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Indomethacin, Alone and Combined With Prochlorperazine and Caffeine, but not Sumatriptan, Abolishes Peripheral and Central Sensitization in In Vivo Models of Migraine

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Abstract: **Recently it has been proposed that the throbbing pain of migraine is mediated by sensitization of peripheral trigeminovascular neurons, and that cutaneous allodynia of migraine is mediated by sensitization of central trigeminovascular neurons, and, moreover, that the triptans are less effective in aborting a migraine attack if the central sensitization is already established. The combination of indomethacin, prochlorperazine, and caffeine (IndoProCaf) is a drug of well-established use in the acute treatment of migraine. The aim of this study was to investigate whether the 3 active principles of IndoProCaf, alone and combined, compared to sumatriptan, were able to abolish the peripheral sensitization induced by kainic acid and the central sensitization induced by N-methyl-D-aspartate (NMDA) in in vivo models of hyperalgesia. The study showed that indomethacin or IndoProCaf is able to abolish both the kainic acid–induced and the NMDA-induced hyperalgesia. If administered at different times, IndoProCaf was always effective in reversing the kainic acid-induced hyperalgesia. Sumatriptan was not able to reverse either the kainic acid–induced or the NMDA-induced hyperalgesia. The efficacy of indomethacin, alone and combined with prochlorperazine and caffeine, in abolishing peripheral and central sensitization in in vivo models of hyperalgesia is a further explanation of the clinical efficacy of IndoProCaf in the treatment of migraine.**

Perspective: ***This study suggests that, although triptans were shown to be able to abort migraine attacks only if given before the establishment of cutaneous allodynia and central sensitization, IndoProCaf should be able to abort migraine attacks independently from the time of administration, because it is able to abolish an already established peripheral and central sensitization.***

Key words: *Indomethacin, prochlorperazine, caffeine, sumatriptan, migraine.*

Migraine is a common, multifactorial, neurovascular disorder, characterized by recurrent disabling attacks of moderate to severe headache, nausea, vomiting, photophobia, and phonophobia, and also, in up to one third of patients, neurologic aura symptoms.[12] It is known that migraine is characterized by a state of central neuronal hyperexcitability,[30] which involves overactivity of the excitatory amino acids. Higher concentrations of excitatory amino acids (mainly glutamic and aspartic acid) were observed in the cerebrospinal fluid,[23] in the saliva,[26] in the plasma,[2,13] and in the platelets[9] of patients with migraine.

Recently it has been proposed that the throbbing pain of migraine, which is the pulsating pain aggravated by routine physical activities, is mediated by sensitization of peripheral trigeminovascular neurons, and that cutaneous allodynia (CA) of migraine, which means pain resulting from a non-noxious stimulus to normal skin, is mediated by sensitization of central trigeminovascular neurons.[4,22]

The development of hyperalgesia has been shown after the injection of kainic acid. The hyperalgesic effect of kainic acid appears to be mediated by activity at amino–methylisoxazole-propionic acid/kainate receptors that are located outside the spinal cord, perhaps on primary afferents in trigeminal ganglia.[27] Intraperitoneal injection of kainic acid induces a persistent hyperalgesia in mice and rats.[18]

Glutamate, an excitatory neurotransmitter, produces

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its action by acting on N-methyl-D-aspartate (NMDA) receptors. It has been demonstrated in rats that NMDA receptors mediate the synaptic transmission of the peripheral trigeminovascular neurons in central trigeminal subnucleus caudalis.[1] NMDA receptors play a key role in the nociceptive transmission within the spinal cord, as evidenced by the short duration hyperalgesia induced by the intrathecal injection of NMDA in mice and rats.[21,29]

Sumatriptan is the first commercially available and most widely prescribed of the serotonin 5-HT1B/1D agonists known as triptans. Sumatriptan is considered the gold standard in controlled trials of drugs used in the acute treatment of migraine.[12] However, it has recently been shown that the triptans are less effective in aborting a migraine attack if the central sensitization is already established.[5]

The combination of indomethacin, prochlorperazine, and caffeine (IndoProCaf) is a drug of well-established use in Italy with a recognized efficacy and a good level of safety in the acute treatment of migraine. Each active ingredient of IndoProCaf has specific antimigraine properties. Differently from other nonsteroidal anti-inflammatory drugs (NSAIDs), indomethacin is structurally related to serotonin and has central analgesic and cranial vasoconstrictor properties.[19,28] Prochlorperazine is a phenothiazine antiemetic, endowed with analgesic properties mediated by the presynaptic inhibition of the D2 heteroreceptor located on the cholinergic neurons.[16] Caffeine induces central cholinergic analgesia.[15]

Recently in a multicenter, randomized, crossover clinical trial, IndoProCaf was shown to be significantly more effective than sumatriptan in the acute treatment of migraine attacks.[10] The aim of this study was to investigate whether the 3 active principles of IndoProCaf, alone and combined, compared to sumatriptan, were able to abolish the peripheral sensitization induced by kainic acid and the central sensitization induced by NMDA in in vivo models of hyperalgesia.

Materials and Methods

Animals

Male albino mice (20 to 25 g) from Morini breeding farm (San Polo d'Enza, Italy) were used. All experiments were carried out according to the guidelines of the European Community Council Directive dated November 24, 1986 (86/609/EEC) for experimental animal care. All efforts were made to minimize suffering and to reduce the number of animals used.

Drugs

The following drugs were used: indomethacin (NSAID; Sigma, Milan, Italy), prochlorperazine (D_2-antagonist; Sigma), caffeine (adenosine antagonist; Sigma), sumatriptan (5-$HT_{1B/1D}$ agonist; GlaxoSmithKline, Verona, Italy), kainic acid (kainate receptor agonist; Sigma), NMDA receptor agonist (Sigma), oxotremorine (muscarinic agonist; Fluka, Milan, Italy), baclofen ($GABA_B$-agonist; R.B.I., Milan, Italy), amitriptyline (tricyclic antidepressant; Sigma), and diclofenac (NSAID; Sigma). The doses of the drugs tested in this study were able to reverse other models of hyperalgesia and were much lower than those shown to be analgesic in the hot plate test[14,17]: indomethacin (0.1 mg/kg intraperitoneal), prochlorperazine (0.1 mg/kg intraperitoneal), caffeine (0.3 mg/kg intraperitoneal), and sumatriptan (1 mg/kg intraperitoneal). The doses of the drugs tested as negative reference drugs were not antinociceptive in the hot plate test[14]: oxotremorine (0.01 mg/kg intraperitoneal), baclofen (0.2 mg/kg intraperitoneal), amitriptyline (0.5 mg/kg intraperitoneal), and diclofenac (1 mg/kg intraperitoneal).

Indomethacin, prochlorperazine, and caffeine (alone and combined), sumatriptan, oxotremorine, baclofen, amitriptyline, and diclofenac were intraperitoneally administered to reach the respective analgesic peak in correspondence with the hot plate test.[14] According to the specific pharmacokinetic properties, the drugs were administered 45 minutes (prochlorperazine), 30 minutes (baclofen, amitriptyline, and diclofenac), or 15 minutes (sumatriptan, caffeine, and oxotremorine) before the hot plate test. Indomethacin, alone and combined with prochlorperazine and caffeine, was administered 15, 30, and 45 minutes before the hot plate test.

Kainic Acid–Induced Hyperalgesia

According to Giovengo et al,[18] kainic acid–induced hyperalgesia was obtained through intraperitoneal administration of kainic acid (20 mg/kg) at least 48 hours before the hot plate test. Because the intraperitoneal injection of kainic acid produces a long-term thermal hyperalgesia, when tested with the hot plate (mice) and tail flick (mice and rats) assays, and mechanical hyperalgesia, when tested with von Frey filaments (rats), whereas, when injected intrathecally, it fails to induce hyperalgesia, it seems unlikely that the brain is the site of action of kainic acid.[18] Thus this model should be considered an example of peripheral sensitization, because of the pharmacologic effects of kainic acid and the route of administration. The reduction of pain threshold reaches the peak 24 hours after the injection of kainic acid, and the effect lasts for several days. The drugs were administered when the hyperalgesia was already established; therefore the kainic acid–induced hyperalgesia should be considered a model of treatment of peripheral sensitization.

NMDA-Induced Hyperalgesia

Hyperalgesia was induced by the intrathecal administration of NMDA (1.64 μg/mouse) 15 minutes before the hot plate test. Intrathecal injections were performed under ether anesthesia as described by Hylden and Wilcox.[20] The mouse was gently restrained, and a 30-gauge, 1/2-inch needle mated to a 50-μL Hamilton syringe was inserted between L5 and L6 of the mouse spinal column. A volume of 5 μL was used for intrathecal injection. Because of the pharmacologic effects of NMDA and the route of administration, this model should be considered

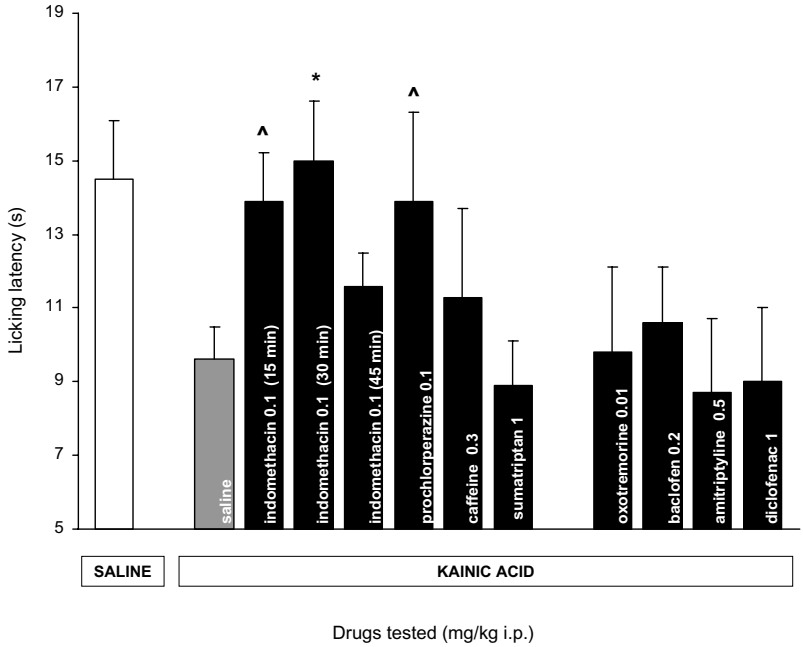

Figure 1. Effect of analgesic drugs (indomethacin, prochlorperazine, caffeine, sumatriptan, oxotremorine, baclofen, amitriptyline, diclofenac) on hyperalgesia induced by kainic acid (20 mg/kg intraperitoneal) in mouse hot plate test. Each column represents the mean of at least 8 mice. All data are mean ± SEM. *$P < .01$, ^$P < .05$ vs kainic acid only treated mice.

an example of central sensitization. The drugs were administered before or simultaneously with the NMDA; however, the maximal effect of NMDA on the licking latency was observed a few minutes after the administration. Therefore, the NMDA-induced hyperalgesia should be considered a model of treatment of central sensitization.

Hot Plate Test

The hyperalgesia was evaluated through the hot plate test. The method adopted was described by O'Callaghan and Holtzman.[24] Mice were placed inside a stainless steel container, thermostatically set at 52.5°C ± 0.1°C in a precision water-bath (KW Mechanical Workshop, Siena, Italy). Reaction times (seconds) were measured with a stopwatch before (pretest) and after treatment. The end point used was the licking of the fore or hind paws. Those mice scoring less than 12 and more than 18 seconds in the pretest were rejected (30%). An arbitrary cutoff time of 45 seconds was adopted.

Statistics

Results are given as the mean ± standard error of mean (SEM); the analysis of variance was used to verify the significance between 2 means, followed by Fisher protected least significant difference procedure for post hoc comparison. *P* values of less than .05 were considered significant. Data were analyzed with the StatView for the Macintosh computer program (1992; SAS Institute, Inc, Cary, NC).

Results

Hyperalgesia Induced by Kainic Acid

Indomethacin was able to abolish the hyperalgesia induced by kainic acid. Indomethacin was able to reverse the decrease of licking latency observed with kainic acid–treated mice when administered 15 minutes ($P < .05$) and 30 minutes ($P < .01$) before the hot plate test (Fig 1).

Prochlorperazine was also able to reverse the kainic acid–induced hyperalgesia ($P < .05$) when administered 45 minutes before the hot plate test. Caffeine was not able to reverse the kainic acid–induced hyperalgesia.

IndoProCaf was able to reverse the kainic acid–induced hyperalgesia when administered 15, 30 ($P < .01$), and 45 minutes ($P < .05$) before the hot plate test (Fig 2).

Sumatriptan was not able to reverse the kainic acid–induced hyperalgesia.

Other analgesic drugs (oxotremorine, baclofen, amitriptyline, and diclofenac), tested as negative reference drugs, were not able to reverse this type of hyperalgesia (Fig 1).

Hyperalgesia Induced by NMDA

Indomethacin was able to abolish the hyperalgesia induced by NMDA. Indomethacin was able to reverse the decrease of licking latency observed with NMDA-treated mice when administered 15 ($P < .05$) and 30 minutes ($P < .01$) before the hot plate test (Fig 3).

Prochlorperazine and caffeine were not able to reverse the NMDA-induced hyperalgesia.

IndoProCaf was able to reverse the NMDA-induced

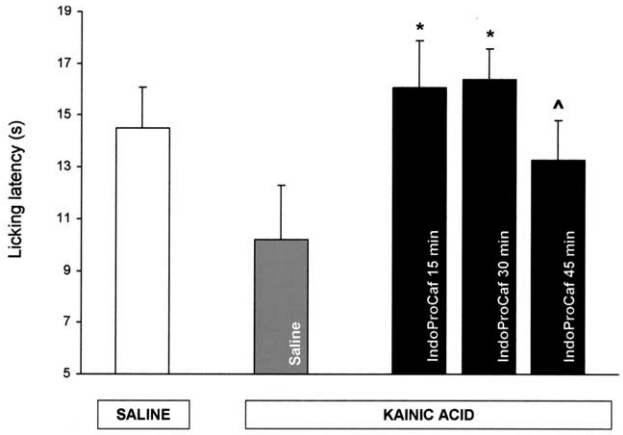

Figure 2. Effect of IndoProCaf (indomethacin 0.1 mg/kg, prochlorperazine 0.1 mg/kg, caffeine 0.3 mg/kg intraperitoneal) on hyperalgesia induced by kainic acid (20 mg/kg intraperitoneal) in mouse hot plate test. Each column represents the mean of at least 10 mice. All data are mean ± SEM. *$P < .01$, ^$P < .05$ vs kainic acid only treated mice.

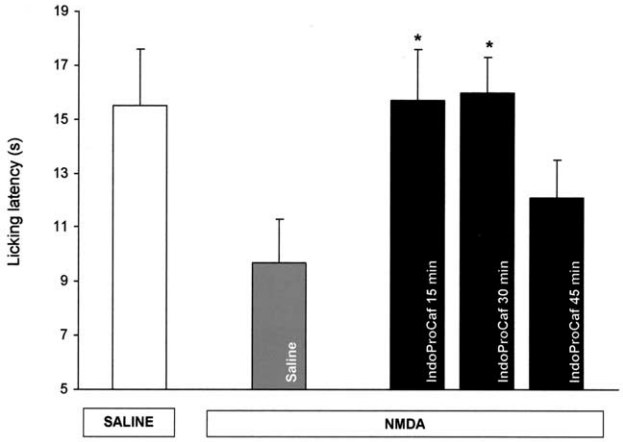

Figure 4. Effect of IndoProCaf (indomethacin 0.1 mg/kg, prochlorperazine 0.1 mg/kg, caffeine 0.3 mg/kg intraperitoneal) on hyperalgesia induced by intrathecal administration of NMDA (1.64 μg/mouse) in mouse hot plate test. Each column represents the mean of at least 8 mice. All data are mean ± SEM. *$P <$ .01 vs NMDA only treated mice.

hyperalgesia when administered 15 and 30 minutes before the hot plate test ($P < .01$) (Fig 4).

Sumatriptan was not able to reverse the NMDA-induced hyperalgesia. Diclofenac was also able to reverse the NMDA-induced hyperalgesia ($P < .05$).

Other analgesic drugs (oxotremorine, baclofen, and amitriptyline), tested as negative reference drugs, were not able to reverse this type of hyperalgesia (Fig 3).

Discussion

The aim of this study was to investigate whether the 3 active principles of IndoProCaf, alone and combined, compared to sumatriptan, were able to abolish the peripheral sensitization induced by kainic acid and the central sensitization induced by NMDA in in vivo models of hyperalgesia.

The study showed that indomethacin, alone and combined with prochlorperazine and caffeine, but not sumatriptan, is able to abolish the peripheral hyperalgesia induced by kainic acid and the central hyperalgesia induced by NMDA.

The study also showed that prochlorperazine is able to abolish the kainic acid–induced hyperalgesia, and that

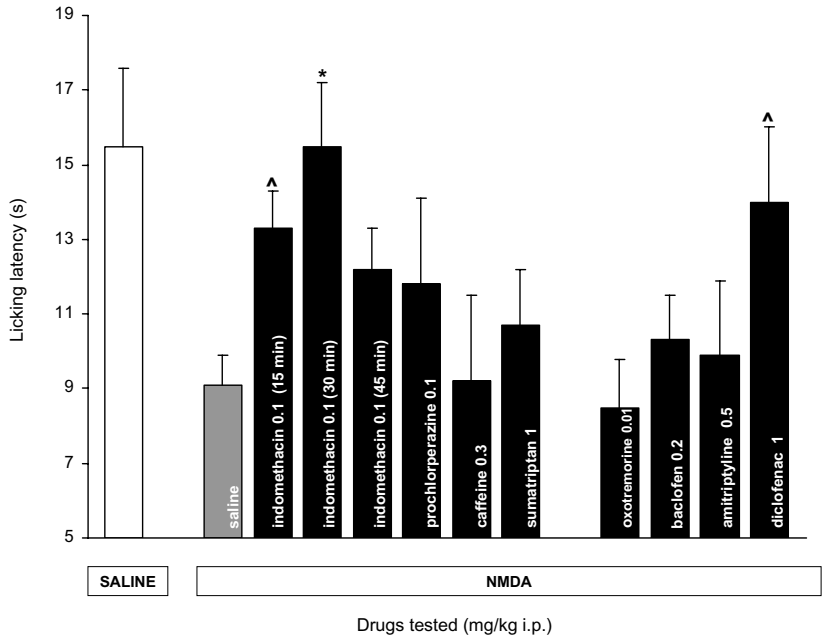

Figure 3. Effect of analgesic drugs (indomethacin, prochlorperazine, caffeine, sumatriptan, oxotremorine, baclofen, amitriptyline, diclofenac) on hyperalgesia induced by intrathecal administration of NMDA (1.64 μg/mouse) in mouse hot plate test. Each column represents the mean of at least 8 mice. All data are mean ± SEM. *$P < .01$, ^$P < .05$ vs NMDA only treated mice.

diclofenac is able to reverse the NMDA-induced hyperalgesia.

Other analgesic drugs, which are not indicated for the treatment of migraine, were not able to abolish this type of sensitization.

These results are consistent with previous data showing that the intrathecal injection of indomethacin is able to reduce the hyperalgesia induced by NMDA.[25,32] However, in these previous experiments indomethacin was centrally (intrathecally) injected, whereas in the present study it was peripherally (intraperitoneally) administered. The efficacy of diclofenac, a NSAID, in reversing the NMDA-induced hyperalgesia could be expected, considering that it is known that prostaglandins and nitric oxide mediate this type of hyperalgesia.[25] Why indomethacin, and not diclofenac, was able to reverse kainic acid–induced hyperalgesia is not known.

If administered at different times, IndoProCaf was always effective in reversing the kainic acid–induced hyperalgesia. In a previous study, IndoProCaf was found to be able to abolish almost completely the abdominal constrictions induced by the intraperitoneal injection of a 0.3% solution of acetic acid, with a significantly higher efficacy compared to the single active principles.[14]

In this study, sumatriptan was not able to reverse the kainic acid–induced peripheral sensitization or the NMDA-induced central sensitization.

This result confirms that triptans cannot abolish already established central sensitization,[5,7] but it also suggests that triptans cannot abolish already established peripheral sensitization.

The in vivo models used in this study are clinically important, because they evidenced different effects of IndoProCaf and sumatriptan. On the contrary, in another in vivo model of migraine both IndoProCaf and sumatriptan, at the same doses used in this study, were active in reversing hyperalgesia induced by morphine withdrawal.[14,17]

The dosages used for IndoProCaf in these experimental models (indomethacin 0.1 mg/kg, prochlorperazine 0.1 mg/kg, and caffeine 0.3 mg/kg) are 10 times lower than those shown to be analgesic in the hot plate test.[14] It is important to note that the therapeutic dose (orally or rectally administered) of indomethacin contained in IndoProCaf as antimigraine drug is 25 to 50 mg, a lower dose compared to the antirheumatic dosage, and that of prochlorperazine is 2 to 8 mg, a lower dose compared to the antipsychotic and antiemetic dosage. These findings confirm that migraine is a particular pain characterized by neuronal hyperexcitability, and that migraine should be treated with specific drugs at dosages that could be subanalgesic but antihyperalgesic.

The efficacy of indomethacin, prochlorperazine, and IndoProCaf in reversing the kainic acid–induced hyperalgesia is clinically important, when considering the hypothesis that peripheral sensitization is a common feature of migraine patients, whose throbbing and pulsating pain is aggravated by routine physical activities such as climbing stairs and bending over or by coughing.[8] The efficacy of indomethacin and IndoProCaf in abolishing the NMDA-induced hyperalgesia is also clinically relevant, when considering that central sensitization is suggested to lead to CA, meaning that in 79% of migraine patients an innocuous stimulation of the skin, such as brushing hair, touching the scalp, or wearing glasses, is perceived as painful during the migraine attack.[8]

Recently the hyperexcitability that develops along the trigeminovascular pain pathway during a migraine attack has been studied in humans. It has been suggested that a few minutes after the onset of migraine, the peripheral nociceptors become sensitized, and the headache starts to throb; afterwards, the barrage of impulses that come from the peripheral nociceptors activate second-order neurons in the nucleus caudalis and initiate their sensitization, mediating the development of CA on the ipsilateral head. Then the barrage of impulses that come from the hyperactive second-order neurons sensitizes third-order thalamic neurons, mediating the development of CA on the contralateral head and ipsilateral forearm over 1 hour after the appearance of allodynia on the ipsilateral head.[6] Moreover, the efficacy of triptan therapy in patients with or without CA, defined by differences between migraine and baseline pain thresholds to mechanical and thermal stimulation of periorbital skin, has been investigated.[5] Triptan treatment of migraine was equally ineffective once CA was present and equally effective in the absence of CA. In patients with CA, triptan treatment was far more effective if given before rather than after CA developed. Furthermore, in an animal model of central sensitization induced by topical application of inflammatory soup on the dura, early endovenous sumatriptan treatment blocked the development of central sensitization; on the other hand, late sumatriptan treatment did not reverse central sensitization such as response threshold to heating of the skin.[7]

The clinical implication of these recent findings is that patients with CA should take triptan therapy as soon as possible after onset of migraine, because triptans were shown to be able to abort migraine attacks only if given before the establishment of CA and central sensitization. On the contrary, IndoProCaf should be able to abort migraine attacks independently from the time of administration, because this fixed combination is able to abolish an already established peripheral and central sensitization. It has to be considered that the model of NMDA-induced central hyperalgesia is not analogous to the animal model used to evaluate the effects of triptans on central sensitization.[7]

The efficacy of indomethacin and IndoProCaf in abolishing the central hyperalgesia induced by NMDA could have a potential therapeutic value, when considering that NMDA is involved in long-term potentiation and that in patients with tension-type headache the development of central sensitization could lead to

chronic headache.[3] Recently in a double-blinded, randomized, nimesulide-controlled, multicenter clinical trial, IndoProCaf was shown to be very effective in the treatment of episodic tension-type headache (Cerbo et al, unpublished data).

Moreover, neuronal hyperexcitability is involved not only in migraine but also in neuropathic pain, a pain caused by a lesion of the peripheral and/or central nervous system.[11] The role that central sensitization and the NMDA receptor have in pathological pain is highlighted by studies that show that the blocking of central sensitization with NMDA antagonists abolishes pain hypersensitivity in patients with neuropathic pain.[31]

In conclusion, the efficacy of indomethacin, alone and combined with prochlorperazine and caffeine, in abolishing peripheral and central sensitization in in vivo models of hyperalgesia is a further explanation of the clinical efficacy of IndoProCaf in the treatment of migraine. Furthermore, these results support the rationale for exploring the clinical efficacy of IndoProCaf in the treatment of other types of pain in humans.

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