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ORIGINAL ARTICLE

Reducing ulcerogenic potential of biphenyl acetic acid: Design and development of chimeric derivatives with amino acids

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Abstract In an attempt to minimize the ulcerogenic potential and associated gastro-intestinal toxicity of bioprecursor fenbufen and its active metabolite biphenyl acetic acid, carrier-linked chimeric derivatives of the latter were designed and synthesized using amino acids as the promoities. DCC coupling method was used for the synthesis of these amides. The chimeras were characterized by IR and ^{1}H NMR. Pharmacological investigations were carried out in animal models for analgesic, anti-inflammatory, anti-arthritic and ulcerogenic activities. The chimeras exhibited high gastro-sparing effect; quick onset and longer duration of analgesia; enhanced/prolonged anti-inflammatory activity and better anti-arthritic effect than fenbufen or biphenyl acetic acid. These derivatives could be useful as a chronotherapy for rheumatoid arthritis due to their prolonged analgesic and anti-inflammatory effects.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune inflammatory disorder, affecting about 1% of the world's population. RA causes irreversible destruction of the cartilage, tendon, and bone that comprise synovial joints (Burrage et al., 2006). Non-steroidal anti-inflammatory drugs (NSAIDs) form the mainstay treatment of RA. Clinical use of most of the available acidic NSAIDs is strongly limited by their GI side effects which range in both severity and frequency from relatively mild to severe gastrointestinal toxicities (Gad, 2011). The latter are more serious and potentially life threatening, such as GI ulceration and hemorrhage (Laine, 2001). Fenbufen is a phenylalkanoic acid class NSAID, used in the treatment of rheumatic and other musculoskeletal disorders (Aronson, 2010; Brogden et al., 1981). It is metabolized into four major metabolites: γ-hydroxy[1,1′-biphenyl]-4-butanoic acid (I), [1,1′-biphenyl]-4-acetic acid (II), 4′-hydroxy[1,1′-biphenyl]-4-acetic

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acid (III), and γ,4′-dihydroxy[1,1′-biphenyl]-4-butanoic acid (IV). Metabolites III and IV are inactive while metabolites I and II are biologically active that circulate to plasma (Brogden et al., 1981). The major metabolite, biphenylacetic acid (BPA; metabolite II); is a potent prostaglandin synthetase inhibitor while the literature reports that fenbufen has no inhibitory effect on cyclooxygenase. Therefore fenbufen fits into the conventional definition of bioprecursor prodrug. The major contributor to the GI injury caused by the oral administration of fenbufen is a direct local irritant effect of free carboxylic group of fenbufen and BPA (Kerwar, 1983). The characteristic drawback of bioprecursors is their susceptibility to more than one metabolic enzyme, some of which might inactivate them while others might biotransform them into active metabolites (Wermuth, 2008). As the enzymatic set up of every individual is different, this scenario of multiple metabolic pathways leads to high incidences of inter-subject variation and poor bioavailability attributed to inactive metabolites. Conversion of a bioprecursor into a carrier-linked, chimeric derivative might improve the inter-subject variation and bioavailability. Several attempts have been tried for enhancing its action and/or minimization of its ulcerogenic potential by various research groups. Conjugates of BPA with one of the primary hydroxyl groups of α, β and γ-cyclodextrins (CyDs) through an ester or amide linkage for colon-specific delivery have been reported (Minami et al., 1998). Mutual hydrazide prodrugs of probenecid and diclofenac with biphenylacetic acid with lower ulcerogenicity and increased anti-inflammatory effect have been reported (Sharma and Khan, 2003). Sharma et al. (2004, 2010) have also reported a number of potential mutual prodrugs of BPA by the attachment of several phytophenols/alcohol as promoieties through ester linkage, directly as well as through spacers, with the objective of obtaining safer NSAIDs devoid of ulcerogenic side effects. Khan et al. (2006) have reported glyceride prodrugs of BPA to reduce its gastrointestinal toxicity. A gastro-sparing prodrug of 4-biphenylacetic acid and quercetin tetramethyl ether has been reported by Madhukar et al. (2010) which was devoid of any ulcerogenic side effects.

In one of our preliminary studies we investigated the potential of L-tyrosine conjugate of BPA to minimize its ulcerogenic effect (Dhaneshwar and Sharma, 2012), the positive results of which motivated us to explore the usefulness of L-tryptophan and L-glutamine as promoities in the design of gastro-sparing chimeric derivatives of BPA. Also we did not find any other reports of BPA-amino acid conjugates in the literature. The amino acids L-tryptophan and L-glutamine were chosen for linking them covalently with BPA due to their inherent anti-inflammatory activity and wound healing effects. Madan and Khanna (1978) have reported that L-tryptophan reduces the pain of arthritis (Dhaneshwar et al., 2011). L-Tryptophan being the precursor of serotonine, results in increased serotonine levels which have an effect on increasing pain tolerance, especially for chronic pain caused from arthritis and lower back problems (Fth, 2012). Glutamine metabolism provides precursors for the synthesis of glutathione, an antioxidant, which protects the body from oxidative stress (Soledad et al., 2005). Glutamine also acts as an immune-nutrient and helps in promoting wound healing (Nora and Fatma, 2011). Based on these findings, we hypothesized that conjugation of these amino acids with BPA might potentiate its effect against inflammation in RA with additional gastro-sparing effect due to temporary masking of carboxylic group of BPA which is thought to be partially responsible for local upper GIT irritation. It was further rationalized that co-administration of BPA and amino acids in the form of single chemical entity as chimeras with complementary activities (anti-inflammatory/antioxidant activities) might prove to be potentially advantageous.

2. Experimental

Biphenylacetic acid (BPA) and dicyclohexyl carbodimide (DCC) were purchased from Sigma–Aldrich, Germany. Amino acids and dimethylamino pyridine (DMAP) were purchased from Loba Chemie, Mumbai, India. Fenbufen was purchased from Shanghai AoBo Bio-pharmaceutical Technology Co., Ltd., Shanghai, China, which was used as a standard for pharmacological studies. All other chemicals used in the synthesis were of A.R. grade and those of the synthetic grade were purified prior to use. ^{1}H NMR spectra of the derivatives were recorded in DMSO-d_6 using ^{1}H NMR Varian Mercury 300 MHz with a super conducting magnet using TMS as internal standard at the Department of Chemistry, University of Pune, Pune. Chemical shift values are reported in ppm downfield on δ scale. The IR spectra of the synthesized compounds were recorded on JASCO, V-530 FTIR in potassium bromide (anhydrous IR grade). The absorbance maxima (λ_{max}) of synthesized prodrugs were determined on JASCO V530, UV–Visible double-beam spectrophotometer. Partition coefficient was determined in *n*-octanol/phosphate buffer (pH 7.4). The reactions were monitored by TLC on pre-coated silica gel plates-60 F264 (Merck) in benzene:ethyl acetate:glacial acetic acid (2:1:0.1 v/v) solvent system. The TLC spots were located by exposing to iodine vapors or UV light. Melting points were recorded on precision melting point apparatus and are uncorrected.

2.1. Synthesis of chimeric derivatives of BPA with amino acids

Amino acids were esterified to methyl ester hydrochloride by Ronald's reported method. These were treated with triethylamine to release free amino acid methyl esters which were then coupled with BPA using DCC. The scheme of synthesis is illustrated in Fig. 1.

2.1.1. Synthesis of amino acid methyl ester hydrochloride (Ronalds et al., 1969; Dhaneshwar et al., 2011)

Freshly distilled thionyl chloride (0.05 mol + 30% excess; 5 mL) was slowly added to methanol (100 mL) with cooling and amino acid (0.1 mol) was added to it. The mixture was refluxed for 8 h at 60–70 °C with continuous stirring on magnetic stirrer, excess of thionyl chloride and solvent was removed under reduced pressure giving the crude product. It was triturated with 2 × 20 mL portions of cold ether at 0 °C until excess of dimethyl sulfide was removed. The resulting solid product was collected and dried under high vacuum to give dried methyl ester hydrochloride of amino acid. It was recrystallized from hot methanol by a slow addition of 30–40 mL of ether, followed by cooling at 0 °C. Crystals were collected the next day and washed twice with ether:methanol mixture (5:1) followed by pure ether and dried under vacuum.

2.1.1.1. L-Tryptophan methyl ester hydrochloride (TME·HCl). % Yield: 88, mp: 202–205 °C (uncorrected), R_f: 0.69 in methanol:water (2:1 v/v), IR (KBr; cm^{-1}): 1748 C═O stretching

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