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Suppression of the Excitability of Rat Nociceptive Primary Sensory Neurons Following Local Administration of the Phytochemical, Quercetin

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Abstract: Although the modulatory effect of quercetin on voltage-gated Na, K, and Ca channels has been studied in vitro, the in vivo effect of quercetin on the excitability of nociceptive primary neurons remains to be determined. The aim of the present study was to examine whether acute local quercetin administration to rats attenuates the excitability of nociceptive trigeminal ganglion (TG) neurons in response to mechanical stimulation in vivo. Extracellular single unit recordings were made from TG neurons of anesthetized rats in response to orofacial non-noxious and noxious mechanical stimulation. The mean firing frequency of TG neurons in response to both non-noxious and noxious mechanical stimuli was dose-dependently inhibited by quercetin, and maximum inhibition of the discharge frequency of both non-noxious and noxious mechanical stimuli was seen within 10 min. The inhibitory effect of quercetin lasted for 15 minutes and was reversible. The mean magnitude of inhibition on TG neuronal discharge frequency with 10 mM quercetin was almost equal to that of the local anesthetic, 2% lidocaine. These results suggest that local injection of quercetin into the peripheral receptive field suppresses the excitability of nociceptive primary sensory neurons in the TG, possibly via inhibition of voltage-gated Na channels and opening voltage-gated K channels.

Perspective: Local administration of the phytochemical, quercetin, as a local anesthetic may provide relief from trigeminal nociceptive pain with smallest side effects, thus contributing to the area of complementary and alternative medicines.

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Key Words: Alternative medicine, extracellular single unit recording, lidocaine, quercetin, trigeminal pain.

Abbreviations: CAM, complementary and alternative medicine; Cav, voltage-gated calcium; Kv, voltage-gated potassium; Nav, voltage-gated sodium; SpVc, trigeminal spinal nucleus caudalis; TG, trigeminal ganglion; TMJ, temporomandibular joint; WDR, wide-dynamic range. Erα, Estrogen α: Erβ, Estrogen β, CYP450, cytochrome P450.

The sensory information of the orofacial area innervated by Aδ- and C-trigeminal ganglion (TG) neurons is sent to second-order neurons in the spinal trigeminal nucleus (SpV). The SpV is functionally subdivided into 3 nuclei from rostral to caudal; oralis, interpolaris, and SpV caudalis (SpVo).16,36,44 Two types of nociceptive neurons are known, nociceptive-specific and wide-dynamic range (WDR) neurons, found in both
primary and secondary neurons. Nociceptive-specific neurons only respond to noxious stimulation of receptive fields, and thus possibly send location-related information to higher centers. In contrast, WDR neurons respond to noxious and non-noxious stimulation. When graded nociceptive stimulation is applied to the most sensitive area of the receptive field, increased firing frequency is induced in proportion to stimulus intensity, suggesting that WDR neurons encode stimulus intensity.

Complementary and alternative medicine (CAM) therapies, such as herbal medicines and acupuncture, are often used in pain management, especially after the failure of conventional Western medicine or when adverse side effects are a concern. Previous studies reported that various dietary constituents can potentially affect protective biological mechanisms, such as those in the cardiovascular, neural and anticancer systems. Before dental operations, such as tooth extraction, local anesthetic agents are often applied to the patient. However, if blood levels rise too high during the dental operation, adverse side effects on the central nervous system (sleepiness, lightheadedness, and visual, and auditory disturbances) or cardiovascular system (ventricular arrhythmias and myocardial infarction) may occur. Therefore, recently, there has been increased interest in the use of CAM for the control of pain symptoms.

Recent studies have revealed that local administration of dietary constituents, such as resveratrol, isoflavone, and chlorogenic acid, attenuate the excitability of TG nociceptive secondary neurons in vivo. For example, subcutaneous injection of the natural polyphenolic compounds, resveratrol, and chlorogenic acid, into the receptive field of rats inhibits the excitability of SpVc WDR neurons via inhibition of voltage-gated sodium (Nav) channels and activation of voltage-gated potassium (Kv) channels in the nociceptive nerve terminals of TG neurons. Recently, we found that local injection of a half-dose of the isoflavone, genistein, replicated a half-dose of the local anesthetic, lidocaine. Therefore, administration of genistein as a local anesthetic may provide relief from trigeminal nociceptive pain without side effects, thus contributing to the use of CAM. However, observation of the local anesthetic effects of dietary constituents acting on nociception was based on changes in the excitability of nociceptive secondary neurons, and not primary afferent activity. Therefore, it still remains to be determined if local administration of such dietary constituents to rats attenuates the excitability of nociceptive primary neurons in response to mechanical stimulation in vivo.

Quercetin is one of the most common flavonoids found in the daily human diet. Flavonoid phytochemicals have a variety of biological functions, including antioxidant, anti-inflammatory, and cardioprotective properties. A modulatory role has been reported for quercetin on Nav, Kv, and voltage-gated calcium (Cav) channels in cardiac muscle. For example, in a rat coronary arterial smooth muscle in vitro preparation, application of quercetin induces vasorelaxation via enhancement of Kv channels and depression of Cav channels. Interestingly, using a whole-cell patch clamp technique in rat cardiac myocytes, Wallace et al. reported that the red grape polyphenols, quercetin, catechin, and resveratrol, inhibited Na currents and their half maximal inhibitory concentration was as follows: quercetin > catechin > resveratrol. Although the question of whether the application of quercetin inhibits Nav in sensory neurons remains to be answered, it can be assumed that quercetin acts as a more potent Nav channel blocker in nociceptive sensory neurons in vivo than the other polyphenols. Taken together, these findings suggest that under in vivo conditions, quercetin inhibits the firing of action potentials in nociceptive TG neurons evoked by a natural nociceptive stimulus via Nav channels. However, the acute effects of quercetin in vivo, particularly on nociceptive transmission in the trigeminal system, remain to be determined.

The aim of the present study was to examine whether acute local administration of quercetin to rats attenuates the excitability of primary sensory TG neurons, in response to mechanical stimulation in vivo. In addition, we also compared the potency of suppressing trigeminal nociception between quercetin and the conventional, clinically-used local anesthetic drug, the Nav channel blocker, lidocaine.

**Methods**

The experiments reported herein were approved by the Animal Use and Care Committee of Azabu University and were performed in accordance with the ethical guidelines of the International Association for the Study of Pain. Every effort was made to minimize the number of animals used and their suffering.

**Extracellular Single Unit Recording of TG Neuronal Activity**

Adult male Wistar rats (weighing 210–260 g) were maintained under fixed light condition (on 07:00 AM–07:00 PM). Room temperature was kept at 25±1°C. Food and water were given ad libitum. Electrophysiological recordings were made in 23 rats. Each rat was anesthetized with 3% isoflurane and a combination of anesthetic (0.3 mg/kg medetomidine, 4.0 mg/kg midazolam, 5.0 mg/kg butorphanol) and maintained with additional doses through a cannula in the jugular vein, as required. During the recording session, the anesthesia level was confirmed by the lack of response to paw pinching. The rectal temperature was maintained at 37.0±0.5°C with a homeothermic blanket during recording. All wound margins were continuously covered with a local anesthetic, 2% lidocaine (Xylocaine), throughout the experiments. The animals were placed in a stereotaxic apparatus and a partial craniotomy was performed 2 to 5 mm posterior to bregma and 1 to 4 mm lateral to the midline. An enamel-coated tungsten microelectrode (impedance = 3 MΩ) was advanced carefully through the cortex about 2.5 to 3.5 mm lateral to midline and 2.5 to 3.5 mm posterior to bregma (depth 8.1–9.9 mm) until an electrode tip reached the TG, as described previously. Then, the electrode was advanced or retracted in 10 μm steps using a micromanipulator. Single unit activity from the TG region was recorded extracellularly...
by means of the tungsten electrode according to the stereotaxic coordinates of Paxinos and Watson. Neuronal activity was amplified (DAM80; World Precision Instruments, Sarasota, FL), filtered (0.3–10 kHz), monitored with an oscilloscope (Iwatsu, SS-7672, Tokyo, Japan), and recorded for off-line analysis by Power lab and Chart 5 software (ADI Instruments, Oxford, UK), as described previously.18,37,47

**Experimental Protocols for Electrophysiological Recording**

Extracellular single-unit TG neuronal activity responding to mechanical stimulation of the whisker pad was recorded using the following protocol. To avoid sensitization of peripheral mechanoreceptors, a paint brush was quickly used as a search stimulus to identify the approximate area of receptive field in the left side of the whisker pad. Next, the left side of the whisker pad was searched for single units that responded to a set of von Frey hairs (Semmes-Weinstein Monofilaments, North Coast Medical, U.S.) with non-noxious (2, 4, 6, 8, 10 g) and noxious (15, 26, 60 g) mechanical stimulation for 5 seconds at intervals of 5 seconds.18,37,47

We have identified criteria for WDR neurons as follows: graded non-noxious and noxious mechanical stimulation applied to the receptive field produces increased firing frequency in proportion to stimulus intensity. After identification of nociceptive TG WDR neurons responding to the whisker pad, we determined the threshold for mechanical stimulation, and the size of the receptive field. The mechanical receptive field of neurons was mapped by probing the facial skin with von Frey hairs, and then outlined on a life-sized drawing of the rat on tracing paper.18,37,47

The TG neuronal discharges induced by mechanical stimulation were quantified by subtracting the background activity from the evoked activity. Spontaneous discharge frequencies were determined over 2 to 5 minutes. Poststimulus histograms (bin = 100 ms) were generated in response to each stimulus. The conduction velocity for each TG neuron was calculated by dividing the distance between the site of electrical stimulation and recording site by the latency between the stimulus artifact and evoked response. TG neuronal activity responding to electrical stimulation (0.3 ms, 1–3 mA, 1 Hz) of the orofacial skin was analyzed and TG neurons with conduction velocities between 2 and 13 m/s were classified as Aδ-fibers.39

The effects of subcutaneous quercetin (Sigma-Aldrich, Milano, Italy; 0.02 mL; 1 mM and 10 mM) and lidocaine (2% injection solution without epinephrine, Lidocaine HCL, 2-Diethylamino-N-(2,6-dimethylpheny) acetamide; MW = 280.1; pH 5.0–7.0; equivalent to 74 mM, 0.02 mL, Aspen Japan), administered through a Hamilton micro syringe, were evaluated 5, 10, 15, 20, 30, and 40 minutes after administration because the peak effect and recovery were thought to occur within this timeframe.

Quercetin was dissolved in 100% dimethyl sulfoxide to create a stock solution of 10 mM. The stock solution was stored at -20°C until use. The stock solution was diluted to the desired concentrations using saline immediately before use. Mean spontaneous and mechanical stimulation-induced discharge rates, and the mechanical threshold before and after subcutaneous administration of quercetin were analyzed in the present study.

**Data Analysis**

Values are expressed as the mean ± standard error. Statistical analysis was performed using 1-way repeated-measures analysis of variance followed by Tukey-Kramer or Dunnett’s tests as posthoc tests, or t-tests for electrophysiological data. Two-sided P < .05 was considered significant.

**Results**

**General Properties of TG Neurons Innervating the Facial Skin**

In this study, extracellular single-unit activity was recorded from 23 TG neurons. The effects of subcutaneous injections of quercetin were tested on 18 TG neurons, while 5 neurons were used to examine neuronal excitability after subcutaneous injection of the Nav channel blocker, 2% lidocaine. A typical example of the receptive field of TG neurons responding to non-noxious and noxious mechanical stimulation in the whisker pad is shown in Fig 1A.25,41 Fig 1B indicates that recording sites were mainly distributed in maxillary branches in the TG.25,41,44 There were no obvious differences in the location of the recording sites between quercetin and lidocaine-injected groups. Typical examples of TG neuronal unit responses are shown in Fig 1C. The nerve conduction velocities of some of the TG neurons were calculated and they were in the range of Aδ fibers (7.2±0.2 m/s, n = 4), as described previously.41,44 Graded mechanical stimulation was applied to the most sensitive area of the receptive field, which resulted in an increase in the firing frequency of SpVc WDR neurons in proportion to stimulus intensity. No TG neurons showed any spontaneous discharges. Typical examples of the action potential waveforms evoked by mechanical stimulation are shown in Fig 1C (inset). The mean mechanical stimulation-induced spike threshold was 3.2±1.8 g. Every neuron recorded belonged to the WDR category of neurons.25,41,44

**Effect of Local Quercetin Injection on the Excitability of TG Neurons in Response to Non-noxious and Noxious Stimuli**

Fig 2A shows a representative example of the effect of local subcutaneous injection of quercetin (1 mM) on the excitability of TG neurons in response to non-noxious mechanical stimulation. Five minutes after subcutaneous injection of 1 mM quercetin into the center of the
receptive field, non-noxious (2−10 g) mechanical stimulation-evoked TG neuronal activity was inhibited, with activity returning to control levels within approximately 15 minutes. No obvious changes in the mechanical threshold were observed after quercetin administration. The effects of quercetin on non-noxious mechanical stimulation-evoked TG neuronal activity are summarized in Fig 2B. Mean firing rates of non-noxious (6 and 10 g) mechanical stimulation-evoked TG neurons decreased significantly after quercetin injection compared with prior to injection (P < .05, n = 10), and returned to control levels within 15 minutes (P < .05, n = 10).

Fig 2 also shows representative examples of the effects of subcutaneous injection of 1 mM quercetin into the receptive field on the excitability of TG neurons in response to noxious mechanical stimulation. Noxious (15, 26, and 60 g) mechanical stimulation-evoked TG neuronal activity was inhibited 5 minutes after injection of quercetin, but neuronal activity returned to control levels within approximately 15 minutes (Fig 2A). As shown in Fig 2B, the mean firing rates of TG neurons evoked by noxious (15 g) mechanical stimulation decreased significantly after injection of quercetin compared with controls (Fig 2B, P < .05; n = 5). Local injection of vehicle (dimethyl sulfoxide) had no significant effect on non-noxious or noxious mechanical stimulation-evoked TG neuronal activity (n = 3, Fig 2A). Quercetin exhibited significant dose-dependent (1 and 10 mM) suppression of non-noxious (6 g) mechanical stimulation-evoked TG neuron firing (Fig 3; 1 mM vs 10 mM, P < .05, n = 12). Quercetin also exhibited significant dose-dependent (1 and 10 mM) suppression of noxious (26 g and 60 g) mechanical stimulation-evoked TG neuronal firing (Fig 3; 1 mM vs 10 mM, P < .05, n = 12).

**TG Neuronal Activity in Response to Noxious vs Non-noxious Stimuli After Quercetin Administration**

We compared the relative inhibitory effect of a 10 mM subcutaneous injection of quercetin on responses to non-noxious and noxious stimuli. As shown in Fig 4, no significant difference between non-noxious and noxious stimuli-induced mean magnitude of inhibition discharge frequency by quercetin was observed (n = 5).
Comparison of the Effects of Quercetin and Lidocaine on Mechanical Stimulus-induced TG Neuronal Activity

Finally, the magnitude of the inhibition of noxious stimulation-induced TG neuronal excitability by quercetin and lidocaine was compared. Representative examples of the effects of 2% lidocaine (74 mM; injected subcutaneously into the center of the receptive field) on the excitability of TG neurons in response to non-noxious and noxious mechanical stimulation are shown in Fig 5A. The response of TG neurons to non-
noxious and noxious mechanical stimulation was inhibited 5 to 10 minutes after lidocaine injection, with responses returning to control levels within 40 minutes (Fig 5). The effects of lidocaine injection on non-noxious and noxious mechanical stimulation-evoked TG neuronal activity are summarized in Fig 5B. The mean firing rates of TG neurons evoked by non-noxious (10 g) and noxious (15, 26, and 60 g) mechanical stimulation decreased significantly after injection of quercetin compared with controls (Fig 5B, \( P < .05; n = 5 \)) and returned to control levels within 40 minutes (\( P < .05, n = 5 \)). The mean magnitude of inhibition of TG nociceptive transmission was no different between 10 mM quercetin and 74 mM lidocaine, with the mean magnitudes of inhibition being almost equal (10 mM quercetin vs 74 mM lidocaine: non-noxious, 74.1±7.3% vs 48.6±21.5%, \( n = 3, \text{NS} \); noxious, 63.3±8.4% vs 67.7±14.5%, \( n = 3, \text{NS} \)).

**Discussion**

The present study provides the first evidence that in vivo local injection of quercetin into the peripheral receptive field suppresses the excitability of nociceptive primary sensory neurons in the TG, possibly via inhibition of voltage-gated Na channels and opening voltage-gated K channels in the nociceptive nerve terminals. Therefore, administration of the phytochemical, quercetin, as a local anesthetic may provide relief from trigeminal nociceptive pain with smallest side effects, thus contributing to the use of CAM.

**Local Application of Quercetin Suppresses the Excitability of Nociceptive Primary TG Neurons**

We recently demonstrated that local administration of some types of dietary constituents attenuate the excitability of trigeminal nociceptive secondary neurons in vivo.\(^6\,^1,^7,^47\) However, these findings were based on nociceptive secondary neuronal activity, and not nociceptive primary afferent activity. Therefore, the aim of the present study was to investigate whether local administration of quercetin to rats attenuates the excitability of TG neurons in response to nociceptive and non-nociceptive mechanical stimulation in vivo, in the absence of inflammatory or neuropathic pain.

The main findings of the present study are as follows: 1) the mean firing rate of TG neurons in response to both non-noxious and noxious mechanical stimuli was dose-dependently reduced by local injection of quercetin (1−10 mM); 2) quercetin inhibition of the discharge frequency in response to both non-noxious and noxious mechanical stimuli was reversible (within 15−20 minutes); and 3) local injection of vehicle had no significant effect on non-noxious or noxious mechanical stimulation-evoked TG neuronal activity. These findings are in agreement with a previous report that found, under in vitro conditions, 0.1 mM quercetin inhibited Nav currents in rat cardiac myocytes.\(^46\) In this study we examined local application of quercetin on the firing rates of extracellular action potentials in the primary sensory TG neurons in response to both noxious and non-noxious stimulation. Our findings, therefore, provide the first evidence that local injection of quercetin into the peripheral receptive field suppresses excitability of primary sensory neurons, possibly via inhibition of voltage-gated Na channels and opening voltage-gated K channels in the nociceptive nerve terminals of TG. However, further confirmational studies, such as in vitro patch-clamp studies of dissociated TG neurons, are needed.\(^21\)

**Peripheral Mechanism Underlies Suppression of TG Neuronal Excitability by Local Administration of Quercetin**

It is well known that the mechanism of nociceptive sensory signaling depends on the following 4 general processes: 1) transduction, whereby the peripheral terminal transduces external stimuli; 2) generation, initiation of action potentials; 3) propagation, via the axon
that conducts action potentials; and 4) transmission, whereby the central terminal forms the presynaptic elements of the first synapse in the sensory pathways of the central nervous system.13,42

Our previous studies18,37,47 on the effect of the local administration of dietary constituents on the excitability of SpVc WDR neurons in vivo have shown: 1) resveratrol and chlorogenic acid inhibit nociceptive neuronal excitability via the modulation of generator potentials, such as with transient receptor potential ankyrin 1 and acid sensing ion channel 2 (ASIC2) (candidates for mechanoreceptors);18,37 2) resveratrol, chlorogenic acid, and isoflavone inhibit action potentials in nociceptive neurons via the suppression of Nav and potentiation of Kv channels;18,37,47 and 3) the relative local analgesic drug potency of the inhibitory effect on the excitability of nociceptive neuronal activity is: resveratrol (10 mM) = chlorogenic acid (10 mM) = isoflavone (10 mM) > 1% lidocaine (37 mM).18,37,47

In the present study, we observed that in vivo local injection of quercetin into the peripheral receptive field suppresses the excitability of nociceptive primary sensory TG neurons. Since quercetin inhibits ASIC currents in the central vestibular neurons,24 it can be assumed that local administration of quercetin works through ASIC channels as candidates for mechanoreceptors in the nerve terminal of TG neurons.11,22 Therefore, local administration of quercetin directly inhibits generator potentials, and subsequently, suppresses the action potential firings of the nociceptive TG neurons.

The Nav channels conducting action potentials for nociception are generally divided into 2 types. Tetrodotoxin-resistant Nav channels appear to be selectively expressed in nociceptive (small- and medium-sized) dorsal root ganglion neurons, corresponding to Aδ/C primary afferent TG neurons, while tetrodotoxin-sensitive Nav channels are expressed in Aβ/Aδ-(large-/medium-sized) sensory neurons.2,39 Our present study showed a reduction in discharge frequency in response to both non-noxious and noxious mechanical stimuli by quercetin. The mean magnitude of inhibition by quercetin on the TG neuronal discharge frequency was not significantly

![Figure 5. Effects of subcutaneous administration of 2% lidocaine (74 mM) into the peripheral receptive field on the response of TG neurons to non-noxious and noxious mechanical stimulation. (A) Typical examples of TG neuronal activity in response to non-noxious (2, 4, 6, 8, and 10 g) and noxious (15, 26, and 60 g) mechanical stimulation before, 10 minutes and 40 minutes after 2% lidocaine administration (74 mM). Receptive field of whisker pad in the facial skin. Blackened area indicates the location and size of the receptive field. (B) Time-course of local administration of lidocaine into the peripheral receptive field on the mean firing frequency of TG neurons responding to non-noxious and noxious mechanical stimulation. *P < .05, before vs 10 minutes after lidocaine administration (n = 5). *P < .05, 10 minutes after lidocaine vs 40 minutes after lidocaine administration (n = 5).]
greater for noxious compared to non-noxious stimuli. Some of TG neurons in this study had nerve conduction velocities belonging to the Aβ range. Taken together, local injection of quercetin possibly suppresses the excitability of TG neurons via both inhibition of tetrodotoxin-resistant and -sensitive Nav channels in the nociceptive nerve terminals.

Kv channels are also involved in several important functions in the nervous system, including setting the resting membrane potential, setting the action potential shape, neuronal repolarization, and neurotransmitter release via slow-inactivating sustained (K-current) and fast-inactivating transient (A-current) channels. We have previously observed that a reduction in fast-inactivating transient (A-current) channel density, but not dominant slow-inactivating sustained (K-current) channel density, contributes to the increased excitability of small-diameter TG neurons in intact rats. In fact, applying an A-type Kv channel blocker to TG neurons in vivo also enhances Aβ/C-TG neuronal activity innervating the temporomandibular joint (TMJ) region in intact rats. Therefore, an A-type Kv channel in Aβ/C-TG neurons innervating the TMJ plays an important role in trigeminal inflammatory pain in TMJ disorders. Hou et al have reported that application of quercetin induced vasorelaxation via enhancement of Kv channels and depression of Cav channels in smooth muscle. Similarly, Granados-Soto et al showed in an in vivo rat formalin test that dietary constituents, such as resveratrol, induced peripheral antinociception via opening of several Kv channels. Since the opening of Kv channels leads to hyperpolarization of resting membrane potentials, and in turn to decreased cell excitability, several types of Kv channels have been proposed as target candidates for therapeutic approaches to pain. Taken together, these findings suggest that local quercetin injection into the peripheral receptive field suppresses the excitability of TG neurons responding to noxious mechanical stimulation, possibly via the activation of Kv channels in the nociceptive nerve terminal of TG neurons. However, further in vitro study is needed to explore this possibility.

It has been demonstrated that T-type Cav channels are dominantly expressed in the small- and medium-diameter sensory neurons. These neurons roughly belong to unmyelinated C- and myelinated Aβ-neurons, respectively. Todorovic and Todorovic, found that the amplitude of T-type Ca2+ currents was increased, causing a reduction in the excitability threshold and consequently an increase in the probability of burst-firing of neurons. Recent study indicated that quercetin related molecules such as flavonoid gossypetin that have strong analgesic properties in peripheral afferents and they act on an intracellular signaling pathway acting on T-type Cav channels. Taken together, it can be assumed that quercetin would inhibit the excitability of trigeminal neuronal firing responding to noxious mechanical stimulation. This assumption was supported by recent report that T-type Cav channels regulate action potential firing and are expressed in TG neurons.

**Functional Significance of Quercetin Suppression of Nociceptive Stimulation-induced TG Neurons**

CAM therapies are often used for pain management, especially after the failure of conventional Western medicine, or when adverse side effects are a concern. The use of local anesthetic agents may have an adverse effect on the central nervous system or cardiovascular system if blood levels rise too high. In the present study, we compared the mean magnitude of inhibition of TG nociceptive transmission achieved by quercetin with that of 2% lidocaine. Surprisingly, the mean magnitude of TG neuronal discharge frequency inhibition was almost equal between quercetin (10 mM) and 2% lidocaine (74 mM), indicating that the potency of quercetin was 7-fold higher than 2% lidocaine (quercetin = 74 mM lidocaine vs resveratrol = 37 mM lidocaine). These findings are supported by evidence in rat cardiac myocytes using a whole-cell patch clamp technique, that the red grape polyphenols, quercetin, catechin, and resveratrol, all inhibit Nav currents, and their inhibitory potency, based on the half maximal inhibitory concentration, is quercetin > catechin > resveratrol.

Since we found that quercetin has higher potency than lidocaine in this study, it is possible to speculate that excitability is equivalently blocked at their effective concentrations. This speculation led us to suggest that the physiological and adverse side effects are likely to be similar. However, there is the possibility that adverse effect of quercetin may be relatively smaller than that of lidocaine because the observation that the duration of suppression of TG neuronal activity was relatively shorter for quercetin than lidocaine in this study (15 minutes vs 40 minutes, respectively). Further studies are needed to confirm this possibility. Although a precise mechanism underlying the difference in duration of inhibition of neuronal activity for quercetin and lidocaine remains unknown, there are several possible explanations. There are different molecular targets and their affinity between quercetin and lidocaine, as above mentioned. Since lidocaine selective potent Nav channel blocker, however quercetin modulates several voltage-gated channels (eg, Nav, Kv, and Cav) and mechanosensitive ionic channels, therefore, it can be assumed that relatively shorter for quercetin than lidocaine may be due to differences in affinity of 2 drugs for Nav channels. However, further in vitro study is needed to explore this possibility.

It has been known that quercetin are classified phytoestrogen because of their capability to bind to both Estrogen (ER) α and ER β and to activate the transcription of several estrogen-responsive genes in vitro. Previous study showed that gender-related differences in the ability of selected flavonoids and phenolic compounds to modify porcine hepatic cytochrome P450 (CYP450)-dependent activity. For example, quercetin inhibited CYP2E1 activity in the microsomes from male pigs, however no effect of quercetin on CYP2E1 activity as observed in the microsomes from female pigs.
study, we have tested whether local administration of quercetin to rats attenuates the excitability of TG neurons in response to mechanical stimulation using only male rats. Although to our knowledge, there are no report showing sex differences in the actions of quercetin on the excitability of nociceptive neurons, further studies are necessary to assess the sex differences in the actions of quercetin on the nociception including voltage-gated ion channel and mechanosensitive ion channels.

In conclusion, we have demonstrated that local administration of quercetin suppresses the excitability of nociceptive primary sensory neurons in the TG, which may provide relief of trigeminal nociceptive pain, with smallest side effects, thus contributing to the area of CAM. Our findings in this study strongly suggest that the phytochemical, quercetin, is a more potent local analgesic than other polyphenols, such as resveratrol, as it inhibited the generation of both generator potentials and action potentials in nociceptive primary nerve terminals. However, further studies are needed to confirm the identity of the analgesic molecular sites of quercetin.

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The Journal of Pain 9


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