HHS Public Access

Author manuscript

Hypertension. Author manuscript; available in PMC 2022 May 17.

Published in final edited form as:

Hypertension. 2020 December ; 76(6): 1924–1934. doi:10.1161/HYPERTENSIONAHA.120.15582.

Acute kidney injury sensitizes the brain vasculature to angiotensin II constriction via FGFBP1

Liang Zhao[1,2,3], **Xiaoyun Cao**[2], **Lingli Li**[4], **Qin Wang**[2], **Suhan Zhou**[2], **Nan Xu**[2], **Shan Jiang**[2], **Limeng Chen**[5], **Marcel O. Schmidt**[6], **Qichun Wei**[7], **Jingwei Zhao**[8], **Robert Labes**[3], **Andreas Patzak**[3], **Christopher S. Wilcox**[4], **Xiaodong Fu**[9], **Anton Wellstein**[6], **En Yin Lai**[1,2,3,4]

[1]Department of Physiology, School of Basic Medical Sciences, Guangzhou Medical University, Guangzhou 511436, China

[2]Department of Physiology, School of Basic Medical Sciences, Zhejiang University School of Medicine, Hangzhou 310003, China

[3]Institute of Vegetative Physiology, Charité–Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin 10117, Germany

[4]Division of Nephrology and Hypertension, Georgetown University, Washington, DC 20007, USA

[5]Department of Nephrology, Peking Union Medical College Hospital, Chinese Academy of Medical Science & Peking Union Medical College, Beijing 100730, China

[6]Lombardi Cancer Center, Georgetown University, Washington, DC 20007, USA

[7]Department of Radiation Oncology, Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310009, China

[8]Department of Anatomy, Histology and Embryology, Institute of Neuroscience, School of Basic Medical Sciences, Zhejiang University School of Medicine, Hangzhou 310058, China

[9]Department of Gynecology and Obstetrics, the Sixth Affiliated Hospital, Guangzhou Medical University, Guangzhou 511518, China

Abstract

Acute kidney injury (AKI) causes multiple organ dysfunction. Here we identify a possible mechanism that can drive brain vessel injury after AKI. We induced 30 min bilateral renal ischemia-reperfusion injury in C57B1/6 mice and isolated brain micro- and macro- vessels 24 h or 1 week later to test their responses to vasoconstrictors and found that after AKI brain vessels were sensitized to angiotensin II (Ang II). Upregulation of fibroblast growth factor 2 (FGF2) and FGF binding protein 1 (FGFBP1) expression in both serum and kidney tissue after AKI suggested a potential contribution to the vascular sensitization. Administration of FGF2 and FGFBP1 proteins

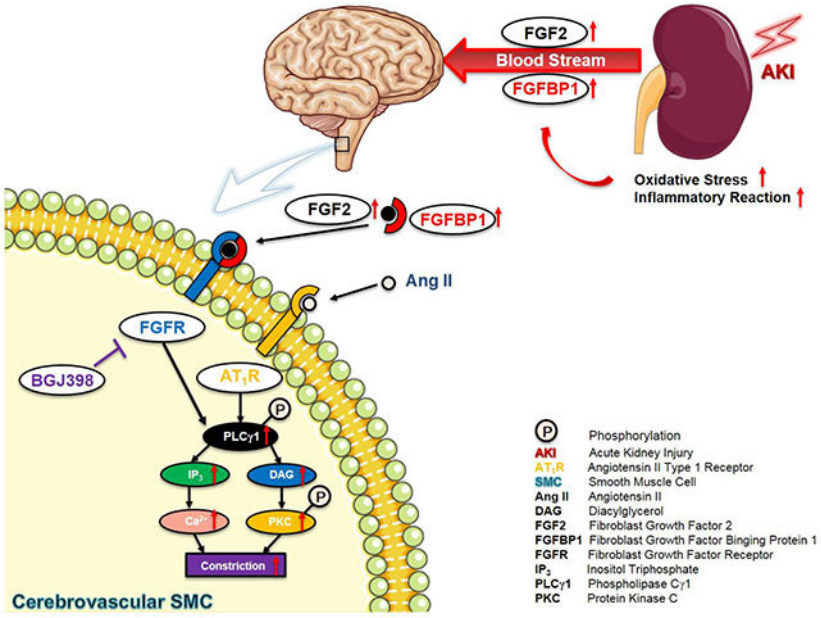
Correspondence: En Yin Lai, Department of Physiology, School of Basic Medical Sciences, Zhejiang University School of Medicine, Hangzhou 310003, China. laienyin@zju.edu.cn; and Anton Wellstein, Lombardi Cancer Center, Georgetown University, Washington, DC 20007, USA. wellstea@georgetown.edu; and Xiaodong Fu, Department of Gynecology and Obstetrics, the Sixth Affiliated Hospital, Guangzhou Medical University, Guangzhou 511518, China. fuxiaod@gzhmu.edu.cn.

Disclosures

None.

to isolated healthy brain vessels mimicked the sensitization to Ang II after AKI. Brain vessels in *Fgfbp1*$^{-/-}$ AKI mice failed to induce Ang II sensitization. Complementary to this, systemic treatment with the clinically used FGF receptor kinase inhibitor BGJ398 (Infigratinib) reversed the AKI-induced brain vascular sensitization to Ang II. All these findings lead to the conclusion that FGFBP1 is especially necessary for AKI-mediated brain vascular sensitization to Ang II and inhibitors of FGFR pathway may be beneficial in preventing AKI-induced brain vessel injury.

Graphical Abstract



Keywords

acute kidney injury; fibroblast growth factor 2; fibroblast growth factor binding protein 1; brain vasculature; ischemia/reperfusion injury

Introduction

Acute kidney injury (AKI) is a common clinical syndrome characterized by a rapid accumulation of end products of nitrogen metabolism such as urea and creatinine and/or decreased urine output, caused by reduction of the kidney's excretory capacity. The incidence of AKI is increasing worldwide,[1,2] and poses a severe clinical challenge in hospital patients.[3-6] Despite considerable efforts,[7,8] an optimal therapy has not been found. Importantly, therapeutic approaches focusing on kidney injury alone are not sufficient since multiple distant organs including lung,[9,10] brain,[11] heart,[12] liver[13] and small intestine are also damaged due to AKI and the damage to these organs further contribute to poor disease outcome.[14] Thus, it is crucial to identify mechanisms by which AKI can affect distant organ function and develop therapies to prevent AKI-associated distant organ injury.

Several studies have explored the crosstalk between kidney and brain after AKI,[15] which are mainly manifested as insufficient blood supply of brain and ischemic stroke. However, the mechanisms of the interaction and the impairment of brain function, respectively, are still poorly understood. The activation of renin-angiotensin system is important in the development of AKI and the interaction of kidney and brain during AKI. Some studies have shown that in the early stages of AKI, the activation of the renin-angiotensin system produces more angiotensin II (Ang II), which causes arteriole constriction and changes in hemodynamics.[16,17] In addition, reactive oxygen species (ROS) and inflammation may also participate in the kidney-brain crosstalk since AKI will induce a high oxidative stress state and systemic inflammatory reactions.[18-20] While excessive ROS can damage endothelial cells and enhance the vascular sensitization to Ang II,[21] it can also promote the secretion of fibroblast growth factor 2 (FGF2).[22] Moreover, after AKI, leukocytes can be recruited to infiltrate the ischemic tissue, that may further increase the secretion of cytokines and induce inflammatory responses. Among them, interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α) are closely related to the formation and damage of inflammation after ischemia, while IL-1β can also drive the secretion of FGF2.[23] In addition to this, administration of antioxidant or anti-inflammatory therapy can significantly reduce kidney damage and distant organ injury.[24,25] According to epidemiological studies, patients with AKI had a significantly higher incidence for developing dementia than the controls,[26] and the patients who recovered from AKI had a higher incidence of developing incident stroke and mortality than the patients without AKI.[27] Due to the importance of maintenance of central nervous system perfusion on treatment of dementia or stroke, we focus on brain vessels during AKI and study the mechanism behind it.

Fibroblast growth factors (FGFs) are widely expressed in various tissues and cells with diverse biological activities such as tissue repair and angiogenic activities.[28] The most abundant FGF protein FGF2 binds to and dimerizes FGF receptors, resulting in phosphorylation receptor tyrosine residues. Phospholipase Cγ1 (PLCγ1), as the main protein in the downstream of FGF pathway, hydrolyze 4,5-bisphosphatidylinositol to produce inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 stimulates cytosolic calcium release by binding to relevant receptors in the cell. In addition, DAG can activate a series of intracellular signaling pathways such as protein kinase C (PKC) and increases cytosolic calcium and calcium sensitivity.[29] Some studies have shown that FGF2 can impact vascular tone and blood pressure.[30,31] and our previous study has shown that renal afferent arterioles from *Fgf2* knockout mice fail to contract in response to Ang II.[32] In addition, some other studies have shown that the expression of FGF2 is significantly increased after AKI, and the increased expression of FGF2 can be used as an indicator of the degree of AKI injury.[33,34] Moreover, Ang II exerts vasoconstriction mainly through angiotensin II type 1 receptors (AT_1R), which trigger the phosphorylation of PLCγ1 and increase the expression of IP_3 and DAG.[35] Thus the FGF2 signaling pathway and the Ang II signaling pathway share the common downstream signaling molecule PLCγ1, suggesting a mechanism for synergistic effect on vasoconstriction.

FGF binding protein 1 (FGFBP1) is a secreted carrier protein that releases locally stored FGFs from the extracellular heparin sulfate proteoglycans (HSPG) matrix to target cells which express FGF receptors (FGFRs).[36-38] A polymorphism in the human FGFBP1 locus

is associated with familial hypertension which results in increased expression of FGFBP1 in kidney macrophages of hypertensive subjects,[39] and conditional FGFBP1 expressing transgenic mice have FGF2 and Ang II dependent hypertension.[32] In the present study we show that FGFBP1 plays a significant role in AKI-mediated cerebral vascular sensitization to Ang II.

Methods

Animals

Male C57B1/6 mice (22-30 g, SLAC laboratory animal company, Shanghai, China) were fed standard food and allowed free access to tap water under standard conditions. Generation and characterization of the *Fgfbp1*$^{-/-}$ mice was described recently.[40] Animal handling procedures and experiment protocols were reviewed and approved by Guangzhou Medical University, Zhejiang University School of Medicine and Georgetown University's institutional animal care and use committee and conducted according to the NIH Guide for the Care and Use of Laboratory Animals.

Renal I/R surgery

Normal mice were randomly divided into five groups: sham-operated mice, 1 day AKI mice, 1 day AKI mice with intragastric administration of BGJ398 (10 mg/kg) 4 h before euthanasia, 1 week AKI mice and 1 week AKI mice treated with intragastric administration of BGJ398 (infigratinib) (10 mg/kg) at the 24 h, 48 h, 72 h and 96 h after surgery. For the *Fgfbp1*$^{-/-}$ mice, only divided into sham-operated mice and 1 day AKI mice. Using ischemia/reperfusion (I/R) to generate the AKI model was as described previously.[41] In brief, mice were anesthetized under inhalational anesthesia with 2% isoflurane and oxygen mixed with room air in a vaporizer, then both renal pedicles were clamped with two non-traumatic microvascular clamps for 30 min after visualizing both renal pedicles through a mid-line abdominal incision. Thereafter, the clamps were released to start reperfusion. Mice were kept on a heating pad (37±0.5 °C) during the surgery until they regained full consciousness. All sham-operated mice and 1 day AKI mice were euthanized 24 h after surgery and brain and kidney tissues were harvested for analyses. One week AKI mice were euthanized on the seventh day after surgery. Sham-operated mice were subjected to the complete surgical procedure without occlusion of the renal pedicles.

Determination of serum creatinine and BUN

Before euthanasia, blood was obtained from the eyes of mice in three groups (sham-operated mice, 1 day AKI mice and 1 week AKI mice) under 2% isoflurane anesthesia. After incubated the blood undisturbed at room temperature for 30 min, tubes were centrifuged it at 3000 rpm at room temperature for 15 min to collect serum. The concentration of serum creatinine and blood urea nitrogen (BUN) were determined by specific enzyme creatinine aminohydrolase and urease respectively and were measured by an automatic biochemical analyzer.

Morphometric studies

Mice were anesthetized by 2% isoflurane. After perfusion via the heart with phosphate-buffered saline (PBS), kidney tissue was fixed in 10% formalin and processed by dehydration and embedded in paraffin. Five microns' kidney tissue sections were stained with hematoxylin-eosin (H&E), periodic acid Schiff reaction (PAS) and Masson's trichrome for histological assessment. Histological changes were observed at 40X and 200X magnification. Renal injury was scored by grading the percentage of affected tubules per 10 randomly chosen nonoverlapping fields (200X magnification) according to the following criteria: tubular dilation, loss of brush border, tubular necrosis, and cast formation using PAS staining. The renal injury scoring was estimated on a scale from 0 to 5 as follows: 0, none; 1, 0-10%; 2, 11-25%; 3, 26-45%; 4, 46-75%, and 5, 76-100%. The renal pathologist quantitatively assessed tubular injury in a blinded fashion.

ELISA for FGF2, FGFBP1, BNP, S100B, TNF-α and IL-1β

Mice were anesthetized by 2% isoflurane. After collection of the whole blood through mice eyes, the blood was allowed to clot by leaving it undisturbed at room temperature. It took about 10-20 min. The clots were removed by centrifuging at 2000-3000 rpm for 20 min to get the serum for enzyme-linked immunosorbent assay (ELISA) assay of FGF2, FGFBP1, brain natriuretic peptide (BNP) and S100 calcium binding protein B (S100B). Kidney and brain tissue samples were cut, weighed, frozen in liquid nitrogen and stored at −80 °C. The tissue samples were homogenized after adding PBS. The supernatants were collected carefully after centrifuging at 2000-3000 rpm for 20 min. Aliquot the supernatant for ELISA assay of FGF2, FGFBP1, TNF-α and IL-1β.

Microperfusion and myograph of brain vasculature

Microperfusion and myograph technology allow analysis of isolated vessels without the confounding influences of the surrounding tissue and systemic cardiovascular regulation in the intact animal. Mice were anesthetized by 2% isoflurane and the brain tissue was gently lifted out of the skull. Brain medulla oblongata arterioles (BMas) ranging from 15-30 μm in diameter of 100 μm length located in the medulla oblongata were gently and rapidly dissected from surrounding brain tissue at 4°C in Dulbecco's modified eagle medium (DMEM) then transferred to an inverted microscope and mounted and perfused via two holding pipettes. One of the holding pipettes contained an internal perfusion pipette with an intraluminal flow (0.2 μL/min) and pressure (60 mmHg) was maintained as described previously.[42] The holding and perfusion pipettes were tailored to the mouse brain medulla arteriole size. The arterioles were perfused and studied in a temperature-controlled tissue chamber and the bath solution was maintained at 37 °C during experiments. Experimental success depended on efficient perfusion. The viability of each arteriole was firstly verified by bath addition of KCl (100 mmol/L). A monochrome video camera attached to a side port on the microscope was used to record the data. The photos were taken every 6 seconds. Each concentration lasted 2 minutes, so 20 photos were taken at each concentration. Arteriolar luminal diameter was calculated from the mean of seven photos for each concentration and no arteriole was reused in each experimental set: (i) Bath addition of Ang II (10^{-12} to 10^{-6} mol/L); (ii) Bath addition of norepinephrine (NE) (10^{-12} to 10^{-4} mol/L); (iii) Bath addition

of endothelin-1 (ET-1) (10^{-12} to 10^{-7} mol/L); (iv) Preincubation with FGF2 (20 ng/mL, 15 min) followed by bath addition of Ang II (10^{-12} to 10^{-6} mol/L); (v) Preincubation with FGFBP1 (20 ng/mL, 15 min) followed by bath addition of Ang II (10^{-12} to 10^{-6} mol/L); (vi) Preincubation with both FGF2 (20 ng/mL, 15 min) and FGFBP1 (20 ng/mL, 15 min) followed by bath addition of Ang II (10^{-12} to 10^{-6} mol/L); (vii) Preincubation with BGJ398 (1000 nmol/L, 15 min) followed by bath addition of Ang II (10^{-12} to 10^{-6} mol/L); (viii) Preincubation with BGJ398 (1000 nmol/L, 15 min) and then incubation with both FGF2 (20 ng/mL, 15 min) and FGFBP1 (20 ng/mL, 15 min) followed by bath addition of Ang II (10^{-12} to 10^{-6} mol/L). Contractions of BMas were presented as relative values of luminal diameters (percentage of the initial diameter). Brain basal arteries (BBAs) or post-communication arterioles (PCoAs) were gently isolated and transferred to a small volume culture dish with ice-cold (0 °C), combination gas-bubbled (95% O_2, 5% CO_2) artificial cerebrospinal fluid of the following composition (mmol/L): 125 NaCl, 3 KCl, 18 $NaHCO_3$, 1.25 NaH_2PO_4, 1 $MgCl_2$, 2 $CaCl_2$, 5 glucoses, PH 7.4 for further dissecting the arterial rings (2 mm in length). The arterial rings were mounted on 25 μm diameter stainless steel wire in myograph chambers and equilibrated for 20 min at 37 °C before experiments. After that, resting tension was set according to the manufacturer's protocol (DMT Wire Myograph Systems, ADInstruments Inc.) and vessel viability was assessed by the response to 80 mmol/L KCl. After two washes, cumulative concentration response for vasoactive agents was obtained. The protocols were as same as for BMas. Only one vessel of each type (BMa/BBA/PCoA) per animal was used and only viable brain vessels with a strong constriction in response to KCl were entered.

Calcium imaging

The BMas were perfused and loaded with calcium fluorescence indicator fluo 3, AM (solved in DMSO, 4 μmol/L) in DMEM for 45 min. Using three washes with DMEM to remove the free probes. The calcium fluorescence of BMas from both sham-operated mice and 1 day AKI mice were measured using a Nikon confocal microscope. The 488 nm excitation and 526 nm emission were used to determine the cytosolic calcium concentration of BMas *in vitro*. The calcium changes were used to investigate the effect of vasoactive substances on cytosolic calcium in five different experimental sets (one arteriole per set): (i) DMEM; (ii) Ang II (10^{-6} mol/L); (iii) Preincubation with both FGF2 (20 ng/mL, 15 min) and FGFBP1 (20 ng/mL, 15 min) followed by Ang II (10^{-6} mol/L); (iv) Preincubation with BGJ398 (1000nmol/L, 15min); (v) Preincubation with BGJ398 (1000 nmol/L, 15 min) and then incubation with both FGF2 (20 ng/mL, 15 min) and FGFBP1 (20 ng/mL, 15 min) followed by bath addition of Ang II (10^{-12} to 10^{-6} mol/L).

Measurement of blood pressure

Blood pressure were monitored by implanted radio telemetry (Data Sciences International, St. Paul, MN, USA).[43] Briefly, the mouse was anesthetized with 2% isoflurane. The telemeter catheter was inserted to the left carotid artery at the aortic arch, with the telemeter body placed in a subcutaneous pocket on the right flank. The telemetric signal was detected using a model RPC-1 receiver, a 20-channel data-exchange matrix, APR-1 ambient pressure monitor, and a Dataquest ART 2.3 acquisition system (Data Sciences International). The

mean arterial pressure for every 5 min periods within 30 min measurement time was measured from the recorded signals at 10 AM in each mouse.

Collection of the cerebral arterioles by iron perfusion

The method was applied as described previously.[44,45] Mice from both sham-operated and 1 day AKI group were anesthetized with 2% isoflurane. The inferior vena cava was isolated with a small incision cut. A catheter was then inserted into the left ventricle of the heart through a incision. Thereafter, the vascular system was flushed with PBS and perfused with 1% iron oxide PBS solution. After removal of the skull, cerebral arterioles were visualized via magnetized iron particles. The vascular structures filled with magnetized iron particles were isolated from non-vascular tissue first by collagenase type IV digestion and then collected by a high-performance magnet. The procedure resulted in isolated free cerebral arterioles which were used for mRNA measurement.

Quantitative RT-PCR analysis

Total RNA was extracted from cerebral arterioles and brain medulla oblongata tissue from both sham-operated and 1 day AKI group following the manufacturer's instruction in AxyPrep multisource total RNA miniprep Kit from AXYGEN, and reverse transcribed and subjected to PCR on the CFX96 System (Bio-Rad). SYBR green was used for the fluorescent detection of DNA generated during PCR. Data were expression as cycle threshold (Ct) and the fold difference in target gene expression between sham-operated and 1 day AKI group was calculated according to the following formula: fold difference=$2^{-\text{difference in dCt}}$. Gene expression was normalized to the expression of reduced glyceraldehyde-phosphate dehydrogenase (GAPDH). Primers, which bridge at least one intron, were designed for PCR amplification on the basis of published sequences for mice. The primers used are shown in Table 1.

Western Blotting

After euthanasia, the brain medulla oblongata tissue was collected and stored at −80 °C. The samples were loaded and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes. The proteins on the membranes were probed with corresponding primary antibodies for AT_1R, AT_2R, FGFR1-4 and β-actin and the second horseradish peroxidase-labeled IgG anti-rabbit/mouse antibody. The probed bands were visualized by enhanced chemiluminescent substrates (ECL, Thermo Scientific) and analyzed by using Image J software (National Institutes of Health, Bethesda, MD).

Detection of antioxidant capacity

Mice were anesthetized by 2% isoflurane and the tissue samples were harvested and used for measurement of O_2^-, H_2O_2, superoxide dismutase (SOD) and catalase (CAT) activity. O_2- and H_2O_2 content in both brain and kidney tissues were analyzed using standard assays according to manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China and Beyotime Biotechnology, Shanghai, China).[46] The supernatants from brain and kidney tissues were collected for SOD and CAT activity according to the

manufacturer's instructions (Beyotime Biotechnology, Shanghai, China).[47] Enzymatic activities of SOD and CAT in supernatants were determined based on its ability to form H_2O_2 and degradation of H_2O_2 respectively.

Pharmacological reagents

Reagent sources were as follows: Mouse FGF2 ELISA Kit, Mouse IL-1β ELISA Kit and Mouse TNF-α ELISA Kit from Wuhan ColorfulGene biological technology Co., Ltd (China); Mouse FGFBP1 ELISA Kit, Mouse BNP ELISA Kit, Mouse S100B ELISA Kit and Mouse Ang II ELISA Kit from Wuhan Elabscience Biotechnology Co., Ltd (China); DMEM with low glucose and no phenol red indicator and FGF2 from Gibco (USA); Ang II, NE, ET-1, collagenase type IV from Sigma-Aldrich (USA); FGFBP1 from R&D Systems (USA); FGFR1/2/3 tyrosine kinase inhibitor BGJ398 from MedChem Express (USA); Fluo 3, AM from Molecular Probes (USA); Kit for O_2^- and H_2O_2 from Nanjing Jiancheng Bioengineering Institute, Nanjing (China); Kit for SOD and CAT activity from Beyotime Biotechnology, Shanghai (China); PrimeScript RT Master Mix Kit and SYBR Premix Ex Taq II Kit from TaKaRa Clontech, Dalian (China); AxyPrep multisource total RNA miniprep Kit from AXYGEN (USA); AT_2R primary antibodies from Bioss, Beijing (China) and the rest of the primary antibodies and the second antibodies from Affinity (USA).

Statistical analyses

Data were presented as Mean ± SEM values. Statistical analyses were mostly performed with GraphPad Prism Software (6.01, La Jolla, CA, USA). One-way ANOVA or two-way ANOVA with repeated measures followed by Sidak's post hoc test or Turkey's post hoc test were performed when multiple groups were compared according to different conditions and unpaired *t* test was performed when two groups were compared. $P<0.05$ was considered statistically significant and all *P* value are two-sided.

Results

Successfully constructed AKI animal model

In the studies presented here, mice had a ninefold increase of serum creatinine (Sham:16.33±0.61 μmol/L *v.s.* 1 day AKI:145.20±14.91 μmol/L; N=6; $P<0.001$) and a threefold increase of BUN (Sham: 13.93 ± 0.78 mmol/L *v.s.* 1 day AKI:45.63 ± 4.08 mmol/L; N=6; $P<0.05$) 24 h after renal I/R compared to sham-operated mice (Figure 1A) and returned to normal values after one week (serum creatinine: 20.67 ± 2.86 μmol/L; BUN:24.50 ± 4.90 mmol/L; N=6). The kidney in 1 day AKI group showed widespread disruption of the tubular architecture such as tubular dilation, swelling and necrosis, luminal congestion and inflammatory cell infiltration in the corticomedullary junction compared to sham-operated mice and returned to normal values after one week (Figure 1B and Figure 1C).

Enhanced cerebral vascular sensitivity to Ang II after AKI

Functional investigation of BMas, BBAs and PCoAs showed enhanced Ang II responses in AKI group compared to sham-operated mice for at least one week ($P<0.001$, Figure 2 and

Figure S1), thus at a time the BUN and creatinine have already returned to normal values (Figure 1A). The response to NE or ET-1 was not altered in the AKI group compared to sham-operated mice (Figure 2 and Figure S1).

Enhanced serum cerebral biomarkers after AKI

To test for cerebral injury after AKI, we analyzed BNP and S100B, which are used as biomarkers for the diagnosis of ischemic stroke.[48] Serum concentration of S100B was significantly increased after 1 day AKI. BGJ398 treatment reversed this increase, whilst BNP was not changed significantly in either group (Figure S2).

Brain FGF2 and FGFBP1 expression were increased after AKI and can promote cerebrovascular contractile to Ang II and calcium release

Protein expression of FGF2 and FGFBP1 in both serum and kidney tissue was significantly increased in 1 day AKI mice compared to sham-operated mice (Figure 3A) suggesting that the increased FGF2 and FGFBP1 in serum could be derived from the damaged kidney tissue and transported throughout the body. In sham-operated mice, only combined application of FGF2 and FGFBP1 enhanced the response to Ang II in all three types of cerebral vessels compared to non-treated animals. Treatment with FGF2 and FGFBP1 together further enhanced the Ang II responses in AKI animals (Figure 3B and Figure S3). The calcium transients in BMas reflect the observations made in the contraction experiments. Ang II did not increase cytosolic calcium measurably in sham-operated mice, but did it after addition of FGF2 and FGFBP1. In 1 day AKI group, Ang II application went along with increases in the cytosolic calcium, which were further enhanced by treatment with FGF2 and FGFBP1, and were effectively blocked by BGJ398. Only treated with BGJ398 did not block the cytosolic calcium release (Figure 3C). The baseline cytosolic calcium was stronger in 1 day AKI group than in the sham-operated group, which may be due to the increased expression of FGF2 and FGFBP1 after AKI.

Inhibition of the FGFR tyrosine kinase reversed AKI-induced increase in cerebrovascular contractile response to Ang II

The FGFR1-3 tyrosine kinase inhibitor BGJ398 has been introduced into human studies and recently shown promising efficacy and manageable side effects in a Phase II trial in cancer patients.[49] Thus, we used BGJ398 to evaluate whether FGFR kinase inhibition can impact the AKI-induced vasoconstriction to Ang II. In the *in vitro* sets, addition of the inhibitor BGJ398 decreased the vasoconstrictive effect of Ang II in BMas from sham-operated mice with both FGF2 plus FGFBP1 treatment or AKI mice (no additions) (Figure 4A). In the *in vivo* studies, BMas from both sham-operated mice and AKI mice with BGJ398 treatment showed no significant contraction to Ang II (Figure 4B and Figure 4C). The Ang II response in BBAs and PCoAs from AKI mice with BGJ398 treatment both *in vitro* and *in vivo* was significantly reduced compared to vessels from the untreated group (Figure S4).

Blood pressure was not changed after AKI

There was no significant difference in blood pressure between sham-operated mice and 1 day AKI mice. (Figure S5).

No AKI-mediated increase in cerebrovascular contractile response to Ang II in Fgfbp1 knockout mice

The BMas from the *Fgfbp1*$^{-/-}$ sham-operated mice did not respond to Ang II. Thus, they behaved similar to the BMas from wild type sham-operated mice. However, the Ang II response after 1 day AKI in the *Fgfbp1*$^{-/-}$ mice was almost completely abolished compared to the wild type 1 day AKI mice, suggesting an important role of this protein for the enhanced Ang II response on AKI. Application of FGF2 and FGFBP1 in *Fgfbp1*$^{-/-}$ mice with AKI enhanced the response, supporting the idea that FGF2 and FGFPB1 are necessary for the enhanced Ang II response during AKI (Figure 5).

Increased expression of PLCγ1 as indicators of FGFR and Ang II pathway activation after AKI

We probed small arterioles of the brain for the expression of PLCγ1 by iron perfusion and used brain tissue for analysis of the expression of Ang II- and FGF-receptors. Notably, the receptor mRNA and protein for AT_1R, AT_2R were not changed significantly in brain tissue after 1 day AKI (Figure 6). The FGFR2 mRNA expression was reduced by about 50% and FGFR3 mRNA expression was increased by about 250% in brain tissue after 1 day AKI (Figure 6A), but there was no change at the protein level (Figure 6B). PLCγ1 is the common key downstream signaling molecule of both FGF2 and Ang II signaling, which can influence calcium release and vasoconstriction. In the cerebral arterioles, the mRNA expression of PLCγ1 was significantly increased after AKI (Figure 6A). These findings matched with the activation of the FGF2 and Ang II pathway after AKI.

The inflammatory response increased and the antioxidant capacity decreased after AKI

TNF-α and IL-1β levels were markedly increased after 30 min ischemia and 24 h reperfusion, while there was no significant change of these inflammatory cytokines in the brain tissue suggesting that the kidney was the major important source of these inflammatory signals (Figure 7A). The analysis of components of ROS metabolism (such as O_2^-, H_2O_2, SOD and CAT) showed patterns related to oxidative stress in the kidney but not in the brain (Figure 7B).

Discussion

The present study reveals an increased reactivity of cerebral arterial vessels to Ang II after AKI. The data indicate an important contribution of both FGF2 and FGFBP1 for this effect. The increased Ang II reactivity may result in disturbed brain blood flow and thus contributes the impairment of the brain function after AKI.

The role of the FGF2 pathway in AKI has not been well explored. Several studies have described a protective function of FGF2 in AKI,[50,51] although renal accumulation of FGF2 was also associated with tubular proliferation and fibrogenic lesions.[52] On the one side, systemic administrations of FGF2 lowered blood pressure and restored nitric oxide synthase activity in spontaneously hypertensive rats.[53,54] Chronic as well as acute intravenous infusion of recombinant FGF2 in normotensive animals induced a significant reduction in blood pressure.[53,55] On the other side, it was surprising that *Fgf2* knockout mice had a lower

blood pressure and their portal veins showed reduced response to vasoconstrictors due to decreased smooth muscle contractility.[30] The convergence of Ang II and FGF2 signaling in rat aortic smooth muscle cells through the extracellular regulated kinases (ERK) pathway towards the stimulation of calcium release and enhanced contraction has already known.[41] We have recently described the crosstalk of FGF2 and Ang II signaling in cultured cells, in isolated renal afferent arterioles *in vitro* and in vessels in intact animals *in vivo*.[32]

The current finding of increased Ang II responses in brain vessels differs from that seen in renal afferent arterioles from AKI mice, which responded less compared to sham animals.[56] Thus, the increased cerebrovascular response to Ang II after AKI may show organ specificity. Remarkably, NE and ET-1 responses did not differ between AKI and sham-operated animals (Figure 2B). The observed increase in serum concentrations of S100B one day after I/R of the kidney indicates an irritation of the brain tissue, which, however, was not severe, because serum BNP concentration and IL-1β and TNF-α levels in the brain tissue did not change significantly (Figure 7 and Figure S4). At the same time, there was no significant change in blood pressure after AKI, excluding a role of systemic cardiovascular reaction in the AKI model (Figure S5). In support of a contribution of the FGF2 pathway to the sensitization to Ang II, the pretreatment of isolated vessels from sham-operated mice with exogenously added FGF2 plus FGFBP1 mimics this sensitization to Ang II. It is noteworthy that neither FGF2 nor FGFBP1 alone can enhance the Ang II induced vasoconstriction effect because FGF2 alone is bound by extracellular HSPGs but can be made available to the receptors by the FGFBP1 chaperone protein and thus induce FGF2 signaling (Figure 3B).[38] Synergism between FGF2 and Ang II pathways had been reported earlier in Ang II-induced cardiomyopathy and cardiac hypertrophy as well as FGFBP1 induced hypertension which utilized c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) pathways.[32,57] Here we describe that the crosstalk between FGF2 and Ang II signaling can also lead to an increase in cytosolic calcium release that mediates brain vessel contraction (Figure 3C). A differential Ang II receptor expression seem not to be a reason for the enhanced Ang II response of cerebral vessels after AKI, because it did not differ compared to sham-operated animals. However, we found strong increase of PLCγ1 mRNA concentration in the cerebral arterioles (Figure 6). PLCγ1 contributes to both the FGF2 and Ang II pathway. However, if this enzyme is an important factor in the FGF2 effect remains questionable, since NE and ET-1, of which pathways include the PLC, do not induce stronger responses in AKI animals. One possible explanation is that the function of NE and ET-1 are more dependent on different subtypes of PLC.

It will be critical to develop targeted therapies to improve the outcomes in AKI. Our studies implicate the activation of the FGF2 pathway via upregulation of FGFBP1 as an important step in the sensitization of brain vessels to Ang II (Figure 5). With the FGFR1-3 kinase inhibitor, we have a clinically used drug (BGJ398, infigratinib) at our disposal to test whether inhibition of FGF2 pathway could impact Ang II sensitization of brain vessels after AKI. But which type of FGF receptor subtypes plays the most important role during AKI requires further research. We opt for treatment model rather than prevention model: BGJ398 treatment was initiated after AKI which had been allowed to progress for several hours. We found that treatment of mice with BGJ398 after initiation of AKI indeed reversed the sensitization of brain vessels to Ang II. Thus, we speculate that an appropriate treatment for

AKI may be FGFR inhibition. Given that BGJ398 is approved for clinical use,[58] this may be a potential candidate for a clinical trial.

From studies on the mechanisms of regulation of FGF2 and FGFBP1 we know that these genes are stress response gene controlled by a defined set of transcriptional activators that are turned on by different stressors including ROS and inflammation,[59-63] One study showed that the inflammation were found dysregulated in the kidney while they were not changed in the brain which were consistent with our study. In this study, animals showed neurological deficits along with increased numbers of pyknotic neurons and activated microglia cell. The data suggest that the impairment of brain function does not require inflammation or oxidative stress in the brain itself.[64] Rather, activation of these systems in the kidney induces the generation of factors such as FGF2 and FGFBP1, which in turn interact with the angiotensin system in brain vessels and may impair brain perfusion. A very recent study in AKI showed that kidney-resident macrophages are reprogrammed towards a developmental state after AKI.[65] It is noteworthy that FGFBP1 is upregulated in macrophages in patients with familial hypertension due to a genetic polymorphism as discussed in the introduction,[39] and that embryonic kidneys show upregulated FGFBP1 relative to adults.[66] Further work will be needed to explore the role of the macrophage FGFBP1 system on the sensitive cerebrovascular responses reported here.

Perspectives

In conclusion, our studies indicate that activation of the FGF2 signaling pathway by induction of the FGFBP1 chaperone protein can drive kidney-brain crosstalk during AKI as summarized in Figure 8. Most importantly, in our opinion, blockade of FGFR tyrosine kinase activation with a clinically used FGFR kinase inhibitor BGJ398 might reset the brain vascular reactivity during AKI and thereby prevent brain vessel injury, but this still require careful clinical trial.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

L. Zhao, X. Cao, L. Li and E.Y. Lai designed, performed and analyzed most of the experiments. L. Zhao, X. Cao, Q. Wang, S. Zhou, N. Xu, S. Jiang, M.O. Schmidt and R. Labes generated the AKI model, did the iron microperfusion experiments, acquired molecular data or performed immunhistological and biochemical analyses. E.Y. Lai, A. Wellstein, L. Li, Q. Wei, L. Chen, J. Zhao, X. Fu, A. Patzak and C.S. Wilcox advised on data interpretation and discussion. L. Zhao, E.Y. Lai and A. Wellstein wrote the manuscript, E.Y. Lai, L. Li, L. Zhao, X. Fu, A. Wellstein, A. Patzak and C.S. Wilcox advised data analysis. E.Y. Lai, X. Fu and A. Wellstein supervised the studies.

Sources of Funding

This study was supported by research grants to E.Y. Lai from the National Nature Science Foundation of China (31671193) and the Key research development program of Ningxia 2018BFG0210; E.Y. Lai is Mercator Fellow of the Deutsche Forschungsmeinschaft (CRC 1365). Support to A. Patzak from the German Research Foundation (DFG PA 479/10-2) and (DFG CRC 1365), to A. Wellstein from the US NIH (HL68686 and CA71508), to C.S. Wilcox support from the NIDDK of the NIH (DK-108272), to X. Fu support from National Natural Science Foundation of China (81871137) and to L. Zhao support from postdoctoral research project of Guangzhou (Q0301-026).

References

1. Yang L. Acute kidney injury in Asia. Kidney Dis (Basel). 2016;2:95–102. doi: 10.1159/000441887 [PubMed: 27921036]
2. Bouchard J, Mehta RL. Acute kidney injury in western countries. Kidney Dis (Basel). 2016;2:103–110. doi: 10.1159/000445091 [PubMed: 27921037]
3. Lameire N, Van Biesen W, Vanholder R. Acute renal failure. Lancet. 2005;365:417–430. doi: 10.1016/S0140-6736(05)17831-34 [PubMed: 15680458]
4. Hsu CY, McCulloch CE, Fan D, Ordoñez JD, Chertow GM, Go AS. Community-based incidence of acute renal failure. Kidney Int. 2007;72:208–212. doi: 10.1038/sj.ki.5002297 [PubMed: 17507907]
5. Lafrance JP, Miller DR. Acute kidney injury associates with increased long-term mortality. J Am Soc Nephrol. 2010;21:345–352. doi: 10.1681/ASN.2009060636 [PubMed: 20019168]
6. Chawla LS, Amdur RL, Amodeo S, Kimmel PL, Palant CE. The severity of acute kidney injury predicts progression to chronic kidney disease. Kidney Int. 2011;79:1361–1369. doi: 10.1038/ki.2011.42 [PubMed: 21430640]
7. VA/NIH Acute Renal Failure Trial Network, Palevsky PM, Zhang JH, O'Connor TZ, Chertow GM, Crowley ST, Choudhury D, Finkel K, Kellum JA, Paganini E, Schein RM, Smith MW, Swanson KM, Thompson BT, Vijayan A, Watnick S, Star RA, Peduzzi P. Intensity of renal support in critically ill patients with acute kidney injury. N Engl J Med. 2008;359:7–20. doi: 10.1056/NEJMoa0802639 [PubMed: 18492867]
8. Ympa YP, Sakr Y, Reinhart K, Vincent JL. Has mortality from acute renal failure decreased? A systematic review of the literature. Am J Med. 2005;118:827–832. doi: 10.1016/j.amjmed.2005.01.069 [PubMed: 16084171]
9. Hassoun HT, Grigoryev DN, Lie ML, Liu M, Cheadle C, Tuder RM, Rabb H. Ischemic acute kidney injury induces a distant organ functional and genomic response distinguishable from bilateral nephrectomy. Am J Physiol Renal Physiol. 2007;293:F30–F40. doi: 10.1152/ajprenal.00023.2007 [PubMed: 17327501]
10. Klein CL, Hoke TS, Fang WF, Altmann CJ, Douglas IS, Faubel S. Interleukin-6 mediates lung injury following ischemic acute kidney injury or bilateral nephrectomy. Kidney Int. 2008;74:901–909. doi: 10.1038/ki.2008.314 [PubMed: 18596724]
11. Liu M, Liang Y, Chigurupati S, Lathia JD, Pletnikov M, Sun Z, Crow M, Ross CA, Mattson MP, Rabb H. Acute kidney injury leads to inflammation and functional changes in the brain. J Am Soc Nephrol. 2008;19:1360–1370. doi: 10.1681/ASN.2007080901 [PubMed: 18385426]
12. Kelly KJ. Distant effects of experimental renal ischemia/reperfusion injury. J Am Soc Nephrol. 2003;14:1549–1558. doi: 10.1097/01.ASN.0000064946.94590.46 [PubMed: 12761255]
13. Park SW, Chen SW, Kim M, Brown KM, Kolls JK, D'Agati VD, Lee HT. Cytokines induce small intestine and liver injury after renal ischemia or nephrectomy. Lab Invest. 2011;91:63–84. doi: 10.1038/labinvest.2010.151 [PubMed: 20697374]
14. Bove T, Calabrò MG, Landoni G, Aletti G, Marino G, Crescenzi G, Rosica C, Zangrillo A. The incidence and risk of acute renal failure after cardiac surgery. J Cardiothorac Vasc Anesth. 2004;18:442–445. doi: 10.1053/j.jvca.2004.05.021 [PubMed: 15365924]
15. Lu R, Kiernan MC, Murray A, Rosner MH, Ronco C. Kidney-brain crosstalk in the acute and chronic setting. Nat Rev Nephrol. 2015;11:707–719. doi: 10.1038/nrneph.2015.131 [PubMed: 26281892]
16. Allred AJ, Chappell MC, Ferrario CM, Diz DI. Differential actions of renal ischemic injury on the intrarenal angiotensin system. Am J Physiol Renal Physiol. 2000;279:F636–F645. doi: 10.1152/ajprenal.2000.279.4.F636 [PubMed: 10997913]
17. Cao W, Li A, Li J, Wu C, Cui S, Zhou Z, Liu Y, Wilcox CS, Hou FF. Reno-cerebral reflex activates the renin-angiotensin system, promoting oxidative stress and renal damage after ischemia-reperfusion injury. Antioxid Redox Signal. 2017;27:415–432. doi: 10.1089/ars.2016.6827 [PubMed: 28030955]
18. Serteser M, Koken T, Kahraman A, Yilmaz K, Akbulut G, Dilek ON. Changes in hepatic TNF-alpha levels, antioxidant status, and oxidation products after renal ischemia/reperfusion injury in mice. JSurg Res. 2002;107:234–240. doi: 10.1006/jsre.2002.6513 [PubMed: 12429181]

19. Ramesh G, Reeves WB. Inflammatory cytokines in acute renal failure. Kidney Int Suppl. 2004;66:S56–S61. doi: 10.1111/j.1523-1755.2004.09109.x
20. Kinsey GR, Li L, Okusa MD. Inflammation in acute kidney injury. Nephron Exp Nephrol. 2008;109:e102–e107. doi: 10.1159/000142934 [PubMed: 18802372]
21. Phillips SA, Pechman KR, Leonard EC, Friedrich JL, Bian JT, Beal AG, Basile DP. Increased ang II sensitivity following recovery from acute kidney injury: role of oxidant stress in skeletal muscle resistance arteries. Am J Physiol Regul Integr Comp Physiol. 2010;298:R1682–R1691. doi: 10.1152/ajpregu.00448.2009 [PubMed: 20335375]
22. Wang X, Zhao T, Huang W, Wang T, Qian J, Xu M, Kranias EG, Wang Y, Fan GC. Hsp20-engineered mesenchymal stem cells are resistant to oxidative stress via enhanced activation of Akt and increased secretion of growth factors. Stem Cells. 2009;27:3021–31. doi: 10.1002/stem.230 [PubMed: 19816949]
23. Lee JG, Kay EP. Common and distinct pathways for cellular activities in FGF-2 signaling induced by IL-1beta in corneal endothelial cells. Invest Ophthalmol Vis Sci. 2009;50:2067–2076. doi: 10.1167/iovs.08-3135 [PubMed: 19136710]
24. Ahmadiasl N, Banaei S, Alihemmati A, Baradaran B, Azimian E. The anti-inflammatory effect of erythropoietin and melatonin on renal ischemia reperfusion injury in male rats. Adv Pharm Bull. 2014;4:49–54. doi: 10.5681/apb.2014.008 [PubMed: 24409409]
25. Emal D, Rampanelli E, Stroo I, Butter LM, Teske GJ, Claessen N, Stokman G, Florquin S, Leemans JC, Dessing MC. Depletion of gut microbiota protects against renal ischemia-reperfusion injury. J Am Soc Nephrol. 2017;28:1450–1461. doi: 10.1681/ASN.2016030255 [PubMed: 27927779]
26. Tsai HH, Yen RF, Lin CL, Kao CH. Increased risk of dementia in patients hospitalized with acute kidney injury: A nationwide population-based cohort study. PLoS One. 2017;12:e0171671. doi: 10.1371/journal.pone.0171671 [PubMed: 28192452]
27. Wu VC, Wu PC, Wu CH, Huang TM, Chang CH, Tsai PR, Ko WJ, Chen L, Wang CY, Chu TS, Wu KD, National Taiwan University Study Group on Acute Renal Failure (NSARF) Group. The impact of acute kidney injury on the long-term risk of stroke. J Am Heart Assoc. 2014;3:e000933. doi: 10.1161/JAHA.114.000933 [PubMed: 25027018]
28. Itoh N, Ornitz DM. Fibroblast growth factors: from molecular evolution to roles in development, metabolism and disease. J Biol Chem. 2011;149:121–130. doi: 10.1093/jb/mvq121
29. Goetz R, Mohammadi M. Exploring mechanisms of FGF signalling through the lens of structural biology. Nat Rev Mol Cell Biol. 2013;14:166–180. doi: 10.1038/nrm3528 [PubMed: 23403721]
30. Zhou M, Sutliff RL, Paul RJ, Lorenz JN, Hoying JB, Haudenschild CC, Yin M, Coffin JD, Kong L, Kranias EG, Luo W, Boivin GP, Duffy JJ, Pawlowski SA, Doetschman T. Fibroblast growth factor 2 control of vascular tone. Nat Med. 1998;4:201–207. doi: 10.1038/nm0298-201 [PubMed: 9461194]
31. Dono R, Texido G, Dussel R, Ehmke H, Zeller R. Impaired cerebral cortex development and blood pressure regulation in FGF-2-deficient mice. EMBO J. 1998;17:4213–4225. doi: 10.1093/emboj/17.15.4213 [PubMed: 9687490]
32. Tassi E, Lai EY, Li L, Solis G, Chen Y, Kietzman WE, Ray PE, Riegel AT, Welch WJ, Wilcox CS, Wellstein A. Blood pressure control by a secreted FGFBP1 (fibroblast growth factor-binding protein). Hypertension. 2018;71:160–167. doi: 10.1161/HYPERTENSIONAHA.117.10268 [PubMed: 29158353]
33. Wai K, Soler-García AA, Perazzo S, Mattison P, Ray PE. A pilot study of urinary fibroblast growth factor-2 and epithelial growth factor as potential biomarkers of acute kidney injury in critically ill children. Pediatr Nephrol. 2013;28:2189–2198. doi: 10.1007/s00467-013-2543-3 [PubMed: 23872928]
34. Hoffman SB, Massaro AN, Soler-García AA, Perazzo S, Ray PE. A novel urinary biomarker profile to identify acute kidney injury (AKI) in critically ill neonates: a pilot study. Pediatr Nephrol. 2013;28:2179–2188. doi: 10.1007/s00467-013-2524-6 [PubMed: 23783654]
35. Schieffer B, Paxton WG, Marrero MB, Bernstein KE. Importance of tyrosine phosphorylation in angiotensin II type 1 receptor signaling. Hypertension. 1996;27:476–480. doi: 10.1161/01.HYP.27.3.476 [PubMed: 8613189]

36. Wu DQ, Kan MK, Sato GH, Okamoto T, Sato JD. Characterization and molecular cloning of a putative binding protein for heparin-binding growth factors. J Biol Chem. 1991;266:16778–16785. [PubMed: 1885605]
37. Aigner A, Butscheid M, Kunkel P, Krause E, Lamszus K, Wellstein A, Czubayko F. An FGF-binding protein (FGF-BP) exerts its biological function by parallel paracrine stimulation of tumor cell and endothelial cell proliferation through FGF-2 release. Int J Cancer. 2001;92:510–517. doi: 10.1002/1097-0215(20010515)92:4<510::AID-IJC1227>3.0.CO;2-H [PubMed: 11304685]
38. Czubayko F, Liaudet-Coopman ED, Aigner A, Tuveson AT, Berchem GJ, Wellstein A. A secreted FGF-binding protein can serve as the angiogenic switch in human cancer. Nat Med. 1997;3:1137–1140. doi: 10.1038/nm1097-1137 [PubMed: 9334727]
39. Tomaszewski M, Charchar FJ, Nelson CP, Barnes T, Denniff M, Kaiser M, Debiec R, Christofidou P, Rafelt S, van der Harst P, Wang WY, Maric C, Zukowska-Szczechowska E, Samani NJ. Pathway analysis shows association between FGFBP1 and hypertension. J Am Soc Nephrol. 2011;22:947–955. doi: 10.1681/ASN.2010080829 [PubMed: 21436287]
40. Schmidt MO, Garman KA, Lee YG, Zuo C, Beck PJ, Tan M, Aguilar-Pimentel JA, Ollert M, Schmidt-Weber C, Fuchs H, Gailus-Durner V, Hrabe de Angelis M, German Mouse Clinic Consortium, Tassi E, Riegel AT, Wellstein A. The role of fibroblast growth factor-binding protein 1 in skin carcinogenesis and inflammation. J Invest Dermatol. 2018;138:179–188. doi: 10.1016/j.jid.2017.07.847 [PubMed: 28864076]
41. Samain E, Bouillier H, Miserey S, Perret C, Renaud JF, Safar M, Dagher G. Extracellular signal-regulated kinase pathway is involved in basic fibroblast growth factor effect on angiotensin II-induced Ca^{2+} transient in vascular smooth muscle cell from Wistar-Kyoto and spontaneously hypertensive rats. Hypertension. 2000;35:61–67. doi: 10.1161/01.HYP.35.1.61 [PubMed: 10642276]
42. Coyne EF, Ngai AC, Meno JR, Winn HR. Methods for isolation and characterization of intracerebral arterioles in the C57/BL6 wild-type mouse. J Neurosci Meth. 2002;120:145–153. doi: 10.1016/S0165-0270(02)00197-8
43. Perry TE, Song M, Despres DJ, Kim SM, San H, Yu ZX, Raghavachari N, Schnermann J, Cannon RO 3rd, Orlic D. Bone marrow-derived cells do not repair endothelium in a mouse model of chronic endothelial cell dysfunction. Cardiovasc Res .2009;84:317–325. doi: 10.1093/cvr/cvp215 [PubMed: 19578071]
44. Chaudhari A, Kirschenbaum MA. A rapid method for isolating rabbit renal microvessels. An J Physiol. 1988;254:F291–F296. doi: 10.1152/ajprenal.1988.254.2.F291
45. Lai EY, Martinka P, Fähling M, Mrowka R, Steege A, Gericke A, Sendeski M, Persson PB, Persson AE, Patzak A. Adenosine restores angiotensin II-induced contractions by receptor-independent enhancement of calcium sensitivity in renal arterioles. Circ Res. 2006;99:1117–1124. doi: 10.1161/01.RES.0000249530.85542.d4 [PubMed: 17038642]
46. Xu N, Wang Q, Jiang S, Wang Q, Hu W, Zhou S, Zhao L, Xie L, Chen J, Wellstein A, Lai EY. Fenofibrate improves vascular endothelial function and contractility in diabetic mice. Redox Biol. 2019;20:87–97. doi: 10.1016/j.redox.2018.09.024 [PubMed: 30296701]
47. Lu MC, Zhao J, Liu YT, Liu T, Tao MM, You QD, Jiang ZY. CPUY192018, a potent inhibitor of the Keap1-Nrf2 protein-protein interaction, alleviates renal inflammation in mice by restricting oxidative stress and NF-κB activation. Redox Biol. 2019;26:101266. doi: 10.1016/j.redox.2019.101266 [PubMed: 31279986]
48. Jickling GC, Sharp FR. Biomarker panels in ischemic stroke. Stroke. 2015;46:915–920. doi: 10.1161/STROKEAHA.114.005604 [PubMed: 25657186]
49. Javle M, Lowery M, Shroff RT, Weiss KH, Springfeld C, Borad MJ, Ramanathan RK, Goyal L, Sadeghi S, Macarulla T, El-Khoueiry A, Kelley RK, Borbath I, Choo SP, Oh DY, Philip PA, Chen LT, Reungwetwattana T, Van Cutsem E, Yeh KH, Ciombor K, Finn RS, Patel A, Sen S, Porter D, Isaacs R, Zhu AX, Abou-Alfa GK, Bekaii-Saab T. Phase II study of BGJ398 in patients with FGFR-altered advanced cholangiocarcinoma. J Clin Oncol. 2018;36:276–282. doi: 10.1200/JCO.2017.75.5009 [PubMed: 29182496]
50. Villanueva S, Cespedes C, Gonzalez A, Vio CP. bFGF induces an earlier expression of nephrogenic proteins after ischemic acute renal failure. Am J Physiol Regul Integr Comp Physiol. 2006;291:R1677–R1687. doi: 10.1152/ajpregu.00023.2006 [PubMed: 16873559]

51. Villanueva S, Cespedes C, Gonzalez A, Roessler E, Vio CP. Inhibition of bFGF-receptor type 2 increases kidney damage and suppresses nephrogenic protein expression after ischemic acute renal failure. Am J Physiol Regul Integr Comp Physiol. 2008;294:R819–R828. doi: 10.1152/ajpregu.00273.2007 [PubMed: 18184769]
52. Kriz W, Hähnel B, Rösener S, Elger M. Long-term treatment of rats with fgf-2 results in focal segmental glomerulosclerosis. Kidney Int. 1995;48:1435–1450. doi: 10.1038/ki.1995.433 [PubMed: 8544400]
53. Cuevas P, Carceller F, Ortega S, Zazo M, Nieto I, Giménez-Gallego G. Hypotensive activity of fibroblast growth-factor. Science. 1991;254:1208–1210. doi: 10.1126/science.1957172 [PubMed: 1957172]
54. Cuevas P, García-Calvo M, Carceller F, Reimers D, Zazo M, Cuevas B, Muñoz-Willery I, Martínez-Coso V, Lamas S, Gimènez-Gallego G. Correction of hypertension by normalization of endothelial levels of fibroblast growth factor and nitric oxide synthase in spontaneously hypertensive rats. P Natl Acad Sci USA. 1996;93:11996–12001. doi: 10.1073/pnas.93.21.11996
55. Lazarous DF, Scheinowitz M, Shou M, Hodge E, Rajanayagam S, Hunsberger S, Robison WG Jr, Stiber JA, Correa R, Epstein SE, Unger EF. Effects of chronic systemic administration of basic fibroblast growth-factor on collateral development in the canine heart. Circulation. 1995;91:145–153. doi: 10.1161/01.CIR.91.1.145 [PubMed: 7805195]
56. Huang Q, Wang Q, Zhang S, Jiang S, Zhao L, Yu L, Hultström M, Patzak A, Li L, Wilcox CS, Lai EY. Increased hydrogen peroxide impairs angiotensin II contractions of afferent arterioles in mice after renal ischaemia-reperfusion injury. Acta Physiol. 2016;218:136–145. doi: 10.1111/apha.12745
57. Pellieux C, Foletti A, Peduto G, Aubert JF, Nussberger J, Beermann F, Brunner HR, Pedrazzini T. Dilated cardiomyopathy and impaired cardiac hypertrophic response to angiotensin II in mice lacking FGF-2. J Clin Invest. 2001;108:1843–1851. doi: 10.1172/JCI13627 [PubMed: 11748268]
58. Sharples EJ, Patel N, Brown P, Stewart K, Mota-Philipe H, Sheaff M, Kieswich J, Allen D, Harwood S, Raftery M, Thiemermann C, Yaqoob MM. Erythropoietin protects the kidney against the injury and dysfunction caused by ischemia-reperfusion. J Am Soc Nephrol. 2004;15:2115–2124. doi: 10.1097/01.ASN.0000135059.67385.5D [PubMed: 15284297]
59. Harris VK, Coticchia CM, Kagan BL, Ahmad S, Wellstein A, Riegel AT. Induction of the angiogenic modulator fibroblast growth factor-binding protein by epidermal growth factor is mediated through both MEK/ERK and p38 signal transduction pathways. J Biol Chem. 2000;275:10802–10811. doi: 10.1074/jbc.275.15.10802 [PubMed: 10753873]
60. Harris VK, Coticchia CM, List HJ, Wellstein A, Riegel AT. Mitogen-induced expression of the fibroblast growth factor-binding protein is transcriptionally repressed through a non-canonical E-box element. J Biol Chem. 2000;275:28539–28548. doi: 10.1074/jbc.M001677200 [PubMed: 10871606]
61. Harris VK, Liaudet-Coopman ED, Boyle BJ, Wellstein A, Riegel AT. Phorbol ester-induced transcription of a fibroblast growth factor-binding protein is modulated by a complex interplay of positive and negative regulatory promoter elements. J Biol Chem. 1998;273:19130–19139. doi: 10.1074/jbc.273.30.19130 [PubMed: 9668098]
62. Liaudet-Coopman ED, Schulte AM, Cardillo M, Wellstein A. A tetracycline-responsive promoter system reveals the role of a secreted binding protein for FGFs during the early phase of tumor growth. Biochem Biophys Res Commun. 1996;229:930–937. doi: 10.1006/bbrc.1996.1904 [PubMed: 8954996]
63. Liaudet-Coopman ED, Wellstein A. Regulation of gene expression of a binding protein for fibroblast growth factors by retinoic acid. J Biol Chem. 1996;271:21303–21308. doi: 10.1074/jbc.271.35.21303 [PubMed: 8702908]
64. Liu M, Liang Y, Chigurupati S, Lathia JD, Pletnikov M, Sun Z, Crow M, Ross CA, Mattson MP, Rabb H. Acute kidney injury leads to inflammation and functional changes in the brain. J Am Soc Nephrol. 2008;19:1360–1370. doi: 10.1681/ASN.2007080901 [PubMed: 18385426]
65. Lever JM, Hull TD, Boddu R, Pepin ME, Black LM, Adedoyin OO, Yang Z, Traylor AM, Jiang Y, Li Z, Peabody JE, Eckenrode HE, Crossman DK, Crowley MR, Bolisetty S, Zimmerman KA, Wende AR, Mrug M, Yoder BK, Agarwal A, George JF. Resident macrophages reprogram

toward a developmental state after acute kidney injury. JCI Insight. 2019;4 doi: 10.1172/jci.insight.125503.

66. Liu HX, Aigner A, Wellstein A, Ray PE. Up-regulation of a fibroblast growth factor binding protein in children with renal diseases. Kindey Int. 2001;59:1717–1728. doi: 10.1046/j.1523-1755.2001.0590051717.x

Novelty and Significance

What is New?

Fibroblast growth factor 2 (FGF2) signaling pathway and angiotensin II (Ang II) signaling pathway share the common downstream signaling molecule phospholipase Cγ1 (PLCγ1) and we found that after acute kidney injury (AKI) brain vessels were sensitized to Ang II and not to other vasoconstrictors. Upregulation of FGF2 and FGF binding protein 1 (FGFBP1) expression after AKI suggested a possible contribution to the vascular sensitization.

What is Relevant?

We demonstrated that treatment with the clinically used FGF receptor kinase inhibitor BGJ398 prevented the AKI-induced brain vascular sensitization to Ang II, which may be beneficial in preventing AKI-induced brain function injury.

Summary

FGF2 pathway activation by induction of the FGFBP1 chaperone protein can drive the kidney-brain crosstalk during AKI. Most importantly, in our opinion, blockade of FGFR tyrosine kinase activation with a clinically used FGF receptor kinase inhibitor BGJ398 may be able to reset the brain vascular reactivity during AKI and may prevent brain vessel injury.

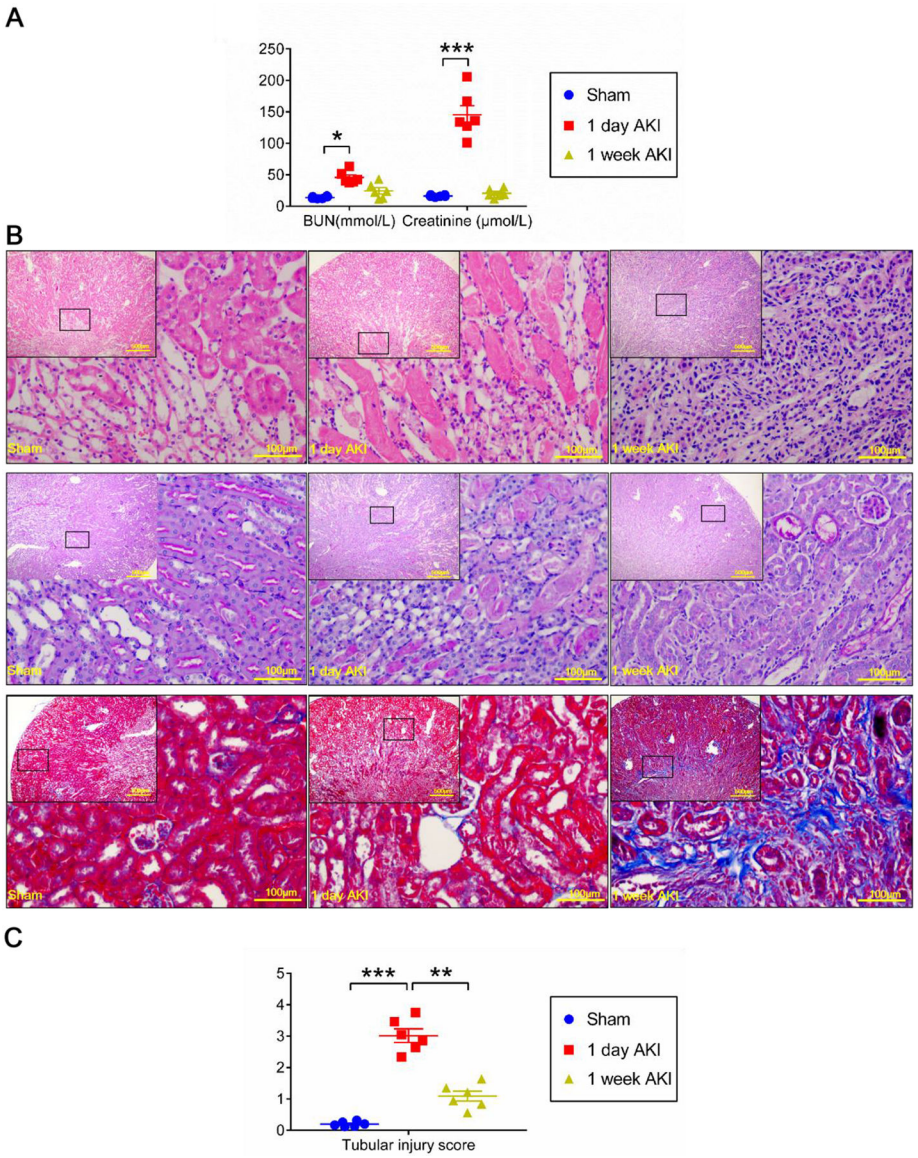


Figure 1.

Short-term effects of acute kidney injury (AKI) on renal function and structure. **A,** Serum creatinine (μmol/L) and blood urea nitrogen (BUN) (mmol/L) in sham-operated mice and AKI mice (Mean ± SEM values; N=6). *, *P*<0.05; ***, *P*<0.001 by two-way ANOVA. **B**, Hematoxylin-eosin (H&E) (above), periodic acid Schiff reaction (PAS) (middle) and Masson's trichrome (below) of renal cortical medullary junction area in sham-operated mice and AKI mice. (Scale bar = 100 μm in the enlarged images and Scale bar = 500 μm in the original images). **C**, Tubular scores based on PAS-stained sections were assessed by a semiquantitative histomorphological scoring system from 0 to 5 (Mean ± SEM values; N=6).**, *P*<0.01; ***, *P*<0.001 by Kruskal-Wallis test.

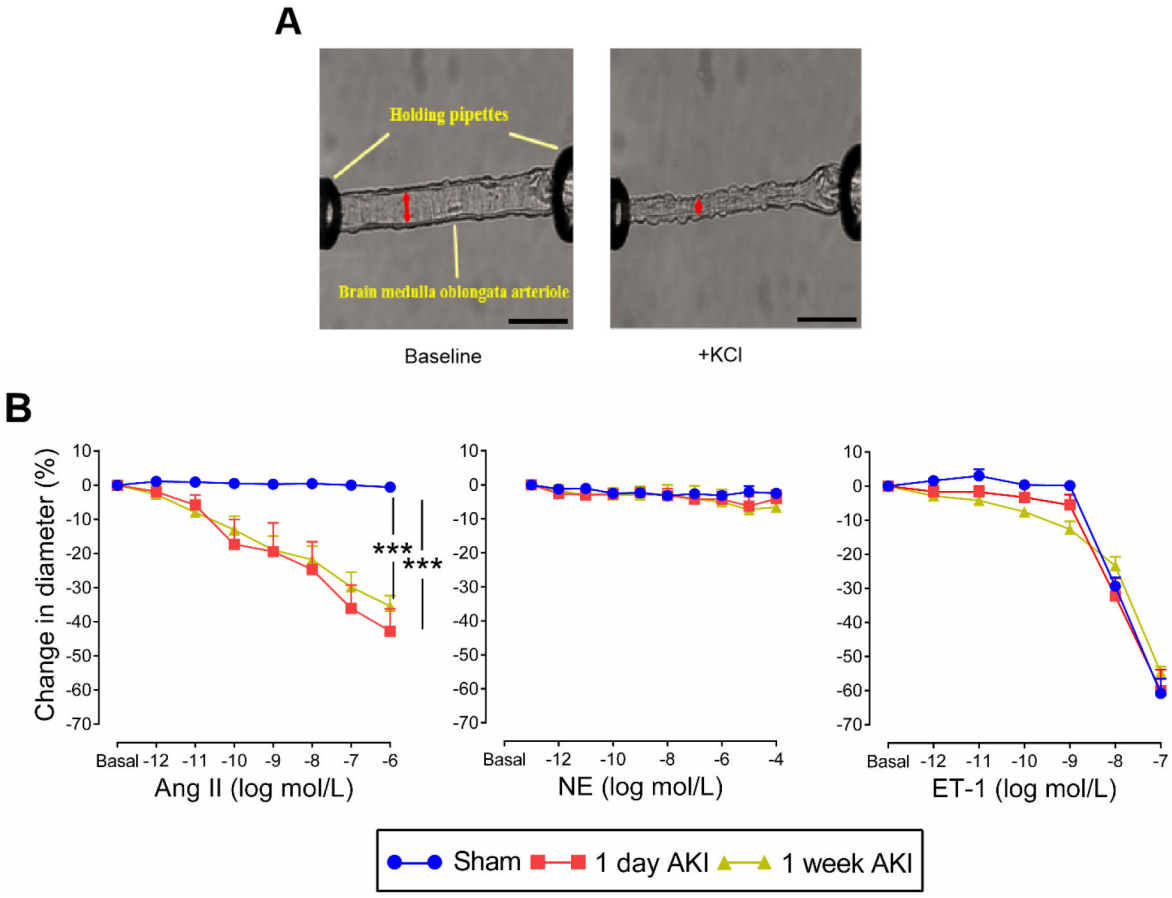


Figure 2.

Increased response of brain medulla oblongata arterioles (BMas) to angiotensin II (Ang II) after acute kidney injury (AKI). **A**, BMa at baseline and after KCl (100 mmol/L). Scale bar=50 μm; The red double sided arrow shows the inner diameter of the arteriole. **B**, Effect of Ang II, norepinephrine (NE) or endothelin 1 (ET-1) on arterioles isolated from sham-operated and AKI mice (Mean ± SEM values; N=6). ***, $P<0.001$ by two-way ANOVA.

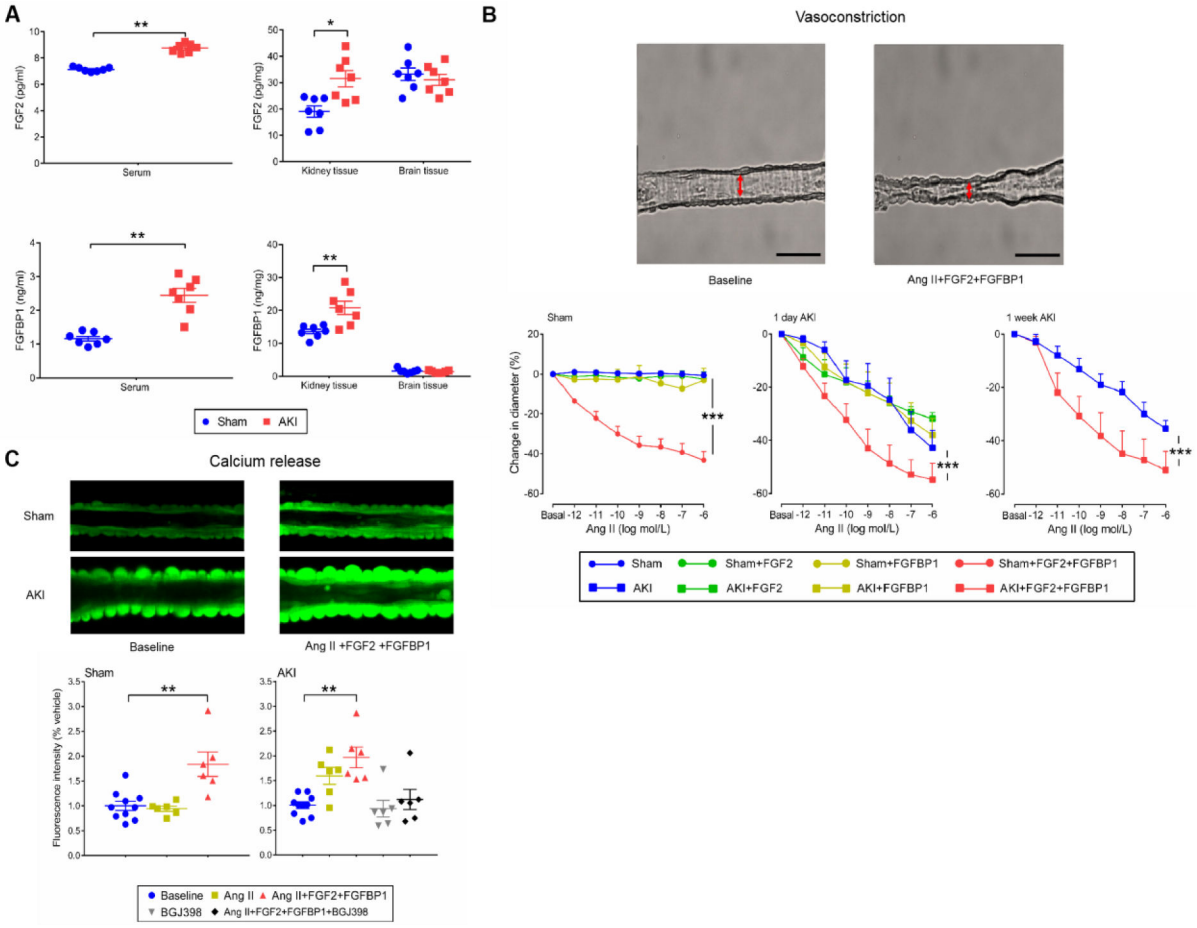


Figure 3.

Expression of fibroblast growth factor 2 (FGF2) and fibroblast growth factor binding protein 1 (FGFBP1), vasoconstriction of brain medulla oblongata arterioles (BMas) to angiotensin II (Ang II) and calcium release of BMas. **A**, FGF2 and FGFBP1 concentration in serum and kidney and brain tissues from sham-operated mice and 1 day acute kidney injury (AKI) mice (Mean ± SEM values; N=7). *, $P<0.05$; **, $P<0.01$ by two-way ANOVA. **B**, Isolation and microperfusion of a single BMa from sham-operated mice at baseline and after preincubation with FGF2 plus FGFBP1 followed by 10^{-6} mol/L Ang II (Scale bar=50 μm; The red double sided arrow shows the inner diameter of the arteriole) and the effect of FGF2 and FGFBP1 in BMs from sham-operated mice, 1 day AKI and 1 week AKI mice. For sham+FGF2+FGFBP1 vs. sham or AKI+FGF2+FGFBP1 vs. AKI (Mean ± SEM values; N=6) ***, $P<0.001$ by two-way ANOVA. **C**, Relative cytosolic calcium fluorescence intensity in BMas from sham-operated mice and 1 day AKI mice (Mean ± SEM values; N=6-10). **, $P<0.01$ by one-way ANOVA.

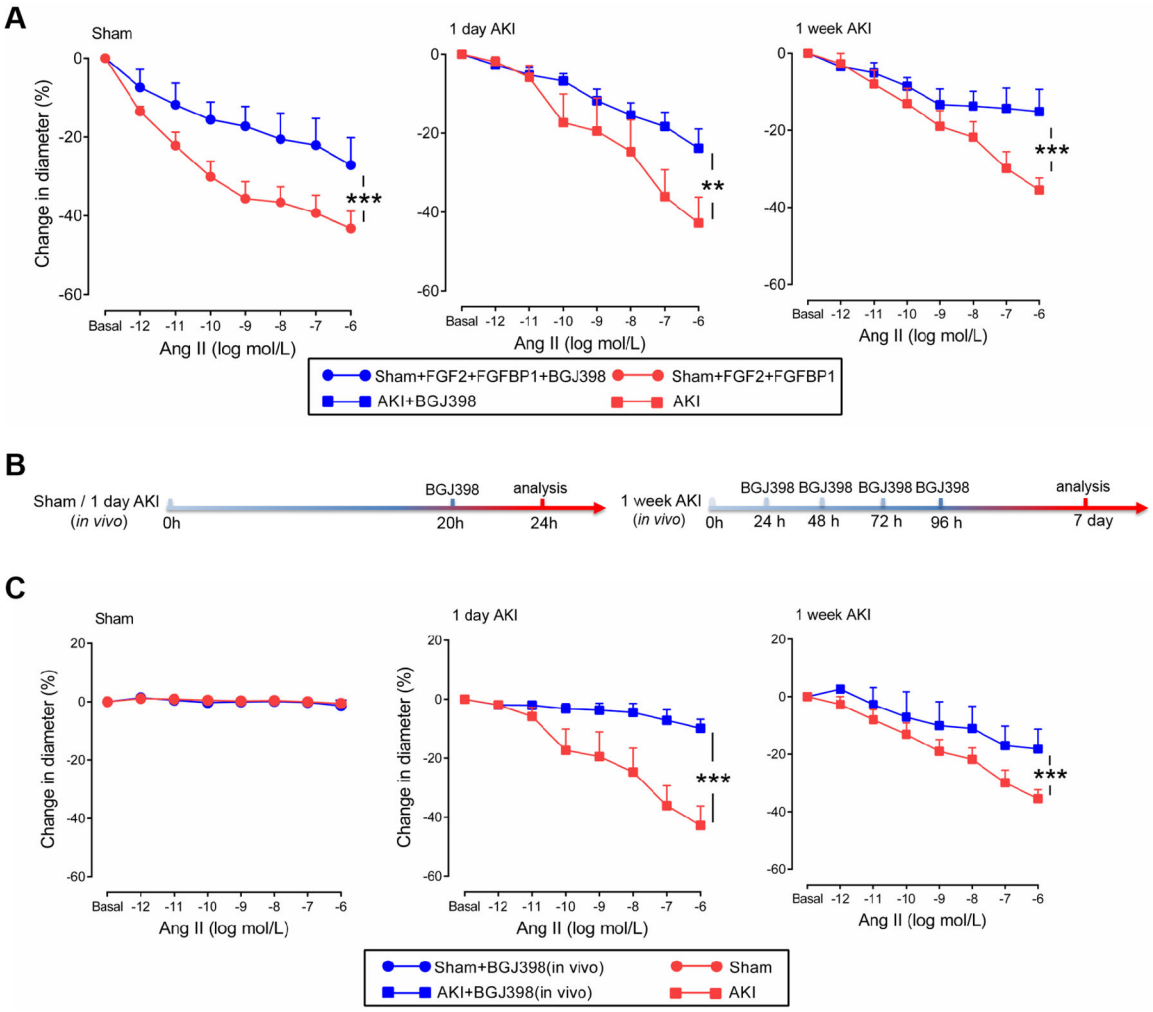


Figure 4.

Effect of fibroblast growth factor receptor (FGFR) tyrosine kinase inhibitor BGJ398 *in vitro* **A** or *in vivo* **C**. Brain medulla oblongata arterioles (BMas) from sham-operated mice, 1 day and 1 week acute kidney injury (AKI) mice were subjected to angiotensin II (Ang II) stimulation and fibroblast growth factor 2 (FGF2) plus fibroblast growth factor binding protein 1 (FGFBP1) were only added to BMas from sham-operated mice *in vitro*. (Mean ± SEM values; N=6). **, $P<0.01$; ***, $P<0.001$ by two-way ANOVA. Panel **B** depicts the time points of BGJ398 treatment in the sham-operated mice, 1 day and 1 week AKI mice.

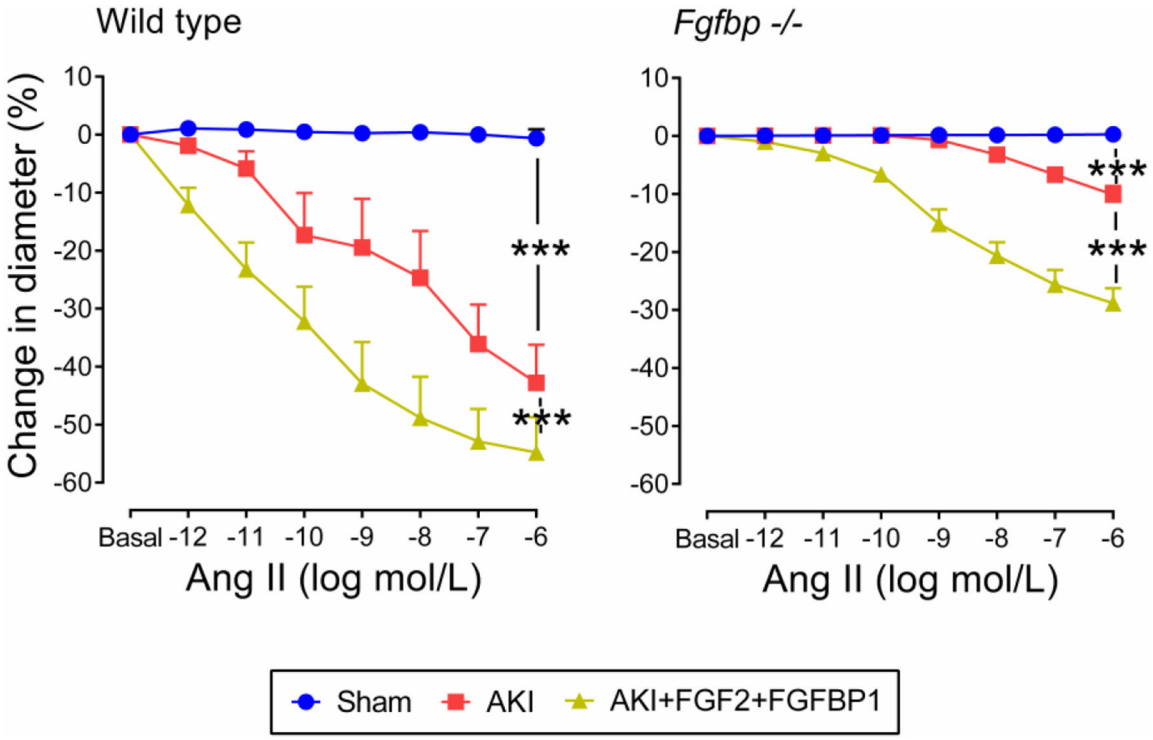


Figure 5.

Contribution of endogenous fibroblast growth factor binding protein 1 (Fgfbp1) to angiotensin II (Ang II) mediated contractility of brain medulla oblongata arterioles (BMas). Response of BMas to Ang II from wide type mice (left) or *Fgfbp1*$^{-/-}$ mice (right) after 1 day acute kidney injury (AKI) versus sham-operated mice; (Mean ± SEM values; N=6). Fibroblast growth factor 2 (FGF2) and FGFBP1 (both at 20 ng/mL, 15 min). ***, $P<0.001$ by two-way ANOVA.

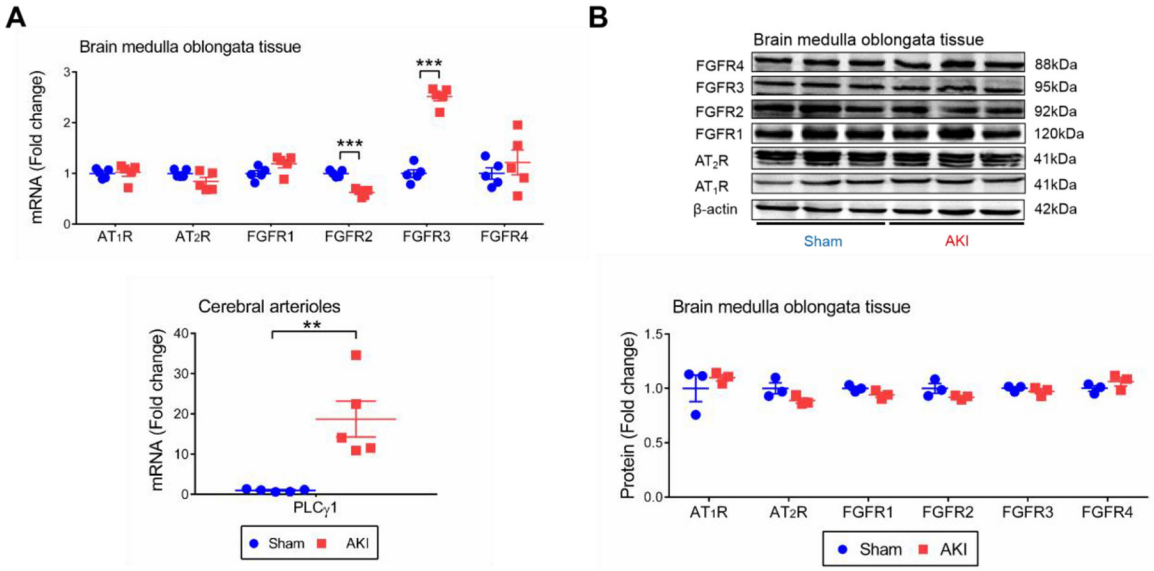


Figure 6.

Relative mRNA and protein expression of candidate signaling molecules. **A**, Relative mRNA expression of Ang II receptor type 1 and type 2 receptor (AT_1R, AT_2R) and fibroblast growth factor receptors (FGFRs) in brain medulla oblongata tissue and expression of phospholipase C γ1 (PLCγ1) in cerebral arterioles. **B**, Relative protein expression of AT_1R, AT_2R and FGFRs in brain medulla oblongata tissue. (Mean ± SEM values; N=5 for mRNA and N=3 for protein). **, $P<0.01$; ***, $P<0.001$ by two-way ANOVA or unpaired *t* test.

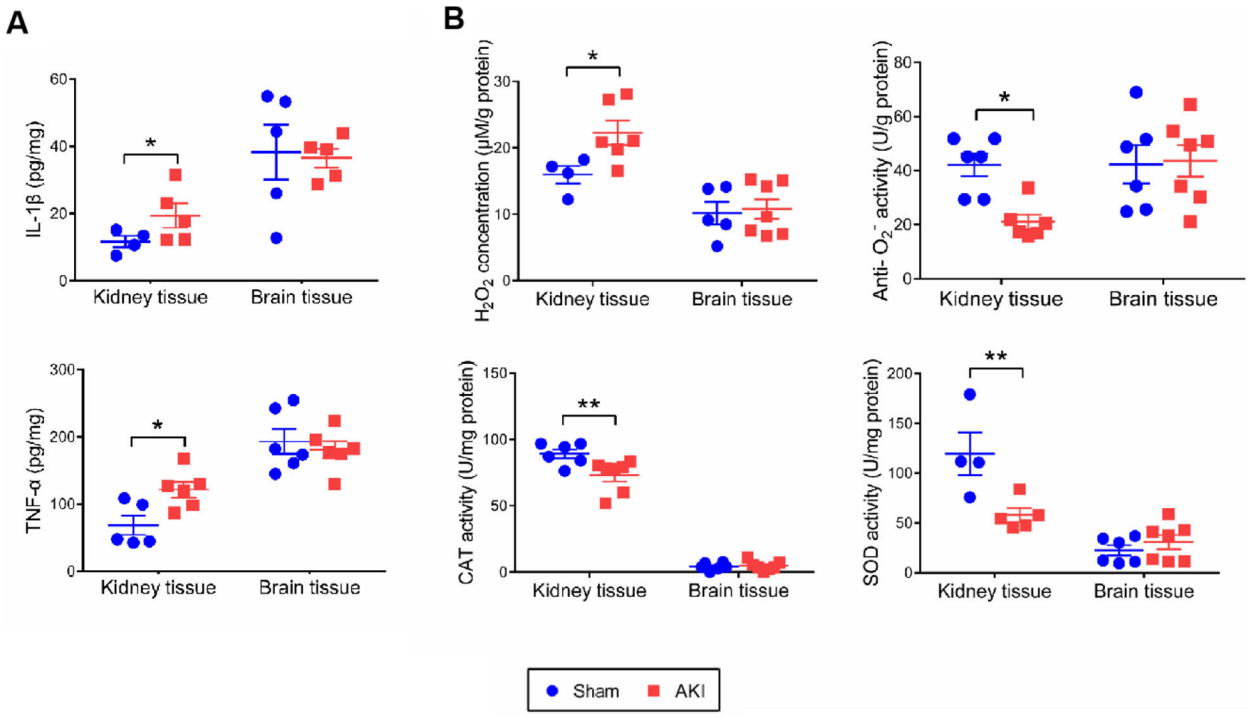


Figure 7.

Concentration of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) (**A**) and the antioxidant ability (**B**) in tissues of kidney and brain after 1 day acute kidney injury (AKI). (Mean ± SEM values; N=6). *, $P<0.05$, **, $P<0.01$ by two-way ANOVA.

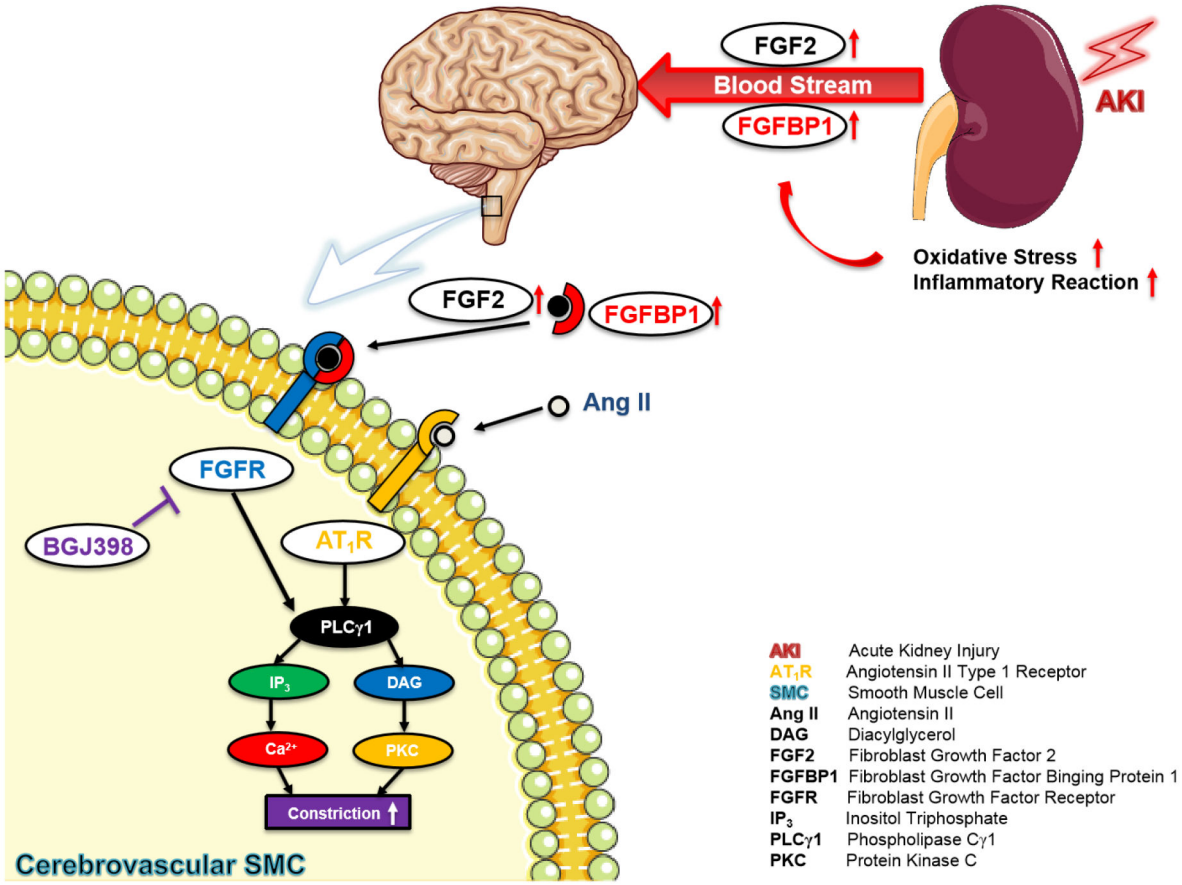


Figure 8.

The increased expression of fibroblast growth factor 2 (FGF2) and fibroblast growth factor binding protein 1 (FGFBP1) induced by acute kidney injury (AKI) lead to increased cerebral vasoconstriction to angiotensin II (Ang II).

Table 1.

Primer sequence:

Gene		5'-3'
GAPDH	Forward primer	AGGTCGGTGTGAACGGATTTG
	Reverse primer	TGTAGACCATGTAGTTGAGGTCA
FGFR1	Forward primer	AGGCTACAAGGTTCGCTATG
	Reverse primer	GTGGTTGATGCTCCCATACT
FGFR2	Forward primer	CAGCCTCTCGAACAGTATTCTC
	Reverse primer	GAGGCAGACAGGGTTCATAAG
FGFR3	Forward primer	GTGGTCATGGCAGAAGCTATT
	Reverse primer	CCTTGTCAGTCGCATCATCTT
FGFR4	Forward primer	CCTGAGGCCAGATACACAGATA
	Reverse primer	GGATGACTTGCCGATGATACAC
PLCγ1	Forward primer	GAGACGCGCCAGATCACAT
	Reverse primer	AAAGTCCCGAGAAGTCTTCCC
AT_1R	Forward primer	CAACTGCCTGAACCCTCTGT
	Reverse primer	TATCTGAGGGGCGGTAGGAG
AT_2R	Forward primer	CCTGGCAAGCATCTTATGTAGTTC
	Reverse primer	CCGGAAATAAAATGTTGGCAAT

GAPDH, reduced glyceraldehyde-phosphate dehydrogenase; FGFR, fibroblast growth factor receptor; PLCγ1, phospholipase C γ1; AT_1R, Ang II receptor type 1; AT_2R, Ang II receptor type 1.