Article

Analgesic Effects of Thiamine in Male Long Evans Rats

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Abstract

Background: The concept of analgesic effects of thiamine along with other B vitamins has been supported since long by various clinical and experimental evidences, though effects of individual thiamine on pain are yet to be clearly demonstrated. **Objective**: To assess the effects of increasing doses of thiamine supplementation on pain. **Methods:** Forty-eight (48) male Long Evans rats (200±20 gm) were given thiamine (100, 200, 250, mg/kg/day; experimental) or normal saline (5 ml/kg/day; control) intraperitonealy (i.p) for 7 consecutive days. The analgesic activity was evaluated by three experimental pain models, hot (52±0.5^{0}C) water tail immersion test, the interphase (6th-15th minutes) of formalin (50 µl, 2.5%, subcutaneous) test and acetic acid (2%, i.p) induced writhing test. Statistical analysis was done by ANOVA followed by Bonferroni post hoc test and $p \leq 0.05$ was considered as significant. **Results:** In tail immersion test, %MPE significantly increased after 200 ($p \leq 0.05$) and 250 ($p \leq 0.001$) mg/kg of thiamine. In the formalin test, thiamine significantly lowered the jerking frequency ($p \leq 0.05$, $p \leq 0.001$, $p \leq 0.001$, respectively) and duration of flexing and licking ($p \leq 0.001$, in all doses), compared to control. In addition, in writhing test, significant increment in latency of appearance of 1st writhe ($p \leq 0.001$, in higher 2 doses) and significant decrement in frequency of writhes ($p \leq 0.01$, $p \geq 0.001$, $p \leq 0.001$, respectively, in all doses) were observed. **Conclusion:** The results of this study conclude that, repetitive administration of thiamine may cause alleviation of pain through central as well as peripheral inhibitory mechanisms, which is dose dependent as well.

Key words: Thiamine, Antinociception, Tail immersion test, Writhing test, formalin test.

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Introduction

Human awareness of nociception is pain which has been defined according to International Association of Study on Pain (IASP) as "an unpleasant sensory and emotional experience associated with tissue damage or described in terms of such damage"[1]. Pain is the effect produced due to arrival of nerve impulses in the brain evoked by noxious stimuli[2]. It can be often interpreted as a suffering that results from the perception of painful stimuli and it is a well-known symptom indicating something is wrong in our body and can give a clue about the nature of disease[3]. On the other hand, management of pain is one of the most important reason to which medications are given[2]. Drugs which alter the pain sensitivity or eliminate pain are called as painkiller or analgesics[2]. The current therapies to relieve pain are having a number of limitations such as, gastro-intestinal irritation by non steroidal anti-inflammatory drugs and dependency by long standing use of opioids[4]. Therefore, novel therapies for pain treatment are essential to overcome the adverse effects of current analgesics used[4]. It is certainly a need of

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hour to treat the pain of specific pathologic origin such as cancer pain, neuropathic pain etc[5].

Thiamine is one of the most important medications needed in basic health care in the present world[6]. Thiamine with B_6 and B_{12}, have been prescribed for long, either alone or with other analgesics, for treatment of various painful conditions, such as polyneuropathy, neuritis, painful vertebral syndrome, rheumatic diseases, carpal tunnel syndrome as well as premenstrual tension[7,8]. However, in several animal studies combined thiamine, B_6 and B_{12} have been found effective to reduce nociceptive and inflammatory pain, both after single (acute) as well as after chronic administrations (7consecutive days)[9-12]. Recently, the analgesic effects of individual thiamine, B_2, B_6, and B_{12} have been demonstrated in different experimental animals[13-15].

Among the different tests used to measure the nociceptive pain in animals, the tail immersion test is one of the specific, simple, and harmless experiments, which is sensitive to centrally acting analgesics[17] (morphine and morphine-like drugs) and are selectively capable of prolonging the reaction time of the typical tail-withdrawal reflex[18]. The test is also useful to differentiate central opioid like analgesics from peripheral analgesics[18].

In addition, formalin test is one of the valid and reliable animal models to assess both the acute as well as chronic form of nociception and is sensitive for both centrally and peripherally acting analgesics[19-20]. Here, subcutaneous injection of formalin at hindpaw induces inflammation, which leads to a response characterized by jerking, flexing followed by licking of the affected hindlimb[19,21-22]. Pain intensity is measured by converting these behavioral responses into numerical values[20,22-24].

Furthermore, the writhing test has been used as a standard tool for measuring visceral pain[25], where a chemical irritant (e.g., acetic acid) is injected into the peritoneal cavity of the test animal[18,23,24], resulting in peritoneal irritation[24,25] and pain due to release of bradykinin, prostaglandins and other inflammatory chemical mediators[26]. This produces writhing, a reflex behavior characterized by abdominal contractions, twisting of the dorso-abdominal muscles,

movements of the body as a whole and a reduction in motor activity and motor incoordination[24,25]. Here nociception is assessed by quantifying the writhing responses[23], that is, the latency time for the appearance of first writhe[25] and the number of writhing responses over a specific time period[18,24,27]. Several studies, using electrophysiological approaches have shown that administration of B vitamins (B1, B6 and B12) may inhibit nociceptive neurons in the spinal dorsal horn[28] and thalamus[29]. Again, repeated administration of aforementioned vitamins daily for one week was shown to be more effective than a single dose[30]. Franca et al. evaluated the acute and chronic antinociceptive effect of thiamine by hot plate test in mice model[27]. These researchers have shown significant effect of thiamine at single dose of 200 mg/kg/body weight, reducing the nociceptive response in acetic acid induced writhing test[27]. In addition, in their study, thiamine at chronic doses with 50 and 100 mg/kg/body weight, reduced only the second phase (16th to 30th minutes) of formalin test, but failed to exhibit any analgesic effect in the early(1st to 5th minutes) as well as interphase (6th to15th minutes) of that test[27].

Despite the fact that analgesic effect of thiamine with different doses was reported but there is lack of information about the potency of aforementioned effect as well as the role of this vitamin on afferent pain inhibitory system in the central nervous system with higher doses. So, this study was aimed to evaluate the analgesic effect of chronic (single daily dose for 7 consecutive days) supplementations of thiamine

in increasing doses (100, 200 and 250 mg/kg of body weight, i.p.) by tail immersion test, inter phase of formalin test and writhing test, in rats. This study was also intended to assess whether this progressive increment of doses links with the more pronounced effect of thiamine on the aforesaid tests.

Methods

This experimental study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka from March 2014 to February 2015, with prior protocol approval from the Institutional Review Board of the University.

Procurement and maintenance of animals[31,32]:

Forty eight male Long Evans rats weighing 180 to 220 gm were obtained from animal house of Bangladesh Institute of Research and Rehabilitation for Diabetes, Endocrine and Metabolic Disorders (BIRDEM), Shahbag, Dhaka, and were kept under a 12/12 hour light/ dark cycle in a room with constant temperature (28±5ºC)[27]. The animals were acclimatized for 7 consecutive days prior to the experiments and had free access to standard laboratory food and cooled boiled water. The experiments were performed during the day time between 8:00 to 14:00 hours, to avoid any circadian influences. All experiments and animal care were performed according to the guidelines set in the 'Manual for Care and Use of Laboratory Animals' by the Animal Experimentation Ethics Committee (AEEC) of the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b).

Dose schedule[13,27,33,34]:

Experimental solutions of thiamine (100, 200, 250 mg/kg) were prepared by dissolving desired amounts of thiamine hydrochloride (THCL) in 5 ml/kg of normal saline (NS). All compounds (THCL for experimental animals and NS for control animals) were administered intraperitoneally for 7 consecutive days and the last dose was administered 1 hour prior to the experiments.

Tail immersion test[24,35,36]:

For acclimatization, all the rats were taken to the experimental laboratory, within 8:00 to 14:00 hours and were kept for 15 minutes in a plexiglass mechanical restrainer every day for 7 consecutive days. Then on the very first day of the study (Day 1), the rats were placed in that plexiglass mechanical restrainer for initial 5 minutes for adaptation, with the tail hanging freely. After that, the distal 10 cm of the tail was immersed into a beaker with preheated (52±0.5ºC) water with a thermometer placed in it and the tail withdrawal latency (time taken for withdrawal of tail from the hot water) was recorded with a stop watch. The mean of 3 similar successive maneuvers (at 5 minutes interval) were noted, as basal latency (BL). Again, on 7th day (Day 7), similar test was done 1 hour after the last dose of NS (for control rats) and THCL (for experimental rats) supplementations and the mean of 3 identical maneuvers was noted, as test latency (TL). To minimize tissue damage, a maximum latency of 15 seconds was considered as cut off time (CT). The antinociceptive effect was calculated by percentage of maximum possible effect (% MPE), as follows, %MPE = [(TL-BL) / (CT-BL)] X 100

Formalin test[22,27,37-39]:

The rats were acclimatized in the experimental laboratory environment, in the observation cage of the plexiglass formalin box (30×30×30 cm^3) for 1 hour daily (within 8:00 to 14:00 hours) for 7 consecutive days prior to the formalin test. Then on the day of experiment (Day 7), 1 hour after last dose of (i.p.) injection of THCL or equal volume of NS, the rats were restrained by a thick towel and 50 µl of dilute formalin (2.5%) was injected subcutaneously into the plantar aspect of the rats' right hindpaw with an insulin syringe. Immediately thereafter, the animal was placed in the observation cage of the formalin box and the pain behaviors were observed for 6th to 15th minutes. Observation was made by counting the total frequency of jerking and total duration of flexing plus licking of the injected paw during

this time through a mirror fixed below the formalin box at 45^{o} angle. A stop watch was used to note the time.

Writhing test[27,40-46]*:*

All the rats of this test were also acclimatized to the laboratory environment as well as to the plexiglass observation chamber (30×30×30 cm^3) for 7 days prior to the final experimental day. Then on the day of experiment (Day 7)), 1 hour after the last dose of i.p. supplementation of THCL or NS, 1 ml of 2% acetic acid was administered (i.p.) and immediately the rat was placed into the observation chamber. Then the writhing appearance latency (time taken for appearance of 1st writhe) was recorded with a stop watch and the frequency of writhes (number of abdominal constrictions along with stretching of the hind limbs) were counted over a period of consecutive 60 minutes.

Data were expressed as mean ± SEM and statistically analyzed by SPSS (version 17.0) using analysis of variance (ANOVA), followed by a Bonferroni Post hoc test. In the interpretation of results, p value ≤0.05 was considered as significant.

Results

Tail immersion test:

The percentage of maximum possible effect (%MPE) of all doses were compared to that of the control and showed significant increment in higher 2 doses (≤0.05 and ≤0.001, respectively) (Figure 1).

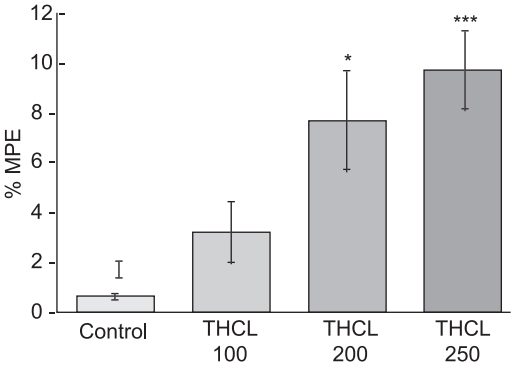

Figure 1: Antinociceptive effects of different doses (i.p) of thiamine hydrochloride (THCL at 100, 200 and 250 mg/kg doses) in tail immersion test. Comparison was done on percentage of maximum possible effect (%MPE). Each bar represents for mean±SE for 6 rats. *= p≤0.05, ***= p≤0.001, compared to control.

Formalin test:

In the interphase of formalin test, THCL significantly lowered the frequency of jerking after 100 mg/kg (p≤0.05), 200 mg/kg (p≤0.001) and 250 mg/kg (p≤0.001) and duration of flexing and licking after 100 mg/kg (p≤0.001), 200 mg/kg (p≤0.001) and 250 mg/kg (p≤0.001), in comparison to those of control (Figure 2).

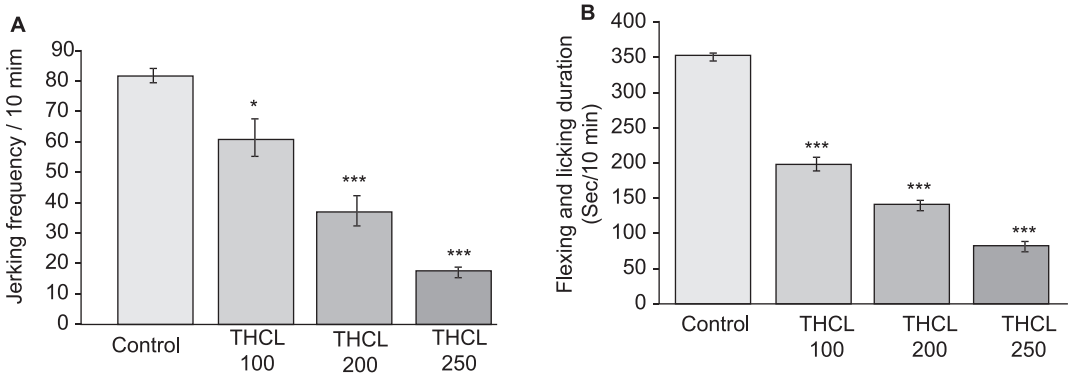

Figure 2: Antinociceptive effects of different doses of thiamine hydrochloride (THCL) in the interphase of formalin test (6th - 15th minutes). THCL lowered jerking (A) as well as flexing and licking (B) responses. Each bar symbolizes for mean ± SE for 6 rats. *= p≤0.05, ***= p≤0.001, compared to control.

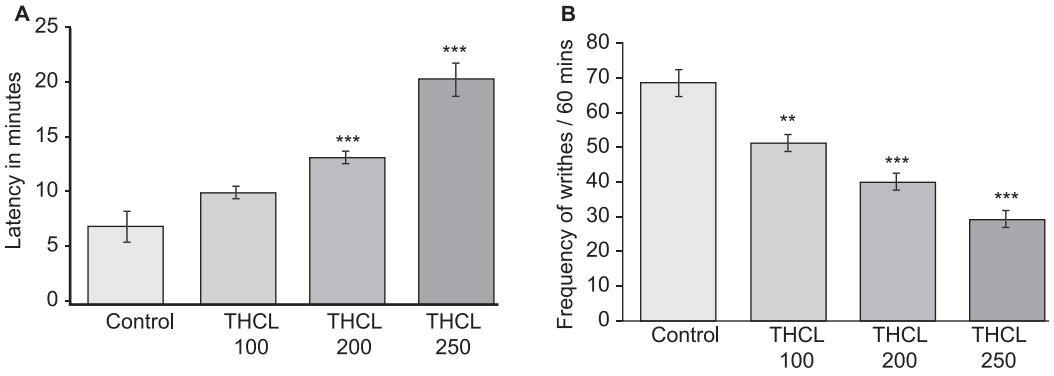

Figure 3: Analgesic effects of different doses of thiamine hydrochloride (THCL at 100, 200 and 250 mg/kg doses) in writhing test. THCL increased the latency of appearance of 1st writhe (A) and decreased the frequency of writhes (B). Each bar represents for mean ± SE for 6 rats. **= p≤0.01, ***= p≤0.001, compared to control.

Writhing test:

As it is shown in figure 3, i.p. administration of THCL showed significant augmentation of the writhing appearance latency ($p<0.001$, in 200 and 250 mg/kg) as well as reduction of the frequency of abdominal writhes in all 3 doses ($p\leq0.01$, $p\leq0.001$, $p\leq0.001$, respectively), in comparison to those of control.

Discussion

In recent years, various researchers have intended to find out alternatives to the conventional analgesics in order to replace them with natural substances, or to decrease their doses to minimize their adverse effects. The results of the present study revealed that, thiamine supplementation elicited potent analgesic effects evident in the three analgesic models, which is suggestive of the presence of both centrally and peripherally mediated mechanisms.

The brain and spinal cord are two main components involving in central pain mechanism[47]. The dorsal horn of the spinal cord is endowed with various neurotransmitters and receptors such as, substance P, somatostatin, neuropeptide Y, nitric oxide, endogenous opoids etc[47]. Amongst the tests used in this study, tail immersion test has been considered to be selective to assess compounds acting via opioid receptors[48] and our results revealed increased pain threshold (%MPE) at higher two doses (200 and 250 mg/kg) of thiamine. So it may be proposed that this vitamin exerts its effect against acute thermal nociception by modulating the somatosensory pain transmission in the central nervous system, which is indicative of centrally mediated analgesic mechanism[48]. Several other investigators also observed similar type of findings in different doses with different experimental models. Fu et al. have reported the dose-dependent reduction of nociceptive pain evoked by heating (at 50 or 52°C for 10 s) of hind foot skin in cat model after acute intrathecal administration of thiamine with B_6 and B_{12}[28]. In other studies, Wang and Galvan-Montano et al. also supported this notion in different models, who observed prolongation of the foot withdrawal latency to heat stimulation after acute supplementation of thiamine at different doses (5, 10, 33 and 100 mg/kg, i.p) and significant antinociceptive effect was reported at highest dose group[39,49].

The descending pain modulation system or central analgesia system can block pain at the initial entry point at the spinal cord and also in

other regions of the brain and spinal cord[50]. The characteristic response in the interphase of formalin test is considered to be a central nociceptive model[19,22,38]. In this test, decrement of these behavioral expressionsresult from inhibition at the central descending pain modulatory system of the brain and spinal cord[28]. In our study, as thiamine supplementation (i.p.) in repetitive administration (at 100, 200 and 250 mg/kg daily for 7 consecutive days) enhanced the central analgesia demonstrated in interphase, it may be proposed that it has the central antinociceptive effect. Moreover, dose dependent increment of the efficacy of THCL was also observed.

Similar trend of finding has been observed by after 7 days supplementation of B vitamins (B_1/B_6/B_{12}, 100 mg/100 mg/0.1 mg in 3 mL) 1 ml/kg (s.c.) daily in rats[30]. Review of literature suggested that this antinociceptive effect of THCL may be due to increased afferent inhibitory control of nociceptive neurons at the spinal cord along with decreased response of thalamic neurons to nociceptive stimulation[51]. Again, the enhancement of afferent inhibition by the vitamin could be due to an increase in the synthesis rate of inhibitory neurotransmitters in the central neurons, as it has been shown that deficiency of B vitamins (B_1/B_6/B_{12}) impairs serotonergic and other nonopioid inhibitory neurotransmitter systems[52]. So, thiamine supplementation may possibly increase the availability or effectiveness of serotonin as well as noradrenaline in the central nervous system[48], which could explain the increase in the efficacy of afferent inhibitory control in our experiment.

On the other hand, the abdominal constriction response induced by acetic acid in writhing model is a sensitive procedure to evaluate peripherally acting analgesics[42]. It has been suggested that, in writhing test, intraperitoneal acetic acid may involve both the direct stimulation of the visceral nociceptive afferent fibers (due to the pH reduction) as well as the synthesis of inflammatory mediators[43]. The abdominal injection of acetic acid in rats has been attributed to the release of arachidonic acid, which results the synthesis of prostaglandin by the cycloxygenase enzyme[44]. On release of prostaglandin the special nerve endings act via prostaglandin E_2 receptor and transmit the signal to the brain and results in visceral writhing response[45]. Moreover, the response is also thought to be mediated by peritoneal mast cells[43] as well as acid sensing ion channels[46]. Here, in this study, as all 3 doses of THCL (100, 200 and 250 mg/kg) significantly reduced both the variables (the latency time for the appearance of first writhe and the number of writhing responses over a specific time period) of this acetic acid induced writhing test. Therefore it may be suggested that, this vitamin may possess analgesic activity acting against inflammation followed by decrement in inflammatory pain.

A number of researchers also observed similar trend of findings. Franca et al. have shown significant decrement in number of writhes after intraperitoneal acetic acid following single administration of combination of B_1, B_6 and B_{12} at 20, 50, 100 and 200 mg/kg[27]. Baroszyk and Wild (1990) also have reported the significant decrement of inflammatory pain in writhing test at higher dose (667 mg/kg oral and 167 mg/kg i.p.) after supplementation of thiamine with B_6 and B_{12} in rat model[53].

Besides the central effects in lowering nociception, thiamine may reduce the inflammatory pain by lowering inflammation as it has been suggested that anti-inflammatory effect of this vitamin may be mediated through regulation of arachidonic acid pathway in macrophage, where it can block the expression of any of the enzymes (cycloxygenase-2, lipoxygenase-2, thromboxane B synthase, prostacycline synthase) as well as transcription factor nuclear factor kappa[54]. Moreover, this vitamin may also decrease the oxidative stress and production of free radicals[55]. Any of the

above-mentioned mechanisms might be responsible for lowering nociceptive pain as well as inflammatory pain in this study.

Conclusion

From this study it can be concluded that, chronic thiamine supplementation exert analgesic effects both via central and peripheral mechanisms. This vitamin can diminish the nociception by enhancing the spinal dorsal horn pain modulation system and also by its anti-inflammatory properties as it inhibits enzymes required for production of the chemical mediators of inflammation. Further studies are recommended to understand the underlying exact mechanisms responsible for the aforesaid effects of this vitamin.

Conflict of interest: none

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