ORIGINAL ARTICLE

JOURNAL OF AD-DIN AKIJ MEDICAL COLLEGE

Vol. 2 | No. 2 | July 2023
JAAMC 2023; 2(2): 19–24

Effects of ketorolac and α-tocopherol combination on formalin-induced paw edema in rats

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Received: 08 Mar 2023
Accepted: 10 Apr 2023

Abstract

Background: *Alpha-tocopherol (αT) is a fat-soluble antioxidant that protects cell membranes and other cellular components from oxidative damage. In addition to its antioxidant properties, alpha-tocopherol has also been found to possess anti-inflammatory activities in many studies. But a comparison of these effects with similar effects of ketorolac tromethamine (KT) and their combination has not been established.* ***Objectives:*** *To assess the impacts of αT and KT on inflammation and compare them with the varieties of αT and KT in rat models.* ***Materials and Methods:*** *This experimental study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. For this, 20 (twenty) Long Evan's rats of both sexes were divided into control (with 5mL/kg normal saline) and experimental (with 500mg/kg αT; with 10mg/kg KT; 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 the formalin test. To evaluate the treatments' effect on inflammation, rats' right hind paws were injected subcutaneously into the plantar surface with formalin (50 µL, 2.5%) except for the control group. The paw volume was measured by using a water plethysmometer. Statistical analysis was done by ANOVA, followed by Bonferroni post hoc test. In the interpretation of results, $p \leq 0.05$ was considered significant.* ***Results:*** *αT mediated reduction in inflammation was not statistically significant. KT lowered the inflammation more than αT, and it was statistically significant. On the other hand, the combination of αT and KT reduced the inflammation more significantly ($p \leq 0.001$).* ***Conclusion:*** *From this study, it may be concluded that the variety of αT with KT is more effective in reducing inflammation than those in their administration.*

Keywords: *Inflammation, α-tocopherol, ketorolac, formalin, paw oedema.*

Introduction

Inflammation is a local response of living mammalian tissues to injury. It is a body's defence reaction to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction injury. Oedema formation, leukocyte infiltration, and granuloma formation represent such components of inflammation (1). Oedema formation in the paw results from a synergism between various inflammatory mediators, increasing vascular permeability and blood flow (2). Even though different allopathic drugs like immunosuppressants, NSAIDs, corticosteroids, and antihistamines have been used till now, their potential side effects limit their use. There is a growing concern about developing a new, safe, potent, and less toxic anti-inflammatory drug. Hence, there is a need to explore more naturally available alternatives so that their therapeutic values can be assessed and expanded (3, 4). Vitamin E is a lipophilic vitamin, and α-tocopherol is the most physiologically active of its eight naturally active forms (5, 6). Alpha-tocopherol (αT) can help to reduce inflammation by inhibiting the production of pro-inflammatory cytokines and enzymes (7). αT's antinociceptive activity is thought to be related to a mechanism that suppresses anti-inflammatory actions, and it is likely to be helpful in treating both acute and chronic pain. Additionally, it has been claimed that αT may operate with NSAIDs to reduce gastrointestinal inflammation and discomfort in people suffering from peptic ulcer disease (5, 8, 9).

Ketorolac tromethamine (KT) is a potent nonsteroidal anti-inflammatory medication (NSAID) that is often used to treat severe acute pain caused by inflammation that needs urgent analgesia, such as postoperative pain, renal colic, arthritis, lumbago, headache, and cancer pain (10, 11). Studies have been conducted worldwide to find analgesic alternatives that can replace or, at the very least, shorten the duration of drug therapy, to minimise any adverse effects of the medicine (12, 13).

Materials and Methods

This experimental study was conducted in the Pain Laboratory of the Department of Physiology after receiving permission from the Institutional Review Board (IRB, No. BSMMU/2015/5994) 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 outlined 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) (14).

Procurement and maintenance of animals:

Twenty (20) healthy adult Long Evans rats weighing 180 to 250 g of both sexes (15, 16, 17) were obtained from the animal house of the 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 six rats per cage under a 12/12 hour light/dark cycle (18, 19). The ambient room temperature was maintained at around 27 to 28°C, corresponding to the thermo-neutral zone for rodents (20, 21). All the rodents had free access to standard laboratory food and cooled, boiled water (22). They were kept there for seven consecutive days for environmental acclimatisation before the experiment. To avoid circadian influences, all the experiments were performed during day time between 08:00 and 16:00 hours (7, 8).

Dose schedule:

The α-tocopherol (Biopharma, Bangladesh) and Ketorolac tromethamine (Novartis, Bangladesh) were obtained in granular form and dissolved in normal saline (5 ml/kg body weight). Based on drugs and vitamin administration, all the rats have divided into four(4) groups (5 rats/group); the control group received only normal saline (5 ml/kg body weight)(22), Vitamin treated group received α T (500mg/kg body weight) (8), ketorolac treated group received ketorolac tromethamine (KT) (10mg/kg body weight) (15), the combination-treated group 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) (8, 23) administration of drug and vitamin, all the rats underwent a formalin test.

Formalin-induced paw oedema test:

To make the rats accustomed to the test environment, all the rats were placed in the observation chamber (34X34X34cm3) of the plexiglass formalin box in pairs for fifteen(15) minutes daily for four (4) consecutive days and singly for three (3) days before the test(10, 24). On the day of the experiment, each rat was intraperitoneally injected with normal saline, or αT or KT or combinations thereof, following 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 subcutaneously (24, 25) into the planter aspect of the right hind paw with an insulin syringe. Immediately after that, the animal was placed in the observation chamber of the plexiglass formalin test box, and pain behaviours were observed for a consecutive sixty (60) minutes. Immediately after completing the formalin test, all the rats were sacrificed. After sacrifice, inflammation was measured by a formalin-induced paw oedema test in all the groups. The hind paws of the sacrificed rat of all the groups were cut at their knee joints by sharp scissors. Then the paw volume was measured using a water plethysmometer (20, 26).

Paw volume = (amount of water column after paw immersion – amount of water column before paw immersion.)
Net oedema volume was calculated by subtracting the left from the right paw volume.
Net oedema volume = right paw volume – left paw volume.

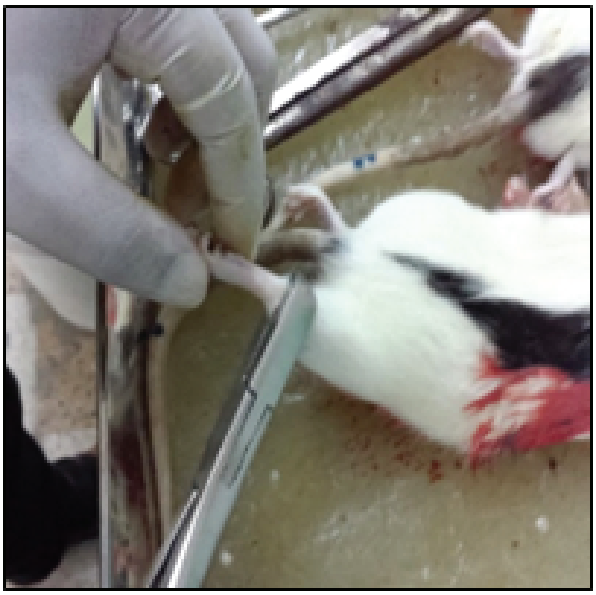

Figure 1

Figure 2

Results were expressed as mean±SEM, and the data were statistically analysed by ANOVA, followed by Bonferroni post hoc test. In interpreting results, $p \leq 0.05$ was accepted as the significance level.

Results

In this study, the differences in the mean values as well as the percent reduction of oedema volume among the groups were statistically not significant except in the control group vs ketorolac treated group and control group vs combinedly (αT+KT) treated group, where the difference of the mean value of this variable was statistically significant ($p \leq 0.01$).

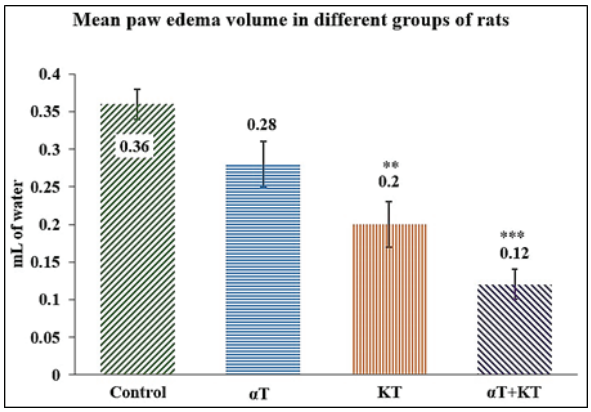

Figure 3: Reduction in oedema volume in paw oedema test in different groups of rats. Each bar symbolises mean±SE for five rats. ** = $p \leq 0.01$, *** = $p \leq 0.001$, compared to those of control. αT = α-tocopherol; KT = Ketorolac tromethamine.

Discussion

Tissue damage and injury are always associated with pain and inflammation. In formalin-induced paw oedema, the inflammatory reactions are mediated by prostaglandin, serotonin, histamine, bradykinin, and cytokines, such as interleukin-1 beta, interleukin-6 tumour necrosis factor-alpha eicosanoids, and Nitric Oxide (27). Inflammation was not significantly lower in αT supplemented group, as evidenced by reduced paw oedema volume compared to that of the control. Many investigators from different countries also reported a similar observation in animal models (28, 29, 30) and human models (31). In this study, inflammation was significantly decreased after combined administration of αT and KT than that of controls, as evidenced by reduced oedema volume in formalin-induced paw edema. Moreover, this variable was significantly lower after combined administration of αT and KT than their individual intervention as shown by more reduction of formalin induced paw edema. However, no published data were available to compare all these findings as mentioned earlier for combined administration of αT with KT.

Conclusion

In summary, single-dose administration of α-tocopherol failed to show a significant anti-inflammatory effect. On the other hand, ketorolac tromethamine and its combination with α-tocopherol showed anti-inflammatory effects. However, their combined administration showed a more significant anti-inflammatory effect than the individual administration of ketorolac. Therefore, it is possible to deduce that a combination of α-tocopherol and ketorolac tromethamine may reduce inflammation to a greater extent than their administration alone. This data may apprise the clinicians and the general population about using α T along with KT for better inflammation management. However, a further experimental study is needed to elucidate these effects' exact components and mechanisms.

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