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ORIGINAL ARTICLE

Pharmacological evidence of involvement of nitric oxide pathway in anti-pruritic effects of sumatriptan in chloroquine-induced scratching in mice

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ABSTRACT

Chloroquine (CQ) induces histamine-independent itch in human and mice. We recently reported the role of intradermal nitric oxide (NO)/cyclic guanosine monophosphate pathway in CQ-evoked scratching in mice. Chloroquine stimulates neuronal nitric oxide synthase (nNOS) activity to over-producing NO in the skin. Sumatriptan, a 5-hydroxytryptamine 1b/1d receptors (5-HTR1b/1d) agonist, is involved in pain and used to treat migraine and cluster headaches. According to previous studies, sumatriptan inhibits NOS activity. Thus, we aimed to investigate the effect of sumatriptan on CQ-induced scratching. We used the rostral back model of itch. Chloroquine was injected intradermally into the rostral back of NMRI mice, and the scratching behavior was evaluated by measuring the number of bouts over 30 min. We evaluated the effect of sumatriptan and combination of sumatriptan and a non-selective NO synthase inhibitor, L-N-nitro arginine methyl ester (L-NAME), on the scratching behavior. Additionally, the changes of skin, hippocampus, and cortical nitrite level after different treatments were studied. Intraperitoneal and intradermal sumatriptan attenuates CQ-induced itch which reversed by GR-127935, the selective 5-HTR1b and 5-HTR1d antagonist. Co-administration of subeffective doses of sumatriptan and L-NAME significantly decreases the scratching behavior. Intradermal injection of CQ significantly increases the intradermal nitrite levels while it does not have any significant effects on hippocampal or cortical nitrite concentrations. Likewise, the effective doses of intraperitoneal and intradermal sumatriptan significantly reduce intradermal nitrite levels. We concluded that sumatriptan suppresses CQ-induced itch most likely by activating 5-HT1b/1d receptors. This effect probably mediates through NO pathway.

INTRODUCTION

Itch (pruritus) is defined as an unpleasant sensation leading to the desire to scratch [1]. Chronic pruritus is the most frequent symptom in dermatology and is common in systemic and metabolic disorders [2,3]. In addition, this debilitating symptom greatly influences the quality of life of many patients worldwide while the current available treatments including antihistamines have limited efficacy [4,5]. Thus, the experimental models of histamine-independent itch are essential to better understand the neuropharmacology of itch to provide new treatment options. The antimalarial drug chloroquine, CQ, is frequently used for non-histaminergic itch studies [6]. Chloroquine induces itch in humans unresponsive to antihistamines [7]. Likewise, intradermal (ID) administration of CQ provokes scratching behavior in mice [6]. Chloroquine excites Mas-related G-protein-coupled receptor A3 (MrgprA3; homologous to human MRGPRX1) on DRG neurons which only mediate an itch sensation [6]. MrgprA3 is a Gαq-coupled G protein-coupled receptor (GPCR) triggers intracellular signaling pathways which are not completely known [8]. Chloroquine affects different transient receptor potential (TRP) ion channels; it activates TRPA1 and TRPC3, inhibits TRPM8, and sensitizes TRPV1 via different mechanisms [9]. Lately, we have reported that stimulation of neuronal nitric oxide synthase (nNOS) activity with subsequent intradermal nitric oxide (NO) production involves in mediating CQ-induce itch in NMRI mice [10,11].

Nitric oxide is a known mediator of itch [12,13] and intradermal NO production increases in patients with psoriasis, atopic dermatitis, irritant dermatitis and allergic dermatitis [14]. Thus, NOS inhibitors would be potential therapeutic target to treat pruritus. Nitric oxide is present in several kinds of cells in the skin such as keratinocytes, melanocytes, macrophages, fibroblasts, endothelial cells, mast cells [15] and is produced by nNOS in peripheral nerves [16].

The 5-hydroxytryptamine 1b/1d receptor (5-HTR1b/1d) agonists, triptans including sumatriptan, are used to treat migraine and cluster headaches [17,18]. The 5-HTR1b/1d are expressed on primary sensory nerves [18] as well as various population of skin cells [19]. There are studies reported that 5-HTR1 agonists inhibit NOS activity to suppress pain signals [20,21]. Despite the known role of 5-HTR1b/1d agonists in pain, limited studies have investigated their involvement in itch. On the other hand, 5-HTR1d co-regulates with itch and has been assumed as a mediator of atopic dermatitis [22]. In this study, we investigated the inhibitory effects of sumatriptan on CQ-induced itch and the possible involvement of NO pathway in this phenomenon.

METHODS AND MATERIALS

Animals

We used 5–6 weeks of age (mean = 5.3) naval medical research institute (NMRI) male mice, each weighing between 23 and 30 g (mean = 24.9). The NMRI mice, an outbred strain, are Swiss-type mouse which were purchased from Pasteur Institute, Tehran, Iran. All the experiments were approved by the committee for animal ethics and experiments at Tehran University of Medical Sciences, Tehran, Iran, and confirmed to the protocol approved by the European Communities Council Directive of 24 November 1986 (86/609/EEC) and National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH publication #80-23). They were maintained under controlled temperature (23–25 °C) and light (lights on from 08:00 AM to 20:00 PM) conditions in cages with an inner floor area of 500 cm^2. Free access to water and a standard rodent diet was available for each animal [23]. Eight animals were randomly selected for each group.

Materials

Chloroquine bisphosphate (Pubchem CID 64927), Sumatriptan succinate (Pubchem CID 59772), N-nitro-L-arginine methyl ester (L-NAME; Pubchem CID 39836), and GR-127935 (Pubchem CID 107780) were purchased from Sigma, St. Louis, MO, USA. All the drugs were prepared freshly for use by dissolving in physiological saline.

Behavioral experiments

We used the rostral back model of itch for our experiments as described previously [10,24,25]. The hair was removed from the rostral back region by depilatory cream 2 days before each experiment. The syringes 24–25G insulin injection needles were used for intradermal drug administration. The drugs injected intradermally to the shaved area in a volume of 50 μL per site, and each mouse was used only once.

Before behavioral experiments, animals were placed in an acrylic box (10 × 10 × 13 cm) at 23 ± 1 °C and allowed to habituate for 1 h. Mice could not see each other during each experiment. A small amount of bedding was placed in each box and vacuum lines

pulled air through the boxes at a rate of about 300–500 mL/min.

Mice were removed briefly from the box for injections, and after substances were applied returned to the same box smoothly. The behavior was recorded using a video camera for 30 min. The scratching behavior could be easily distracted and inhibited; thus, efforts were made to perform experiments in unmanned conditions to reduce distractions to a minimum. The experimenter was present briefly to start the video recording and to inject the chemicals.

The video was played back to score the scratching bouts directed at the site of injection. A bout of scratching is initiated by lifting of the hind paw to the region of injection, and ended when the hind paw was returned to the floor or to the mouth [26].

Drug administration

Chloroquine was injected intradermally at dose of 400 μg in volume of 50 μL per site for induction of the scratching behavior.

Sumatriptan succinate, a selective 5-HTR1b/1d agonist [27], was administered intraperitoneally (IP) at doses of 0.1, 0.3, and 1 mg/kg 15 min before CQ. We also injected intradermal sumatriptan at dose of 1 nmol simultaneously with CQ in a volume of 50 μL per site.

To clarify the involvement of 5-HTR1b and 5-HTR1d in anti-pruritic effects of sumatriptan, GR-127935, a selective 5-HTR1b and 5-HTR1d antagonist [28], was injected at dose of 1 pmol/site concurrently with CQ (400 μg/site).

One milligram per kilogram of L-NAME, a non-selective NOS inhibitor, was considered as a subeffective dose and injected intraperitoneally. The subeffective doses of L-NAME and sumatriptan were administered 30 min and 15 min before CQ (400 μg/site) to determine the involvement of NO pathway through the scratching behavior induced by CQ.

Nitrite assay

To determine the NO levels in the skin, hippocampus, and the frontal cortex, NO metabolite, nitrite was measured after injection of 400 μg CQ (ID), saline, or combination of CQ and effective doses of ID or IP sumatriptan (1 nmol/site and 1 mg/kg). Mice were sacrificed 15 min after injection of CQ (the time of maximum scratching behavior and nitrite concentration) by cervical dislocation. Then, the pruritogen-injected skin, hippocampus, and the frontal cortex were dissected on ice-cold surface and immediately immersed into liquid nitrogen. The tissues were homogenized in 0.1 M phosphate buffer (0.072 M Na2HPO4, 0.028 M NaH2PO42H2O, pH = 7.4), and the homogenate was centrifuged. Nitrite levels were determined by a colorimetric assay based on the Griess reaction [29]. One-tenth milliliter of washout samples were pipetted into a 96-well microtiter plate, and then, 0.1 mL of Griess reagents was added. Following 15-min incubation at room temperature, the absorbance was measured at 540 nm in an automated plate reader. Concentration of nitrite was determined by reference to a standard curve of sodium nitrite (Sigma) and normalized to the weight of each sample.

Data analysis

GraphPad Prism 6.1, the graphing and statistics software, was used for data analysis and drawing the figures. The irrelevant data of behavioral experiments are excluded. All data are presented as mean ± SEM (the standard error of the mean) of 6–8 animals. The obtained data were evaluated by one-way or two-way analysis of variance (ANOVA) along with Dunnett's test or Tukey's multiple comparisons tests. Likewise, $P < 0.05$ was considered significant.

RESULTS

Sumatriptan attenuates CQ-induced scratching behavior

Figure 1 demonstrates the effect of IP sumatriptan on CQ-induced scratching behavior. A two-way ANOVA showed that intradermal injection of CQ (400 μg/site) elicited scratching of the injected site by the hind paws in mice ($F_{1,54} = 146.3$, $P < 0.0001$). In addition, IP administration of sumatriptan 15 min before CQ markedly reduces the scratching behavior ($F_{3,54} = 36.17$, $P < 0.0001$). Tukey's multiple comparisons test showed that administration of sumatriptan at doses of 0.3 and 1 mg/kg IP significantly decreased the itch responses by CQ (400 μg/site; $P < 0.0001$). However, 0.1 mg/kg of intraperitoneal sumatriptan was not effective to reduce the scratching behavior ($P > 0.05$). No significant pruritic effect was observed with sumatriptan per se ($P > 0.05$).

In addition, ID treatment of sumatriptan (1 nmol/site) with CQ rescued scratching levels to those of mice treated with saline alone. No significant pruritic effect was observed with ID sumatriptan injection ($F_{3,25} = 23.89$, $P < 0.0001$; *Figure 2*).

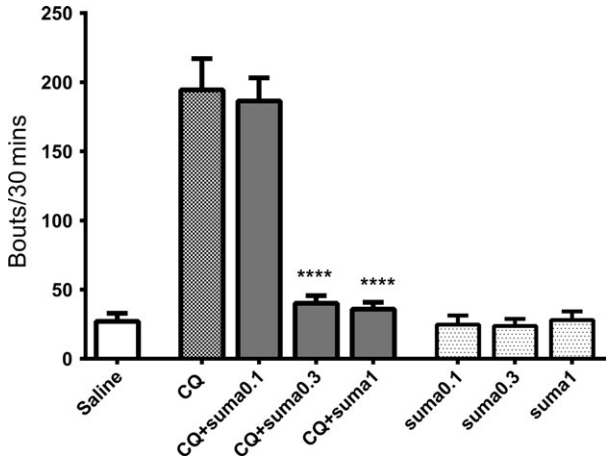

Figure 1 Intraperitoneal injection of sumatriptan suppresses chloroquine (CQ)-induced scratching. Sumatriptan was injected at doses of 0.1, 0.3, and 1 mg/kg 15 min before 400 μg CQ (ID). Sumatriptan per se did not have any significant pruritic effects. Values are expressed as the mean ± SEM (n = 6–8) and were analyzed using two-way ANOVA followed by Tukey's multiple comparisons test. ****$P < 0.0001$.

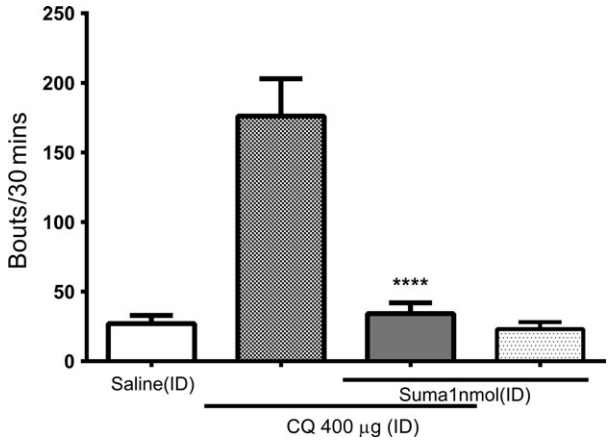

Figure 2 Intradermal injection of sumatriptan suppresses the scratching behavior elicited by chloroquine (CQ) (400 μg/site). Sumatriptan (1 nmol/site) was injected simultaneously with CQ (400 μg/site). Intradermal sumatriptan per se did not have any significant pruritic effects. Each group consisted of 6–8 animals. Values were analyzed using a one-way ANOVA followed by Dunnett's test. ****$P < 0.0001$.

We used GR-127935, a selective 5-HTR1b/1d antagonist [28] to investigate the involvement of these receptors through anti-pruritic effects of sumatriptan on CQ-induced itch. *Figure 3* shows that treatment with GR-127935 (1 pmol/site) completely reversed the inhibitory effects of sumatriptan (1 mg/kg, IP) was injected 15 min before CQ (400 μg/site; $F_{3,26} = 31.69$, $P < 0.0001$).

Combined subeffective doses of sumatriptan and L-NAME decreases CQ-induced scratching behavior

Intraperitoneal administration of L-NAME at dose of 1 mg/kg 30 min before CQ (400 μg/site) had no significant effects on the scratching behavior. While treatment with subeffective doses of L-NAME (1 mg/kg, IP) and sumatriptan (0.1 mg/kg, IP) 30 min and 15 min prior to CQ markedly reduced the itch behavior ($F_{3,27} = 17.74$, $P < 0.0001$; *Figure 4*).

Sumatriptan reduces the CQ-induced increase in intradermal NO levels

Our results show that CQ (400 μg/site, ID) significantly increased the concentration of intradermal nitrite compared to the saline group at 15 min after CQ administration, while CQ had no effects on the hippocampal or cortical nitrite levels. Furthermore, the effective doses of intraperitoneal and intradermal sumatriptan (1 mg/kg and 1 nmol/site) significantly decreased the nitrite levels 15 min after CQ injection. A two-way ANOVA showed the effect of the site of the nitrite levels ($F_{2,60} = 14.9$, $P < 0.0001$), the effect of treatments ($F_{3,60} = 5.98$, $P = 0.0012$), and the effect of site of the

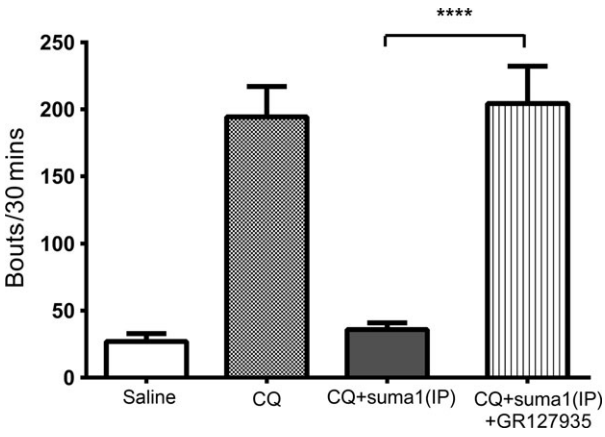

Figure 3 GR-127935 significantly reversed the anti-pruritic effects of sumatriptan. GR-127935 (1 pmol/site) was administered simultaneously with intradermal chloroquine (400 μg/site). The effective dose of sumatriptan (1 mg/kg, IP) was injected 15 min beforehand. Values were analyzed using a one-way ANOVA followed by Dunnett's test and each group consisted of 6–8 animals. ****$P < 0.0001$.

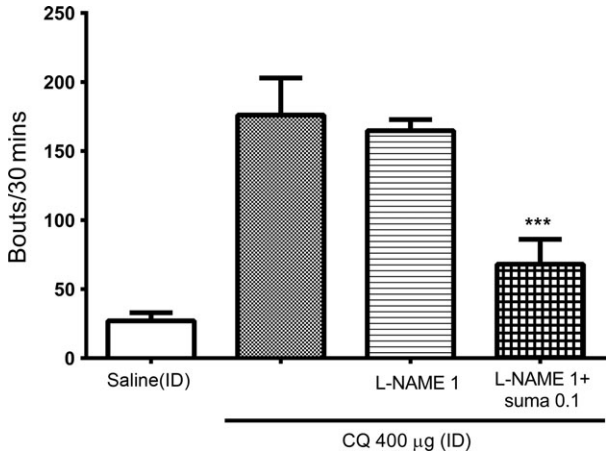

Figure 4 Combined treatment with subeffective doses of sumatriptan and L-NAME attenuates the chloroquine (CQ)-evoked scratching behavior. Administration of L-NAME (1 mg/kg, IP) 30 min before and sumatriptan (0.1 mg/kg < IP) 15 min before CQ 400 μg (ID) significantly reduce the scratching behavior ($P = 0.0004$). Values were analyzed using a one-way ANOVA followed by Dunnett's test and each group consisted of 6–8 animals. ***$P < 0.001$.

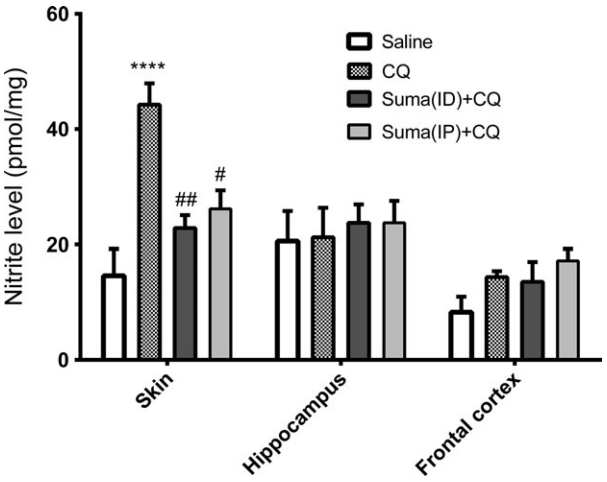

Figure 5 Effect of different treatments on intradermal, hippocampal, and cortical nitrite levels. The pruritogen-injected skin, hippocampus, and the frontal cortex were dissected 15 min after chloroquine (CQ) (400 μg/site, ID) injection. The concentration of intradermal, but not the hippocampal or cortical nitrite, is increased compared to the saline group ($P < 0.0001$). The effective doses of intradermal ($P = 0.0043$) and intraperitoneal ($P = 0.0313$) sumatriptan significantly reduce the chloroquine-induced nitrite levels in the skin. Values are expressed as mean ± SEM ($n = 8$) and were analyzed using two-way ANOVA followed by Tukey's multiple comparisons test. ****$P < 0.0001$ compared with the intradermal nitrite of the saline-treated group. ##$P < 0.01$ and #$P < 0.05$ compared with the intradermal nitrite of the CQ-treated group.

nitrite levels × treatments interaction ($F_{6,60} = 3.75$, $P = 0.0031$; *Figure* 5).

DISCUSSION

We demonstrated that intraperitoneal and intradermal administration of sumatriptan attenuates CQ-induced itch which is reversed by GR-127935, the selective 5-HTR1b/d antagonist [28]. Our results also show that co-administration of subeffective doses of sumatriptan and L-NAME significantly decreases the scratching behavior induced by CQ. While CQ promotes the generation of intradermal nitrite, a proxy for NO production, the hippocampal or cortical nitrite concentrations has not been changed after CQ administration. Consistent with the findings with sumatriptan, the production of intradermal nitrite is decreased after intradermal and intraperitoneal injections of sumatriptan.

Serotonin is a known pruritogen in humans and animals [30]. The impaired serotonergic system is associated with a variety of pruritic skin diseases and systemic disorders [25]. Serotonin directly activates 5-HT receptors on sensory neurons to evoke histamine-independent itch [30,31]. A variety of 5-HT receptors subtypes expressing in sensory nerves participate in itch pathway. It has been reported that ondansetron and tropisetron, the 5-HT3 antagonists, reduce 5-HT-induced scratching behavior in mice [32]. While recent studies demonstrate that 5-HT3 antagonists have no anti-itch effects on 5-HT-evoked scratching behavior in rodents and itch in humans [33–35]. The 5-HT2 antagonist, ketanserin, also reduces 5-HT-evoked scratching in mice [35]. Likewise, 5-HT7 receptor has been associated with 5-HT-induced itch in mice [22]. However, there are no human studies show the effectiveness of 5-HT7 or 5-HT2 antagonists on itch [36].

The 5-HT1b/1d receptors have role in pain, and their agonists, triptans, are widely used to treat migraine and cluster headaches [17,18], although they are not well-known to be involved in itch. The 5-HTR1d is among the top transcriptional correlates of chloroquine itch and also has been proposed as a candidate mediator of atopic dermatitis [22].

The 5-HT1 receptors are classified as GPCRs, a group of integral membrane proteins [37,38], involving in intracellular signal transduction [17]. Activation of 5-HTR1b/1d causes decrease of cyclic adenosine

monophosphate (cAMP) signaling via Gi/Go [39–41] which inhibits release of different neuropeptides [42].

The 5HTR1b/1d are predominantly expressed in the trigeminal ganglia and afferent nerve fibers terminating in various tissues of human and rodents [43,44]. Furthermore, these receptors are detected in cultures of mice skin [19]. The 5-HTR1b is expressed in melanocytes and hair follicles and has been a novel target to treat skin hypopigmentation in mice [19]. Thus, the skin might be the possible site of inhibitory actions of sumatriptan in itch. It is evident that action of triptans is not limited to trigeminal vascular system in nociception and sumatriptan attenuates inflammatory or non-inflammatory visceral pain [45]. It has been shown that local administration of sumatriptan suppresses vasodilatation induced by capsaicin which is prevented by GR-127935 [46]. Thermal hypersensitivity induced by intraplantar injection of carrageenan decreases by systemic injection of sumatriptan in mice [47]. Furthermore, sumatriptan inhibits the evoked release of calcitonin gene-related peptide from the rat isolated spinal cord [48] as well as capsaicin-induced hyperemia in the sciatic nerve [49].

Chloroquine induces itch independent of histamine in human and mice through activating the CQ receptor, MrgprA3 [6]. Chloroquine-evoked scratching reduces by 70% in mice lacking MrgprA3 [50]. Binding of CQ to MrgprA3 activates the downstream ion channel TRPA1 by Gβγ [30,51]. Chloroquine does not induce itch in mice lacking TRPA1 [51]. We recently reported the role of intradermal NO/cyclic guanosine monophosphate (cGMP) pathway and nNOS but not endothelial NOS (eNOS) or inducible NOS (iNOS) activity in CQ-elicited itch in mice [10,11]. It is evident that NO functions as a neurotransmitter in itch [12,52,53] and the production of NO increases in the skin of patients with pruritic skin diseases including psoriasis and atopic dermatitis [54,55]. Likewise, nNOS is present in various types of dermal cells including in human and murine keratinocytes and melanocytes [54,56,57]. Moreover, the primary sensory neurons express nNOS [16]. The increased intracellular Ca^+ leads to activating nNOS [58]. Previously, it was shown that sumatriptan inhibits NOS enzymes activity including nNOS [20,21,44]. It suppresses the increase in NOS level induced by intracisternal carrageenan and the activity of NOS in guinea-pig cerebral blood vessels [59]. Additionally, sumatriptan inhibits Ca^+ signaling which regulates release of neuropeptides from sensory neurons [60] and can be concomitant with reduced nNOS activity. Nitric oxide increases through various mechanisms in CQ-evoked scratching. It was suggested that activation of TRPA1 by CQ triggers Ca^+ influx with subsequent nNOS stimulation [10]. Moreover, opioid system is known to be involved in CQ-induced itch [61–63] which induces the production of neuronal cGMP and nNOS expression via opioid receptors [64–66]. Therefore, sumatriptan probably attenuates nNOS activity to reduce itch evoked by CQ.

We concluded that sumatriptan suppresses CQ-induced itch most likely by activating 5-HT1b/1d receptors. This effect is probably being associated partly with inhibition of NO pathway in the skin. This may provide the insight for development of new target therapeutics to treat CQ-induced itch as well as better understanding of pharmacological interaction of this phenomenon. However, the potency of drugs to reduce itch in human is difficult to be interpreted by mouse models due to species differences. Thus, further studies will be significant to clarify the role for triptans in pruritic diseases in humans.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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