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Synthesis and Investigation of Colon Specific Polymeric Prodrug of Ibuprofen with Cyclodextrin

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The carboxyl group of ibuprofen was conjugated to one of the hydroxyl groups of β-cyclodextrin using a coupling agent, carbonyl-diimidazole. The direct coupling produced ibuprofen appended cyclodextrin conjugate in which the drug is selectively introduced at one of the secondary hydroxyl groups of cyclodextrin through an ester linkage. The aqueous solubility (0.1 % w/v) was much higher than that of ibuprofen and no appreciable release of drug was observed in the stomach and small intestinal homogenates of rats. On the other hand fast disappearance of conjugate and appearance of the drug was observed in the cecal and colon contents of rats. The present ibuprofen-cyclodextrin conjugate may be of the value as an orally administered colon specific polymeric prodrug and hence the direct effect of carboxyl group on the gastric mucosa can be avoided.

Key Words: Ibuprofen, β-Cyclodextrin, Colon specific, Polymer-prodrug.

INTRODUCTION

Recently, number of cyclodextrin (CyD) derivatives have been prepared for many purposes, such as the construction of enzyme models, chiral separators, or recognizing agents[1-3]. Among these purposes, application of drug-CyD conjugates to drug targeting is important. For example, CyD derivatives bearing small saccharide, such as glucose, galactose, mannose, fructose, *etc.*, work as carriers for transporting active drugs to sugar receptors such as lectins on cell surfaces[4-7]. Cyclodextrins are cyclic oligo saccharides consisted of 6-8 glucose units through α-1,4-glycosidic bonds and have been utilized for improvement of certain properties of drugs, such as solubility, stability, bioavailability, *etc.*, by formation of inclusion complexes. Cyclodextrins are barely known to be hydrolyzed and only slightly absorbed in passage through the stomach and small intestine. Most bacteroids isolated from the human colon are capable of degrading CyDs, as evidenced by their ability to grow on CyDs using them as sole carbon source and by the stimulation of cyclodextranase activity by exposure to CyDs. This biodegradable property makes CyDs useful as a colon-targeting carrier. Thus, CyD prodrugs may serve as a source of site specific delivery of drugs to the colon[8].

Ibuprofen is a non-steroidal antiinflammatory drug (NSAID) and the major drawback of this drug, which limits the use is its gastric effects due to local irritation of gastric mucosa by free -COOH group of the drug[9]. In the present study attempt has been made to overcome the direct effect of -COOH group on the gastric mucosa by conjugating with the hydroxyl group of β-CyD. Furthermore, enzymatic degradation of the conjugate was investigated to gain insight into the hydrolysis mechanism in colonic contents.

EXPERIMENTAL

Melting points were taken in open capillaries in liquid paraffin bath and are uncorrected. IR spectra was recorded in Shimadzu Perkin-Elmer 8201 PC IR spectrometer using a thin film on potassium bromide pellets. The ^{1}H NMR spectra was recorded on Bruker Avance II 400 NMR spectrometer using $CDCl_3$. Chemical shift values are reported as values in ppm relative to TMS ($\delta = 0$) as internal standard. The FAB Mass spectra was recorded on Jeol SX-102/DA-6000 Mass spectrometer using Argon/Xenon (6Kv, 10Ma) as the FAB gas.

Preparation of CyD ester conjugates: To the solution of ibuprofen (1 mmol, 0.206 g) in 2 mL dimethyl formamide (DMF), 1 mmol (0.173 g) of CDI (carbonyldiimidazole) was added slowly in a stoppered flask and reacted for 1 h by stirring on a magnetic stirrer at room temperature. To the reaction mixture, 1 mmol (1.135 g) of β-CyD was added, followed by addition of triethyl amine (TEA, 0.8 mL) and stirred for 24 h at room temperature. The reaction was carried out in presence of nitrogen atmosphere and monitored by thin layer chromatography (TLC) using a normal phase plates precoated with silica gel 60 F_{254} obtained from Merck, Germany and an eluent of acetic acid/2-propanol/water (7:7:5 v/v). Large amount of acetone was added at the end of the reaction and filtered the precipitate[8]. Thus obtained precipitate was dried at room temperature for 24 h, followed by in oven at 50 ºC for 2 h.

Measurement of solubility: The screw capped vials containing the conjugate 20 mg in 10 mL of water were shaken at 25 ºC for 5 min. It was centrifuged at 2000 rpm for 5 min and the supernatant was analyzed by UV-visible double beam spectrophotometer for the conjugate at 273 nm[8].

Estimation of drug content of the conjugation: Though, there is no official method to estimate the drug content of cyclodextrin prodrugs, in the present study the hydrolysis was brought about in presence of rat cecal matter, as the chemical hydrolysis path way may not result in complete hydrolysis of conjugation. For the purpose of the complete hydrolysis of the prodrug, 5 mL solution of 0.05×10^{-3} M was prepared in isotonic phosphate buffer of pH 7.4 and incubated with the rat cecal matter preparation under nitrogen atmosphere for 48 h at 37 ºC. The rat cecal matter preparation was made by mixing the contents of rat cecal matter (2 % w/v) with chilled pH 7.4 isotonic phosphate buffer and then filtered through gauge to remove the visible particles. After centrifugation at 2000 rpm for 5 min the supernatant was analyzed for the free drug content at 264 nm after suitable dilution.

Hydrolysis in rat gastro intestinal tract contents or homogenates: Male Wistar rats, 150-200 g, were fed a standard diet. The rats were killed by decapitation. Stomach, small intestine, cecum and colon were removed and the contents were diluted by 20 % w/v with the following chilled isotonic buffers: (pH 4.4 acetate buffer), small intestine (pH 7.4 phosphate buffer) and cecum and colon (pH 6.8 phosphate buffer). The dispersions of the contents were filtered through gauge to remove the large visible particles. The conjugate solution (5 mL of 1 mg/mL strength in the corresponding isotonic buffers) was added to the filtrates (5 mL) in air tight containers. The pH of the contents were adjusted to 4.4 (stomach), 7.4 (small intestine) and 6.8 (cecum and colon) by the addition of 0.1 N NaOH and nitrogen gas was introduced into the containers and incubated at 37 ºC. At appropriate time intervals samples were withdrawn and equal amount of corresponding buffers were replaced. The withdrawn samples were diluted suitably and centrifuged. Supernatant liquid was subjected to spectral analysis for determining the release of free ibuprofen from conjugation by UV-visible spectrophotometer at wavelength of 264 nm[9].

Antiinflammatory activity: *In vivo* antiinflammatory activity was determined using carrageenan-induced paw oedema on rat, after obtaining the permission from Institutional Animal Ethics Committee (K.S. Hegde Medical Academy). Wistar rats of either sex weighing between 150-200 g were taken and divided into 3 groups comprising of six animals in each group. First group was given 1 % w/v solution of carboxymethyl cellulose (CMC). Second group was given ibuprofen suspension in CMC (20 mg/kg). Third group was given solution of conjugate (quantity equivalent to 20 mg/kg of free ibuprofen) in CMC. Oedema was induced by injecting subcutaneously 0.1 mL of 1 % carrageenan solution in subplantar region of right hind paw. The measurement of the hind paw volume was carried out using Plethysmometer (IITC 520, USA,) before any treatment (V_o) and at any interval (V_t) after the administration of the drugs. The percentage swelling inhibition were calculated using the equation

$$\text{Inhibition (\%)} = \{[(V_t\text{-}V_o)_{control} - (V_t\text{-}V^o)_{treated}]/(V_t\text{-}V_o)_{control}\} \times 100$$

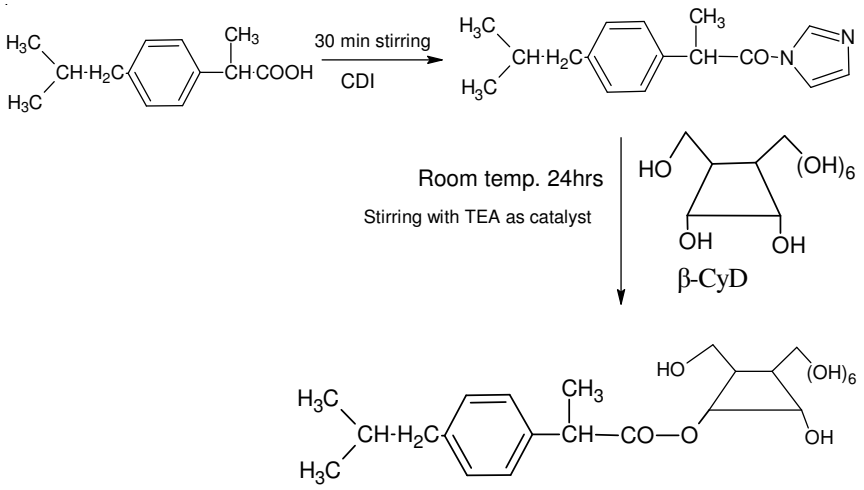
where V_o and V_t relates to the average volume in the hind paw of rats (n = 6) before any treatment and after antiinflammatory agent treatment, respectively[10]. The data was analyzed using student 't' test and the level of significance was determined at $p < 0.05$.

RESULTS AND DISCUSSION

From IR spectra, the carbonyl ester (C=O) peak was observed at 1737 cm^{-1}, 3327 cm^{-1} (-OH *str.*), 3122 cm^{-1}, 3033 cm^{-1} (aromatic -CH *str.*), 2929 cm^{-1}, 2850 cm^{-1}, 2790 cm^{-1}, 2663 cm^{-1} (aliphatic -CH *str.*), 1573 cm^{-1}, 1533 cm^{-1} (aromatic -CH *bend.*), 1434 cm^{-1}, 1463 cm^{-1} (aliphatic -CH *bend.*), 1344 cm^{-1}, 1311 cm^{-1} (aliphatic $-CH_3$ *bend.*), 1087 cm^{-1}, 1047 cm^{-1} (mono substitution). ^1H NMR spectra; δ 7.00-7.25 (d, 4H, ibuprofen phenyl), δ 5.6-5.8 (m, 14 H, CyD 2,3-OH), δ 4.7-4.9 (d, 7 H, CyD 1-H), δ 4.3-4.5 (t, 6H, CyD 6-OH), δ 3.5-3.7 (m, 30 H, CyD 3,5,6H), δ 3.25-

3.4 (m, CyD 2,4-H), δ 2.4-2.5 (d, 2H, ibuprofen CH_2), δ 1.6-2.0 (m, 3 H, ibuprofen CH_3). Mass spectral data indicated that the molecular ion peak at 1333 (M^+) and M+1 peak at 1334. Peak corresponding to 1135 is that of cyclodextrin and at 207 belonging to ibuprofen. Thin layer chromatography; R_f value: 0.64. UV-Visible λ_{max} 273 nm. Melting range: by open capillary in liquid paraffin bath method was found to be 265-267 ºC.

Preparation of CyD-ibuprofen was achieved as shown in **Scheme-I** of synthesis. Initially ibuprofen carboxyl group was activated by reacting with carbonyldiimidazole at room temperature and then conjugated with cyclodextrin in presence of triethylamine as catalyst. Finally acetone was added to precipitate the conjugation.



Conjugation of Ibuprofen with β-CyD (IBC)

Scheme-I

It was observed that the property of conjugate was found to be different from ibuprofen as the drug is insoluble in water. It is of interest to note that the conjugate was found to be soluble (0.1 % w/v, 1.0 mg/mL) in water. This result was in sharp contrast to the decreased solubility of β-CyD derivatives conjugated at the primary hydroxyl positions. For example, the aqueous solubility of biphenylylacetic acid and *n*-butyric acid conjugated at the primary hydroxyl position of β-CyD was approximately 1/10 that of the parent compound. The decreased solubility has been attributed to strong intermolecular association between the drug moieties and the neighboring β-CyD cavity, forming a stable columnar packing structure in the solid state[8]. It was found that the conjugate was more soluble than the drug ibuprofen. Further, it was found that 0.14 mg of drug present in 1 mg of conjugate from drug content study *i.e.* 140 µg/1000 µg of conjugation.

Hydrolysis in rat gastro intestinal tract contents or homogenates: The amount of free ibuprofen released from the conjugates over a period of time in presence of stomach, small intestine and colon and cecum were given in Table-1.

TABLE-1
HYDROLYSIS KINETICS IN RAT GASTRO INTESTINAL TRACT CONTENTS OR HOMOGENATES

Time (min)	Percentage hydrolysis of conjugate in intestinal segment		
	Stomach	Small intestine	Cecum and Colon
0.5	1.5	2.5	6.7
1.0	6.5	6.5	10.5
1.5	9.5	10.5	45.2
2.0	9.8	14.8	61.5
2.5	9.2	22.2	73.3
3.0	8.9	23.0	78.6
3.5	8.9	26.0	88.2
4.0	8.8	28.8	89.3
24.0	8.5	31.8	90.8

It can be observed that the drug ibuprofen in the contents of stomach and small intestine released marginally around 10 and 30 %, respectively, which could be accounted for spontaneous hydrolysis in aqueous solution in the stomach and presence of smaller number of organisms in the small intestine. On the other hand, in the cecal and colonic contents the conjugates released the drug significantly (*ca.* 90 %) within 12 h. The results indicate that the ibuprofen activation took place site-specifically in the rat cecal and colonic contents. This activation of drug from the conjugates may be attributed to the presence of glycosidase enzymes released from the bacteria present in the cecum and colonic area of rat.

Antiinflammatory activity determination: Antiinflammatory activity data is given in Table-2. It can be observed from the data, the conjugates exhibited maximum activity (% inhibition of oedema) after some time delay which may be due to the reason that the free drug was released only after its cleavage in the colon. However, the activity was maintained for longer period of time (81 % at the end of 24 h), where as ibuprofen suspension in CMC showed faster action, though, could not sustain for long period of time. The delayed (after 7 h) antiinflammatory activity is attributed to the inability of the stomach environment to cleave the conjugation, hence the drug action was observed only after some time delay.

TABLE-2
ANTIINFLAMMATORY ACTIVITY OF CONJUGATION OF IBUPROFEN

Compound	Percentage reduction of oedema	
	6 h	24 h
Control	NIL	NIL
Ibuprofen in CMC	44.45*	11.23*
Conjugation of ibuprofen in CMC	25.30*	81.25*

*$p < 0.05$.

Conclusion

Colonic specific drug delivery can be achieved with carriers by making prodrugs that survive passage through stomach and small intestine, but active moiety is released by enzymes specifically produced in colon. A well known demonstration of this concept is the delivery of 5-aminosalicylic acid by the use of azo linked prodrugs[9]. Further, Friend and Cheng[11] demonstrated the colon targeting of steroids by the use of glycoside prodrugs. The present study indicated that the ester type prodrug of ibuprofen with β-CyD released preferentially in cecal and colonic contents of rats after fermentation of CyD to smaller saccharides, suggesting that β-CyD can serve as a new class of site-specific drug carrier. Thus, the β-CyD conjugation approach can avoid the ulcerogenic effect of the drug in the stomach and may provide a versatile means for construction of colon specific delivery systems for drugs.

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