ORIGINAL ARTICLE

Antinociceptive and Anti-inflammatory Effects of Combined Administration of α-tocopherol and Morphine in Long Evans Rats

*T Habib[1], S Begum[2], T Ali[3], KN Afreen[4], N Nahar[5]

[1] Dr. Tamanna Habib, Assistant Professor, Department of Physiology*
Anwer Khan Modern Medical College, Dhaka, Bangladesh
[2]Prof. Dr. Shelina Begum, Professor and Chairman, Department of Physiology
Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh
[3]Dr.Taskina Ali, Associate Professor, Department of Physiology
Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh
[4]Dr. Khandaker Nadia Afreen, Assistant Professor, Department of Physiology
Z H Sikder Women's Medical college
[5]Dr. Mst. Nasrin Nahar, Assistant Professor, Department of Physiology
Armed Forces Medical College, Dhaka Cantonment

**Corresponding Author*

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ABSTRACT

Background: Morphine is an opioid analgesic which is used to treat moderate to severe pain but has a number of side effects. This study is aimed to explore that combination of morphine and α-tocopherol (αT) are better analgesic as well as anti-inflammatory effect than that of morphine alone.

Objective: To assess the effects of combination of morphine with α-tocopherol on pain and inflammation.

Methods: This prospective experimental study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka from January 2013 to December 2013. For this purpose, 15 male Long Evans rats were studied. On the basis of vitamin and drug administrations, the rats were divided into three (3) groups (5 rats in each). Control group received normal saline, one experimental group received morphine sulphate (MS) at a dose of 3 mg/kg body weight and another experimental group received combination of MS with αT at a dose of 3 mg/kg body weight and 500 mg/kg body weight, respectively. All the groups received single dose and equal volume (1 ml) through intraperitoneal route 1 hour before the test. Just one hour after administrations, they were subjected to formalin test followed by formalin induced paw edema test. The data were statistically analyzed by ANOVA followed by Bonferroni Post Hoc test.

Results: Combined administration of MS and αT lowered the variables for nociceptive pain, central analgesic activity, inflammatory pain as well as inflammation than individual administration of MS.

Conclusion: From this study it may be concluded that combined administration of morphine sulphate and α-tocopherol were more effective in lowering pain and inflammation than individual administration of morphine.

Key Words: Analgesic, formalin test, inflammatory pain, inflammation, morphine, pain, α-tocopherol, paw

Introduction

The International Association for Study of Pain (IASP) has been defined pain as- 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage[1]. It involves not only the mere recognition of the sensation of tissue damage, it is also affected by emotional and cognitive state of an individual[2]. It is a major presenting symptom in many medical conditions which can significantly interfere with a person's quality of life. As it is protective in nature so it acts as a warning device which becomes

active due to any ongoing damage of the tissue or organism[3]. But it also causes discomfort, which brings the patient to physician. Pain is not a uniform entity and can be classified on the basis of etiological characteristics into nociceptive, inflammatory, neuropathic and functional pain[4]. Nociception is a neural process by activation of nociceptors due to exposure to noxious stimuli such as mechanical, chemical or thermal stimuli[5]. Inflammatory pain originates from the combination of nociceptor activation and sensitization of the nervous system by the inflammatory mediators[6]. Inflammation is the body's natural response to injury. Inflammation occurs frequently because the world around us consists of a variety of microbes and injurious agents[7]. It is a sequence of events that work to defend the body by bringing plasma protein and phagocytes to the injured area for the purpose of initiating tissue repair[8]. It is protective in nature due to its healing property but excessive inflammation is harmful because of its tissue damaging perspective. Sometimes inflammation also spirals out of control or becomes harmful which 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 carried out 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 pain medications.

In the world of analgesics Morphine sulphate is regarded as the gold standard or benchmark to relieve pain and sufferings. It is a worldwide recognized highly potent opiate analgesic used to relieve both acute and chronic severe pain by directly acting on the central nervous system. It is also used for pain due to myocardial infarction and labor pain[9], reduces shortness of breath and also in acute pulmonary edema[10]. It is also beneficial for reducing the symptoms of acute shortness of breath due to both cancer and non cancer causes[11]. According to the WHO Model list of essential Medicine, it is one of the most important medications in basic health system[12]. But it can frequently causes nausea, stomach upset, vomiting, constipation, drowsiness[13]. It has a high potential for addiction and abuse. If the dose is reduced after long term use, withdrawal may occur. Therefore, to minimize the side effects of this drug, now a days, different animal studies have been experimented to observe the beneficial effect of different vitamins on pain and inflammation with traditional analgesics[14]. Recently, the analgesic and anti-inflammatory effects of several members of the Vitamin B complex such as B_1[15], B_2[16], B_6[17] as well as B12 and folic acid[18] and α-tocoherol [19-20] have been demonstrated in different experimental animals. α-tocoherol is a well known anti-oxidant which is the most biologically active form of vitamin E. It was suggested to perform various functions in human body including antioxidation and prevention of infertility by preserving the sperm in male as well as by protecting the zygotes in female[21] , also involves in CD[36] gene expression[22], enzyme regulation[23], prevention of ataxia[24]. It has been experimented that deficiency of αT causes spinocerebellar ataxia, dysarthia, absence of deep tendon reflex, anemia, retinopathy[25-26].

As far as we know, no experiment has been done regarding the antinociceptive and anti-inflammatory effect of combination of morphine sulphate and α-tocopherol as a single loading dose and compares these effects with individual administration of morphine. Different investigators of different countries have observed significant reduction of the nociceptive pain, inflammatory pain and inflammation after supplementation of this vitamin in different doses in different animal model. In his study, as we used αT and its combination with morphine sulphate, so we used 500 mg/kg of αT through intraperitoneal route as a single loading dose.

Meterials and Methods

This prospective experimental study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka from 1st January 2013 to 31st December 2013. The study was approved by the Institutional Review board (IRB) of BSMMU.

Experimental Animals

For this study, fifteen (15) male long Evans rats, weighting about 180 to 250 gram were obtained from 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[27] with the room temperature of $28^0C \pm 5^0C$ (which was corresponded to the thermo-neutral zone of rats[28]) at the Pain laboratory of the Department of Physiology, BSMMU. The animals were there for consecutive

7 days prior to the experiments for acclimatization and had free access to standard laboratory food and boiled water after cooling. All the experiments were performed during the day time between 8:00 AM to 1:00 PM, to avoid the circadian influences.

Grouping

On the basis of vitamin and drug administrations, the rats were divided into three (3) groups (5 rats / each). Control group received normal saline, one experimental group received MS (3 mg/kg body weight) and another experimental group received combination of MS with αT (3 mg/kg body weight and 500 mg/kg body weight, respectively). All the groups received single dose and equal volume (1 ml) through intraperitoneal route 1 hour before the test. Just one hour after administrations, they were subjected to formalin test followed by immediate sacrifice and then formalin induced paw edema test.

All the experiments were conducted according to the guidelines for the Animal Experimentation Ethics Committee, Institute of Cholera and Diarrheal Disease Research, Bangladesh.

Formalin Test

On the day of experiment the rat was adminstered by NS or MS or combined dose of MS and αT intraperitonealy. One hour after adminstration, the rat was restrained by a thick towel and the right hind paw was exposed. Fifty (50) μl of dilute formalin (2%) was injected subcuteneously into the planter aspect of the rat's right hind paw with an insulin syringe. Immediately the animal was placed in the observation cage of the plexiglas formalin box(30×30×30 cm^3) and the pain behaviors (total frequency of jerking and total duration of flexing and licking) was observed for consecutive 60 minutes. Within this time the first 5 minutes (1^{st}-5^{th}) was considered as the early phase, middle 10 minutes (6^{th}-15^{th}) as the interphase and last 45 minutes (16th-60th) as the late phase[29]. Observation was made by counting the total frequency of jerking and total duration of flexing plus licking of the injected paw through a mirror fixed below the formalin box at 45^0 angle. A stop watch was used to count the time.

Formalin Induced Paw Oedema Test

Immediately after the completion of formalin test, the rat was sacrificed by using 10-12 ml of di- ethyl ether (99%) and both the hind paws of the sacrificed rat were cut at their knee joints by a sharp scissor. Then the volume of both the paws were measured using a water plethysmometer[30]. The paw volume was measured by using the following formula:

Paw volume=Height of water column after paw immersion-height of water column before paw immersion.

Net oedema volume=right paw volume-left paw volume

The results were expressed as mean±SE and the data were statistically analyzed by ANOVA followed by Bonferroni's Post Hoc test. In the interpretation of results $p \leq 0.05$ was accepted, as the level of significant.

Results

The effects of intraperitoneal (i.p) administration of MS and its combination with αT in early, inter and late phase were observed. In all the phases the study variables were observed as total frequency of jerking and total duration of flexing and licking in the formalin injected paw.

Nociceptive Pain

In the early phase of formalin test, All the mean values of this variable were significantly ($p \leq 0.001$) lowered in the study groups in comparison to that of control group. This variable was lowered in the combined administered group in comparison to morphine administered group but the difference was statistically non significant (Figure: 1)

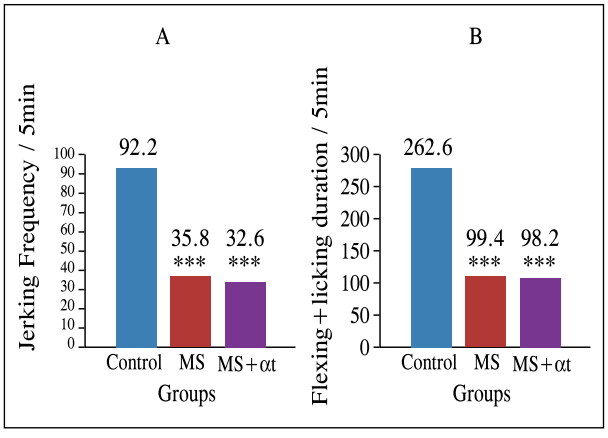

Figure 1: frequency of jerking (A) and duration of flexing and licking (B) in early phase of formalin test in different groups of rats. Each bar symbolizes for mean±SE for 5 rats. ***= $p \leq 0.001$, compared to control

Central Analgesic Activity

Again the frequency of jerking and the duration of flexing and licking in the interphase of formalin test were significantly ($p \leq 0.001$) lowered in the study groups in comparison to the control group. Moreover, this study variables were lowered in the combined administered group than that of morphine administered group but significantly ($p \leq 0.05$) lowered the duration of flexing and licking in the intrerphase of formalin test (figure: 2)

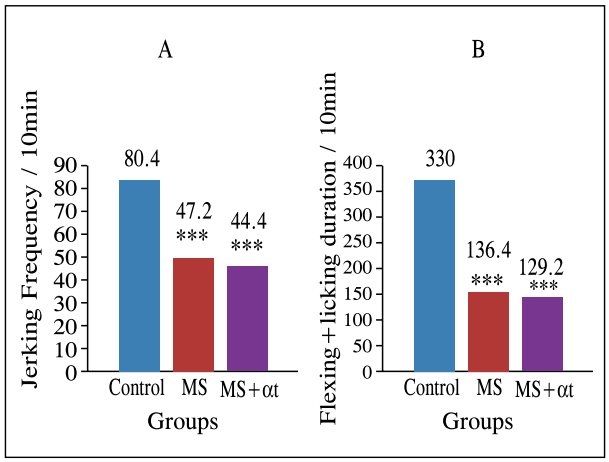

Figure 2: frequency of jerking (A) and duration of flexing and licking (B) in inter phase of formalin test in different groups of rats. Each bar symbolizes for mean ±SE for 5 rats. ***= $p \leq 0.001$, compared to control and # = $p \leq 0.05$, compared between MS vs MS+αT

Inflammatory Pain

In the late phase of formalin test, both test groups shows significant ($p = \leq 0.001$) reduction in the study variables in comparison to the control groups. Besides this, combined administration of morphine with aT reduced this pain variables than those of morphine alone but only significant ($p = \leq 0.01$) in duration of flexing and licking in late phase of formalin test (figure 3)

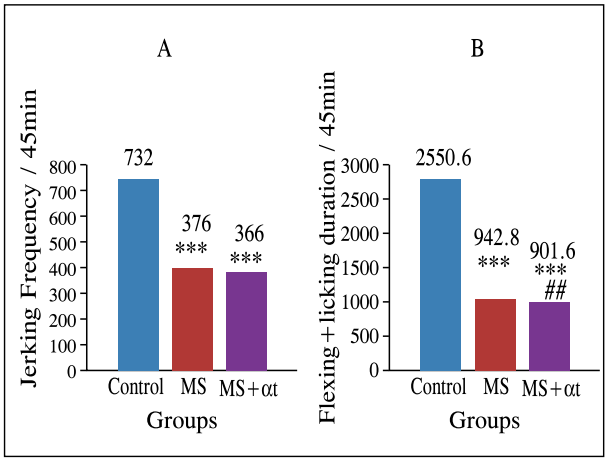

Figure 3: frequency of jerking (A) and duration of flexing and licking (B) in inter phase of formalin test in different groups of rats. Each bar symbolizes for mean±SE for 5 rats. ***= p?0.001, compared to control and ## = $p \leq 0.01$, compared between MS vs MS+αT

Anti- inflammatory effect:

The amount of paw edema volume was measured after the completion of formalin test. All the mean values were significantly ($p = \leq 0.01$) lowered in the study groups than the control group. In addition, this value was lowered in combined administered group than morphine alone but it was not statistically significant (figure 4)

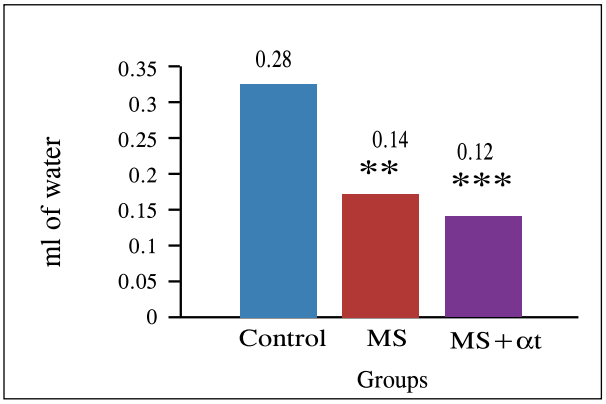

Figure 4: formalin induced paw edema voluin different groups of rats. Each bar symbolizes for mean±SE for 5 rats. ***=$p \leq 0.001$ and **= $p \leq 0.01$, compared to control.

Discussion

Pain and inflammation are the body's protective mechanism but from the most ancient period of time human have been trying to conquer pain and inflammation as they are the most unpleasant sensation among all the sensory perceptions. Our body itself has different mechanism for treatment of pain and inflammation but their discomfortness bring the patient to physicians and its management exceeds billion of dollars every year. So its our great responsibility to manage the pain in an appropriate way. From this point the present study was undertaken to assess the analgesic and anti inflammatory effect of a traditional analgesic morphine and compare its effects with the combination of αT

The formalin test is a useful model for the screening of both the nociceptive and inflammatory pain[31]. The centrally acting analgesic like morphine inhibits both early and late phase of formalin test[32]. Pain intensity in this test is dependent on some objective behavioral catagories which are converted to numerical values[33]. In this test the early phase results from the direct chemical stimulation of the nociceptive afferent fibers while the late phase results from the action of locally released inflammatory mediators and also by the facilitation of synaptic transmission in spinal cord[34]. In our study combined administration of morphine and vitamin lowered the nociceptive pain as well as inflammatory pain and enhanced the central analgesic activity in comparison to that of morphine alone but only significantly lowered the duration of flexing and licking in interphase ($p \leq 0.05$) and late phase ($p \leq 0.01$) of formalin test. Though the exact mechanisms of these effects could not be elucidated from this study, but several investigators of different countries suggested different mechanisms for the decrement of nociceptive pain and enhancing the central analgesic activity like increased activity of endogenous canabinoid or serotonergic pathway or closure of Ca^{2+}channel in presynaptic membrane or opening of K+channel in the post synaptic membrane, as the possible causes[35-37].

For measuring inflammation in animal study, paw edema test is an accurate and simple method[38]. In this study combined administration of morphine and α-tocopherol lowered the inflammation than individual administration of morphine but they are statistically non significant. Several investigators suggested several mechanisms like inhibition of COX, decrement of production of NO, TNF-α, free radicals, PGE_2 and bradykinin might be the possible mechanisms for lowering inflammation[39-40].

Though the exact mechanism of these more effectiveness of the combined administration could not be understand directly from this study, however, the concomitant activation of different pain lowering pathways at the same time might be the possible cause.

Conclusion

Therefore, from this result, it may be concluded that combination of morphine sulphate and αT reduced pain and inflammation more effectively, than individual administration of morphine sulphate. This study is help to reduce the adverse effect of this drug and also help the general population to achieve a better management for pain.

Conflict of Interest: Authors declared that they have no conflict of interest.

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