Original Research Article

Abietic acid ameliorates neuroinflammation and blood-brain barrier disruption in traumatic brain injury by inhibiting MAPK pathway

Kongxue Xing¹, Wenrong Jiang²*
¹Trauma Center, The Second People's Hospital of Ledong County, Ledong Li Autonomous County, Hainan Province 572536, China
²Department of Neurosurgery, Haikou People's Hospital, Haikou, Hainan Province 570208, China

*For correspondence: Email: jwenrong0419@163.com; Tel: +86-18189850718

Sent for review: 9 May 2022 Revised accepted: 27 August 2022

Abstract

Purpose: To investigate the effects of abietic acid (AA) on traumatic brain injury (TBI) in a rat model, and the underlying mechanisms of action.

Methods: Twenty male Sprague-Dawley (SD) rats were randomly divided into four groups of 5 animals each: Sham, TBI, TBI + AA (14 mg/kg), and TBI + AA (28 mg/kg). Controlled cortical impact (CCI) model was used to induce TBI in rats. Western blot was used to determine the protein levels of Claudin 3, Occludin, ZO-1, Bax, Bcl-2, cleaved-caspase3, p65 NF-κB, p-p65 NF-κB, ERK, p-ERK, JNK, p-JNK, p38 and p-p38, while the expressions of TNF-α, IL-6 and IL-1β were determined using the applicable assay kits. Neurological deficit was assessed based on mNSS and brain water content.

Results: Treatment with AA significantly reduced TBI-induced blood-brain barrier (BBB) damage, as well as apoptosis and neuroinflammation in a concentration-dependent manner (p < 0.001). The neuroprotective effect of AA was associated with the inhibition of the MAPK signaling pathways and subsequent suppression of NF-κB (p < 0.001).

Conclusion: Abietic acid serves as a potential novel candidate for the treatment of TBI, and its anti-inflammatory and anti-apoptotic effects are related to the suppression of MAPK signaling pathways. Therefore, abietic acid may serve as a novel candidate for the treatment of TBI in humans.

Keywords: Abietic acid, MAPK pathway, Traumatic brain injury, Blood brain barrier, Neuroinflammation, Neurological deficit

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

INTRODUCTION

Traumatic brain injury presents various symptoms such as mild cognitive impairment, coma and even death and about 60 million individuals sustain TBI annually. It has been identified as the leading cause of death and disability, especially in adolescents. It comprises both primary and secondary injuries. The primary injury represents direct injury to the blood-brain barrier (BBB), while secondary injury is used to describe neuronal loss and central nervous system dysfunction [3]. Moreover, multiple mechanisms play important roles in the secondary injury.
Mitogen-activated protein kinase (MAPK) cascades which contain extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinases (JNK) and p38, have been reported to promote neuronal damage in TBI via the activation of downstream pathways such as the NF-kB pathway. It has been reported that the inhibition of the MAPK pathway could effectively ameliorate neuroinflammation and BBB damage in TBI, and serve as a potential therapeutic target of TBI. Abietic acid (AA) is abietane diterpene which is isolated mainly from Pimenta racemosa var. grisea. It has various pharmacological effects on multiple diseases. It has been reported that AA inhibits cancer cell growth through suppressing the expression of proliferation of oncogenic genes, such as VEGF, TGF-β and NF-κB in breast cancer, and non-small-cell lung cancer. Abietic acid also possess anti-inflammatory and anti-oxidative properties which it exhibits by suppressing the production of nitric oxide (NO), and activating peroxisome proliferator-activated receptor-γ (PPARγ) [9]. Abietic acid reduces LPS-induced osteoporosis by suppressing the phosphorylation of MAPKs, and the nuclear translocation of NF-κB [10]. Furthermore, it has been shown that AA can protect against Cryptococcus neoformans-induced fungal meningitis through the inhibition of Mpr1-mediated BBB crossing of Cn [11]. However, the effect of AA on TBI is still unknown. Therefore, the purpose of this study was to investigate the effects of abietic acid on traumatic brain injury using a rat TBI model. These results would provide a novel therapeutic strategy for TBI and potential targets of AA.

EXPERIMENTAL

Animals

All animal experiments were approved by the Ethics Committee of The Second People’s Hospital of Ledong County for the use of animals (approval no. ZY-IRB-FOM-054), and conducted in accordance with the National Institutes of Health, Laboratory Animal Care and Use Guidelines [12]. Twenty male Sprague-Dawley (SD) rats were purchased from Charles River Laboratories. The rats were randomly divided into four groups of five animals per group. The groups include: sham, TBI, TBI + AA (14 mg/kg), and TBI + AA (28 mg/kg).

TBI model

Controlled cortical impact (CCI) model was used to induce TBI in rats. Firstly, SD rats weighing 250 - 280 g were anesthetized with 1 % pentobarbital sodium. The soft tissue of the head was surgically operated upon to expose the skull, and then a cranial window was created using a pneumatic grinding drill. The parameters of impact were set to the following conditions: 1 mm in depth, 2 m/s in speed, 3 mm of impact tip diameter and 85 milliseconds of impact time. Finally, the rats skull were sutured and the rats kept for further experiments.

Neurological deficit assessment

Traumatic Brain Injury in the rats was assessed according to the mNSS score [13], and the water content of their brains was evaluated by weighing the wet weight of the brain and the dry weight (120 °C, 24 h). Then the neurological deficit was calculated according to Eq 1.

\[
BW = \frac{(Ww - Dw) \times 100}{Ww} \quad (1)
\]

where BW is the brain water content, Ww is the wet weight and Dw is the dry weight.

Immunohistochemical staining

The rat brains were fixed in 4 % paraformaldehyde, embedded using paraffin, and cut for further staining. The hematoxylin-eosin (H&E) staining kit (Boster, Wuhan, China) was utilized according to the manufacturer’s guidelines.

Western blot assay

Brain tissues were lysed with RIPA buffer (Beyotime, Hangzhou, China), which contained protease and phosphatase inhibitors. The BCA Protein Assay kit (Beyotime, Hangzhou, China) was used for assessing protein concentration. Then the proteins were separated by SDS-PAGE, and subsequently transferred to polyvinylidene fluoride membranes. The proteins were incubated with its primary antibodies against claudin 3, occludin, ZO-1, cleaved caspase3, p65 NF-Kb, p-p65 NF-Kb, ERK, p-ERK, JNK, p-JNK, p38, p-p38, Bcl-2, and Bax (Abcam, Cambridge, UK) and β-actin (internal control, Santa Cruz, California, USA) for 12 h at 4 °C. Finally, the membranes were incubated with the corresponding hors eradish peroxidase (HRP)-conjugated secondary antibodies (Beyotime, Jiangsu, China). The blots were analyzed using Molecular Imager ChemiDoc XRS+ System (Bio-Rad, Philadelphia, PA).

Statistical analysis

Data were analyzed using SPSS and presented as mean ± standard error of the mean (SEM).
Statistical analyses among the different groups were performed via one-way ANOVA test, and p < 0.05 was considered statistically significant.

**RESULTS**

**Abietic acid attenuated TBI-induced blood-brain barrier damage**

To evaluate the effects of AA on TBI, the controlled cortical impact model of the rats was used to induce the TBI. The assay of hematoxylin-eosin (H&E) staining showed that AA exhibited neuroprotective effects in CCI rats at 14 and 28 mg/kg (Figure 1 A). Moreover, Figures 1 B and C suggested that AA improved neurological deficits and manifested significant decrease in mNSS score and brain water content in a dose-dependent manner. As shown in Figures 1 D and E, AA effectively increased the expression of Claudin3, Occludin and ZO-1 in a dose-dependent way, indicating that AA may serve as a potential treatment for BBB damage in TBI.

**Abietic acid suppressed neuronal apoptosis in TBI rats**

Figure 2 A reveals the influence of AA on neurons. The TBI caused an increase of TUNEL-positive staining cells, which indicated an apoptosis of the proliferated neurons. Compared with the TBI group, AA attenuated neuronal apoptosis in a dose-independent way. While TBI consistently resulted in increased expression of Bax and cleaved-caspase3, and decreased protein levels of Bcl-2, these were reversed by AA (Figure 2 B and C). These results revealed that AA inhibited neuronal apoptosis in TBI rats.

**Abietic acid inhibited neuroinflammation in TBI rats**

To determine the effect of AA on neuroinflammation in TBI rats, the expressions of inflammatory factors such as TNF-α, IL-6 and IL-1β, and NF-κB were determined. As shown in Figure 3 A and B, TBI induced the release of TNF-α, IL-6 and IL-1β, and phosphorylation of P65 NF-κB. Compared with the TBI group, AA treatment protected the brain from the damage of TBI-induced inflammation. This was shown by the inhibition of the levels of TNF-α, IL-6, IL-1β, and decreased phosphorylation of P65 NF-κB. Therefore, AA treatment possessed anti-inflammatory effect in TBI rats.
Abietic acid restrained MAPK pathway in TBI rats

To confirm the effect of AA on MAPK pathway, the protein expression of p-ERK, ERK, p-JNK, JNK, P38 and p-P38 were assessed (Figure 4). Traumatic brain injury leads to increased phosphorylation of ERK, JNK and P38. However, the increased phosphorylation of ERK, JNK and P38 were significantly reversed by the treatment of AA in a dose-dependent manner. These results show that AA regulates the activation of MAPK pathway in TBI rats.

**Figure 4:** Effect of abietic acid on MAPK pathway in TBI rats (A) Representative images of Western blot results. (B) Optical density values for the protein blots of p-ERK versus ERK, p-JNK versus JNK and p- P38 versus P38. Data were expressed as mean ± SEM. ***P < 0.01 vs Sham, ###P < 0.001 vs TBI

**DISCUSSION**

Traumatic brain injury is an injury caused by a blow to the head, and it ranges from a simple blow to a penetrating injury which serve as a primary cause of morbidity and mortality worldwide [3]. The secondary injury is the major reason for neurological deficits [4]. Seeking novel potential therapeutic strategies for TBI treatment are focused on attenuating the secondary injuries. In this study, the protective effect of Abietic acid on TBI rats was determined. The data revealed that TBI resulted in neurological dysfunction accompanied by the up-regulation of inflammatory factors via the MAPK/NF-κB axis. Abietic acid also reversed the influence of TBI in rats. To the best of our knowledge, this study is the first evidence to indicate the neuroprotective effect of Abietic acid on TBI through the suppression of the MAPK pathway. The physical injury destroys the neuronal structures and neuronal network in the damaged area, resulting in the release of cellular contents, including ions and cytokine-altering cell statues [14]. Multiple dysfunctions result from TBI secondary injuries, such as neuroinflammation, oxidative stress, metabolic dysfunction and even cell death.

Numerous studies have demonstrated that neuronal fate is determined by various cell signaling pathways, and the activation of MAPK pathway is the arbiter of neuronal fate [4]. The ERK, JNK and P38 MAPK pathways play key roles in the MAPK pathway, and they contribute to inflammation, oxidative stress and apoptosis in TBI [15]. With cellular stress, the up-regulation of p-ERK, p-JNK and p-P38 activates pro-caspase family [16]. The MAPK-induced apoptosis induces ERK, JNK and P38 [17]. The data showed that Abietic acid restrained the MAPK-mediated apoptosis by inhibiting the expressions of Bax and cleaved-caspase3, thus increasing the expression of Bcl-2 and suppressing neuronal apoptosis in TBI rats. The activation of MAPKs also results in inflammation through the enhancement of the expression of NF-κB, and it has been reported that sustained activation of MAPK leads to NF-κB-dependent inflammatory stress response [18]. The nuclear translocation of NF-κB contributes to the up-regulation of inflammatory cytokines. In an osteolysis model, abietic acid inhibited the nuclear translocation of NF-κB and attenuated the phosphorylation of IKKα/β [10]. These results indicate that abietic acid affected the phosphorylation of p65 NF-κB, and decreased the expressions of TNF-α, IL-6 and IL-1β in TBI in rats.

**CONCLUSION**

The findings of this study demonstrate the neuroprotective effect of abietic acid against TBI in rats. The data further indicate that abietic acid’s anti-TBI activity occurs through the inhibition of MAPK-dependent apoptosis and inflammation. Therefore, abietic acid may serve as a novel candidate for the treatment of TBI in humans.

**DECLARATIONS**

**Acknowledgements**

None provided.

**Funding**

None provided.

**Ethical approval**

This study was approved by the Ethics Committee of The Second People's Hospital of Ledong County for the use of animals (approval no. ZY-IRB-FOM-054).
Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Kongxue Xing and Wenrong Jiang designed the experiments and carried them out; Kongxue Xing analyzed and interpreted the data; and Wenrong Jiang prepared the manuscript with contributions from all co-authors.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/read), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES

1. Menon DK, Schwab K, Wright DW, Maas AI. Position statement: definition of traumatic brain injury. Arch Phys Med Rehabil 2010; 91(11): 1637-1640.
2. Mollayeva T, Mollayeva S, Colantonio A. Traumatic brain injury: sex, gender and intersecting vulnerabilities. Nat Rev Neurol 2018; 14(12): 711-722.
3. Pavlovic D, Pekic S, Stojanovic M, Popovic V. Traumatic brain injury: neuropathological, neurocognitive and neurobehavioral sequelae. Pituitary 2019; 22(3): 270-282.
4. Tanniverdi F, Schneider HJ, Aimaretti G, Masel BE, Casanueva FF, Kelsestum F. Pituitary dysfunction after traumatic brain injury: a clinical and pathophysiological approach. Endocr Rev 2015; 36(3): 305-342.
5. Li G, Duan L, Yang F, Yang L, Deng Y, Yu Y, Xu Y, Zhang Y. Curcumin suppress inflammatory response in traumatic brain injury via p38/MAPK signaling pathway. Phytother Res 2022; 36(3):1326-1337. doi: 10.1002/ptr.7391.
6. Morganti JM, Goulding DS, Van Eldik LJ. Deletion of p38alpha MAPK in microglia blunts trauma-induced inflammatory responses in mice. J Neuroinflamm 2019; 16(1): 98.
7. Liu X, Chen W, Liu Q, Dai J. Abietic acid suppresses non-small-cell lung cancer cell growth via blocking IKKbeta/NF-kappaB signaling. Onco Targets Ther 2019; 12: 4825-4837.
8. Hafez H, Osman S, Ebrahim HY, Hassan ZA. Growth inhibition and apoptotic effect of pime tac and abietic acid on MCF-7 breast cancer cells via alteration of multiple gene expressions using in vitro approach. Molecules 2022; 27(1): 293. doi: 10.3390/molecules27010293.
9. Kang S, Zhang J, Yuan Y. Abietic acid attenuates IL-1beta-induced inflammation in human osteoarthritis chondrocytes. Int Immunopharmacol 2018; 64: 110-115.
10. Thummuri D, Guntuku L, Challia VS, Ramavat RN, Naidu VGM. Abietic acid attenuates RANKL induced osteoclastogenesis and inflammation associated osteolysis by inhibiting the NF-kB and MAPK signaling. J Cell Physiol 2018; 234(1): 443-453.
11. Aaron PA, Vu K, Gelli A. An anti-virulence approach for preventing cryptococcus neoformans from crossing the blood-brain barrier via novel natural product inhibitors of a fungal metalloprotease. mBio 2020; 11(4): e01249-20. doi: 10.1128/mBio.01249-20.
12. Guide for the Care and Use of Laboratory Animals. The National Academies Collection: Reports funded by National Institutes of Health. Washington (DC) 2011.
13. Yuan J, Zhang J, Cao J, Wang G, Bai H. Geniposide alleviates traumatic brain injury in rats via anti-inflammatory effect and MAPK/NF-kB inhibition. Cell Mol Neurobiol 2020; 40(4): 511-520.
14. Ren KD, Liu WN, Tian J, Zhang YY, Peng JJ, Zhang D, Li N, Yang J, Peng J, Luo X. Mitochondrial E3 ubiquitin ligase 1 promotes brain injury by disturbing mitochondrial dynamics in a rat model of ischemic stroke. Eur J Pharmacol 2019; 861: 172617.
15. Sharma HS, Sahib S, Tian ZR, Muresanu DF, Nozari A, Castellani RJ, Lafuente JV, Wiklund L, Sharma A. Protein kinase inhibitors in traumatic brain injury and repair: new roles of nanomedicine. Prog Brain Res 2020; 258: 233-283.
16. Luan Y, Ren KD, Luan Y, Chen X, Yang Y. Mitochondrial dynamics: pathogenesis and therapeutic targets of vascular diseases. Front Cardiovasc Med 2021; 8: 770574.
17. Abdelzaher WY, Bahaa HA, Elkhatteeb R, Atta M, Favzy MA, Ahmed AF, Rofaeil RR. Role of JNK, ERK, and p38 MAPK signaling pathway in protective effect of sildenafil in cyclophosphamide-induced placental injury in rats. Life Sci 2022; 293: 120354.
18. Ramalingam P, Poulos MG, Lazzari E, Gutkin MC, Lopez D, Kloss CC, Crowley MJ, Katsnelson L, Freire AG, Greenblatt MB, et al. Chronic activation of endothelial MAPK disrupts hematopoiesis via NFkB dependent inflammatory stress reversible by SCGF. Nat Commun 2020; 11(1): 666. doi: 10.1038/s41467-020-14478-8.