Evaluation Of Pharmacological Activities Of Ibuprofen Loaded Interpenetrating Polymer Network (IPN) Beads From Sodium Carboxymethyl Xanthan and Sodium Alginate On Rats

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Summary

Ibuprofen (IBP) is a non-steroidal anti-inflammatory agent (NSAID) used in the treatment pain and inflammation particularly against rheumatoid arthritis and osteoarthritis. It is commonly associated with side effects including a high incidence of gastric and duodenal ulceration, Peptic ulceration, perforation, and gastrointestinal bleeding. The associated side effects can be successfully addressed by developing a proper controlled release delivery system. The present study was designed to evaluate and compare the ulcerogenic effect and the anti-inflammatory effect of IBP loaded novel Interpenetrating Network (IPN) beads developed by cross linking sodium carboxymethyl xanthan (SCMX) and sodium alginate (SAL) using aluminium chloride ($AlCl_3$)as cross-linking agent with that of pure IBP in 150-200 g adult male albino Wistar rats. Results indicate that ulcerogenicity decreases significantly with IBP loaded Interpenetrating Network Polymer (IPN) beads in comparison to the pure IBP. The duration of action was also found to be more prolong for the IPN beads in comparison to pure IBP.

Key words : IPN , ulcerogenicity, anti- inflammatory action, polymer ,cross-link

Introduction

Ibuprofen (IBP), derived from propionic acid by the research arm of Boots Group[1], is being used to treat pain and inflammation since long. The Pharmacological efficacy of Ibuprofen (IBP) is through the nonselective inhibition of cyclo-oxygenase 1(COX-1) and cyclo-oxygenase 2(COX-2) enzymes[2] and has been established as one of the most useful anti-inflammatory, analgesic and antipyretic agent in the treatment of rheumatoid arthritis, osteoarthritis[3]. In case of rheumatoid arthritis and osteoarthritis, where patients generally remember morning and evening medication but tend to forget doses in between, thus leading inaccurate dosing or coverage. On the other hand, short biological half-life of IBP gives rise to valley effect if

repeated dosing becomes necessary. Ideally once or twice daily dosing improves therapy by maintaining steady state plasma concentration of the drug in the blood, avoiding the peaks of high plasma concentration as well as troughs of low plasma concentration. For that reason IBP often cannot be recommended for use in patients suffering from rheumatoid arthritis, osteoarthritis and others joint pains in spite of its versatilities. Controlled release or sustained release formulations with non-irritant bio-compatible polymers not only provide protection to the Gastro Intestinal Tract (GIT) but also enable this versatile drug to be used in the treatment of rheumatoid arthritis, osteoarthritis and others joint pains. Natural polymers like sodium alginate (SAL), chitosan, xanthan gum, gellangum are used for because of their bio-compatibility and capacity to absorb large quantities of water or biological fluid[4,5,6,7]. Modification and derivatization of these polymers help in modulating the release of the drug by controlling their swelling capacity. Beads prepared by cross linking a homopolymer or heteropolymer gives rise to sustained release formulation, release from which can be modulated as per requirement by varying extent of cross links. These polymers are often called smart polymers which are design to delivery of drug at specific organ.

The sustained release formulation of ibuprofen loaded Interpenetrating Polymer Network (IPN) bead has the potential to minimize its toxicity and extend the duration of pharmacological efficacy. IBP loaded Interpenetrating Polymer Network (IPN) bead has been formulated to reduce gastrointestinal tract irritation and to release the drug over a prolong period. In vitro study of IPN beads, prepared from SCMX, SA and IBP in the weight ratio 1.5:1.5:1.5 respectively in the presence of 2% w/v $AlCl_3$ as cross linking agent and gelled for half an hour, has shown to release 13-14% IBP in two hours in Gastro Intestinal Fluid (data not shown). In simulated intestinal fluid however 98% - 99.5% drug is released in 4-5 hours (data not shown). As in vitro

studies suggested that the IBP loaded IPN beads control and restrict the release of the drug in gastro intestinal fluid and release the drug in a sustain manner in simulated intestinal fluid for 4 to 5 hours (data not shown) , the delivery system can be considered to be used for treatment of rheumatoid arthritis, osteoarthritis and others joint pains after evaluating the pharmacological effects of the IPN beads in animal system. Thus ,the objective of these study was to evaluate the ulcerogenicity property and anti-inflammatory property in biological system.

Materials and Methods:

Chemicals and reagents

IBP (Indian Pharmacopoeia) and xanthan gum were obtained as gift samples from respectively M/S Albert David Limited and M/S Deys Medical Stores (Mfg). Pvt. Limited, Kolkata, India. Sodium alginate (Mol.wt. 240 kDa), $AlCl_3.6H_2O$ (SD Fine Chem Pvt. Ltd, Mumbai, India), monochloro acetic acid (Loba Chemie Pvt. Ltd, Mumbai, India) , carrageenin (Spectrochem Pvt. Ltd.Mumbai,/INDIA), dextran and 5-HT (Sigma Aldrich®) and all other analytical grade reagents were obtained commercially and used as received.

Animals:

Male Wistar albino rats (8 weeks), weighing 150–200g were purchased from M/S BN Ghose, Kolkata. The animals were grouped and housed in poly acrylic cages(38x23x10 cm) with not more than six animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2^{o}C) with 14h dark : 10 h light cycle. They were allowed free access to standard dry pellet diet (Hindustan Lever , Kolkata) and water ad libitum. The animals were acclimatized to laboratory conditions for one week before commencement of the experiment.

The ethical clearance was obtained from Jadavpur University Ethical Committee for using animals in the present study.

Experimental design

The pure drug, the IPN beads loaded with IBP and the blank beads were suspended in 1% carboxy methyl cellulose (CMC). The required volume was administered orally through gavages with Fr 8 x 23-inch feeding tube. Fasted rats that were deprived of food but not water for 24 hours prior to experiment were used to assess the effect of IBP ,IPN beads and CMC on gastric mucosa.

(A) Comparative ulcer study of pure IBP with the IBP loaded IPN beads:

Preparation of IBP Loaded IPN Beads

The required quantity of IBP (1.5%), accurately weighed, was uniformly dispersed in an aqueous solution containing required proportions of sodium carboxymethyl xanthan gum and sodium alginate(1.5:1.5) and the dispersion was mixed homogeneously using a magnetic stirrer. The IPN beads were prepared by extruding the polymeric dispersion (10 ml) containing IBP (15mg), into 2%w/v solution of aluminum chloride at a rate of 2 ml/min, using 21G flat-tip hypodermic needle. These beads were allowed to harden for 30 minutes. The beads were collected by filtering aluminium chloride solution, washed with deionized water and dried to a constant weight at 45°C in hot air oven and then collected in desiccators.

In-vivo ulcerogenicity study:

Ulcerogenicity studies were conducted according to the procedure reported [8,9,10,11] . Adult male Wister albino rats fasted for 24 hours but given with water *ad libitum* were randomly divided into three groups, each containing 6 animals. Pure IBP , IPN beads loaded with IBP were

fed after dispersing them in 1 % CMC suspension. While the first group was administered with 1 ml CMC suspension(1%), the second and third groups were fed with pure IBP and IPN beads loaded with IBP in 1ml 1% suspension of CMC respectively. After oral administration of the suspensions, all animals were kept for 6 hours and then sacrificed. The stomach of the sacrificed animals were removed and opened along the greater curvature. Mucosal damages (ulcer formation) of the stomachs were examined under the microscope. The severity of mucosal damage was assessed in terms of a rating scale proposed by Tammara *et al. (*1993). The rating scale used was as follows:

Observation	**score**
No lesion	0.0
Punctiform lesions (less than 1 mm)	1.0
Five or more punctiform lesions	2.0
One to five small ulcers (1-2 mm)	3.0
More than five small ulcers or one large ulcer	4.0
More than one large ulcer (greater than 4 mm)	5.0

Based on the severity of mucosal damage, each specimen was assigned a score. The scores were averaged and the mean score was tabulated. Statistical Dunnett's tests were performed to test the significance of difference in severity index between IBP and IBP loaded IPN beads.

(B) Comparative study of Anti-inflammatory activity of IBP and the IBP loaded IPN Beads in male Wistar albino rats:

Carrageenin induced paw edema model:

This model was based on the principle of release of various inflammatory mediators by carrageenin[12]. The Wistar albino rats (n=6) were divided into three groups. Each animal of the first group received 1ml CMC(1%). The second group received the suspension of pure IBP(25mg/kg bw) in 1ml 1% CMC and the last group received suspension of IPN beads (75mg/kg bw, equivalent to 25 mg/ kg bw pure IBP) orally. The right hind paw was marked with the marker at the level of lateral melleolus. The basal paw volume were measured by volume displacement method using Plethysmometer by immersing the paw up to the mark at the level of lateral malleolus. One hour after dosing, 0.1 ml of 1.0 % carrageenin was injected into the right hind paw of each rat. The paw volume was measured again at 1, 2, 3, 4 and 5hours after challenge. The increase in paw volume was calculated as percentage compared with the basal volume. The percentage of inhibition was calculated using the following formula of Lanher (1992) [13].

Percentage Inhibition = $(V_t - V_o)$ control – $(V_t - V_o)$ treated / $(V_t - V_o)$ control X 100

Where,

V_t is the final average volume (after carrageenin treatment).

V_O is the initial average volume (before carrageenin treatment).

Dextran induced rat paw edema model :

The animals were treated exactly the same way as in the carrageenin-induce paw edema model but instead of carrageenin, here 0.1 mL of Dextran (1%w/v in normal saline) was used as the edemogen[14].The paw edema was measured as mentioned in the carrageenin-induced paw edema model.

Serotonin (5-HT) induced rat paw edema model :

5-HT acts as a mediator of inflammation. The animals were treated exactly the same as in the carrageenin-induced model but instead of carrageenin, here 0.1ml 5-HT (1%) was used as edemogen [15]. The paw edema was measured as mentioned in the carrageenin-induced paw edema model. In the edema models, animals who did not receive any drug were used as control. The data obtained for the various groups were reported as mean±SEM. Percentage edema inhibition was calculated.

Result and Discussion:

Evaluation of Ulcer study:

Results for evaluation of ulcer index have been shown in **table 1** and photograph for the extent of mucosal damage in different groups of animals treated with CMC, pure IBP and IBP loaded IPN beads have been shown in **figure 1, figure 2** and **figure 3** respectively.

After removing the stomach of sacrificed animals along their greater curvature, mucosal damages were found in all groups, with a very small value of ulcerogenicity for the group treated with 1% CMC suspension that has been designed as control group. However the severity of mucosal damages were varied from groups treated with pure IBP with that of the IPN beads loaded with IBP (**figure 2** and **figure 3**). The severity of the ulcer was measured on a rating scale followed by Tammara et al(1993) showed that animals treated with pure IBP forms an average

ulcer index of 3.1666±0.30 whereas the animals treated with IBP loaded IPN beads has an average ulcer index of 1.8333±0.30. The stomachs of the rats which were given with 1% CMC suspension show slight hemorrhage (0.5±0.22). Carboxy methyl cellulose is a non irritant polysaccharide and being widely used as pharmaceutical adjuvant for long. There is no report available that CMC is a gastro irritant agent. Therefore the small value of ulcer index shown may be attributed to stress factor that the animals undergo during the experiment. The results clearly indicate that the ulcer index reduce significantly for group treated with the IBP loaded IPN beads compared with the group treated with pure IBP.

The findings are in line with the observation of Payam Khazaeli *et al*. (2008) [16],who had reported that calcium alginate IBP beads reduce gastric ulcerogenicity of IBP. From the above observation it can be concluded that IBP loaded IPN beads developed through dispersion of SCMX: SA: IBP in the ratio 1.5:1.5:1.5 in 2% aluminum chloride solution which acts as cross-linking agent,can be safely used as gastro protective anti inflammatory agent for treating rheumatoid arthritis, osteoarthritis and others joint pains. Control release alginate beads of IBP was evaluated for in vivo trial in mice and was found to prevent gastric lesions [17].

Evaluation of Anti inflammatory study:

The anti inflammatory effect of the IBP and the test formulation, IBP loaded IPN beads, were compared by measuring the percentage inhibition of paw edema, which were separately induced by carrageenin, dextrin and 5-HT. The results for evaluation of anti inflammatory effect of IBP, IPN beads loaded with IBP, and 1% CMC on the carrageenin induced, dextrin induced and 5-HT induced animals have been shown in table 2, table 3 and table 4 respectively.

From the data presented in **table 2**, it is seen that the Percentage inhibition of carrageenin induced paw edema for pure IBP was higher at the 1^{st},2^{nd}, and 3^{rd} hours when compared to IBP

loaded IPN beads. In the later stage however i.e., after 3 hours the phenomenon is reversed and the percentage inhibition for IBP loaded IPN beads becomes higher in comparison to pure IBP. It is also seen that the maximum percentage inhibition value is 64.89±8.89 for the pure IBP and 69.79±6.21 for IBP loaded IPN beads, reaches after 3rd and 4th hour respectively. From the experimental data it is evident that IBP loaded IPN beads provide a sustained action prolonging the therapeutic action of IBP while retaining degree of action more or less. Statistical difference determined by ANOVA followed by Dunnett's test done on mean paw volume increase of the groups treated with IBP and IBP loaded IPN beads gave significant value ($P<0.01$) when compared with CMC group at the maximum percentage inhibition value.

From the data presented at **table 3**, it is seen that the percentage inhibition of dextrin induced paw edema by pure IBP generally increases after first, second and third hour of administering drug and starts falling abruptly after third hour, whereas the IBP loaded IPN beads inhibit in a steady condition, raising efficacy slowly and start falling slowly after the 4 hour. Maximum percentage inhibition value is 57.36±5.15 for the pure IBP and is 68.48±4.34 for IBP loaded IPN bead that reaches after 3 and 4 hour respectively. Statistical difference determined by ANOVA followed by Dunnett's test done on mean paw volume increase of the groups treated with IBP and IBP loaded IPN beads gave significant value($P<0.01$) when compared with CMC group at the maximum percentage inhibition value.

From the data presented at **table 4**, it is seen that the percentage inhibition of 5-HT induced paw edema for pure IBP was higher at the first three hours in comparison to IBP loaded IPN beads. In the later stage however e.g. after 3 hours the phenomenon is reversed and the percentage inhibition for IBP loaded IPN beads was higher in comparison with pure IBP. It is also seen that the maximum percentage inhibition value is

66.34±7.80 for the pure IBP and 69.58±6.84 for IBP loaded IPN beads that reaches at 4th and 5th hour respectively. Statistical difference determined by ANOVA followed by Dunnett's test done on mean paw volume increase of the groups treated with IBP and IBP loaded IPN beads gave significant value($P<0.01$) when compared with CMC group at the maximum percentage inhibition value.

The above observations are in agreements with the observation of other research workers. Ibuprofen Loaded Nanoparticles has been found to improve the anti-inflammatory activity [18].

Table1: A comparative ulcer -index study of CMC, pure IBP and IBP loaded IPN beads.

Group	**Dose**	**Ulcer-index**
CMC	1ml 1%	0.5±0.22
Pure IBP	25mg/Kg bw	3.1666±0.30**
IBP loaded IPN beads	75mg/Kg bw[a]	1.8333±0.30**

[a] 75 mg/Kg bw of IBP loaded IPN bead is equivalent to 25mg/Kg bw of Pure IBP

Values are expressed as mean±SEM for six independent observations (n=6)

Statistical differences were determined by ANOVA followed by Dunnett's test.

**P<0.01 when all treated groups are compared with CMC group.

Table2. Anti-inflammatory effect of Pure IBP and IBP loaded IPN beads on carrageenin induced rat paw edema.

Groups	Dose	Mean increase in paw volume (mL)					
		0 hr (initial paw volume)	1hr	2 hr	3hr	4hr	5hr
Control (CMC)	1ml (1%)	2.91±0.05	0.33±0.02	0.38±0.03	0.47±0.05	0.80±0.05	0.51±0.04
Pure IBP	25mg/kg bw	2.82±0.04	0.18±0.02** (43.43±5.45)	0.17±0.02** (54.82±5.10)	0.16±0.04** (64.89±8.89)	0.34±0.03** (56.87±4.74)	0.26±0.04** (47.38±8.68)
IBP loaded IPN Beads	75mg/kg bw[a]	2.79±0.03	0.23±0.02** (31.31±5.22)	0.22±0.02** (39.91±6.01)	0.20± .04** (56.38±8.23)	0.24± 0.05** (69.79±6.21)	0.21±0.03** (58.82±6.36)

[a] 75 mg/Kg bw of IBP loaded IPN bead is equivalent to 25mg/Kg bw of Pure IBP

Values are expressed as mean±SEM for six independent observations (n=6)

The data with in the bracket indicate the percentage inhibition value.

Statistical differences were determined by ANOVA followed by Dunnett's test.

**P<0.01 when all treated groups are compared with CMC group.

Table 3. Anti-inflammatory effect of Pure IBP and IBP loaded IPN beads on Dextran induced rat paw edema

Groups	Dose	Mean increase in paw volume (mL)					
		0 hr (initial paw volume)	1hr	2 hr	3hr	4hr	5hr
Control (CMC)	1ml (1%)	2.75±0.04	0.24±0.06	0.33±0.05	0.43±0.05	0.55±0.06	0.41±0.08
Pure IBP	25mg/kg bw	2.74±0.05	0.14±0.02 (40.27±8.91)	0.17±0.03* (45.95±8.76)	0.18±0.02** (57.36±5.15)	0.26±0.02** (51.81±4.89)	0.21±0.04* (47.15±10.76)
IBP loaded IPN Beads	75mg/kg bw[a]	2.76±0.07	0.16±0.01 (32.63±5.83)	0.20±0.02* (37.37±6.43)	0.19± .03** (53.87±8.19)	0.17±0.02** (68.48±4.34)	0.18±0.02* (56.09±6.20)

[a] 75 mg/Kg bw of IBP loaded IPN bead is equivalent to 25mg/Kg bw of Pure IBP

Values are expressed as mean±SEM for six independent observations (n=6)

The data with in the bracket indicate the percentage inhibition value.

Statistical differences were determined by ANOVA followed by Dunnett's test.

**P<0.01,*P<0.05 when all treated groups are compared with CMC group.

Table4. Anti-inflammatory effect of Pure IBP and IBP loaded IPN beads on 5-HT induced rat paw edema

Groups	Dose	Mean increase in paw volume (mL)					
		0 hr (initial paw volume)	1hr	2 hr	3hr	4hr	5hr
Control (CMC)	1ml (1%)	2.75±0.04	0.39±0.02	0.46±0.05	0.52±0.05	0.40±0.02	0.32±0.03
Pure IBP	25mg/kg bw	2.76±0.05	0.23±0.03** (40.17±9.70)	0.20±0.009** (55.07±2.07)	0.17±0.04** (66.34±7.80)	0.18±0.03** (53.75±8.50)	0.19±0.03** (38.02±8.74)
IBP loaded IPN Beads	75mg/kg bw[a]	2.80±0.05	0.25±0.02** (35.04±4.17)	0.24±0.04** (47.10±8.56)	0.23±0.03** (55.44±6.53)	0.12±0.03** (69.58±6.84)	0.13±0.02** (56.77±8.16)

[a]75 mg/Kg bw of IBP loaded IPN bead is equivalent to 25mg/Kg bw of Pure IBP

Values are expressed as mean±SEM for six independent observations (n=6)

The data with in the bracket indicate the percentage inhibition value.

Statistical differences were determined by ANOVA followed by Dunnett's test.

**P<0.01 when all treated groups are compared with CMC group.

Figure 1. :Ulcerogenicity effect of CMC treated rat

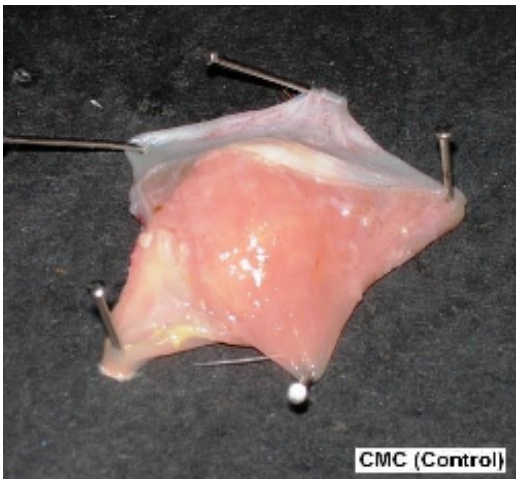

Figure 2. : Ulcerogenicity effect of Pure IBP treated rat

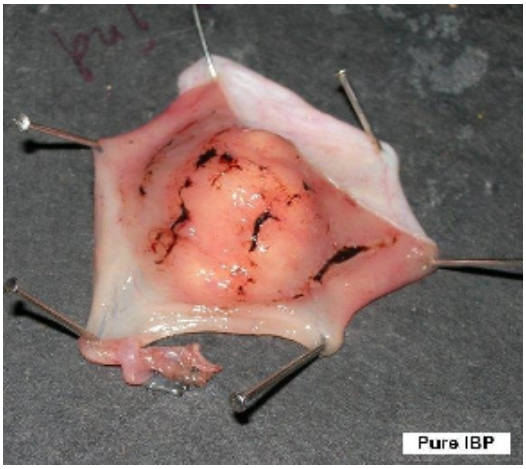

Figure 3. : Ulcerogenicity effect of IBP loaded IPN beads treated rat

Conclusions

From the in vivo studies it is revealed that the IPN beads loaded with IBP provide better gastric tolerance than pure IBP itself . The IPN beads loaded with IBP have been found to be eliciting prolonged anti inflammatory action when compared with the pure IBP. This proves that IPN beads loaded with IBP sustain anti- inflammatory action for a longer period with less side effects.

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