Bioorganic & Medicinal Chemistry Letters 22 (2012) 7582–7587

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and pharmacokinetic profile of rhein- boswellic acid conjugate

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ARTICLE INFO

Article history:
Received 18 August 2012
Revised 21 September 2012
Accepted 2 October 2012
Available online 11 October 2012

Keywords:
Diacerein
Inflammation
In vitro/vivo kinetics
Mutual prodrug
Osteoarthritis

ABSTRACT

Rhein, an active metabolite of diacerein, down-regulates the gene-expression and production of pro-matrix metalloproteinases and up-regulates the tissue inhibitors of metalloproteinase-1 production. The therapeutic effects of diacerein on osteoarthritis are, at least in part, due to the chondroprotective effect of rhein. Boswellic acid is a specific, non-redox inhibitor of leukotriene synthesis. It is claimed to possess good anti-inflammatory, anti-arthritic, analgesic, and anti-ulcer activities. It prevents the destruction of articular cartilage by decreasing degradation of glycosaminoglycans. Therefore, rhein and boswellic acid were linked chemically through a bioreversible ester linkage to synthesize their mutual prodrug by reported procedure. In vitro release profile of this prodrug was extensively studied in aqueous buffers of varied pH, upper GIT homogenates and 80% human plasma. In vivo release studies were undertaken in blood, urine and feces of rats. The prodrug was stable in HCl buffer (pH 1.2) and stomach homogenates of rats. However; in phosphate buffer (pH 7.4) and in intestinal homogenates the prodrug exhibited 91% and 96% release of rhein and 27.5% and 38% release of boswellic acid respectively over a period of 6 h following first order kinetics. In 80% human plasma (in vitro) and rat blood (in vivo) also 96.35% and 91% release of rhein and 78% and 86.41% release of boswellic acid respectively was observed. The 24 h pooled samples of rat urine revealed presence of 6.2% intact prodrug, 7.1% of rhein and 8.9% of boswellic acid indicating their renal excretion. Samples of rat feces pooled over a period of 24 h showed absence of rhein and presence of 3.1% of intact boswellic acid and 4.6% of boswellic acid emphasizing their intestinal excretion. The in vivo release kinetics of prodrug in rat clearly indicated activation of prodrug to be occurring in blood, being catalyzed by the weak alkaline pH of blood (7.4) in combination with esterases present therein.

Osteoarthritis (OA) is a multifactorial, complex disease of synovial joints.[1] It is characterized by loss of articular cartilage due to degradation resulting in reduced joint space, remodeling of the subchondral bone, formation of osteophytes, cyst formation, joint misalignment and inflammation of the synovial membrane; the later being the main cause of chronic pain which is the major symptom of OA.[2–4] Ligament laxity, meniscal degradation and bone marrow edema are other important characteristics which lead to joint impairment and disability. All of these are responsible more or less for the generation of local, chronic pain with insidious onset characterized by aching with episodic stabbing.[5–8] OA generally occurs when the normal cartilage matrix is overloaded or when cartilage matrix is vulnerable. Interleukin- 1 (IL-1) is principally involved in degradation while transforming growth factor (TGF-β) is implicated in the excessive repair of synovium and chondrocytes. Degradation and repair are simultaneously occurring phenomenon.[9]

A large number of palliative treatment modalities are available for OA which mainly use non-steroidal anti-inflammatory drugs (NSAIDs) but steroidal anti-inflammatory agents and immunosuppressants are also equally important components of OA therapy.[10] Long-term administration of NSAIDs has deleterious effects on the vital organs like gastrointestinal tract (GIT), kidney, liver, central nervous system and immune system.[11–13] Intra-articular, long acting corticosteroid injections although used in the management of knee OA, can be given only three to four times in year as repeated injections lead to progressive cartilage damage in weight-bearing joints.[14–17] Chondro-protective or disease-modifying drugs preserve normal joint function by slowing down the rate of anatomical progression of OA.[18] Chondroitin sulfate, glucosamine, diacerein, doxycycline and minocycline are the frontline disease modifying anti-osteoarthritic drugs (DMOADs).[19]

Diacerein [4,5-bis(acetyloxy)-9, 10-dioxo-2-anthracene carboxylic acid], a low molecular weight anthraquinone derivative has been introduced as an effective chondroprotective for the management of OA. Since then it has emerged as a better and safer alternative for the treatment of the OA, which provides symptomatic treatment along with modification of underlying pathological process.[20,21] It has demonstrated efficacy on the functional and structural manifestations of OA by inhibition of IL-1 which is mainly involved in cartilage destruction and acting on the synthesis of

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0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.10.017

principal components of cartilage like proteoglycans and hyaluronic acid.[22] Diacerein inhibits interleukin-1β (IL-1β) and down-regulates the IL-1β-induced inflammatory pathways and cartilage breakdown in OA as opposed to inhibiting the cyclooxygenase (COX) pathway as NSAIDs do. In addition, it down-regulates the gene-expression and production of pro-matrix metalloproteinases (proMMPs) that are involved in cartilage degradation and up-regulates the tissue inhibitor of metalloproteinase-1 (TIMP-1) production.[10,23–25] Orally administered diacerein is completely converted into its active metabolite rhein by deacetylation before reaching systemic circulation.[26] Rhein being an IL-1 inhibitor, reduces collagenase production in articular cartilage, dose-dependently inhibits superoxide anion production, chemotaxix and phagocytic activity of neutrophils, macrophage migration and phagocytosis.[27] The therapeutic effects of diacerein on OA may be due, at least in part, to the chondroprotective effect of rhein.[28]

Therefore in the present work, rhein was chosen as potential candidate for development of a mutual prodrug which would have quick onset of action with improved absorption and bioavailability than diacerein. Boswellic acid was selected as the promoiety in this mutual prodrug design owing to its diverse pharmacological activities that would potentiate the chondroprotetive, disease modifying effect of rhein.

Boswellic acid and its acetates are ursane-type pentacyclic triterpene acids isolated from the gummy exudates of *Boswellia serrata* Roxb and *Boswellia carterri* Birdw.[29] The resin of *Boswellia serrata* is used in India for the treatment of chronic inflammatory arthritis. Ethanolic extract of the resin contains boswellic acid as the main active constituent.[30,31]Gum resin extracted from *Boswellia serrata* Roxb possesses good anti-inflammatory, anti-arthritic and analgesic activities.[32] Boswellic acid is reported to possess anti-ulcer property as well.[33] It also has protective effect on articular cartilage by the virtue of its inhibitory effect on degradation of glycosaminoglycans.[34]

We have reported the synthesis and the chondroproctective effect of mutual prodrugs of diacerein with glucosamine[35,36] and essential amino acids in monosodium iodoacetate-induced osteoarthritis in Wistar rats.[37] Two hydrolytically activated antraquinone-diclofenac prodrugs have been designed and synthesized by Daan et al. (2009) for bone targeting.[38] Synthesis and pharmacological activities of anthraquinone-ibuprofen prodrugs targeting osseous tissues were also reported by Duan et al. (2009).[39] Singh et al. (2007) have revealed the synergistic effect of mixture of boswellic acid and glucosamine for anti-inflammatory and anti-arthritic activities in rats.[40]

There are no reports of any prodrugs of rhein in the literature so far. The present work reports synthesis, in vitro and in vivo release kinetics of mutual prodrug of rhein with boswellic acid with aims of decreasing local irritant effect, potentiating anti-inflammatory activity, anti-arthritic effect and increasing lipophilicity of rhein in order to increase its bioavailability.

Diacerein was generously gifted by Glenmark Pharmaceuticals Ltd., Mumbai, India. Rhein was synthesized in our lab from diacerein by reported procedure. Boswellic acid was purchased from Jai Rhade Sales, Ahmedabad, Gujrat, India. All chemicals and solvents used in the study were purchased from Merck Chemical Corporation, USA and were of analytical reagent grade (AR) or higher purity and were used as it is. Thin layer chromatography was performed on precoated silica gel plates-60 F264 (Merck) for purity check and monitoring of reactions. The IR spectrum of the synthesized compound was recorded on Jasco V-530 FTIR in anhydrous IR grade potassium bromide. Proton and ^{13}C NMR spectra of the synthesized prodrug were recorded in DMSO-d_6 using Bruker Avance II 400 NMR spectrometer at Sophisticated Analytical Instrumentation Facility (SAIF), Panjab University, Chandigarh, India. Chemical shifts are reported in ppm downfield on δ scale. The mass spectrum was recorded employing Waters Q-Tof Macromass spectrometer, LC–MS system at SAIF, Panjab University, Chandigarh, India. The elemental analysis of synthesized prodrug was performed on Elemental Analyzer (Vario MICRO CUBE, Germany) at Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Pune (India). The absorbance maxima (λ_{max}) of synthesized compound in various solvents were determined on Jasco V-530 UV–Visible double-beam spectrophotometer. Partition coefficient was determined in *n*-octanol/phosphate buffer (pH 7.4) whereas the aqueous solubility was determined in distilled water at room temperature 25 ± 1 °C using Jasco V-530 UV–Visible double-beam spectrophotometer. For release kinetic studies, a new HPLC method was developed for simultaneous estimation of prodrug and its active metabolites (rhein and boswellic acid). In vitro release was studied in aqueous buffers of varied pH, rat's upper GIT homogenates and 80% human plasma. In vivo release was studied in rat blood, feces and urine. Human plasma was procured from Bharati Vidyapeeth Medical College, Dhankavadi, Pune. The HPLC system used for this purpose consisted of a pump (Jasco PU model 2080), with autosampler programmed at 20 μL capacity per injection and a UV/VIS detector (Jasco UV 2075). Data was integrated using Jasco Borwin version 1.5, LC-net-II/ADC system. XTerra RP18 column (4.6 × 150 mm; 3.5 μM) in the reversed phase partition chromatographic condition was used for HPLC analysis. The system was used in an air-conditioned HPLC laboratory atmosphere (20 ± 1 °C). Before analysis, the mobile phase was degassed using sonicator and filtered through a 0.45 μM Millipore filter. Sample solutions were also filtered through the same. The system was equilibrated before making an injection. The column was monitored for UV absorbance at a detection wavelength selected after taking the overlay spectra of all the components.

Rhein **1** was synthesized from diacerein by standard procedure.[41] The mutual ester prodrug **3** of rhein **1** with boswellic acid **2** was synthesized by using *N*,*N*′-dicyclohexylcarbodiimide (DCC) coupling (Fig. 1) and the structure was confirmed by spectral analysis.[42–44]

In vitro hydrolysis kinetics of prodrug was studied in aqueous buffers pH 1.2 and 7.4. A reversed-phase HPLC method was developed for the simultaneous estimation of prodrug and its hydrolytic metabolites rhein and boswellic acid. The mobile phase composed of a phosphate buffer: acetonitrile (55:45 v/v, pH 3.0 ± 0.1) at a flow rate of 1.0 mL/min and the column effluent was monitored at 211 nm. The retention times for rhein, boswellic acid and prodrug were found to be 7.1, 5.4 and 8.2 min respectively. Aqueous buffers of pH 1.2 (simulating the pH of stomach) and pH 7.4 (corresponding to physiological pH of small intestine and blood) were prepared to study the release profile of prodrug. The prodrug was incubated in these media at 37 ± 0.5 °C with continuous stirring at 100 rpm. Prodrug (10 mg) was introduced in the medium. Aliquots (5 mL) were withdrawn and replaced with an equal volume of fresh incubation medium by a sampler at different time intervals up to 7 h. The release studies were performed in triplicate.

In vitro release of rhein from the prodrug was further studied in stomach and small intestinal homogenates of rats. A solution of prodrug was prepared in respective buffers (250 μg/mL) and 0.8 mL of it was added to 0.2 g of stomach or small intestinal homogenate placed in 1 mL centrifuge tubes that were pre-equilibrated at 37 ± 0.5 °C. Samples were withdrawn at appropriate time intervals, centrifuged at 10,000 rpm for 10 min. The supernatants were filtered through membrane filter (0.45 μM) and estimated by HPLC for the amount of prodrug remaining and also rhein and/or boswellic acid that might have released after hydrolysis of prodrug. Rate constants for the hydrolysis of prodrug were determined from the slopes of linear plots of the logarithm of residual prodrug concentration versus time. These studies were performed in triplicate and the mean of the rate constant was calculated.

In vitro release kinetic studies were performed in human plasma also. For this purpose 80% human plasma was prepared in