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Original Article

Pharmacology Section

Evaluation of Analgesic and Anti-Inflammatory Activity of Ibuprofen-Pregabalin in Animal Models

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ABSTRACT

Introduction: Despite an enhanced recognition of the molecular mechanisms of nociception, existing analgesic drugs continue to remain restricted in terms of efficacy. Since all single analgesic drugs are not effective in all patients at all times, there is a need either to develop new and more effective drugs or to identify favourable combinations of drugs that are already available.

Aim: To evaluate the analgesic and anti-inflammatory activity of combined use of ibuprofen and pregabalin in animal models of pain and inflammation.

Materials and Methods: The animals (Swiss albino mice and Wistar rats) were randomly divided into eight groups with six animals in each group. Analgesia was assessed by acetic acid induced writhing and tail immersion methods in mice and hot plate method in rats. Paw oedema model in rats after induction with 0.1 mL of 1% carrageenan was used to assess the anti-inflammatory activity. The percentage inhibition of writhes and prolongation of reaction time were used for assessing analgesic activity and reduction in paw volume was used for assessing anti-inflammatory activity. The results obtained were analysed by ANOVA followed by Tukey HSD Post-hoc Test.

Results: There was a significant diminution in the amount of writhing in all the groups tested when compared to the control group and the highest percentage inhibition of pain was seen with high dose combination group. There was a statistically significant increase in reaction time at all time points compared to baseline values in all treatment groups in the hot plate and tail immersion methods. A notable observation was that the degree of inhibition of paw oedema was greater in the combination groups than ibuprofen alone or the standard anti-inflammatory drug diclofenac while pregabalin alone exhibited negligible anti-inflammatory activity.

Conclusion: The present study displayed prominent analgesic effect and serves as a proof-of-principle study for considering the combination of ibuprofen and pregabalin as a lead for the development of new dual-action analgesic drugs. If confirmed, in additional models of acute and/or chronic pain this combination might be useful in the clinical management of pain associated with inflammation.

Keywords: Anti-nociception, Paw oedema, Pain

INTRODUCTION

Despite an enhanced recognition of the molecular mechanisms of nociception, existing analgesic drugs continue to remain restricted in terms of efficacy since several mechanisms act in tandem to produce pain. Drugs acting either on the opioid receptor system or inflammatory cascade have been the only successful molecules over the past few decades. At this time, the marketed analgesic drugs are at best modestly effective and many of them are known to cause unacceptable side effects or have been linked to long-term safety issues [1]. Among the various marked oral analgesic drugs ibuprofen has emerged as the safest and is available as an OTC product in many countries [2]. Specific drugs directed at individual molecular targets are often found to be less effective than multi-target therapeutics since they have one mechanism of action. Hence, combining drugs from different classes, with different and complementary mechanism(s) of action, provides a better opportunity for effective analgesia at reduced doses of individual agents. Analgesic combinations are therefore recommended by several organizations and are used in clinical practice [3].

At present, many diverse classes of drugs serve as an efficient complement to Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)-acetaminophen, gabbapentinoids, anticonvulsants or opioids in the management of pain. Tramadol is a synthetic, centrally acting analgesic agent with two distinct, synergistic mechanisms of action [4]. The efficacy and safety profile of tramadol make it suitable as a long-term treatment of moderate to severe chronic pain in patients for whom paracetamol (acetaminophen), NSAIDs or COX-2 inhibitors are no longer effective or in whom treatment with NSAIDs is contraindicated [5]. Previous works carried out in our laboratory to evaluate the analgesic and anti-inflammatory activity of tramadol and ibuprofen or pregabalin when used alone or in combination demonstrated an antinociceptive activity, independent of the animal models of nociception or the nociceptive stimulus in a dose dependent manner [6,7]. However, pregabalin was found to be devoid of significant anti-inflammatory activity. Hence in the present study the combination of ibuprofen-pregabalin in standard animal models of pain and inflammation was evaluated using adult Wistar rats and Swiss albino mice.

MATERIALS AND METHODS

Animals and Ethics

The present study was carried out using male adult *Wistar* rats, (weighing 180 to 230 g) and *Swiss albino* mice (weighing 25 to 30 g) between 09.00 AM and 2.00 PM. The animals were kept in a separate temperature-regulated room in cages (six animals in each cage) with air-cooling and 12 hours light and dark cycle. They had free access to water and standard laboratory diet. All experiments were conducted on separate groups of animals and each animal was used only once and only in a single test to avoid interference between tests.

Rats and mice were randomly divided into groups with six members in each group. Control groups: (Group I) were administered normal

saline at the dose of 10 mL/kg and diclofenac 100 mg/kg (Group II). Ibuprofen was administered at dosage of 10 mg/kg (Group III), 30 mg/kg (Group IV) and the dosage for pregabalin was 6 mg/kg (Group V), 12 (Group VI) mg/kg while the dosage for combination of ibuprofen and pregabalin was 10/6 mg/kg (Group VII) and 30/12 mg/kg (Group VIII). The combination ratio of pregabalin and ibuprofen was determined considering the maximum recommended human dose of pregabalin and ibuprofen, respectively. Diclofenac is often used as reference drug to compare other analgesic drugs for inflammatory pain. All the drugs were administered through oral route after dissolving them in normal saline. All chemicals and solvents (pregabalin, Ibuprofen, λ-carrageenan and saline) used were at least of analytical grade and were obtained commercially.

The study was approved by the Institutional Animal Ethics Committee. All experiments and animal care were as per the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and Good Laboratory Practice (GLP) Guidelines, between January 2018 and April 2018 and no animal was sacrificed at the end of the study.

Acetic Acid-induced Abdominal Writhing in Mice

The analgesic activity was evaluated in mice by injecting 0.6% acetic acid (10 mL/kg) intraperitoneally (ip) into the lower right quadrant of the abdominal cavity at an angle of 30° and a depth of 5 mm [8]. The test drugs were administered orally to the mice 30 minutes before acetic acid injection. Immediately after injection, the mice were observed for writhing reflex, which is characterized by abdominal muscle contractions associated with inward outstretching of the hind limbs, a hind paw reflex, or whole body extension. The number of writhing reflexes was observed over a period of twenty min for each animal after acetic acid injection and the results were expressed as the mean±SD. Analgesic effect was quantified as the percent reduction in the number of writhes produced by each drug dose. For a given dose, percentage inhibition was calculated using the following formula: % inhibition={(Wc-Wt)×100}/Wc where, Wc=No. of writhes in control group, Wt=No. of writhes in test group.

Tail-Immersion Test in Mice

Mice were placed into individual restraining cages leaving the tail hanging out freely. The animals were allowed to adapt to the cages for 30 minutes before testing. The lower 5 cm portion of the tail was marked. This part of the tail was immersed in a cup of freshly filled water of exactly 55°C. The reaction time was recorded with the help of a stopwatch. After each determination the tail was carefully dried. The reaction time was determined before (0 hour) and thereafter at one and two hour following oral administration of the test substances. The cut off time of the immersion was 15 seconds. Tail immersion latency was measured as the time between tail immersion and tail withdrawal [9]. Change in tail immersion latency, Dt (s), was calculated for each animal according to the formula {Dt (s)=post drug latency-pre drug latency} [10]. For each animal, the % maximum possible effect (%MPE) was calculated using the following formula: {(Post drug latency-Pre drug latency)/(15-Pre drug latency)} X100.

Hot Plate Method

The analgesic activity was also assessed using hot plate method of Eddy and Leimbach in rats [11]. In this experiment, the hot-plate apparatus was maintained at 55±0.1°C. Rats were placed in an acrylic cylinder (20 cm in diameter) on the heated surface, and the time between placement and licking of their hind paws or jumping was recorded as the response latency. A 20 seconds cut-off was used to prevent tissue damage. The response latency was recorded before (0 min) and thereafter at 1 and 2 hour following oral administration of drugs. Analgesia was defined as prolongation of latency without licking or flicking of hind limb or jumping. Animals presenting with latencies higher than 15 seconds at 0 minute were excluded. For each animal, the % maximum possible effect (%MPE) was calculated using the following formula: {(Post drug latency-Pre drug latency)/ (20-Pre drug latency)} X100.

Carrageenan Induced Paw Oedema

We followed the method adopted by Winter CA et al., subsequently modified by Singh H and Ghosh MN, for evaluating the acute anti-inflammatory activity in rats. Paw oedema was induced in the right hind paw of each rat by intra-plantar injection of 100 µl of 1% (suspension in saline) lambda carrageenan [12,13]. The paw volume of rats was measured by traditional mercury plethysmometer, before the injection of carrageen, 2.5 hours after the injection of carrageen (just before the administration of the test drugs and then again one and two hours after test drug administration. The degree of oedema induced was assessed by the volume of the right hind paw before and after carrageenan treatment, respectively. Mean baseline paw volume ranged from 1.3 to 1.4 mL among the different treatment groups. When measured 2.5 hours after the injection of carrageenan, mean paw volume ranged from 2.4 to 2.7 mL among the different treatment groups. Drug effects were expressed as a difference score in which the paw volume measured 2 hours after administration of the drug was subtracted from that determined immediately before (i.e., 2.5 hours after carrageenan). Negative values, therefore, represent a reduction in inflammation.

STATISTICAL ANALYSIS

All data were expressed as mean±SD. Data were evaluated by means of one-way analysis of variance (ANOVA), post-hoc comparisons were made using Dunnett's t-test or Tukey HSD post-hoc test, wherever appropriate to establish the statistical difference between groups. The criterion for statistical significance was fixed at $p<0.05$. Statistical analyses were accomplished using Graph Pad Prism 5 (Graph Pad Software, San Diego, CAUSA) for Windows (Microsoft Corporation, USA).

RESULTS

Acetic Acid-induced Abdominal Writhing in Mice

There was a significant diminution in the amount of writhing in all the groups tested when compared to the control group (vehicle 10 mL/kg) as shown in [Table/Fig-1]. Although the combination of ibuprofen plus pregabalin (both lower and higher groups) were effective in reducing the number of writhing, there was no significant difference in the amount of analgesia between these groups (60.6 and 73.2%) as compared to the standard analgesic diclofenac (62.4%) indicating possibly an analgesic ceiling effect.

Group	Drug/Dose (mg/kg)	No. of writhing Mean±SD	Percent Inhibition
I	Saline (10 mL/kg)	35.2±3.8	-
II	Diclofenac (100)	13.2±2.6***	62.4±7.5
III	Ibuprofen (10)	23.3±2.6**	33.1±9.8
IV	Ibuprofen (30)	19.3±3.1***	44.4±11.9
V	Pregabalin (6)	24.7±7.9*	27.4±19.5
VI	Pregabalin (12)	18.5±3.3**	46.9±10.7
VII	Ibuprofen (10)+Pregabalin (6)	13.7±3.6***	60.6±12.2
VIII	Ibuprofen (30)+Pregabalin (12)	9.3±1.0***	73.2±3.8

[Table/Fig-1]: Antinociceptive activity on acetic acid-induced abdominal writhing in mice.
Each value represents the Mean±SD of observations on six animals
ANOVA F=36.78 ***p<0.001 ** p<0.01 *p<0.05 as compared to control group

Tail-immersion Latency in Mice

In the tail-immersion test, the treatment with ibuprofen and pregabalin increased the latency to thermal stimulation at one hour after the treatment when compared to the control group (vehicle 10 mL/kg) and was maintained until two hours after treatment [Table/Fig-2].

Group	Drug /Dose (mg/kg)	Tail Immersion Latency			% MPE	
		0 hour	1 hour	2 hour	1 hour	2 hour
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
I	Saline (10 mL/kg)	5.0±0.8	4.9±0.4	5.1±1.1	0.9±0.8	0.7±0.9
II	Diclofenac (100)	4.9±1.0	7.6±1.1***	7.8±0.9***	25.9±16.4	28.9±10.9
III	Ibuprofen (10)	5.3±0.9	8.0±0.7***	7.9±1.5***	27.8±9.0	26.3±20.2
IV	Ibuprofen (30)	5.1±1.0	8.6±1.7***	8.1±0.6***	35.0±16.9	30.3±8.7
V	Pregabalin (6)	5.1±0.8	5.9±0.8**	6.9±1.0**	8.1±7.5	11.0±6.4
VI	Pregabalin (12)	5.0±0.7	7.4±0.9**	7.1±0.7**	24.1±9.6	20.9±4.5
VII	Ibuprofen (10)+Pregabalin (6)	5.0±0.8	10.9±2.3***	11.3±3.0***	62.9±21.9	63.3±29.8
VIII	Ibuprofen (30)+Pregabalin (12)	5.0±0.8	11.2±2.1***	11.6±2.5***	58.6±23.9	66.3±24.0

[Table/Fig-2]: Analgesic activity in albino mice on tail immersion latency.
Each value represents the Mean±SD of observations on six animals
ANOVA F=69.1 **p<0.01 ***p<0.001 as compared to 0 hour
MPE=Maximum possible effect

Hot Plate Test

The results of the analgesic effect of the drugs using hot plate method are presented in [Table/Fig-3]. The results revealed that there was no significant difference on the thermal stimulus in rats treated with normal saline (control group) throughout the observation period. There was a statistically significant increase in reaction time at all time points compared to baseline values in all treatment groups and was greatest for combination treated group at 1 hour after treatment. The experimentally-derived value of the maximum possible antinociceptive effect for low and high dose combination at 1 hour were 81.5±6.8 and 83.1±6.1% while at 2 hours they were 71.9±6.1 and 76.6±4.3% respectively.

Group	Drug/Dose (mg/kg)	Response Latency (sec)		
		0 hour	1 hour	2 hours
		Mean±SD	Mean±SD	Mean±SD
I	Saline (10 mL/kg)	3.8±0.2	4.7±0.1	4.2±0.6
II	Diclofenac (100)	3.7±0.2	12.3±4.0***	9.3±2.1***
III	Ibuprofen (10)	4.0±0.2	9.0±0.8***	9.4±0.5***
IV	Ibuprofen (30)	4.1±0.5	9.6±0.4***	10.0±0.4***
V	Pregabalin (6)	4.0±0.6	11.4±0.8***	11.1±0.8***
VI	Pregabalin (12)	4.2±0.6	13.0±0.3***	11.9±0.2***
VII	Ibuprofen (10)+Pregabalin (6)	4.5±0.5	13.2±0.6***	12.1±0.4***
VIII	Ibuprofen (30)+Pregabalin (12)	4.0±0.8	13.9±0.8***	12.6±0.8***

[Table/Fig-3]: Analgesic activity in Eddy's Hot Plate model in rats.
Each value represents the Mean±SD of observations on six animals
ANOVA F=292.18 ***p<0.001 as compared to 0 h

λ-carrageenan-induced Paw Oedema

Mean baseline paw volume ranged from 1.28 to 1.35 mL among the different treatment groups [Table/Fig-4]. When measured 2.5 hours after the injection of carrageenan, mean paw volume ranged from 2.5 to 2.7 mL among the different treatment groups. Drug effects are expressed as a difference score in which the paw volume measured 1 and 2 hours after administration of the drug was subtracted from that determined immediately before (i.e., 2.5 hours after carrageenan). Negative values, therefore, represent a reduction in inflammation. The results of λ-carrageenan-induced paw oedema indicated that the diclofenac, ibuprofen and the low or high dose combination significantly inhibited the development of paw oedema after 1 and 2 hours of treatment. A notable observation was that the degree of inhibition of paw oedema was greater in the combination groups (37.7 and 37.8%; 39.5 and 42%) than ibuprofen alone (21.3 and 21.8%; 23.6 and 25.2%) or the standard anti-inflammatory drug diclofenac (23.4 and 28%) while pregabalin alone exhibited negligible anti-inflammatory activity.

Group	Drug	Dose (mg/kg)	Paw volume (mL) Mean±SD			
			Carrageenan		1 hour after test drug	2 hour after test drug
			Before	2.5 hour after		
I	Saline (10 mL/kg)		1.35±0.03	2.56±0.06	2.52±0.06	2.60±0.07
II	Diclofenac (100)		1.35±0.03	2.59±0.04	1.98±0.08**	1.86±0.07**
III	Ibuprofen (10) 10		1.28±0.04	2.64±0.05	2.07±0.02**	2.06±0.02**
IV	Ibuprofen (30)		1.32±0.06	2.62±0.08	2.00±0.20**	1.95±0.08**
V	Pregabalin (6)		1.35±0.03	2.49±0.06	2.47±0.12	2.43±0.07
VI	Pregabalin (12)		1.35±0.02	2.57±0.10	2.52±0.04	2.52±0.09
VII	Ibuprofen (10)+Pregabalin (6)		1.32±0.08	2.66±0.17	1.67±0.20***	1.54±0.11***
VIII	Ibuprofen (30)+Pregabalin (12)		1.28±0.02	2.61±0.18	1.61±0.12***	1.57±0.14***

[Table/Fig-4]: Effect of ibuprofen or pregabalin alone or in combination on carrageenan induced paw oedema in rats.
Each value represents the Mean±SD of observations on six animals
ANOVA F=132.05 **p<0.01 ***p<0.001 as compared to 0 hour

DISCUSSION

Despite the vast amount of research, pain management still relies on the "one drug fits all" model. The ability to develop new analgesic drugs has been disappointing and the commonly used analgesics today are derivatives of drug classes known to be analgesics for centuries or decades-NSAIDs, opioids, cannabinoids, and tricyclics. The present study carried out to evaluate the analgesic and anti-inflammatory activity of ibuprofen and pregabalin when used either alone or in combination demonstrated a potential antinociceptive activity, independent of the animal models of nociception or the nociceptive stimulus in a dose dependent manner with significant anti-inflammatory activity.

Recent animal studies suggested that pregabalin was found to possess analgesic activity in the formalin test on paw licking/late phase-corresponding to inflammatory pain with a central sensitization component [14]. Since persistent and uncontrolled pain may transform into chronic or neuropathic pain where peripheral and sensitization components play a significant role, use of combination of drugs with pregabalin as in present study may aid in the prevention of sensitization component in acute pain settings.

Ibuprofen has been shown to have analgesic and anti-inflammatory properties through central and peripheral blockade of COX-1 and COX-2 isoenzymes and can also have an effect through cox-independent pathways [15]. An isobolographic approach to characterize the nature of the interaction of gabapentin or pregabalin with naproxen in an animal model of inflammatory pain indicated that pregabalin was found to interact in a synergistic manner with naproxen [16]. The results of present study indicate that the combination is effective in inhibiting nociception at different levels of the central nervous system, as the hot-plate test predominantly

measures supraspinal response to a painful stimulus while the tail-flick test primarily measures the spinal response. It is likely that the enhanced analgesic effect of the combination may be due to the activation of different antinociceptive pathways that are inhibited by these drugs. Taken together, findings in the present study suggested that the combination appears to be a good candidate for multimodal coverage of a wider spectrum of pain.

CONCLUSION

The present study displayed prominent analgesic effect and serves as a proof-of-principle study for considering the combination of ibuprofen and pregabalin as a lead for the development of new dual-action analgesic drugs. Isobolographic analysis is warranted to delineate whether the enhanced analgesic efficacy observed in this study was due to synergistic or supra-additive interactions of ibuprofen and pregabalin and efforts are under way to conduct the same. Further, more pharmacological and chemical studies are necessary in order to characterize the mechanism(s) responsible for the antinociceptive and anti-inflammatory action of this combination.

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