Imatinib attenuates cerebrovascular injury and phenotypic transformation after intracerebral hemorrhage in rats

William J. Pearce,1,5 Coleen Doan,1,5 Desirelys Carreón,1,5 Dahlin Kim,1,5 Lara M. Durrant,1,5 Anatol Manaenko,1 Lauren McCoy,4 Andre Obenaus,4 John H. Zhang,1,2,3 and Jiping Tang1

1Department of Physiology and Department of Pharmacology, Loma Linda University School of Medicine, Loma Linda, California; 2Department of Anesthesiology, Loma Linda University School of Medicine, Loma Linda, California; 3Department of Neurosurgery, Loma Linda University School of Medicine, Loma Linda, California; 4Department of Pediatrics, Loma Linda University School of Medicine, Loma Linda, California; and 5Center for Perinatal Biology, Loma Linda University School of Medicine, Loma Linda, California

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Pearce WJ, Doan C, Carreón D, Kim D, Durrant LM, Manaenko A, McCoy L, Obenaus A, Zhang JH, Tang J. Imatinib attenuates cerebrovascular injury and phenotypic transformation after intracerebral hemorrhage in rats. Am J Physiol Regul Integr Comp Physiol 311: R1093–R1104, 2016. First published October 5, 2016; doi:10.1152/ajpregu.00240.2016.—This study explored the hypothesis that intracerebral hemorrhage (ICH) promotes release of diffusible factors that can significantly influence the structure and function of cerebral arteries remote from the site of injury, through action on platelet-derived growth factor (PDGF) receptors. Four groups of adult male Sprague-Dawley rats were studied (n = 8 each): 1) sham; 2) sham + 60 mg/kg ip imatinib; 3) ICH (collagenase method); and 4) ICH + 60 mg/kg ip imatinib given 60 min after injury. At 24 h after injury, sham artery passive diameters (+3 mM EGTA) averaged 244 ± 7 μm (at 60 mmHg). ICH significantly increased passive diameters up to 6.4% and decreased compliance up to 42.5%. For both pressure- and potassium-induced contractions, ICH decreased calcium mobilization up to 26.2% and increased myofilament calcium sensitivity up to 48.4%. ICH reduced confocal colocalization of smooth muscle α-actin (αActin) with nonmuscle myosin heavy chain (MHC) and increased its colocalization with smooth muscle MHC, suggesting that ICH promoted contractile differentiation. ICH also enhanced colocalization of myosin light chain kinase (MLCK) with both αActin and regulatory 20-kDa myosin light chain. All effects of ICH on passive diameter, compliance, contractility, and contractile protein colocalization were significantly reduced or absent in arteries from animals treated with imatinib. These findings support the hypothesis that ICH promotes release into the cerebrospinal fluid of vasoactive factors that can diffuse to and promote activation of cerebrovascular PDGF receptors, thereby altering the structure, contractile protein organization, contractility, and smooth muscle phenotype of cerebral arteries remote from the site of hemorrhage.

cerebral arteries; Gleevec; myosin; platelet-derived growth factor; STI-571

SPONTANEOUS INTRACEREBRAL HEMORRHAGE (ICH) is a high-mortality injury that occurs at a rate of up to 37 per 100,000 in developed countries (40) and accounts for up to 15% of all strokes (13). Studies of ICH have been ongoing for more than 100 years (39), with a primary focus on risk factors, edema, and neuronal injury (5, 21, 31). Collectively, this work has clearly demonstrated that the extent of ICH-induced injury is closely related to the volume of the hematoma produced, which typically expands over time and induces multiple neuroinflammatory and neurotoxic effects (13, 44). As early as 1997, however, evidence began to accumulate indicating that the blood-brain barrier, and possibly small arteries, were also injured by ICH (4). Further evidence demonstrated that intracerebral blood could alter cytoskeletal organization within cerebral arteries (17) and even induce contractile dedifferentiation (36). Current evidence strongly implies that phenotypic transformation of cerebrovascular smooth muscle and altered contractile function are prominent consequences of ICH (42, 52), although the mechanisms mediating this vascular injury remain unclear. This uncertainty is due, in large part, to the relative absence of studies that have examined the direct effects of ICH on cerebrovascular phenotype and contractility.

At the cellular level, smooth muscle differentiation and contractile capacity are controlled by a broad variety of growth factors and trophic influences that together dynamically govern the phenotypic characteristics of all vascular smooth muscle (37). Among the most prominent of these factors are the platelet-derived growth factors (PDGFs) (19, 22). With particular relevance to ICH, the levels of PDGFs in cerebrospinal fluid (CSF) increase after intracranial hemorrhage (16, 45). These findings confirm the hypothesis first advanced by Iwasa et al. (26) that peptide growth factors released into the interstitium and ultimately into the CSF contribute to hemorrhagic cerebral injury. In particular, the overall evidence supports the hypothesis that hemorrhagic brain injury promotes the release of multiple vasoactive factors including PDGF and chemically related factors into the CSF, which then diffuse throughout the brain’s interstitial compartment and modulate the phenotype of smooth muscle in cerebral arteries. Most importantly, the evidence in aggregate advances the idea that a PDGF receptor-dependent mechanism could help explain not only the altered cerebrovascular contractility associated with subarachnoid hemorrhage, where it has been most widely studied, but also that associated with ICH, which has received far less attention. In light of the combined evidence, the present study explored the hypothesis that ICH promotes the local release of vasoactive factors, which in turn diffuse throughout the brain interstitial space and facilitate phenotypic transformation of cerebrovascular smooth muscle remote from the site of initial injury via influence on PDGF receptors.

The compound imatinib, also known as Gleevec, was first developed as a small-molecule tyrosine kinase inhibitor for use in treatment of chronic myelogenous leukemia (11). Subsequent use of imatinib for treatment of gliomas (38) and ulti-
imately stroke (43) established that this drug could be used effectively to inhibit activation of PDGF receptors expressed in cerebral tissues. The strategy of the present study was to test the ability of PDGF receptor antagonism with imatinib to influence the vascular injury produced in a rat model of collagenase-induced ICH. Vascular injury was assessed functionally via myographic measurements of contractility, and structurally by quantitation of contractile protein colocalization, in isolated rat middle cerebral arteries.

**METHODS**

**General preparation.** All experimental procedures were approved by the Institutional Animal Use and Care Committees at Loma Linda University. Sprague-Dawley rats (Charles River Laboratories, Hollister, CA) were housed at constant temperature and humidity with a 12:12-h light-dark cycle. The overall study design included four groups of rats: 1) sham operated (Sham); 2) Sham + 60 mg/kg ip imatinib; 3) ICH; and 4) ICH + 60 mg/kg ip imatinib. Imatinib, also known as Gleevec, is a tyrosine kinase inhibitor with high activity against the PDGF receptor (7), particularly in vascular smooth muscle (29). In previous experiments we have explored the effects of 30, 40, 60, and 120 mg/kg ip imatinib, and on the basis of these studies we have found that 60 mg/kg is the optimum dose to attenuate the effects of ICH without significant, nonspecific extracranial effects (30, 51).

Slightly, we have also examined the effects of ICH at multiple times after injury, including 3, 6, 12, 24, and 72 h, and have found that the direct vascular effects are first fully developed at 24 h after injury (30, 51). Given these results, the present study was designed to examine the mechanisms of the initial, 24-h vascular response in relation to contractility and smooth muscle phenotype.

Adult male rats weighing from 250 to 300 g underwent ICH produced by collagenase injection, as previously described (28). After induction of anesthesia with ketamine and xylazine, each animal was placed prone in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) and positioned on an electronic thermostat-controlled warming blanket that maintained core temperature at 37.0 ± 0.5°C. A midline incision was made over the scalp, and a hole was drilled 0.2 mm anterior to, and 2.9 mm lateral to, the bregma. A 27-gauge needle was inserted to a depth of 4.7 mm, after which 0.2 U of bacterial collagenase (Sigma-Aldrich, St. Louis, MO) dissolved in 1 ml of sterile saline was injected over 5 min at a rate of 0.2 μl/min. The needle remained in place for an additional 10 min after injection to prevent back-leakage of collagenase. Sham-operated animals received the same manipulation, but instead of collagenase injection they received needle insertion alone. After needle removal, the incision was sutured closed and the animal was allowed to recover in the warming cage. One hour after needle insertion, 1 ml of 0.1% DMSO in saline either with or without imatinib (60 mg/kg body wt) was administered via an intraperitoneal injection. On resumption of spontaneous movements, each animal was returned to its feeding cage and allowed to recover for 24 h.

**Myography.** At the end of the 24-h postsurgical recovery period, rats were anesthetized with 100% CO2 (1 min) and then decapitated. The brains were rapidly removed and placed in ice-cold physiological salt solution (PSS) containing (in mM) 130 NaCl, 10.0 HEPES, 6.0 glucose, 4.0 KCl, 4.0 NaHCO3, 1.8 CaCl2, 1.2 KH2PO4, 1.2 MgSO4, and 0.025 EDTA, at pH 7.4. The main branch of the middle cerebral artery (MCA) was taken beginning at its origin adjacent to the circle of Willis, cleaned of all connective tissue, denuded of endothelium as previously described (8), and cut into lengths of 3–5 mm that were mounted on glass cannulas in an organ chamber positioned on the stage of an inverted microscope (Living Systems, Burlington, VT). The proximal cannula was connected to a pressure transducer and a windkessel reservoir of PSS, whose pressure was controlled by a pressure servo-control system used to precisely set and change the transmural pressures of the arteries. The distal cannula was connected to a Luer-Lock valve that was opened to gently flush the lumen during the initial equilibration. After subsequent equilibration, the outflow valve was closed and all measurements were conducted under no-flow conditions. Arterial diameters were recorded with the SoftEdge Acquisition Subsystem (IonOptix, Milton, MA). All methods used in this study have been previously described in detail (8, 12).

**Measurement of smooth muscle calcium concentration.** Once cannulated and equilibrated at 21°C, the artery segments were loaded with fura-2 AM (Thermo-Fisher, Waltham, MA) for 20 min at a concentration of 1 μM as previously described (8, 12). After loading, the arteries were washed with PSS and the bath temperature was increased to 37°C. Photons emitted at 510 nm were detected at a sampling rate of 3 Hz with an IonOptix photomultiplier system that automatically corrected for background fluorescence. K0 values obtained from separate in vitro calibrations were used to convert the experimental fluorescence intensity ratios (R) to intracellular Ca2+ concentration ([Ca2+]i) over the physiological range by iterative fit to the Grynkiewicz equation: [Ca2+]i = Kd[(R − Rmin)/(Rmax − R)]SF (18). Our calculations using this equation employed the following averaged values: SF (4.7), Rmin (0.4), Rmax (5.7), and Kd (251 nM).

All arteries were first exposed to PSS containing 120 mM K+ for 1–2 min at 60 mmHg to verify contractility. When contractile responses had stabilized, the 120 mM K+ was replaced with normal PSS and the arteries were reequilibrated until baseline diameters were reestablished (~10 min). Next, a series of pressure steps at 20, 40, 60, 80, and 100 mmHg were applied to each artery. At each pressure, artery diameter and wall [Ca2+]i were recorded first during equilibration in normal PSS and then in PSS containing 120 mM K+. After stabilization of the diameter and Ca2+ responses to 120 mM K+, the arteries were returned to normal PSS and the arteries were reequilibrated until initial diameters were reestablished (~10 min). The pressure was then raised to the next level, and the measurements were repeated. Once responses were measured at 100 mmHg, the arteries were equilibrated in PSS containing zero calcium with EGTA (3 mM) to determine the maximum passive diameter for each artery. This diameter was recorded at each of the previously applied pressure steps.

**Fluorescent immunohistochemistry.** Artery segments immediately adjacent to those used for myography were fixed in 4% neutral buffered EM-grade formaldehyde (Electron Microscopy Sciences, 15713-S) overnight and then dehydrated, embedded in paraffin, and cut into coronal sections 5 μm thick. The sections were then deparaffinized, rehydrated, immersed in citrate buffer at pH 6.03, and microwaved for 5 min to facilitate antigen recovery. The sections were then incubated in 1% bovine serum albumin (BSA, Santa Cruz Biotechnology, SC-2323) with Triton X-100 (Sigma-Aldrich, T-8877) blocking buffer for an hour. Next, the sections were double-stained in various combinations of primary antibodies reactive with α-Actin (Sigma-Aldrich, A5228; 1:200), anti-smooth muscle myosin heavy chain (SM-MHC) (Abcam, ab683; 1:400), anti-nonmuscle myosin heavy chain (NM-MHC) (Biologend, PRB-445P; 1:500), anti-myosin light chain kinase (MLCK) (Santa Cruz Biotechnology, SC-25428; 1:50), or anti-regulatory 20-kDa myosin light chain (MLC20) (Sigma-Aldrich, M4401; 1:300) overnight at 4°C. The following day, the sections were washed in PBS, equilibrated in darkness for 2 h at room temperature with two secondary antibodies labeled with DyLight 488 and DyLight 633, and then examined with our Olympus FV1000 using a ×60 oil immersion objective (NA = 1.4) that enabled a lateral resolution between 146 and 185 nm and an axial resolution between 545 and 693 nm, depending on wavelength of excitation.

The extent of colocalization between the marker pairs was determined with Colocalizer Pro (www.Colocalizer.com), which calculated a modified Mander’s coefficient independent of absolute marker intensities (32). For the colocalization analysis, coronal sections were extracted from the parent images by using a masking routine to eliminate background contributions to the measurement statistics. For
each pixel in each double-immunostained image, each marker was automatically graded as 1) below threshold; 2) above threshold but <50% maximum intensity (low intensity); or 3) >50% maximum intensity (high intensity). All pixels above threshold for both markers were grouped into three categories: low marker 1 and low marker 2; low marker 1 and high marker 2; and high marker 1 and high marker 2. The total of all pixels above threshold for both markers was taken at 100%, and the number of pixels in each of the three categories was calculated as a percentage of that total. These methods have previously been described in detail (2, 8, 12).

Magnetic resonance imaging and analysis. Once the MCAs had been removed, the brains were immersed in 4% paraformaldehyde for fixation. Ex vivo neuroimaging was undertaken at 2–4 wk after fixation on a Bruker Advance (Bruker Biospin, Billerica, MA) 11.7-T MRI (89-mm bore) for high-resolution volumetric imaging. Two imaging modalities were utilized for hemorrhage analysis: 1) a T2-weighted imaging (T2WI) sequence for brain and hemorrhage volumes (34) and 2) a susceptibility-weighted imaging (SWI) sequence that is hypersensitive to extravascular blood and iron-containing molecules (6). The T2WI imaging sequence parameters were TR/TE = 2,357.9/10.2 ms; matrix size = 256 × 256; field of view (FOV) 2 cm with 10 echoes, and 25 slices to span the entire cerebrum. The SWI sequence comprised a TR/TE = 617.7/7 ms; matrix size = 256 × 256; FOV 2 cm, with 48 slices to image the entire brain.

T2WI were processed for quantitative T2 maps with in-house software written in MATLAB (MathWorks, Natick, MA) as previously described (35). Brain volumes were derived with Cheshire software (Hayden Image/Processing Group, Waltham, MA) using semiautomated routines. SWI images were postprocessed with SPIN (Signal Processing in Nuclear Magnetic Resonance; MRI Institute, Detroit, MI) to enhance SWI magnitude images using minimum-intensity-projection (MIP) filters that are computed from SWI phase images. The minimum intensity projections (MIPs) were generated to increase contrast and enhance areas containing hemorrhage, and the computed SWI MIPs were then analyzed for hemorrhage. SWI MIPs were imported into Cheshire for hemorrhage volume analysis based on defined thresholds for hemorrhage. Any apparent hypointense signal within the cortex above the corpus callosum was defined as being due to needle insertion and was excluded from volumetric analysis. All data were extracted and summarized for brain and hemorrhage volumes.

Data analysis and statistics. All values are given in the text as means ± SE, and statistical significance implies P < 0.05. In all cases, n refers to the number of animals studied. Values of artery diameter, wall calcium, and colocalization coefficients were analyzed via two-way ANOVA with repeated measures using treatment (Sham, Sham + imatinib, ICH, and ICH + imatinib) and pressure as factors with SPSS (v23). Post hoc between-group comparisons were performed using the Fisher protected least significant difference analysis. Where significant between-group differences were observed, post hoc comparisons between individual means at the same pressure were performed with a Behrens-Fisher analysis with pooled variance. For the myography results, statistical differences between treatment groups were determined by comparing the area beneath the curve for each parameter (diameter, calcium, and diameter-to-calcium ratio) plotted against transmural pressure; this approach both simplified and clarified group-to-group comparisons. All data sets were normally distributed, as verified with a D’Agostino-Pearson K2 test, and for each distribution values outside the mean ± 2SD were excluded. Homogeneity of variance within ANOVA was verified with a Bartlett-Cochran test (49).

RESULTS

A total of 32 rats were used in this study, of which 8 were in the Sham group, 8 were in the Sham+imatinib group, 8 were in the ICH group, and 8 were in the ICH+imatinib group. MRI-derived hemorrhage volumes as percentage of brain volumes averaged 1.15 ± 0.32% and 0.55 ± 0.28% in the Sham and Sham+imatinib groups, respectively; these values were not significantly different and indicated that some hemorrhage was produced by needle insertion alone. The hemorrhage volumes averaged 6.05 ± 1.34% and 5.37 ± 0.75% in the ICH and ICH+imatinib groups, respectively. These latter values did not differ significantly from one another, were both significantly greater than the values in either Sham group (ANOVA), and agreed well with previous results from this model (28).

Passive diameter and incremental compliance. Across all animals, the average passive outside artery diameters ranged from 221 ± 7 μm at 20 mmHg to 263 ± 4 μm at 100 mmHg (Fig. 1, top). Between groups, passive diameters at corresponding pressures were significantly greater in the ICH group than in the Sham group. In contrast, there were no significant differences in average passive diameters at corresponding pressures between Sham+imatinib and ICH+imatinib groups.

Average values of arterial compliance (Fig. 1, bottom), calculated as the derivative of the relation between passive diameter and pressure (Fig. 1, top), ranged from 2.71 ± 0.45% diameter/mmHg at 20 mmHg to 0.74 ± 0.13% diameter/mmHg at 100 mmHg, and all group values decreased progressively with increasing pressure. Arterial compliance values were significantly less in arteries from ICH animals than in those from Sham animals. Conversely, compliance values at corresponding values were not significantly different in Sham+imatinib and ICH+imatinib arteries, except at 60 mmHg, where the ICH+imatinib arteries exhibited a greater compliance. At all pressures, compliance values were significantly less in Sham+imatinib arteries than in untreated Sham arteries. Overall, ICH increased passive diameters and reduced compliance through mechanisms that were negated by treatment with imatinib.

Changes in diameter, calcium, and calcium sensitivity induced by pressure and K+. Pressure-induced changes in diameter (ΔD) were calculated as the difference between diameter in PSS and diameter in 3 mM EGTA. Plots of these values against intraluminal pressure were used to calculate the areas beneath the ΔD-pressure curves, and the results of these calculations are shown in Fig. 2, top left. No significant differences were observed between any of the four groups studied for pressure-induced contractions. Similarly, potassium-induced changes in diameter were calculated as differences between diameter in PSS and diameter in PSS + 120 mM KCl, and plots of these values against intraluminal pressure were used to calculate the areas beneath the ΔD-pressure curves for potassium-induced contractions. As shown in Fig. 2, top right, no significant differences were observed between any of the four groups studied for potassium-induced contractions.

Pressure-induced changes in wall calcium concentration were calculated as the difference between wall calcium in PSS and wall calcium in PSS with 3 mM EGTA. Plots of these values against intraluminal pressure were used to calculate the areas beneath the Δwall calcium-pressure curves, and the results of these calculations are shown in Fig. 2, middle left. In untreated animals, ICH significantly depressed pressure-induced increases in wall calcium by 26%. This effect was absent in animals treated with imatinib. Correspondingly, potassium-induced changes in wall calcium concentration were calculated...
as the difference between wall calcium in PSS and calcium in PSS + 120 mM KCl, and plots of these values against intraluminal pressure were used to calculate the areas beneath the Δwall calcium-pressure curves for potassium-induced contractions. As shown in Fig. 2, middle right, ICH significantly depressed potassium-induced increases in wall calcium by 14% in untreated animals. This effect was absent in animals treated with imatinib. Imatinib alone also significantly decreased the responses of wall calcium to both pressure and potassium in Sham arteries.

The product of cytosolic calcium concentration and myofilament calcium sensitivity determines contractile force in smooth muscle. To estimate the effects of pressure on myofilament calcium sensitivity, the ratios of the pressure-induced ΔD response divided by the corresponding Δwall calcium response were calculated at each pressure. Plots of these ratios against intraluminal pressure were used to calculate the areas beneath the ΔD/Δwall calcium-pressure curves for pressure-induced contractions, and the results of these calculations are shown in Fig. 2, bottom left. In untreated animals, ICH elevated estimated pressure-induced increases in myofilament calcium sensitivity by 29%. This effect was absent in animals treated with imatinib. In a similar manner, the ratios of potassium-induced ΔD/Δwall calcium were calculated, plotted against pressure, and used to calculate the areas beneath the ΔD/Δwall calcium-pressure curves for potassium-induced contractions. In untreated animals, ICH elevated potassium-induced increases in myofilament calcium sensitivity by 48%. Once again, this effect was absent in animals treated with imatinib (Fig. 2, bottom right). Imatinib alone also significantly increased the responses of myofilament calcium sensitivity to potassium but not to pressure in Sham arteries.
Effects of ICH ± imatinib on colocalization of MHC isoforms with smooth muscle α-actin. To complement measurements of the effects ICH and imatinib on contractile function, the parallel effects on contractile protein organization were assessed by using confocal microscopy to determine the extent of colocalization for five different protein pairs including 1) NM-MHC and α-Actin; 2) SM-MHC and α-Actin; 3) MLCK and α-Actin; 4) MLC20 and α-Actin; and 5) MLCK and MLC20 (Fig. 3). For each protein pair examined, only pixels for which both proteins were above threshold intensity were analyzed, and the total number of such pixels was defined as 100%.

These pixels were then categorized into three groups: 1) both markers were above threshold but below half-maximal intensity (Low/Low); 2) one marker was above threshold but below half-maximal intensity and the other was greater than half-maximal intensity (Low/High); and 3) both markers were greater than half-maximal intensity (High/High). The effects of ICH and imatinib on contractile protein organization were then assessed by examining how these treatments influenced the percentage of total pixels in each group.

In sections stained for both NM-MHC and α-Actin (Fig. 4, top), ICH without imatinib significantly increased the fraction of pixels...
Sham ICH Sham + Imatinib ICH + Imatinib

| Protein Pair             | Sham | ICH   | Sham + Imatinib | ICH + Imatinib |
|--------------------------|------|-------|-----------------|----------------|
| NM-MHC/α-Actin           | ![Image](image1) | ![Image](image2) | ![Image](image3) | ![Image](image4) |
| SM-MHC/α-Actin           | ![Image](image5) | ![Image](image6) | ![Image](image7) | ![Image](image8) |
| MLCK/α-Actin             | ![Image](image9) | ![Image](image10) | ![Image](image11) | ![Image](image12) |
| MLC20/α-Actin            | ![Image](image13) | ![Image](image14) | ![Image](image15) | ![Image](image16) |
| MLCK / MLC20             | ![Image](image17) | ![Image](image18) | ![Image](image19) | ![Image](image20) |

Fig. 3. Effects of ICH ± imatinib on contractile protein colocalization. The effects of ICH with and without imatinib on the organization of contractile proteins were assessed in 5-μm sections cut from paraformaldehyde-fixed, paraffin-embedded middle cerebral arteries. Colocalization was determined via confocal microscopy for 6 different protein pairs including 1) nonmuscle myosin heavy chain (NM-MHC) and smooth muscle α-actin (α-Actin); 2) smooth muscle myosin heavy chain (SM-MHC) and α-Actin; 3) myosin light chain kinase (MLCK) and α-Actin; 4) regulatory 20-kDa myosin light chain (MLC20) and α-Actin; and 5) MLCK and MLC20. Shown above is a single image from each of the 40 different colocalization groups that represent a total of 320 images. For all colocalization groups, the most abundant protein of the pair was imaged at 633 nm (red channel) and the least abundant protein was imaged at 488 nm (green channel).
in the Low/Low category by 6.9% compared with Sham sections, and this increase was associated with decreases in the Low/High and High/High categories; the 5.1% decrease in the High/High group was significant. In contrast, ICH followed by imatinib treatment significantly decreased the fraction of pixels in the Low MHC/Low αAActin category for NM-MHC/αAActin colocalization but caused a rightward shift toward the High MHC/High αAActin category for SM-MHC/αAActin colocalization. Treatment with imatinib opposed both of these effects. In Sham arteries, imatinib alone significantly increased colocalization in the Low NM-MHC/Low αAActin category and increased it in the High NM-MHC/High αAActin category but had no effect on any category of SM-MHC/αAActin colocalization. Error bars indicate SE for n values of 8 in all groups. *Significant differences (P < 0.05) between Sham and ICH groups; †Sham + imatinib values that were significantly different (P < 0.05) from corresponding untreated Sham values.

Whereas NM-MHC is expressed more abundantly in immature smooth muscle, the SM-MHC isoform is the main MHC isoform expressed in mature, fully differentiated contractile smooth muscle. In sections stained for both SM-MHC and αAActin (Fig. 4, bottom), ICH without imatinib significantly decreased the fraction of pixels in the Low/Low group by 9.3%, and this decrease was associated with significant increases of 3.5% and 5.8% in the Low/High and High/High categories, respectively. In animals treated with imatinib, ICH had no significant effects in the Low/Low and Low/High groups but significantly increased the fraction in the High/High group by 4.1%. Again, imatinib appeared to reverse the effects of ICH on SM-MHC/αAActin colocalization. More importantly, the MHC results support the view that ICH promotes loss of NM-MHC colocalization and gain of SM-MHC colocalization, which is a
pattern consistent with enhanced contractility. In Sham arteries, imatinib alone had no effect on any category of SM-MHC/α-actin colocalization. Imatinib, however, appeared to oppose ICH-induced contractile differentiation, as indicated by both the NM-MHC and SM-MHC results.

**Effects of ICH ± imatinib on colocalization among MLCK, MLC20, and α-actin.** MLCK is the rate-limiting kinase for the development of contractile force, and its colocalization with α-actin is essential for the initiation of contraction. ICH significantly increased the fraction of pixels in the Low MLCK/High α-actin category by 6.1%, apparently by drawing pixels from both the Low MLCK/Low α-actin and High MLCK/High α-actin categories (Fig. 5, top). Treatment with imatinib reversed this pattern and significantly decreased the fraction of pixels in the Low MLCK/High α-actin category by 3.9%. In Sham arteries, imatinib alone significantly decreased colocalization in the Low MLCK/Low α-actin category and increased it in the Low MLCK/High α-actin category.

MLC20 is the sole target for MLCK-mediated phosphorylation, and its colocalization with α-actin is also essential for the initiation of contraction. ICH had no significant effects on the colocalization of MLC20 with α-actin, but treatment with imatinib significantly increased the fraction of pixels in the Low MLC20/Low α-actin category by 7.1% and, correspondingly, significantly decreased the fraction of pixels in the High MLC20/High α-actin category by 5.1% (Fig. 5, middle). In Sham arteries, imatinib alone had no significant effect on any category of colocalization between MLC20 and α-actin.

Colocalization of MLCK with its target substrate MLC20 is strongly correlated with maximum contractile capacity and is a good marker for contractile differentiation in smooth muscle. ICH significantly decreased the fraction of pixels in the High...
MLCK/High MLC\textsubscript{20} category by 3.5% and simultaneously significantly increased the pixel fraction in the Low MLCK/High MLC\textsubscript{20} category by 8.3% (Fig. 5, bottom). In addition, ICH also significantly decreased the fraction of pixels in the Low MLCK/Low MLC\textsubscript{20} category by 4.8%. All these effects were absent in arteries from animals treated with imatinib. In Sham arteries, imatinib alone significantly decreased colocalization in the Low MLCK/Low MLC\textsubscript{20} category and increased it in the Low MLCK/High MLC\textsubscript{20} category.

Altogether, the changes in colocalization among MLCK, MLC\textsubscript{20}, and α-Actin support the idea that ICH generally decreased contractile protein organization by significantly increasing pixel fractions in the Low MLCK/High α-Actin and Low MLCK/High MLC\textsubscript{20} categories, with corresponding significant decreases in the High MLCK/High MLC\textsubscript{20} category. In contrast, after treatment with imatinib, contractile organization appeared better preserved after ICH as demonstrated by the absence of any changes in MLCK/MLC\textsubscript{20} colocalization despite significant decreases in the High MLCK/High MLC\textsubscript{20} category. This study offers three main findings. First, ICH can significantly alter cerebrovascular structure and contractility, even in circle of Willis arteries remote from the site of injury. Second, ICH can induce changes in contractile protein organization consistent with phenotypic transformation of cerebrovascular smooth muscle. Third, the effects of ICH on cerebral artery structure and function can be largely, but not completely, reversed by treatment with the PDGF receptor antagonist imatinib. Together, these findings support the hypothesis that blood entering the CSF promotes the release of vasoactive factors, which in turn can diffuse throughout the cranial compartment and act on PDGF signaling to help mediate changes in cerebrovascular structure and function that are characteristic of ICH.

**DISCUSSION**

PDGFs are an important family of growth factors for fibroblasts, vascular smooth muscle, vascular endothelium, and pericytes as well as nonvascular cell types found in the central nervous system including astrocytes, neurons, and Schwann cells (20). PDGF molecules are expressed as four different polypeptide monomers (A, B, C, and D) of ~100 amino acids each, which combine into dimers that serve as active ligands. Dimers of PDGF can originate from more than a dozen different cell types but are particularly abundant in platelets, as the name implies. The cellular effects of PDGF dimers are mediated by two separate tyrosine kinase receptors, the PDGF-α receptor and the PDGF-β receptor, which form either homodimers or heterodimers upon binding to PDGFs. The exact cellular responses to PDGFs, in turn, depend upon the composition of the PDGF dimer and combination of receptors bound. In vascular cell types, activated PDGF receptors are typically coupled to changes in gene expression through the phosphatidylinositol 3-kinase, phospholipase C\textgamma, and mitogen-activated protein kinase pathways, as is the case for many other tyrosine kinase receptors (3). In vascular smooth muscle, the signaling pathways activated by PDGFs generally influence contractile dedifferentiation and cytoskeletal remodeling (37).

The compound imatinib mesylate, also known as Gleevec or STI-571, has been highly useful in identifying the biological effects of PDGFs (23). Imatinib was originally designed as a highly specific inhibitor of BCR-ABL, an EGFR mutation that is constitutively active and expressed only in cancer cells, particularly those of chronic myeloid leukemia (11, 14). This strategy proved effective, and imatinib remains a first-line orally effective therapy for most patients with chronic myelogenous leukemia. As suggested by more than 9,000 published studies including ~800 clinical trials, imatinib is tolerated well, is effective against several different cancers, and has modest side effects (38, 46), most of which appear to be vascular in nature. Subsequent work has demonstrated that imatinib is a potent PDGF receptor antagonist, which is its primary effect in healthy tissues (33). The idea that imatinib might be effective after ICH was first published in 2008 by Su et al. (43), who reported that administration of imatinib reduced the effects of tPA on cerebrovascular permeability during treatment of ischemic stroke. Independent of thrombolytic therapy, the use of imatinib to attenuate the effects of ICH on blood-brain barrier permeability was first reported by Ma et al. in 2011 (30). Subsequent studies have further shown that imatinib can attenuate cerebral vasospasm (41) and preserve blood-brain barrier permeability (51) after subarachnoid hemorrhage. Imatinib also can reduce the transformation of cerebral microvascular smooth muscle into an inflammatory phenotype after ICH (48). Together, these findings support the hypothesis that the presence of blood in the intracranial compartment has important PDGF receptor-dependent vascular effects. The extent to which these effects include changes in cerebrovascular structure and contractility, however, remains largely unreported.

In the present study, the intracerebral injection of collagenase caused hemorrhage in the right caudate putamen as previously described (28) but also increased the passive diameter of second branch segments of the MCA (Fig. 1, top). In these MCA segments, which were remote from the site of hemorrhage, ICH also decreased incremental compliance (Fig. 1, bottom), raising the possibility that ICH released one or more diffusible factors that both stiffened and enlarged the MCA. Given that both the increase in passive diameter and the decrease in incremental compliance were absent in animals treated with imatinib, the results argue that the diffusible remodeling factor released by ICH ultimately influenced activation of PDGF receptors. One notable effect of imatinib, however, was that it decreased arterial compliance in sham arteries with no effect on arterial diameter (Fig. 1, bottom), suggesting that endogenous activation of PDGF receptors helped maintain arterial compliance. More importantly, the qualitative effects of imatinib and ICH on compliance were similar (both caused a decrease), raising the possibility that factors released by ICH may attenuate endogenous PDGF receptor activity.

ICH also significantly altered the contractile behavior of the MCA segments taken for study. Responses to increased intraluminal pressure and potassium-induced depolarization both exhibited depressed calcium mobilization but increased myofilament calcium sensitivity after ICH (Fig. 2). Because these effects largely offset one another, ICH had relatively little effect on pressure-induced and potassium-induced changes in diameter, hinting that changes in either calcium mobilization or myofilament calcium sensitivity may have been a homeostatic
response to ICH; further experiments will be needed to determine which, if either, of these parameters were directly influenced by ICH. More importantly, the ICH-induced changes in both calcium mobilization and myofilament calcium sensitivity were absent in arteries from animals treated with imatinib, consistent with the hypothesis that a diffusible remodeling factor released by ICH influenced the activity of PDGF receptors. Again, imatinib had significant effects in Sham arteries, where it decreased the responses of wall calcium to increased pressure or potassium and increased myofilament calcium sensitivity responses to potassium depolarization (Fig. 2, middle and bottom). As for the effects of imatinib on arterial compliance, the effects of imatinib and ICH on wall calcium and myofilament calcium sensitivity were qualitatively similar, reinforcing the idea that factors released by ICH may attenuate endogenous PDGF receptor activity.

Previous work from many laboratories, including our own (8, 25), has demonstrated that significant changes in vascular structure and function are often associated with changes in vascular smooth muscle phenotype (37). To explore the possibility that ICH-induced changes in MCA structure and function might have involved shifts in smooth muscle phenotype, the present study employed quantitative confocal microscopy to examine segments of MCA arteries adjacent to those used for contractility measurements (Fig. 3). These measurements quantified changes in the colocalization of α-Actin with NM-MHC and SM-MHC isoforms of MHC to identify transitions in phenotype, as previously reported (25, 37). Complementary measurements detailed changes in colocalization among the contractile proteins MLCK, MLC20, and α-Actin to reveal shifts in contractile protein organization, also as previously reported (1, 2). A unique strength of the nonparametric colocalization analysis employed in these studies was that all colocalized pixels fell into one of three main phenotypic categories depending on the relative intensities of the marker pairs examined. This assignment emphasized shifts among the phenotypic categories in response to ICH and imatinib.

As indicated by changes in colocalization between α-Actin and MHC isoforms, ICH appeared to promote contractile differentiation (Fig. 4). ICH significantly decreased the fraction of colocalized pixels with high intensities for both NM-MHC and α-Actin and correspondingly increased the fraction with low intensities for both markers; this leftward shift in pixel distribution implies a decrease in the proportion of cells in a noncontractile phenotype (Fig. 4, top). Conversely, ICH significantly increased the fractions of colocalized pixels with high intensities for α-Actin and either low or high intensities for SM-MHC (Fig. 4, bottom). ICH also correspondingly decreased the fraction of colocalized pixels with low intensities for both α-Actin and SM-MHC. This rightward shift in pixel distribution was consistent with an increase in the proportion of smooth muscle cells in a contractile phenotype. Most importantly, the patterns of phenotypic shifts indicated by ICH-induced changes in colocalization between α-Actin and MHC isoforms were not evident in arteries from imatinib-treated animals. After treatment with imatinib, ICH produced a rightward shift in colocalization between NM-MHC and α-Actin (Fig. 4, top) but yielded no significant changes in the fractions of pixels with low intensities for SM-MHC and either low or high intensities for α-Actin (Fig. 4, bottom). ICH with imatinib did still increase the fraction of colocalized pixels with high intensities for both SM-MHC and α-Actin, but overall the response to ICH was greatly attenuated by imatinib.

In Sham arteries, imatinib alone increased the fraction of pixels with low intensities for both NM-MHC and α-Actin and decreased the fraction of pixels with high intensities for both markers, effects that were both qualitatively and quantitatively similar to the effects of ICH on this marker combination. This similarity again suggests that at least some effects of ICH may be attributable to attenuation of endogenous PDGF receptor activity. Other effects must be involved, however, because in Sham arteries imatinib had no effects on the fractions of pixels in the Low NM-MHC/High α-Actin category or in any category of colocalization of SM-MHC with α-Actin (Fig. 4), even though ICH alone had significant effects in all of these categories. Together, these results support the hypothesis that diffusible factors released during ICH ultimately exerted multiple effects on PDGF receptor signaling, which in summation promoted phenotypic contractile differentiation after ICH.

In addition to its effects on smooth muscle phenotype, ICH also influenced organization among the contractile proteins MLCK, MLC20, and α-Actin. ICH had no significant effect on colocalization between MLC20 and α-Actin (Fig. 5, middle) but significantly increased colocalization among pixels with low intensity for MLCK and high intensity for MLC20 (Fig. 5, bottom). These ICH-induced increases in the Low MLCK/High MLC20 fraction were supported by significant decreases in both the Low MLCK/Low MLC20 and the High MLCK/High MLC20 fractions. ICH also stimulated a similar but more modest increase in colocalization among pixels with low intensity for MLCK and high intensity for α-Actin (Fig. 5, top). Together, these results support the interpretation that ICH generally enhanced integration of MLCK into the contractile apparatus, albeit at the expense of a modest decrease in High MLCK/High MLC20 colocalization. Interestingly, these changes in contractile protein organization occurred in tandem with a shift toward phenotypic contractile differentiation (Fig. 4), a decrease in calcium mobilization, and an increase in apparent myofilament calcium sensitivity (Fig. 2). Most importantly, the effects of ICH on contractile protein organization were largely negated by treatment with imatinib. In arteries from imatinib-treated animals, ICH had no significant effect on MLCK colocalization with MLC20, decreased High MLC20/High α-Actin colocalization in favor of Low MLC20/Low α-Actin, and decreased Low MLCK/High α-Actin colocalization.

Treatment of Sham arteries with imatinib again produced effects qualitatively similar to those produced by ICH alone, including decreased fractions of pixels in the Low MLCK/Low α-Actin and Low MLCK/Low MLC20 categories and increased fractions of pixels in the Low MLCK/High α-Actin and Low MLCK/High MLC20 categories. As for other end points, however, imatinib and ICH did not produce parallel effects in all categories, again emphasizing that ICH exerted multiple effects on the phenotype of cerebrovascular smooth muscle that included, but were not limited to, antagonism of PDGF receptors. More generally, this pattern of results further implied that ICH significantly influenced contractile protein organization, and possibly function, through the action of diffusible factors that influenced PDGF receptor signaling.
Perspectives and Significance

As a whole, the results of this study emphasize that ICH can alter the structure, contractile protein organization, contractility, and smooth muscle phenotype of cerebral arteries remote from the site of hemorrhage. Given that many of these effects can be attenuated or ablated by imatinib, these findings reinforce the hypothesis that ICH promotes the release of factors into the CSF that can diffuse to, and influence, PDGF signaling throughout the cerebral vasculature. Consistent with this idea are multiple findings that subarachnoid hemorrhage can increase levels of PDGFs in CSF (16, 27, 42, 47). Evidence that subarachnoid hemorrhage also increases PDGFs in circulating blood (10) raises the possibility that some extracranial vascular effects of ICH might also be explained by remote action on vascular PDGF receptors. Alternatively, the present results are also consistent with the hypothesis that hemorrhagic insults release factors other than PDGF that could diffuse to vasculature remote from the site of injury and influence local synthesis and activity of PDGFs (47). In particular, the qualitative similarities between the effects of ICH and imatinib on multiple end points suggest that at least some of the effects of ICH are mediated by antagonism of PDGF receptor signaling. Independent of the sources and types of molecules that influence PDGF signaling after ICH, the finding that the structural and functional characteristics of cerebral arteries were highly susceptible to the influence of a PDGF receptor antagonist emphasizes that the phenotypic makeup of cerebrovascular smooth muscle, and probably vascular smooth muscle in general, is highly labile and dynamic (37). Whereas the results are highly consistent with the possibility that multiple molecules that interact with PDGF receptors are released into the CSF by ICH, it is highly likely that numerous other vasoactive factors are also released, including endothelins, TNF-α, interleukins, and many others, some of which may act intracellularly (27). Indeed, receptor cross talk is a common feature of cellular responses to simultaneous stimulation by multiple ligands, particularly for the tyrosine kinase family of receptors (9, 50). How the actions of these factors combine to influence vascular structure and function remains a worthy topic for further study, particularly in relation to the molecular mechanisms whereby changes in contractile protein organization govern contractility. More detailed studies of protein-protein interactions using super-resolution microscopy (24), proximity ligation assays (15), and other methods with high spatial resolution will be required to illuminate these mechanisms. In the meantime, it seems likely that PDGF receptors are important mediators of cerebrovascular responses to ICH, which in turn suggests that imatinib may be useful in the clinical management of post-ICH patients.

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No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

W.J.P., J.H.Z., and J.T. conception and design of research; W.J.P., C.D., D.C., D.K., L.M.D., A.M., L.M., A.O., J.H.Z., and J.T. analyzed data; W.J.P. and D.K. prepared figures; W.J.P. drafted manuscript; W.J.P., L.M.D., and A.M. edited and revised manuscript; W.J.P., C.D., D.C., D.K., L.M.D., A.O., J.H.Z., and J.T. approved final version of manuscript; C.D., D.K., L.M.D., and A.M. performed experiments.

REFERENCES

1. Adeoye OO, Bothurs V, Hubbell MC, Williams JM, Pearce WJ. VEGF receptors mediate hypoxic remodeling of adult ovine carotid arteries. J Appl Physiol (1985) 117: 777–787, 2014. doi:10.1152/japplphysiol.00012.2014.
2. Adeoye OO, Butler SM, Hubbell MC, Semotnik A, Williams JM, Pearce WJ. Contribution of increased VEGF receptors to hypoxic changes in fetal ovine carotid artery contractile proteins. Am J Physiol Cell Physiol 304: C656–C665, 2013. doi:10.1152/ajpcell.00110.2012.
3. Andrae J, Galliani R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. Genes Dev 22: 1276–1312, 2008. doi:10.1101/gad.1653708.
4. Armado D, Kornfeld M, Estrada EY, Grossette M, Rosenberg GA. Neutral proteases and disruption of the blood-brain barrier in rat. Brain Res 767: 259–264, 1997. doi:10.1016/S0006-8993(97)00567-2.
5. Badjatia N, Rosand J. Intracerebral hemorrhage. Neurologin 11: 311–324, 2005. doi:10.1097/01.wnl.0000178757.68551.26.
6. Belaey L, Obenaus A, Zhao W, Saul I, Busto R, Wu C, Vgidorchik A, Lin B, Ginsberg MD. Experimental intracerebral hematoma in the rat: characterization by sequential magnetic resonance imaging, behavior, and histopathology. Effect of albumin therapy. Brain Res 1157: 146–155, 2007. doi:10.1016/j.brainres.2007.04.077.
7. Capdeville R, Silberman S, Dimitrjevic S. Imatinib: the first 3 years. Eur J Cancer 38, Suppl 5: S77–S82, 2002. doi:10.1053/jes.2002.00098-00409/02080607-4.
8. Charles SM, Zhang L, Cipolla MJ, Buchholz JN, Pearce WJ. Roles of cytotoxic Ca2+ concentration and myofilament Ca2+ sensitization in age-dependent cerebrovascular myogenic tone. Am J Physiol Heart Circ Physiol 299: H1034–H1044, 2010. doi:10.1152/ajpcell.00214.2010.
9. Chen PY, Qin L, Li G, Tellides G, Simons M. Smooth muscle FGF/ TGFβ cross talk regulates adherscrosis progression. EMBO Mol Med 8: 712–728, 2016. doi:10.15252/emmm.201506181.
10. Cui HK, Yan RF, Ding XL, Zhao P, Wu QW, Wang HP, QinHX, Tu JF, Yang RM. Platelet-derived growth factor-β expression in rabbit models of cerebral vasospasm following subarachnoid hemorrhage. Mol Med Rep 10: 1416–1422, 2014.
11. Druker BJ, Tanura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J, Lydon NB. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 2: 561–566, 1996. doi:10.1038/nm0596-561.
12. Durrant LM, Khorrorn O, Buchholz JN, Pearce WJ. Maternal food restriction modulates cerebrovascular structure and contractility in adult rat offspring: effects of metyrapone. Am J Physiol Regul Integr Comp Physiol 306: R401–R410, 2014. doi:10.1152/ajpregu.00436.2013.
13. Eliovich I, Patel PV, Hemphill JC III. Intracerebral hemorrhage. Semin Neurol 28: 657–667, 2008. doi:10.1055/s-0028-1105974.
14. Fausel C, Targeted chronic myeloid leukemia therapy: Seeking a cure. Am J Health Syst Pharm 64, Suppl 15: S9–S15, 2007. doi:10.2146/ajhp070482.
15. Fredriksson S, Gullberg M, Jarvius J, Olsson C, Pietras K, Gустafsdottir SM, Ostman A, Landegren U. Protein detection using proximity-dependent DNA ligation assays. Nat Biotechnol 20: 473–477, 2002. doi:10.1038/nbt0502-473.
16. Gaetani P, Tancioni F, Gregnani G, Tartara F, Merlo EM, Brocchieri A, Rodriguez y Baena R. Intracerebral hematoma: a study on cisternal cerebrospinal fluid. Histochem J 39: 319–324, 1997. doi:10.1007/BF01088827.
17. Gomis P, Kacem K, Sercombe C, Seylaz J, Sercombe R. Confocal microscopic evidence of decreased alpha-actin expression within rabbit cerebral artery smooth muscle cells after subarachnoid haemorrhage. Histochem J 32: 673–678, 2000. doi:10.1023/A:1004115432660.
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18. Grynkiewicz G, Poenio M, Tisen RY. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 260: 3440–3450, 1985.

19. Ha JM, Yun SJ, Kim YW, Jin SY, Lee HS, Song SH, Shin HK, Bae SS. Platelet-derived growth factor regulates vascular smooth muscle phenotype via mammalian target of rapamycin complex 1. Biochem Biophys Res Commun 464: 56–62, 2015. doi: 10.1016/j.bbrc.2015.05.097.

20. Helden CH, Westermark B. Mechanism of action and in vivo role of platelet growth factor. Physiol Rev 79: 1283–1316, 1999.

21. Hemphill JC III, Greenberg SM, Anderson CS, Becker K, Bendok BR, Cushman M, Fung GL, Goldstein JN, Macdonald RL, Mitchell PH, Scott PA, Selim MH, Woo D; American Heart Association Stroke Council; Council on Cardiovascular and Stroke Nursing; Council on Clinical Cardiology. Guidelines for the Management of Spontaneous Intracerebral Hemorrhage. Stroke 46: 2032–2060, 2015. doi: 10.1161/STR.116.1525.

22. Holycross BJ, Blank RS, Thompson MM, Peach MJ, Owens GK. Platelet-derived growth factor-BB-induced suppression of smooth muscle cell differentiation. Circ Res 71: 1525–1532, 1992. doi: 10.1161/01.RES.71.6.1525.

23. Hu W, Huang Y. Targeting the platelet-derived growth factor signalling in cardiovascular disease. Clin Exp Pharmacol Physiol 42: 1221–1224, 2015. doi: 10.1111/1440-1681.EP2015.73276.X.

24. Huang B, Wang W, Bates M, Zhuang X. Three-dimensional superresolution imaging by stochastic optical reconstruction microscopy. Science 319: 810–813, 2008. doi: 10.1126/science.1153529.

25. Hubbell MC, Semotiuk AJ, Thorpe RB, Adeoye OO, Butler SM, McDonald JH, Dunn KW. Targeting the platelet-derived growth factor signalling in cardiovascular disease. Ann Neurol 71: 1525–1532, 2012. doi: 10.1002/ana.22549.

26. Iwasa K, Bernanke DH, Smith RR, Yamamoto Y. Platelet-derived growth factor regulates vascular smooth muscle phenotype in vasospasm after subarachnoid hemorrhage via inhibiting tenascin-C expression. Neurosci Biol Dis 46: 172–179, 2012. doi: 10.1016/j.wsnb.2012.01.005.

27. Jeltsch T, Kraft D, Lekic T, Ma Q, Zajic J, Winglee R, Quigley ME, Yanamoto H, Matsumoto M, Kaki T, Wang W, Bokoch GM. Targeting the platelet-derived growth factor signalling in cardiovascular disease. Acta Neurochir (Wien) 155: 781–789, 2013. doi: 10.1007/s00701-013-1725-x.

28. Ji Y, Li J, Wang Y, Wang X, Wang H, Wang J, Zhang J, Zhang H, Zhang J, Zhang J. Fibrinogen fragment mediates platelet aggregation in intracerebral hemorrhage. J Stroke Cerebrovas Dis 21: 30–41, 2012. doi: 10.1016/j.jstrokecerebrovasdis.2010.04.004.

29. Kadotani H, Lederer C, Takahashi N, Katayama H, Kojima K, Tanaka M, Nakaya H, Tominaga S. Platelet-derived growth factor signaling regulates vascular smooth muscle cell phenotype and anti-inflammatory activity in intracerebral hemorrhage. J Neurotrauma 27: 267–269, 2010. doi: 10.1089/neu.2009.1163.

30. Lindschog H, Atthley E, Larsson E, Lundin S, Hellström M, Lindahl P. New insights to vascular smooth muscle cell and pericyte differentiation of mouse embryonic stem cells in vitro. Acta Neurochir (Wien) 152: 303–309, 2012. doi: 10.1007/s00701-012-2045-4.

31. Ma Q, Huang B, Khatibi N, Rolland W II, Suzuki H, Zhang JH, Tang J, PDGFR-α inhibition preserves blood-brain barrier after intracerebral hemorrhage. Transl Stroke Res 5: 357–364, 2014. doi: 10.1007/s12975-013-0310-1.

32. Maw NC, Chen CH, Yeoh GJ, Young JC, Tyrrell JAD, Meehan MC, Baker IA, Tipton KF, Bell J, Gray MA, Blundell LE, Blandford L, Jobson AD, et al. Platelet-derived growth factor regulates vascular smooth muscle cell phenotype in vasospasm after aneurysmal subarachnoid hemorrhage. Neuroimage 210: 1–8, 2014. doi: 10.1016/j.neuroimage.2013.09.005.

33. Taylor RA, Sansing LH. Microglial responses after ischemic stroke and intracerebral hemorrhage. Clin Dev Immunol 2013: 2013: 746068, 2013. doi: 10.1155/2013/746068.

34. Weiß WW, Schramm J, Urbach H. Platelet-derived growth factor (PDGF-AB) like immune reactivity in serum and in cerebral spinal fluid following experimental subarachnoid haemorrhage in dogs. Acta Neurochir (Wien) 141: 861–866, 1999. doi: 10.1007/s00701-005-0388-5.

35. Waller CF. Imatinib mesylate. Recent Results Cancer Res 201: 1–25, 2010. doi: 10.1007/978-3-642-54490-3_1.

36. Yamamoto H, Kataoka H, Nakajo Y, Iihara K. The role of the host defense system in the development of cerebral vasospasm: analogies between atherosclerosis and subarachnoid hemorrhage. Eur Neurol 68: 229–343, 2012. doi: 10.1159/000341336.

37. Yang P, Wu J, Xiao L, Manaenko A, Matei N, Zhang Y, Xu L, Parent RL, Hartman RE, Obenaus A, Zhang JH, Xu F, Tang J. Platelet-derived growth factor receptor-β regulates vascular smooth muscle cell phenotype transformation and neuroinflammation after intracerebral hemorrhage in mice. Crit Care Med 44: e390–e402, 2016. doi: 10.1097/CCM.0000000000003125.

38. Zafar BH, Bostwick J. Biostatistical Analysis. Upper Saddle River, NJ: Prentice Hall, 1999.

39. Zeng L, Li Y, Yang J, Wang G, Margarit A, Xiao Q, Zapataki A, Yin X, Mayr M, Mori K, Wang W, Hu Y, Xu Q. XBP-1 deficiency abrogates neonatal lesion of injured vessels via cross talk with the PDGF signaling. Arterioscler Thromb Vasc Biol 35: 2134–2144, 2015. doi: 10.1161/ATVBAHA.115.304220.

40. Zhan Y, Kratke PR, Lekic T, Ma Q, Souvenir R, Zhang JH, Tang J. Imatinib preserves blood-brain barrier integrity following experimental subarachnoid hemorrhage in rats. J Neurosci Res 93: 94–103, 2015. doi: 10.1002/jnr.23475.

41. Zhang JH, Badwat J, Tang J, Obenaus A, Hartman R, Pearce WJ. The vascular neural network—a new paradigm in stroke pathophysiology. Nat Rev Neurol 8: 711–716, 2012. doi: 10.1038/nrneurol.2012.210.