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Dietary constituent genistein inhibits the hyperexcitability of trigeminal nociceptive neurons associated with mechanical hyperalgesia following orofacial inflammation

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ABSTRACT

Objectives: Genistein, a dietary constituent, modulates voltage-dependent and ligand-gated ionic channels, suggesting that it could also attenuate inflammatory hyperalgesia. However, the mechanism underlying how genistein affects inflammation-induced hyperexcitability of nociceptive neurons *in vivo* remains to be determined. The present study therefore investigated whether administration of genistein could attenuate the inflammation-induced hyperexcitability of trigeminal spinal nucleus caudalis (SpVc) neurons associated with mechanical hyperalgesia *in vivo*.

Methods: Inflammation was induced by injection of complete Freund's adjuvant into the whisker pad. The mechanical thresholds for escape behavior and electrophysiological single-unit recording of SpVc neurons responding to mechanical stimulation were then conducted in naïve rats, inflamed rats, and inflamed rats with genistein administered intraperitoneally.

Results: The lowered mechanical threshold in the inflamed rats was returned to control level following administration of genistein for 2 days. The mean number of discharge frequencies of SpVc neurons in inflamed rats was significantly decreased after genistein administration with both non-noxious and noxious mechanical stimuli. The increased spontaneous discharges of SpVc neurons in inflamed rats were significantly decreased after genistein administration. Noxious pinch-evoked after-discharge frequency and occurrence in inflamed rats was also significantly diminished after genistein administration, and expansion of the receptive field was significantly returned to control levels in inflamed rats.

Conclusion: Herein, we present the first evidence that genistein attenuates hyperexcitability of SpVc neurons associated with inflammatory mechanical hyperalgesia. These findings suggest that genistein could be a potential therapeutic agent in complementary alternative medicine for the prevention of trigeminal inflammatory hyperalgesia.

1. Introduction

Complementary alternative medicine (CAM) therapies such as herbal medicines and acupuncture are often used for pain control when other medical treatments are ineffective [1–3]. The isoflavone, genistein, is a naturally occurring polyphenol found in high concentrations in soy products that also shows a variety of medically useful biological actions, including cardioprotection, neuroprotection, and anticancer properties [4,5]. Genistein has been shown to modulate neuronal excitability via various voltage-dependent [6–9] and ligand-gated ion channels [8–10], such as inhibiting glutaminergic synaptic transmission *in vitro* via *N*-methyl-D-aspartate (NMDA) receptors [9,10]. Specifically, modulating peripheral nociceptors can trigger central sensitization, such as that seen with phosphorylation of NMDA receptor subunits, and genistein has been reported to modulate NMDA receptor 2B (NR2B) phosphorylation following tissue inflammation [11,12]. In addition, genistein can also decrease the production of prostaglandin E_2 (PGE_2) by inhibiting cyclooxygenase (COX)-2 cascades [13,14]. Together, these findings suggest that chronic administration of

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genistein is a potential therapeutic strategy for moderating or preventing inflammatory hyperalgesia.

For orofacial sensory processing, the spinal trigeminal nucleus caudalis (SpVc) provides important relay stations for trigeminal nociceptive inputs following inflammation and tissue injury [15,16]. Chronic pathological conditions, including tissue inflammation, can change the properties of somatic sensory pathways, leading to hyperalgesia [17,18]. Complete Freund's adjuvant (CFA) models of inflammation in the orofacial region have been developed in rats to study trigeminal pathological pain [16,19]. Previous studies have reported CFA inflammation-induced hyperexcitability of SpVc wide-dynamic range (WDR) neurons in response to mechanical stimuli [16,20,21]. In addition, SpVc WDR neurons can contribute to the mechanism of hyperalgesia and/or referred dental pain [22,23]. Chronic administration of dietary constituents including polyphenols, polyunsaturated fatty acids, and carotenoids has been shown to attenuate inflammation-induced mechanical hyperalgesia, primarily by suppressing SpVc WDR neuronal hyperexcitability via both peripheral and central Cox-2 cascade signaling pathways [24–26]. Based on these studies, we hypothesized that genistein administration may attenuate inflammation-induced hyperexcitability of SpVc WDR neurons associated with trigeminal hyperalgesia. The present study therefore investigated whether chronic genistein administration to rats attenuates the inflammation-induced hyperexcitability of SpVc WDR neurons associated with mechanical hyperalgesia *in vivo*.

2. Materials and methods

2.1. Induction of inflammation and genistein administration

The experiments were performed on adult male Wistar rats (body weight 250–290 g, n = 30). Rats were divided into three groups, as follows; naïve (n = 8), inflamed (n = 8), and inflamed rats with genistein treatment (100 mM; n = 4 and 200 mM; n = 10, intraperioneally; Sigma-Aldrich, Milano, Italy). Previous studies indicated 100 μM genistein significantly suppresses the excitability of trigeminal ganglion neurons *in vitro* [7]. Since intraperioneally (i.p.) administered 100 mM genistein entered the general circulation and was diluted into blood stream to a calculated concentration of approximately 100 μM in this study, we used a concentration of genistein that was >100 mM (100 and 200 mM). Each animal was anesthetized with sodium pentobarbital (45 mg/kg, i.p.), and then CFA (0.05 ml 1:1 oil/saline suspension) was injected into the left side of the facial skin, as described previously [24–26]. For naïve rats, vehicle only (0.9% NaCl) was injected into the left side of the facial skin. Genistein was dissolved in dimethyl sulfoxide (DMSO) and administered chronically to the rats over three days (injection time: 13:00). Behavioral experiments (20 min) were conducted immediately prior to genistein administration daily. Based on the behavioral analysis for escape threshold, electrophysiological experiments were conducted only on day 2 in inflamed-group animals (recording time: 13:00–17:00). In some experiments, we also tested systemic administration of vehicle (DMSO) on day 2 in inflamed-group animals.

2.2. Mechanical threshold for escape behavior

Mechanical threshold for escape behavior was conducted as described in previous studies [24–26]. In brief, from one to three days after CFA or vehicle injection into the facial skin, the ipsilateral and contralateral skin regions were tested to assess mechanical hyperalgesia using a set of von Frey hairs (Semmes-Weinstein Monofilaments, North Coast Medical, CA). To evaluate the rat's escape threshold, the von Frey mechanical stimuli were applied to the whisker pad in ascending series of trials. Each von Frey stimulation was applied three times in each series of trials. Escape threshold intensity was determined when rats moved their heads away from at least one of three stimuli.

2.3. Extracellular single-unit recording of SpVc WDR neuronal activity

Electrophysiological recordings were conducted two days after CFA or vehicle injection as described in previous studies [24–26]. Each animal was first anesthetized with pentobarbital sodium (45 mg/kg, i.p.) and maintained with additional doses of 2–3 mg/kg/h through a cannula in the jugular vein, as necessary. Single-neuron activity was recorded through a glass micropipette filled with 2% pontamine sky blue and 0.5 M sodium acetate, and recording location was determined by stereotaxic coordinates. Neuronal activity was amplified (WPI, DAM 80), filtered (0.3–10 KHz), and monitored with an oscilloscope (Iwatsu, SS-7672, Tokyo) for off-line analysis by Power Lab and Chart 5 software (ADI Instruments, UK).

2.4. Experimental protocols

Recordings of the extracellular SpVc WDR unit activity were carried out as follows. Mechanical stimulation (with a paint brush) was used as a search stimulus to quickly identify receptive fields and to avoid sensitizing the peripheral receptors. The single unit that responded to left side of whisker pad were search for with the brush and a set of von Frey hairs. Noxious pinch stimulation was applied to the whisker pad with calibrated forceps (5s) that evoked a pain sensation when applied to a human subject. After identification of SpVc WDR neurons responding in the whisker pad, we determined whether there was a spontaneous discharge by comparing the discharge rates induced by mechanical stimulation in naïve and inflamed rats. The threshold for mechanical stimulation was determined by using non-noxious (0.07, 0.16, 0.2, 1, 4, 6, and 10 g) and noxious (15, 26, and 60 g) mechanical stimulation (duration: 5s) using von Frey hairs for intervals of 5 s. The mechanical receptive fields of neurons were mapped by probing the facial skin with von Frey hairs, and a peristimulus histogram (bin = 100 ms) was generated in response to each stimulus. Recording discharges were recorded for 10 s after pinching the receptive fields, and then the mean spontaneous, mechanical stimulation-evoked discharges frequencies, after-discharge frequencies, and mean mechanical thresholds of SpVc WDR neurons were compared among the three groups (naïve, CFA, and CFA rat with genistein treatment).

2.5. Identification of recording site

At the end of recording sessions, anodal DC currents (30 μA, 3 min) were passed through a recording micropipette before the animals were transcardially perfused with saline and 10% formalin. Frozen coronal sections were cut into 30-μm sections and stained with hematoxylin-eosin. Recording sites were identified as blue spots.

2.6. Data analysis

Values are expressed as means ± SEM. Statistical analysis was performed using one-way repeated measure analysis of variances (ANOVAs) followed by the Tukey-Kramer tests (*post hoc* test) for behavioral and electrophysiological data. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Inflammation-induced hyperalgesia

To test for hyperalgesia, the animals received a CFA injection followed by mechanical stimulation of the injected site and/or the orofacial skin using von Frey filaments. The inflamed rats showed a significantly reduced threshold for escape from stimulation compared with control animals at 1 day and at 3 days after CFA injection with mechanical stimulation of the whisker pad area (Fig. 1, n = 8, $P < 0.05$). In the contralateral whisker pad, no significant changes in threshold were observed between the two groups (naïve vs. inflamed; 56.9 ± 3.8 g vs. 58.2 ± 5.2 g, n = 8).

3.2. Chronic administration of genistein for hyperalgesia

Genistein administration (200 mM, i.p) also reduced escape thresholds from mechanical stimulation in inflamed rats compared to naïve rats (Fig. 1). The effect was not significant at day 1 after administration, but the thresholds were significantly returned to control levels by chronic administration of 200 mM genistein after 2 and 3 days of inflammation (n = 10, $p < 0.05$). Conversely, administration of low-dose genistein (100 mM) did not significantly reduce escape thresholds from mechanical stimulation back control levels in day-2 inflamed rats (n = 4, NS). Thus, the effect of genistein on escape threshold from mechanical stimulation was concentration-dependent in inflamed rats. Vehicle administration had no significant effect on the escape threshold in day-2 inflamed rats (data not shown).

3.3. Changes in excitability of SpVc WDR neurons following inflammation

The response of 26 SpVc WDR neurons to mechanical stimulation of the whisker pad was analyzed in naïve, CFA, and CFA rats with genistein administration. These SpVc neurons responding to non-noxious and noxious mechanical stimulation exhibited a somatic receptive field in the whisker pad (Fig. 2A). The recording sites were typically distributed in the maxillary branch (Fig. 2B), with no obvious difference across recording sites among the three groups. As shown in Fig. 2C and D, every neuron recorded was of the WDR category, as graded mechanical stimulations of the most sensitive receptive field area showed increased firing frequency of SpVc neurons proportional to stimulus intensity. The mechanical stimulus-response curve of SpVc WDR neurons is shown in Fig. 2D. Naïve rats showed spontaneous discharges in 12.5% (1/8) of SpVc neurons (Fig. 3A), whereas all WDR neurons (8/8; 1.2 ± 0.4 Hz) were spontaneously active in inflamed rats (Fig. 3A). In inflamed rats, SpVc WDR neurons also showed significantly stronger responses to non-noxious mechanical stimulation compared with naive rats (Fig. 3A), significantly greater mean firing frequencies in response to mechanical stimuli (n = 8, $P < 0.05$; Fig. 3B), and significantly decreased mean mechanical thresholds (n = 8, $P < 0.05$; Fig. 3C). The mean spontaneous discharge frequency in inflamed rats was also significantly increased compared to that in naïve rats (Fig. 3D), as was the mean receptive size (n = 8, $P < 0.05$; Fig. 3E). Most of the SpVc neurons in inflamed rats (4/8: 50%) showed after-discharges following noxious pinch stimulation (Fig. 3F) compared to no after-discharges in naïve rats.

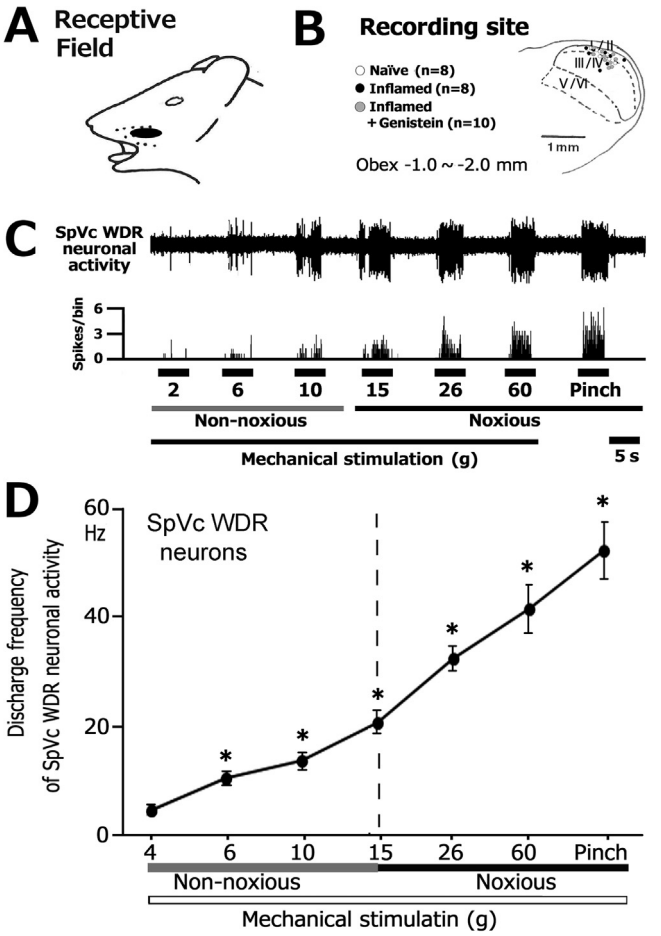

Fig. 2. General characteristics of spinal trigeminal nucleus caudalis (SpVc) wide-dynamic range (WDR) neuronal activity in orofacial skin. **(A)** Receptive field of whisker pad in the facial skin. **(B)** Distribution of SpVc WDR neurons responding to non-noxious and noxious mechanical stimulation of facial skin (n = 26). The number below each drawing indicates the frontal plane in relation to the obex. **(C)** Example of non-noxious- and noxious mechanical stimulation-induced firing of SpVc WDR neurons. **(D)** Stimulus-response curve of SpVc WDR neurons (n = 8). * represents $P < 0.05$–4 g vs. 6 g, 10 g, 15 g, 26 g, 60 g, and pinch.

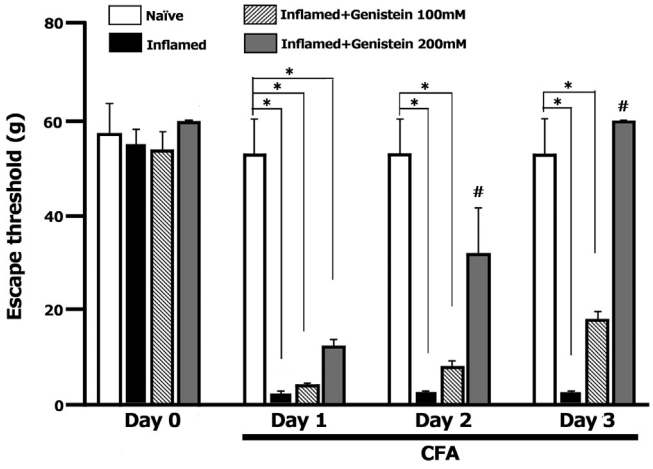

Fig. 1. Comparison of changes in the escape threshold among naïve, inflamed, and inflamed with genistein rats. Mechanical stimulation using von Frey hairs was applied to the ipsilateral whisker pad of naïve (saline) (n = 8), complete Freund's adjuvant (CFA)-inflamed (n = 8), and CFA-inflamed with genistein (100 mM; n = 4, 200 mM; n = 10, i.p) rats to assess hyperalgesia. Data are mean ± SEM; # represents $P < 0.05$ - Inflamed vs. inflamed with genistein.

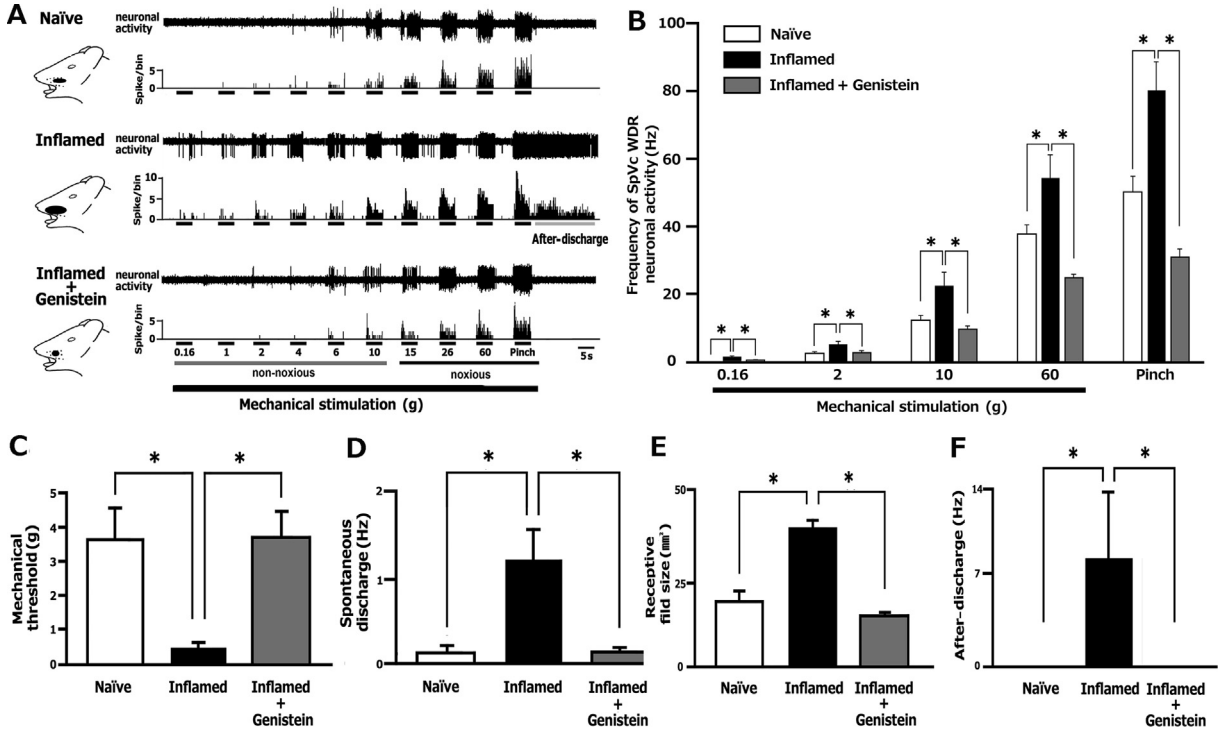

Fig. 3. Chronic genistein administration reverses the hyperactivity of SpVc WDR neuronal activity after orofacial CFA inflammation. **(A)** Example of non-noxious- and noxious mechanical stimulation-induced discharge of SpVc WDR neurons in naïve (n = 8), inflamed (n = 8), and inflamed with genistein administration (200 mM, n = 10, i.p.) rats for 2 days. Note that the decreased mechanical stimulation threshold required to evoke neuronal firing, increased spontaneous discharges, increased size of receptive field, and occurrence of noxious pinch-evoked discharges in the inflamed rats were reversed to control levels following genistein administration. **(B)**: Comparison of mean discharge frequency of SpVc WDR neurons evoked by mechanical stimulation (non-noxious and noxious) of orofacial skin among the three rat groups. *represents $P < 0.05$ - naive vs. inflamed rats and inflamed vs. inflamed with genistein. * represents $P < 0.05$ -naive vs. inflamed rats and inflamed vs. inflamed with genistein. **(C)**: Comparison of mean mechanical threshold of SpVc WDR neurons among the three rat groups. * represents $P < 0.05$ - naive vs. inflamed rats and inflamed vs. inflamed with genistein. * represents $P < 0.05$ - naive vs. inflamed rats and inflamed vs. inflamed with genistein. **(D)** Comparison of mean spontaneous discharges of SpVc WDR neurons among the three rat groups. * represents $P < 0.05$ - naive vs. inflamed rats and inflamed vs. inflamed with genistein. **(E)** Comparison of mean noxious pinch-evoked after-discharge frequency of SpVc WDR neurons among the three rat groups. * represents $P < 0.05$ - naive vs. inflamed rats and inflamed vs. inflamed with genistein. **(F)** Comparison of mean receptive field size of SpVc WDR neurons among the three rat groups. * represents $P < 0.05$ - naive vs. inflamed rats and inflamed vs. inflamed with genistein.

3.4. Chronic administration of genistein inhibits hyperexcitability of SpVc WDR neurons in inflamed rats

Based on our behavioral analysis for escape threshold, we next tested how chronic administration of genistein (200 mM, i.p.) affected the hyperexcitability of SpVc WDR neurons in day-2 inflamed rats. Fig. 3A represents a typical example of the discharge rates of SpVc WDR neurons responding to non-noxious and noxious mechanical stimulation in inflamed rats. Genistein administered over 2 days decreased the discharge frequency of SpVc WDR neurons to control levels following both non-noxious and noxious mechanical stimulation, while the reduced mechanical threshold and augmented spontaneous, noxious, and non-noxious firing frequencies were returned to the levels of naïve rats ($P < 0.05$; Fig. 3A and B). In inflamed rats, genistein also significantly reversed the mean threshold to mechanical stimulation to control levels in inflamed rats (Fig. 3C), as well as significantly reversed the SpVc WDR neuronal spontaneous discharges in inflamed rats (Fig. 3D, $P < 0.05$). Genistein administration also decreased the noxious pinch-evoked after-discharge frequency (inflamed vs. inflamed with genistein: 6/8; 75% vs. 2/10; 20%). Additionally, both the mean size of the receptive field and noxious pinch-evoked after-discharge frequency in inflamed rats were significantly returned to control levels ($P < 0.05$; Fig. 3E and F). Chronic vehicle administration had no significant effect on spontaneous, non-noxious-, noxious mechanical-, or pinch stimulation-evoked hyperexcitability of SpVc WDR neurons in inflamed rats (data not shown).

4. Discussion

The present behavioral study showed the following: *(i)* a significantly lower threshold of escape from mechanical stimulation applied orofacially in inflamed rats compared to naïve rats, as reported previously [24–26]; *(ii)* a dose-dependent reversal of the reduced mechanical threshold to control levels in inflamed rats on days 2 and 3 of chronic genistein administration; and *(iii)* vehicle administration had no significant effect on the escape threshold in day-2 inflamed rats. Together, these findings confirm that genistein treatment suppresses inflammation-induced hyperalgesia, possibly via previously described mechanisms [24–26]. In this study, the higher dose of genistein significantly attenuated hyperalgesia after only 2 days of inflammation, in agreement with previous findings of a significantly decreased CFA inflammation-induced mechanical hyperalgesia after application of a soy diet in a rat neuropathic pain model [28]. Although the precise mechanism underlying the effects of genistein on inflammation-induced hyperalgesia remains unknown, several possibilities exist. Genistein decreases the production of PGE_2 by inhibiting Cox-2 cascades [13,14], and in a previous study, injection of CFA into the whisker pads significantly increased the mean number of Cox-2-immunoreactive cells in inflamed rats compared with naïve rats. However, in inflamed rats administered the dietary constituent, lutein, Cox-2 immunoreactivity of the whisker pads reached control levels by day 3 [26]. Together, these observations support that daily genistein use reduces inflammation-induced

hyperalgesia in whisker pads via Cox-2 suppression resulting in inhibition of PGE_2 production.

In vitro, genistein inhibited action potentials in capsaicin-sensitive nociceptive trigeminal ganglion neurons via lowered voltage-dependent tetrodotoxin-resistant (TTX-R) and sensitive (TTX-S) Na^+ and K^+ currents [7]. TTX-R Na^+ channels are selectively expressed in small- and medium-sized dorsal root ganglion neurons, such as nociceptive neurons [29,30]. In addition, inflammation upregulated voltage-dependent Na^+ channels [31,32] and downregulated K^+ channels [33,34] in primary sensory ganglion neurons. In this study, the high-dose systemic administration of genistein reversed the decreased mean mechanical stimulation threshold in inflamed rats, while both the non-noxious- and noxious mechanical stimuli-evoked mean discharge frequency of SpVc WDR neurons was returned to control levels in inflamed rats after genistein treatment. These findings indicated that systemic genistein may modulate inflammation-induced peripheral sensitization and SpVc WDR neuronal hypersensitivity in peripheral nerve terminals, as suggested by previous *in vitro* findings in trigeminal neurons [7].

Conversely, genistein directly inhibits central glutaminergic synaptic transmission and tyrosine kinase signaling via NMDA mechanisms [10]. In addition, sensitizing peripheral nociceptors can trigger central sensitization, and genistein can modulate NR2B phosphorylation following tissue inflammation [11,12]. Further, a recent study in chronic migraine model rats implicated tyrosine phosphorylation of NR2B in central sensitization mechanisms [35], specifically via protection against threshold dysfunction and migraine attack through regulating synaptic plasticity. It is therefore likely that the central antinociceptive effect of systemic genistein occurred via suppression of the tyrosine kinase-dependent phosphorylation of NMDA receptor subunits, specifically NR2B, in addition to effects on postsynaptic mechanisms.

Moreover, this study showed that genistein reversed the increase in mean spontaneous discharge frequency of SpVc WDR neurons following inflammation, thus complementing recent findings of ongoing activity of WDR neurons in the SpVc being restricted from the periphery [36]. Together, these findings suggest that genistein attenuates spontaneous discharge activity in SpVc WDR neurons that innervate the facial skin due to trigeminal ganglion sensitization [37], and that via this mechanism, genistein is likely to modulate spontaneous pain. Previous studies reported after-discharge in SpVc WDR neurons undergoing noxious mechanical stimulation in a chronic inflammation model and associated these changes with neuronal sensitization during persistent pain [24–26,38,39]; this data supports the potential importance of after-discharges for central sensitization. Herein, we found that genistein abolished after-discharges following noxious pinch stimulation in inflamed rats, although the precise mechanism underlying this effect remains unclear. In addition, administration of a substance P (SP) neurokinin-1 (NK_1) receptor antagonist inhibits pinch-evoked after-discharges in WDR neurons of the spinal cord [40]. Thus, because genistein can also dose-dependently inhibit SP-induced responses [41], chronic administration of genistein might attenuate the NK_1 receptor-mediated after-discharges of WDR neurons in the SpVc under inflammatory conditions. We previously suggested that a local GABAergic mechanism could control nociceptive transmission in SpVc neurons, thus impacting the overall properties of mechanical receptive fields [22]. In the present study, the expanded receptive field size in inflamed rats was returned to control levels after genistein administration, although the mechanisms underlying this effect remain unclear. It is possible that genistein modulates local GABAergic tonic control of nociceptive mechanoreceptive transmission and inhibits central mechanisms through excitatory synaptic transmission. Further studies are warranted to investigate this possibility.

Recently reports implicate dietary constituents such as resveratrol, docosahexaenoic acid, and lutein in attenuating CFA-induced inflammatory mechanical hyperalgesia and associated nociceptive neuronal hyperexcitability, possibly through Cox-2 cascade inhibition [24–26]. The toxic side effects associated with most commonly prescribed analgesic drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs), Cox-2 inhibitors, and opioids, have increased interest in CAM agents for the treatment of persistent chronic pain [2,42]. Thus, further studies need to examine the potential effects of dietary components, including supplements, on conditions associated with pain [3,43]. In the current study, systemic administration of dietary genistein attenuated inflammation-induced hyperexcitability of trigeminal SpVc WDR neurons associated with mechanical hyperalgesia in rats. These results therefore contribute to the development of analgesic drugs with fewer side effects for the treatment of pathological pain, including orofacial pain.

5. Conclusion

Herein, we provide the first evidence that genistein attenuates the hyperexcitability of SpVc WDR neurons associated with inflammatory mechanical hyperalgesia. These findings support genistein as a potential CAM therapeutic agent for the prevention of trigeminal inflammatory hyperalgesia.

Ethical statement

The experiments were approved by the Animal Use and Care Committee of Azabu University and complied with the ethical guidelines of the International Association for the Study of Pain [27]. Each experiment was performed such that the experimenter was blind to the conditions. Every effort was made to minimize the number of animals used and their suffering, as described in our previous study.

Conflicts of interest

The authors declare no conflicts of interest.

CRediT authorship contribution statement

Shuho Arakawa: Investigation, Formal analysis. **Mogami Inoue:** Investigation, Formal analysis. **Rina Kinouchi:** Data curation, Formal analysis. **Shiori Morizumi:** Data curation, Formal analysis. **Manaka Yamaguchi:** Data curation, Formal analysis. **Yoshihito Shimazu:** Formal analysis, Methodology. **Mamoru Takeda:** Supervision, Project administration, Methodology, Writing - original draft.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.job.2019.08.003.

References

[1] Konvicka JJ, Meyer TA, McDavid AJ, Roberson CR. Complementary/alternative medicine use among chronic pain clinic patients. J. Perianesth Nurs. 2008;23:17–23.

[2] Rosenberg EI, Genao I, Chen I, Mechaber AJ, Wood JA, Faselius CJ. Complementary and alternative medicine use by primary care patients with chronic pain. Pain Med. 2008;9:1065–72.

[3] Tall JM, Raja SN. Dietary constituents as novel therapies for pain. Clin. J. Pain 2004;20:19–26.

[4] Kuzer MS, Xu X. Dietary phytoestrogens. Annu. Rev. Nutr. 1997;17:353–81.

[5] Li J, Gang D, Yu X, Hu Y, Yue Y, Cheng W, Pan X, Zhnag P. Genistein: the potential for efficacy in rheumatinoid arthritis. Clin. Rheumatol. 2013;32:535–40.

[6] Paillart C, Carlier E, Guedin D, Dargaent B, Couraud F. Direct block of voltage-sensitive sodium channels by genistein, A tyrosine kinase blocker. J. Pharmacol. Exp. Ther. 1997;280:521–6.

[7] Liu L, Yang T, Simon SA. The protein tyrosine kinase inhibitor, genistein, decreases excitability of nociceptive neurons. Pain 2004;112:131–41.

[8] Jia Z, Jia Y, Liu B, Zhao Z, Jia Q, Liang H, Zhang H. Genistein inhibits voltage-gated sodium currents in SGC neurons through protein kinase-dependent and kinase-independent mechanisms. Pflüg. Arch. 2008;456:857–66.

[9] Belevych AE, Warrier S, Harvey RD. Genistein inhibits cardiac L-type Ca^{2+} channel activity by a tyrosine kinase-independent mechanism. Mol. Pharmacol. 2002;62:554–65.

[10] Huang R, Singh M, Dillon GH. Genistein directly inhibits native and recombinant NMDA receptors. Neuropharmacology 2010;58:1246–51.

[11] Guo W, Zou S, Guan Y, Ikeda T, Tal M, Dubner R, Ren K. Tyrosine phosphorylation of the NR2B subunit of the NMDA receptor in the spinal cord during the development and maintenance of inflammatory hyperalgesia. J. Neurosci. 2002;15:6208–17.

[12] Luo Y, Wang SX, Zhou ZQ, Wang Z, Zhang YG, Zhang Y, Zhao P. Apoptotic effect of genistein on human colon cancer cells via inhibiting the nuclear factor-kappa B (NF-κB) pathway. Tumour Biol 2014;35:11483–8.

[13] Ye F, Dum WT, Yi J, Tong X, Zhang D. Inhibition of cyclooxygenase-2 activity in head and neck cancer cells by genistein. Cancer Lett. 2004;211:39–46.

[14] Swami S, Krishnan AV, Moreno J, Bhattacharyya RB, Peehl DM, Feldman D. Calciriol and genistein action to inhibit the prostaglandin pathway: potential combination therapy to treat prostate cancer. J. Nutr. 2007;205S-10S.

[15] Iwata K, Takeda M, Oh S, Shinoda M. Neurophysiology of orofacial pain. In: Farah C, Balasubramaniam R, McCullough M, editors. Contemporary Oral Medicine. Cham: Springer; 2017. p. 1–23.

[16] Takeda M, Takahashi M, Mastumoto S. Suppression of neurokinin-1 receptor in trigeminal ganglia attenuates central sensitization following inflammation. J. Peripher. Nerv. Syst. 2012;17:69–81.

[17] Scholz J, Woolf CJ. Can we conquer pain? Nat. Neurosci. 2002;5(Suppl):1062–7.

[18] Millan MJ. The induction of pain: an integrative review. Prog. Neurobiol. 1999;57:1–164.

[19] Imbe H, Iwata K, Zhou Q-Q, Zou S, Dubner R, Ren K. Orofacial deep and cutaneous tissue inflammation and trigeminal neuronal activation. Cells Tissues Organs 2001;169:238–47.

[20] Nakajima R, Uehara A, Takehana S, Akama Y, Shimazu Y, Takeda M. Decanoic acid attenuates the excitability of nociceptive trigeminal primary and secondary neuron associated with hypoalgesia. J. Pain Res. 2018;II:2867–76.

[21] Iwata K, Tashiro A, Tsuboi Y, Imai T, Sumino R, Morimoto T, Dubner R, Ren K. Medullary dorsal horn neuronal activity in rats with persistent temporomandibular joint and perioral inflammation. J. Neurophysiol. 1999;82:1244–53.

[22] Takeda M, Tanimoto T, Matsumoto S. Changes in mechanical receptive field properties induced by $GABA_A$ receptor activation in the trigeminal spinal nucleus caudalis neurons in rats. Exp. Brain Res. 2000;134:409–16.

[23] Takeda M, Tanimoto T, Ikeda M, Nasu M, Kadoi J, Shima Y, Matsumoto S. Temporomandibular joint inflammation potentiates the excitability of trigeminal root ganglion neurons innervating the facial skin in rats. J. Neurophysiol. 2005;93:2723–38.

[24] Sekiguchi K, Takehana S, Shibuya E, Matsuzawa N, Hidaka S, Kanai Y, Inoue M, Kubota Y, Shimazu Y, Takeda M. Resveratrol attenuates inflammation induced hyperexcitability of trigeminal spinal nucleus caudalis neurons associated with hyperalgesia in rats. Mol. Pain 2016;12. 1744806916643082.

[25] Nakazaki S, Tadokoro K, Takehana S, Syoji Y, Shimazu Y, Takeda M. Docosahexaenoic acid attenuates inflammation-induced hyperexcitability of trigeminal spinal nucleus caudalis neurons associated with hyperalgesia in rats. Eur. J. Oral Sci. 2018;126:458–65.

[26] Syoji Y, Kobayashi R, Miyamura N, Hirohara T, Kubota Y, Uotsu N, Yui K, Shimazu Y, Takeda M. Suppresssion of hyperexcitability of trigeminal nociceptive neurons associated with inflammatory hyperalgesia following systemic administration of lutein via inhibition of cyclooxygenase-2 cascade signaling. J. Inflamm. 2018;15:24.

[27] Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–10.

[28] Borzan J, Tall JM, Zhaao C, Meyer R, Gaja SN. Effect of soy diet on inflammation-induced primary and secondary hyperalgesia in rats. Eur. J. Pain 2010;14:792–8.

[29] Takeda M, Matsumoto S, Sessle BJ, Shinoda M, Iwata K. Peripheral and central mechanisms of trigeminal neuropathic and inflammatory pain. J. Oral Biosci. 2011;53:318–29.

[30] Akopian AN, Sivilotti L, Wood JN. A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons. Nature 1996;379:257–62.

[31] Stewarts T, Beyak MJ, Vanner S. Ileitis nodulates potassium and sodium currents in Guinea pig dorsal root ganglia sensory neurons. J. Physiol. (Lond.) 2003;523:797–807.

[32] Villarreal CF, Sachs D, Funez MI, Pareade CA, de Queiroz Cunha F, Ferreira SH. The peripheral pro-nociceptive state induced by repetitive inflammatory stimuli involves continuous activation of protein kinase A and protein kinase C epsilon and its Na(v) 1.8 sodium channel functional regulation in primary sensory neurons. Biochem. Pharmacol. 2009;77:867–77.

[33] Yoshimura N, de Grout WC. Increased excitability of afferent neurons innervating rat urinary bladder inflammation. J. Neurosci. 1999;19:4644–53.

[34] Takeda M, Tanimoto T, Ikeda M, Nasu M, Kadoi J, Yoshida S, Matsumoto S. Enhanced excitability of rat trigeminal ganglion neurons via decrease in A-type potassium currents following temporomandibular joint inflammation. Neuroscience 2006;136:621–30.

[35] Wang X-Y, Zhou H-R, Wang S, Liu C-Y, Qin G-C, Fu Q-Q, Zhou J-Y, Chen L-X. NR2B-Tyr phosphorylation regulates synaptic plasticity in central sensitization in a chronic migraine rat model. J. Headache Pain 2018;19:102.

[36] Roch M, Messlinger K, Kulchitsky V, Tichonovich O, Azev O, Koulchitsky S. Ongoing activity in trigeminal wide-dynamic range neurons is driven from the periphery. Neuroscience 2007;150:681–91.

[37] Takeda M, Takahashi M, Matsumoto S. Contribution of the activation of satellite glia in sensory ganglia to pathological pain. Neurosci. Biobehav. Rev. 2009;33:784–92.

[38] Kitagawa J, Kanda K, Sugiura M, Tsuboi Y, Ogawa A, Shimizu K, Koyama N, Kamo H, Watanabe T, Ren K, Iwata K. Effect of chronic inflammation on dorsal horn nociceptive neurons in aged rats. J. Neurophysiol. 2005;93:3594–604.

[39] Tsuboi Y, Iwata K, Dostrovsky JO, Chiang CY, Sessle BJ, Hu J. Modulation of astroglial glutamine synthetase activity affects nociceptive behaviour and central sensitization of medullary dorsal horn nociceptive neurons in a rat model of chronic pulpitis. Eur. J. Neurosci. 2011;34:292–302.

[40] Radhakrishnan V, Henry JL. Antagonism of nociceptive responses of cat spinal dorsal horn neurons in vivo by the NK-1 receptor antagonists CP-96,345 and CP-99,994, but not by CP-96,344. Neuroscience 1995;64:943–58.

[41] Rigler M, Castagliuolo G, So PTC, Lotz M, Wang C, Wlk M, Sogukoglu T, Cosentini E, Bishof C, Hamilton G, Teleky B, Wenzl E, Matthews J, Porthoulakis C. Effects of substance P on human colonic mucosa in vitro. Am. J. Physiol. 1999;276:G1473–83.

[42] Kessler RC, Davis RB, Foster DF, Van, Rompay MI, Walters EE, Wilkey SA, Kaptchuk TJ, Eisenberg DM. Long-term trends in the use of complementary and alternative medical therapies in the United States. Ann. Intern. Med. 2001;135:262–8.

[43] ShirY Raja SN, Weissman CS, Campbell JN, Seltzer Z. Consumption of soy diet before nerve injury preempts the development of neuropathic pain in rats. Anesthesiology (Hagerst.) 2001;95:1238–44.