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Research Article

Synthesis and biological evaluation of amino acid conjugates of tolmetin

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Abstract

Synthesis and biological evaluation of amino acid conjugates of tolmetin was carried out to improve pharmacokinetic properties and to minimize undesirable side effect of ulcer genesis. Hydrolysis studies follow first order kinetics. The quantitative structure property relationship study reveals that the rate of hydrolysis of the compounds is inversely related to partition coefficient values. The study of acute and chronic anti-inflammatory and ulcerogenic activity gave statistically significant results and it concluded that the compounds minimize the gastric side effects of tolmetin remarkably.

Introduction

Tolmetin, one of non-steroidal anti-inflammatory drugs, could not be used as up to its potential because of its adverse reactions offered due to presence of free carboxylic acid group. The non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for indications extending from inflammation and pain to cardiovascular and genitourinary diseases. Gastrointestinal side effects constitute the most frequent of all the adverse reactions of NSAIDs and often these reactions lead to gastrointestinal ulceration and hemorrhage.[1] Gastrointestinal mucosal injury produced by NSAIDs is generally believed to be caused by two different mechanisms. The first mechanism involves a local action composed of a direct contact while the other has indirect effect on the gastrointestinal mucosa. The direct contact effect can be attributed to a combination of a local irritation produced by acidic group of NSAIDs and local inhibition of prostaglandin synthesis in the gastrointestinal tract. The indirect effect can be attributed to combination of an ion trapping mechanism of NSAIDs from the lumen into the mucosa. The second mechanism is based on a generalized systemic action occurring after absorption, which can be demonstrated following intravenous dosing.[2,3] Recently considerable attention has been focused in the development of bio-reversible derivatives, by temporarily masking the acidic group of NSAIDs, as a promising mean of reducing the gastrointestinal toxicity.[4]

The present work aims to synthesize amino acid conjugates of tolmetin using amino acid ester with the expectation to get nontoxic conjugates with minimized gastrointestinal disturbances while maintaining the useful anti-inflammatory and analgesic activities.

Experimental

Materials

Synthetic grade chemicals were used for synthesis of conjugates and analytical grade chemicals were used for analytical works.

Chemistry

Amino acid conjugates of tolmetin were synthesized by conversion of acidic group of drug to acid chloride

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using phosphorous pentachloride with continuous stirring at 40-45 ºC for 5 min. Alcoholic thionyl chloride and amino acid were refluxed for 6-14 h at 60-70 ºC with continuous stirring on magnetic stirrer to obtain methyl ester hydrochloride of amino acid. Acid chloride of drug and methyl ester hydrochloride of amino acid formed an amide bond between the drug and the amino acid with continuous stirring for 6 h in ice cooled basic solution (Figure 1).

Procedure of synthesis of conjugates

Ice cooled, 10% w/v potassium carbonate solution (150 ml) or pyridine 3 ml (to dissolve pyridine 20 ml benzene) was taken in 250 ml beaker and TGME (2.45 g) or TCME (2.1 g) or TTME (2.67 g) or TLME (2.25 g) or TPME (1.4 g) was added to it. The reaction mixture was stirred for 30 min at room temperature. The reaction temperature was then reduced and maintained at 30 ºC. Tolmetin acid chloride (5 g) was added, in small portions with continuous stirring. The reaction mixture was stirred for 4-6 h. The compound, so obtained was washed with 0.5% cold potassium carbonate and then recrystallized from methanol.

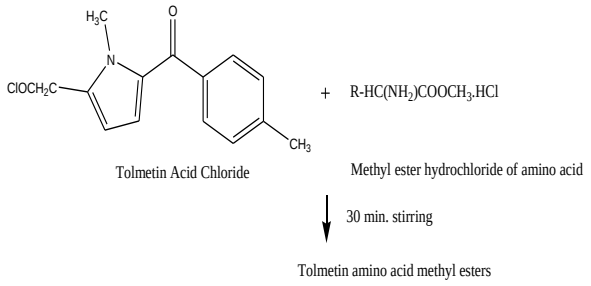

Figure 1. Scheme of synthesis of amino acid conjugates of tolmetin

Dissolution study

Dissolution studies were performed on USP XXI dissolution apparatus using simulated gastric fluid (50 ml of 0.2 M potassium chloride and hydrochloride at pH 1.2) and simulated intestinal fluid (pH 7.4). Conjugated derivatives remain as such in simulated gastric fluid up to 2 h but the synthesized conjugates attain a peak concentration after 60 min in simulated intestinal fluid. The hydrolysis studies were done in simulated intestinal fluid (pH 7.4) (USP 2003). The amount of drug released and the remaining amount of product was estimated quantitatively by reverse phase HLPC using RP5C-18 column and methanol: phosphate buffer (60:40) as mobile phase with flow rate of 0.8 ml/min.

Biological activity

Anti-inflammatory activity

The suspension of test compounds was prepared in distilled water using 2% gum acacia and in all cases, control received the same amount of gum acacia. The anti-inflammatory activity of synthesized compounds was determined by hind paw edema method utilizing carrageenan as phlogistic agent. Wistar rats (150-200 g) were divided into twelve groups of six rats each including control (vehicle treated), standard (drug treated), and test (drug conjugates treated) groups. At 0 h, the test conpounds were administered orally and after half an hour, carrageenan (0.05 ml, 1%) was injected into the planter tissue of paw. The paw edema was measured, before and at regular interval of 1 h, for three hours after the injection of carrageenan.[5,6]

Anti-inflammatory activity = 1-(Vt – Vc) × 100

where Vc = Mean relative change of the thickness of the right hind paw of rats in the control group; Vt = Mean relative change of the thickness of the right hind paw of rats in the test group.

Cotton pellet implantation

Surgical cotton pellet of 100 mg were sterilized in an autoclave at 15 lb pressure at 121 ºC for 30 minutes. The sterilized pellets were aseptically implanted in both the groins of each rat. Drugs were given once in seven days. On eighth day, rats were sacrificed and the pellets were taken out and dried overnight at 60 ºC and weighed. The percent anti inflammatory activity was calculated by the formula

Anti-inflammatory activity = 1-(Vt – Vc) × 100

Vt= mean weight of granulation tissue in drug treated group; Vc= mean weight of granulation tissue in controlled group.

Ulcerogenic index

Gastrointestinal toxicity of the synthesized conjugates was measured and compared with the drug by measuring ulcer index. Wistar rats (150-200 g) were divided into twelve groups of six rats each including control (vehicle), standard (drug treated) and test (drug conjugates treated) groups. The drug/conjugate was administered orally in 1% suspension of sodium carboxymethylcellulose to the overnight fasted rats. Eight hours after treatment, rats were sacrificed by decapitation and the stomach was extracted and dipped in 1% formaldehyde solution for about 15 min and then cut out along its great curvature. The lesions on the gastric mucosa were counted by visual examination using a microscope. A score for the ulcer was made as no ulcer (0.0), spot ulcer (1.0), deep ulcers (2.0) and perforation (3.0). The overall total of scores was designated as the 'ulcer index' (UI).[7,8]

The UI was calculated as: UI = [1 × (number of lesions of score 1) + 2 × (number of lesions of score 2) + 3 × (number of lesions of score 3)]/10

Results and Discussion

Conjugates of tolmetin with amino acid were synthesized and the yield was found between 60-75%. The physicochemical properties were calculated (Table 1). The structure of the synthesized conjugates were confirmed by IR and mass spectroscopy. The result of molecular weight determination coincided with the calculated values of the conjugates. The IR spectra obtained from Perkin Elmer spectrophotometer using KBr pellets showed peaks of amino acid conjugates. Jeol mass spectrometer used for molecular weight confirmation of the synthesized conjugates. Partition coefficient of tolmetin and its conjugates of amino acid was determined in octanol/simulated intestinal fluid system. The values of half life and hydrolysis constant of the synthesized conjugates are given in Table 1. The biological evaluation including anti-inflammatory and anti-ulcerogenic activities have been shown in table 2 and 3.

Table 1. Physical constants & physicochemical properties of synthesized conjugates.

Conjugate code	Molecular formula	Molecular weight	M.P (ºC)*	Partition coefficient	Hydrolysis constant in phosphate buffer (pH 7.4)	$T_{1/2}$
Control	$C_{15}H_{15}NO_3$	257	192	2.64	-	-
TOL_1	$C_{18}H_{20}N_2O_4$	328	140	1.66	0.016	52.51
TOL_2	$C_{19}H_{22}N_2O_4S$	374	90	0.24	0.019	41.25
TOL_3	$C_{27}H_{27}N_3O_4$	457	96	1.26	0.038	33.43
TOL_4	$C_{22}H_{29}N_3O_4$	399	140	2.32	0.018	42.82
TOL_5	$C_{25}H_{26}N_2O_4$	418	170	1.08	0.024	31.25

*Melting point uncorrected, TOL_1- Tolmetin Glycine methyl ester, TOL_2- Tolmetin Cysteine methyl ester, TOL_3- Tolmetin Tryptophan methyl ester, TOL_4- Tolmetin Lysine methyl ester, TOL_5- Tolmetin Phenylalanine methyl ester.

Table 2. Anti inflammatory activity (chronic and acute) of amino acid conjugates of tolmetin.

Conjugate code	Oral dose (mg/kg)	Percent anti inflammatory activity (chronic)	Oral dose (mg/kg)	Percent anti inflammatory activity (Acute)			
				1 h	2h	3h	4h
Control	20	63.52	20	40.22	58.38	69.31	66.45
TOL_1	20	46.52	20	39.34	36.41	35.23	31.16
TOL_2	20	50.58	20	38.21	49.84	66.87	64.71
TOL_3	20	66.34	20	36.73	52.89	68.36	67.41
TOL_4	20	33.76	20	44.12	45.41	43.64	40.27
TOL_5	20	41.87	20	58.31	57.96	55.38	53.31

TOL_1- Tolmetin Glycine methyl ester, TOL_2- Tolmetin Cysteine methyl ester, TOL_3- Tolmetin Tryptophan methyl ester, TOL_4- Tolmetin Lysine methyl ester, TOL_5- Tolmetin Phenylalanine methyl ester.

Table 3. Ulcerogenic index of amino acid conjugates of tolmetin.

Conjugate code	Dose (mg/kg)	Ulcer index
Control	20	28.46
TOL_1	20	7.274
TOL_2	20	12.36
TOL_3	20	9.33
TOL_4	20	6.52
TOL_5	20	10.22

TOL_1- Tolmetin Glycine methyl ester, TOL_2- Tolmetin Cysteine methyl ester, TOL_3- Tolmetin Tryptophan methyl ester, TOL_4- Tolmetin Lysine methyl ester, TOL_5- Tolmetin Phenylalanine methyl ester.

The results from analytical methods provide the structural conformity of the amino acid conjugates of tolmetin. NH stretching of amides (3406-3426 cm^{-1}) and C(=O) NH carbonyl (1560-1550 cm^{-1}) stretching was observed in all conjugates. An additional stretching of SH was observed in tolmetin cysteine methyl ester because of cysteine (2560 cm^{-1}).

The parent peak (M^+) in tolmetin was observed at 257. The m/z of 328, 374, 457, 399 and 418 gives information about the molecular weight of amino acid conjugates of tolmetin. The partition coefficient of tolmetin and its amino acid conjugates was determined in octanol/simulated intestinal fluid (pH 7.4) and the value of partition coefficient of amino acid conjugates of tolmetin was found to be less than that of tolmetin indicating the ionization of the conjugates in intestine thus reducing the gastric irritation and ulcer formation in the stomach. Amino acid conjugates of tolmetin showed reduced ulcerogenic index than that of tolmetin with an ulcer index of 28.46. TOL_1 and TOL_4 have lower ulcer index comparable with the other conjugates suggesting that

the amino acid conjugates have the anti inflammatory activity along with less ulcer causing ability. The amino acid conjugation reduces the partition coefficient thus dissociation takes place in intestine resulting into reduced ulcer formation.

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