



Comparative Efficacies of Antimigraine Drugs Using Nitroglycerin Induced Migraine Rats Model

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Authors' contributions

This work was carried out in collaboration between both authors. Authors ASM and VP designed the study and protocol. Author ASM performed the all experimental part, statistical analysis and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The migraine pathology is still not explained effectively. There is a common relationship between anxiety, depression, and migraine. So the aim of the study to illustrate effectively the behavioural and biomarker changes in the migraine condition.

Methods: Nitroglycerin (NTG) induced migraine rats model was used the present study. Twenty-five male Wistar rats were randomly divided into five groups. Ergotamine, sumatriptan, and BIBN4096 were used as antimigraine drugs. The behavioural activity was measured by scratching head, body shaking, and social interaction task. ELISA detected biomarkers like interleukin 6 (IL-6), Substance P (SP) and 5-hydroxytryptamine (5-HT) in various rats brain regions such as cortex, brain stem, trigeminal ganglion.

Results: A significant reduction in hyperalgesic response and behavioral changes like scratching head, body shaking and social interaction task. Biomarkers like 5-HT, SP and IL-6 were significantly reduced in the various brain regions such as prefrontal cortex, brain stem and trigeminal ganglia of the rats in the BIBN4096 treated groups.

Conclusion: The present study showed a good antimigraine efficacy with a calcitonin gene-related

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peptide (CGRP) antagonistic agent BIBN4096 than ergotamine and sumatriptan but still lack of behavioural pattern, need to explore nonpharmacological intervention along with the drug treatment.

Keywords: Migraine; nitroglycerin; BIBN4096; social interaction task; 5-HT.

1. INTRODUCTION

Migraine is a common, multi-factorial, neurovascular disorder with significant individual and societal effects [1-3]. Epidemiological and clinical studies have shown that primary headaches, particularly migraine, have a bi-directional relationship with depression and anxiety [4–8].

Nitroglycerin (NTG) reliably causes headaches in normal subjects. NTG-evoked migraine is a commonly used human experimental paradigm [9-12]. Hyperalgesia evoked by NTG in rodents has been developed as a model for sensory hypersensitivity associated with migraine. Acute NTG produced thermal and mechanical allodynia in mice that had been reversed by sumatriptan anti-migraine therapy [13,14]. Overall, these results indicate that the effects of NTG may effectively modulate migraine-like symptoms in rodents.

Ergotamine, which was previously the mainstay of therapeutic management, can no longer be considered the preferred treatment for acute migraine [15]. Ergotamine is very helpful in particular situations, such as treating very long attacks with recurrence of headaches. In addition to many vascular problems, overuse can lead to severe headaches [16]. Triptans, which are serotonergic 5-HT_{1B/1D} receptor activators, have revolutionised many patients with chronic migraine attacks, which are the most potent way of stopping migraine attacks. The main concern for triptans is their rare but serious cardiovascular side-effects, so it is not used in patients with the cerebrovascular or cardiovascular conditions [17].

The neuropeptide calcitonin gene-related peptide (CGRP) is well documented to play a critical role in pain's pathophysiology. Specifically, the significance of CGRP to migraine pain has also been extensively studied preclinically [18-20] and clinically [21-24]. BIBN4096BS was the first CGRP receptor antagonist to be evaluated in clinical trials [25] and was shown to be effective in migraine therapy [26]. It also decreases migraine pain and neuronal trigeminal activity.

Since migraine is well correlated with depression and anxiety, the authors are trying to provide insight and influence of BIBN4096, a CGRP antagonist, towards behavioural and biomarker changes in the nitroglycerin-induced migraine model.

2. MATERIALS AND METHODS

2.1 Drugs and Vehicles

Experiments were performed using a commercially available preparation of nitroglycerin at 5 mg/ml (Neon Laboratories Ltd, Mumbai, India), intraperitoneal (i.p) at a dose of 10 mg/kg. The drugs ergotamine and sumatriptan were received as gift samples from Dr Reddy's laboratory, Hyderabad. BIBN4096 was purchased from Tocris Bioscience (Bristol, UK), which was diluted in saline with dimethyl sulfoxide (DMSO) and slowly injected subcutaneously (1 mg/kg).

2.2 Collection

Adult male Wistar rats weighing 180–250 g, were purchased from the Central Animal House, Annamalai University. All rats had free access to food and water except during behavioural observations, and a constant temperature (25±1°C) and a 12:12 hr cyclic lighting schedule were maintained.

2.3 Experimental Design

Twenty-five Wistar rats were randomly divided into five groups consisting of five animals each: (A) Control group, where the Wistar rats received an i.p injection of normal saline with DMSO; (B) NTG group, where the Wistar rats received an i.p injection of 10 mg/kg body weight of NTG in alternative days consecutively for the first week (five doses) and followed by weekly once up to four weeks; (C) NTG + Ergotamine; (D) NTG + Sumatriptan; (E) NTG+ BIBN4096.

2.4 Tail-Flick Test

The test was performed with an Ugo Basile tail flick instrument (model 7360) that allowed

automatic recording of the latency of the tail-flick response to radiant heat. The number of seconds elapsing between activation of the heat source and the rat flicking its tail away (latency) was recorded. Each evaluation was calculated as the mean of three measurements in three different parts of the tail [27].

2.5 Behavioural Research

The behavioural evaluation was a vital examination in experimental migraine models. The behavioural characteristics such as reddening of ears, cage climbing, body shaking, scratching the head frequently lasted for 2h [28]. In the present study, we observed and evaluated the number of scratching the head and body shaking after modelling in unit time.

2.6 Social Interaction Task

The social interaction task for rats was tested in various experimental conditions by placing them into a 120 × 80 cm plexi glass box divided into three equal chambers. The rats received different experimental conditions set into the center of the chamber for 5 min, and they were allowed to freely move between the chambers through a small opening of a 20-cm square in each divider. The unfamiliar, same-sex rats were placed in a wire cage with 11-cm height and a bottom diameter of 10-cm. The space between each bar is 1cm for one side of the chamber. Another empty wire cage was placed on the opposite side of the chamber, which was active as a non-social object. The behaviour of the rats was recorded for 10 min. The testing chambers were cleaned thoroughly, using 70% ethanol before and after the experiment with rats [29].

2.7 Detection of Biomarkers in Various Brain Regions

After scarification of the rats, the whole brains were rapidly dissected, placed on dry ice and cortex, brain stem and trigeminal ganglia were isolated. The expression of 5-HT, SP and IL-6 levels were measured by ELISA with detection kits, following the manufacturer's instructions.

2.8 Statistical Analysis

The statistical analyses of the data were conducted using Graph pad prism 7.0 software. The data are expressed as mean ± SEM. Analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple

comparisons test. Statistically significance were considered as a $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Tail-Flick Test (TFT)

Peripheral and central sensitizations are implicated in the hyperalgesia and allodynia that accompany migraine [30]. The tail-flick test reflects acute physiological pain and outcomes from the activation of A δ primary afferents and unmyelinated C-fibers. Nitroglycerin is a lipid-soluble substrate that quickly passes the blood-brain barrier and accumulates in brain tissue and reflecting the endogenous nitric oxide (NO) effects [31] and lasts for several hours [32]. Fig. 1 shows nitroglycerin induced hyperalgesia; the NTG group showed a significant reduction in the tail-flick latency compared with the control group ($P < 0.01$). Four hours later tail-flick latency was evaluated in all groups after NTG or NTG with drug administration. There was no significant difference in NTG + Ergo ($P < 0.75$) and NTG + Suma ($P < 0.20$) group. BIBN4096 group showed a significant difference when compared with the NTG group ($P < 0.05$). Therefore it is speculated that nitroglycerin-induced hyperalgesia may be connected to NO-mediated enablement of nociceptive transmission in peripheral tissue and spinal cord and it is well correlated with previous findings [33].

3.2 Migraine-Like Behavioural Response in Rats by NTG

The brain's areas involved in the body's behaviour and head shaking are not known but are linked to the perception of pain. NTG treated rats did not show a significant head-scratching affect up to 20 min compared to control animals. However, statistically increased head-scratching time detected when compared with the control group. This effect started within 20 min, peaked at 20–40 min (15.33 ± 1.20 , $p < 0.001$), and lasted for 80 min. Animals in both NTG+Ergo and NTG+Suma groups showed a timing peak scratching head of 0 to 20 min and decreased at 60 min. NTG+BIBN4096 significantly lower head scratches during 20–40 min (5.00 ± 0.58 , $p < 0.001$) and inactive at 40 min (Table 1) compared to the NTG group.

Table 2 showed several body shakings in 0–40 min after NTG administration compared to control rats. NTG+ BIBN4096 treatment significantly reduced body shaking times from 20

to 40 min compared with the NTG group ($p < 0.001$). The result observed that NTG-induced migraine rats were effectively established and that NTG+BIBN4096 reduced migraines like NTG-induced pain and reduced pain responses.

In this model, the changes were observed in pain perception, like reduction in the latency of the tail-flick test and a typical manifestation of

migraine-like scratching head and body shaking. The increased level of Nitric Oxide in a person's blood indicates that this individual has a head discomfort. NTG significantly increased the expression of two pain-related peptides CGRP and neuropeptide Y (NPY) in sera [34]. These reports agree with the earlier study that NO, CGRP was detected in plasma and various parts of the brain region [33].

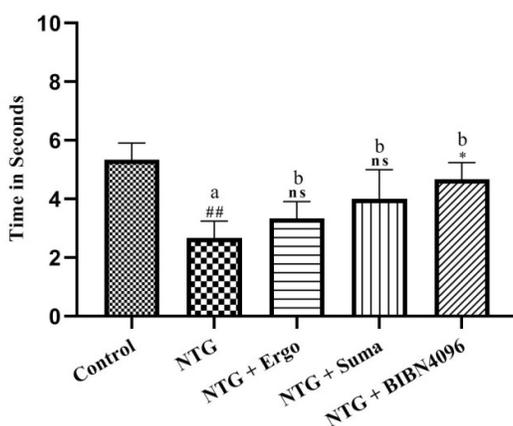


Fig. 1. The hyperalgesic response induced by nitroglycerin in the tail-flick test

The data represented are Mean ± SEM. *a* denotes comparing with control group vs NTG group, *b* denotes comparing NTG group vs treated groups. Where, ## $p < 0.01$, * $p < 0.05$, *ns* denotes nonsignificant. $p < 0.05$ are considered as statistically significant. One-way ANOVA with Tukey's post hoc test was used for statistical analysis

Table 1. The numbers of head scratching during various time periods

Groups	0-20 min	20-40 min	40-60min	60-80 min	80-100 min
Control	7.00 ± 0.58	3.30 ± 0.88	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
NTG	8.00 ± 0.58 ^{a,ns}	15.33 ± 1.20 ^{a,###}	11.67 ± 0.68 ^{a,###}	2.00 ± 0.58 ^{a,ns}	0.00 ± 0.00
NTG+ Ergo	11.00 ± 0.58 ^{b,**}	11.33 ± 0.33 ^{b,ns}	3.33 ± 0.67 ^{b,***}	0.00 ± 0.00	0.00 ± 0.00
NTG+Suma	10.67 ± 1.02 ^{b,*}	7.33 ± 0.88 ^{b,**}	2.00 ± 0.58 ^{b,***}	0.00 ± 0.00	0.00 ± 0.00
NTG+BIBN4096	10.00 ± 0.58 ^{b,ns}	5.00 ± 0.58 ^{b,***}	0.00 ± 0.00 ^{b,***}	0.00 ± 0.00	0.00 ± 0.00

Data were presented as mean ± SEM. ^a denotes comparing with control group vs NTG group, ^b denotes comparing NTG group vs treated groups. Where, ### $p < 0.001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, *ns* denotes nonsignificant. $p < 0.05$ are considered as statistically significant. One-way ANOVA with Tukey's post hoc test was used for statistical analysis

Table 2. The numbers of body shaking during various time periods

Groups	0-20 min	20-40 min	40-60min	60-80 min	80-100 min
Control	4.00 ± 1.12	0.67 ± 0.33	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
NTG	5.33 ± 1.45 ^{a,ns}	10.00 ± 0.58 ^{a,###}	2.33 ± 0.67 ^{a,ns}	0.33 ± 0.33 ^{a,ns}	0.00 ± 0.00
NTG+Ergo	6.00 ± 1.00 ^{b,ns}	8.00 ± 0.58 ^{b,ns}	1.33 ± 0.67 ^{b,ns}	0.00 ± 0.00	0.00 ± 0.00
NTG+Suma	5.33 ± 0.88 ^{b,ns}	5.33 ± 0.67 ^{b,**}	1.00 ± 0.58 ^{b,ns}	0.00 ± 0.00	0.00 ± 0.00
NTG+BIBN4096	3.67 ± 0.67 ^{b,ns}	2.00 ± 0.58 ^{b,***}	0.67 ± 0.33 ^{b,ns}	0.00 ± 0.00	0.00 ± 0.00

Data were presented as the mean ± SEM. ^a denotes comparing with control group vs NTG group, ^b denotes comparing NTG group vs treated groups. Where, ### $p < 0.001$, *** $p < 0.001$, ** $p < 0.01$, *ns* denotes nonsignificant. $p < 0.05$ are considered as statistically significant. One-way ANOVA with Tukey's post hoc test was used for statistical analysis

3.3 Effect of BIBN4096 in Social Interaction Task

Many studies have found a relationship between migraine and anxiety/depression [35,36]. A recent study has reported that repeated NTG in rats elicits clinically relevant responses to behavioural endpoints of migraine [37]. The social behaviour of the animals was assessed using the 3-chamber social interaction instrument. There was a significant decrease in interaction with the inanimate object in treated animals with NTG ($p < 0.001$) compared to the control group. However, a significant increase in active connection was observed in animals treated with NTG+Suma and NTG+BIBN4096 ($p < 0.05$) when compared with the NTG group (Fig. 2A). In sociability, significantly decreased active contact with foreign rats in NTG ($p < 0.001$) group, but no significant effect in NTG+Ergo ($p < 0.12$), NTG+Suma ($P < 0.09$), and NTG+BIBN4096 ($p < 0.06$) compared with the NTG group (Fig. 2B).

We observed rats' behaviours after NTG injection, including peripheral, central, grooming and rearing in the open field tests, time spend in open arm and closed arm in plus elevated maze [33] and social interaction test as well. All behaviour patterns of anxiety and depression show symptoms similar to migraine headaches. Corticosterone binds with mineralocorticoid and glucocorticoid receptors in the brain. It regulates various cellular and molecular systems involved in neurogenesis, synaptic plasticity, and dendritic remodeling [38], leading to deficits in

affective behavior and impaired social cognition. The neurochemical imbalance of the HPA axis has been well researched in human anxiety and depression [39]. The result of the report agreed with the finding of this present study.

3.4 Effect of BIBN4096 in Various Biomarkers

The 5HT level in the prefrontal cortex of NTG group rats were significantly ($p < 0.001$) decreased when compared with the control group. Whereas, the treatment of NTG + Suma ($p < 0.05$) and NTG + BIBN4096 ($p < 0.01$) significantly improved the level of 5HT (Fig. 3A), but in NTG + Ergo group showed nonsignificant ($p < 0.23$) when compared with NTG induced group. After NTG induction, the SP level in the brain stem (BS), cerebral cortex (CT) and trigeminal ganglia (TG) showed significantly increased ($p < 0.001$) when compared with control group. But there is no significant difference in NTG + Ergo group, while in NTG + Suma ($p < 0.05$) and NTG + BIBN4096 ($p < 0.001$) exhibit a significantly decreased amount of SP in the brain region such as BS and CT. Whereby in TG there is no significant difference between NTG + Ergo, NTG + Suma and NTG + BIBN4096 when compare with positive control group (Fig. 3B). In IL-6 detection, the NTG induced group showed a significant increased ($p < 0.05$) in BS, CT and TG. Interestingly, all the groups indicate significantly decreased ($p < 0.001$) expression of IL-6 in BS. However, in CT and TG, no significant difference was observed when compared with the induced group (Fig. 3C).

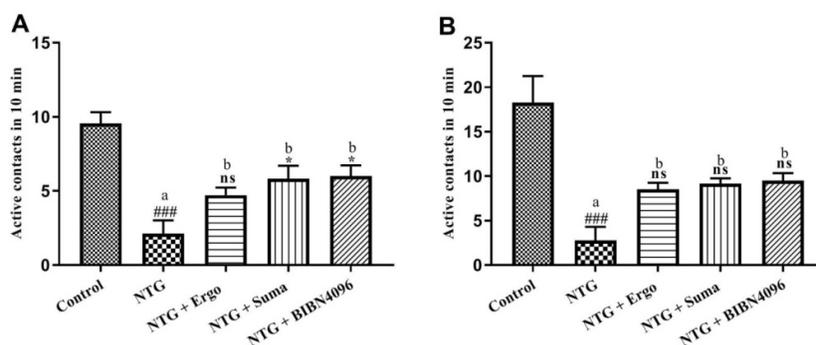


Fig. 2. Effect of environmental enrichment on social interaction task. (A) Sociability test with an inanimate object; (B) Sociability test with stranger rat

The data represented are the Mean \pm SEM. Where, ^acomparing with control vs NTG, ^bcomparing NTG vs treated groups. ### $p < 0.001$, * $p < 0.05$, ^{ns}denotes nonsignificant. $p < 0.05$ considered as statistically significant. One-way ANOVA with Tukey's post hoc test was used for statistical analysis

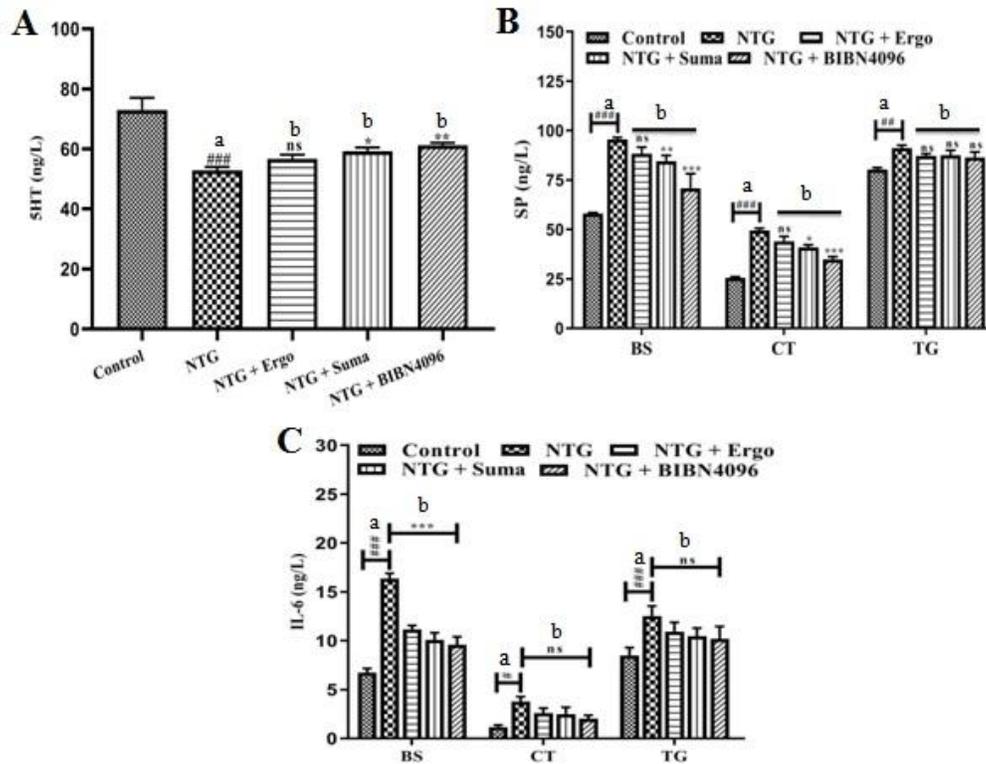


Fig. 3. BIBN4096 in nitroglycerin induced biomarkers expression in various regions of rat's brain (A) 5HT (Prefrontal cortex), (B) SP, (C) IL-6

The results are the Mean \pm SEM. Where, ^adenotes comparing with control and NTG, ^bdenotes comparing NTG with treated groups. Where, ^{###} $p < 0.001$, [#] $p < 0.05$, ^{***} $p < 0.001$, ^{**} $p < 0.01$, and ^{*} $p < 0.05$, ^{ns}denotes nonsignificant. $p < 0.05$ considered as statistically significant. One-way ANOVA with Tukey's post hoc test was used for statistical analysis. Where, BS-Brain Stem, CT-Cerebral Cortex, TG-Trigeminal Ganglion

There is now much evidence to suggest that 5-HT may play a crucial role in migraines [40]. The lowered concentration of 5-HT in blood cannot sustain blood vessels contraction, resulting in hemangiectasis. The decrease in 5-HT also leads to a decrease in pain threshold within the thalamencephalon. Migraines are caused by these two factors [41]. This finding well correlated with our finding that NTG + BIBN4096 showed the restoration of the 5-HT concentration. Elevated CGRP and SP levels were found in migraine patients [42,43], which implied a vital role of these neuropeptides in migraine. The present finding both the neuropeptide was elevated in the NTG induced migraine animal model and decreased in the BIBN4096 treated group. IL-1b, IL-6, and TNF may indirectly induce hyperalgesia by releasing prostaglandins and thromboxanes, modulating sympathetic fibres, or increasing the nerve growth factor and bradykinin receptors [44]. These inflammatory cytokines contribute to the

discomfort as well as the inflammation in migraine sufferers [45,46]. This evidence supports our report that during migraine condition, CGRP, TNF- α and IL-6 increased in the model and declined in the BIBN4096 treatment.

4. CONCLUSION

In conclusion, the present study shows CGRP antagonist drug-like BIBN4096 ameliorates the level of biomarkers like 5-HT, SP and IL-6 than the other antimigraine like sumatriptan and ergotamine. But there is a lack of significant difference in the behavioural changes like head-scratching, body shaking and social interaction task. Further experimental studies are needed to check the behavioural pattern because migraine also correlates with anxiety and depression. So, a drug with nonpharmacological adjuvant therapy may be helpful to improve the behavioural characteristics in migraine condition.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The Institutional Animal Ethics Committee approved all protocols (Registration No.: 160/199/CPCSEA and proposal no. 1152). The study was performed as per the health guidelines for the care and use of laboratory animals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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