Abstract

Introduction: Migraine is a complex neurovascular disorder. Gabapentin is a structural analogue to Gamma-Amino Butyric Acid (GABA), with strong anticonvulsant and analgesic activities, while caffeine is an adjuvant in analgesic formulations.

Aim: The aim of this study was to evaluate the potential therapeutic effect of combined GABA analogue, gabapentin and caffeine in glyceryl trinitrate (NTG) migraine rat model and the possible underlying mechanisms.

Methods: Thirty male albino rats were received either (NTG, 10mg/kg, i.p.) or saline (NTG vehicle). NTG treated rats received combined gabapentin/caffeine or their vehicles. Normal rats were taken as control group. The analgesic activity was evaluated by Formalin Test (FT). Dural inflammation was assessed by Evans Blue Dye (EBD) extravasation. Serum total tryptase was measured as an indicator of Mast Cells (MCs) activation. Glutamic acid and GABA levels in Trigeminal Nucleus Caudalis (TNC) tissue homogenate were assayed.

Results: Gabapentin/caffeine treatment significantly alleviated the nociception during both phases of the FT with greater reduction in the second phase possibly through MCs stabilization, anti-inflammatory effect with restored neurotransmitters balance.

Conclusion: These data suggest that GABA analogue, gabapentin together with caffeine may abort migraine attack and represent a potential therapeutic tool for the treatment of migraine.

Key Words: Migraine – Caffeine – GABA analogue – Gabapentin – Formalin test – Tryptase – Glutamate – GABA.

Introduction

MIGRAINE is a common debilitating neurovascular disorder, typically characterized by recurrent episodes of headaches, mostly throbbing, unilateral, moderate to severe, aggravated by physical activity to an intensity that may incapacitate the patient from performing daily activities, and frequently accompanied by other symptoms like nausea, phonophobia and/or photophobia. Migraine attacks are triggered by a variety of conditions including endogenous and exogenous factor.

The pathophysiology of migraine is complex and not fully elucidated. It is thought that during a migraine attack primary afferent meningeal nociceptive neurons, the peripheral arm of the trigeminovascular system are activated and release vasoactive neuro inflammatory peptides, including Calcitonin Gene-Related Peptide (CGRP), substance P, and Nitric Oxide (NO) from the nerve endings within the meninges, constitutes one of the earliest events promoting the intracranial pain of migraine. These neurotransmitters result in vasodilatation of meningeal blood vessels via receptors on the Smooth Muscle Cells (SMCs) consequently plasma extravasation and Mast Cells (MCs) degranulation. They also facilitate the transmission of pain signals from the trigeminal ganglion as the primary neurons of the migraine pain, centrally via the secondary neurons, in Trigeminal Nucleus Caudalis (TNC) in the brain stem, which are relayed to the thalamus then to higher cortical pain regions.

The distribution of GABAergic/glutamergic neurons are abundant in migraine pain-relay centers, including the thalamus and the trigeminal ganglion, reaching approximately 30% of interneurons in caudal part of the TNC in the rat trigeminal nuclei, especially in areas related to the convergence of sensory stimuli. So they could play a critical role in the transmission and modulation of oro-facial nociceptive information conveyed via primary afferents in the trigeminal nerve. Moreover, GABA transporters and glutamate transporters...
have been shown to be co-localized on the same cells in the Central Nervous System (CNS) [7].

Gabapentin is a lipophilic orally active an Amino Acid (AA) that was initially synthesized to mimic the chemical structure of GABA by addition of a cyclohexyl to its backbone. Although it is rapidly absorbed, readily crosses the blood-brain barrier and other lipid membranes via system L amino acid transporters, which allows influxed gabapentin to act on its target in neurons [8].

Gabapentin was originally approved by the Food and Drug Administration for use as an adjuvant medication to anti-seizure drugs, but it has demonstrated analgesic effect in patients with acute post-operative and chronic neuropathic pain and in several animal models of hyperalgesia, diabetic neuropathy, post-herpetic neuralgia and neuropathic pain. Gabapentin prevents neuronal death in vitro and in vivo in models of the neurodegenerative disease [9].

Gamma-Amino Butyric Acid (GABA), the major neurotransmitter for fast inhibitory synaptic transmission and tonic inhibitory control, is present in 25-50% of all synapses, while glutamate is responsible for fast excitatory neurotransmission and is present at approximately 80% of brain synapses [10]. GABA is synthesized from glutamate by removal of an $\alpha$-carboxyl group by Glutamic Acid Decarboxylase (GAD). A disturbance in the actions of these major transmitters is implicated in the pathophysiology of migraine [11].

Migraines attacks occur more frequently in patients with allergy and asthma implying involvement of meningeal and/or brain MCs [3]. These MCs are located perivascular in close association with neurons especially in the dura. Up on degranulation by migraine triggers, they release several neuroactive and vasoactive substances that potentially generate a positive-feedback loop onto sensory neurons, inducing prolonged sensitization of the primary sensory neurons that innervate the dura, thereby have ability to modulate neural activity and nociception and contribute to migraine pathogenesis [3].

Tryptase is a neutral mast cell specific trypsin-like serine protease, the most abundant mediator stored in MC granules, released only after MCs degranulation. It is stored in small amounts in stem cells and basophils [12].

Caffeine is the most widely consumed psychoactive agent in the world. It is a xanthine with various effects and mechanisms of action [13].

Caffeine is considered as nonselective Adenosine Receptor (AR) blocker. Four (ARs) subtypes (AR1, AR2A, AR2B and AR3) have been cloned, all belong to the G Protein-Coupled Receptor (GPCR) family. The effects of caffeine on CNS appear to be mediated primarily by its antagonistic actions at the AR1 and AR2A subtypes [14].

The current study aimed to elucidate the potential effects and the mechanisms of action of a GABA analogue, gabapentin when combined with caffeine in Glyceryl Trinitrate (GTN)-induced brain injury rats, a reliable method to provoke migraine-like headaches in experimental animals and humans.

Material and Methods

Animals: Thirty male albino rats weighing 250-300 grams, were obtained from Faculty of Science, Tanta University. This study was conducted at Faculty of Medicine, Tanta University, on the period between May to July, 2015. Animals were individually housed in a temperature-controlled environment under a 12-hour light/dark cycle and allowed free access to food and tap water.

Ethics statement: All animal care and experimental procedures were carried out in accordance with the National Institutes of Health Animal Care Guidelines (NIH publications No. 80-23), and conducted following the protocol approved by the Committee of the Ethical Use of Animals of the Faculty of Medicine, Tanta University. All efforts were made to minimize the number of animals used and their suffering [15].

Drugs: GTN (Sigma Aldrich, Egypt) was dissolved in isotonic saline; caffeine (Sigma Aldrich, Egypt), gabapentin (Gaptin 400mg, Deltapharm, Co., Egypt). Gabapentin was dissolved in 8ml of warm saline. Solutions were prepared immediately before use. Formalin solution was prepared at 5% in saline from a formalin stock (an aqueous solution of 37% formaldehyde, Sigma-Aldrich, Egypt); evans blue (Sigma, St. Louis, MO, USA). Evans blue was reconstituted in 0.9% saline to obtain the required dose (4ml/kg).

Migraine model was induced by GTN (10 mg/kg, i.p.), a pro-drug for NO, that induces sponta-
neous-like migraine attacks in migraine sufferers and a condition of hyperalgesia in the rats about 4h after its systemic administration. Successful model establishment was confirmed by the presence of ear flushing, scratching the head frequently using forelimbs, increased activities to climb the cage, biting tails, and a reciprocating motion [16].

Experimental groups: The rats were randomly divided into three groups, ten rats each, and underwent to the FT. Rats were assigned to one of the following groups.

Control group: The rats received GTN vehicle (normal saline, i.p.), 4h before the FT. Migraine model (GTN) group: The rats received GTN (10mg/kg, i.p.), 4h before the FT and received (gabapentin/caffeine)’s vehicle. Gabapentin/caffeine treated migraine group: The gabapentin was given at a dose of (200mg/kg), intragastrically by stomach feeding tube [17,18], combined with caffeine at a dose of (3mg/kg, i.p.) [19], two hours after NTG injection and two hours before FT.

Formalin test: Is a well-established rat model of persistent somatic pain, used for the evaluation of possible analgesic effects of combined gabapentin/caffeine [16]. The animals were habituated to the screening chamber for at least 30 minutes prior to testing. One animal was tested at a time. For the FT, 50 µl of 5% formalin solution was injected subcutaneously, as rapidly as possible while the animal was immobilized, into the center of the plantar surface of the right hind paw. The animal was then moved to an elevated clear plastic cage, bitten tails, and a reciprocating motion [20].

Immediately following injection, the animal’s nociceptor behavior was recorded over a period of 60 minutes during which the total number of flinches and shakes were counted during the period from 1 to 5min (phase 1 or early phase) and, subsequently, every 5-min intervals during the period from 15 to 60min (phase 2 or late phase) after formalin injection [21].

Flinches/shakes were readily discriminated as rapid and brief withdrawal movements or flexion of the injected paw [20]. The anti-nociceptive effect was determined by evaluating combined (gabapentin/caffeine)’s ability to quantitatively attenuate the number of the formalin evoked nociceptive responses.

Evan's Blue Dye dural (EBD) extravasation:

Evans blue (dye which complexes with plasma proteins) technique is routinely used as an indicator of Plasma protein extravasation [2,3]. Animals were injected with 4ml/kg, i.p., of 2% EBD in saline, immediately after NTG or vehicle injection. After FT, the animals were deeply anesthetized by ketamine/xylazine (50/5mg/kg). The thorax was opened and the blood was collected immediately from heart. The rats were perfused with 37°C oxygenated phosphate-buffered saline through the left ventricle, to remove the intravascular dye, until colorless perfusion fluid outflowed from the right atrium.

The brain was carefully removed and the cranial cavity rinsed with saline to remove residual blood and Cerebrospinal Fluid (CSF) prior to dissection of the dura. The dura covering supra-tentorial region of the brain, primarily innervated by trigeminal nerve [22], was harvested. To assess the dural extravasation, dural tissue was collected, weighed and homogenized in 1ml of 50% trichloroacetic acid (wt/vol). After centrifugation (12,000 X g, 20 minutes), supernatant was collected and mixed with ethanol (1:3). The concentration of EBD was carried out with a spectrophotometer (Biotech Engineering Ltd., UK) at 610nm absorbance and tissue content of EBD was quantified from a linear standard curve and expressed in terms of Evans blue (µg/tissue (g)).

Serum total tryptase assay: Blood samples were collected. Allow samples to clot for 2 hours. The serum samples were centrifuged at 1000 X g for 15 minutes at room temperature and stored at –80°C until analysis. Serum tryptase level, as a selective marker of MC activation, was determined by a sandwich enzyme immunoassay (ELISA) using Rat Tryptase ELISA Kit (Medico Trade Co., Cairo, Egypt). The lower limit of sensitivity using 50 µl of serum per assay was 0.1ng/ml [23].

GABA and glutamate levels in TNC were assayed by high performance liquid chromatography-electrochemical detector (HPLC-ECD), as described previously by the modified method of Allison LA et al., [24].

The trigeminal system is strongly implicated in the initiation of the headache pain. No matter whether a peripheral or a central site is the prevailing source for activation; all painful trigeminal sensations are conveyed by the Trigeminal Nucleus Caudalis (TNC), serving as rationale to examine neuronal dysfunction associated with migraine [24].
Brains and Brainstems were quickly dissected out on an ice-cold plate as previously described [25]. The TNC samples were dissected corresponding the caudal part of spinal trigeminal nucleus in the brain stem according to brain atlas Paxinos and Watson [26].

The TNC samples were removed, weighed and placed in microcentrifuge tubes, which contained 1 ml of chilled homogenisation buffer (0.1M citric acid, 0.1M sodium dihydrogen phosphate monohydrate, 5.6mM octane sulfonic acid, 10_m EDTA in 10% (v/v) methanol solution, pH 2.8 with 4M NAOH). Each sample was centrifuged at 14,000rpm at (4ºC) for 15min and the supernatant stored at –80ºC until derivatisation for GABA/glutamate analysis [27].

Small aliphatic amino acids, such as Glutamate and γ-Amino-Butyric Acid (GABA) are not naturally electroactive and do not possess fluorescent or strong UV absorbance characteristics, rendering their analysis by HPLC problematic. Precolumn derivatisation can overcome this drawback [28]. One of the most commonly used derivatising agents is o-phthalaldehyde (OPA) which reacts with primary amines in the presence of a thiol and generates derivatives which are both electroactive and fluorescent [29].

GABA and glutamate were identified by their characteristic retention times as determined by standard injections which were run at regular intervals during sample analysis. Sample peak heights were measured and compared with standard injections in order to quantify the amino acids. The GABA and glutamate levels were represented in nmol/mg tissue protein. The total protein in brain tissue homogenate was assayed by Bradford [30].

Statistical analysis: Statistical analysis was performed using GraphPad Prism version 4 (Graphpad Software Inc., La Jolla, CA, USA), using one-way analysis of variance (ANOVA). A value of p<0.05 was considered to be statistically significant. All data are expressed as the means ± Standard Deviation (SD). The number of formalin-induced flinches was recorded within 5 minute bins per animal. Analyses of sums of flinches within each phase of the test were performed by ANOVA test followed by Tukey-Kramer post-hoc analysis.

The effects of the gabapentin/caffeine on formalin-evoked pain-related behaviors were expressed as a percent inhibitory effect on the total behaviors in phase 1 and phase 2 [31].

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\text{Inhibition} \% = \frac{\text{Total number of vehicle behavior} - \text{Total number of drug behavior}}{\text{Total Number of vehicle behavior}} \times 100
\]

Results

Effect of combined gabapentin/caffeine on Formalin induced nociception Fig. (1):

The formalin injection in the control group resulted in a highly reliable, typical, biphasic pattern of flinches/shakes of the injected paw, being characterized by an initial acute phase of nociception within the first 5min, followed by a prolonged tonic response from 15 to 60min after formalin injection, with a quiescent phase between them.

NTG administration significantly increased the total number of flinches/shakes in both phases specially phase II of FT, when compared to control group, reflecting NTG-mediated hyperalgesia and confirming previous reports [16].

The gabapentin/caffeine significantly inhibited the formalin induced nociceptive behaviors during phase I, by about 30.4%, and proved effective in counteracting NTG induced hyperalgesia in phase II, as suggested by the marked reduction of the nociceptive behavior by about 77% of animal pretreated with NTG. In general, the pain behavior response in the gabapentin/caffeine group was significantly lower than in all other groups throughout the period of test.

Effect of combined gabapentin/caffeine on serum total tryptase Fig. (2):

The serum tryptase was significantly elevated in migraine model by about 3-fold compared to the control group, suggesting that MC activation may be involved in the migraine pathophysiology. Gabapentin/caffeine resulted in marked stabilization of the MCs with significant reduction in tryptase value to be comparable to that of the control group.

Effect of combined gabapentin/caffeine on NTG induced EBD leakage Fig. (3):

Plasma protein extravasation, measured as EBD leakage, is a component of neurogenic inflammation, frequently used endpoint for quantification of the effects of trigeminal stimulation [22].

GTN injection induced marked plasma protein leakage within rat dura mater by about 2.7-fold. While it is markedly attenuated by combined gabapentin/caffeine by about 2.3-fold.
Effect of combined gabapentin/caffeine on GABA and glutamate levels in TNC tissue homogenate Fig. (4):

With induction of migraine model, there were up regulated glutamate and GABA levels by about 1.4- and 1.1- folds respectively. Combined gabapentin/caffeine treatment of migraine group resulted in profound modulation of these neurotransmitters, with reduction in glutamate level by about 1.3-fold and still further increase in GABA level by about 1.1-fold.

Fig. (1): Effect of gabapentin/caffeine on pain-related behavior in the formalin test in rat migraine model (A). The horizontal axis is time post-formalin injection (min) over 1 hour and the vertical axis is total number of formalin-induced behaviors counted every 5 minutes. Total number of formalin-induced behaviors during phase 1 (1-5min) (B) and phase 2 (15-60min) (C). Data are presented as the mean ± SD of 10 rats at each time point.

*: p<0.05 vs. control group.
#: p<0.05 vs. GTN group.
GTN: Glyceryl Trinitrate.

Fig. (2): Box and Whisker Plot of the effect of gabapentin/caffeine on serum total tryptase. Lines in boxes are means, ends of boxes are quartiles, and whiskers show the range of values.
*: p<0.05 vs. control group.
#: p<0.05 vs. GTN group.
GTN: Glyceryl Trinitrate.

Fig. (3): Effect of gabapentin/caffeine on dural evan blue extravasation. Data are presented as the mean ± SD of 10 rats each group.
*: p<0.05 vs. control group.
#: p<0.05 vs. GTN group.
GTN: Glyceryl Trinitrate.

Fig. (4): Effect of gabapentin/caffeine on GABA and glutamate levels in TNC tissue homogenate. Data are presented as the mean ± SD of 10 rats each group.
*: p<0.05 vs. control group.
#: p<0.05 vs. GTN group.
TNC : Trigeminal Nucleus Caudalis.
GTN : Glyceryl Trinitrate.
GABA : Gamma-Amino Butyric Acid.
Discussion

Migraine is ranked as the third most prevalent disorders and the seventh highest cause of disability in the world. It is a complex primary headache, with multiple phenotypes that reflect disturbances in both brain and/or vascular functions. It seems to involve different pathophysiological mechanisms named as "low" serotonin, neurogenic inflammation and dopaminergic hypersensitivity while disrupted balance between inhibitory and excitatory neurotransmitters is a critical step in migraine circuits. Which implies that addressing one of the involved biological systems will always stand below the ideal and offers multiple targets for therapeutic intervention [2].

To address this issue, we used, caffeine with gabapentin, GABA analogue in GTN induced migraine rat model.

There are two behavioral phases following hind paw formalin injection which are markedly distinct in terms of neurological mechanisms. The early phase is mediated by an intense barrage of peripheral nociceptor activity, followed by the quiescent phase, and the subsequent late phase is mediated by sustained spinal dorsal horn neuron hyperactivation, driven by the combined effects of changes to dorsal horn synaptic neurochemistry and prolonged nociceptor afferent activity. Both quiescent and late part of phase II could be mediated via possible recruitment of active inhibitory mechanisms [20].

NTG induce hyperalgesia possibly attributed to the activation of spinal and brainstem structures involved in nociception directly via NO involving transcriptional processes by upregulation of NO-producing neurons and CGRP or indirectly via the activation of NOS synthesis at the meningeal level as a consequence of a sensitization of the TNC. NTG-potentiated FT is a relevant model for investigating migraine circuit [16].

The combined gabapentin/caffeine exhibited profound anti-nociceptive effect especially in phase II. Gabapentin' analgesic activity has been previously demonstrated in a wide range of animal pain models [32,33] and in patients with acute postoperative and chronic pain [9] and became one of 1st choice treatments for chronic pain.

The mechanisms underlying the gabapentin/caffeine-mediated modulation of NTG-induced hyperalgesia are not clearly understood. It seems that gabapentin/caffeine abrogate the final common pathway of migraine mechanism. They may act centrally rather than peripherally and promptly inhibit incoming activity from the trigeminal system involved in meningeal nociception. However, the trigeminal ganglion itself has not been excluded as an important receptor site and may function as a central component in the postulated mechanism [32,33].

Initially, the mechanisms of gabapentin's action thought to involve the modulation of GABA-ergic transmission, later it seems more related to high affinity of gabapentin to bind to the alpha-2 delta-1 (α2δ-1) auxiliary subunit of voltage-sensitive Ca2+ channels, in brain tissues, the blockade of neuronal calcium influx [34].

Indeed, gabapentin, as an N-methyl-D aspartate receptor and voltage-gated calcium channel blocker, can decrease intracellular Ca2+ and diacylglycerol levels and inhibit the activation of protein kinase C, thereby inhibiting the positive feedback loop and reducing excitatory AA, glutamate release, reducing spinal trigeminal nucleus neuronal excitability and aborting formation of peripheral and central sensitization during migraine [35].

The gabapentin evoked analgesia could be enhanced by caffeine, as an AR blocker evoked antinociception results from an increase in K+ conductance and presynaptic inhibition of sensory nerve terminals to inhibit the release of substance p and perhaps glutamate [14].

The intrinsic analgesic activity of caffeine is well documented in multiple controlled randomized double-blind [36] and experimental studies [14]. It has direct analgesic effects by elevating nociceptive thresholds, mostly centrally mediated by binding in a non-selective way to ARs in the brain [14].

Caffeine is used in several analgesic preparations due to its central cholinergic analgesic properties, through up regulation of muscarinic and nicotinic receptors by about 40-50%, enhancing central cholinergic transmission, that is blocked by atropine [37].

Modulation of serotonergic neurotransmission might be one mechanism of the analgesic effects of gabapentin/caffeine. Both gabapentin [38] and caffeine increased brain levels of both serotonin and 5HT1/5HT2 receptors protein by 26-30%, with particularly pronounced inhibition of serotonin re-uptake, rising the synaptic availability of serotonin [39]. Thus mimicking triptans (5HT1B/1D) receptor
agonist), the gold standard in acute migraine therapy, to date [1].

Independently of adenosine antagonism, caffeine is able to downregulate the brain beta-adrenergic receptors by up to 25% [40]. β-adrenoceptors are implicated in migraine pathophysiology and their antagonists are effective preventive treatments for migraine [1].

Through induced upregulation of delta the opioid receptor subtypes in some brain areas, caffeine can effectively relieve pain [41].

The repeated stress is one of the well-known migraine triggers, that elicits neurochemical and morphological changes that negatively affect brain functioning. Caffeine has a unique mechanism of action among all centrally stimulating drugs; it alleviates the fatigue and asthenia with its light mood-elevating effects. It emerges as candidate therapeutic target for migraine associated depression and memory deterioration because they control aberrant synaptic plasticity and afford neuroprotection associated with stress [42].

Despite demonstration of α2δ I subunits as molecular targets of gabapentin action, the identity and neuronal circuits essential to gabapentin analgesia remain obscure. To investigate the potential role of central neurotransmitters in the pathogenesis of migraine, and their contribution in gabapentin/caffeine analgesia, we measured glutamate and GABA levels in TNC tissue homogenates in our migraine model.

Effective brain and behavior function depends on a carefully orchestrated balance of inhibition and excitation in the CNS. That dynamic is largely dependent on the actions of glutamate and GABA.

The results of the present work pointed to increased GABA/glutamate levels in migraine group.

It has been reported that migraineurs had substantially higher plasma [43] and CSF [44] glutamate levels, between attacks, than did controls and tension headache patients. Which became still further elevated during a migraine attack, most probably attributed to defective cellular reuptake mechanism for glutamate at the neuronal/glial cell levels, and possibly favored by impaired mitochondrial function [45], predisposing the brain of migraineurs to develop spreading depression, that may participate in the triggering of attacks and central sensitization possibly by favoring a state of neuronal hyperexcitability [46].

The elevated GABA concentrations observed with migraine induction could reflect the tonic extrasynaptic inhibition as a compensatory negative feedback response to neural hyperexcitability, that will established in migraine, triggered by elevated glutamate level and increased release of substance p, that known to be up regulated in TNC in migraine [47]. Where previous results pointed out that inhibitory interneurons play an important role in regulating the excitatory neuronal activities in the TNC by releasing GABA [48]. GABA is believed to be involved in both pre-synaptic and post-synaptic inhibition in the spinal dorsal horns and TNC [6].

Our results referred to increase in the GABA content along with the increase of glutamate, possibly to maintain the dynamic homeostasis between two transmitters. Previous data suggest that brain GABA and glutamate transmission maintain a functional balance by reciprocal modulation of excitatory and inhibitory neurotransmitter release [7].

It appears that concentrations of either GABA or glutamate may be influenced by the other, where the increase in extracellular GABA may, by activating GABA transporters, provoke glutamate release by opening anion channels or by reversing glutamate transporters [7]. Similarly, an increase in extracellular glutamate may stimulate nonvesicular GABA release by activating glutamate transporters located on GABAergic cells [7].

The combined gabapentin/caffeine treatment of migraine group resulted in enhancing GABAAergic with concomitant damping of glutamergic levels. Research regarding gabapentin’s effects on GABA and glutamate synthetic and metabolizing enzymes reveals a complex pattern of activity [49].

Gabapentin has been shown to increase in vivo GABA concentration in the brain of both rodents and humans [38], in a concentration dependent manner [49]. This gabapentin enhancing effect on GABA release was enhanced by caffeine [50] and blocked by the GABAA receptor antagonists, implicating GABAA receptors in its effect [49].

Human subjects studies suggest that gabapentin administration leads to an overall increase in central GABA levels [8]. Gabapentin stimulates GAD, the enzyme responsible for the majority of GABA synthesis and its levels correlate strongly with GABA tissue levels. Moreover, gabapentin inhibits the GABA-catabolizing enzyme, GABA-transaminase, which may enhance non-vesicular GABA release [8].
At the same time, the decreased glutamate level obtained with gabapentin/caffeine treatment could be attributed to inhibition of glutamate synthesis by branched-chain amino acid aminotransferase [38], a mechanism that may be responsible for neuroprotective effect of gabapentin. Gabapentin activates of glutamate dehydrogenase, the central enzyme in the metabolism of glutamate [49].

Gabapentin blocked the substance \( p \)-mediated increase of glutamate release from the rat spinal TNC in vitro, ensuring the possible mechanistic relevance to the anti-hyperalgesic/allodynic gabapentin’ actions [34].

We detected augmented plasma protein extravasation in dura mater, a marker of inflammation, together with elevated serum tryptase, a surrogate marker of MC degranulation, with GTN injection. These data suggest that GTN, either directly or indirectly, produces a delayed inflammation within rat dura mater with significant MCs degranulation [51]. The degranulating dural MCs contribute to activation of trigeminal pain pathway via sensitization of meningeal and spinal-level trigeminal nociceptors, possibly through lowering the local pH in the dura and sensitization of the dural afferent nerves via acid-sensing ion channels [52].

Inflammation within dura mater, as one of the important pain-producing intracranial structures, has been proposed as fundamental to the pathogenesis of migraine headaches. This idea is supported by increased intracranial levels of inflammatory mediators and the efficacy of anti-inflammatory drugs during migraine attacks [53].

Several biological and immunological investigations have implicated tryptase in the pathophysiology of headaches. It has an important role in inflammation, is implicated as a mediator in the vascular leakage and sensitization of meningeal nociceptors. Moreover tryptase cleaves and activate proteinase activated receptor-2 of meningeal nociceptors amplifying the initial vasodilatation caused by neuropeptide and possibly the central transmission of nociceptive signals [54].

One of the proposed anti-migraine mechanisms for the gabapentin/caffeine is through their direct effects on dural arteries. Caffeine block Adenosine induced A2aR dependent middle meningeal artery relaxation, leading to preserved expression levels of tight junction proteins, inhibition of cAMP phosphodiesterase activity, by affecting the release of calcium from intracellular stores [55].

Signs of dural neurogenic inflammation were effectively attenuated by combined gabapentin/caffeine. Caffeine proved to decrease prostaglandin-E2 synthesis in microglia cells and mitigates the protein synthesis of the cyclooxygenases-2, both play a key role in pain and inflammation adding a further mechanism leads to substantial pain inhibition [56].

**Conclusion:**

The combined gabapentine/caffeine have robust ability to alleviate formalin induced nociceptive behaviors and abolished NTG induced hyperalgesia possibly through abrogating the neurogenic inflammation and restoration of brain mitochondrial bioenergetics. This combination provides a guarantee of a precise therapeutic multi-target approach, in which the active substances act on different but distinct molecular targets and thus are able to act on more signaling cascades involved in pain than most single analogics. So they can work efficiently to provide consistent rapid relief, could represent a valid strategy for the migraine treatment.

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**Conflicts of interest:**

There are no actual or potential conflicts of interest.

**References**


الملخص العربي

الخلاضية العلمية: يعد الصداع النصفي مرعب معقد يتضمن خلل في الأوعية الدموية والتقنية العصبية. يعتبر جابابينتين نظير تركيب لحمض جاما بييتركل الأميني مضاد للتشنجات ومسكن قوى. بينما يعد الكافيين مادة مساعدة في تركيب المسكنات.

الهدف من البحث: تقييم التأثير العلاجي المحتمل لحمض جاما بييتركل الأميني، جابابينتين، والكافيين معاً في تموذج الفئران للصداع النصفي المستحدث بالنيتروجيلسرين والآليات الكامنة وراء هذا التأثير.

طريق البحث: تم تقسيم ثلاثين فئراً من ذكور الفئران البيضاء إلى ثلاث مجموعات متساوية، تعالج إما بالنيتروجيلسرين (NTG) 10 مجم لكل كيلو جرام من وزن الجسم عن طريق الحقن بالغشاء البريتوني أو المحلول الملح. تعتمد الفئران المعالجة بالنيتروجيلسرين إما جابابينتين والكافيين على المحلول الملقى. اختبرت الفئران الـ 63 مجموعة الضابطة. تم إجراء اختبار الفيسيولوجي (FT) لتقييم القدرة على تسكن الألم. تم تقييم الالتهاب بالبطاقة البلازما عن طريق قياس مدى تسرب صبغة ليفانز الزرقاء (EBD). تم قياس كل من المستوى الكلي لغرانتازيا وسبريم مؤشر لتفتيت الخلايا الباطنية ويعاب باستخدام الجلوكوزيك ومستويات كيميائية للحمض جاما بييتركل الأميني المستخلصات للنواة المثلثة الدندنية.

النتائج: أظهر العلاج بكل من جابابينتين/الكافيين القدرة على تخفيض الألم إلى حد كبير في مراحل إختبار الفيسيولوجي خصوصاً في مرحلة الثانية، بينما من خلال إستقرار الخلايا الباطنية، التأثير المضاد للالتهاب مع استعادة التوازن بين الناقلات العصبية.

الخلاصة: تشير هذه النتائج إلى أن قدرة من كل من جابابينتين/الكافيين معاً على إنهاء نوبة الصداع النصفي وعمليات إغاثة علاجية محتملة لعلاج الصداع النصفي.