Basic Research

Pain & Inflammation: Effects of short term daily administration of Vitamin B₁₂ & Folic acid in long evans rats

Masud Imtiaz¹, Noorzahan Begum², Taskina Ali³, Richmond Ronald Gomes⁴, Sanjoy Saha⁵, Riza Faridun Tasfi⁶, Khan Shakil Ahmed⁷, Nahid Kamal⁸

Abstract

Background: Vitamin B₁₂ & Folic acid (FA) are used with other B vitamins to relieve various painful and inflammatory conditions. But combined effects of vitamin B₁₂ & FA on nociceptive pain, inflammatory pain and inflammation are yet to be clearly demonstrated.

Objective: To assess the effectiveness of short term daily administration of B₁₂ & FA on reducing pain and inflammation.

Methods: This prospective experimental study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka from 1st January 2011 to 30th June 2012. For this purpose, 12 male Long Evans rats, weighing 200 to 250 grams were collected from the animal house of BIRDEM, Shahbag, Dhaka. All the rats received a daily intraperitoneal injection of either combination of B₁₂ (15mg/kg) & FA (5mg/kg) or equal volume of normal saline for 7 consecutive days. To evaluate the effects on pain, tail immersion test for nociceptive pain and formalin test for nociceptive & inflammatory pain were done. In addition, to evaluate their effects on inflammation formalin induced hind paw oedema was measured.

Results: Combination of B₁₂ & FA Supplementation significantly lowered the variables for inflammatory pain & inflammation.

Conclusion: This study revealed that, combined short term daily supplementation of B₁₂ & FA is effective in lowering inflammatory pain & inflammation.

Key words: Pain, Nociceptive pain, inflammatory pain, Inflammation, B₁₂, Folic acid, Tail immersion test, Formalin test, Formalin induced paw oedema test.

Introduction:

Pain is a complex sensory & emotional experience which warns the brain about actual or potential tissue damage.

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Though protective, it is also the most unpleasant sensation. As a result, human have been trying to conquer pain from the most ancient period of time. Pain is not a uniform entity rather it is classified on the basis of etiological characteristics into nociceptive, inflammatory, neuropathic and functional pain¹-³. Direct activation of the nociceptor by noxious stimuli results in nociceptive pain². When painful stimuli lead to peripheral inflammation; resultant inflammatory soup amplifies the ongoing nociception & results in inflammatory pain². Inflammation is the local reactions of vascularized living tissues to microbial invasion or injury. It has both protective & harmful potential which often spirals out of control and needs medical attention. Traditional analgesic and anti-inflammatory drugs which are being used to treat painful and inflammatory conditions have many side effects. Now a days, many studies are being conducted throughout the world to replace or at least to reduce the dose or duration of traditional analgesics or anti-inflammatory drugs, by inventing alternate or adjunct medications.

Earlier, it was reported that deficiency of B vitamins causes painful disorders, which were relieved by their deficiency correction⁴. In the present decade, different researchers reported high dose supplementations of these vitamins relieve painful and inflammatory conditions not associated with their deficiency⁵-⁷.

Recently, the analgesic and anti-inflammatory effects of several members of the Vitamin B complex such as B₁, B₂, B₆, B₁₂,
and B$_6$ have been demonstrated in different experimental animals.

Among the B vitamins, B$_{12}$ & FA are used as mutually exclusive supplements in various hematological conditions. B$_{12}$ supplementation in combination with B$_6$ and B$_2$ lowered nociceptive$^{8-14}$, inflammatory$^{10, 12, 13, 15-18}$ & neuropathic pain$^{19-20}$ and also inflammation$^{11, 18}$ after single or repeated administrations. On the other hand, single dose or short term (7 days) supplementation of vitamin B$_{12}$ alone failed decrease pain in rodents$^{10-11}$. Though no study was found on the effect of FA on pain, supplementation of this vitamin alone was found to improve the availability of NO$^{21}$ and serotonin$^{22}$ in the human brain, where they act as analgesic agents$^{23}$. On the other hand, 2-12 weeks supplementation of FA showed anti-inflammatory effect in different animal & human models$^{24-26}$.

Combined supplementation of multivitamins comprising aforementioned two vitamins significantly reduced inflammatory pain after 12 days supplementation in a group of patients with dysfunction pain syndrome, chronic cephalgia and facial pain$^{1}$. Similar finding was also reported by Flynn, Irvin and Krause$^{27}$ in another group of osteoarthritic patients after 2 months supplementation with these 2 vitamins.

In addition, decrement of inflammation was also reported after supplementation of combination of B$_{12}$ and FA (with other B vitamins) in a number of clinical trials abroad$^{28-29}$.

Therefore, on the basis of the background the present study has been designed to evaluate the effects of combined supplementation of B$_{12}$ (15 mg/kg) & FA (5mg/kg) daily for 7 days on nociceptive pain, inflammatory pain and inflammation in male Long Evans rats.

**Materials and Methods :**

This prospective experimental study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka from 1st January 2011 to 30th June 2012. All the experiments were conducted according to the guidelines for the Animal Experimentation Ethics Committee, Institute of Cholera and Diarrhoeal Disease Research, Bangladesh (icddr,b; 2003) and was approved by the Ethical review committee, BSMMU.

**Experimental animal :**

A total number of 12 male Long Evans rats, weighing 200 to 250 grams were collected from the animal house of Bangladesh Institute of Research and Rehabilitation for Diabetic Endocrine and Metabolic Disorders (BIRDEM), Shahbag, Dhaka. They were kept under a 12/12 hour light/dark cycle in a standard laboratory condition for 7 days prior to testing for acclimatization. The experiments were performed during the day time between 8:00 to 13:00 hours to avoid the circadian influences. All the rats had free access to standard laboratory food and cooled boiled water. The room temperature was kept around 27° to 28° C which corresponds to the thermo neutral zone of rats. All the rats were regularly inspected for their wellbeing.

**Grouping :**

The rats received intra-peritoneal combined supplementation with 15mg/kg of B$_{12}$ & 5mg/kg of FA (B$_{12}$+FA- 6 rats) or equivalent amount of normal saline (control - 6 rats) daily for 7 consecutive days. One hour after the last dose of supplementation, they were subjected to tail immersion test followed by formalin test & then formalin induced paw oedema test.

**Tail immersion test :**

To assess the thermal nociception tail immersion test was done$^{30-31}$. For this, each rat was placed in a Plexiglas mechanical restrainer, with the tail hanging freely and kept there for initial 5 minutes for acclimatization. Then 400 ml of heated water (52±0.5°C) was taken in a 500 ml glass beaker with a thermometer placed in it. Then the distal 10 cm of the tail was immersed into the heated water and the tail withdrawal latency and the mean of similar 3 successive maneuvers (at 5 minutes interval) noted as baseline latency (BL). Again, another tail immersion measurement was done 1 hour after the last dose of vitamin supplementation. The mean of similar 3 successive maneuvers at 5 minutes interval were noted as test latency (TL). To minimize tissue damage, a maximum latency of 15 seconds was considered as cut-off time. Antinociceptive effect was calculated as percentage of maximum possible effect (% MPE) as follows:

\[
\% \text{ MPE} = \frac{[\text{TL}-\text{BL}]}{(\text{Cut off time-BL})} \times 100
\]

**Formalin test :**

Formalin test$^{32}$ was done to assess nociceptive & inflammatory pain just after completing the tail immersion test. The rat was restrained by a thick towel and the right hind paw was exposed. Fifty (50) µl of dilute formalin (2%) was injected subcutaneously into the planter aspect of the rats right hind paw with an insulin syringe. Then the animal was placed in the observation cage of the plexiglas formalin box and the pain behaviors were observed for consecutive 60 minutes. Within this time the first 5 minutes (1st – 5th) were considered as the early phase (nociceptive pain), middle 10 minutes (6th -15th) as the interphase and last 45 minutes (16th-60th) as the late phase (inflammatory pain)$^{10, 15, 33}$. 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° angle.

**Formalin induced paw oedema test :**

After completing the formalin test, the animal were sacrificed and formalin induced paw oedema test$^{15}$ was done to measure inflammation. Both hind paws were cut at knee joint & their volumes were measured using a water plethysmometer. Paw volume was calculated as the difference of the amount of water volume after & before paw immersion. Net oedema volume was calculated by subtracting the left paw volume from the right paw volume.

**Drugs :**

B$_{12}$ (Jayson Pharmaceuticals Limited, Bangladesh) & FA
Statistical analysis:
The results were expressed as mean ± SE and were statistically analyzed by independent sample’s ‘t’ test. In the interpretation of results, p≤0.05 was accepted, as the level of significance.

Results:
Nociceptive pain:
The effects of intraperitoneal (i.p.) administration of B12+FA or normal saline were observed in tail immersion test & in early phase of formalin test. In tail immersion test % MPE & in early phase of formalin test total jerking frequency as well as total duration of flexing & licking were analyzed as nociceptive pain behaviors.

All the nociceptive pain variables where improved in B12+FA supplemented group though the results were statistically non-significant (Table I).

Table I: Nociceptive pain variables in different groups (n=12)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>B12+FA</th>
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</thead>
<tbody>
<tr>
<td>Maximum possible effect (%)</td>
<td>4.27±1.59</td>
<td>4.9±1.58</td>
</tr>
<tr>
<td>Jerking in early phase of formalin test</td>
<td>101.5±4.40</td>
<td>90.83±5.73</td>
</tr>
<tr>
<td>(frequency/ 5min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flexing &amp; licking in early phase of formalin test</td>
<td>260.33±6.93</td>
<td>260±11.51</td>
</tr>
<tr>
<td>(seconds/ 5min)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data were expressed as mean±SE. Independent sample ‘t’ test was done in between control & B12+FA supplemented groups.

Inflammatory pain:
Inflammatory pain behaviors were observed as frequency of jerking & total duration of flexing & licking in the late phase of formalin test.

All the inflammatory pain variables where lower in the study group than that of controls, though the results were statistically non-significant (Table II).

Discussion:
Pain & inflammation, though two important alarm systems of body, are the major causes of physician consultation worldwide. The annual cost for their management exceeds billions of dollars in developed countries. Search for newer drugs with fewer side effects for treating these conditions is a cornerstone of research now a days. Combinations of B vitamins were previously associated with treating painful & inflammatory conditions. With this view, the present study was undertaken to assess the efficacy of combination of B12 & FA on pain & inflammation.

Inflammation:
The amount of oedema in the formalin injected paw was measured as inflammatory variable at the end of formalin test. This variable was significantly (p≤0.05) lower in B12+FA supplemented group compared to controls (Figure 2).

![Figure 1: Formalin induced paw oedema volume in different groups of rats. Each bar symbolizes for mean ± SE for 6 Rats. * = p≤0.05, compared to control.](image)

Figure 1: Frequency of jerking (A) & duration of flexing & licking (B) in late phase of formalin test in different groups of rats. Each bar symbolizes for mean ± SE for 6 Rats. ** = p≤0.01 & *= p≤0.05, compared to control.

![Figure 1](image)

Inflammation:

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  ![Figure 1](image)
observations were observed in two clinical trials, though duration of suppletions were much longer (2month & 6 month) & they also administered vitamin B₆ along with these two vitamins. Although mechanism of this decrement of inflammation can't be elucidated from our study, decreased production of TNF-α, IL-6, IL-8, CRP, free radicals or NF-κB by the inflammatory cells are proposed as mechanism for their anti-inflammatory effects by several researchers.

**Conclusion:**

From this study, it may be concluded that short term supplementation of B₃⁺ FA alleviates formalin induced inflammatory pain & inflammation.

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**References:**