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Research Article

EVALUATION OF DICLOFENAC POTASSIUM MICROSPHERE FOR ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

Inflammation is a response of vascularized living tissue to the local injury. The severe side effects of steroidal and non-steroidal anti-inflammatory drugs existed marketed formulation evoked us to search for new anti-inflammatory dosage form design for better management of inflammatory conditions.The present investigation was carried out to find the anti-inflammatory effect of Diclofenac Potassium microspheres in albino rats. The anti-inflammatory activity was evaluated using acute inflammatory model like carrageenan induced paw edema and chronic inflammatory model like cotton pellet induced granuloma respectively. The Diclofenac Potassium blank microspheres and drug loaded formulation exhibited and significant anti-inflammatory activity comparision to pure drug diclofenac potassium, in acute (carageenan induced hind paw edema, $p< 0.05$) and chronic (cotton pellet granuloma formation, $p<0.05$) model of inflammation.

Keywords: Microspheres, Diclofenac Potassium, Carrageenan induced paw edema, Cotton pellet granuloma.

INTRODUCTION

The history of inflammation is as old as man's existence in this planet. It is one of the most fundamental response of the cells and tissue to injury. It is essentially a defense reaction but sometimes it over do itself either in intensity or in duration and cause lot of suffering and pain. Steroidal and Non steroidal anti-inflammatory drugs are available but their prolonged administration is known to be associated with various adverse effects.

Inflammation is a pathophysiological response of living tissue to injury that leads to local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism that helps body to protect itself against infection, burns, toxic chemicals, allergens or other noxious stimuli, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases [1].

Pain has been defined by International Association for the Study of Pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [2]. Failure to relieve pain is morally and ethically unacceptable. Drugs that are currently used for the management of pain are opioids or non-opioids and that for inflammatory conditions are non-steroidal antiinflammatory drugs (NSAIDs) and corticosteroids. All these drugs carry potential toxic effects. One study suggests that risk of gastrointestinal bleeding was significantly associated with acute use of non-steroidal anti-inflammatory drugs (NSAIDs) like regular-dose aspirin, diclofenac, ketorolac, naproxen or nimesulide. Piroxicam increased the risk of bleeding in both acute and chronic therapy [3]

Drug research and development (R & D) is comprehensive, expensive, time-consuming and full of risk. It is estimated that a drug from concept to market would take approximately 12 years and capitalizing out-of-pocket costs to the point of marketing approval at a real discount rate of 11% yields a total pre-approval cost estimate of US$ 802 million .

EXPERIMENTAL SECTION

Materials

Diclofenac Potassium was obtained as a gift sample from ISV Pharma Hyderabad. Ethyl cellulose was obtained as gift sample from Kopran pharmaceutical Ltd. Mumbai. Polyvinyl alcohol, ethanol, dichloromethane, tween 20 were purchased from SD Fine Chemicals Ltd. All chemicals/reagents used were of analytical grade. A UV/Vis spectrophotometer (Shimadzu 1700 pharma spec) was used for drug analysis.

Preparation of microspheres

Ethyl cellulose microspheres were prepared using a w/o/w multiple emulsion solvent evaporation technique. Ethyl cellulose dissolved in 20 ml Dichloromethane. 500 mg diclofenac potassium dissolved in 5 ml water. Drug solution was added into the polymeric solution and stirring with high speed using magnetic stirrer. So formation of primary emulsion. Primary emulsion was drop wise added into the 1% PVA solution (1 gm of PVA dissolved in 100 ml distilled water) and stirring the resulting solution at1600 RPM using the Remi propeller till the dichloromethane evaporate. of microspheres were formed and collected by vacuum filtration, washed the microspheres with 2 times 100 ml distilled water and remove the PVA residue. The microspheres collected in filter paper dried at room temperature.

Test Animals

Adult Swiss male albino rats (150-200gms) were obtained from animal house, Department of Pharmacology,R.C.P.H.S,, Berhampur and used throughout the study. They were housed in microlon boxes in a controlled environment (temperature25±2°C and 12hr dark/light cycle) with standard laboratory diet and water *adlibitum*. All experimental procedures and protocol used in this study were reviewed and approved by institutional animal ethical committee (Reg no 1018/C/06/CPCSEA), R.C.P.H.S,Berhampur, orissa.

Anti-inflammatory activity

a)Acute inflammatory model

Carrageenan induced paw edema in rats [4]

In the present study, antiinflammatory activity was determined in albino rats of either sex according to the method of Winter [4]. All drugs were given orally to the respective groups as a suspension in gum acacia one hour before carrageenan injection. The procedure followed was, acute inflammation produced by injection of carrageenan (0.1 ml of 1% w/v suspension) [5], in the right hind paw of the rats under the plantar aponeurosis. It was injected +1h after the oral administration of the drug. The inflammation was quantitated in terms of ml i.e. displacement of water by edema using a digital plethysmometer immediately before and after carrageenan injection at +1, +2, +3, +4, +5 and +6 h. The percentage inhibition of edema was calculated for each group with respect to its vehicle-treated control group [6, 7, 8].

Percentage inhibition of paw edema = (1–Vt/Vc) 100

Where Vc represent average increase in paw volume (average inflammation) of the control group of rats at a given time; and Vt was the average inflammation of the drug treated (i.e. DKM0,DKM1 or DK) rats at the same time. The difference in the initial 0h and volume at +1h indicate paw edema at 1h followingcarrageenan administration. Accordingly paw edema at +2, +3, +4, +5 and +6h was calculated. Then percentage inhibition of paw edema was calculated.

Table-I

control group	
Group A	Carrageenin (Injected)+Normal Saline (Orally administered)
Test groups	
Group B	Carrageenin (Injected)+DKM0 10mg/Kg (Orally administered)
Group C	Carrageenin (Injected)+DKM1 10mg/Kg (Orally administered)
Standard group	
Group D	Carrageenin (Injected)+Diclofenac potassium100mg/Kg (Orally administered)

b) Sub acute inflammatory model

Cotton pellet induced granuloma model[9]

Sub acute inflammation was produced by cotton pellet induced granuloma model in rats [9, 10]. On day 1, with aseptic precautions sterile cotton pellets (50±1 mg) were implanted subcutaneously, along the flanks of axillae and groins bilaterally under ether anaesthesia. All drugs were given orally to the respective group of rats as a suspension in gum acacia daily for six consecutive days from day 1. The animals were sacrificed on the 7th day. The granulation tissue with cotton pellet was dried at 60°C overnight and then the dry weight was taken. Weight of the cotton pellet before implantation was subtracted from weight of the dissected dried pellets. Only dry weight of the granuloma formed was used for statistical analysis.

c) Chronic inflammatory model[12]

The anti-inflammatory activity against chronic inflammation was tested by the adjuvant arthritis method in albino rats. On day 1, the animals were injected into the subplantar region of the left hind paw with 0.1 ml of complete Freund's adjuvant. Dosing with the test compounds or the standards to the respective groups was started on the same day and continued for 12 days. Paw volumes of both sides and the body weights were recorded on the day of injection. On day 5, the volume of the injected paw was measured again, indicating the primary lesion. On day 21, the noninjected paw volume and the body weight were determined again. [11] Polyarthritis severity was graded on a scale of 0-4: 0 = no swelling; 1 = isolated phalanx joint involvement; 2 = involvement of the phalanx joint and digits; 3 = involvement of the entire region down to the ankle; and 4 = involvement of the entire paw, including the ankle. The maximum joint score was 12 including 3 secondary arthritic paws for each rat. [12] An "arthritic index" was calculated as the sum of the scores as indicated above for each animal.

Statistical Analysis

All values were shown as mean±SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnet's t test. $P<0.05$ was considered statistically significant.

RESULTS

Antiinflammatory activity in rats

Effect on carrageenan induced paw edema (Table II, Fig. 1)

Pretreatment with Diclofenac potassium and its microspheres reduction in carrageenan evoked hind paw edema and differed significantly ($P<0.001$) among the different groups of rats . In carrageenan induced rat paw edema test, Diclofenac potassium microspheres showed statistically significant ($P<0.001$) inhibitory effect on "mean increase in paw volume" at all the time intervals (+1 h, +2 h,+ 3 h, +4 h, +5 h, and +6 h) as shown in (Table I). After +2 h and +3 h of carrageenan administration, Diclofenac potassium pure drug exhibited maximum % inhibition of paw volume ($P<0.01$) by 68%, 87% and 60%, 45%, at the higher time intervals**.** In figure 1 the volume displaced is clearly mentioned for all the treated group at a interval of 1,2,3,4,6,8 and 12h respectively**,** however % inhibition of paw volume was greater than that of standard drug, 77.43%,and 87.09% ($P<0.001$) at 4th hr and 6th hr of DKM1.(Table II).

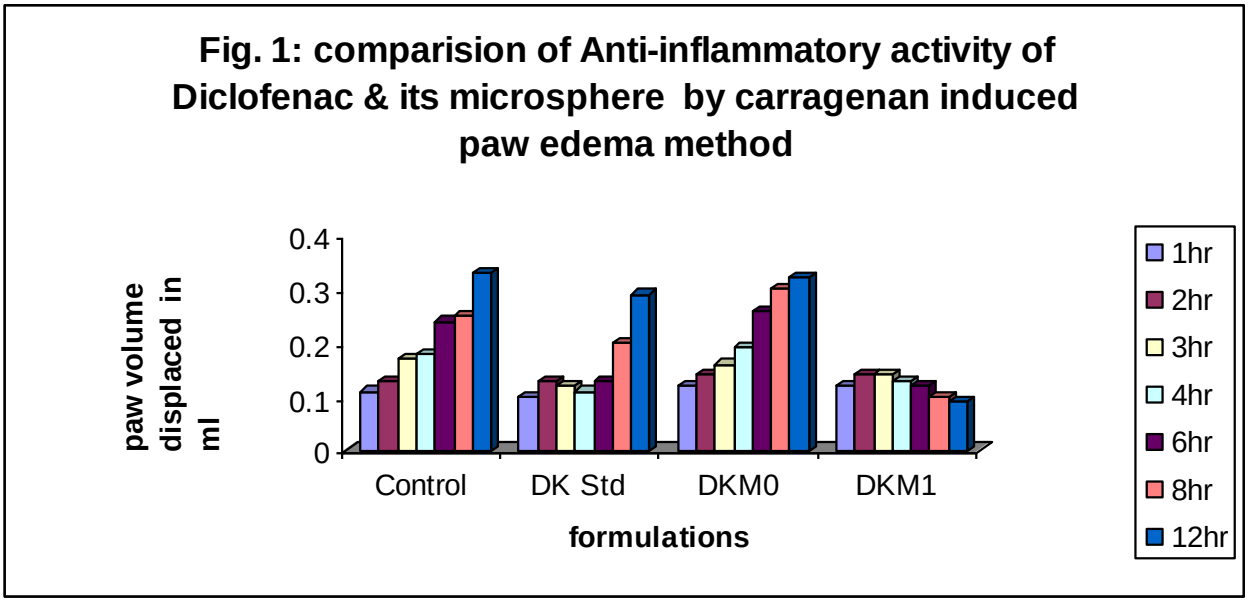

Table 2: Acute anti-inflammatory activity of Diclofenac and its microspheres on carrageenan-induced rat paw edema

Group	Drug dose p.o	Mean increase in the paw volume (mean±SEM;ml) (% inhibition)				
		1h	2h	3h	4h	6h
A(control)	10ml/Kg	0.33±0.012	0.43±0.01	0.64±0.013	0.68±0.01	0.55±0.01
B (DKM0)	10mg/Kg	0.32±0.03	0.41±0.02	0.59±0.021	0.65±0.04	0.51±0.02
C (DKM1)	10mg/Kg	0.30±0.02	0.28±0.005 (56.10)	0.18±0.004[a,b] (56.23)	0.07±0.01[a,b] (77.43)	0.04±0.004[a,b] (87.09)
D (DK)	100mg/Kg	0.28±0.004[a] (55.06)	0.13±0.006[a] (68.27)	0.04±0.01[a] (87.54)	0.21±0.02[a] (60.37)	0.29±0.04[a] (45.23)
one way ANOVA	F	88.55	74.87	108.87	76.87	87.34
	df	20,3	20,3	20,3	20,3	20,3
	p	<0.05	<0.05	<0.05	<0.05	<0.05

Values are expressed as mean±ESM for six animals; [a]P< 0.05 when compared to the control; [b]P< 0.05 when compared with the standard.

Antigranulation effect in rats (Table III)

The dry weight of cotton pellet granuloma in control, two different formulations (DKM0,DKM1)of diclofenac microsphere and diclofenac potassium one formulations of diclofenac i.e DKM1 and diclofenac std drug showed significant (P<0.001) activity in inhibiting dry weight of granuloma. The diclofenac microsphere administered at 10 mg/kg, p.o. had a greater anti-granulation (67.08%) effect than that of diclofenac potassium standard drug (55.17%).

Table 3: Anti-inflammatory activity of Diclofenac and its microspheres on subacute inflammation by the granuloma pouch method

Group	Drug dose p.o	Mean volume of exudates (ml) (mean±SEM;ml)	Inhibition of exudates formation (%)
A(control)	10ml/Kg	3.98±0.043	-
B (DKM0)	10mg/Kg	3.19±0.032	-
C (DKM1)	10mg/Kg	1.30±0.034[a,b]	67.08
D (DK)	100mg/Kg	1.35±0.025[a]	55.17
one way ANOVA	F	341.5	
	df	20,3	
	p	<0.05	

Values are expressed as mean±ESM for six animals; [a]P< 0.05 when compared to the control; [b]P< 0.05 when compared with the standard.

Table 4: Anti-inflammatory activity of Diclofenac and its microspheres on chronic inflammation by adjuvant-induced arthritis

group	drug dose p.o	increase in paw volume in (ml) (% inhibition)		weight change on the 21st day (% change)	Arthritis index
		on the 5th day	on the 21st day		
A(control)	10 ml/Kg	0.95±0.05	0.32±0.02	-12.6±0.75	7.6±0.19
B (DKM0)	10 mg/Kg	0.92 ±0.09	0.28±0.01	-11.4±0.65	7.2±0.16
C (DKM1)	10 mg/Kg	0.67±0.035(27.86)[a,b]	0.18±0.014(38.16)	10±0.86(20)	3.0±0.51[a,b]
D (DK)	100mg/Kg	0.49±0.013(46.21)[a]	0.14±0.088(54.86)	0±0.56(100)	5.0±0.11[a]
One way ANOVA	F	31.23	31.09	73.76	37.87
	df	20,3	20,3	20,3	20,3
	p	<0.05	<0.05	<0.05	<0.05

Values are expressed as mean±ESM for six animals; [a]P< 0.05 when compared to the control; [b]P< 0.05 when compared with the standard

Antiarthritic effect in rats (Table IV)

The results obtained as mean increase in paw volume and arthritis index are shown in table-3. the values of arthritis index was 3% for DKM1 and 5% for diclofenac potassium standard drug.

SEM study All the microspheres were spherical in nature its surface was smooth observed in SEM report. The best formulation selected for anti-inflammatory study that is coded as DKM1, and another blank microsphere coded as DKM0 selected for further studies. (Figure 2)

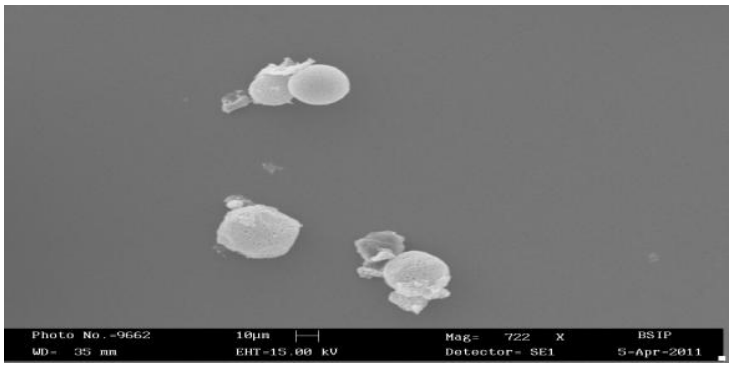

Figure 2 : SEM of formulation DKM1

DISCUSSION

The carrageenan-induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal antiinflammatory agents, which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis [13].

Carrageenan-induced hind paw edema is the standard experimental model of acute-inflammation. The time course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve [14]. The first phase of inflammation occurs within an hour of carrageenan injection and is partly attributed to trauma of injection and also to histamine, and serotonin components [15]. The second phase is associated with the production of bradykinin, protease, prostaglandin, and lysosome [15]. Prostaglandins (PGs) play a major role in the development of the second phase of inflammatory reaction which is measured at +3h [16]. diclofenac microspheres produced a significant inhibition of carrageenan induced paw edema at +3h and +6h. Therefore, it can be inferred that the inhibitory effect of diclofenac microspheres on carrageenan induced inflammation could be due to inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis. Significant inhibition of paw edema in the early hours of study by diclofenac could be attributed to the inhibition of histamine [17] and/or serotonin. The increase in paw edema inhibition at +6h may be attributed to the sustained drug action. (table-2)

Cotton pellet granuloma model was used to evaluate the antiinflammatory activity of diclofenac microspheres in sub acute inflammation. Three phases of the inflammatory response to a subcutaneously implanted cotton pellet in the rats have been described: (A) a transudative phase, that occurs during the first 3 h; (B) an exudative phase, occurring between 3 and 72h after implanting the pellet; (C) a proliferative phase, measured as the increase in dry weight of the granuloma that occurs between 3 and 6 days after implantation [18]. The suppression of proliferative phase of sub acute inflammation could result in decrease in the weight of granuloma formation [19,20]. The dry weight of cotton pellet granuloma was significantly reduced ($P<0.001$) by 10mg/ kg and 100 mg/kg doses of diclofenac potassium and its microspheres, however the antiproliferative effect of diclofenac microspheres was more than that of the standard drug.(table-3)

Adjuvant-induced arthritis model was used to evaluate the antiinflammatory activity of diclofenac microspheres in chronic inflammation**.** For the anti-inflammatory activity against chronic inflammation, again the diclofenac potassium microspheres showed a significantly better anti-inflammatory activity than the standard drug ($P < 0.05$) for parameters of chronic inflammation including primary lesion, secondary lesion, and arthritis index ($P < 0.05$), a change in weight, where a significant difference was observed ($P < 0.05$;(table-4).

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