 DOI: 10.18231/2393-9087.2016.0014

Evaluation of Anti-inflammatory and Immunosuppressive Activities of Indomethacin in Experimental Animal Models

Chandan Chatterjee

Associate Professor, Dept. of Pharmacology, ESI-PGIMSR, ESIC Medical College, Kolkata

Email: rivuc2006@gmail.com, soriasis_2004@yahoo.co.in

Abstract

Background: A potent non selective non-steroidal anti-inflammatory drug(NSAIDs) is indomethacin commonly not used as first line analgesic in clinical practice due to its adverse drug reactions. Acute anti-inflammatory and Immunomodulatory activity of the drug has already been studied. But we had set forward our study to establish these properties in experimental animals.

Objectives: Evaluation of anti-inflammatory and immunosuppressive activities of Indomethacin in experimental animal models.

Methods: In each group 12 rats were taken as experimental animals**.** Dexamethasone was used as standard drug in model of chronic inflammation and water as vehicle control. Indomethacin in different doses was used as standard drug. The following models of inflammation were employed: Carrageenan-and Dextran induced acute oedema (measured between 30 min and 4 h) in rat paw, and Freund's adjuvant induced arthritis (oedema measured on 21st day).

Results: Indomethacin at a dose of 10 mg/Kg of body weight showed significant inhibitory response in carrageenan and dextran induced rat paw edema in comparison to vehicle control in our study (87.3% and 91.5%, respectively). In arthritic model 1 mg/kg of Indomethacin showed significant chronic anti-inflammatory effect (29%) in comparison to vehicle control .Standard drug dexamethasone showed (84.6%) of immunosuppressive effects on 21st day.

Conclusion: These data support the fact that Indomethacin has both acute anti-inflammatory and chronic anti-inflammatory (immunosuppressive) activity.

Keywords: Indomethacin, Immunosuppressant, Inflammation

Introduction

Three common characteristics, variable anti-inflammatory, anti-pyretic and analgesic activities are shared by all Non-steroidal anti-inflammatory drugs (NSAIDs). They inhibit cyclooxygenase enzyme 1 and 2 (COX-1 and COX-2) following its binding with the enzyme, lead to significant decrease in the level of central and peripheral prostaglandins.[1] Though suppression of inducible COX-2 is primarily responsible for its anti-inflammaory action. Moreover, most NSAIDs except the selective ones also share gastrointestinal complications like gastritis, gastric irritation, gastric bleeding and peptic ulcer.[2] Chronic inflammatory diseases (such as rheumatoid arthritis) commonly being treated with NSAIDs both selective and non-selective type show relief of painful symptoms on many occasions. But they also increase risk of adverse drug reactions like gastric or duodenal ulcers and bleeding.[3]

Indomethacin is a nonselective non-steridal anti-inflammatory drugs (NSAIDs) acts by inhibiting isoforms of cyclooxygenase 1 and 2 with equal affinity and. It is effective in period pains and pains after surgery and fever. This drug is also used to treat in closure of patent ductus arteriosus following birth and also cases of Barter Syndrome. Antipyretic effect of indomethacin is explained by its action on hypothalamus, resulting in an increased peripheral blood flow, vasodilatation with subsequent heat dissipation.[4]

Objectives

Evaluation of anti-inflammatory and immunosuppressive activities of Indomethacin in experimental animal models.

Materials and Methods

The study was carried out in a tertiary care hospital (Reg No: of animal ethics committee is CPCSEA/544) using Sprague Dawley rats, weighing between 100-150 g. The animals were maintained under standard laboratory conditions. They were provided with free access to commercial pellet feed and water *ad libitum*. The animals were housed for a period of seven days for acclimatization.

Chronic or Immunological induced inflammation

Adjuvant induced arthritis: The method of adjuvant arthritis in rats as described by Pearson et al. (1959)[5] exhibits many similarities to human rheumatoid arthritis and was accordingly followed as a model of chronic or immunologically induced inflammation. Male Sprague Dawley rats with an initial body weight of 130- 150 g were used. Thirty six rats were taken and divided into three groups of twelve rats each. The groups were treated as follows:

Group (n=12)	Treatment
Group I	Vehicle Control
Group II	Standard drug, Indomethacin (1mg/kg)
Group III	Standard drug, Dexamethasone (0.1mg/kg)

0.1 ml of complete Freund's adjuvant was injected into the left hind paw (sub plantar region) of each rat on day 1. (contains 1 mg Mycobacterium tuberculosis (H37RA, ATCC 25177) heat killed and dried 0.85 ml. mineral oil and 0.15 ml Mannide mono oleate is present in each ml. of complete Freund's adjuvant). Dosing with a standard or test compound (Indomethacin) started on the same day via oral route of administration and continued daily for fourteen days. Body weight and paw volumes of both legs were recorded on the day of injection. The volume of the injected paw was measured again on day 5, indicating the influence of therapeutic agents on that paw and primary lesions. A plethysmometer was used to understand severity of the adjuvant induced disease by measurement of the non-injected paw (secondary lesions). Body weight of the animal was determined again on day 21 and the severity of the secondary lesions was graded according to the following scheme and evaluated visually.

Arthritic Index:[6]

Site of lesion	Nature of lesion	Score
Ears	Absence of nodules and redness Presence of nodules and redness	0 1
Nose	No swelling of connective tissue Intense swelling of connective tissue	0 1
Tail	Absence of nodules Presence of nodules	0 1
Forepaw	Absence of inflammation Inflammation of at least one joint	0 1
Hind paw	Absence of inflammation Slight inflammation Moderate inflammation Marked inflammation	0 1 2 3

Evaluation

a) **For primary lesions:** The percent inhibition of paw volume of the injected left paw over control was measured at day 5.
b) **For secondary lesions:** The percent inhibition of paw volume of the non-injected right paw over control was measured at day 21.
c) An arthritic index was calculated as the sum of the scores as indicated above for each animal. The average of the treated animals was compared with the control group.

Statistical Analysis: SPSS (version 20) is used to calculate statistical significance and mean±S.D is used as a mode of expression of result. Student's t-test(unpaired) is used to calculate the difference between two groups, with $p<0.05$ implying significance.

Results

The standard drug indomethacin (87.3% and 91.5%, respectively) showed remarkable inhibitory response in carrageenan and dextran induced rat paw edema (Table 1, Table 2) in comparison to vehicle control. It suggests that Indomethacin possesses potent acute anti-inflammatory activity possibly due to the inhibition of the synthesis and release of mediators of inflammation, principally the prostaglandins. The dextran induced edema has been reported to be mediated mainly by histamine and serotonin released by the mast cells.[7]

Table 1: Effects of Indomethacin in Carrageenan induced rat paw edema

Group (n=12)	Dose(mg/Kg)	Edema volume(ml, Mean ± SEM) (% inhibition)		
		1st h	2nd h	3rd h
Group I	Vehicle control(1 ml)	0.15±0.03	0.45±0.05	0.63±0.08
Group II	Indomethacin 10 mg/kg	0.1±0.04 (33%)	0.25±0.07 (44%)	0.08±0.04 (87.30%)*

*p<0.01, NI- No inhibition

Table 2: Effects of Indomethacin in Dextran induced rat paw edema

Group (n = 12)	Dose (mg/Kg b.w.)	Mean edema volume (ml, Mean ± SEM) (% inhibition)		
		1st h	2nd h	3rd h
Group I	Vehicle control (1ml)	0.54±0.05	0.58±0.05	0.59±0.05
Group II	Indomethacin 10 mg/kg b.w.	0.39±0.12 (27.70%)	0.35±0.04 (39.65%)	0.09±0.03* (91.50%)

*p<0.001 as compared to control

In arthritic model 1 mg/kg of Indomethacin showed significant chronic anti-inflammatory effect (29%) in comparison to the standard drug dexamethasone(84.6%)(Table 3, 4) on 21st day. But on 5th day suppression of primary lesions was 48.9% with Indomethacin in comparison to 73% with dexamethasone. All data were statistically

significant. The severity of the adjuvant induced disease was followed by measurement of the non-injected paw (secondary lesions) and by arthritic index as mentioned in material and methods. Suppression of secondary lesions was best observed with dexamethasone. Indomethacin showed significant suppression of secondary lesions (2.97±0.20) but lesser in effect in comparison to dexamethasone (1.25±0.50).

Table 3: Effects of Indomethacin on Freund's adjuvant induced arthritis in rats

Gr (n=12)	Dose (mg/kg)	Edema Volume(ml) (% inhibition)		
		5th day	13th day	21st day
Vehicle Control(water)	1mi	1.37±0.40	1.36±0.3	1.3±0.1
Indomethacin	1mg/kg	0.70±0.16(48.9%)#	0.8±0.04(27.65%)	0.9±0.13 (29%)
Dexamethasone	0.7mg/kg	0.37±0.1(73%)	0.37±0.12(73%)	0.2±0.1 (84.6%)©

Table 4: Effect of Indomethacin on arthritic index in a model of Freund's adjuvant induced arthritis

Treatment groups(n=12)	Dose	Arthritic index on 21st day
Vehicle Control(water)	1ml	4±0
Dexamethasone	0.7 mg/kg	1.25± 0.5Ъ
Indomethacin (1%)	1ml(p.o)	2.97±0.2¥

Ъ P<0.001 ¥ P<0.05

Discussion

The present study was conducted as a part of dissertation to investigate the potential anti-inflammatory and immunoregulatory actions of Indomethacin in experimental models of inflammation in rats. In a study conducted by Bernadi et al, three classical models of inflammation *in vivo* were employed to evaluate the short and long-term effects of Indomethacin nanocapsule (IndOH-NC), in comparison with Indometacin in carrageenan-induced acute oedema, CFA-induced sub-chronic inflammation and CFA-induced arthritis.[8]

The injection of carrageenan into the hind paw of rat usually used as a model to study acute inflammation and pain. The application of carrageenan causes a rapid formation of oedema, allied to an exacerbated sensitivity to thermal and mechanical stimuli.[9] In this regard, carrageenan-induced rat paw oedema is widely used to characterize the mechanisms of action of new anti-inflammatory drugs or formulations, including NSAIDs.[10,11,12]

The standard drug indomethacin (87.3% and 91.5%, respectively) showed remarkable inhibitory response in carrageenan and dextran induced rat paw edema (Table 1, Table 2) in comparison to vehicle control in our study. Therefore statistically significant difference was observed between the anti-inflammatory effect of Indomethacin and vehicle control in the carrageenan and dextran induced model of inflammation.

Though immunosuppressive action was more predominant with dexamethasone both in primary and secondary phase of the event in Freund's adjuvant induced arthritis model indomethacin showed significant effect in the primary phase. On 5th day suppression of primary lesions was 48.9% with Indomethacin in comparison to 73% with dexamethasone. In arthritic model Indomethacin (1 mg/kg) showed significant chronic anti-inflammatory effect (29%) in comparison to the standard drug dexamethasone(84.6%)(Table 3, 4) on 21st day. Other doses of Indomethacin (Dose dependent effect) were rejected because of significant gastric toxicity inspite of taking effective precautionary measures with cytoprotective drugs like ranitidine and Antacids.

Conflict of Interest: Nil

References

1. Burian M, Geisslinger G. COX-dependent mechanisms involved in the anti-nociceptive action of NSAIDs at central and peripheral sites. Pharmacol Ther. 2005;2:139–154.
2. Kean WF, Buchanan WW. The use of NSAIDs in rheumatic disorders 2005: a global perspective. Inflammopharmacology. 2005;4:343–370.
3. Langford R, McKenna F, Ratcliffe S, Vojtassák J, Richarz U. Transdermal fentanyl for improvement of pain and functioning in osteoarthritis: a randomized, placebo-controlled trial. Arthritis Rheum. 2006;6:1829–1837.
4. Vogel HG, Vogel WH. Analgesic, Anti-inflammatory and antipyretic activity. In: Vogel HG, Vogel WH, editors. Drug Discovery and Evaluation. Pharmacological Assays. New York: Berlin Heidelberg: Springer-Verlag; 1997b. p. 382.
5. Pearson CM, Wood FD. Studies on polyarthritis and other lessions induced in rats by injection of mycobacterium adjuvant. I. General clinic and pathological characteristics and some modifying factors. *Arthr Rheum* 1959;2:440.
6. Schleyerbach R. Antiarthrotic and Immunmodulatory Activity. Vogel, G H, eds. Drug Discovery and Evaluation. 2nd edition. Berlin, Germany. Springer – 2002:802.
7. Lo, T.N., Almeida, A.P., Beaven M.A. Dextran and carrageenan evoke different inflammatory responses in rat with respect to composition of infiltrates and effect of indomethacin. Journal of Pharmacology and Experimental Therapeutics.1982;221:261-267.
8. A Bernardi, ACCV Zilberstein, E Jäger, MM Campos, FB Morrone, JB Calixto, et al. Effects of indomethacin-loaded

nanocapsules in experimental models of inflammation in rats. Br J Pharmacol. 2009;158:1104–1111.
9. Rocha AC, Fernandes ES, Quintão NL, Campos MM, Calixto JB. Relevance of tumour necrosis factor-alpha for the inflammatory and nociceptive responses evoked by carrageenan in the mouse paw. Br J Pharmacol. 2006;5:688–695.
10. Velo GP, Dunn CJ, Giroud JP, Timsit J, Willoughby DA. Distribution of prostaglandins in inflammatory exudates. J Pathol. 1973;111:149–158.
11. Kawamura M, Hatanaka K, Saito M, Ogino M, Ono T, Ogino K, et al. Are the anti-inflammatory effects of dexamethasone responsible for inhibition of the induction of enzymes involved in prostanoid formation in rat carrageenan-induced pleurisy? Eur J Pharmacol. 2000;400:127–135.
12. Quintão NL, Medeiros R, Santos AR, Campos MM, Calixto JB. The effects of diacerhein on mechanical allodynia in inflammatory and neuropathic models of nociception in mice. Anesth Analg. 2005;6:1763–1769.