Journal of Pain Research

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Indolepropionic Acid Attenuates CFA-Induced Inflammatory Pain in Mice

Rui-Feng Ao[1], Heng-Rui Yong[1], Ying-Tao Hu[1], Yu-Sheng Huang[1], Jia-Wen Gao[1], Chen Tu[1,2], Jing-Shen Zhuang[1], Zhao-Ming Zhong[1]

[1]Division of Spine Surgery, Department of Orthopaedics, Nanfang Hospital, Southern Medical University, Guangzhou, People's Republic of China; [2]Department of Orthopedics, Academy of Orthopedics, The Third Affiliated Hospital, Southern Medical University, Guangzhou, People's Republic of China

Correspondence: Jing-Shen Zhuang; Zhao-Ming Zhong, Division of Spine Surgery, Department of Orthopaedics, Nanfang Hospital, Southern Medical University, Guangzhou, People's Republic of China, Email zjs119@smu.edu.cn; zhongzm@smu.edu.cn

Background: Chronic pain is a global health issue that affects as many as 20% of the population. Inflammatory pain, an important form of chronic pain, negatively impacts patients' quality of life. Indolepropionic acid (IPA), a metabolite derived from the gut microbiota, has anti-inflammatory properties. However, its effect on inflammatory pain has not yet been explored. This study aims to investigate the impact of IPA on CFA-induced inflammatory pain.

Methods: A mouse model of inflammatory pain was established by injection of Complete Freund's Adjuvant (CFA) into the hind paw, and treated with the IPA supplement. Behavioral assessments were conducted using the Von Frey test, cold or hot plate tests. The expression of pain-related transcripts, such as transient receptor potential vanilloid 1 (TRPV1) and calcitonin gene-related peptide (CGRP) was evaluated. Degree of inflammation was assessed by the thickness of paws, degree of inflammatory infiltration and the changes of serum tumor necrosis factor (TNF)-α, interleukin(IL)-6 and IL-1β.

Results: IPA supplement improved the CFA-induced decrease of the mechanical withdrawal threshold and cold and thermal withdrawal latency. Meanwhile, IPA inhibited the CFA-induced upregulation of TRPV1 and CGRP in DRGs. In addition, IPA treatment also suppressed the CFA-induced local and systemic inflammation, including the swelling and thickening of the paw, local infiltration of inflammatory cells, and increased serum levels of TNF-α, IL-6, and IL-1β.

Conclusion: Our results show that IPA can improve pain-related behavior and alleviate inflammation in the CFA–treated mice, which provides new insight into potential strategies for inflammatory pain management.

Keywords: indolepropionic acid, inflammatory pain, complete freund's adjuvant, TRPV1, CGRP

Introduction

Chronic pain affects more than 30% of people worldwide and imposes a substantial burden on individuals and society.[1,2] Accumulating evidence suggests that inflammation plays an important role in the pathogenesis of chronic pain conditions.[3] Currently, oral nonsteroidal anti-inflammatory drugs (NSAIDs) are considered as the first-line treatment for chronic inflammatory pain, such as neck pain, back pain and osteoarthritis.[4,5] However, the adverse effects of NSAIDs and poor patient compliance often limit their long-term efficacy.[5] It is still urgent to find novel therapeutic strategies for inflammatory pain.

Indolepropionic acid (IPA), a gut microbiota-produced tryptophan metabolite, has attracted increasing attention for its beneficial role in health and diseases.[6,7] IPA has shown good safety and low toxicity in both in vitro and in vivo studies.[8] and it is involved in the regulation of various physiological functions, including inflammation, metabolism, immune responses, redox state and neurological function.[9,10] Recent evidence suggests that IPA exerts not only an anti-inflammatory influence but also antioxidative action against oxidative damage.[11–13] IPA may have a beneficial effect on inflammatory pain, but this possibility has not been thoroughly investigated. Therefore, a mouse model of inflammatory pain was established by injection of Complete Freund's Adjuvant (CFA) into hind paw as previously described.[14] Our study aimed to examine the role of IPA in inflammatory pain.

Received: 2 March 2025
Accepted: 13 May 2025
Published: 23 May 2025

Materials and Methods

Animal and Drug

Male C57BL/6J mice, aged 10 weeks, were purchased from the Animal Experimental Center of Nanfang Hospital, Southern Medical University, China. The animals were maintained at standard conditions with a strict 12 h:12 h light: dark cycle, humidity at 50 ± 15%, temperature at 22 ± 2 °C. Food and water were available ad libitum. Mice were allowed to adapt to these conditions for at least 7 days before all experiments. The experiments were conducted following the Animal Research Reporting In Vivo Experiments (ARRIVE) guidelines. All animal experiments were approved by the Nanfang hospital animal ethic committee (IACUC-LAC-20241210-003). CFA was purchased from Sigma-Aldrich (St. Louis, MO). IPA was purchased from Macklin Biochemical Corp. (Shanghai, China), and diluted in sterile phosphate buffered saline (PBS) at a concentration of 0.5 mg per 200 μL.[15] The welfare and treatment of laboratory animals follow the GUIDE FOR THE CARE AND USE OF LABORATORY ANIMALS and the China and Nanfang hospital policies for health and well-being of laboratory animals.

Experimental Procedures

Mice were randomly divided into three groups (n=7 per group): (1) The Control group, in which the animals were injected 10μL of saline subcutaneously into the right paw. (2) The CFA group, in which mice were injected 10μL of CFA subcutaneously into the right paw for three days and received daily PBS treatment by gavage for the next seven days (3) The CFA + IPA group, in which mice were injected 10μL of CFA subcutaneously into the right paw for three days, and received 20 mg/kg IPA per day by gavage for next seven days. The experiment was conducted as shown in Figure 1A.

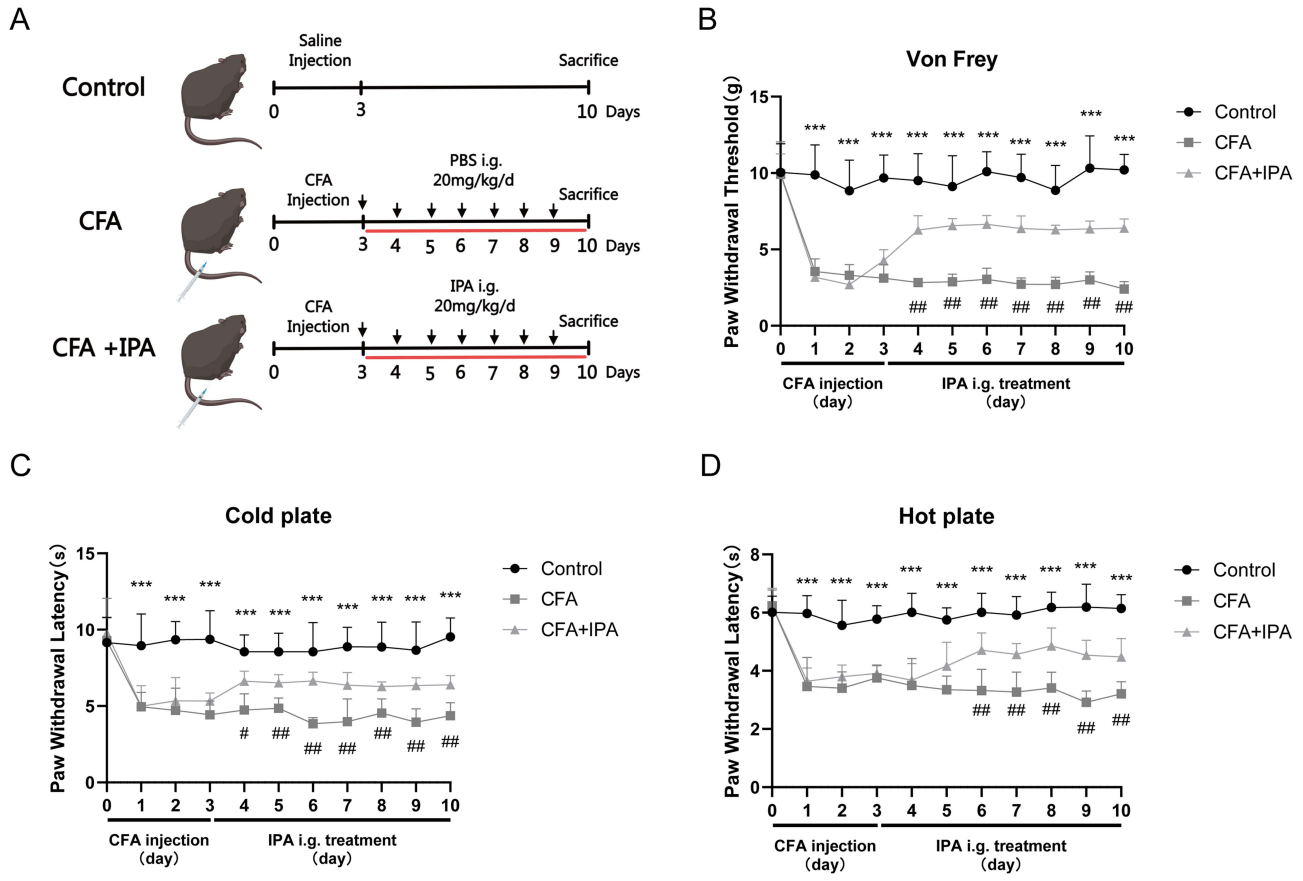

Figure 1 IPA alleviated inflammatory pain induced by CFA. (**A**) Schematic diagram of the experimental procedure. (**B**)The alterations in the mechanical withdrawal threshold. (**C** and **D**) The alterations in the paw withdrawal latency of cold plate and hot plate. ***p < 0.001, Control group vs CFA group; #p < 0.05, ##p < 0.01, CFA group vs CFA + IPA group.

Assessment of Behavior

The mechanical withdrawal threshold was tested by using electronic Von Frey filaments (Ugo Basile, Italy) as previously described.[16] Briefly, mice were placed in a plastic box on a metal frame for 15 minutes to get used to their surroundings before testing. A filament was then pressed on their paw, and the peak force of stimulation was recorded. A positive response was defined as abrupt paw away or licking. This procedure was repeated five times with an inter-test period of 15 min, and the average value of the peak force was regarded as the paw withdrawal threshold (PWT) of the mice. The Von Frey tests were conducted daily after a three-day injection of CFA.

Cold or hot sensitivity was assessed by measuring the latency of the hind paw withdrawal response to cold or hot stimulation as previously described.[16] Briefly, mice were put on a platform for 30 minutes to get used to their surroundings before testing. They were subjected to cold and hot stimulation on a ZH-6C Plate at 4°C or 50°C, with a wall to stop them from escaping. Any behavioural changes like paw licking or jumping were noted, and the latency to display such behaviour was recorded. The cutoff time was set at 20s, and the procedure was repeated 5 times at 30-minute intervals, and the average value was taken as the paw withdrawal latency (PWL). All behavioral baseline data were assessed before the injection of CFA.

Hematoxylin and Eosin (H&E) Staining

Right hind foot tissue was collected from mice, fixed in 4% paraformaldehyde (PFA) for 24 hours, and then stored in PBS for 24 hours. Samples were then subjected to dehydration and paraffin embedding. Sections of 5-μm thickness were cut from the paraffin-embedded tissue, and the paraffin sections were dewaxed and rehydrated according to standard procedures, followed by H&E staining as previously described.[17]

Measure of Footpad Thickness

The foot pad thickness was measured by a vernier calliper as previously described.[18] Briefly, the foot pad thickness was measured in the area 3mm away from the distal end of the ankle tip and the base of the first toe. Each animal was measured five times and the average value was taken as the pad thickness. The baseline data of the foot pad thickness were assessed before the injection of CFA.

Immunofluorescence Staining

L3-L5 dorsal root ganglia (DRGs) were collected from mice and fixed in 4% PFA for 4–6 hours. They were then transferred to 30% sucrose for a 16–18 hour dehydration process. Subsequently, samples were embedded in OCT and immediately frozen at −80°C to solidify the OCT. The OCT-embedded tissues were sectioned into 10μm slices using a cryostat (Leica CM1950). Immunofluorescence staining included the following steps: First, the tissues were blocked with 10% goat serum at room temperature for half an hour. Then, they were incubated overnight at 4°C with primary antibodies: TRPV1 (1:100, Absin, Shanghai, China), CGRP (1:100, Cell Signaling, USA), and co-incubated with NeuN (1:250, Cell Signaling, USA). Afterwards, the sections were incubated with secondary antibodies for 1 hour at room temperature: Goat anti-Rabbit Alexa Fluor 488 (1:200, Abcam, UK) and Goat anti-Rat Alexa Fluor 594 (1:200, Abcam, UK). Images were acquired using a Zeiss Imager D2 microscope and analyzed with ZEN software (Zeiss). The analysis was performed using the arithmetic mean intensity recorded in Zeiss Zen 3.8. The overall data were tested to determine whether they followed a normal distribution. If the fluorescence signal distribution was uneven or if there were outliers, it might be necessary to consider using the median or geometric mean to calculate the relative fluorescence intensity. The data from the Control group were used as the reference value "1" for comparison of relative fluorescence intensity.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA kits were used to detect the concentrations of tumor necrosis factor (TNF) – α (Jingmei, Beijing, China), interleukin (IL)-6 (Abebio, Wuhan, China), and IL-1β (Mreda, Beijing, China) in serum. All tests were performed according to the manufacturer's instructions. Six hippocampal samples were measured in each group, and each sample was tested in triplicate.

Statistical Analysis

All statistical data in this study are presented as Mean ± Standard Deviation. We conduct statistical analysis of the data using one-way ANOVA, two-way ANOVA, or two-sample *t*-test, with data being paired whenever possible. The P value < 0.05 was considered to indicate a statistically significant difference. Statistical analyses were performed using GraphPad Prism version 10.4.

Results

IPA Alleviated the Inflammatory Pain Induced by CFA

CFA-induced inflammatory pain lasts for at least several weeks.[19] To explore the effects of IPA on inflammatory pain, the behavioral performance of mice was observed within one week after a three-day injection of CFA (Figure 1A). As shown in Figure 1B–D, the mechanical withdrawal threshold, thermal withdrawal latency and cold withdrawal latency were significantly reduced in the CFA group compared with the Control group. However, after receiving IPA treatment, the CFA-induced decrease of mechanical withdrawal threshold was noticeably improved, and both thermal withdrawal latency and cold withdrawal latency were significantly prolonged in the CFA + IPA group. These results indicated that IPA is effective in alleviating inflammatory pain induced by CFA.

IPA Inhibited CFA-Induced Upregulation of TRPV1 and CGRP in DRGs

The TRPV1 channel and CGRP play a crucial role in pain response. The activation of the TRPV1 channel and up-regulation of CGRP in DRGs are often associated with increased sensitivity to pain.[20–22] As shown in Figure 2A–D, the fluorescence intensity of TRPV1 and CGRP in the DRGs was significantly higher in the CFA group than in the Control group, suggesting that CFA induced the up-regulation of TRPV1 and CGRP expression. Meanwhile, IPA treatment significantly inhibited the CFA-induced up-regulation of TRPV1 and CGRP expression. The downregulation of these pain mediators indicated a potential neuromodulatory mechanism underlying the analgesic effect of IPA.

IPA Attenuated the CFA-Induced Inflammation

Reducing inflammation can potentially help relieve pain.[23,24] As shown in Figure 3A, the injection of CFA triggered an inflammatory response, including redness and edema. CFA injection increased the footpad thickness of mice compared with the control mice, and the IPA treatment reduced the CFA-induced swelling of the paw (Figure 3B). Meanwhile, the massive infiltration of inflammatory cells in the plantar region was observed in the CFA group, but these local inflammatory manifestations were alleviated after IPA treatment (Figure 3C and D). Furthermore, serum levels of TNF-α, IL-6, and IL-1β were significantly higher in the CFA group than in the Control group, and IPA treatment decreased the serum levels of TNF-α, IL-6, and IL-1β (Figure 3E–G). These results indicated that IPA can aid in alleviating inflammation and pain.

Discussion

Inflammatory pain is a prevalent condition, leading to progressive dysfunction and reduced quality of life.[25] Inflammation and overexcitation of DRG neurons that innervate inflamed tissues were the main causes of inflammatory pain.[26–28] In our study, CFA injection induced swelling of hind paws, invasion of inflammatory cells and hyperalgesia. The local release of inflammatory mediators, such as inflammatory cytokines and neurogenic factors, can also trigger neurogenic inflammation in peripheral and central sensitization, eventually resulting in pain.[29,30] Reducing inflammation played a crucial role in relieving pain conditions.[23,24] In this study, we demonstrated that IPA administration can alleviate CFA-induced pain and inflammatory response in mice.

The activation of the TRPV1 channel and the release of CGRP play a key role in inflammatory, neuropathic, and cancer pain.[31–33] In our study, CFA injection led to increased expression of TRPV1 and CGRP in the DRGs, which are associated with decreased mechanical, hot and cold pain thresholds in the CFA model mice. Activation of TRPV1 can cause a burning sensation and plays an important role in inflammatory pain. However, reports of adverse reactions in human trials have hindered the widespread efforts to develop TRPV1 antagonists as new types of analgesic drugs in

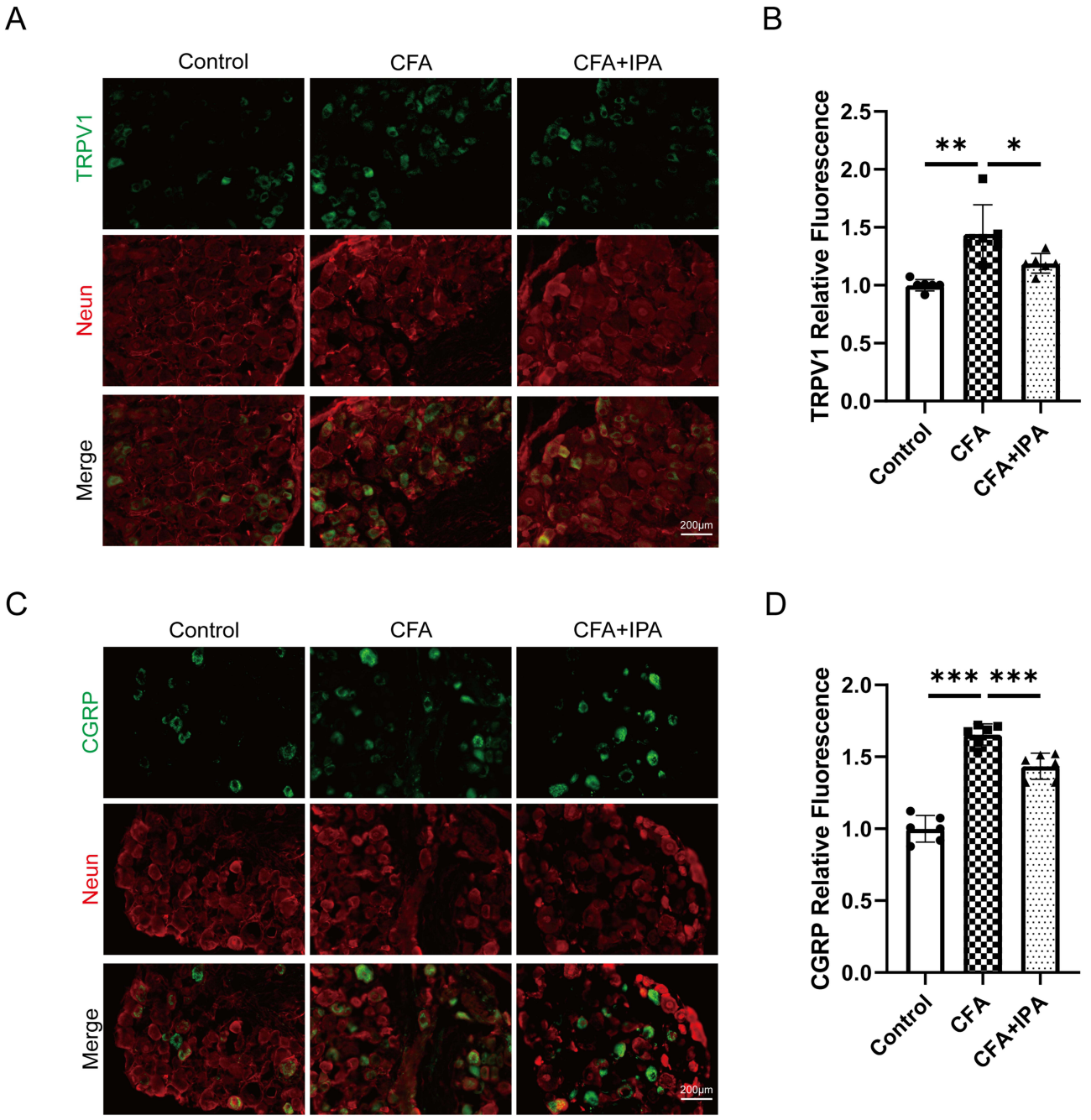

Figure 2 IPA inhibited CFA-induced upregulation of TRPV1 and CGRP in DRGs. (**A**) Representative images of TRPV1 co-stained with Neun in the L3-L5 DRGs of the Control, CFA and CFA + IPA groups. Scale bar, 200 μm. (**B**) Relative fluorescence of TRPV1 among the three groups. (**C**) Representative images of CGRP co-stained with Neun in the L3-L5 DRGs of the Control, CFA and CFA + IPA groups. Scale bar, 100 μm. (**D**) Relative fluorescence of CGRP among the three groups. *p < 0.05, **p < 0.01, ***p < 0.001.

clinical settings.[34,35] Meanwhile, CGRP is primarily released from sensory nerves and is an important substance associated with pain pathways. CGRP antagonists were proven to have the ability to alleviate migraines and were also involved in the treatment of other types of pain.[36]

IPA can exert its anti-inflammatory effects by reducing the release of inflammatory factors and regulating inflammatory responses. It was reported that IPA markedly decreases the level of serum inflammatory cytokines, reduces cartilage destruction and synovitis inflammation, thus showing a protective effect on the progression of osteoarthritis.[13] IPA can also improve intestinal barrier damage, and alleviate the reactivity of intestinal fibroblasts to injurious stimuli,

https://doi.org/10.2147/JPR.S525859

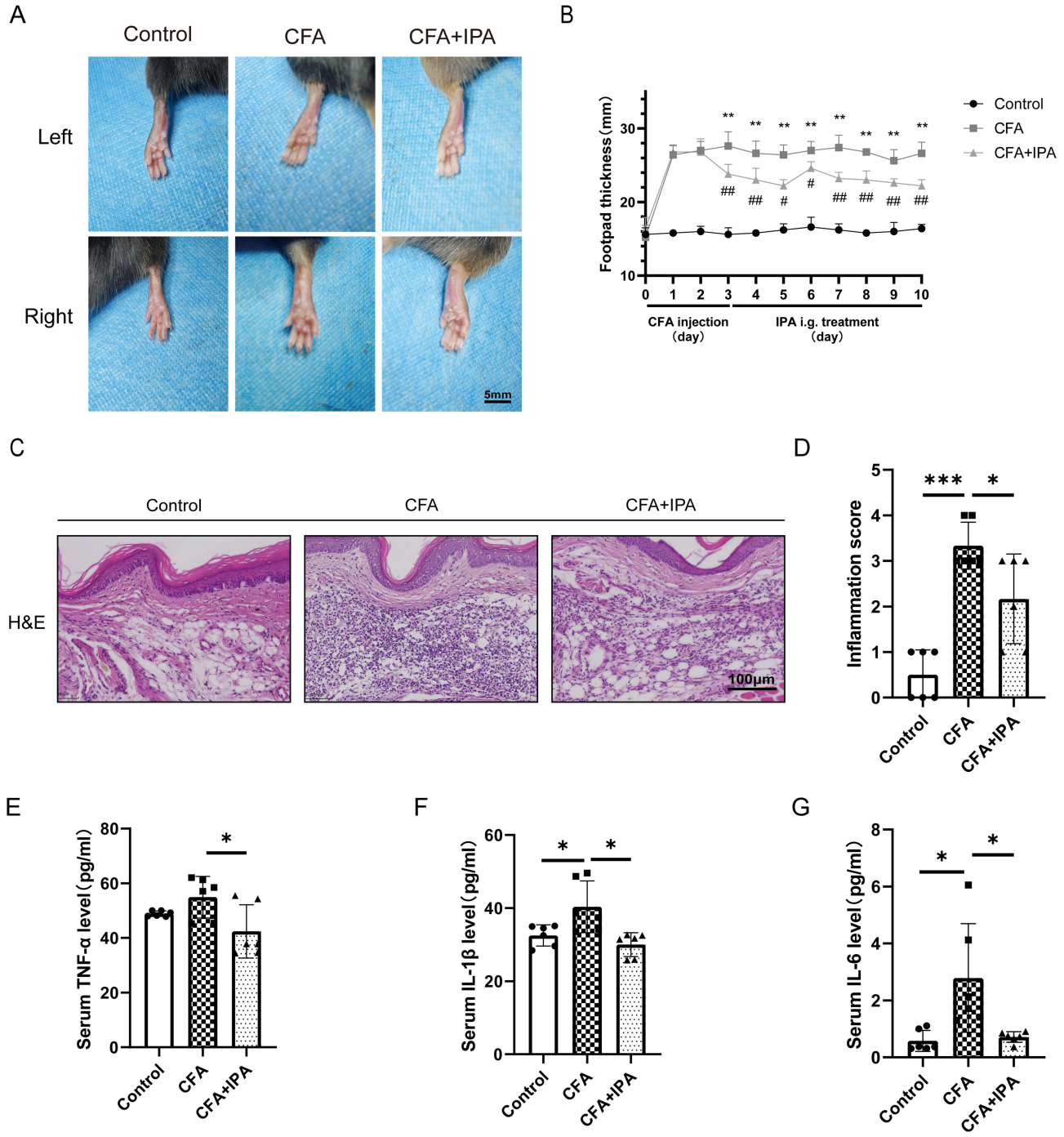

Figure 3 IPA attenuates CFA-induced inflammation. (**A**) Macroscopic images of both left and right hind paws of mice from the Control, CFA and CFA + IPA groups. Scale bar, 5mm. (**B**) Changes in footpad thickness of mice during the experimental process. (**C**) H&E staining of hind paw tissues. Scale bar, 100 μm. (**D**) Scores of inflammation in the hind paw tissues. (**E–G**) Serum levels of TNF-α, IL-6 and IL-1β among the three groups. *p < 0.05, **p < 0.01, ***p < 0.001, Control group vs CFA group; #p < 0.05, ##p < 0.01, CFA group vs CFA + IPA group.

thereby reducing the release of inflammatory mediators and alleviating the manifestations of intestinal inflammation and fibrosis.[37,38] Furthermore, IPA can act in conjunction with IFN-I to suppress inflammation in the central nervous system.[39] In our study, IPA supplement can alleviate the local and systemic inflammation induced by CFA, which is similar to the anti-inflammatory effects of IPA shown in other studies.

Recent research shows that NGF is a crucial pain-inducing substance that transmits pain signals by binding to the TrkA receptor. Anti-NGF drugs are considered one of the key targets for the treatment of chronic pain. A novel anti-NGF

monoclonal antibody, DS002, has shown significant pain-relieving effects. DS002 primarily affects the metabolism of aromatic amino acids, and targeted metabolomics analysis in studies has indicated that changes in indolepropionic acid may be associated with the analgesic effects of DS002.[40] Additionally, in a study on rosacea, it was found that inflammatory symptoms of rosacea, such as stinging and itching, are related to the increased expression of TRPV1, NGF, and TrkA in DRGs, and the expression levels of CGRP are also elevated. The activation of the NGF-TrkA-TRPV1 pathway can lead to cutaneous neurogenic inflammation and angiogenesis.[41] In this study, the downregulation of TRPV1 and CGRP in DRGs by IPA was observed, and whether this effect is achieved through the inhibition of the NGF-TrkA pathway, which may be one mechanism by which IPA alleviates pain caused by CFA, requires further research to confirm.

Above all, our results show that IPA can improve pain-related behavior and alleviate inflammation in a mouse model of CFA-induced inflammatory pain. These findings also offer new insight into potential strategies for inflammatory pain management.

Acknowledgments

The author would like to express gratitude to the Spinal Cord Injury Laboratory of Southern Hospital and the Central Laboratory of Southern Hospital for providing experimental facilities.

Funding

This work was partly supported by the Natural Science Foundation of Guangdong Province, China (grant No. 2023A1515011943), and the National Natural Science Foundation of China (No. 82304154).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Cohen SP, Vase L, Hooten WM. Chronic pain: an update on burden, best practices, and new advances. *Lancet*. 2021;397(10289):2082–2097. doi:10.1016/S0140-6736(21)00393-7
2. St Sauver JL, Warner DO, Yawn BP, et al. Why patients visit their doctors: assessing the most prevalent conditions in a defined American population. *Mayo Clin Proc*. 2013;88(1):56–67. doi:10.1016/j.mayocp.2012.08.020
3. Fang XX, Zhai MN, Zhu M, et al. Inflammation in pathogenesis of chronic pain: foe and friend. *Mol Pain*. 2023;19:17448069231178176. doi:10.1177/17448069231178176
4. Murray CJ, Atkinson C, Bhalla K, et al. The state of US health, 1990-2010: burden of diseases, injuries, and risk factors. *JAMA*. 2013;310(6):591–608. doi:10.1001/jama.2013.13805
5. Yabuki S, Ip AKK, Tam CK, et al. Evidence-based recommendations on the pharmacological management of osteoarthritis and chronic low back pain: an Asian consensus. *Asian J Anesthesiol*. 2019;57(2):37–54. doi:10.6859/aja.201906_57(2).0003
6. Roager HM, Licht TR. Microbial tryptophan catabolites in health and disease. *Nat Commun*. 2018;9(1):3294. doi:10.1038/s41467-018-05470-4
7. Wikoff WR, Anfora AT, Liu J, et al. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci U S A*. 2009;106(10):3698–3703. doi:10.1073/pnas.0812874106
8. Anastassova N, Stefanova D, Hristova-Avakumova N, et al. New indole-3-propionic acid and 5-methoxy-indole carboxylic acid derived hydrazone hybrids as multifunctional neuroprotectors. *Antioxidants*. 2023;12(4):977. doi:10.3390/antiox12040977
9. Sivaprakasam S, Bhutia YD, Ramachandran S, Ganapathy V. Cell-surface and nuclear receptors in the colon as targets for bacterial metabolites and its relevance to colon health. *Nutrients*. 2017;9(8):856. doi:10.3390/nu9080856
10. Dodd D, Spitzer MH, Van Treuren W, et al. A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites. *Nature*. 2017;551(7682):648–652. doi:10.1038/nature24661
11. Hwang IK, Yoo KY, Li H, et al. Indole-3-propionic acid attenuates neuronal damage and oxidative stress in the ischemic hippocampus. *J Neurosci Res*. 2009;87(9):2126–2137. doi:10.1002/jnr.22030
12. Karbownik M, Stasiak M, Zygmunt A, Zasada K, Lewiński A. Protective effects of melatonin and indole-3-propionic acid against lipid peroxidation, caused by potassium bromate in the rat kidney. *Cell BiochemFunct*. 2006;24(6):483–489. doi:10.1002/cbf.1321
13. Zhuang H, Ren X, Jiang F, Zhou P. Indole-3-propionic acid alleviates chondrocytes inflammation and osteoarthritis via the AhR/NF-κB axis. *Mol Med*. 2023;29(1):17. doi:10.1186/s10020-023-00614-9
14. Xiang HC, Lin LX, Hu XF, et al. AMPK activation attenuates inflammatory pain through inhibiting NF-κB activation and IL-1β expression. *J Neuroinflammation*. 2019;16(1):34. doi:10.1186/s12974-019-1411-x
15. Serger E, Luengo-Gutierrez L, Chadwick JS, et al. The gut metabolite indole-3 propionate promotes nerve regeneration and repair. *Nature*. 2022;607(7919):585–592. doi:10.1038/s41586-022-04884-x
16. Tu C, Wu DZ, Huang YS, et al. Oxidative stress contributes to hyperalgesia in osteoporotic mice. *J Pain Res*. 2020;13:131–142. doi:10.2147/JPR.S234334

17. Li HZ, Han D, Ao RF, et al. Tanshinone IIA attenuates osteoarthritis via inhibiting aberrant angiogenesis in subchondral bone. *Arch Biochem Biophys*. 2024;753:109904. doi:10.1016/j.abb.2024.109904
18. Chia WT, Lin CF, Yeh LT, Sytwu HK. A noncontact footpad thickness assay to evaluate rheumatoid disease. *Rheumatol Int*. 2010;30(4):547–550. doi:10.1007/s00296-009-1261-4
19. Fan XC, Fu S, Liu FY, Cui S, Yi M, Wan Y. Hypersensitivity of prelimbic cortex neurons contributes to aggravated nociceptive responses in rats with experience of chronic inflammatory pain. *Front Mol Neurosci*. 2018;11:85. doi:10.3389/fnmol.2018.00085
20. Xie YK, Luo H, Zhang SX, et al. GPR177 in A-fiber sensory neurons drives diabetic neuropathic pain via WNT-mediated TRPV1 activation. *Sci Transl Med*. 2022;14(639):eabh2557. doi:10.1126/scitranslmed.abh2557
21. Landini L, Souza Monteiro de Araujo D, Chieca M, et al. Acetaldehyde via CGRP receptor and TRPA1 in Schwann cells mediates ethanol-evoked periorbital mechanical allodynia in mice: relevance for migraine. *J Biomed Sci*. 2023;30(1):28. doi:10.1186/s12929-023-00922-6
22. Liu D, Chang L, Wang J, et al. Effect of Xiongmatang extract on behavioral and TRPV1-CGRP/CGRP-R pathway in rats with migraine. *Front Pharmacol*. 2022;13:835187. doi:10.3389/fphar.2022.835187
23. Matsuda M, Huh Y, Ji RR. Roles of inflammation, neurogenic inflammation, and neuroinflammation in pain. *J Anesth*. 2019;33(1):131–139. doi:10.1007/s00540-018-2579-4
24. Gao F, Xiang HC, Li HP, et al. Electroacupuncture inhibits NLRP3 inflammasome activation through CB2 receptors in inflammatory pain. *Brain Behav Immun*. 2018;67:91–100. doi:10.1016/j.bbi.2017.08.004
25. Andrews P, Steultjens M, Riskowski J. Chronic widespread pain prevalence in the general population: a systematic review. *Eur J Pain*. 2018;22(1):5–18. doi:10.1002/ejp.1090
26. Sun X, Li X, Zhou Y, Wang Y, Liu X. Up-regulating TIPE2 alleviates inflammatory pain by suppressing microglial activation-mediated inflammatory response via inhibiting Rac1/NF-κB pathway. *Exp Cell Res*. 2021;404(1):112631. doi:10.1016/j.yexcr.2021.112631
27. Yuan Y, Zhang Y, He X, Fan S. Protective effects of sinomenine on CFA-induced inflammatory pain in rats. *Med Sci Monit*. 2018;24:2018–2024. doi:10.12659/msm.906726
28. Djouhri L, Al Otaibi M, Kahlat K, Smith T, Sathish J, Weng X. Persistent hindlimb inflammation induces changes in activation properties of hyperpolarization-activated current (Ih) in rat C-fiber nociceptors in vivo. *Neuroscience*. 2015;301:121–133. doi:10.1016/j.neuroscience.2015.05.074
29. He W, Long T, Pan Q, et al. Microglial NLRP3 inflammasome activation mediates IL-1β release and contributes to central sensitization in a recurrent nitroglycerin-induced migraine model. *J Neuroinflammation*. 2019;16(1):78. doi:10.1186/s12974-019-1459-7
30. Fu X, Zhu ZH, Wang YQ, Wu GC. Regulation of proinflammatory cytokines gene expression by nociceptin/orphanin FQ in the spinal cord and the cultured astrocytes. *Neuroscience*. 2007;144:275–285. doi:10.1016/j.neuroscience.2006.09.016
31. Yu L, Yang F, Luo H, et al. The role of TRPV1 in different subtypes of dorsal root ganglion neurons in rat chronic inflammatory nociception induced by complete Freund's adjuvant. *Mol Pain*. 2008;4:61. doi:10.1186/1744-8069-4-61
32. Bigal ME, Walter S, Rapoport AM. Calcitonin gene-related peptide (CGRP) and migraine current understanding and state of development. *Headache*. 2013;53(8):1230–1244. doi:10.1111/head.12179
33. Sun R-Q, Tu Y-J, Lawand NB, et al. Calcitonin gene-related peptide receptor activation produces PKA- and PKC-dependent mechanical hyperalgesia and central sensitization. *J Neurophysiol*. 2004;92(5):2859–2866. doi:10.1152/jn.00339.2004
34. Iftinca M, Defaye M, Altier C. TRPV1-targeted drugs in development for human pain conditions. *Drugs*. 2021;81(1):7–27. doi:10.1007/s40265-020-01429-2
35. Katz B, Zaguri R, Edvardson S, et al. Nociception and pain in humans lacking a functional TRPV1 channel. *J Clin Invest*. 2023;133(3):e153558. doi:10.1172/JCI153558
36. Russell FA, King R, Smillie SJ, Kodji X, Brain SD. Calcitonin gene-related peptide: physiology and pathophysiology. *Physiol Rev*. 2014;94(4):1099–1142. doi:10.1152/physrev.00034.2013
37. Wang A, Guan C, Wang T, Mu G, Tuo Y. *Lactobacillus*-derived indole derivatives ameliorate intestinal barrier damage in rat pups with complementary food administration. *Food Funct*. 2024;15(17):8775–8787. doi:10.1039/d4fo02230k
38. Flannigan KL, Nieves KM, Szczepanski HE, et al. The pregnane X receptor and indole-3-propionic acid shape the intestinal mesenchyme to restrain inflammation and fibrosis. *Cell Mol Gastroenterol Hepatol*. 2023;15(3):765–795. doi:10.1016/j.jcmgh.2022.10.014
39. Rothhammer V, Mascanfroni ID, Bunse L, et al. Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. *Nat Med*. 2016;22(6):586–597. doi:10.1038/nm.4106
40. Jin D, Yang H, Chen Z, et al. Effect of the novel anti-NGF monoclonal antibody DS002 on the metabolomics of pain mediators, cartilage and bone. *Front Pharmacol*. 2024;15:1396790. doi:10.3389/fphar.2024.1396790
41. Lee SG, Kim J, Lee YI, et al. Cutaneous neurogenic inflammation mediated by TRPV1-NGF-TRKA pathway activation in rosacea is exacerbated by the presence of demodex mites. *J Eur Acad Dermatol Venereol*. 2023;37(12):2589–2600. doi:10.1111/jdv.19449