A Prodrug Approach Involving *In Situ* Depot Formation to Achieve Localized and Sustained Action of Diclofenac After Joint Injection

METTE THING,[1] LI ÅGÅRDH,[1] SUSAN LARSEN,[1] RUNE RASMUSSEN,[1] JAKOB PALLESEN,[1] NINA MERTZ,[1] JESPER KRISTENSEN,[2] MARTIN HANSEN,[2] JESPER ØSTERGAARD,[1] CLAUS SELCH LARSEN[1,3]

[1]Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[2]Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[3]DepXplora Aps, Gammelbyvej 17, Lejre DK-4320, Denmark

Received 15 August 2014; revised 15 September 2014; accepted 30 September 2014

Published online 29 October 2014 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24221

ABSTRACT: Long-acting nonsteroidal anti-inflammatory drug formulations for intra-articular injection might be effective in the management of joint pain and inflammation associated sports injuries and osteoarthritis. In this study, a prodrug-based delivery system was evaluated. The synthesized diclofenac ester prodrug, a weak base (p*K*a 7.52), has relatively high solubility at low pH (6.5 mg mL^{-1} at pH 4) and much lower solubility at physiological pH (4.5 μg mL^{-1} at pH 7.4) at 37°C. In biological media including 80% (v/v) human synovial fluid (SF), the prodrug was cleaved to diclofenac mediated by esterases. *In situ* precipitation of the prodrug was observed upon addition of a concentrated slightly acidic prodrug solution to phosphate buffer or SF at pH 7.4. The degree of supersaturation accompanying the precipitation process was more pronounced in SF than in phosphate buffer. In the rotating dialysis cell model, a slightly acidic prodrug solution was added to the donor cell containing 80% SF resulting in a continuous appearance of diclofenac in the acceptor phase for more than 43 h after an initial lag period of 8 h. Detectable amounts of prodrug were found in the rat joint up to 8 days after knee injection of the acidic prodrug solution. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:4021–4029, 2014
Keywords: drug delivery systems; joint injection; injectables; non-steroidal anti-inflammatory drugs; precipitation; prodrugs; suspensions; physicochemical properties

INTRODUCTION

Generally, the rationale for intra-articular (IA) depot injectables is to maintain high therapeutic drug concentrations in the joint over extended time periods while keeping systemic drug levels low to minimize side effects.[1] A significant dose reduction (by a factor of 3000 or more), as compared with intravenous administration, can be achieved by direct injection into the joint target area.[2] Local joint injection of aqueous microcrystalline suspensions of anti-inflammatory corticosteroid esters, for example, methylprednisolone acetate (Depo-Medrol®), is common practice in the relief of pain- and inflammation-associated rheumatoid arthritis[3,4] as well as osteoarthritis.[5] Also, oral nonsteroidal anti-inflammatory drugs (NSAIDs) are used for these indications but their use may be prohibited because of the emergence of severe adverse outcomes even after short-term use.[6] As this class of drugs possesses anti-inflammatory as well as antinociceptive properties,[7,8] long-acting NSAID formulations for IA injection might afford optimal alleviation of joint associated pain and inflammation arising from minor arthroscopic procedures and sports injuries that do not require surgery. Also, as regards flare up pain episodes in osteoarthritis, IA NSAID depot-based therapy may be feasible and may possibly replace corticosteroid injections.[9]

Like most dissolved low-molecular-weight drugs, NSAIDs disappear relatively fast from the synovial space after IA injection.[1] IA injections of aqueous solutions of NSAIDs may, at best, provide pain relief for up to 6 h after arthroscopic procedures.[10] In comparison, the duration of the action of the long-acting steroid ester prodrugs is influenced by the intrinsic solubility of the ester derivatives as well as their enzyme-mediated cleavage rate in the synovial fluid (SF).[11,12] In analogy to these steroid suspensions, depot NSAID injectables might be developed in the form of suspensions comprising poorly soluble NSAID ester prodrugs. Parenteral suspensions have attractive attributes, for example, a high drug load involving a minimum of pharmaceutical excipients.[1] On the contrary, this formulation type may pose major challenges as regards the manufacturing process (terminal sterilization[13]) and the physical stability on storage (particle size distribution[14]). A means to circumvent such barriers to dosage form development is to use a liquid formulation (a preformulation), which after IA injection forms the depot suspension *in situ* upon contact with the SF. Previously, various approaches to provide *in situ* depot formation after other routes of administration have been investigated.[15,16]

Here, we introduce a prodrug approach[17] to enable local and sustained action of diclofenac after joint injection. The potential utility of the prodrug approach taken is illustrated mainly by *in vitro* characterization but also preliminary *in vivo* data in the rat are presented, demonstrating substantially extended joint residence times of the diclofenac prodrug. A sketch of the contemplated prodrug strategy is presented in Figure 1. The role of the promoiety or immobility promoting unit (IPU) is to confer the synthesized diclofenac ester prodrug, a pH-dependent

Correspondence to: Claus Selch Larsen (Telephone: +45-35336466; E-mail: claus.larsen@sund.ku.dk)
This article contains supplementary material available from the authors upon request or via the Internet at http://onlinelibrary.wiley.com/.

Journal of Pharmaceutical Sciences, Vol. 103, 4021–4029 (2014)

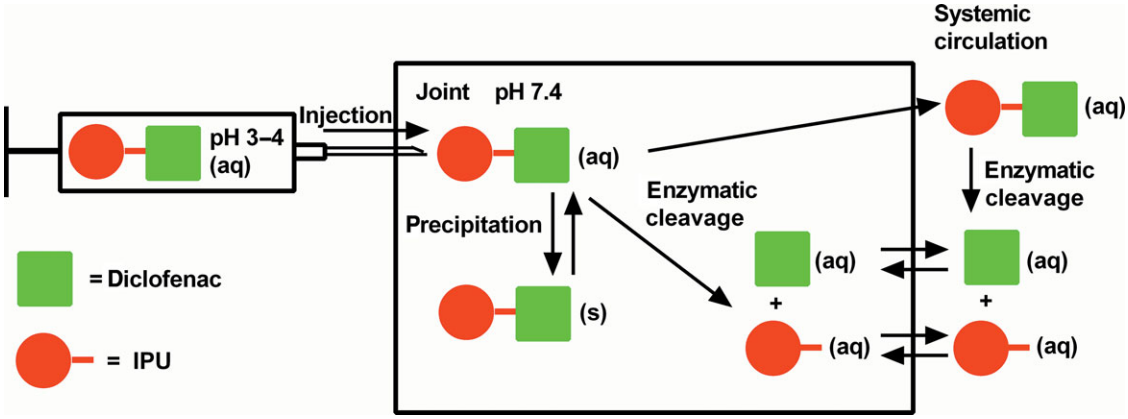

Figure 1. Schematic representation of the *in situ* precipitation formulation principle. After injection of a slightly acidic solution of the prodrug, the prodrug precipitates in the joint because of the lower solubility at pH 7.4. The prodrug in solution is either enzymatically cleaved to the active compound and the IPU or transported intact out of the joint.

solubility, that is, a suitable high solubility at slightly acidic pH and a low solubility in the (patho) physiological pH range close to 7.4. This can be accomplished by employing IPUs that in addition to an OH group comprise a weak base functionality with a p*K*a value of about 5–8. In this manner, it is possible to obtain a concentrated slightly acidic diclofenac prodrug solution (the preformulation), which after injection into the joint leads to *in situ* precipitation of the neutral form of the prodrug on contact with the SF (pH 7.4). To exert pharmacological activity, the ester prodrug has to be converted to active diclofenac by enzyme cleavage of the prodrug bond mediated by esterases and other hydrolases residing in the SF. This activation process only takes place for the small amount of dissolved prodrug that is in equilibrium with precipitated prodrug in the SF.

The objective of the current work was to investigate *in vitro* the potential suitability of the above outlined prodrug concept through characterization of ionization and solubility properties, prodrug stability in aqueous solution, parent compound regeneration in biological media, *in situ* precipitation behavior, and release in biological media. A further objective was to investigate the synovial joint residence time of the prodrug after IA injection in the rat.

MATERIALS AND METHODS

Materials

The following chemicals were purchased from Sigma–Aldrich (Munich, Germany): diclofenac for synthesis, 2-(1-methyl-1*H*-imidazol-2-yl)ethanol, 4-dimethylaminopyridine (DMAP), dichloromethane, dicyclohexylcarbodiimide (DCC), methanol, acetonitrile, Tween 80, bovine serum albumin (BSA) > 98%, and NH_3. Diclofenac > 98% was obtained from TCI (Tokyo, Japan). All other chemicals used were of analytical grade or highest grade possible. Demineralized water was used throughout the study. Diclofenac sodium, fluid for injection, 25 mg mL^{-1} (Novartis Healthcare A/S) was purchased from Nomeco (Copenhagen, Denmark). Outdated human plasma was obtained from Rigshospitalet (Copenhagen, Denmark). SF from arthritis patients was obtained from the Parker Institute, Frederiksberg Hospital (Frederiksberg, Denmark). SF samples from several patients with different diagnoses were pooled and frozen in smaller portions for later use. Rat serum was obtained from untreated Sprague–Dawley rats immediately after execution.

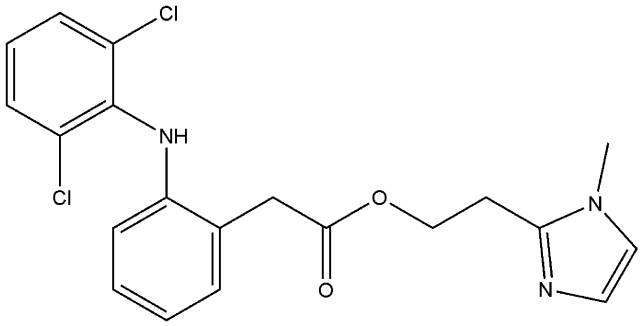

Figure 2. Chemical structure of the diclofenac prodrug DPX-1-0011, 2-(1-methyl-1H-imidazol-2-yl)ethyl 2-(2-(2,6-dichlorophenyl)amino)phenyl)acetate.

Synthesis of DPX-1-0011 [2-(1-Methyl-1H-Imidazol-2-yl)Ethyl 2-(2-(2,6 Dichlorophenyl)Amino)Phenyl)Acetate]

Diclofenac-free acid (2.96 g, 10 mmol), 2-(1-methyl-1*H*-imidazol-2-yl)ethanol[18] (10 mmol) and DMAP (122 mg, 1 mmol) were dissolved in dichloromethane (25 mL) and cooled to 0°C in an ice bath. A solution of DCC (4.12 g, 20 mmol) in dichloromethane (25 mL) was added dropwise over the course of 30 min. After complete addition, the reaction was allowed to reach room temperature over 3 h. The reaction mixture was filtered and the filtrate evaporated to give a pale yellow oil, which was purified by flash chromatography (0%–10% 2 M methanolic NH_3 in ethyl acetate) to give DPX-1-0011 (Fig. 2) in 67% yield as colorless crystals. See Supplementary Information for methods and results of the characterization of the prodrug.

Solubility Studies

Excess DPX-1-0011 was added to either 67 mM phosphate buffer (PBS) pH 7.4 or water to which 0.1 M hydrochloric acid was added to lower pH. Mixtures were rotated at 37 ± 0.5°C in an incubation hood and samples were withdrawn after approximately 24 and 48 h or later. Withdrawn samples were filtered through a 0.45-μm Millex®–HV (Millipore, Billerica, MA) low protein-binding filter. After discarding the first 0.5 mL, filtrates