Asian Journal of Pharmaceutical and Clinical Research
Vol. 4, Issue 1, 2011 ISSN - 0974-2441

Research Article

AN EXPERIMENTAL STUDY OF ANALGESIC ACTIVITY OF SELECTIVE COX-2 INHIBITOR WITH CONVENTIONAL NSAIDs

DEBASIS MISHRA[1*], GOUTAM GHOSH[1], P. SUDHIR KUMAR[1] AND PRASANNA KUMAR PANDA[2]

[1]School of Pharmaceutical Sciences, Siksha O Anusandhan University, Bhubaneswar, Orissa, India
[2]University Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar, Orissa, India.
E-mail: mdebasis7@gmail.com

ABSTRACT

The present study was carried out to investigate the analgesic activity of Etoricoxib (10 mg) for individual drug therapy and etoricoxib (5 mg) for combination therapy with diclofinac potassium (10 mg) using Acetic acid induce writhing, Hot plate and Tail immersion methods. The test and standard drugs significantly ($p<0.001$) reduced the number of abdominal constriction and stretching of hind limb induce by the injection of acetic acid in a dose dependent manner. The Hot plate and Tail immersion test useful in the elucidating centrally mediated antinociceptive responses, which focused mainly on changes above the spinal cord level. All the test and standard drugs significantly ($p<0.001$) reduced the pain as compare to the control group. The results of pharmacological tests performed in the present studies suggest the combination of Etoricoxib and diclofenac potassium possess potent analgesic activity.

Key words: Etoricoxib, cox-2 inhibitor, diclofenac potassium, analgesic.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) reduce pain and edema by suppressing the formation of prostaglandins, by inhibiting the activity of the enzyme Cyclooxygenase (COX-1 and COX-2). However, prostaglandins are key mediators of several components of GI mucosal defense, so suppression of synthesis of prostaglandins (PGs) by NSAIDs greatly reduces the resistance of the mucosa to injury as well as interfering with repair processes. Selective COX-2 inhibitors were thought to be the solution to this conundrum as it is required that NSAIDs suppress prostaglandin synthesis at sites of inflammation, and not in the GI tract.

However, it is now clear that both COX-1 and COX-2 isoforms contribute to mucosal defense. Selective COX-2 inhibitors elicit less GI damage and bleeding than conventional NSAIDs, although the magnitude of this reduction continues to be contested in the literature. As widely reported in the lay-press, the selective COX-2 inhibitors also cause significant adverse effects in the renal and cardiovascular systems, possibly more serious than those caused by conventional NSAIDs. The market for NSAIDs is expanding rapidly because of an aging population in developed countries and the associated increase in the prevalence of diseases like arthritis. Use of Aspirin is also increasing because of its utility in reducing the incidence of a number of common disorders including stroke, myocardial infarction, Alzheimer's disease and cancer1.

However, their use is limited by their significant side effects upon the stomach and the kidney. Their side effects as well as their therapeutic actions are related to their ability to inhibit cyclooxygenase enzymes involved in the first step of the arachidonic acid cascade.[2-3] In addition, the damaging effect of some NSAIDs upon the stomach and intestine is in part due to their acidic nature, as with indomethacin, ibuprofen, diclofenac, naproxene, aspirin, etc.[4] Although basic NSAIDs such as glafenine and floctafenine are expected to be devoid of the primary insult effect, their damaging effect upon the stomach and kidney is still prominent as they inhibit prostaglandin biosynthesis as strongly as indomethacin.[5-6]

In the recent years, several novel approaches for reducing the GI toxicity of NSAIDs with promising results have been reported. These mainly involve structural modification of existing NSAIDs such that inhibition of COX is maintained, but other attributes are added that diminish GI (and other) toxicity, and in some cases boost efficacy and/or potency[1]. The mortality rate for NSAID induced GI bleeding is 5-10% in the world population.

MATERIALS AND METHODS

Selection of Drugs and Chemicals

For the purpose of this work we selected Etoricoxib (Selective COX – 2 inhibitor), Diclofenac potassium (Conventional NSAID), Ibuprofen (Standard drug) and Glacial acetic acid.

Preparation of drugs and Chemical solutions

Etoricxib (10mg/kg body weight) was dissolved in sufficient quantity of solvent in normal saline and use in the treatment. Etoricoxib (5mg/kg) and Diclofenac potassium (10mg/kgbody weight) was dissolved together in sufficient quantity of solvent(normal saline)& Glacial acetic acid was prepared by using normal saline of strength of 1%v/v.

Selection of Experimental Animals

Healthy Swiss albino rats of either sex weighing 220-250g were used in this study. All the animals were obtained from Animal house of the School of Pharmaceutical Sciences, SOA University, Bhubaneswar, Orissa. The animals were housed comfortably in a group of six in a single clean plastic cage with a metal frame lid on its top. They were housed under standard environmental conditions of temperature (24±1°C) and relative humidity of 30-70 %. A 12:12 h light dark cycle was followed. All animals had free access to water and standard pelletized laboratory animal diet ad libitum. All the experimental procedures and protocols used in this study were reviewed and approved via the Approval No. 17/09/IAEC/SOAU by the Institutional Animal EthicalCommittee (IAEC) of School of Pharmaceutical sciences, S 'O' A University, Bhubaneswar, Orissa (Regd. No. 1171/C/08/CPCSEA) constituted in accordance with the guidelines of the CPCSEA, Government of India.

Evaluation of Analgesic activity[7-10]

Pain is not easily or satisfactorily defined and therefore is often interpreted as a suffering that results from the perception of painful stimuli. It's a common symptom and it indicates that something is wrong in the body and may give a clue to the nature of disease. Hence, "pain is a specific sensation with its own peripheral and central mechanisms independent of other five senses." Pain itself is not a disease; it is by far the most common medical complaint. It is usually perceived as an indication of ill health and most diseases have a component of pain. The control of pain is one of the most important uses to which drugs are put. Pain can be defined as the effect produced in consciousness by the arrival of nerve impulses generated by noxious stimuli in the brain. Drugs, which alter the pain sensitivity or remove pain, are called as painkiller or analgesics.

Acetic acid induced writhing in mice[11]

Acetic acid induced writhing method was adopted for evaluation of analgesic activity. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, turning of trunk (twist). Any writhing is considered as a positive response. Swiss albino mice weighing between 15-35g were used for evaluation of analgesic activity; in each group six albino mice were kept.

A solution of acetic acid (1% v/v) in distilled water was prepared. A solution of Ibuprofen (dose-100mg/kg/10ml) was prepared in normal saline water. Test - 1 : A solution of Etoricoxib (10mg/kg/10ml), Test - 2 : A solution Etoricoxib (5mg/kg) in combination with Diclofenac potassium (10mg/kg) was prepared in 10ml of normal saline water.

Wistar albino mice of either sex were divided into four different groups each containing six animals, the animals were marked individually. Food was withdrawn 12 hours prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately. The test and standard drugs were given orally. After 60 minuteswrithing was induced by intraperitonial injection of 1% acetic acid in volume of 0.1 ml/10g body weight. The writhing episodes were recorded for 30 minutes; stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted.

Percentage of inhibition was evaluated using following formula: The results of Acetic acid induced writhing method in mice was tabulated in Table-1.

Hot plate method in rats[12]

The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws.

The hot plate, which is commercially available, consists of a electrically heated surface. The temperature is controlled for 55° to 56 °C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop-watch.

Swiss albino rats weighing between 100-150g were used for evaluation of analgesic activity; in each group six albino rats were kept. A solution of Ibuprofen (dose-100mg/kg/10ml) was prepared in normal saline water. Test - 1 : A solution of Etoricoxib (10mg/kg/10ml), Test - 2 : A solution Etoricoxib (5mg/kg) in combination with Diclofenac potassium (10mg/kg) was prepared.
Wistar albino rats of either sex were divided into four different groups each containing six animals, the animals were marked individually. Food was withdrawn 12 hours prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately.
The test and standard drugs were given orally. After 60 minutes, the animals are placed on the hot plate andthe observations were recorded and at the time interval of 90, 120 and 180 minutes. The results of Hot plate method in rats was tabulated in Table-2.

Tail immersion test in mice[13]

The procedure is based on the observation that morphine-like drugs are selectivelycapable of prolonging the reaction time of the typicaltail-withdrawal reflex in rats induced by immersingthe end of the tail in warm water of 55 °C.

The lower 5 cm portion of the tail is marked. This part of the tail is immersed in to the water bath of exactly 55 °C. Within a few seconds the rat reacts by withdrawing the tail. The reaction time is recorded in 0.5 s units by a stopwatch. After each determination the tail is carefully dried. The reaction time is determined before and periodically after oral administration of the test and standard substance.

The cut off time is 15sec. Swiss albino mice weighing between 15-35g were used for evaluation of analgesic activity; in each group six albino mice were kept. A solution of Ibuprofen (dose-100mg/kg/10ml) was prepared in normal saline water. Test - 1 : A solution of Etoricoxib (10mg/kg/10ml), Test - 2 : A solution Etoricoxib (5mg/kg) in combination with Diclofenac potassium (10mg/kg) was prepared in 10ml of normal saline water.

Wistar albino mice of either sex were divided into four different groups each containing six animals, the animals were marked individually. The animals were weighed and numbered appropriately. The test and standard drugs were given orally. After 60 minutes, the observations were recorded and at the time interval of 90, 120 and 180 minutes.The results of tail immersion test in mice was tabulated in Table-3.

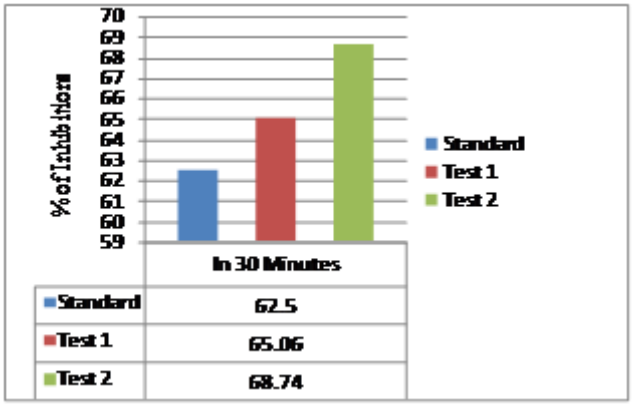

Figure -1: % age of Inhibition in Acetic Acid Induced Writhing in Mice [Standard – Ibuprofen (100mg/kg), Test 1 – Etoricoxib (10mg/kg), Test 2 – Etoricoxib (5mg/kg) + Diclofenac potassium (10mg/kg)]

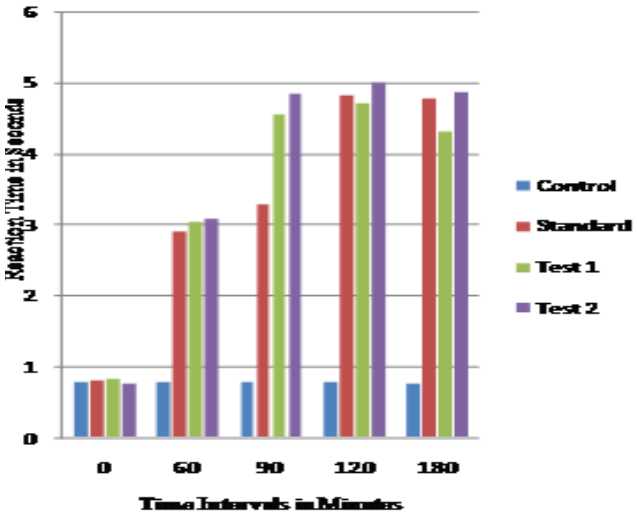

Figure –2: Analgesic Activity by Hot Plate Method in Rats [Standard – Ibuprofen (100mg/kg), Test 1 – Etoricoxib (10mg/kg), Test 2 – Etoricoxib (5mg/kg) + Diclofenac potassium (10mg/kg)]

RESULTS

All the test and standard drugs significantly ($p<0.001$) reduce the number of abdominal constriction and stretching of hind limb induce by the injection of acetic acid in a dose dependent manner. (Table-1)

As all the drugs are standard analgesics, by applying Student Newman-Keuls test, it was shown that no significant difference betwwen the tests and standard. The standard drug exhibited a

Table –1: Analgesic Activity by Acetic Acid Induced Writhing in Mice

Group	Treatment	Dose (mg/kg)	No. of Writhes in 30 min. (mean ± sem)	Inhibition (%)
Control	Normal Saline	--------	72.5 ± 1.118	--------
Standard	Ibuprofen	100	27.16 ± 1.887 [c]	62.5
Test - 1	Etoricoxib	10	25.33 ± 0.802 [c]	65.06
Test - 2	Etoricoxib + Diclofenac Potassium	5 + 10	22.66 ± 0.494 [c]	68.74

Each value is the mean ± SEM for 6 rats, [a] P < 0.05; [b] P < 0.01; [c] P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

Table – 2 : Analgesic Activity by Hot Plate Method in Rats

Group	Treatment	Dose (mg/kg)	Reaction time in seconds at time (minutes) (mean ± sem)				
			0	60	90	120	180
Control	Normal Saline	--------	3.17 ± 0.040	3.24 ± 0.039	3.96 ± 0.148	3.55 ± 0.144	3.98 ± 0.254
Standard	Ibuprofen	100	3.33 ± 0.081	6.635 ± 0.062 [c]	7.86 ± 0.249 [c]	8.24 ± 0.266 [c]	7.945 ± 0.290 [c]
Test – 1	Etoricoxib	10	3.42 ± 0.040	7.78 ± 0.184 [c]	8.16 ± 0.296 [c]	8.57 ± 0.274 [c]	8.10 ± 0.293 [c]
Test - 2	Etoricoxib + Diclofenac Potassium	5 + 10	3.25 ± 0.054	7.41 ± 0.297 [c]	8.28 ± 0.328 [c]	8.64 ± 0.314 [c]	8.19 ± 0.293 [c]

Each value is the mean ± SEM for 6 rats, [a] P < 0.05; [b] P < 0.01; [c] P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test

Table – 3 : Analgesic Activity by Tail Immersion Test in Mice

Group	Treatment	Dose (mg/kg)	Reaction time in seconds at time (minutes) (mean ± sem)				
			0	60	90	120	180
Control	Normal Saline	--------	0.78 ± 0.021	0.78 ± 0.020	0.78 ± 0.021	0.78 ± 0.021	0.77 ± 0.029
Standard	Ibuprofen	100	0.81 ± 0.021	2.92 ± 0.249 [c]	3.29 ± 0.279 [c]	4.84 ± 0.283 [c]	4.80 ± 0.234 [c]
Test – 1	Etoricoxib	10	0.82 ± 0.022	3.04 ± 0.161 [c]	4.57 ± 0.180 [c]	4.72 ± 0.175 [c]	4.33 ± 0.201 [c]
Test - 2	Etoricoxib + Diclofenac Potassium	5 + 10	0.75 ± 0.043	3.10 ± 0.059 [c]	4.85 ± 0.232 [c]	5.015 ± 0.267 [c]	4.88 ± 0.235 [c]

Each value is the mean ± SEM for 6 rats, [a] P < 0.05; [b] P < 0.01; [c] P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

writhing inhibition percentage of 62.5% , test-1 (65.06) and test-2 (68.74) as comparision to control group.

The Hot plate and Tail immersion test useful in the elucidating centrally mediated antinociceptive responses, which focuses mainly

on changes above the spinal cord level. All the test and standard drugs significantly ($p<0.001$) reduce the pain as compare to the control group. (Table-2)

By applying Student Newman-Keuls test, it was shown that there is significant ($p<0.01$) effect of test-2 as compare to the standard at 60 and 90 minutes. But there is no significant difference betwwen test-1 and standard.

In Tail immersion method all the test and standard drugs significantly ($p<0.001$) reduce the pain as compare to the control group. (Table-3)

By applying Student Newman-Keuls test, it was shown that there is significant ($p<0.01$) effect of test-1 &test-2 as compare to the standard at 90 minutes and there is significant ($p<0.05$) effect of test-2 and standard group at 90 and 120 minutes.

DISCUSSION

The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics.[14] In general, acetic acid causes pain by liberating endogenous substances such as serotonin histamine, prostaglandins (PGs), bradykinins and substance P, endings. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response.[15] The method has also been associated with prostanoids in general that is, increased

levels of PGE2 and PGF2α in peritoneal fluids as well as lipoxygenase products.[16]

The significant increase in pain threshold produced by tests and standard in these models suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic descending noradrenergic and serotonergic systems.[17-19]. The analgesic effect produced by the tests and standards may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes, and other endogenous substances that are key players in pain.

The selective cox-2 inhibitor has high effective than the conventional NSAIDs and has low GI and high cardivascular side effects than to the conventional NSAIDs.Etoricoxib is a cox-2 inhibitor with a high degree of selectivity of its target. It provides an alternative to other selective and traditional NSAIDs in tratingpateints with arthritis and other painful conditions.

Here in this research work we found that Etoricoxib is more effective than the conventional NSAIDs. The low dose combination of etoricoxib with conventional NSAIDs has more effective for analgesic activity as comapare to the etoricoxib and conventional NSAIDs. Here we conclude that the combination product was more effective than the single drug, it may be due to different mechanism of actions of different drugs in combined products. But the chances of side effects of combination products are more as compare to the single drug. More study on combination drug therapy may overcome these problems.

ACKNOWLEDGEMENT

The authors are thankful to the authorities of Siksha O Anusandhan University, Bhubaneswar for providing necessary facilities to carry out this research work.

REFERENCES

1. Dubois RW, Melmed GY, Laine L, Henning JM. Guidelines for the appropriate use of non-steroidal anti-inflammatory drugs, COX-2 Specific inhibitors and proton pump inhibitors in patients requiring Chronic anti-inflammatory therapy. Ailment Pharmacol. Ther. 2004; 19:197.
2. Hardman JG, Limbird LE, Molinoff PA. Analgesic-antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout., 9th ed., The Pharmacological Basis of Therapeutics, McGraw-Hill, New York. 1996; 617.
3. Wolfe MN, Lichtestein DR, Singh GN. Gastrointestinal Toxicity of Non-Steroidal Anti-inflammatory Drugs. N. Engl. J. Med. 1999; 128.
4. Cena C, Lolli ML, Lazzarato L, Guaita E, Morini G, Coruzzi G, McElroy S P, Megson I L, Fruttero R, Gasco A. Antiinflammatory, gastrosparing, and antiplatelet properties of new NO-donor esters of aspirin. J. Med. Chem. 2003; 46: 747.
5. Lequesne M, Fannius J, Reginster JY, Verdickt W, Laurier M V. Revuedurhumatisme. 1997; 64: 327.
6. Van Kolfschoten AA, Zandberg P, Jager LP, Van Noordwijk J. Protection by paracetamol against various gastric irritants in the rat. Toxicol. Appl. Pharmacol. 1983; 69: 37.
7. Kulkarni SK. Handbook of experimental Pharmacology. 3rd ed. Vallabh Prakashan, New Delhi. 2005;127.
8. Kenji O. Pain signalling pathways: from cytokines to ion channels. Int.J.B.C.B. 2007; 39: 490.
9. Tripathi KD, Essentials of Medical Pharmacology. 5th ed., Jaypee Brothers Medical Publishers (P) LTD, New Delhi. 2003; 167.
10. Satoskar RS, Bhandarkar SD, Ainapure SS. Pharmacology and Pharmacotherapeutics. 16th ed., Popular Prakashan, Mumbai. 1998; 151.
11. Kaneria MS, Naik SR, Kohli RK. Anti-inflammatory,antiarthritic and analgesic activity of a herbal formulation. Indian J. Experimental Biol. 2007; 45: 279.
12. Shanmugasundaram P, Venkataraman S. Anti-nociceptive activity of *Hygrophilaauriculata* (SCHUM) Heine. Afr. J. Trad. CAM. 2005; 2: 62.
13. Olaleye SB, Farombi EO, Adewoye EA, Owoyele BV, Onasanwo SA, Elegbe RA. Analgesic and anti-inflammatory effects of Kolaviron (a garcinia kola seed extract). Afr. J. Biomed. Res. 2000; 3: 171.
14. Gené RM, Segura L, Adzet T. *Heterothecainuloides*: anti-inflammatory and analgesic effects. J. Ethnopharmacol. 1989; 60: 157.
15. Bentley GA, Newton SH, Starr J. Studies on the Anti-nociceptive Action of Agonist Drugs and their Interaction with Opioid Mechanisms. Br. J. Pharmacol. 1983; 79: 125.
16. Derardt R, Jongney S, Delevalcee F. Release of Prostaglandin E and F in an analgesic Reaction and its Inhibition. Eur. J. Pharmacol. 1980; 51: 17.
17. Bensreti MM, Sewell RD. Selective effects of dopaminergic modifiers on antinociception produced by different opioid receptor agonists. Pro. Br. Pharmacol. Soc. 6th – 8th July. 1983; 70.
18. Headley PM, Shaughnessy CT. Evidence for opiate and dopamine interaction in striatum. Br. J. Pharmacol. 1985; 86: 700.
19. Wigdor S, Wilcox GL. Central and systemic morphine-induced antinociception in mice: of Contribution descending serotonergic and noradrenergic pathways. J. Pharmacol. Exp. Ther. 1987; 242: 90.