Celecoxib Alleviates Radiation-Induced Brain Injury in Rats by Maintaining the Integrity of Blood-Brain Barrier

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Abstract
The underlying mechanisms of radiation-induced brain injury are poorly understood, although COX-2 inhibitors have been shown to reduce brain injury after irradiation. In the present study, the effect of celecoxib (a selective COX-2 inhibitor) pretreatment on radiation-induced injury to rat brain was studied by means of histopathological staining, evaluation of integrity of blood-brain barrier and detection of the expressions of inflammation-associated genes. The protective effect of celecoxib on human brain microvascular endothelial cells (HBMECs) against irradiation was examined and the potential mechanisms were explored. Colony formation assay and apoptosis assay were undertaken to evaluate the effect of celecoxib on the radiosensitivity of the HBMECs. ELISA was used to measure 6-keto-prostaglandin F1α (6-keto-PGF1α) and thromboxane B2 (TXB2) secretion. Western blot was employed to examine apoptosis-related proteins expressions. It was found that celecoxib protected rat from radiation-induced brain injury by maintaining the integrity of the blood-brain barrier and reducing inflammation in rat brain tissues. In addition, celecoxib showed a significant protective effect on HBMECs against irradiation, which involves inhibited apoptosis and decreased TXB2/6-keto-PGF1α ratio in brain vascular endothelial cells. In conclusion, celecoxib could alleviate radiation-induced brain injury in rats, which may be partially due to the protective effect on brain vascular endothelial cells from radiation-induced apoptosis.

Keywords
celecoxib, radiation-induced brain injury, blood-brain barrier, apoptosis

Introduction
Radiation-induced brain injury is a serious complication that can occur after radiation therapy for head and neck cancers and brain tumors as well as metastases in general.1,2 Radiation-induced brain injury, particularly following stereotactic radiosurgery,3-7 accounts for 68% of such complications, including radiation necrosis, with a frequency of 3%-24%.4,8-10 Risk factors for radiation necrosis include the radiation dose, fractionation, the use of chemotherapy (especially concurrent chemotherapy), re-irradiation, and additional boost irradiation.4,11

The pathogenesis of radiation-induced brain injury is still poorly understood but is known to involve vascular endothelial cell injury, glial cell damage, and autoimmune reaction.2 Animal studies showed that radiation decreased the microvessel density of the brain, induced decreased spinal blood flow,12 and increased blood vessel permeability.13 Radiation also decreases the production of endothelial progenitor cells.14 Eventually, tissue inflammation and ischemia will promote the infiltration of macrophages and lymphocytes, which secrete interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α, maintaining and enhancing the inflammatory state.15

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It has been reported that the content and the ratio of thrombox-
ane A2 (TXA2) and prostaglandin I2 (PGI2) play key roles in the
vascular system. TXA2 causes vasoconstriction, induces platelet
aggregation and thrombosis and promotes inflammation.16 PGI2
inhibits platelet aggregation and is a vasodilator. Under normal
circumstances, the effects of PGI2 are dominant.17 Ionizing radia-
tions can break the balance between TXA2 and PGI2, resulting in
increased secretion of TXA2 and decreased secretion of PGI2,
impairing the cerebral vasculature and promoting thrombosis.18
Thromboxane B2 (TXB2) and 6-keto-prostaglandin F1α (6-keto-
PGF1α) are metabolic products of TXA2 and PGI2, which are
relatively stable and easy to measure.19,20
Cyclooxygenase (COX), prostacyclin synthase, and throm-
oxane synthase participate in the synthesis of TXA2 and
PGI2, among which COX is the most important.21 COX-2
expression is increased by inflammation, and leads to increased
production of TXA2 from arachidonic acid, and low levels of
PGI2.22 Celecoxib is a selective COX-2 inhibitor that has been
shown to reduce the levels of inflammatory cytokines induced
by radiation in rats.23 Meloxicam, another selective COX-2
inhibitor, has been shown to reduce brain injury after irradia-
tion, partly through the relief of vascular endothelial cell
injury.24 Hence, we supposed that inhibiting COX-2 by cele-
oxib might also be an appropriate approach to prevent
radiation-induced brain injury.

On the other hand, radiation can stimulate the formation of
ceramide, thereby reducing the stability of the mitochondrial
membrane and promoting apoptosis through the c-Jun N-terminal kinase (JNK) pathway.25,26 JNK can be activated by a
variety of stimuli, such as oxidative damage, DNA and endo-
plasmic reticulum damage.27 The activated form of JNK is phos-
horylated JNK (p-JNK), which translocates to the cytoplasm,
activating caspase-3 and the mitochondrial apoptotic path-
way.25,26 However, whether the radiation-induced brain injury
involves JNK-mediated apoptosis signaling remains unclear.

The present study aims to examine the effect of celecoxib
pretreatment on radiation-induced brain injury in rats and
explore the related molecular mechanisms using human brain
microvascular endothelial cells (HBMECs).

Materials and Methods
Cell Culture, Grouping, and Irradiation
HBMECs were purchased from Shanghai Biological Technol-
ogy Co., Ltd. (Shanghai, China) and cultured in Dulbecco’s
Modified Eagle Medium (DMEM) supplemented with 10%
fetal bovine serum (Gibco, Grand Island, NY, USA), 1% peni-
cillin sodium and 100 µg/mL streptomycin, at 37°C in 5% CO2
in a humidified incubator (Thermo Scientific, NC, USA). Cells
in the logarithmic growth phase were divided into the control
group, irradiation (IR) group, and celecoxib pretreatment
before IR group (IR+C). The IR+C group was pretreated with
celecoxib for 24 h at an appropriate concentration selected
according to the cytotoxicity pre-experiment (see below). Ira-
diation of the IR group and IR+C group was carried out using a
RS-2000 X-ray irradiator (Rad Source Technologies Inc.,
Suwanee, GA, USA) at a dose rate of 1.2 Gy/min. Field size
was 20 × 30 cm and the focus-surface distance was 20 cm.

Animal and Irradiation Treatment
Two hundred and forty 8-week-old male Sprague-Dawley (SD)
rats, SPF-housed in the Laboratory Animal Center of Soochow
University with access to water and food ad libitum, were
randomly divided into 3 groups, the Control group (anesthe-
tized only), the Irradiation group (irradiated after anesthetiza-
tion) and celecoxib-plus Irradiation group (celecoxib
administered twice before and once after exposure for 3 con-
secutive days), with 80 rats in each group. Animals were sub-
jected to cranial irradiation of 20 Gy X-rays from a linear
accelerator (Siemens Mevatron MD2, Erlangen, Germany) at
a dose rate of 2 Gy/min after being anesthetized by 10% chloral
hydrate intraperitoneally (400 mg/kg body weight). A dose of
20 Gy was selected since it was reported that the late delayed
effect of whole brain radiation, cognitive dysfunction, can
occur with a dose as low as 20 Gy in adults.28 No signs of
peritonitis were observed following the administration of the
10% chloral hydrate. Celecoxib (Pfizer, New York, NY, USA),
was administered intraperitoneally at a dose of 30 mg/kg/day.
The permeability of the blood-brain barrier (BBB) was exam-
ined by Evans Blue (EB) assay. Briefly, 1 mL/kg EB (2% in
0.9% saline) was administered to rats by tail vein injection.
After 3 hours of EB circulation, all rats were deeply intraperi-
toneally anesthetized by 10% chloral hydrate (400 mg/kg) and
transcardially perfused with ice-cold saline to flush away the
blood and EB from the blood vessels. The brain tissues were
quickly harvested by decapitation and weighed, cut into small
pieces and homogenized with 3 mL of formamide at 37°C for
72 h for EB extraction. After centrifuged at 15000 g for 15 min
at 4°C, the supernatant was collected and diluted with 100%
ethanol at a ratio of 1:3. The EB content in brain tissues was
quantified at 620 nm using a spectrophotometer (BioTek
Instruments, VT, USA)) and expressed as micrograms of per
gram of brain tissue (µg/g). The rats were sacrificed by cervical
dislocation after intraperitoneally anesthetized by 10% chloral
hydrate (400 mg/kg body weight) at a series of indicated time-
points from 3 h post-irradiation to 1 month and the brain water
content (BWC) and genes expression were measured. Death
was verified by cessation of the heartbeat and respiration and
absence of reflexes. During the study, the animals were docu-
mented by the laboratory group at least twice a week and the
following endpoints were employed for humane removal from
this study: the animal reached a body condition score of 2/5, it
was unable to right itself within 30 s, or it had severe clinical
signs including evidence of lethargy, changes in ambulation,
diarrhea, or increased respiratory effort. All experiments were
performed in strict accordance with the U.K. Animals (Sci-
etific Procedures) Act and associated guidelines as well as
AVMA Guidelines for the Euthanasia of Animals 2013. All
animal studies were reviewed and approved by the Soochow
University Institutional Animal Care and Use Committee.
**Cytotoxicity of Celecoxib Examined by CCK8 Assay**

Celecoxib (Sigma, St Louis, MO, USA) was dissolved in DMSO (Sigma, St Louis, MO, USA) at the storage concentration of 0.5 mol/L, and further diluted with the culture medium to achieve different working concentrations (10, 30, 40, 50, and 100 μmol/L). HBMECs in 96-well plates were exposed to different drug concentrations for 24 h. Then, 10 μL of CCK-8 solution (Sigma, St Louis, MO, USA) was added to each well. Absorbance was measured at 450 nm using a microplate reader (Synergy2; BioTek Instruments, VT, USA) 2 hours later. Three independent experiments were performed, each in sextuplicates. The IC50 was calculated from the growth inhibition curve.

**Colony Formation Assay**

After exposed to gradient radiation doses of 0, 2, 4, 6, 8, 10 Gy with or without celecoxib pretreatment, the cells were harvested, counted and plated into 96 mm dishes. Cells were fixed with 70% ethanol for 5 min and stained with crystal violet 14 days later. Colonies containing more than 50 cells were counted as survivors. At least 3 parallel dishes were scored for each treatment.

**Apoptosis Rate of HBMECs Determined by Flow Cytometry**

The celecoxib pretreatment and irradiation were carried out in sequence. At 24 h and 48 h after irradiation, the HBMECs were collected by centrifugation of 450 g for 5 min at room temperature. The supernatant was also collected and 300 μL of Annexin V-APC binding buffer was added, followed by 5 μL of Annexin V-APC (Keygentec, Nanjing, China). After 15 min of incubation, 5 μL of PI was added (Beyotime Institute of Biotechnology, Haimen, China) and apoptosis was detected by a flow cytometer (FC500, Beckman Coulter, Brea, CA, USA).

**ELISA of 6-Keto-PGF1α and TXB2**

The celecoxib pretreatment and irradiation were carried out in sequence. At 6, 12, 24, and 48 h after irradiation, the culture media were collected and centrifuged at 2350 g for 10 min at 4°C. Then, 10 μL of supernatant was mixed with 40 μL of dilituents, and ELISA was performed according to the manufacturer’s instructions (Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China).

**qRT-PCR**

Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol. Reverse transcription (RT) of total RNA was performed using RNA to cDNA EcoDry™ Premix Kit at 42°C for 60 min and the RT reaction was terminated by heating at 70°C for 10 min (Takara, Japan). The real-time PCR analysis was carried out using a SYBR Premix Ex Taq II Kit (Takara, Japan) in a 96 wells plate and done with ABI 7500 Real-Time PCR System (Applied Biosystems, CA, USA). The program was run by initial denaturing for 2 min at 95°C before 40 cycles, each at 95°C for 15 s and 57°C for 30 s. Melting curve plotting was performed at the end of the PCR cycles to validate the correct generation of PCR product. The expression levels of genes were normalized to GAPDH and calculated by C(t) comparison method.29 The primers are listed in Table 1.

**Western Blot**

The celecoxib pretreatment and irradiation were carried out in sequence. The HBMECs were collected at the indicated time-points and lysed with RIPA buffer to extract the proteins (Invitrogen, Carlsbad, CA, USA). An amount of 30 μg of proteins was loaded in each lane for polyacrylamide gel electrophoresis. The proteins were transferred to PVDF membranes, which were then blocked at room temperature for 2 h with PBST (PBS with 0.1% Tween-20) containing 5% non-fat milk. The specific antibodies were incubated at 4°C for 1 h and 3 h at room temperature after thorough TBST washing. The bands were revealed using an ECL reagent kit (Pierce Chemical, Dallas, TX, USA) and observed using a chemiluminescence gel imager (Carestream Health, Inc., Rochester, NY, USA). Anti-Caspase-3 (ab184787), anti-phospho-JNK (ab124956), anti-JNK (ab208035) and anti-COX-2 (ab15191) were purchased from Abcam (Cambridge, MA, USA) while anti-β-actin (4970 S) was purchased from Cell Signaling Technology (Beverly, MA, USA).

**Immunohistochemistry**

The hippocampus of the brain was collected for immunohistochemical analysis. Sections (3 μm in thickness) of formalin-fixed and paraffin-embedded samples were used to analyze the brain morphological changes induced by radiation and the expression of nuclear factor kappa-B (NF-kB) and vascular endothelial growth factor (VEGF). After deparaffinization, the antigen retrieval was performed in citric acid buffer (pH6.0) using an autoclave oven, then the naturally cooled sections were incubated in PBS containing 10% FBS overnight and incubated with NF-kB/VEGF polyclonal antibodies (ab16502 for NF-kB and ab1316 for VEGF, Abcam, Cambridge, MA, USA) at 4°C overnight. After thorough washing in PBS the
sections were incubated with the HRP-conjugated secondary antibodies for 30 min. Then the antibody-antigen complex was visualized using DAB reaction and hematoxylin staining for cell nuclei. The stained sections were examined under a DM2000 optical microscope (Leica, Germany) and the images were captured at a magnification of 100×.

**Statistical Analysis**

SPSS 19.0 (IBM, Armonk, NY, USA) was used to calculate the IC50 and to perform the statistical analyzes. Data were expressed as mean ± standard deviation (SD) and analyzed by ANOVA. Two-sided *P* values <0.05 were considered statistically significant.

**Results**

**Protective Effects of Celecoxib on Radiation-Induced Brain Injury of Rats**

A radiation-induced brain injury model was established using rats. It was found by H&E staining that there were no obvious pathological changes in the brain tissues of control group, where the cell morphology and vascular structure were intact. While in the irradiation group, a small number of cells showed cytoplasmic edema, nuclear fragmentation and pyknosis 3 hours post-irradiation. From 0.5 to 3 days after irradiation, the number of edema cells increased further, accompanied by vacuolated cytoplasm, increased steatosis, widened capillary space, and thrombosis in some vessels. On the 7th day after irradiation, the number of pathological cells peaked, and the brain tissues around vessels became disordered. 15 days after irradiation, necrosis of the cells, and fragmentation of nucleus was observed. Vascular embolism still existed, leading to infarction of some brain tissues. Thirty days after irradiation, most embolic vessels were recanalized, cell edema disappeared, and necrotic areas increased slightly. However, in the celecoxib plus irradiation group, the pathological changes of brain tissue at each indicated timepoint were similar to those in the irradiation group, but the lesion degree was significantly lighter than that in the irradiation group, especially on the 15th and 30th day. The number of necrotic cells and the disordered tissue structure areas were significantly smaller than those in the irradiation group (Figure 1A). Besides, we detected the integrity of blood-brain barrier by Evans Blue (EB) assay as well as the brain water content. As shown in Figure 1B, the permeability of blood-brain barrier was increased significantly by irradiation treatment, especially between 3 hours and 15 days post-irradiation (*P* < 0.05). However, celecoxib pretreatment decreased the EB content in brain tissues at 3 h, 12 h, and 1 day post-irradiation (*P* < 0.05). As shown in Figure 1C, the brain water content was also increased significantly at all indicated timepoints post-irradiation (*P* < 0.05), which was decreased by celecoxib significantly from 3 h through 15 days post-irradiation (*P* < 0.05).

**Effects of Celecoxib Pretreatment on the Expressions of Both $\text{NF-κB}$ and $\text{VEGF}$ in Brain Tissues**

Since $\text{NF-κB}$ and $\text{VEGF}$ are 2 key regulators participating in the inflammation and repair of vascular injury and repair, we investigated whether celecoxib pretreatment has a role in the expressions of both these 2 genes. Brain tissues from 3 hours through to 30 days post-irradiation were collected for both mRNA and protein detection. As shown in Figure 2A, the mRNA levels of $\text{NF-κB}$ were up-regulated at all indicated timepoints, which peaked on the 3rd day and then reduced. However, they were still higher than that of the control group at the 30th day. Celecoxib pretreatment significantly decreased the induced $\text{NF-κB}$ mRNA levels at all timepoints except for the 7th and the 30th day (*P* < 0.05 at the 3, 12, 24, 72, 360 hours post-irradiation). As to the $\text{VEGF}$ mRNA, it was also up-regulated at all indicated timepoints, which peaked on the 7th day and then reduced. However, celecoxib decreased VEGF mRNA induced by irradiation from 3 hours through to 7 days post-irradiation except for at 24 hours (*P* < 0.05 at 3, 12, 72, 168 hours post-irradiation), while it didn’t change VEGF mRNA significantly on the 15th or 30th day (*P* > 0.05) (Figure 2B). Western blot assay also indicated celecoxib significantly decreased both $\text{NF-κB}$ and $\text{VEGF}$ protein levels induced by irradiation (Figure 2C and D). To further verify that celecoxib...
Effects of Celecoxib on the Radiosensitivity of HBMECs

Based on the above achievements, it was found that serious BBB damage was caused by irradiation. As is known, human brain microvascular endothelial cells, the major component of the BBB, play an essential role in the maintenance of the integrity of the BBB and comprise the primary limitation to passage of both soluble and cellular substances from the blood into the brain. Thus we want to know whether celecoxib affects the radiosensitivity of this kind of cells. HBMECs were employed to conduct colony formation assay and apoptosis assay after irradiation. The growth inhibition rates of HBMECs by celecoxib at concentrations of 10, 30, 40, 50, and 100 μmol/L were 4.52% ± 0.13%, 6.31% ± 0.07%, 9.01% ± 0.10%, 15.26% ± 0.70%, and 70.65% ± 0.89%, respectively (Supplementary Figure 1), based on which the IC50 was calculated as 81.50 ± 0.75 μmol/L. A concentration of 30 μmol/L was used in the in vitro experiments. As shown in Figure 3A and B, compared with the irradiation group, celecoxib pretreatment resulted in a significantly higher survival rate of HBMECs exposed to X-rays of 6 Gy, 8 Gy and 10 Gy (P < 0.05), while no appreciable radioprotective effects were observed when 2 Gy or 4 Gy was used (P > 0.05). The apoptosis assay found that 8 Gy X-ray irradiation caused severe apoptosis in HBMECs (P < 0.05), approaching an apoptosis rate of 91.28% ± 4.95% 48 hours post-irradiation. However, celecoxib pretreatment decreased this rate to 78.58% ± 4.36% (P < 0.05). At 24 h post-irradiation, celecoxib pretreatment also inhibited the radiation-induced apoptosis (Figure 3C and D, P < 0.05). These results suggested that celecoxib considerably decreased the radiosensitivity of HBMECs.

Celecoxib Pretreatment Decreased the Secretion of TXB2 by HBMECs After Irradiation

Since TXA2 promotes thrombosis by inducing platelet aggregation, maintains a specific balance with its antagonist PGI2 and plays a vital role in radiation-induced brain injury. We detected TXB2 (a metabolite of TXA2) and 6-keto-PGF1α (a metabolite of PGI2) secreted by the irradiated HBMECs. As shown in Figure 4A, 8 Gy X-ray irradiation increased the
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Figure 4. Celecoxib pretreatment decreased the secretion of TXB2 by HBMECs exposed to irradiation. A. The concentrations of TXB2 in the culture medium of HBMECs at indicated timepoints post 8 Gy X-rays irradiation were measured with ELISA. B. The concentrations of 6-keto-PGF1α in the culture medium of HBMECs at indicated timepoints post 8 Gy X-rays irradiation were measured with ELISA. C. The ratios of TXB2/6-keto-PGF1α at indicated timepoints post 8 Gy X-rays irradiation were calculated. Error bars denote the mean ± SD derived from 3 independent experiments. a: P < 0.05 compared with the control group at the same timepoint post-irradiation; b: P < 0.05 when compared with the IR group at the same timepoint post-irradiation.

Celecoxib Pretreatment Decreased p-JNK and COX-2 in HBMECs After Irradiation

As an essential member of MAPK family, JNK plays a key role in regulating intracellular and extracellular stress response, and its phosphorylated form could activate the mitochondrial apoptotic pathway. As shown in Figure 5, the protein amounts of phosphorylated JNK, cleaved caspase-3 and COX-2 were increased substantially after 8 Gy X-ray irradiation at all indicated timepoints (P < 0.05), and the expression of both cleaved caspase-3 and COX-2 increased from 6 h through to 48 h post-irradiation. Compared with the IR group, the amounts of phosphorylated JNK and cleaved caspase-3 in the IR+C group was decreased significantly (all P < 0.05) with celecoxib pretreatment, which also reduced the protein expression of COX-2 induced by irradiation (all P < 0.05). These results are in accordance with the apoptosis assay.

Discussion

The mechanisms of radiation-induced brain injury are poorly understood. It has been reported that COX-2 inhibition can reduce radiation-induced brain necrosis and edema.30 This study aimed to examine the effect of celecoxib on radiation-induced injury both in vivo and in vitro and to explore the potential mechanisms. The results showed that celecoxib might help protect both brain tissues and HBMECs from irradiation.

Previously research indicated that vascular endothelial cells are sensitive to X-rays and that after irradiation, endothelial cells shed from the basement membrane present cytoplasmic vacuolization and apoptosis, leading to damage to the blood-brain barrier, vascular wall remodeling, lumens stenosis, and thrombosis.31,32 Radiation-induced endothelial cell apoptosis is a critical factor in radiation-induced brain injury.33 The present study confirms that irradiation increased the apoptosis rate of HBMECs and caused damage to the BBB.

Recent investigation revealed that COX-2 inhibition could protect against radiation-induced damage.34,35 Indeed, Khayyal et al34 showed that celecoxib, a selective COX-2 inhibitor, reduced the damaging effects of radiation in animals. Han et al35 demonstrated that meloxicam reduces brain injury after...
irradiation, partly through relief of vascular endothelial cell injury. Yang et al. showed that celecoxib can reduce the degree of brain edema in rats and glial cell injury after irradiation, which plays a protective role against radiation-induced brain injury. The present study showed that celecoxib suppressed the radiation-induced brain injury by alleviation of radiation-induced BBB damage, which may involve the apoptosis inhibition of brain microvascular endothelial cells, suggesting a potential protective role of celecoxib against radiation-induced brain injury and that celecoxib decreases COX-2 expression in addition to the selective inhibition. However, celecoxib was shown to inhibit HBMECs growth at higher concentrations as shown in Supplementary Figure 1, which suggests that celecoxib at higher concentrations may present toxicity on both HBMECs and the integrity of BBB. Thus the protective role of celecoxib should be measured at a relatively safe concentration.

Our in vivo experiments confirmed a significant increase of NF-kB and VEGF. It has been reported that radiation-induced COX-2 can promote prostaglandin synthesis, which facilitates the secretion of various kinds of inflammatory cytokines, such as TNF-α, IL-1β, IL-6, ICAM-1. Additionally, COX-2 could promote the expression of the inflammatory cytokines through activation of AP-1 and NF-kB. The up-regulated inflammatory cytokines will play important roles in the late stage of radiation-induced brain injury. Besides, radiation-induced up-regulation of COX-2 will increase the expression of VEGF, which will cause increased capillary permeability, resulting in increased BBB permeability, vascular endothelial dysfunction, vasodilation, wall thickening, decreased vascular density and length, as well as increased vascular permeability. As shown by the results, celecoxib treatment significantly reduced the radiation-induced up-regulation of NF-kB and VEGF on both mRNA and protein levels. Thus it can be concluded that the alleviation of radiation-induced BBB damage by celecoxib is partially caused by the inhibition of NF-kB and VEGF signaling, which play key roles in radiation-induced brain injury.

Radiation stimulates the formation of ceramide, thereby reducing the stability of the mitochondrial membrane and promoting apoptosis through the JNK pathway. JNK can be activated by various stimuli, controls the intracellular and extracellular stress reactions of cells, which is called the stress-activated protein kinase (SAPK), and it plays a very important role in regulating cell apoptosis. In the present study, irradiation increased the p-JNK and cleaved caspase-3 expression in HBMECs, while celecoxib partially reversed these increases. The results further suggested that the protective effects of celecoxib are associated with the regulation of apoptosis.

The content and ratio of TXA2 and PGI2 play crucial roles in thrombosis and inflammation. Ionizing radiations can break the balance between TXA2 and PGI2 by inducing COX-2 expression while inactivating prostacyclin synthase, resulting in decreased PGI2 production while increasing TXA2 production, impairing the cerebral vasculature and promoting thrombosis as well as inducing apoptosis. The present study showed that irradiation increased the ratio of TXB2 to 6-keto-PGF1α, which were stable metabolic products of TXA2 and PGI2. COX-2 is involved in the synthesis of TXA2 and PGI2. However, compared with the IR group, there was no significant reduction of 6-keto-PGF1α in the IR+C group. Thus the decrease in the ratio of TXB2/6-keto-PGF1α may be due to the reduction of TXA2, which can induce an antagonistic effect on PGI2 secretion, then increase the secretion of PGI2 through the prostaglandin synthesis pathway. As shown in Figure 6, celecoxib pretreatment decreased the amount of TXB2 after irradiation by inhibiting COX-2, which suggests a reduction in PGI2 as well as TXA2, leading to the inhibited apoptosis of endothelial cells. Furthermore, inhibited production of PGI2 resulted in decreased VEGF and NF-kB production, leading to the remission in increased BBB permeability and vascular endothelial dysfunction, finally alleviated radiation-induced brain injury.

In conclusion, the results from this study demonstrated that celecoxib helps alleviate radiation-induced brain injury by maintaining the integrity of BBB and reducing the inflammation in the rat brain tissues, and the mechanisms involve the inhibited apoptosis and decreased TXB2/6-keto-PGF1α ratio in brain vascular endothelial cells. However, we just focused on mitigation effect of celecoxib on BBB damage in the present study by measuring certain cellular, biochemical and histopathological parameters. As is known, neurogenesis, neurons and neural functions are important to the radiation-induced cognitive impairment, thus additional experimental studies are required to determine the protective effect of celecoxib on the neurocognitive functions in the future.
Authors' Note
Xiaoting Xu, Hao Huang and Yu Tu contributed equally to this paper. XTX, HH, YT, JXS, YZX and CYM conducted the experiments and SBQ performed data analysis. JYZ and WTH designed the research and wrote the paper. All authors contributed substantially to this research and reviewed this manuscript.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material
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