Synthesis, in-vitro characterization and pharmacological evaluation of conjugates of flurbiprofen and polysaccharides for colon specific drug delivery

P. Soni, L. K. Soni, G. P. Choudhary *
School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore (MP), India

ABSTRACT

The aim of the study was to prepare site specific drug delivery of flurbiprofen using polysaccharides by the formation of glycosidic linkage which is hydrolysed by the microflora present in colon. This approach prevents drug release in the upper gastrointestinal environment. Due to the minimal degradation of conjugates in upper GIT, the in vitro drug release in SGF, SIF and SCF was found upto 4.26±0.03%, 12.41±0.08% and 92.72±3.33% respectively.

Keywords: Colon specific drug delivery, Conjugates, Flurbiprofen, Microbial system.

INTRODUCTION

Colon specific drug delivery technologies (CSDDT) are being enormous improved in current years. The major impediments in drug targeting to the colon are degradation pathways and systemic absorption of the drugs in the upper GI tract. Many research studies are being done in CSDDT to meet the needs of ever-increasing gastrointestinal complications such as Crohn’s disease and ulcerative colitis, amebic colitis, to treat colon cancer, early morning arthritis, nocturnal angina and hypertension, influenced by circadian rhythms. A range of strategies/systems have been developed/practiced for better colonic drug delivery. Among all other strategies, The traditional approaches for colon targeting are produg formulation, pH-sensitive drug delivery, time-dependent systems and microbial degradation methods to formulate different dosage forms like tablets, capsules, multiparticulates, microspheres, liposomes. A microbial degradation system is more frequent and acceptable because A number of synthetic azo polymers and natural or modified polysaccharides (chondroitin sulphate, guar gum, xanthan gum, locust gum, inulin, dextrins, starch, amylose,pectins) degraded by the human colonic flora, have thus been investigated as colonic drug delivery carriers. The human colon has over 400 distinct species of bacteria as resident flora, a possible population of up to millions of bacteria per gram of colonic contents. Among the reactions carried out by these gut flora are azoreduction and enzymatic cleavage i.e. glycosides. These metabolic processes may be responsible for the metabolism of many anti-inflammatory drugs and may also be applied to colon targeted delivery of them. These systems are designed to reduce/avoid the degradation and absorption of the drugs in the starting portion of the gastrointestinal tract and reduce side effects of drugs. In oral colon-specific drug delivery system, colon has a large amount of lymphoma tissue (facilitates direct absorption in to the blood), negligible brush boarder membrane activity, and much less pancreatic enzymatic activity as compared with the small intestine to treat local diseases associated with colon. Nonsteroidal anti-inflammatory drugs (NSAIDs) are extensively used in the management of pain and inflammation associated with osteoarthritis, rheumatoid arthritis and ulcerative colitis. They also preferred for the management of inflammatory bowel disease and large intestine cancers. Flurbiprofen, a nonsteroidal anti-inflammatory drug, successfully used for the management of inflammation, pain associated with rheumatoid arthritis, was selected as a model drug. It has plasma half-life 3-6 h. Its high dose frequency is due to its shorter half-life. The polysaccharides as carrier of NSAIDs to target colon is much better approach by formation of

CODEN (USA): JDDTAO
ISSN: 2250-1177
[316]
glycosidic conjugates. It is biocompatible, biodegradable, non-toxic and shows mucoadhesive properties as well. Due to its mucoadhesion nature, its residence time in the colon can be increased, which subsequently results in maximum bioavailability. Taking the above information into account, a study was designed for the preparation and characterization of flurbiprofen conjugates for the colon drug delivery system.

**METHODS AND MATERIALS**

**Materials**

Flurbiprofen selected for the project was procured as gift samples from FDC, Ltd, Mumbai, India. Polysaccharides like dextran was purchased from Himedia, Mumbai, India; chitosan was obtained as gift sample from Central Institute of Fisherish Technology, Cochin, India. Pectin and Inulin was purchased from Loba Chemical Pvt. Ltd, Mumbai. All other chemical and reagents were used of analytical grade.

**Methods**

**Preparation of Drug Conjugate**

**Chemical modification of flurbiprofen**

**Reduction of flurbiprofen**

Flurbiprofen (0.03 mole) and LiAlH₄ (0.03 mole) in diethyl ether were refluxed in a water bath for 2 hr; filtered, washed with 10 mL diethyl ether for three times and then finally washed with water, dried and purified by recrystallization using ethanol water. Yield 71%; M.P. 107-109°C.

**Chlorination of the [2-(2-Fluorobiphenyl-4-yl)] Propenol:**

The compound [2-(2-Fluorobiphenyl-4-yl)propanol] (0.015 mole) and thionyl chloride (1.6 mL, 0.015 mole) were mixed and allowed to get reacted in a round bottom flask at 0°C for 1 hr, refluxed for 2 hr and allowed to cool and poured onto to ice cold water. The compound was washed with distilled water several times to remove any unreacted thionyl chloride, purified by recrystallization using ethanol-water and dried. Yield 62%; M.P. 120-122°C.

**Conjugation of modified naproxen with polysaccharides**

**Synthesis of Dextran-Flurbiprofen Conjugate (DFI)**

The solution of dextran in 25 mL of DMF in a round bottom flask, [2-(2-Fluorobiphenyl-4-yl)] 1- Chloro propane (0.008 mole), mercuric bromide (0.008 mole) and yellow mercuric oxide (0.004 mole) were added and refluxed for 2 hr under stirring. The obtained suspension was filtered and the filtrate was dried in a rotary vacuum evaporator at 80°C; 100 rpm; 400 mm Hg. The product was recrystallized using methanol-distilled water, filtered using Whatmann filter paper, and dried. Yield 70%; M.P. 246-248°C.

**Synthesis of Pectin-Flurbiprofen Conjugate (PFI)**

The solution of pectin in 25 mL DMF in a round bottom flask [2-(2-Fluorobiphenyl-4-yl)] 1- Chloro propane (0.008 mole) was added slowly. Then mercuric bromide (0.008 mole) and yellow mercuric oxide (0.004 mole) were added, stirred and refluxed for 2-3 hr. The obtained suspension was filtered, dried in a rotary vacuum evaporator at 75°C; 100 rpm; 300 mm Hg and recrystallized using methanol-distilled water. The product was filtered using Whatmann filter paper and dried. Yield 51%; M.P. 245-247°C.

**Synthesis of Inulin-Flurbiprofen Conjugate (IFl)**

Inulin in minimum quantity of water was added to [2-(2-Fluorobiphenyl-4-yl)] 1- Chloro propane (2g; 0.008 mole) in 25 mL DMF. Then mercuric bromide (0.008 moles) and yellow mercuric oxide (0.004 mole) were added, stirred and refluxed for 2-3 hr. The obtained suspension was filtered, dried in a rotary vacuum evaporator at 90°C; 100 rpm; 400 mm Hg and recrystallized using methanol-distilled water. The product was filtered using Whatmann filter paper and dried. Yield 56%; M.P. 248-249°C.

**Synthesis of Chitosan-Flurbiprofen Conjugate (CFI)**

The chitosan dissolved in minimum quantity of 2% glacial acetic acid, 25 mL DMF containing (0.008 mole) [2-(2-Fluorobiphenyl-4-yl)] 1- Chloro propane was added under stirring. Then mercuric bromide (0.008 mole) and yellow mercuric oxide (0.004 mole) were also added to the solution, stirred and refluxed for 2 hr and then finally stirred at room temperature for 12 hr. The obtained suspension was filtered, dried in a rotary vacuum evaporator at 90°C; 100 rpm; 400 mm Hg and recrystallized using methanol-distilled water. The product was filtered using Whatmann filter paper and dried. Yield 52%; M.P. 254-256°C.

**Characterization**

**Thin Layer Chromatography (TLC):** the purity of the compound was checked by TLC using silica gel G, suitable solvent systems and detecting agent; iodine vapors

**FT-IR and 1 HNMR:** the IR spectrum of the synthesized conjugates was recorded on jasco V-530 FTIR in potassium bromide (anhydrous , IR grade).The 1 HNMR spectrum of conjugates was recorded in DMF, using a 1 HNMR Varian Mercury 300 Hz, with super conducting magnet.

**Swelling Study of Conjugates**

Accurately weighed amount of each of the conjugates (Wᵢ) was dipped in the swelling medium SGF pH 1.2, SIF-1 pH 4.5, SIF-2 pH 6.8 and SCF pH 7.0 for 2 hr. At predetermined time intervals, the gel was removed from the swelling medium, blotted with filter paper to remove excess water from the gel surface, and the swollen hydrogels (Wᵢ) was weighed. The swelling ratio (SR) is calculated according to the following equation

\[ SR = \frac{W_i - W_0}{W_0} \]

Where \( W_i \) the weight of the swollen gel and \( W_o \) the weight of the conjugate

**In-Vitro Drug Release Studies**

**In-vitro** drug release studies were carried out according to Sounders and Ellenbogen (1985) extraction techniques using USP dissolution test apparatus (apparatus 2). The dissolution studies were carried out in different dissolution medium (900 mL) including simulated colonic fluid (4.0%/w/v), which was stirred at 100 rpm at 37±0.2°C.

Samples were withdrawn periodically and compensated with an equal volume of fresh dissolution media. The drug content in the withdrawn samples was estimated spectrophotometrically at \( \lambda_{max} = 247 \) nm for conjugates of flurbiprofen.
In-Vivo Animal Study

Ulcerogenic Activity

The ulcerogenic activity was determined by the Rainsford’s cold stress method, which is used to determine ulcerogenic potency of a drug at ten times higher dose. Albino rats were distributed in healthy control, standard group and test groups. Doses of each of the synthesized compounds were first calculated on equimolar basis of pure drug and then were converted into ten times higher doses. Formulation of synthesized compounds and standard drug were administered orally. After oral administration of 5 mL of the aqueous drug suspensions (at 10 times the normal dose), the animals were stressed by exposure to −15° for 1 h. The animals were placed in separate cages, to ensure equal cold exposure. After 2 hrs of drug administration, the rats were sacrificed using isoflurane anaesthesia, the stomach and duodenum were dissected out of the body along with the first 5 cm of the intestine, then rinsed with saline and the contents of the stomach were emptied. The stomach and the intestine were then excised open along the greater curvature and gently wiped clean with a swap dipped in saline. The mucosal damage was examined grossly using a magnifier. A score for the ulcer was studies similar to pyloric ligation induced ulcer model.[27]

Scoring of ulcer will be made as follows:

Normal stomach/intestine——— (0.0)
Red coloration--------------- (0.5)
Spot ulcer------------------- (1.0)
Hemorrhagic streak----------- (1.5)
Ulcers------------------------ (2.0)
Perforation------------------- (3.0)

Mean ulcer score for each animal will be expressed as ulcer index (UI):

\[ UI = \frac{UN + US + UP}{10} \]

UN = Average of number of ulcer per animal
US = Average of severity score
UP = Percentage of animal with ulcer

Colonic edema study

A section of inflamed colon of healthy control, colitis control, standard control and test control group on twelfth day in TNBS induced animal model after treatment with flurbiprofen and its conjugates, was weighed then dried in an oven (80 °C) for 24 hr and then reweighed to determine the wet-to-dry weight ratio, an indicator of colon edema.[27]

RESULT AND DISCUSSION

TLC

Reduced compound of Flurbiprofen [2-(2-Fluorobiphenyl-4-yl) Propenol, The purity of the compound was checked by the TLC using silica gel G, solvent system: n hexane: ethyl acetate: glyceric acid (63:1) detecting agent; iodine vapors. Only one spot was obtained Rf 0.77.

After chlorination of the [2-(2-Fluorobiphenyl-4-yl)] Propenol, The purity of the compound was checked by the TLC using silica gel G, solvent system; n hexane: ethyl acetate: glyceric acid (63:1), detecting agent; iodine vapors. Only one spot was obtained Rf 0.78.

The purity of Dextran – Flurbiprofen Conjugate was checked by TLC using silica gel G, solvent system; ethyl acetate: water (10:1), detecting agent; iodine vapors. Only one spot was obtained, Rf 0.74. The purity of Inulin – Flurbiprofen Conjugate was checked TLC using silica gel G, solvent system; ethyl acetate: water (10:1), detecting agent, iodine vapors. Only one spot was obtained, Rf 0.67. The purity of Chitosan- Flurbiprofen Conjugate was checked by TLC using silica gel G, solvent system ethyl acetate: acetic acid: water (5:1:2), detecting agent, iodine vapors. Only one spot was obtained, Rf 0.71.

Structural elucidation

Reduced compound of Flurbiprofen IR (KBr) spectrum: 3422.62(OH str), 3072.43(CH str aromatic), 2941.35(CH str, aliphatic), 2823.61(CH str, aliphatic), 1602.07, 1584.34, 1492, 1423.16(C=C str in benzene) 1071.17(C-F str cm-1). The disappearance of peak at 1710.21 cm-1, and appearance of peak at 3422.62 cm-1 (m; OH), confirms that the carboxyl group has been reduced to methyl hydroxy group. [H NMR: δ(ppm): 1.47 (CH3, 3H d), 2.1 (CH, 1H, q), 7.2 - 7.4 (benzenem), 4.6 (OH, 1H, s), 3.4(CH2, 2H, d). Chlorinated product of flurbiprofen ((2-(2-Fluorobiphenyl-4-yl) 1-Chloro propane) IR (KBr) spectrum: 3073.43(CH str aromatic), 2942.34(CH str, aliphatic), 2823.61(CH str, aliphatic), 1602.6, 1582.34, 1490.25, 1420.16 C=C str in benzene), 1071.17(C-F str), 702(C-Cl str) (cm-1). IR: The disappearance of Cl str peak at 702.63 cm-1 and appearance of peak at 1140.20 cm-1 (m; characteristic peaks in glycoside). [H NMR: 3.6 ppm (d) indicate formation of glycosidic bond between drug and polysaccharide. IR spectrum of Pectin – Flurbiprofen Conjugate was shown some characteristic peaks at 3454.70(m; OH), 3014.65(CH str aromatic), 2922.07(CH str, aliphatic), 2804.87(CH str, aliphatic), 1749.39(C=O str), 1610.52, 1564.23, 1487, 1410.76(C=C str in benzene), 1116.76(glycosidic linkage str), 1071.76(C-F str) (cm-1)] IR: The disappearance of Cl str peak at 702.63 cm-1 and appearance of peak at 1116.76 cm-1 (m; characteristic peaks in glycoside). [H NMR: 3.40 ppm (d) indicate formation of glycosidic bond between drug and polysaccharide. IR spectrum of Chitosan- Flurbiprofen Conjugate was shown some characteristic peaks at: 3508.41(m; OH), 3014.65(CH str aromatic), 2922.07(CH str, aliphatic), 2873.31(CH str, aliphatic), 1608.53, 1595.09, 1533.37, 1440.79 C=C str in benzene), 1178.15(glycosidic linkage str), 1071.76(C-F str) (cm-1)] IR: The disappearance of Cl str peak at 702.63 cm-1 and appearance of peak at 1163.05 cm-1 (m; characteristic peaks in glycoside). [H NMR: 3.52 ppm (d) indicate formation of glycosidic bond between drug and polysaccharide. IR spectrum of Dextran – Flurbiprofen Conjugate was shown some characteristic peaks at: 3539.31(m; OH), 3390.31, 3308.67 NH str), 3014.65(CH str aromatic), 2922.07(CH str, aliphatic), 2849.39(CH str, aliphatic), 1602.81, 1523.18, 1470.79, 1407.35(C=C str in benzene), 1193.91(glycosidic linkage str), 1071.76(C-F str) (cm-1)] IR: The disappearance of Cl str peak at 702.63 cm-1 and appearance of peak at 1193.91 cm-1 (m; characteristic peaks in glycoside). [H NMR: 3.40 ppm (d) indicate formation of glycosidic bond between drug and polysaccharide.

Swelling Index:

ISSN: 2250-1177 [318] CODEN (USA): JDDTAO
Drug release from swellable conjugates depends on the degree of gelation, hydration, chain relaxation and erosion of the polymer. Swelling studies were performed in simulated fluids to evaluate drug release kinetics of DFI, PFI, IFl, and CFl and DFl, PFI, IFl, and CFl showed drug release up to 4.2±0.26, 6.09±0.26, 4.81±0.19, 3.80±0.11 and 2.81±0.08 swelling ratio were found respectively in SGF. Conjugates synthesized with dextran showed higher swelling than the conjugates with chitosan in SGF. DFI, PFI, IFl, and CFl showed 6.09±0.24, 5.08±0.22 and 3.89±0.15 swelling ratio respectively in SIF I. Drug conjugates with pectin and dextran showed higher swelling than conjugates with chitosan in SIF II. Swelling studies of the drug conjugate were also performed in SCF the swelling ratio 30.62±1.11, 25.08±1.08, 27.14±1.12 and 35.29±1.31 were found in case DFI, PFI, IFl and CFl respectively. Drug conjugates with chitosan showed maximum swellability while conjugates with pectin showed minimum swellability in SCF.

Table 1: Swelling Ratio of the Synthesized Conjugates in Simulated Fluids

<table>
<thead>
<tr>
<th>Conjugates</th>
<th>Simulated gastric fluid</th>
<th>Simulated intestinal fluid I</th>
<th>Simulated intestinal fluid II</th>
<th>Simulated colonic fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFI</td>
<td>6.09±0.26</td>
<td>7.19±0.32</td>
<td>8.06±0.34</td>
<td>30.62±1.11</td>
</tr>
<tr>
<td>PFI</td>
<td>4.81±0.19</td>
<td>6.09±0.24</td>
<td>7.45±0.29</td>
<td>25.08±1.08</td>
</tr>
<tr>
<td>IFl</td>
<td>3.80±0.11</td>
<td>5.08±0.22</td>
<td>7.02±0.34</td>
<td>27.14±1.12</td>
</tr>
<tr>
<td>CFl</td>
<td>2.81±0.08</td>
<td>3.89±0.15</td>
<td>4.09±0.18</td>
<td>35.29±1.31</td>
</tr>
</tbody>
</table>

Values represent Mean ±SD, n=6

In-Vitro Drug Release

In-vitro drug release studies showed drug release up to 4.26±0.03% in SGF, 12.41±0.08% in SIF and 25.08±1.08% from conjugates in SCF which confirm the stability of drug conjugates in SGF and would have more potential for colon specific delivery. The initial drug release in SGF might be due to the fact that a small number of charges present in SGF might have allowed a faster drug release as a result of higher solvent penetration into the polymeric network. This would have resulted into a faster rate of polymer hydration in acidic pH of SGF. Once the outer layer is hydrated/gelled, it acts as a barrier for the drug release and the drug then slowly diffuses out independent of pH. The drug release in SIF I and SIF II could be attributed to the fact that there might be an ion exchange phenomenon between these fluids, but release of drug from conjugates in colon is due to presence of microflora enzymes which degrade polysaccharide as well as hydrolysis of glycosidic linkage in conjugates. The results of this study revealed that the conjugates would be suitable for colonic delivery system by the formation of conjugates of naproxen with polysaccharides.

Colonic edema study

A section of affected colon was weighed, dried in an oven (80 °C) for 24 hr and then reweighed to determine the wet-to-dry weight ratio, an indicator of colon edema. This pharmacological evaluation shows that the wet-to-dry weight ratio of animals of test groups was very close to healthy group. This study confirms better anti-inflammatory activity of conjugates as compared to parent drug. The Wet/dry weight ratio of Healthy, Colitis control, Flurbiprofen, DFI, PFI, IFl and CFl was 4.2±0.22, 6.4±0.23, 6.1±0.12, 4.41±0.19, 5.09±0.21, 4.56±0.23 and 4.28±0.17 respectively.

Table 2: Wet/Dry Weight Ratio of the Albino Rats Colon

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Wet/dry weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Healthy</td>
<td>4.2±0.22</td>
</tr>
<tr>
<td>2</td>
<td>Colitis control</td>
<td>6.4±0.23</td>
</tr>
<tr>
<td>3</td>
<td>Flurbiprofen</td>
<td>6.1±0.12</td>
</tr>
<tr>
<td>4</td>
<td>DFI</td>
<td>4.41±0.19</td>
</tr>
<tr>
<td>5</td>
<td>PFI</td>
<td>5.09±0.21</td>
</tr>
<tr>
<td>6</td>
<td>IFl</td>
<td>4.56±0.23</td>
</tr>
<tr>
<td>7</td>
<td>CFl</td>
<td>4.28±0.17</td>
</tr>
</tbody>
</table>

Values represent mean±SD (n=6)
In-vivo ulcerogenic activity

The ulcerogenic activity was determined by the Rainsford’s cold stress method, which is used to determine ulcerogenic potency of a drug at a ten times higher dose. Albino rats were distributed in healthy control, standard group and test groups. Doses of each of the synthesized compounds were first calculated on equimolar basis of Flurbiprofen (10 mg /kg) and then were converted into ten times higher doses. The conjugates of drug shown very less ulcer index as compared to pure drug which were Healthy (2.12 ± 0.57), Flurbiprofen (29.62 ± 0.31), DFI (4.72 ± 0.56), PFI (5.16 ± 0.72), IFI (6.32 ± 0.22) and CFI (3.21 ± 0.51).

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Group</th>
<th>Ulcer Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Healthy</td>
<td>2.12 ± 0.57</td>
</tr>
<tr>
<td>2</td>
<td>Flurbiprofen</td>
<td>29.62 ± 0.31</td>
</tr>
<tr>
<td>3</td>
<td>DFI</td>
<td>4.72 ± 0.56</td>
</tr>
<tr>
<td>4</td>
<td>PFI</td>
<td>5.16 ± 0.72</td>
</tr>
<tr>
<td>5</td>
<td>IFI</td>
<td>6.32 ± 0.22</td>
</tr>
<tr>
<td>6</td>
<td>CFI</td>
<td>3.21 ± 0.51</td>
</tr>
</tbody>
</table>

CONCLUSION

The ileo-colonic region of the GIT has become an important site for drug targeting and drug absorption. Colon drug delivery system provides local and systemic treatment to the patients suffering from colon infections and colon diseases. However more commonly; systems that use natural materials that degraded by colonic bacterial enzymes are used now a days for ileo-colon specificity. Different diseases such as ulcerative colitis, colon cancer, and diarrhea can be treated by using delivery of drug through colon method and by this route different drugs of different nature and molecular weight can be administered effectively. This method is good for poorly absorbed drugs because colon provides long retention time and shows good absorption. By comparing the dissolution data of test formulations and standard %age drug release was predicted. It was observed that release of drug in gastric media was minimum and it was also observed that release of drug in microbial media with phosphate buffer pH 7.0 was maximum. Conclusively an appropriate dosage form of conjugate can be considered as therapeutically efficacious system for treatment of colitis, with reduced gastric intolerance.

Conflict of interest

Authors have reported no conflict of interest.

REFERENCES


