lower in the imatinib-treated group than in the vector-treated group. As reported previously,[22] a high interindividual variation was observed for vascular leakage in liver and the lungs, which may account for the lack of significance in post hoc analyses.

The optimal protective effect of imatinib on endothelial barrier function was already achieved at concentrations be-

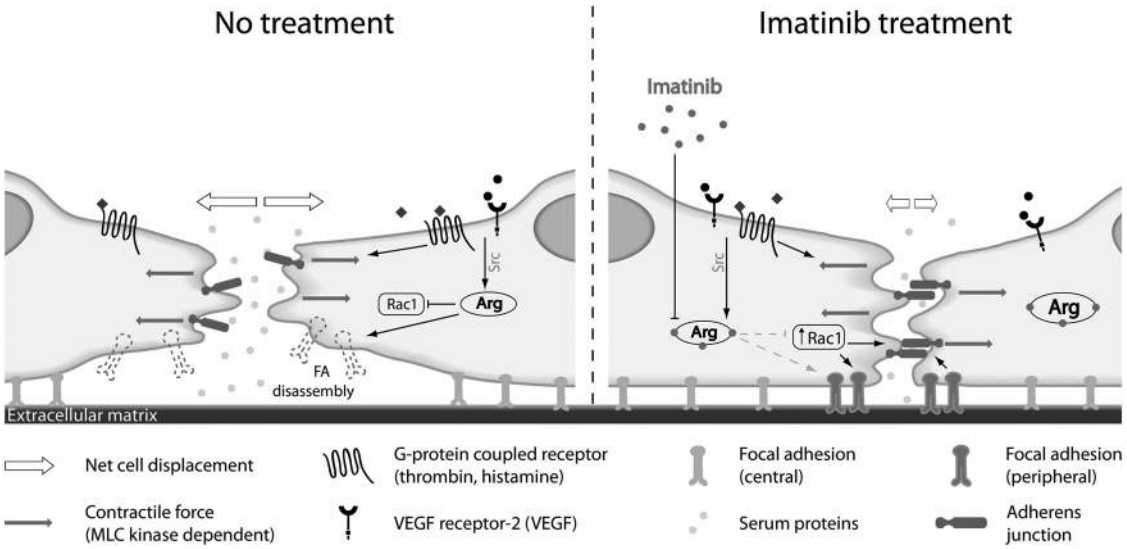

Figure 8. Overview of the protective effect of imatinib during endothelial barrier dysfunction. Imatinib inhibits Abl-related gene (Arg), which is activated upon binding of barrier-disruptive agents to their receptor. Arg activation leads to disassembly of peripheral focal adhesions (FAs) and can be inhibited with imatinib. Peripheral FAs improve cell–matrix interaction and contribute to endothelial barrier integrity by counteracting contractile forces and supporting cell–cell contacts. In addition, Arg inhibition with imatinib enhances Rac1 activity, which supports both cell–cell contacts and cell–matrix interaction.

tween 5 and 10 μmol/L in vitro. These concentrations correlate with plasma levels in patients treated with imatinib for chronic myeloid leukemia[23] or gastro-intestinal stromal tumors.[24] Also, the dosage used in our in vivo experiments resembles imatinib dosages used in the clinical setting. The slight difference in treatment concentration between our in vivo experiments (20–50 mg/kg) and clinical treatment dosage (5–10 mg/kg) is compensated by the higher metabolism and the lower half-life of imatinib in mice ($T_{1/2}$=2–4 hours in mice)[25] compared with human ($T_{1/2}$=18 hours). Therefore, this study not only explains how imatinib may protect against edema, but also proposes imatinib administration as promising approach to edema resulting from endothelial barrier dysfunction.

Role of Arg in Endothelial Barrier Dysfunction

The protective effects of imatinib on endothelial barrier function resulted predominantly from inhibition of the nonreceptor tyrosine kinase Arg. Knock-down of Arg mimicked the effect of imatinib on endothelial barrier function, and imatinib did not have an additive effect in Arg-depleted cells. To the best of our knowledge, this is the first report showing that Arg is involved in endothelial barrier dysfunction. The importance of Arg as mediator of endothelial barrier dysfunction was illustrated by the fact that Arg inhibition with imatinib reduced the thrombin response up to 44%, whereas the finding that Arg is activated on endothelial stimulation with the barrier-disruptive agents thrombin, VEGF, and histamine stresses its relevance.

In search for signaling pathways underlying the barrier-disruptive actions of Arg, we found that inhibition of Arg by genetic knock-down or imatinib treatment prevented the loss of cell–matrix interaction during endothelial stimulation. This was accompanied by enhanced formation of focal adhesions (FAs), particularly at the periphery of the cell. As proposed by Ingber,[20] adhesion of cells to the subcellular matrix is one of the ways for a cell to remain cell shape and counteract contractile forces during cell retraction. Cell–matrix interaction is mainly achieved through FAs, multifaceted protein complexes that connect extracellular matrix proteins to the intracellular cytoskeleton.[2] The spatial distribution of FAs is an important determinant of endothelial barrier function, as redistribution of FAs to the cell periphery has previously been associated with improved endothelial barrier integrity.[21,26] Fibroblast studies have demonstrated that Arg inhibits this redistribution by reducing formation and increasing turnover of peripheral FAs.[27] Compared with wild-type, Arg-deficient fibroblasts show larger and denser FAs, mainly located at the cell periphery.[28] These studies support our finding that Arg inhibition with imatinib increases the number of FAs at the cell periphery.

In addition, we found that imatinib enhanced the activity of Rac1, a GTPase known to enforce both cell–cell interaction[1,2] and cell–matrix interaction.[2,21] Rac1 activity may enforce endothelial cell–cell junctions via mediators like angiopoietin-1.[29] Of note, Rac1 was also described to mediate peripheral accumulation of FAs, thereby enhancing endothelial barrier function.[21] A direct interrelation between Arg, Rac1, and integrin-mediated adhesion was recently suggested in a fibroblast study that demonstrated that Arg inhibits Rac1 activity and integrin function.[30]

Figure 8 shows an overview of the protective effect of imatinib during endothelial barrier dysfunction as proposed in this study. Arg is activated upon binding of barrier-disruptive agents to their receptor. A likely mediator of Arg activation is Src, a tyrosine kinase that is activated by the thrombin receptor and the VEGF receptor[2] and that is able to bind and activate Arg.[31] Arg activation leads to disassembly of peripheral FAs,[27,28] thereby reducing cell–matrix interaction. Imatinib inhibits Arg, which directly leads to preservation of peripheral FAs and improved cell–matrix interaction. In

addition, imatinib enhances Rac1 activation, which in turn improves cell–cell contact and cell–matrix interaction. The enhanced cell–matrix interaction, by supporting cell–cell contacts and counteracting contractile forces, limits cell retraction and gap formation.[20]

Potential Further Improvement of Endothelial Barrier Function by Imatinib Derivatives

Considering previous reports on subcutaneous edema as side effect of imatinib, it is also important to note that in the concentrations used in this study, imatinib did not affect basal endothelial barrier integrity. This difference might first of all be explained by treatment duration. Subcutaneous edema as side effect may result from chronic PDGFR inhibition (several months to years) in pericytes and consequent disturbed vascular support.[32] Second, because c-Abl inhibition impairs endothelial barrier function[33] and imatinib inhibits both Arg and c-Abl, the protective effect of imatinib may depend on the balance of Arg and c-Abl expression in a specific vascular bed. In none of the various macro- and microvascular endothelial cell types that we tested, c-Abl inhibition with imatinib impaired barrier function. This suggests that c-Abl inhibition by imatinib has a limited effect on barrier function. However, the opposing effects of Arg/PDGFR/c-KIT versus c-Abl on endothelial barrier function suggest that imatinib-derivatives lacking c-Abl as target may further improve treatment of endothelial barrier dysfunction.

Clinical Implications

For several reasons, this study may have direct clinical value. Imatinib had an optimal protective effect at 10 μmol/L, which correlates with plasma concentrations in patients on imatinib treatment.[23,24] The barrier protective effect observed in our study was independent of anatomic location, species, endothelial phenotype, and barrier-disruptive agent, indicating a broad applicability of imatinib. Endothelial barrier protection was already achieved after short-term treatment (30 minutes pretreatment in vivo), whereas initiation of imatinib treatment after induction of sepsis was also shown to be effective. This may facilitate edema treatment in acute conditions like sepsis, but also limit side-effects. As noted before, this study elucidates previous clinical observations favoring imatinib treatment in edema.[5,11] Combining our study with these clinical observations provides bench-to-bedside evidence for a protective effect of imatinib on endothelial barrier dysfunction and supports clinical development of imatinib as therapeutic approach to edema.

Conclusion

Thus, imatinib prevents endothelial barrier dysfunction and edema formation via inhibition of Arg. These findings identify imatinib treatment as a promising approach to permeability edema and indicate Arg as novel target for edema treatment.

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Disclosures

None.

References

1. Goldenberg NM, Steinberg BE, Slutsky AS, Lee WL. Broken barriers: a new take on sepsis pathogenesis. *Sci Transl Med*. 2011;3:8825.
2. Mehta D, Malik AB. Signaling mechanisms regulating endothelial permeability. *Physiol Review*. 2006;86:279–367.
3. Weis SM, Cheresh DA. Pathophysiological consequences of VEGF-induced vascular permeability. *Nature*. 2005;437:497–504.
4. Matthay MA, Zemans RL. The acute respiratory distress syndrome: pathogenesis and treatment. *Annu Rev Pathol*. 2011;28:147–163.
5. Overbeek MJ, van Nieuw Amerongen GP, Boonstra A, Smit EF, Vonk Noordegraaf A. Possible role of imatinib in clinical pulmonary veno-occlusive disease. *Eur Respir J*. 2008;32:232–235.
6. Waller CF. Imatinib mesylate. *Recent Results Cancer Res*. 2010; 184:3–20.
7. Daniels CE, Wilkes MC, Edens M, Kottom TJ, Murphy SJ, Limper AH, Leof EB. Imatinib mesylate inhibits the profibrogenic activity of TGF-beta and prevents bleomycin-mediated lung fibrosis. *J Clin Invest*. 2004;114:1308–1316.
8. Schermuly RT, Dony E, Ghofrani HA, Pullamsetti S, Savai R, Roth M, Sydykov A, Lai YJ, Weissmann N, Seeger W, Grimmiger F. Reversal of experimental pulmonary hypertension by PDGF inhibition. *J Clin Invest*. 2005;115:2811–2821.
9. Kerkelä R, Grazette L, Yacobi R, Iliescu C, Patten R, Beahm C, Walters B, Shevtsov S, Pesant S, Clubb FJ, Rosenzweig A, Salomon RN, van Etten RA, Alroy J, Durand JB, Force T. Cardiotoxicity of the cancer therapeutic agent imatinib mesylate. *Nat Med*. 2006;12:908–916.
10. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med*. 2001;344:1031–1037.
11. Carnevale-Schianca F, Gallo S, Rota-Scalabrini D, Sangiolo D, Fizzotti M, Caravelli D, Capaldi A, Anselmetti G, Palesandro E, D'Ambrosio L, Coha V, Obert R, Aglietta M, Grignani G. Complete resolution of life-threatening bleomycin-induced pneumonitis after treatment with imatinib mesylate in a patient with Hodgkin's lymphoma: hope for severe chemotherapy-induced toxicity? *J Clin Oncol*. 2011;29:e691–e693.
12. Su EJ, Fredriksson L, Geyer M, Folestad E, Cale J, Andrae J, Gao Y, Pietras K, Mann K, Yepes M, Strickland DK, Betsholtz C, Eriksson U, Lawrence DA. Activation of PDGF-CC by tissue plasminogen activator impairs blood-brain barrier integrity during ischemic stroke. *Nat Med*. 2008;14:731–737.
13. Armulik A, Genové G, Mäe M, Nisancioglu MH, Wallgard E, Niaudet C, He L, Norlin J, Lindblom P, Strittmatter K, Johansson BR, Betsholtz C. Pericytes regulate the blood-brain barrier. *Nature*. 2010;468:557–561.
14. Lo CM, Keese CR, Giaever I. Cell-substrate contact: another factor may influence transepithelial electrical resistance of cell layers cultured on permeable filters. *Exp Cell Res*. 1999;250:576–580.
15. Moy AB, Winter M, Kamath A, Blackwell K, Reyes G, Giaever I, Keese C, Shasby DM. Histamine alters endothelial barrier function at cell-cell and cell-matrix sites. *Am J Physiol Lung Cell Mol Physiol*. 2000;278: L888–L898.
16. Vogel SM, Orrington-Myers J, Broman M, Malik AB. De novo ICAM-1 synthesis in the mouse lung: model of assessment of protein expression in lungs. *Am J Physiol Lung Cell Mol Physiol*. 2006;291:L496–L501.
17. Rittirsch D, Huber-Lang MS, Flierl MA, Ward PA. Immunodesign of experimental sepsis by cecal ligation and puncture. *Nature Protocols*. 2009;4:31–36.
18. Xu Z, Castellino FJ, Ploplis VA. Plasminogen activator inhibitor-1 (PAI-1) is cardioprotective in mice by maintaining microvascular integrity and cardiac architecture. *Blood*. 2010;115:2038–2047.
19. Smith-Pearson PS, Greuber EK, Yogalingam G, Pendergast AM. Abl kinases are required for invadopodia formation and chemokine-induced invasion. *J Biol Chem*. 2010;285:40201–40211.
20. Ingber DE. Opposing views on tensegrity as a structural framework for understanding cell mechanics. *J Appl Physiol*. 2000;89:1663–1670.

21. Birukova AA, Alekseeva E, Cokic I, Turner CE, Birukov KG. Cross talk between paxillin and Rac is critical for mediation of barrier-protective effects by oxidized phospholipids. *Am J Physiol Lung Cell Mol Physiol*. 2008;295:L593–L602.
22. Raven K. Rodent models of sepsis found shockingly lacking. *Nat Med*. 2012;18:998.
23. Singh N, Kumar L, Meena R, Velpandian T. Drug monitoring of imatinib levels in patients undergoing therapy for chronic myeloid leukaemia: comparing plasma levels of responders and non-responders. *Eur J Clin Pharmacol*. 2009;65:545–549.
24. Demetri GD, Wang Y, Wehrle E, Racine A, Nikolova Z, Blanke CD, Joensuu H, von Mehren M. Imatinib plasma levels are correlated with clinical benefit in patients with unresectable/metastatic gastrointestinal stromal tumors. *J Clin Oncol*. 2009;27:3141–3147.
25. Oostendorp RL, Buckle T, Beijnen JH, van Tellingen O, Schellens JH. The effect of P-gp (Mdr1a/1b), BCRP (Bcrp1) and P-gp/BCRP inhibitors on the in vivo absorption, distribution, metabolism and excretion of imatinib. *Invest New Drugs*. 2009;27:31–40.
26. Shikata Y, Birukov KG, Garcia JG. S1P induces FA remodeling in human pulmonary endothelial cells: role of Rac, GIT1, FAK, and paxillin. *J Appl Physiol*. 2003;94:1193–1203.
27. Peacock JG, Miller AL, Bradley WD, Rodriguez OC, Webb DJ, Koleske AJ. The Abl-related gene tyrosine kinase acts through p190RhoGAP to inhibit actomyosin contractility and regulate focal adhesion dynamics upon adhesion to fibronectin. *Mol Biol Cell*. 2007;18:3860–3872.
28. Peacock JG, Couch BA, Koleske AJ. The Abl and Arg non-receptor tyrosine kinases regulate different zones of stress fiber, focal adhesion, and contractile network localization in spreading fibroblasts. *Cytoskeleton (Hoboken)*. 2010;67:666–675.
29. Hoang MV, Nagy JA, Senger DR. Active Rac1 improves pathologic VEGF neovessel architecture and reduces vascular leak: mechanistic similarities with angiopoietin-1. *Blood*. 2011;117:1751–1760.
30. Li R, Pendergast AM. Arg kinase regulates epithelial cell polarity by targeting beta1-integrin and small GTPase pathways. *Curr Biol*. 2011;21:1534–1542.
31. Bradley WD, Koleske AJ. Regulation of cell migration and morphogenesis by Abl-family kinases: emerging mechanisms and physiological contexts. *J Cell Sci*. 2009;122:3441–3454.
32. Hellberg C, Ostman A, Heldin CH. PDGF and vessel maturation. *Recent Results Cancer Res*. 2010;180:103–114.
33. Dudek SM, Chiang ET, Camp SM, Guo Y, Zhao J, Brown ME, Singleton PA, Wang L, Desai A, Arce FT, Lal R, Van Eyk JE, Imam SZ, Garcia JG. Abl tyrosine kinase phosphorylates nonmuscle myosin light chain kinase to regulate endothelial barrier function. *Mol Biol Cell*. 2010;21:4042–4056.

CLINICAL PERSPECTIVE

Endothelial barrier dysfunction is a major contributor to morbidity and mortality in the critically ill. Loss of the endothelial barrier follows exposure of the endothelium to inflammatory mediators and drives vascular leakage and edema formation. To date endothelial barrier function and vascular leakage still lack appropriate therapy. This study shows that imatinib—an US Food and Drug Administration–approved tyrosine kinase inhibitor—directly protects the endothelial barrier under inflammatory conditions. With the use of endothelial cells isolated from various vascular beds, it was shown that imatinib attenuates the loss of endothelial barrier on stimulation with inflammatory mediators. Imatinib protects against endothelial barrier dysfunction predominantly by inhibition of the tyrosine kinase Abl-related gene (Arg), a novel mediator of endothelial barrier disruption. The effect of imatinib on endothelial barrier was established in various mouse models of vascular leakage. Notably, imatinib attenuated vascular leakage in a murine model of sepsis, even when imatinib treatment was initiated considerable time after induction of sepsis. This study carries important clinical implications. First, imatinib may form a suitable therapy for treatment of diseases characterized by vascular leakage. The longstanding experience with imatinib, together with the fact that imatinib concentrations used in this study parallel plasma values in cancer patients, are apparent advantages in this case. Logical first steps in further development of imatinib involve Phase I and II trials to evaluate safety and efficacy of imatinib in patients with profound vascular leakage. Second, the identification of Arg as a novel and drugable target opens perspectives for more specific pharmaceutical interventions.