Research Article

Possible Anti-nociceptive Action of Either Vitamin B_1 or B_{12} Against Writhing Response Induced in Mice by Glacial Acetic Acid: Comparative Study with Tramadol.

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ABSTRACT

Objective: to evaluate whether or not vitamin B_1 or B_{12} possess anti-nociceptive effect against writhing response induced in mice by glacial acetic acid; to compare that effect of each vitamin with tramadol and to clarify the mechanism of the proposed anti-nociceptive effect centrally of only vitamin B1 utilized in this study by administering naloxone (as opioid antagonist). **Methods:** 42 mice (*Mus musculus*) of both sexes are divided into 4 groups as follows: **Group I**. ***Six*** animals were given intraperitoneal injection (IP) of normal saline, then after 30 minutes they were given 1 ml I.P. injection of glacial acetic acid (0.6%.). This group served as untreated control. **Group II**. ***Eighteen*** *mice* were allocated as follows: **IIA**. **Six** animals were injected Vitamin B_1 I.P 30 minutes prior to IP injection of 1 ml of glacial acetic acid (0.6%). **IIB**. **Six** animals were injected Vitamin B_{12} I.P 30 minutes prior to IP injection of 1 ml of glacial acetic acid (0.6%). **IIC**. **Six** animals were injected tramadol (20 mg/Kg) IP 30 minutes prior to IP injection of 1 ml of glacial acetic acid (0.6%). **Group III**. **Twelve** animals were allocated as follows: **IIIA**. ***Six*** animals treated with IP injections of a combination of tramadol (20mg / Kg) and Vitamin B_1 (100mg/kg) given to mice 30 minutes prior to 1 ml I.P. injection of glacial acetic acid (0.6%.). ***IIIB. Six*** animals treated with IP injections of a combination of tramadol (20mg / Kg) with Vitamin B_{12} (1mg/Kg) given to mice 30 minutes prior to 1 ml I.P. injection of glacial acetic acid (0.6%.). ***Group IV.*** **Six** animals were injected IP with naloxone (0.73mg/Kg) were given to mice followed by I.P. infection of vitamin B_1 (1mg/Kg) and after 30 minutes they were given 1 ml I.P. injection of glacial acetic acid (0.6%.). The number of writhes (abdominal constriction and full extension of hind limb) of each mouse was recorded after 5minutes of glacial acetic acid for a period of 10 min. **Results:** there were significant reduction in number of writhes produced by either vitamin B_1 or B_{12} compared to untreated group; also there was significant difference produced by the combination of tramadol and B_1 compared to vitamin B_1 alone, and there was no significant difference between the combination of naloxone and vitamin B_1 and untreated group. **Conclusion:** both vitamin B_1 and B_{12} possess an anti-nociceptive effect in this animal module. Vitamin B_1 acts via opioid mechanism (as pain modulator) manifested by returning to base line of pain when using naloxone (opioid antagonist) and the synergistic effect with the use of vitamin B_1 with tramadol (opioid agonist).

Keywords: Vitamin B_1, vitamin B_{12}, anti-nociception, glacial acetic acid, writhing, mice.

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INTRODUCTION

Pain is a sensorial modality and primarily protective in nature, but often cause discomfort. Typically, it is evoked as a response to tissue(s) injury. Whatever the cause; it is result from the activation of the peripheral afferent nociceptor, and central mechanism by which the afferent input generates a pain or both [1-3].

Vitamins B are water-soluble complex, composed of variety of subtypes, they required in small amounts to assist a variety of biochemical functions as enzymes

cofactors, and must be supplied adequately in diet. Thiamine (B_1) has a central role in energy-yielding metabolism, where it is phsphorylated to thiamin triphosphate**,** which has a role in nerve conduction. Its deficiency cause peripheral neuritis (beriberi) and Wernicke's encephalopathy with Korsakoff's psychosis. Cyancobalamine (vitamin B_{12}) is essential for growth and replication. Its deficiency cause megaloblastic anemia and de-myelination of the posterior and lateral columns of the spinal cord [4-6].

This study was designed to evaluate whether or not vitamin B_1 or B_{12} possess anti-nociceptive effect against writhing response induced in mice by glacial acetic acid; to compare that effect of each vitamin with tramadol and to clarify the mechanism of the proposed anti-nociceptive effect centrally of only vitamin B1 utilized in this study by administering naloxone (as opioid antagonist).

MATERIALS AND METHODS

Drugs:

Drugs used in this study, their doses and route of administration [7] are listed in (T**able 1**).

Table 1: Drugs used, their doses and Route of administration [7]

Drugs (Injection)	***Dose (mg/kg)***	***Route of administration***
Vitamin B_1	100	I.P.
Vitamin B_{12}	1	I.P.
Naloxone	0.73	I.P.
Tramadol	20	I.P.

Animals: **Forty two** *Mus musculus* mice of both sexes weighing 30-35g were obtained from and maintained in the Animal House of the College of Pharmacy, University of Baghdad under conditions of controlled temperature. The animals were fed commercial pellets and had free access to water except when starvation needed during the investigation. Animal groups were divided as follows:

Group I. ***Six*** mice were injected normal saline intraperitoneally (IP), then after 30 minutes they were given 1 ml of IP injection of glacial acetic acid (0.6%). This group served as untreated control to investigate the writhing response.

Group II. ***Eighteen*** *animals* were allocated to investigate the possible anti-nociceptive effects of each of B vitamins and tramadol as follows:

IIA. Six animals were injected (100mg/kg) Vitamin B_1 I.P 30 minutes prior to IP injection of 1 ml of glacial acetic acid (0.6%).

IIB. Six animals were injected (1mg/kg) Vitamin B_{12} I.P 30 minutes prior to IP injection of 1 ml of glacial acetic acid (0.6%).

IIC. Six animals were injected tramadol (20mg/Kg) IP 30 minutes prior to IP injection of 1 ml of glacial acetic acid (0.6%).

Group III. **Twelve** animals were allocated as follows:

3A. ***Six*** animals treated with IP injections of a combination of tramadol (20mg / Kg) and vitamin B_1 (100mg/kg) given to mice 30 minutes prior to 1 ml of glacial acetic acid (0.6%) IP injected.

3B. Six animals treated with IP injections of a combination of tramadol (20 mg / Kg) with vitamin B_{12} (1mg/Kg) given to mice 30 minutes prior to 1 ml of glacial acetic acid (0.6%) IP injected.

Group IV. **Six** mice were injected IP with naloxone (0.73 mg/Kg), followed by I.P. infection of vitamin B_1 (100 mg/Kg) and after 30 minutes, the animals were injected IP with 1ml glacial acetic acid (0.6%).

The number of writhes (abdominal constriction and full extension of hind limb) of each mouse was recorded after 5minutes of glacial acetic acid for a period of 10 min.

Statistical analysis: Results were expressed as the means ± SEM, utilizing Student's t-test, one-way ANOVA followed

by Turkey's test. Differences in mean were considered to be significant when $p< 0.05$.

RESULTS:

Table 2 and figure 1 showed that there were significant decrease in the number of writhes in group of mice treated with IP injection of either B vitamins and tramadol alone ($p<0.05$) compared to untreated group. The percent of inhibition in the intended number were 61.24%, 44.54% and 62.2 %, respectively. Additionally, no significant difference was observed in group of mice treated with either vitamin B_1 or tramadol ($P>0.05$), but a significant decrease in the number of writhes was observed in group of mice treated with vitamin B12 alone compared to tramadol ($P<0.05$).

Besides, the results of table 2 and figure 1 showed that, significant differences were observed in group of animals treated with combination of each B vitamins with tramadol compared to group of mice treated with each B vitamin given alone ($P< 0.05$).

Furthermore, table 2 and figure 1 showed that, there was a significant difference in the number of writhes in group of mice treated with vitamin B_1+naloxone compared to vitamin B_1-treated group ($P<0.05$); and there was no significant difference ($P>0.05$) in the intended number in group of mice treated with vitamin B_1+naloxone compared to untreated control group.

Table 2: The effects of treatment with normal saline, vitamin B1, vitamin B12, tramadol each alone or in combination, and vitamin B1+naloxone on the number of writhes in mice.

Group	*No. of writhes*	*% of inhibition*
Normal saline+ glacial acetic acid (n=6)	45.8 ± 5.92 [a]	0
Vitamin B_1 (100mg/Kg) (n=6)	17.75 ± 2.84 [b]	61.24
Vitamin B_{12} (1mg/Kg) (n=6)	25.4 ± 1.63 [c]	44.54
Tramadol (20mg/Kg) (n=6)	17.4 ± 3.14 [b]	62.20
Tramadol + B_1 (n=6)	7.6 ± 0.75 [d]	83.41
Tramadol + B_{12} (n=6)	23.33 ± 5.85 [c]	49.06
Naloxone (0.73mg/Kg) + B_1 (100 mg/kg) (n=6)	52.33 ± 2.75 [a]	0

- Data were expressed as mean ± SEM.
- Values with non-identical superscripts (a, b and c) among different groups is considered significant.
- n= No. of animals.

DISCUSSION

Glacial acetic acid-induced writhing reflex in mice represents pain sensation by triggering localized inflammatory response; such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids [8]. Writhing response is a sensitive procedure to evaluate the peripherally-acting analgesics and for detecting central analgesia [9]. The response is thought to be mediated by peritoneal mast cells [10], acid sensing ion channels [11] and the prostaglandin pathways [12].

Experiments in animals have shown that both vitamins B1 (thiamin) and vitamin B12 (cyanocobalamine) has anti-nociceptive activity against chemical- and heat-induced pain [7, 11-14]. An anti-inflammatory effect had also been reported using the carrageenan-induced edema test [11, 15]. Furthermore, B vitamins have been evaluated as analgesic drugs to treat painful disorders such as neuropathic pain and

there were evidences to postulate the role of B vitamins in antagonizing pain nociception [7, 13, 16]. In the present study it is clearly demonstrated that the anti-nociceptive activity of the B vitamins (B1 and B12) injected IP prior to acetic acid-induced writhing response. Our data agree with previous observations about the anti-nociceptive activity of the B vitamins given by acute administration [17, 18]. However, other investigators [19-20] have reported that B vitamins, alone are devoid of anti-nociceptive action in several models of pain, this controversy could be due to the use of different pain stimuli.

In other reports, vitamin B1 produced anti-nociception and potentiated the anti-nociceptive effect of diclofenac in adjuvant arthritis [6]. In addition, vitamin B12 was effective in the carrageenan-induced edema [7].

The neurophysiologic mechanism of the anti-nociceptive effect of vitamin B1 is still unknown. In this study we try to explore the possible centrally anti-nociceptive mechanism of vitamin B1 against glacial acetic acid-induced writhing response utilizing naloxone (0.73 mg/kg, IP) as opioid receptor antagonist. The data obtained from this study indicated that the intended antagonist was able to block the anti-nociceptive effect of vitamin B1 in group IV of mice. Thus, the suggestion is that vitamin B1 could produce anti-nociception by either releasing endogenous opioids or activating opioid receptors. Since there is no evidence that B vitamins can bind to opioid receptors, the effect of naloxone on B vitamins-induced anti-nociception warrants more studies.

In conclusion, this study has been shown that B vitamins may have an anti-nociceptive effect in the 0.6% acetic acid-induced writhing response in mice, an effect seems to be due to the activation of opioid receptors especially after utilizing vitamin B1.

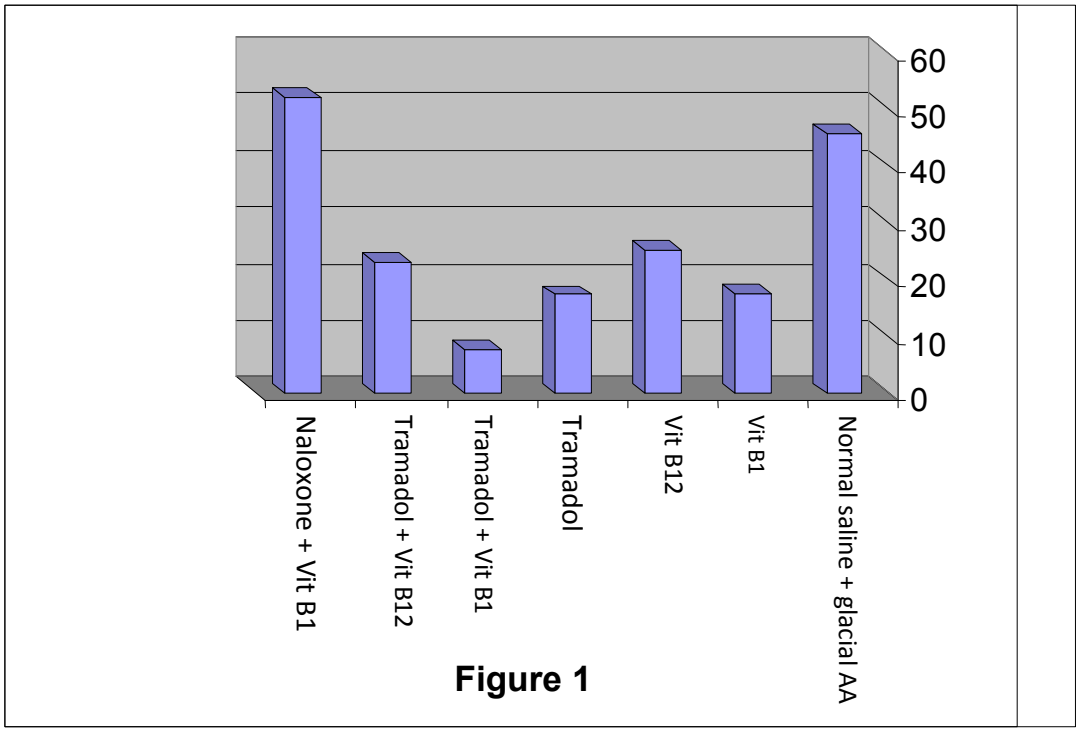

Figure 1: Histogram represents the effects of treatment with normal saline, vitamin B1, vitamin B12, tramadol each alone or in combination, and vitamin B1+naloxone on the number of writhes in mice.

Y- axis: represents the number of writhes.

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