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Comparison of analgesic effects of Thiocolchicoside and Ketoprofen, and their combination in animal models

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Abstract
Objectives: To compare the analgesic activity of thiocolchicoside and ketoprofen alone and their combination in animal models for the degree of analgesia and the time course of action.
Materials and Methods: Analgesia was studied in albino rats using formalin test and in albino mice using writhing test and in rats radiant heat method. For each test, four groups of six animals each were orally fed with a single dose of thiocolchicoside and ketoprofen, and combination of thiocolchicoside and ketoprofen and normal saline as control, respectively.
Results: In all the three test models, all three drug treatments showed significant analgesia (P<0.001) as compared to control, but there was a significant difference in the analgesia produced by combination of thiocolchicoside and ketoprofen. The radiant heat method, writhing test and formalin test demonstrated a quicker onset of action with thiocolchicoside and ketoprofen alone, but greater degree of analgesia (synergism) was seen with the combination of drugs than individual drugs, though this difference was a statistically significant.
Conclusions: Combination of thiocolchicoside and ketoprofen offers advantage over thiocolchicoside and ketoprofen alone, either in terms of degree of analgesia or onset of action. Therefore, our study supports the reports claiming rationality of the fixed dose combination of thiocolchicoside and ketoprofen.

Keywords: skeletal muscle relaxants, Analgesia and reaction time

Introduction
Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely prescribed drugs for pain, fever, and inflammation. Worldwide, over 73 million prescriptions of NSAIDs are written yearly, and approximately 30 million people take NSAIDs daily [1, 2]. The data of global and Indian studies showed that the total number of NSAIDs prescription ranges from 15% to 40% [3, 4, 5]. Various fixed dose combinations of NSAIDs and skeletal muscle relaxants are available in the market, particularly those of thiocolchicoside and ketoprofen. Following this trend, many drug companies have introduced thiocolchicoside and ketoprofen combination and claim that it has faster onset and longer duration of analgesic and then either drug alone [6]. So, some authors have claimed that the combination of thiocolchicoside and ketoprofen does offer advantage and is therefore rational. Thiocolchicoside is skeletal muscle relaxant and mode of action includes modulation of chemokine and prostanoid production. It is an analgesic and anti-inflammatory properties [7, 8, 25]. In addition, thiocolchicoside does not have significant gastro duodenal side effects [9, 10]. Ketoprofen is NSAID with analgesic anti-inflammatory and antipyretic actions and It inhibits the COX enzyme which is required for the synthesis of inflammatory mediators [1, 2].

Materials and methods
Animals
Healthy male albino Wistar rats (3 months old) weighing 200-250 g were used for ant nociceptive tests. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Lipton India Laboratories, Bangalore) and water ad libitum and were fasted overnight before the day of experiment. Animals were housed within the departmental animal house, and the room temperature was maintained at 27°C.

The protocol was approved and carried out after the permission of Institutional Animal Ethics Committee. Animal house registration No-1392/ac/10/CPCSEA.

Investigational drugs and dosage preparation

Tablet thiocolchicoside 4 mg (Glaxo- Smithkline, Dr. Annie Besent Road, Worli, Mumbai) was purchased from the hospital pharmacy counter. Ketoprofen 400 mg and thiocolchicoside and ketoprofen combination 404 mg (Emcure Pharmaceuticals Ltd., Dopadi, Pune) were also procured from the same hospital outlet. The appropriate body weight adjusted doses of test drugs as extrapolated from doses used in similar studies conducted previously to be 0.09µg/250 g rat for thiocolchicoside, ketoprofen 10µg/250 g rat and 10.38 µg/250 g rat for thiocolchicoside and ketoprofen combination were used [6].

Formulations were made as suspension prepared in 10 ml of DMSO uniformly mixed. The formulations were fed to the animals through gastric tube (9 mm) for rat. The normal saline alone was used as a control group.

Experimental protocol

Animals (n = 24) were allocated to four main groups (GI, GII, GIII and GIV) of 6 animals each. Depending on the treatment design, each receiving group 1 thiocolchicoside, group II ketoprofen, group III combination of thiocolchicoside and ketoprofen, group IV normal saline as the control, respectively.

Radiant heat method

Prior to subjecting rats to their individual analgesic response to test drugs, animals were subjected to a preliminary screening and rats showing tail flick response in 10 s were selected. Test drugs were administered. Each mouse was restrained, and radiant heat was applied to a portion of tail (about 5 cm from the tip) placed 2 mm above (5A) heating wire of Digital Analgesiometer (INCO). The current was allowed to flow through heating wire and the time taken for the mouse to show tail flick response was recorded every 30 min up to 90 min maximum [11].

Formalin test

Twenty minutes after p.o. administration of control or investigational drugs, all the rats (n = 24) in GI received 50 µL of 5% formalin subcutaneously into the plantar portion of left hind paw by using tuberculin syringe to produce chemically induced pain. Each animal was put in a transparent cage made of perspex material and observed for behavioral scoring for 30 min, starting 20 min after formalin injection [12]. Pain severity was graded from g0 to g3 and time spent in each grade was recorded as t0 to t3 using following indices: g0 × t0 (grade 0 for t0 s)= pain free (injected paw not favored) g1 × t1 (grade 1 for t1 s) = mild pain (injected paw raised high on floor)g2 × t2 (grade 2 for t2 s) = moderate pain (injected paw was elevated)g3 × t3 (grade 3 for t3 s) = severe pain (injected paw was licked and bitten or shaken)Pain score was calculated as:

$$= g0t0 + g1t1 + g2t2 + g3t3\ t0 + t1 + t2 + t3$$

Writhing test

Calculate, weighe adjusted, doses of test drugs, i.e. thicolchicoside 0.09 µg and ketoprofen 10 µ g/mL in the vehicle, i.e. 2% gum acacia and the control were given p.o. 20 min prior to their antinociceptive effect as tested by counting the number of writhes by calculating the average number of writhes recorded every 30 min up to 240 min for each test group after injection of 1% (1 mL/100 g) acetic acid i.p. [13] The antinociceptive activity was calculated as percent maximum possible effect (% MPE).

Radiant heat method

Prior to subjecting mice to their individual analgesic response to test drugs, animals were subjected to a preliminary screening and mice showing tail flick response in 10 s were selected. Test drugs were administered and antinociceptive response checked as done for the writhing test. Each mouse was restrained, and radiant heat was applied to a portion of tail (about 5 cm from the tip) placed 2 mm above (5A) heating wire of Analgesiometer (INCO). The current was allowed to flow through heating wire and the time taken for the mouse to show tail flick response was recorded every 30 min up to 90 min maximum [11].

Statistical analysis

The results of all the three methods namely, formalin test, writhing test, and the radiant heat method are expressed as mean ± SEM. Statistical analysis for the formalin test was done using Wilcoxon-Mann-Whitney test, and data of the other two methods (writhing test and radiant heat method) were statistically analyzed by using ANOVA (two-way classification analysis). A probability value of less than 0.05 (P<0.05) was considered to be statistically significant.

Results

Formalin-induced pain

Significant analgesia was seen in all three treated groups compared to control [Figure 1]. Analgesia in groups treated with thicolchicoside and ketoprofen alone was lesser than that treated with with thicolchicoside and ketoprofen in combination. However, this difference was statistically significant. {Figure 1}

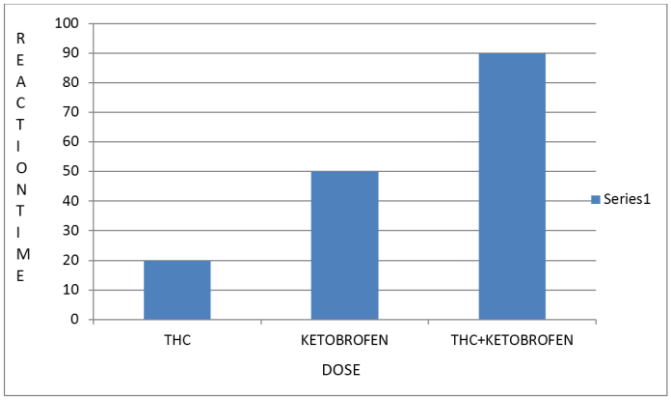

Fig 1

Acetic acid-induced writhing in mice

The drug-treated groups showed significant reduction in number of writhes compared to control [Table 1]. Studying the time course of action, thiocolchicoside action started within 20 min (less than 50% reduction in number of writhes) with complete abolition of writhes from 60 min onwards and ketoprofen reduction the number of writhes to 50% at 60 min and has taken 90 min to completely abolish the writhes. The combination (thicolchicoside and ketoprofen) complete abolition of writhes has occurred within 60 min. However,

this difference in time course of action of the drug treatment was statistically significant. {Table 1}

Table 1

Compound	Dose(mg/Kg)	No of writings	% of inhibition
Control	--	36 ±2.16	--
THC	0.37	19.6 ±1.15	42.5
KETOPROFEN	6.66	20 ± 2	50
THC + KETOPROFEN	6.73	8.33 ±2.51	100

Discussion

This study was undertaken to compare the analgesic efficacy of thiocolchicoside and ketoprofen alone and in combination in different animal models. The comparison was done in terms of degree of analgesia and time course of action. The combination had been introduced in the market on the pretext that it will have the benefits of thiocolchicoside and ketoprofen. As thicolchicoside is faster acting and ketoprofen is longer acting, it was presumed that their combination will have synergistic effects [14]. However, our study showed that in writhing test and in radiant heat method the onset of action of thiocolchicoside was the fastest. Both these methods showed that the combination of thiocolchicoside and ketoprofen is definitely greater than either drugs used alone. The formalin test also indicated slightly greater degree of analgesic activity with combination drugs than with their individual drug alone. Similar observations have been made by other authors [15]. Hence, the popular belief that combining the two drugs would give a faster onset does not seem to be substantiated. However, since our observation period was only 90 min, we cannot make any comment about the other advantages of longer duration claimed by the combination. Further studies with longer periods of observation would probably throw more light on the validity of this combination. In addition, pain is a symptom with substantial subjective component as well. It is, therefore, difficult to comment on the effectiveness of an analgesic purely on the basis of animal studies. Hence, it would also be necessary to test the combination on human subjects, both on experimental pain in healthy volunteers as well as clinical pain, before commenting on the appropriateness of this combination. Many studies have compared the analgesic efficacy of ketoprofen with other NSAIDs; [16, 17, 18]. however, there is no such study, which compares thiocolchicoside with the ketoprofen combination. Usually, the fixed dose combinations are introduced in the market to generate prescriptions and make profit with no consideration of the rationality [19, 20]. Combinations of analgesics are more effective if they act through different analgesic mechanisms and act synergistically [21]. In contrast, the components of the fixed dose

Combination of thiocolchicoside and ketoprofen act by the deferent mechanism of inhibition of prostaglandin biosynthesis. It has been seen that ketoprofen is as good analgesic as any other NSAIDs, but its combination with thiocolchicoside increases its efficacy. Usage of many available fixed dose combinations is controversial, and it is the need of hour to sensitize all practitioners and consumers against this practice.

Conclusion

Our study shows that thiocolchicoside and ketoprofen combination was more efficacious analgesics as compared to the thiocolchicoside and ketoconazole alone. Both the drugs potentiate or synergize the action of the each other. Therefore, there is pharmacological rationale for the combined administration of thiocolchicoside and ketoprofen. However, further studies with longer periods of observation are warranted to evaluate the validity of the combination. In addition, studies of the combination on human subjects are necessary to assess the subjective degree of pain and appropriateness of the combination.

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