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Dextrans – potential polymeric drug carriers for suprofen

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Received January 14, 2003, accepted April 28, 2003

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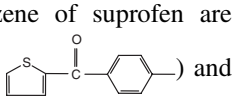
Pharmazie 58: 804–806 (2003)

Dextrans are clinically useful biodegradable polysaccharide macromolecules and have been utilized as carriers for suprofen. Conjugates of suprofen were synthesized by preparing their acylimidazol derivatives which were condensed *in situ* with dextrans of different molecular weights (40000, 60000, 110000 and 200000). The structures of the synthesized conjugates were confirmed by IR and NMR spectroscopy. The degrees of substitution obtained were between 7.5 and 9.0%. The molecular weight was determined by the Mark-Howin Sakurada viscosity equation. Hydrolysis was studied in different buffer solutions (pH 1.2, 7.4, 9.0) and 80% human plasma (pH 7.4) and followed first order kinetics. Much faster hydrolysis was observed at pH 9.0 compared to pH 7.4 buffer solution and 80% human plasma (pH 7.4). Biological evaluation for acute and chronic anti-inflammatory activity was performed and the results were found to be comparable with the parent drug. The ulcerogenic index of conjugates showed a remarkable reduction compared to the parent suprofen.

1. Introduction

Nonsteroidal anti-inflammatory drugs are among the most frequently used groups of drugs to treat are disorders due to inflammation. Gastro-intestinal side effects are the most frequent of all adverse reactions of NSAIDs [1–4]. Conjugation of the drugs with polymers can temporarily mask the acidic function of NSAIDs and decrease gastro-intestinal toxicity due to a direct contact effect. The literature reveals that in most macromolecular or polymeric prodrug approaches, the drug is either linked by physical entrapment or by chemical linkage to polymeric carriers [5–7]. Dextrans being biopolymers can be used as promoieties due to their excellent physicochemical properties and physiological acceptance [8–9]. Suprofen has a tendency to cause gastro-intestinal disturbance, peptic ulceration and GIT bleeding [10, 11]. The concept of a polymeric prodrug has been adopted to prepare dextran conjugates of suprofen with the expectation of improved physico-chemical properties, colon site specificity and reduced gastrointestinal side effects of suprofen.

2. Investigations and results

The NMR spectra of SD conjugates showed characteristic stretching of anomeric proton signals from δ 4.91 (H, d, H-1) to δ 5.17 (H, S, H-1), H-2 Proton from δ 3.42 (H, m, H-2) to δ 3.94 (H, S, H-2) indicates the formation of an ester linkage at position C-2. The signals of the thienyl carbonyl benzene of suprofen are found at δ 7.27–8.0 (7 H, s, s, d, s, ) and are in agreement with the anticipated structure.

The IR spectra of SD conjugates showed characteristic stretching at 1720 cm^{-1} confirming the formation of the 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 1025 cm^{-1} and 1350 cm^{-1} for the thienyl skeleton and C–S stretching. respectively.

The conjugates synthesized were found to be sparingly soluble in water and 0.1 N HCl but soluble in 0.1 N NaOH. Absorption maximum in phosphate buffer (pH 9.0) was observed at 296 nm, which was same as that of suprofen. The degree of substitution was determined by the UV spectrophotometry method [12, 13] and was found between 7.5 and 9.0%. This is the percentage of mg suprofen released per mg conjugate. The average molecular weight was calculated by the Mark-Howink Sakurada equation [14] of the viscosity method (Table 1). It is expressed as:

$$[\eta] = KM^{\alpha}$$

Where $[\eta]$ = intrinsic viscosity, M = molecular weight, K and α are consant.

Table 1: Physico-chemical parameters of dextran conjugates of suprofen

Compound code	Yield (%)	Degree of substitution[a]	Intrinsic viscosity	Molecular weight	
				Calculated	Found
SD_1	86	9.0	0.023	42343	52900
SD_2	84	8.4	0.026	62187	67600
SD_3	87	8.0	0.035	112082	122500
SD_4	80	7.5	0.046	20152	211600

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

Table 2: Hydrolysis data for dextran-suprofen conjugates in phosphate buffer at 37 °C

Compound Code	pH 9.00 buffer $t_{1/2}$(h)	pH 7.4 (80% Human plasma) $t_{1/2}$(h)	pH 7.4 buffer $t_{1/2}$(h)
SD_1	3.55	36.64	47.80
SD_2	3.60	45.23	49.14
SD_3	3.96	46.10	52.90
SD_4	3.96	50.80	52.50

$t_{1/2}$ = half life is the average of 4 trials.

Hydrolysis of SD conjugates was determined by an HPLC method. The hydrolyzed suprofen was detected at 296 nm. The quantitation of hydrolyzed SD conjugates was done from measurement of the peak height in relation to those of suprofen standard chromatographed under the same conditions.

SD conjugates did not show any hydrolysis in acidic medium (pH 1.2) for 4 h. The hydrolysis of SD conjugates at pH 7.4 demonstrated a slow rate of hydrolysis following first order kinetics and relatively much faster hydrolysis was observed at pH 9.0, also following first order kinetics. The half lives were found to be 47.8, 49.1, 52.9, 52.5 h. (pH 7.4 buffer), 36.6, 45.2, 46.1, 50.8 h. (80% human plasma pH 7.4) and 0.195, 0.193, 0.175, 0.174 h. (pH 9.0) for SD_1, SD_2, SD_3 and SD_4 respectively (Table 2).

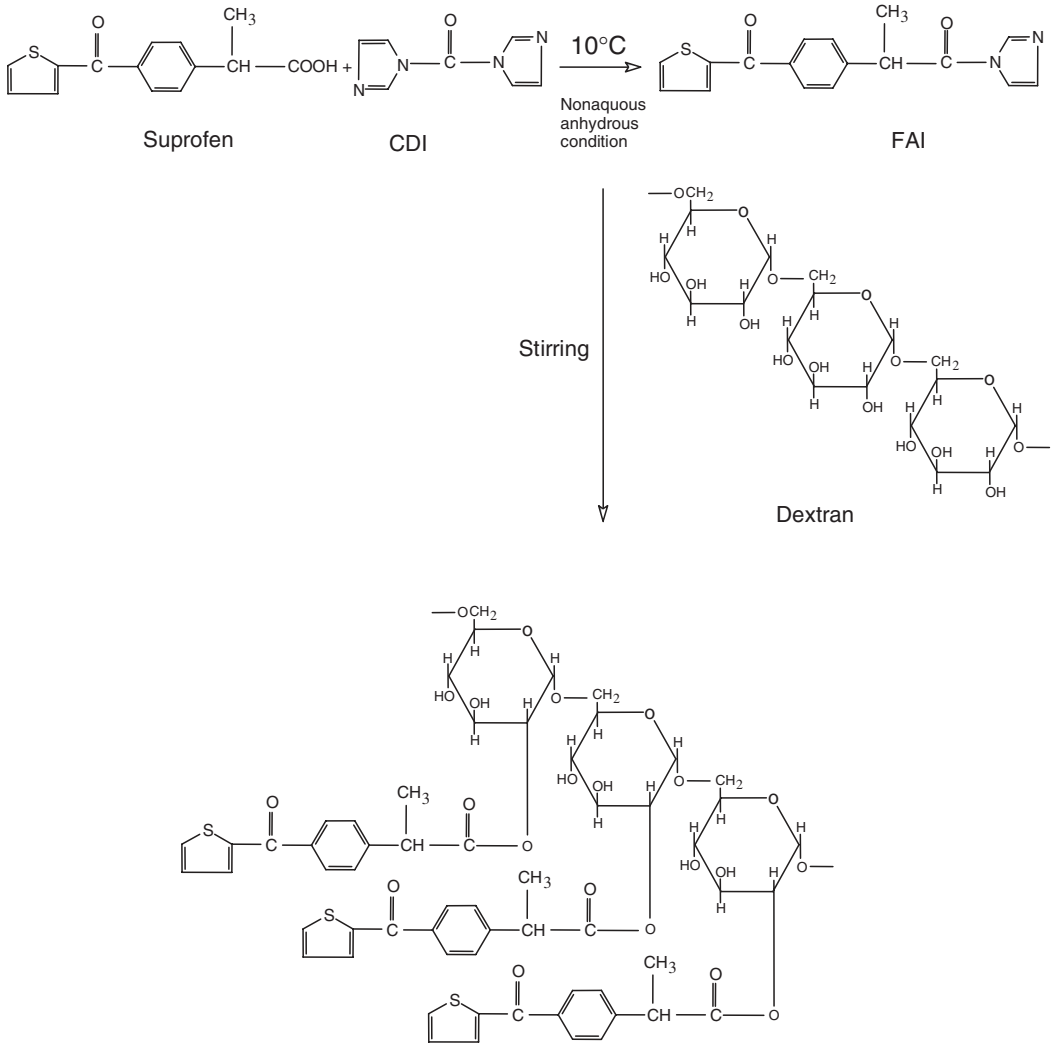
The synthesized conjugates were subjected to biological evaluation for anti-inflammatory (acute and chronic) and ulcerogenic activity. The ulcerogenic index was calculated by the equation described by Robert et al. [15].

Ulcerogenic index = number of ulcer + ulcer score + % incidence / number of animals.

The percentage reduction in oedema at 4 h and the percentage anti-inflammatory activity in comparison to standard suprofen are presented in Table 3. All the SD conjugates synthesised show anti-inflammatory activity comparable with that of the parent drug.

The ulcerogenic index is shown in Table 3. All the SD conjugates synthesized showed a remarkable reduction in the ulcerogenic index as compared to their parent drug suprofen. The ulcerogenic index indicates the parent drug

Scheme



CDI = N,N^1 Carbonyldiimidazole
SAI = Suprofen acylimidazole
SD = Suprofen-dextran conjugate

Table 3: Biological activity of dextran-suprofen conjugates (SD)

Gr.	Treatment	Oral Dose (mg/kg)	Acute anti-inflammatory activity				Chronic anti-inflammatory activity		Ulcerogenic index
			Percent increase in anti-inflammatory activity				Wt. of granulation tissue (mg) Mean ± S. E.	Percent anti-inflammatory activity	
			1 h	2 h	3 h	4 h			
1	Control	–	–	–	–	–	38.55 ± 0.298	–	–
2	Suprofen	5.00	31.00	37.39	40.97	38.70	23.00 ± 0.0375	40.33	31.00
3	SD_1	55.55	23.80	26.37	33.88	31.10	25.74 ± 0.452	33.22	9.83
4	SD_2	59.52	17.87	25.87	35.67	31.94	25.35 ± 0.648	34.22	9.90
5	SD_3	62.50	23.80	28.69	38.29	35.64	23.93 ± 0.355	37.92	6.055
6	SD_4	66.66	20.67	23.98	34.62	30.12	26.08 ± 0.197	32.33	9.16

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

Suprofen has a high ulcerogenic index (31.00) where as the Dextran-suprofen prodrug SD_3 (Dextran molecular weight 1,10,000) had an ulcerogenic index of 6.055 and the others were below 10.0.

The results of anti-inflammatory activity studies suggest that dextran can be successfully employed as a promoiety/carrier for compounds containing a carboxylic function. The present investigation also suggests that dextran can be used as a polymeric carrier to achieve colon site specificity due to the presence of enzymes and alkaline pH in the colon, improved physicochemical properties and reduced gastro-intestinal side effects.

3. Experimental

3.1. Synthesis of the compounds

Synthetic grade chemicals were used throughout the synthetic work. The N, N^1 carbony1 diimidazol (CDI) (Sigma) is moisture sensitive and therefore dry solvents were used throughout and anhydrous conditions were maintained during the experiments.

Dextran conjugates of suprofen were prepared by first activating the carboxylic group using CDI to obtain acylimidazol (SAI) which was then condensed with dextrans of different molecular weight (40000, 60000, 110000 and 20000) *in situ* (16–17) to get SD_1, SD_2, SD_3 and SD_4 respectively (Scheme). The progress of the reaction was monitored by TLC, which was performed on silica gel (Merck No 5554).

3.2. Equipment

^{1}H NMR spectra of synthesized dextran conjugates were recorded in DMSO-d6 on a Brucker DRX 300 MHz instrument using TMS as internal standard. Chemical shift values are reported in ppm downfield on the δ scale. IR spectra were recorded on a Shimadzu 8300 FTIR spectrophotometer in KBr pellets. The IR spectra of SD conjugates showed characteristic stretching at 1720 cm^{-1}, which confirms the formation of an ester linkage. Degree of substitution was determined with a Shimadzu 16O-A, UV spectrophotometer and hydrolysis of SD 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 10 cm stainless steel (4.6 mm. id.) octadicyl-bonded silica (5- µgm. Particil ODS-3; Whatman Inc. Clifton, N. J.) along with a 5 cm. guard column of the same material with Partical 10 µm. The mobile phase consisted of water (+1 ml phosphoric acid): acetonitrile (55 : 45) and flow rate was 1 ml/min.

3.3. Determination of degree of substitution

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

3.4. Anti-inflammatory activity

Acute and chronic anti-inflammatory activity were determined by the methods of Winter et al. [18] and Mier et al. [19] against carrageenan induced rat paw oedema and cotton pellet granuloma respectively in albino rats (weighing 100–120 gm). The oedema volume was measured by mercury displacement in a plethysmograph for acute anti-inflammatory activity determination whereas weights of dried granulated pellets were measured for chronic anti-inflammatory activity determination.

3.5. Gastrointestinal toxicity

Sub-acute gastrointestinal toxicity studies were done by the method of Wilhemi et al. [20]. The animals were divided in to six groups with six animals in each group. 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. Four hours after the last dose, the animals were sacrificed using chloroform. The abdomen was opened at the midline and the stomach 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 mean 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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