Journal of Enzyme Inhibition and Medicinal Chemistry ›
Volume 26, 2011 - Issue 5

Free access

Research Article

Synthesis, hydrolysis studies and phamacodynamic profiles of amide prodrugs of dexibuprofen with amino acids

Arun Rasheed, C. K. Ashok Kumar & Ashutosh Mishra

Pages 688-695 | Received 28 Mar 2010, Accepted 09 Dec 2010, Published online: 21 Jan 2011

Cite this article https://doi.org/10.3109/14756366.2010.548327

Abstract

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Keywords:: NSAIDs dexibuprofen amide prodrugs pharmacokinetics ulcerogenicity

Introduction

Dexibuprofen (Dex), a novel non-steroidal anti-inflammatory drug (NSAID), is a single and pharmacologically effective enantiomer of racemic ibuprofen. Racibuprofen and dexibuprofen differ in their physicochemical properties, pharmacological profiles and metabolic activities[1]. The efficacy of dexibuprofen was found to be same as that of other NSAIDs, such as diclofenac, naproxen and celecoxib. Generally, the NSAIDs are associated with various gastrointestinal (GI) side effects like stomach ulceration, bleeding and perforation due to the presence of its acidic group. The GI toxicity is produced either by direct contact mechanism or by generalized systemic action, which occur after absorption. To overcome the GI side effects, prodrugs of NSAIDs can be developed by hiding its acidic group through conjugation with amino acids. Many amino acids possess site specificity, marked anti-inflammatory activity and exhibit profound healing effect on gastric toxicity[2]. During conjugation, the NSAIDs having free carboxylic group can be derivitized into corresponding amides of amino acid, and it results in altering the physical properties of parent drug with one or more of hydrolase enzymes serving as the in vivo reconversion sites. The new prodrugs were expected to have better lipophilicity, reduced gastric irritancy, improved therapeutic index through the prevention of GI irritation and bleeding, improved anti-inflammatory activity, reduced G nt drug[3,4].

The synt buprofen, etc., wit n ethyl ester, methyl e y so far. The objective methyl ester , Dex-2, Dex-3 various physicoc ies. The compou administ matic or non-enz

Materials and methods

Materials

The amino acids L-tryptophan, L-phenylalanine, glycine and L-tyrosine were obtained from M/s Hi-Media Ltd., India, and drug dexibuprofen was obtained as a gift sample from Alkem Laboratories, India. Other reagents and solvents were of analytical grade. The melting points were recorded using melting point determination apparatus by Sigma Instrument, India, and are uncorrected. The elemental analysis was performed using Carlo-Erba Model 1108 Analyzer (Italy). ^{1}H NMR and ^{13}C NMR spectra were recorded in diemthylsulphoxide (DMSO) on NMR spectrophotometer (Bruker DRX 300, USA). Chemical shifts are expressed as δ (ppm) values. IR spectra were recorded using IR spectrophotometer (Shimadzu FTIR-8201PC (Kyoto, Japan)) in KBr phase and mass spectra were recorded on mass spectrophotometer (Jeol SX-102 (FAB), Japan). The hydrolysis data and drug content determination were performed by ELICO Double Beam UV-VIS Spectrophotometers (Hyderabad, India).

Synthesis of amide prodrugs of dexibuprofen

Dexibuprofen is 2-[4-(2-methyl propyl) phenyl] propanoic acid and the synthesis of its amide prodrugs was carried out by Schotten Baumann technique[8] as explained below. The purity of the compounds was checked by thin-layer chromatography on pre-coated silica GF_{252} plates using iodine vapour as a detecting agent. The structures of all the synthesized prodrugs were confirmed by elemental analysis and spectral analysis such as IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy.

Step 1:

Dex (1) c roform and freshly c The mixture was refl viscous liqui ellow colour

Step 2: n, L-phenyla

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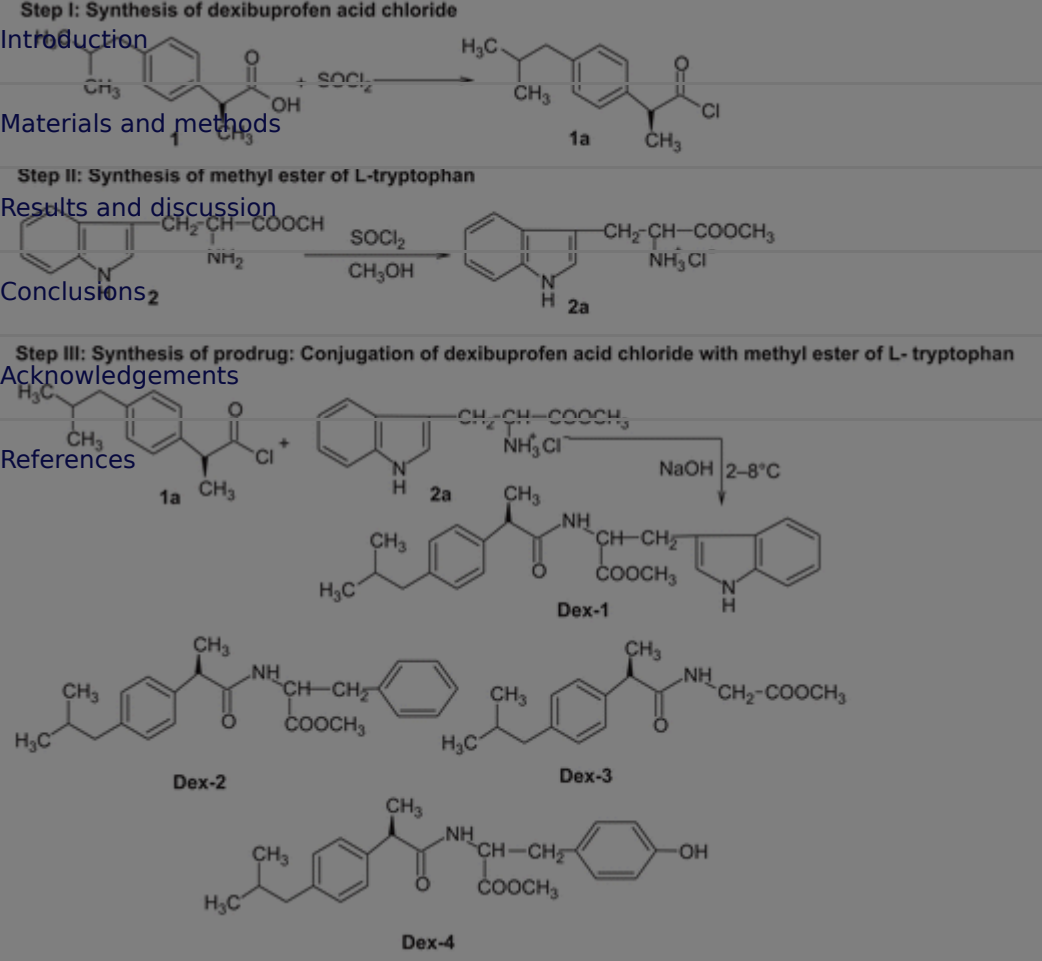
mixture was refluxed for 6–8 h at 60–70°C with continuous stirring on magnetic stirrer. Excess thionyl chloride and solvent was removed under reduced pressure giving crude tryptophan methyl ester hydrochloride. It was treated with 20 mL portion of cold ether at 0°C. The resulting solid product was collected and dried under vacuum. It was recrystallized from hot methanol by slow addition of 15–20 mL ether followed by cooling at 0°C. The crystals were collected next day and washed twice with ether: methanol mixture at 5:1 ratio followed by pure ether and dried under vacuum to give pure tryptophan methyl ester hydrochloride (2a). The same procedure was followed to synthesize phenylalanine methyl ester hydrochloride, glycine methyl ester hydrochloride and tyrosine methyl ester hydrochloride.

Step 3: Synthesis of prodrugs of dexibuprofen with methyl esters of L-tryptophan, L-phenylalanine, glycine and L-tyrosine

Ice cold, aqueous sodium hydroxide solution (5 %) was taken in 250 mL beaker and 12.6 g (0.05 mol L^{-1}) of methyl ester of tryptophan hydrochloride was added to it. The reaction mixture was mechanically stirred for 30 min at room temperature, after which the beaker was transferred to an ice bath kept on the mechanical stirrer, maintaining the temperature at 10°C. To this, 22.3 g (0.01 mol L^{-1}) of dex acid chloride was added in small portions with continuous stirring for 7–8 h. The solid that separated out was filtered off. The crude prodrug was recrystallized from methanol. The same procedure was followed for L-phenylalanine, glycine and L-tyrosine. The schematic representation of synthesis of tryptophan-conjugated dexibuprofen (Dex 1), phenylalanine-conjugated dexibuprofen (Dex 2), glycine-conjugated dexibuprofen (Dex 3) and tyrosine-conjugated dexibupr

Scheme



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Spectral analysis

The spectral data obtained for Dex 1, Dex 2, Dex 3 and Dex 4 are given as follows.

Dex 1: S(+) Methyl 3-(1H-indol-3-yl)-2-(2-(4-isobutylphenyl)propanamido)propanoate—UV Spectra (λ_{max}) in simulated gastric fluid (SGF) 205 nm, in simulated intestinal fluid (SIF) 225 nm, in 80% human plasma 218 nm; IR (KBr, cm^{-1}) 3290 (NH str. of amide), 3075 (ar .of ester); ^1H NMR (δ , CH), 3.72 (d, J = 7. , 9.77 (s, 1H, NH), 2, 38.2, 43.5, 47 .3, 128.2, 129.

Dex 2: S te—UV Spectra ; IR (KBr, cm^{-1}) 3 O str. of ester), 1 (d, J = 7.1

4H, Ar. H), 7.32 (m, 4H, Ar. ring), 9.77 (s,1H, NH); ^{13}C NMR (δ ppm) (DMSO-d_6) 17.3, 17.3, 18.3, 35.2, 35.2, 42.3, 47.3, 47.8, 51.9, 119.2, 120.2, 125.2, 125.3, 125.3, 126.2, 126.6, 127.2, 127.3, 127.5, 128.3, 128.4, 171.3, 172.9; Mass (m/z) 367 (M+).

Dex 3: S(+) Methyl-2-(2-(4-isobutyl phenyl)propanamido)acetate—UV Spectra (λ_{max}) in SGF 212 nm, in SIF 219 nm, in 80% human plasma 222 nm; IR (KBr, cm^{-1}) 3280 (NH str. of amide), 3043 (aromatic CH str.), 2932 (C=O str. of ester), 1630 (C=N), 1290 (CO str.of ester); ^{1}H NMR (δ ppm) (DMSO-d_6) 1.38 (d, J = 6.7 Hz, 6H, CH_3), 2.42 (d, J = 7.3 Hz, 2H, CH_2), 3.80 (q, J = 7.1 Hz, 1H, CH), 3.82 (q, J = 7.1 Hz, 1H, CH), 7.48 (d, J = 8 Hz, 4H, Ar.), 9.68 (s, 1H, NH); ^{13}C NMR (δ ppm) (DMSO-d_6) 17.3, 17.3, 17.8, 20.2, 35.2, 42.3, 47.8, 51.9, 63.2, 120, 120.8, 128.6, 128.9, 139.6, 172.9; Mass (m/z) 277 (M+).

Dex 4: S(+) Methyl 3-(4-hydroxyphenyl)-2-(2-(4-isobutylphenyl)propanamido) propanoate—UV Spectra (λ_{max}) in SGF 208 nm, in SIF 221 nm, in 80 % human plasma 218 nm; IR (KBr, cm^{-1}) 3362 (NH str. of amide), 3046 (aromatic CH str.), 1745 (C=O str. of ester), 1532 (C=N), 1280 (CO str.of ester); ^{1}H NMR (δ ppm) (DMSO-d_6) 1.28 (d, J = 6.7 Hz, 6H, CH_3), 3.62 (q, J = 7.1 Hz, 1H, CH), 7.28 (d, J = 8 Hz, 4H, Ar. ring), 7.63 (d, J = 8 Hz, 4H, Ar. ring), 9.68 (s, 1H, NH); ^{13}C NMR (δ ppm) (DMSO-d_6) 17.8, 18.2, 18.3, 36.2, 47.3, 48.2, 48.3, 51.9, 120, 120.2, 120.6, 122, 122.2, 125, 125.6, 126, 126.2, 128.2, 128.4, 135.2, 171.2, 172.1; Mass (m/z) 383 (M+).

Physicochemical characterization of the synthesized prodrugs

Solubility

Approxi ± 1°C in
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7.4). It was tied at the opening end of dialysis tube; the dialysis tube containing (6%) egg albumin was dipped into the drug solution and covered. The whole assembly was placed on a magnetic stirrer and switched at low revolutions per minute. The temperature was maintained at 37 ± 0.5°C. After every 1 h, 1 mL of the PBS containing drug solution was replaced with fresh 1 mL of PBS. Withdrawn sample was diluted further with 1 mL phosphate buffer and the concentration of prodrug was estimated using spectrophotometer at 223 nm. Table 1 indicates the physicochemical properties of the synthesized prodrugs.

Table 1. Physicochemical properties of prodrugs.

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Hydrolysis rate determination in SGF and SIF[9]

In vitro hydrolysis studies of synthesized prodrugs were carried out in SGF at pH 1.2 (USP 1970) and SIF at pH 7.4 (USP 1970). A solution of 10 mg of prodrug was prepared in 90 mL of SIF (pH 7.4) or SGF (pH 1.2). An aliquot of 15 mL of this solution was withdrawn repeatedly and kept in test tubes maintained at 37 ± 0.5°C. At a definite interval of time (0.5 h, 1–8 h), an aliquot was withdrawn from different test tubes and was transferred to micro-centrifuge tubes followed by addition of methanol to make up the volume. The tubes were placed in freezing mixture in order to arrest further hydrolysis, followed by vortexing at high speed for 5 min. After vortexing, the tubes were ce ant obtained from eac free Dex released etics of hydrolys and order of reaction calculated using

r = (2.30

where r oncentration of prodru t of prodrug remainin

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A solution of 10 mg of prodrug was prepared in methanol (2 mL) and was added to 88 mL of 80% human plasma (pH 7.4 prepared by mixing 80 portion of plasma and 20 portion of phosphate buffer at pH 7.4). An aliquot of 15 mL of this solution was withdrawn and kept in test tubes maintained at 37 ± 0.5°C. At definite interval of time (0–8 h), an aliquot of 22 mL was withdrawn and mixed with 0.5 % $ZnSO_4$ solution. Samples were centrifuged at 6000g for 10 min and a clear supernatant solution was analyzed spectrophotometrically at 223 nm.

Hydrolysis rate determination in rat faecal matter[11]

The prodrug was dissolved in phosphate buffer so that final concentration of the solution was 250 µg/mL. Fresh faecal material of rats were weighed (about 1 g) and placed in different sets of test tubes. To each test tube, 2 mL of the prodrug solution was added and diluted to 5 mL with phosphate buffer. The sets of test tubes were incubated at 37 ± 0.5°C for different intervals of time (0–8 h). For analysis, the free drug was extracted with 5 mL of methanol and estimated on UV spectrophotometer at 210 nm.

Pharmacological evaluations

Dex and the synthesized prodrugs were evaluated for anti-inflammatory activity, analgesic activity, ulcerogenicity and histopathology, and a comparative study was performed. Test compounds and standard drugs were administered in the form of a suspension by oral route of administration for anti-inflammatory and analgesic studies (1% carboxymethylcellulose as a vehicle), as well as for ulcerogenicity studies (2% acacia a… a standard group, e… sed in acrylic c… midity of 45–55%, in … ith standard rodent d… eek before exper… elines of the Com… val of the Institutio… nacy, Tirupati,…

Anti-in…

The anti-inflammatory activity was determined by hind paw oedema method[12] using carrageenan as phlogistic agent. Wistar rats (100–200 g) were divided into six groups, each comprising of six rats, including a control and a standard group. The initial volume of right hind paw of rat was measured by plethysmometer without administration of the prodrug. A 1% sodium carboxymethylcellulose suspension containing a dose equivalent to the 2.4 mg or 12 mg kg^{-1} body weight of Dex was administered orally. An equivalent quantity of prodrugs 4 mg of Dex-1, 4.36 mg of Dex-2, 3.52 mg of Dex-3 and 4.61 mg of Dex-4 were administered to the test groups. After 30 min of administration of the drugs, carrageenan (0.1 mL, 1% m/v) solution in normal saline was injected into the planter surface of right hind paw of each animal. The volume of right hind paw of albino rat was measured after 2, 4 and 6 h. The mean difference in the volume of the right hind paw of rats was compared with control and standard. The percent inhibition of paw oedema was calculated as

$$\text{Percent inhibition} = (1 - V_t/V_c) \times 100$$

where V_c is the mean relative change in paw edema volume in control group and V_t is the mean relative change in paw edema volume in test group.

Analgesic activity

The analgesic activity was determined by thermal stimulus using tail flick method[13]. Analgesiometer was used for the determination of pain threshold of albino mice. Cold water was circulated through the water jackets of the instrument to avoid heating of the area around the hot wire. The rat (100–200 g) was placed in a holder through which the tail c , 3 and 4 h after the the time taken to ejected. The drug (do d orally in 1% susp f prodrugs 4 mg of adm ted by the formula

% Analg

where T e reaction

Ulcerogenicity

Ulcerogenicity of the synthesized prodrugs was measured and compared with the parent drug by measuring mean ulcer index (UI)[14]. The control group was administered orally in 2% acacia suspension. Test compounds and standard were administered orally (at 10 times higher dose) as a suspension in 2% acacia daily for 5 days. The rats (130–150 g) were fasted after the administration of last dose, thereafter they were killed by decapitation and the stomach was removed, opened and washed with distilled water. The lesions on the gastric mucosa were counted by visual examination using a binocular magnifier. Ulcers greater than 0.5 mm were recorded. The mean UI was calculated by severity of gastric mucosal lesions that are graded as (i) grade 1: >1 mm erosions (ii) grade 2: 1–2 mm erosions and (iii) grade 3: <2 mm erosions.

The UI was calculated as:

$$UI = [1 \times (\text{number of lesions of grade 1}) + 2 \times (\text{number of lesions of grade 2}) + 3 \times (\text{number of lesions of grade 3})]/10.$$

Histopathological studies[15]

The histopathological studies of stomach of rats were carried out using haemotoxylin and eosin stain at Pathology Department, Sri Venkateswara Veterinary University, Tirupati, India. The stomach tissues were removed from the rats and fixed in 10% normal saline for at least 48 h. These were then processed routinely and the tissues were embedded in paraffin wax. Histological sections were cut at 5–6 µm and stained with rou ultant histopat osal atrophy, cytes and plasma cations were tak solution of 10 × 45×

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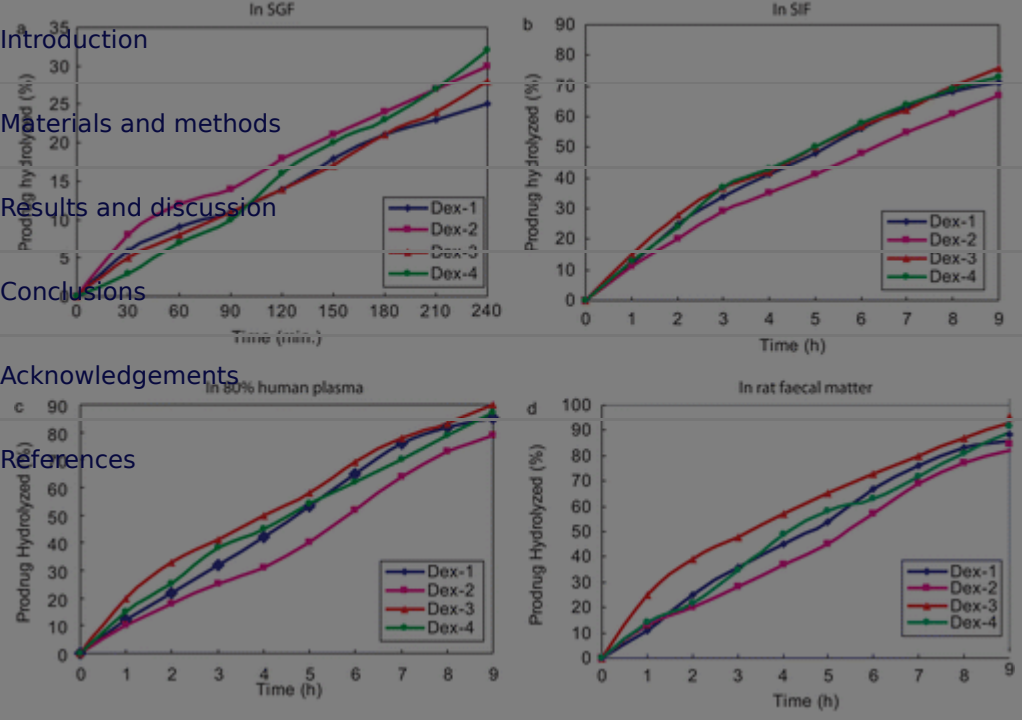
Results and discussion

Synthesis and characterization

This study describes the synthesis of amide prodrugs of Dex as Dex-1 {S(+) methyl-3-(1H-indol-3-yl)-2-(2-(4-isobutylphenyl)propanamido)propanoate}, Dex-2 {S(+) methyl 2-(2-(4-isobutylphenyl)propanamido)-3-phenylpropanoate}, Dex-3 {S(+) methyl-2-(2-(4-isobutyl phenyl)propanamido)acetate} and Dex-4 {S(+) methyl 3-(4-hydroxy phenyl)-2-(2-(4-isobutyl phenyl)propanamido)propanoate}as portrayed in Scheme 1. The prodrugs were subjected to solubility, physicochemical characterization, protein binding and hydrolytic studies. All the prodrugs were observed to be highly soluble in organic solvents, sparingly soluble in alkaline solution and insoluble in acidic solutions. The high solubility of the prodrugs in organic solvents indicates their lipophillic behaviour. The physicochemical properties of synthesized prodrugs were determined, as well as the IR, ^{1}H NMR, ^{13}C NMR and mass spectral analyses were carried out. The results of elemental analysis of synthesized prodrugs were in all case within ±0.4% of theoretical value and were in confirmation of desired structure.

In vitro hydrolysis of Dex-1, Dex-2, Dex-3 and Dex-4 were carried out in SGF (pH 1.2), SIF (pH 7.4), 80% human plasma (pH 7.4) and rat faecal matter (pH 7.4) and their respective half life ($t_{1/2}$) values in SIF were observed as 4.5, 4.8, 4.5 and 4.6 h, respectively. The comparative hydrolysis patterns of the synthesized prodrugs are depicted in Figure 1. None of the prodrugs showed significant hydrolysis in SGF. The amount [illegible] in SIF was found as [illegible] spectively, in 80% h[illegible]natter. The hydrolys[illegible]y encoura[illegible] prodrugs followed[illegible]2, Dex-3 and Dex-[illegible]rugs showed[illegible] be 85%. This incr[illegible]equired dose wil[illegible]

Figure 1.



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Pharmacological studies

Anti-inflammatory activity

Table 2 shows that after 6 h of administration of Dex, the percentage anti-inflammatory activity was found as 43.3%, whereas an improved value of 73.4, 77.3, 72.8 and 64.5% were observed for prodrugs Dex-1, Dex-2, Dex-3 and Dex-4, respectively. The maximum anti-inflammatory activity of prodrugs was observed at 6 h and remained practically constant upto 8 h. The anti-inflammatory activity of free Dex decreased with time, whereas that of its prodrugs increased with time due to its higher bioavailability compare ANOVA showed ve in compari a suitable promoie

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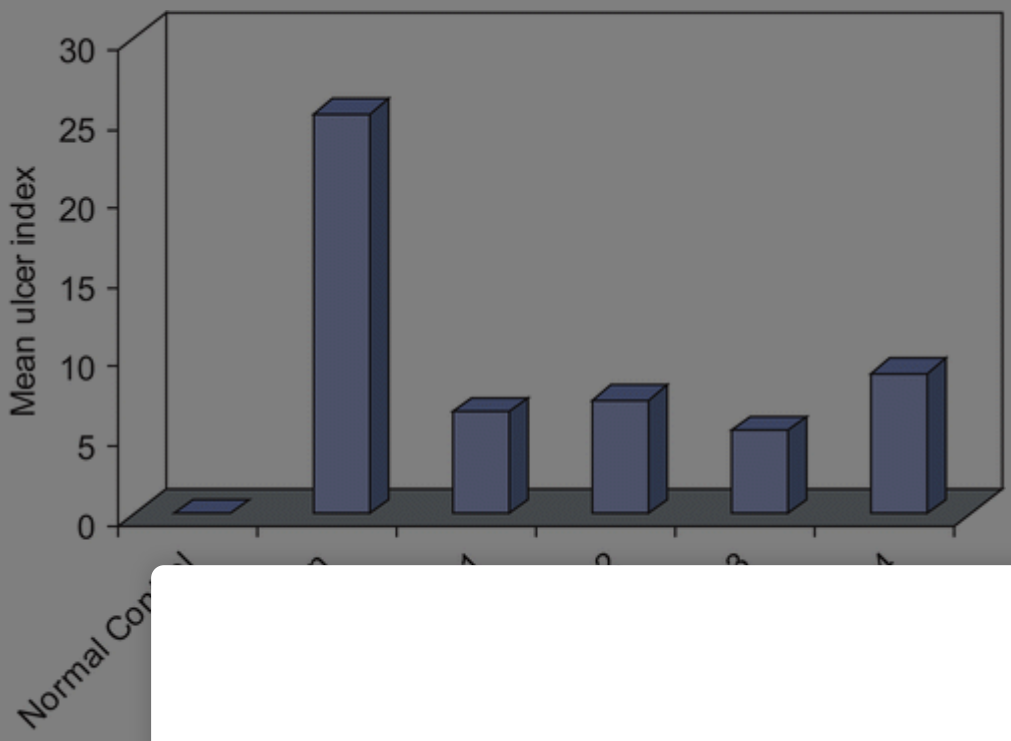
The percentage protection in mice brought about by administration of drug is shown in Table 2. After 4 h of the administration of Dex, the percentage analgesia was observed as 46.4%, whereas for Dex-1, Dex-2, Dex-3 and Dex-4, corresponding values of 61.4, 59.7, 63.6 and 52.7% were observed. The study revealed that analgesic activity of Dex decreased with time, while the prodrugs showed an improved analgesic activity.

Ulcerogenicity

Dex has produced a mean UI of 25.3 while that of its prodrugs resulted in producing very less values and is shown in Figure 2. The study revealed that the minimized side effect obtained in the prodrugs might be due to the inhibition of direct contact of carboxylic acid group of the drug to the gastric mucosa, which is mainly responsible for the damage.

Figure 2. Comparative ulcer index of dexibuprofen and its prodrugs.

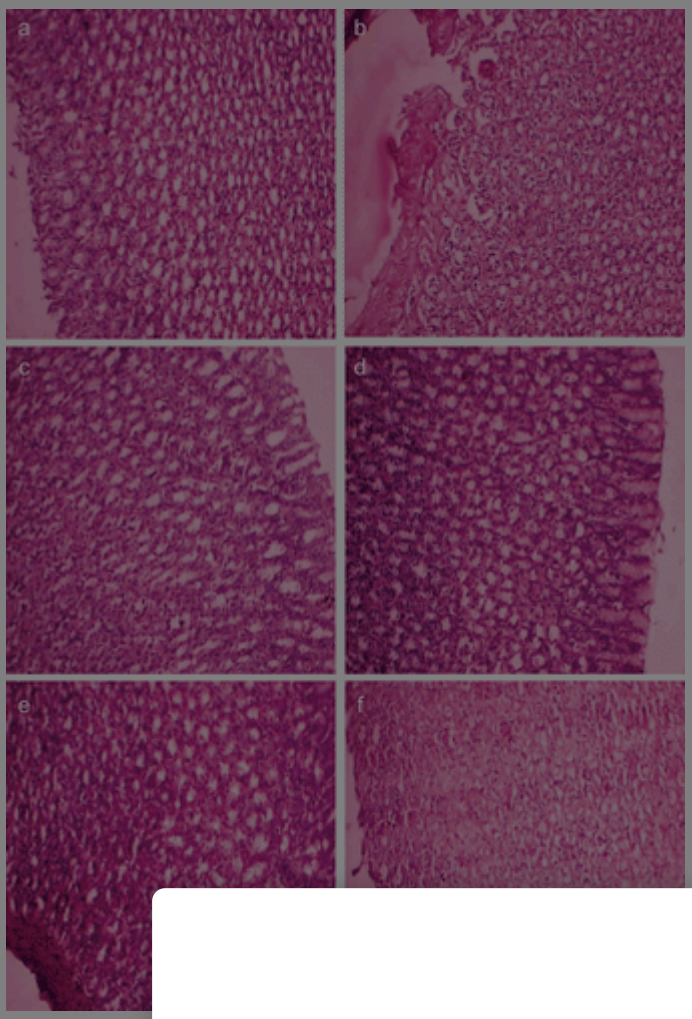


Hist

The hist… The gastric tissues w… group rats showed … Dex group revealed … he basal

stomach lumen. In this zone, the structures of the gland were destroyed. They had disintegrated from the basal lamina and fallen into the lumen. The nuclei of these cells became smaller and dense, and their cytoplasms were stained as dark eosinophilic bodies. Small hemorrhagic areas and patches of inflammatory cell infiltrations were present in the lumen of the glands and lamina propria. Normal histological findings were displayed for Dex-1, Dex-2, Dex-3 and Dex-4 revealing that the prodrugs are not producing any ulceration in the gastric region.

Figure 3. Histopathological studies of dexibuprofen and its prodrugs on rat stomach.



quantitatively in 80% human plasma, rat faecal matter and SIF, but were resistant to hydrolysis at SGF indicating that the prodrugs are resistant to acidic conditions. in vivo studies showed sustained release of Dex upto 8 h as also evident from the longer anti-inflammatory activity observed with prodrugs treated animals compared with Dex-treated animals. The prodrugs were found to be significantly less ulcerogenic and with more analgesia than parent drug. The histopathological study revealed limited ulcer formation in stomach by the prodrugs. These findings suggested that the prodrugs are better in action as compared with dexibuprofen and are advantageous in having less GI side effects without loss of desired anti-inflammatory and analgesic activity of the drug.

Acknowledgements

The authors thank M/s. Alkem Laboratories, Mumbai, India, for providing gift sample of dexibuprofen. The authors are grateful to Padmashree Dr. M. MohanBabu, Chairman, Sree Vidyanikethan Educational Trust, Tirupati, India, for providing the necessary facilities to carry out this work.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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