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Studies on synthesis, stability, release and pharmacodynamic profile of a novel diacerein-thymol prodrug

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ABSTRACT

Involvement of oxidative stress, leading to chondrocyte senescence and cartilage ageing has been implicated in the pathogenesis of osteoarthritis (OA). New efforts to prevent the development and progression of OA include strategies and interventions aimed at reducing oxidative damage in articular cartilage using antioxidants as adjuncts to conservative therapy. Diacerein is an anthraquinone derivative with a marked disease modifying effect on OA owing to IL-1 β inhibition. In the present work an attempt was made at design and development of a co-drug of diacerein with antioxidant thymol. Structural elucidation was carried out by spectral analysis. When release kinetics of prodrug was studied in phosphate buffer (pH 7.4) and small intestinal homogenates of rats, 91% and 94% diacerein was available respectively at the end of 4.5 h. Chemical linkage of thymol with diacerein improved its lipophilicity and hence bioavailability. Screening of prodrug in Freud's adjuvant-induced arthritis and ulcerogenic potential by Rainsford's cold stress model exhibited significant reduction in paw volume, joint diameter and ulcer index with superior anti-inflammatory/anti-arthritic activities than the standards. Results of histopathology of tibio-tarsal joint indicated that animals treated with diacerein exhibited moderate synovitis while thymol and physical mixture-treated animals showed mild synovitis. Interestingly in prodrug-treated animals synovitis was not observed. The results of this study underline the promising potential of co-drug of diacerein and thymol in the management of OA.

Osteoarthritis (OA) is a degenerative type of arthritis in which the biomechanical properties of cartilage are altered due to its break down in synovial joints by local proteases. The involvement of mechanical and cytokine-mediated pathways has been emphasized in cartilage degeneration and pathogenesis of OA.[1] Signs and symptoms of inflammation, joint pain, swelling and stiffness causing significant functional impairment and disability are the highlights of OA. A common feature is infiltration of activated B cells and T lymphocytes and overexpression of proinflammatory mediators in early and late OA causing synovitis. This contributes to dysregulation of chondrocyte function that disturbs the balance between the catabolic and anabolic activities of the chondrocytes which is normally involved in remodeling the cartilage. There is a direct co-relationship between release of inflammatory mediators like prostaglandins, nitric oxide, IL-1β and tumor necrosis factor-α (TNF)-α in OA synovial fluid and joint tissue. The underlying mechanism is not well documented but involvement of abnormal mechanical, and oxidative stresses is indicated.[2]

Cartilage matrix component's degeneration and excessive production of different cytokines are the most prominent features of OA. Inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), microsomal prostaglandin E synthase-1 (mPGEs-1), and matrix metalloproteinases are the important pro-inflammatory mediators whose synthesis is stimulated by interleukin IL-1β, promoting the release of nitric oxide (NO) and prostaglandin E2 (PGE2).[3–6] Anabolic activities in chondrocytes are also retarded by IL-1 β causing decreased proteoglycan and collagen synthesis.[7–9]

The treatment is aimed at relieving pain and suffering, maintenance and restoration of function and ultimately prevention of disease progression.[10] The current treatment modalities for OA are either conservative or surgical. Conservative measures prominently involve pharmacological intervention in the form of nonsteroidal anti-inflammatory drugs (NSAIDs), intra-articular steroid injections or relatively new option of injection of hyaluronan which improves joint lubrication and can decrease pain. All these current treatments are basically symptomatic and do not retard destruction of articular cartilage. Therefore, there is an urgent need for therapeutic modalities to protect or induce regeneration of cartilage on a cellular level by restoring its structural integrity and function. Disease modifying anti-rheumatic drugs (DMARDS) are drugs that not only manage symptoms but also favorably affect joint structure changes over long-term treatment periods and thus

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slow or arrest disease progression, so also called structure modifying drugs.[11] Therefore, out of the critical need to develop alternative agents that prevent the destruction of cartilage and/or stimulate its proper repair, DMARDS came into picture.[12]

The pathophysiology of OA is complex with interplay of many converging mechanisms. The results of study performed by Surapaneni et al. suggest higher oxygen-free radical production and oxidative stress in OA.[13] Numerous reports have demonstrated that oxidative damage due to the over-production of nitric oxide (NO) and other reactive oxygen species (ROS) may be playing a significant role in the pathogenesis of OA and also in the formation of gastric mucosal lesions associated with NSAIDs therapy.[14–16]

The current, modern OA therapy has its own limitations in preventing joint destruction which is grossly focused on inhibiting formation of inflammatory mediators such as prostaglandins and leukotrienes with anti-inflammatory agents. With the understanding of the significant role of antioxidants in arthritis, they are gaining increasing importance as an adjunct therapy as they would help in protecting the biological tissues below a critical threshold of reactive oxygen species[17,18] by forming a mutually supportive defense team against ROS in OA. Impaired antioxidant defense and increased lipid peroxidation suggest that treatment with antioxidants at the initial stages of illness may prevent further oxidative injury and deterioration of associated musculoskeletal deficits in OA.[19] Oxidative stress leads to increased risk for OA but the precise mechanism remains unclear. The findings of Yudoh et al. (2005) clearly show that the presence of oxidative stress induces dysfunction of chondrocytes in OA cartilage, suggesting that oxidative stress, leading to chondrocyte senescence and cartilage ageing, might be responsible for the development of OA.[20] New efforts to prevent the development and progression of OA may include strategies and interventions aimed at reducing oxidative damage in articular cartilage.

In the last decade scientists have proven that some antioxidants have anti-inflammatory properties. In addition to scavenging free radicals, there are antioxidants that actually block inflammation. The antioxidant effect (the blocking of certain oxidizing proteins) lowers the activation of inflammatory signals.[21]

Diacerein is a very recently introduced, symptomatic slow acting disease modifying IL-1β inhibitor, known to possess antiarthritic and moderate anti-inflammatory, antipyretic and analgesic activities.[22] The mechanism of action of diacerein differs from those of NSAIDs or corticosteroids. Neither diacerein nor its active metabolite rhein, inhibit prostaglandin biosynthesis; indeed, cyclooxygenase/lipoxygenase pathways. This unique feature seems to be the reason for the excellent gastric safety profile of diacerein during OA treatment. But according to the theory of Cioli et al. diacerein might produce local irritant effect on the gastric mucosa due to its free carboxylic group.[23] It is reported that diacerein's disease modifying effect is more prominent while its anti-inflammatory effect is mild to moderate with a late onset.[22]

Dhaneshwar et al. (2009, 2012) have reported mutual prodrugs of diacerein with aminosugar as well as amino acids possessing lowered ulcerogenic tendency and more aqueous solubility. These prodrugs had quicker onset of action as compared to diacerein.[24–26] The present work was inspired by promising results of our earlier work. The role of increased levels of ROS and oxidative stress is well documented in the literature. Antioxidants have been suggested as a secondary therapy aimed at limiting tissue destruction.[18] Therefore, an antioxidant carrier; thymol was chosen as a carrier for its covalent linkage with –COOH group of diacerein so as to develop its mutual prodrug with reduced local irritant effect, improved absorption, prolonged release of drug and enhanced anti-inflammatory effect. It has been suggested that co-administration of antioxidants and NSAIDs in formulated dosage forms may possibly suppress the progression of OA and decrease the risk of NSAIDs-induced GI toxicity.[27] Thymol is reported to possess anti-oxidant/anti-inflammatory activity.[28–31] Thymol is listed by the US Food and Drug Administration (US-FDA) as a food additive on the 'generally recognized as safe' (GRAS) list therefore it would be nontoxic. Thymol is hydrophobic in nature[30] that might enhance lipophilicity of diacerein, in turn increasing its transcellular absorption.

In the present work, carboxylic group of diacerein was masked in a transient manner with antioxidant thymol. We expected that this conjugation would increase lipophilicity and absorption of diacerein, decrease the gastric irritant effect and enhance it is anti-inflammatory activity by reducing the oxidative stress in OA. Evaluation and comparison of the efficacy of this novel mutual prodrug with individual drugs and their physical mixture was also one of the objectives of this work.

Thymol was purchased from LOBA Chemie, Mumbai, India while diacerein was obtained as a gift sample from Glenmark Pharmaceutical Pvt. Ltd, Mumbai, India. Synthetic procedures were optimized on Radley's 6-station parallel synthesizer. Thin layer chromatography was performed on pre-coated 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. The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance II 400 NMR at Sophisticated Analytical Instrument Facility (SAIF), Panjab University, Chandigarh. Chemical shift values are reported in ppm downfield on δ scale. The absorbance maximum (λ_{max}) was determined on Jasco V-530 UV–Visible double-beam spectrophotometer. Partition coefficient was determined experimentally in *n*-octanol/phosphate buffer (pH 7.4) by flask shake method. In vitro release of diacerein from its prodrug and log*P* values were determined using Jasco V-530 UV–Visible double-beam spectrophotometer. Institutional Animal Ethical Committee's approved experimental protocols were followed for pharmacological screening of synthesized prodrug which was carried out with at the CPCSEA-approved animal facilities of Poona College of Pharmacy, Pune, India. Anti-arthritic activity was evaluated by Freund's adjuvant-induced arthritis using Complete Freund's adjuvant (F-5881, Sigma–Aldrich Corporation, USA). The body weight, joint diameter and paw volumes (both the paws) were measured using weighing balance, digital vernier caliper and UGO BASILE Plethysmometer 7140, Italy respectively. The photomicrographs were taken on inverted microscope attached with digital olympus model no: E-PL1 camera with 40× resolution.

Prodrug of diacerein with thymol (DTH) was synthesized by DCC coupling method[32] (Fig. 1) and then characterization of its physico-chemical properties was performed by spectral analysis.[33]

In vitro release profile of synthesized prodrug was investigated in aqueous buffers (HCl buffer pH 1.2 and phosphate buffer pH 7.4) and tissue homogenates of upper gastro-intestinal tract (GIT). As the difference between λ max of diacerein (258 nm) and DTH (230 nm) was substantial (28 nm), a new method was developed for simultaneous estimation of diacerein in presence of its prodrug DTH by employing UV spectrophotometer. The method was validated as per ICH guidelines and carried out in triplicate. The *K* values from the plots were calculated separately and average *K* and SD value was determined.

Release kinetics of the prodrug was studied in aqueous buffers of varied pH. DTH (10 mg) was introduced in 100 ml of HCl buffer[34] taken in different beakers kept in a constant temperature bath at 37 ± 1 °C. The solutions were occasionally stirred and 5 ml aliquot portions were withdrawn at various time intervals (0–180 min) each time replenishing with fresh 5 ml HCl buffer (pH 1.2). The aliquots were directly estimated on UV spectrophotometer at 232 nm for the amount of DTH remaining and at 259 nm for released diacerein. Same procedure as above was followed to study release