Evaluation of Analgesic and Anti-inflammatory Activity of a Combination of Tramadol-ibuprofen in Experimental Animals

Abstract

Background: Pain is the major concern of patients attending dental clinics, and satisfactory pain relief has always been difficult to achieve. Since the pathophysiology of pain is a complex, central and peripheral nervous system process, combined analgesic regimens with different mechanisms of action as a multimodal approach are becoming popular among the clinicians and dentists. Objectives: The aim of the present study was to evaluate the analgesic and anti-inflammatory activity of ibuprofen and tramadol when used alone or in combination in animal models of pain and inflammation. Animals and Methods: The animals were divided into six groups with six animals in each group. Analgesic activity was assessed by hot plate method in rats and by acetic acid-induced writhing test in mice. Paw edema model in rats after induction with 0.1 mL of 1% carrageenan was used to assess the anti-inflammatory activity. Statistical Analysis: Analysis of variance followed by Tukey’s honestly significant difference post hoc test was used for statistical analysis. Results and Conclusion: Combined use of tramadol and ibuprofen provided enhanced analgesic and anti-inflammatory effects in animal models of pain and inflammation.

Keywords: Analgesia, anti-inflammatory activity, ibuprofen, tramadol

Introduction

Pain is the major concern of patients attending dental clinics, and satisfactory pain relief has always been difficult to achieve. An experience of poorly managed pain related to dental treatment may lead patients to avoid or postpone treatment. A recent update on relative efficacy of oral analgesics after third molar extraction indicated that no single drug is effective in all patients and that even the best drugs failed to provide good levels of pain relief in at least 30%.\(^1\)

Ibuprofen, a 2-proprionic acid derivative, is a peripherally acting nonsteroidal anti-inflammatory drug (NSAID) that works through a reversible and balanced COX-1/COX-2 inhibition. It has been evaluated extensively in postoperative dental pain, and several studies support its efficacy.\(^2\) Despite its clinical efficacy, some patients do not receive adequate relief from a normal therapeutic dose because of its relatively flat dose–response relationship. The traditional approach to overcome this well-recognized limitation is to combine a therapeutic dose of ibuprofen with another agent such as paracetamol or codeine that provides additive analgesia but without an unacceptable increase in the incidence of adverse effects.\(^3\)

Tramadol is a synthetic, centrally acting analgesic agent with two distinct, synergistic mechanisms of action, acting as both weak opioid agonist and inhibitor of monoamine neurotransmitter re-uptake. The two enantiomers of racemic tramadol, (+) & (−), function in a complementary manner to enhance the analgesic efficacy and improve the tolerability profile of tramadol.\(^4\)

Since the pathophysiology of pain is a complex, central and peripheral nervous system process, combined analgesic regimens with different mechanisms of action as a multimodal approach are becoming popular, with the aim of reducing reliance on opioids and its complications.\(^5\) Although combinations offer the possibility of several desirable outcomes, the combinations must be rigorously tested before being introduced into clinical practice.\(^6\) Hence, the aim of the present study was to evaluate the analgesic and anti-inflammatory activity of ibuprofen and tramadol when used

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alone or in combination in animal models of pain and inflammation.

Animals and Methods

Animals and ethics

The entire study was carried out using adult Wistar rats (weighing between 180 g and 230 g) and Swiss albino mice (weighing between 25 g and 30 g) of both sexes between 09.00 am and 12.00 noon. Animals were kept in cages in temperature-regulated rooms with air-cooling, 12 h light and dark cycle and had free access to water and standard laboratory diet. The rats and mice were divided into six groups with six members in each group. Ibuprofen was administered at dosage of 10 and 30 mg/kg while the dosage for tramadol was 5 and 10 mg/kg. Control group was administered normal saline at the dose of 10 mL/kg, while the combination group received ibuprofen 10 mg/kg and tramadol 5 mg/kg. All the drugs were administered through oral route after dissolving them in distilled water. The study was approved by the Institutional Scientific Research Committee and Animal Ethics Committee. All chemicals and solvents (ibuprofen sodium, tramadol hydrochloride, and λ-carrageenan saline) used were of analytical grade and were obtained commercially.

Analgesic effect

The analgesic activity of ibuprofen or tramadol alone or in combination was evaluated in rats by a modified hot plate procedure\textsuperscript{[7]} with hot plate maintained at 49.5°C ± 0.1°C before (0) and 30 and 60 min after administration of the test drugs, and analgesia was defined as prolongation of latency without licking or flicking of hind limb or jumping. A cutoff time of 20 s was used to avoid tissue injury. The analgesic activity was also evaluated in mice using acetic acid-induced writhing (0.6% acetic acid 10 mL/kg)\textsuperscript{[9]} over a period of 20 min. The test drugs were administered orally to the mice 30 min before acetic acid injection. Based on the number of writhes, percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition} = \left(1 - \frac{(W_t - W_c)}{W_c}\right) \times 100$$

where $W_c$ = number of writhes in control group and $W_t$ = number of writhes in test group.

Anti-inflammatory activity

We followed the method adopted by Winter et al.\textsuperscript{[9]} and subsequently modified by Singh and Ghosh\textsuperscript{[10]} for evaluating the acute anti-inflammatory activity in rats. Inflammation was induced by subplantar injection of 0.1 mL of 1% suspension of λ-carrageenan in normal saline, in the right hind paw of the rats. Just before administration of carrageenan, the test drugs were administered. The paw volume was measured using a plethysmometer before (0) and 30 and 60 min after administration of the test drugs.

Statistical analysis

The data were expressed as mean ± standard deviation. All data were subjected to analysis of variance and individual comparisons were made using Tukey’s honestly significant difference post hoc test. The differences were considered statistically significant if $p < 0.05$.

Results

The results of the hot plate test [Table 1] indicated that latency in hot plate testing was significantly increased in all the test groups and that pain threshold was highest in those rats administered with the combination of tramadol plus ibuprofen as compared to ibuprofen or tramadol alone. In the peripheral analgesic model, there was a significant dose-dependent decrease in the number of writhes in all the treated groups as compared to the control group ($P < 0.05$) and highest percent inhibition was observed with the combination group [Table 2].

The results of λ-carrageenan-induced mice paw edema [Table 3] indicated that ibuprofen (10 and 30 mg/kg) significantly inhibited the development of paw edema induced by carrageenan after 30 and 60 min of treatment, which was partially dose dependent. The combination of lower doses of ibuprofen and tramadol showed a slightly greater reduction in paw volume than the higher dose of ibuprofen (52.8 and 54.4% vs. 47.8 and 48.9%) at 30 min and 60 min.

Discussion

Most pain conditions involve multiple pain generating processes, and pain is conducted through a large number of

| Table 1: Effect of ibuprofen or tramadol alone or in combination on time latency in hot plate test in rats |
|-----------------------------------------------|
| Group | Drug            | Dose mg/kg | Pretreatment | Time latency (s), mean±SD |
|       |                 |            | 0 min        | 30 min | 60 min |
| I     | Saline          | 10 mL/kg   | 4.45±0.62    | 4.48±0.77 | 4.46±0.59 |
| II    | Ibuprofen       | 10         | 3.97±0.30    | 10.4±1.01*** | 9.37±0.65*** |
| III   | Ibuprofen       | 30         | 4.35±0.52    | 9.73±0.77*** | 9.23±0.45*** |
| IV    | Tramadol        | 5          | 4.47±0.45    | 13.05±0.94*** | 10.7±0.98*** |
| V     | Tramadol        | 10         | 4.30±0.76    | 13.98±0.64*** | 12.01±0.21*** |
| VI    | Ibuprofen + tramadol | 10±5   | 4.41±0.56    | 15.03±0.92*** | 12.81±0.52*** |

ANOVA: $F=102.55$. *$p<0.0001$ as compared to control. SD=Standard deviation, ANOVA=Analysis of variance.
Table 2: Effect of ibuprofen or tramadol alone or in combination on acetic acid-induced writhing in mice

| Group | Drug           | Dose mg/kg | Mean±SD | Number of writhing | Percentage inhibition |
|-------|----------------|------------|---------|-------------------|----------------------|
| I     | Saline         | 10 mL/kg   | 32.5±1.9 | 0                 | 0                    |
| II    | Ibuprofen      | 10         | 24.2±2.9** | 25.7±6.4**        |
| III   | Ibuprofen      | 30         | 20.3±2.9*** | 37.0±11.1***      |
| IV    | Tramadol       | 5          | 20.2±5.7*** | 38.2±16.2***      |
| V     | Tramadol       | 10         | 18.5±1.9*** | 42.9±6.9***       |
| VI    | Ibuprofen + tramadol | 10+5 | 12.3±4.1*** | 61.6±14.7***      |

ANOVA F=22.08. **p<0.01 as compared to control, ***p<0.001 as compared to control. SD=Standard deviation, ANOVA=Analysis of variance

Table 3: Effect of ibuprofen or tramadol alone or in combination on carrageenan-induced paw edema in mice

| Group | Drug           | Dose mg/kg | Mean±SD | 0 min | 30 min | 60 min |
|-------|----------------|------------|---------|-------|--------|--------|
| I     | Saline         | 10 mL/kg   | 0.52±0.02 | 0.48±0.03 | 0.51±0.02 |
| II    | Ibuprofen      | 10         | 0.48±0.02 | 0.32±0.02*** | 0.30±0.03*** |
| III   | Ibuprofen      | 30         | 0.47±0.01 | 0.25±0.03*** | 0.24±0.04*** |
| IV    | Tramadol       | 5          | 0.47±0.02 | 0.43±0.02 | 0.41±0.02 |
| V     | Tramadol       | 10         | 0.48±0.02 | 0.40±0.64 | 0.41±0.03 |
| VI    | Ibuprofen + tramadol | 10+5 | 0.47±0.01 | 0.22±0.02*** | 0.21±0.02*** |

ANOVA F=117.66. ***p<0.0001 as compared to 0 min. SD=Standard deviation, ANOVA=Analysis of variance

diverse pathways. A practical treatment approach therefore is using drugs or drug combinations with different mechanisms of action and thus different targets. However, not all combinations are ideal since it might lead to a higher than anticipated side effect rate and hence thorough testing is warranted. Waite et al.[11] while evaluating the efficacy of common analgesics in rats found ibuprofen at 30 mg/kg to be significantly effective in hot plate assay and 10 mg/kg in reducing postsurgical pain. Tramadol (10 mg/kg) was found to have no effect on pain threshold levels by tail flick latency in mice while 20 or 40 mg/kg was found to enhance pain threshold levels.[12] Hence, 10 and 30 mg/kg of ibuprofen and 5 and 10 mg/kg of tramadol and the lower dose of each drug were considered as the doses to be used in this study.

The present study carried out to evaluate the analgesic and anti-inflammatory activity of ibuprofen and tramadol when used alone and in combination in animal models of pain and inflammation indicated that combined use of ibuprofen and tramadol at low doses provided a better analgesic and anti-inflammatory effect. Since combination analgesics offer the possibility of efficient analgesia with a decrease in side effects as a result of reduced dosages of one or both compounds,[13] the combination of ibuprofen and tramadol may offer a worthwhile option to the practicing dentist.

Experimental studies carried out in rats to study the effects of tramadol administration on experimental inflammation (yeast injection and formalin administration) indicated that tramadol significantly reduced the edema.[14,15] El-Sharrawy et al.[16] while assessing the anti-inflammatory effects of ibuprofen and tramadol by measuring the C-reactive protein (CRP) levels, an acute phase reactant and a useful marker for the amount of tissue injury and inflammation, after surgical removal of third molar tooth in humans, found CRP levels to be elevated both in ibuprofen and tramadol treated groups and that elevation was higher in the tramadol group, indicating that tramadol has less anti-inflammatory effect than ibuprofen. Our results indicate that tramadol is devoid of significant anti-inflammatory effect.

Kalra et al.[17] while evaluating the gastric tolerability, antinociceptive and anti-inflammatory activity of combination NSAIDs in rats opined that fixed-dose combinations NSAIDs do not possess additional analgesic activity over their individual components. A later study[18] assessed the anti-inflammatory and antinociceptive activity of commonly used NSAIDs - paracetamol, ibuprofen or diclofenac sodium alone or the latter two in combination with paracetamol, in animal models of pain and inflammation found the addition of paracetamol to ibuprofen increased both analgesic and anti-inflammatory activities. Our results of ibuprofen-tramadol combination having more analgesic, anti-inflammatory activities are in agreement with the latter part of the study. Our results of combination having more analgesic, anti-inflammatory activities are in agreement with the later study.

While the constriction response of the abdomen induced by acetic acid is a sensitive method for evaluating peripheral analgesic agents, tail flick response is a sensitive method for central analgesic agents and carrageenan-induced inflammation model is a predictive test for anti-inflammatory agents acting against mediators of acute inflammation. The results obtained in our study indicated that the combination of ibuprofen and tramadol...
had peripheral and central analgesic actions as well as anti-inflammatory activity. Since the current therapy for pain relief is inadequate for some patients, newer drugs or combination of existing drugs are warranted. Hence, use of tramadol which has both central opioid and monoaminergic effects with peripherally acting NSAIDs such as ibuprofen may provide enhanced analgesia and anti-inflammatory effects in clinical situations associated with pain and inflammation such as extraction of third molar tooth. We have not explored the optimal dosage of the combination drug to be used. It remains to be seen whether combining ibuprofen with tramadol is additive or synergistic in clinical situations; clinical studies are therefore warranted before any firm conclusion can be made about this combination.

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**Conflicts of interest**

There are no conflicts of interest.

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