α-Tocopherol and Ketorolac on Pain in Long Evans Rats

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Abstract

Background: Alpha-tocopherol (αT) is a form of vitamin E that is widely known to be one of the reactive oxygen species scavengers (ROS) and it has been shown to have analgesic effects in different animal and human studies. But comparison of these effects with similar effects of ketorolac tromethamine (KT) and their combination have not been established. Objective: To assess the effects of αT on pain and also compare them with those of the combinations of αT with KT in rat models. Methods: This experimental study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from March 2015 to February 2016. For this, 20 (twenty) Long Evans rats (215±35 gm) of both sexes were divided into control (A, with 5mL/kg normal saline) and experimental (B1, with 500mg/kg αT; B2, with 10mg/kg KT; B3, with αT+KT) groups with 5 (five) rats in each group. All the drugs and vitamins were administered intraperitoneally in a single dose, just one hour before formalin test. To evaluate the effect of treatments on nociceptive pain, early phase (1st - 5th minutes); on central analgesic system, interphase (6th - 15th minutes); and on inflammatory pain, late phase (16th-60th minutes) of the formalin test, were observed. In all phases, total frequency of jerking and total duration of flexing and licking of the right hind paw were counted after administration of subcutaneous formalin (50 µL, 2.5%) injection. Statistical analysis was done by ANOVA, followed by Bonferroni post hoc test. In the interpretation of results, p≤0.05 was considered as significant. Results: αT lowered flexing and licking duration significantly (p≤0.001) in early and late phases. KT lowered flexing and licking duration significantly (P<0.001) in late phase of formalin test. On the other hand, combination of αT and KT significantly (p≤0.001) lowered both the study variables in early and late phase of formalin test. Conclusion: From this study it may be concluded that, αT possesses analgesic effects and combination of αT with KT is more effective than those of their individual administration.

Keywords: Pain, Analgesic, α-tocopherol, Ketorolac, Formalin Test.
To measure the efficacy of different analgesics, pain assessment is very crucial. For the measurement of nociceptive and inflammatory pain behaviors in animal model, the formalin test is one of the valid and reliable assessment tool. This test shows an early phase (1st - 5th minutes) which reflects direct activation of nociceptors, an interphase (6th - 15th minutes) showing the activation of central analgesic system and a late phase (16th - 60th minutes) reflecting pain due to inflammation.

Ketorolac tromethamine (KT) is an extremely effective and potent non-steroidal anti-inflammatory drug (NSAID) commonly used for short term management of severe acute pain associated with inflammation, that requires immediate analgesia. Long term use of KT is not indicated due to its association with peptic ulcer and many other systemic manifestations like, coagulation disorder, nephrotoxicity as well as severe impairment of cardiac, cerebral and hepatic functions.

In recent years, many studies have been conducted throughout the world with an aim to find alternatives to the traditional analgesic drugs in order to replace them or at least to reduce the duration of drug therapy, to minimize their adverse effects. Therefore, to minimize harmful side effects of traditional analgesics, different vitamins are being used concomitantly.

In 1922, Evans and Bishop discovered vitamin E, a fat soluble vitamin and α-tocopherol (αT) is the most biologically active among its eight naturally active forms. α-tocopherol, which is a relatively easy reactive oxygen species (ROS) scavenger, has also proved its antinociceptive effects in a animal experimental study. This vitamin’s antinociceptive effect is thought to arise from anti-inflammatory mechanism and it is anticipated to be effective in controlling both acute and chronic pain. Moreover, it has been suggested that, αT might act synergistically with NSAID to reduce gastric inflammation and pain of peptic ulcer disease.

However, it has also been reported that, single dose of 10 mg/kg KT was a sub effective dose against nociceptive as well as inflammatory pain. It has also been proposed that combined administration of analgesics with antioxidants in pain treatment might be applied to decrease the doses of analgesics and to prevent negative impact of ROS as well.

But, still the information regarding this matter is not sufficient enough to reach any final conclusion. Furthermore, no reported data was available to compare the combined analgesic effects of αT with KT to that of their individual administration on nociceptive and inflammatory pain.

On the basis of this background, the present study has been designed to evaluate the analgesic effects of single administration of Ketorolac as well as its combination with α-tocopherol to compare their combined analgesic effects with their individual administration in Long Evans rats.

**Methods**

This experimental study was conducted in the Pain laboratory of the Department of Physiology of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from March 2015 to February 2016. 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 Center for Diarrhoeal Disease Research, Bangladesh (icddr,b 2002).

**Procurement and maintenance of animals**

Twenty (20) healthy adult Long Evans rats weighing 180 to 250 gm were obtained from the animal house of Bangladesh University of Health Sciences (BUHS), Dhaka. All the rats were kept in the Pain laboratory of the Department of Physiology, BSMMU, where they were housed in specially built plastic cages with 6 rats per cage under a 12/12 hour light/dark cycle. The ambient room temperature was maintained at around 27 to 28°C, corresponding to the thermo-neutral zone for rodents. All the rodents had free access to standard laboratory food and cooled boiled water. They were kept there for a period of seven (7) consecutive days for environmental acclimatization, prior to the experiment. To avoid circadian influences...
all the experiments were performed at day time between 08:00 and 16:00 hours.34

Dose schedule
The α-tocopherol (Biopharma, Bangladesh) and Ketorolac tromethamine (Novartis, Bangladesh) were obtained in granular form and were dissolved in normal saline (5 ml/kg body weight). On the basis of drugs and vitamin administration, all the rats were divided into four (4) groups (5 rats/group). Of them control group (A) received only normal saline (5 ml/kg body weight)34, Vitamin treated group(B1) received αT (500mg/kg body weight)15, ketorolac treated group(B2) received ketorolac tromethamine (KT) (10mg/kg body weight)24, combination treated group (B3) received αT (500mg/kg body weight) and KT (10mg/kg body weight) in equal volume to that of normal saline, respectively. Just an hour after intraperitoneal (i.p)15, 22 administration of drug and vitamin, all the rats underwent formalin test.

Formalin test
In order to make the rats accustomed to the test environment, all the rats were placed in the observation chamber (34X34X34 cm³) of the plexiglass formalin box in pairs for fifteen (15) minutes daily for four (4) consecutive days, and singly for three (3) days prior to the test9, 35. On the day of experiment, each rat was intraperitoneally injected with normal saline or αT or KT or combinations thereof in accordance with the experimental paradigm being followed. Just one (1) hour later, the rat was restrained manually by a thick towel and fifty (50) µl of dilute (2.5%) formalin was injected subcutaneously35, 36 into the planter aspect of the right hind paw with an insulin syringe. Immediately thereafter, the animal was placed in the observation chamber of the plexiglass formalin test box, and pain behaviors were observed for consecutive sixty (60) minutes. A mirror fixed at an angle of 45° beneath the transparent floor of the chamber was used to facilitate unhindered observation. The first five (5) minutes (1st - 5th) was considered as the early phase, the next ten (10) minutes (6th - 15th) as the interphase, and the last 45 minutes (16th - 60th) as the late phase. During observation, the total number of jerking and the total duration of flexing plus licking of the injected paw (in seconds) per 5-minutes time block was also recorded. A stopwatch was used to count the time9, 30, 35.

Results were expressed as mean±SEM and the data were statistically analyzed by ANOVA, followed by Bonferroni post hoc test. In the interpretation of results p ≤ 0.05 was accepted as the level of significance.

Results
Antinociceptive effect
In this study, in the early phase of formalin test, the mean values of both variables were lowered in all the study groups than those of control group. However, this decrement was statistically significant (p ≤ 0.001) in the rats with single αT as well as in the rats with combined treatment of αT and KT, in both variables (Figure 1).

**Figure 1:** Frequency of jerking (A) and duration of flexing and licking (B) in early phase(1st-5th minutes) of formalin test in different groups of rats. Each bar symbolizes for mean±SE for 5 rats. ***=p≤0.001, compared to those of control. αT=α-Tocopherol; KT=Ketorolac tromethamine.
Similarly in the interphase (6th – 15th minutes) of formalin test, combined administration of KT and aT showed significant lowering of the jerking frequency \((p \leq 0.005)\) (Figure 2A) as well as of the duration of flexing and licking \((p \leq 0.001)\) (Figure 2B) when compared to control (Figure 2).

**Figure 2:** Frequency of jerking (A) and duration of flexing and licking (B) in the interphase (6th – 15th minutes) of formalin test in different groups of rats. Each bar symbolizes for mean±SE for 5 rats. \(* *=p \leq 0.001\) and \(*=p \leq 0.05\), compared to those of control. \(\alpha T=\alpha\)-Tocopherol; KT=Ketorolac tromethamine.

However, in late phase of formalin test, both the study groups showed significant reduction \((p \leq 0.001)\) in both pain variables compared to that of the control group (Figure 3). In addition, rats treated with combined administration showed statistically significant \((p \leq 0.001)\) reduction in the duration of flexing and licking than that of individual administration of KT in this phase (not shown in figure 3).

**Figure 3:** Frequency of jerking (A) and duration of flexing and licking (B) in the late phase (16th – 60th minutes) of formalin test in different groups of rats. Each bar symbolizes for mean±SE for 5 rats. \(* *=p \leq 0.001\), compared to those of control and \(* **=p \leq 0.001\). \(\alpha T=\alpha\)-Tocopherol; KT=Ketorolac tromethamine.
Discussion
In this study, individual administration of αT lowered the nociceptive pain as evidenced by reduction of frequency of jerking as well as duration of flexing and licking in the early phase of formalin test. Similar findings were observed by Majagi et al. (2011) and Juaira et al. (2014) in different animal models. On the contrary, individual administration of KT did not lower the nociceptive pain in our study. Similar finding was observed by Banode et al. (2012). However, the combined administration of αT and KT lowered this pain, significantly. No similar study was found to support this observation. It has been suggested that, αT increases K⁺ conductance in post synaptic membrane causing hyperpolarization of dorsal horn neuron. Hence, decrease pain transmission. On the other hand, KT also has a role on increasing K⁺ conductance, which might be synergized by administration of αT and caused more hyperpolarization of dorsal horn neuron and decreased pain conduction in our study as evidenced by significant difference in nociceptive pain variables in rats with individual αT administration and combined administration of αT with KT.

Moreover in the present study, individual administration of αT showed increment of central analgesic activity as evidenced by reduction of jerking in the interphase of formalin test. Similar findings were observed by Majagi et al. (2011) and Juaira et al. (2014) in different animal models. Again, individual administration of KT as well as its combination with αT also showed to increase the central analgesic activity as evidenced by reduction of both its variables. However, the combined administration of αT and KT showed more effectiveness in central analgesia in comparison to the single administration of vitamin and drug. No similar study was found to support this observation. It has been suggested that, αT might enhance the activity of GABA (gamma-Aminobutyric acid) in the CNS to diminish pain transmission. On the other hand KT might inhibit the NMDA (N-methyl-D-aspartate) induced excitation of NMDA receptors in the CNS to diminish pain transmission. It is assumed that any of the above mentioned mechanisms might be activated together and synergistically acted to cause more central analgesic effect after combined administration of αT and KT.

Furthermore in our study, individual administration of αT lowered inflammatory pain as evidenced by reduction of frequency of jerking as well as duration of flexing and licking in the late phase of formalin test. Similar findings were observed by Majagi et al (2011) and Juaira et al (2014) in different animal models. In addition, individual administration of KT also lowered inflammatory pain as evidenced by reduction of frequency of jerking as well as duration of flexing and licking in the late phase of formalin test. Similar response was also reported by other researchers after single administration of ketorolac at different doses in different pain models. However, the combined administration of αT and KT lowered this pain, significantly. No similar study was found to support this observation. It has been suggested that, αT might reduce inflammatory pain by decreasing plasma NO, TNF-α, PGE₂ (Prostaglandin E type 2) and other inflammatory mediators by inhibiting the prostaglandin synthesis by competitively blocking cyclooxygenase (COX). So, combined administration of αT and KT might play a synergized role in reducing inflammatory mediators hence, inflammatory pain.

Conclusion
From this study, it can be concluded that combined administration of αT and KT may be more effective in lowering pain than the individual
administration of KT. This data may apprise the clinicians and general populations to used αT along with KT for better management of pain. Although further experimental study is needed to elucidate the exact component and mechanism for these effects.

Conflict of interest: None

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