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Dextrans – potential polymeric drug carriers for flurbiprofen

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Dextrans have been used as carrier for flurbiprofen. Conjugates of flurbiprofen were synthesized by preparing their acylimidazol derivatives which were condensed *in situ* with dextrans of different molecular weight (40000, 60000, 110000 and 200000). The structures of the synthesized conjugates were confirmed by IR and NMR spectroscopy. The degrees of substitution were obtained between 8.0 to 9.5% and molecular weight was determined by Mark-Howin Sakurada viscosity equation. A hydrolysis study was performed in different buffer solutions (pH 1.2, 7.4, 9.0) and 80% human plasma (pH 7.4). The hydrolysis followed first order kinetics. Much faster hydrolysis was observed at pH 9.0 compared to buffer solution pH 7.4 and 80% human plasma (pH 7.4). The biological evaluation for acute and chronic anti-inflammatory activity was performed and the results were found to be comparable with the parent drug. The conjugates showed remarkable reduction in ulcerogenicity compared to parent flurbiprofen.

1. Introduction

Gastro-intestinal side effects constitute the most frequent of all adverse reactions of NSAIDs [1–4]. The conjugation of a drug with polymers can temporarily mask acidic function of NSAIDs and decrease gastro-intestinal toxicity due to direct contact effect. The literature reveals that in most of macromolecular or polymeric prodrug approaches, the drug is either linked by physical entrapment or by chemical linkage to polymeric carriers [5–7]. Dextrans can be used as promoiety due to their excellent physico-chemical properties and physiological acceptance [8, 9]. Flurbiprofen has a tendency of gastro-intestinal disturbance, peptic ulceration and GIT bleeding properties [10, 11].

The concept of polymeric prodrug has been adopted for preparation of dextran conjugates of flurbiprofen (FD) in order to improve its physico-chemical properties, colon site specificity and reduced gastrointestinal side effects.

2. Investigations, results and discussion

The NMR spectra of FD conjugates showed characteristic shifting of signals of anomeric proton from δ 4.91 (H, d, H-1) to δ 5.2 (H, S, H-1), H-2 proton from δ 3.42 (H, m, H-2) to δ 3.89 (H, S, H-2) which indicates the formation of an ester linkage at position C-2. The signals of the biphenyl aromatic ring of flurbiprofen were found at δ 7.27–7.52 (8 H, d, m) and are in agreement with anticipated structure.

The IR spectra of FD conjugates show characteristic stretching at 1720 cm^{-1} and confirm the formation of ester linkage. A strong O–H stretching vibration of polymeric association at 3400–3200 cm^{-1} and weak C–H stretching of alkane at 2970 cm^{-1} were also found. It also showed the characteristic absorption stretching at 1580–1510 cm^{-1} and 1010 cm^{-1} for biphenyl and C–F moieties, respectively.

The synthesized conjugates were found to be sparingly soluble in water and 0.1 N HCl but soluble in 0.1 N NaOH. An absorption maximum in phosphate buffer (pH 9.0) was observed at 247 nm which was same as that of flurbiprofen. The degree of substitution was determined by UV spectrophotometry [12, 13] and was found between 8.0 to 9.5. The average molecular weight was calculated by the Mark-Howink Sakurada equation [14] of viscosity method (Table 1). It is expressed as:

$$[\eta] = KM^{\alpha} \qquad (1)$$

where [η] intrinsic viscosity, M = molecular weight, K and α are constants.

Hydrolysis of FD conjugates was determined by HPLC. The hydrolyzed flurbiprofen was detected at 247 nm. The quantitation of hydrolyzed FD conjugates was done from measurement of the peak height in relation to those of flurbiprofen standard chromatographed under the same conditions.

Table 1: Dextran conjugates of flurbiprofen

Compd.	Yield (%)	Degree of substitution[a]	Intrinsic viscosity	Molecular weight	
				Calculated	Found
FD_1	88	9.5	0.022	42350	48400
FD_2	83	9.0	0.027	62226	72000
FD_3	85	8.5	0.036	112102	129600
FD_4	82	8.0	0.047	201976	218225

a = amount of parent drug in mg per 100 mg of conjugate

Table 2: Hydrolysis data of flurbiprofen dextran conjugates in phosphate buffer at 37 °C

Compd.	pH 9.00 buffer		pH 7.4 (80% human plasma)		pH 7.4 buffer	
	Average K (h^{-1})	$t_{1/2}$ (h)	Average K (h^{-1})	$t_{1/2}$ (h)	Average K (h^{-1})	$t_{1/2}$ (h)
FD_1	0.714	0.97	0.054	12.77	0.050	13.80
FD_2	0.648	1.06	0.044	15.71	0.039	17.65
FD_3	0.613	1.13	0.032	31.34	0.032	21.17
FD_4	0.493	1.40	0.028	24.62	0.030	22.50

k = rate constant
$t_{1/2}$ = half life is the average of 4 trails

FD conjugate did not show any hydrolysis in acidic medium (pH 1.2) for 4 h. The hydrolysis of FD conjugates at pH 7.4 demonstrated a slow rate of hydrolysis following first order kinetics. A relatively much faster hydrolysis was observed at pH 9.0 also following first order kinetics. The half lives were found to be 13.8, 17.6, 21.1, 22.5 (pH 7.4 buffer), 12.7, 15.7, 31.3, 24.6 (80% Human plasma pH 7.4) and 0.9760, 1.06864, 1.1301, 1.4031 h^{-1} (pH 9.0) for FD_1, FD_2, FD_3 and FD_4 respectively (Table 2).
The synthesized conjugates were subjected for biological evaluation such as anti-inflammatory (acute and chronic) and ulcerogenic activity. The ulcerogenic index was calculated by the expression described by Robert et al. [15]:

$$\text{Ulcerogenic index} = \text{number of ulcer} + \text{ulcer score} + \%\ \text{incidence/number of animals} \quad (2)$$

Acute and chronic anti-inflammatory activity was determined by the method of Winter et al. [16] and Mier et al. [17]. The percentage reduction in oedema at 4 h and percent anti-inflammatory activity in comparison to standard drug flurbiprofen are presented in Table 3. All synthesized FD conjugates show that their anti-inflammatory activity is comparable with the parent drug.
Sub-acute gastrointestinal toxicity studies were done by the method of Wilhemi et al. [18]. The ulcerogenic index is presented in Table 3. All the synthesized FD conjugates showed a remarkable reduction in the ulcerogenic index as compared to their parent drug. The parent drug flurbiprofen (20.6) has a high ulcerogenic index whereas the dextran-flurbiprofen prodrug FD_3 (dextran molecular weight 1,10,000) has an ulcerogenic index of 5.8 and others showed values below 10.0.
The results of the anti-inflammatory activity study suggest that dextran can successfully employed as promoiety/carrier for compounds containing a carboxylic function. The present investigation also suggests that dextran can be used as polymeric carrier to achieve colon site specificity due to the presence of enzymes and alkaline pH in the colon, improved physico-chemical properties and reduced gastro-intestinal side effects.

3. Experimental

3.1. General

^{1}H NMR spectra of synthesized dextran conjugates were recorded in DMSO-d6 on an BRUCKER DRX 300 MHz instrument using TMS as internal standard. Chemical shift values are reported in ppm downfield on δ scale. IR spectra were recorded on a Shimadzu 8300 FTIR IR spectrophotometer in KBr pellets. The degree of substitution was determined by a Shimadzu 160-A, UV spectrophotometer. Hydrolysis of FD conjugates was studied on a water HPLC system (Rexdale, Canada) consisting of a model 6000 A pump, a 710 B WISP auto injector, and a 490 multiple-wave length UV detector operated at ambient temperature. The column was of 10 cm stainless steel (4.6 mm id.) octadecyl-bonded silica (5 µm particil ODS-3, Whatman Inc. Clifton, N.J.) along with a 5 cm guard column of the same material with partical size 10 µm. The mobile phase consisted of acetonitrile : 0.67 M KH_2PO_4 : triethylamine (35 : 65 : 0.02 v/v) and the flow rate was 1 ml/min.

3.2. Degree of substitution

20 mg of FD conjugates were dissolved in 20 ml of phosphate buffer (pH 9.0). The reaction mixture was maintained at 70 °C for 1 h and left a side for 24 h for complete hydrolysis. It was then neutralized with 1 N HCl. The amount released on hydrolysis was extracted with chloroform. The amount of drug extracted in the chloroform layer was estimated by UV spectrophotometry.

3.3. Compounds

Synthetic grade chemicals were used N,N^1-carbonyl-dimidazol (CDI) (SIGMA) is moisture sensitive. Therefore dry solvents were used throughout and an anhydrous condition was maintained during the experiment.
Dextran conjugates of flurbiprofen were prepared by first activating the COOH functional group by using CDI to obtain acylimidazol (FAI) which then condensed with dextrans of different molecular weight (40,000, 60,000, 110,000 and 20,000) *in situ* [19–21] to get FD_1, FD_2, FD_3 and FD_4 respectively (Scheme). The progress of the reaction was monitored on TLC, which was performed on silica gel (Merck No 5554).

3.4. Antiinflammatory activity

Acute and chronic anti-inflammatory activy was determined by the method of Winter et al. [16] and Mier et al. [17] against carrageenan induced rat paw oedema and cotton pallet grannuloma respectively in albino rats (weighing 100–120 g). The oedema volume was measured by mercury displacement in a plethysmograph in acute anti-inflammatory activity determination whereas weighing of dried granulated palletes were measured in chronic anti-inflammatory activity determination.

3.5. Gastrointestinal toxicity

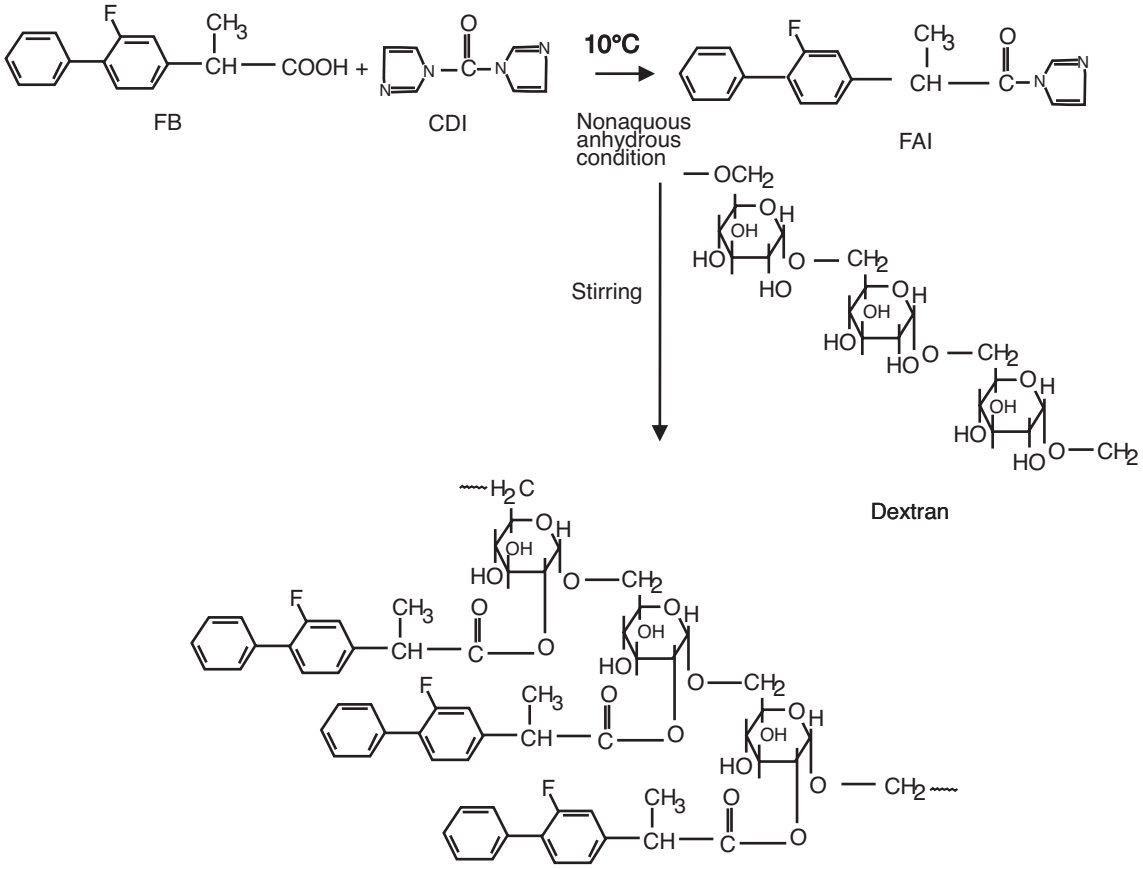
Sub-acute gastrointestinal toxicity studies were done by the method of Wilhemi et al. [18]. The animals were divided in groups with six animals in each group. To the control group was given only 0.5% CMC suspension. Compounds were administered orally once a day for 10 days. The animals were fasted for 8 h prior to dosing and for 4 h post dosing. Food was available at all other time, free access to water was provided throughout the experiment. 4 h after the last dose, the animals were sacrificed using chloroform. The abdomen was opened at the midline and the sto-

Table 3: Biological activity of flurbiprofen dextran conjugates (FD)

Treatment	Oral dose (mg/kg)	Acute antiinflammatory activity				Chronic antiinflammatory		Ulcerogenic index
		Percent increase in antiinflammatory activity				Wt. of granulation tissue (mg) mean ± S.E.	Percent antiinflammatory activity	
		1 h	2 h	3 h	4 h			
Control	–	–	–	–	–	38.55 ± 0.298	–	–
FB	2.00	37.17	41.74	48.20	47.10	19.58 ± 0.465	49.20	29.69
FD_1	21.05	36.29	38.27	45.83	43.11	22.62 ± 0.176	41.32	9.16
FD_2	22.20	34.28	38.70	44.44	42.58	21.71 ± 0.118	43.68	7.055
FD_3	23.52	35.23	40.86	46.39	44.51	20.77 ± 0.132	46.12	5.88
FD_4	25.00	33.33	37.39	40.27	40.00	23.16 ± 0.591	39.92	8.83

Number of rats in each group = 6
Statistical significance at $p < 0.05$ in relation to control

Scheme



Flurbiprofen-dextran conjugate (FD)

FB = Flurbiprofen; CDI = N-N[1]-Carbonyldiimidazole; FAI = Flurbiprofen acylimidazole; FD = Flurbiprofen-dextran conjugate

mach and the first 3 cm of the duodenum were removed. The stomach was opened along the larger curvature and washed with distilled water. The mucus was wiped off and the number of ulcers were examined by means of a magnifying glass. All ulcers were counted and recorded as average number of ulcers per animal and assessed as score [No ulcers (0.0), less than 2 ulcers (1.0), 2–5 ulcers (2.0), 5–10 ulcers (3.0) more than 10 ulcers (4.0)].

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