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The Hydrolysis of Diclofenac Esters: Synthetic Prodrug Building Blocks for Biodegradable Drug–Polymer Conjugates

Feng Wang [1], Joshua Finnin [1], Cassandra Tait [1], Stephen Quirk [2], Igor Chekhtman [1], Andrew C. Donohue [1, 3], Sarah Ng [1, 3], Asha D'Souza [1, 3], Russell Tait [3], Richard Prankerd [1, *]

[1] Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), Parkville, Victoria 3052, Australia
[2] Kimberly-Clark Corporation, Roswell, Georgia 30022
[3] PolyActiva P/L, Melbourne, Victoria 3000, Australia

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ABSTRACT

Degradation reactions on diclofenac-monoglycerides (**3a,b**), diclofenac-(*p*-hydroxybenzoate)-2-monoglyceride (**3c**), diclofenac (**1**), and diclofenac lactam (**4**) were performed at 37°C in isotonic buffer solutions (apparent pH range 1-8) containing varying concentrations of acetonitrile (ACN). The concentration remaining of each analyte was measured versus time. Diclofenac-monoglycerides and diclofenac-(*p*-hydroxybenzoate)-2-monoglyceride (**3c**) were both found to undergo facile and complete hydrolysis in pH 7.4 isotonic phosphate buffer/10% ACN. Under mildly acidic, neutral or alkaline conditions, diclofenac-(*p*-hydroxybenzoate)-2-monoglyceride (**3c**) had the fastest hydrolysis rate ($t_{1/2} = 3.23$ h at pH 7.4), with simultaneous formation of diclofenac lactam (**4**) and diclofenac (**1**). Diclofenac-monoglycerides (**3a,b**) hydrolyzed more slowly under the same conditions, to again yield both diclofenac (**1**) and diclofenac lactam (**4**). There was also transesterification of diclofenac-2-monoglyceride (**3b**) to its regioisomer, diclofenac-1-monoglyceride (**3a**) across the pH range. Diclofenac was shown to be stable in neutral or alkaline conditions but cyclized to form the lactam (**4**) in acidic conditions. Conversely, the lactam (**4**) was stable under acidic conditions but was converted to an unknown species under alkaline or neutral conditions.

Introduction

This article reports the synthesis, hydrolytic behavior, and product formation for two diclofenac esters, diclofenac-monoglyceride [as an equilibrated mixture of two regioisomers DCF-1-MG, **3a**, and DCF-2-MG, **3b** (throughout this manuscript, the mixture of regioisomers of DCF-MG will be referred to as DCF-MG (**3a,b**), whereas DCF-1-MG (**3a**) or DCF-2-MG (**3b**) will be used when referring to a specific individual regioisomer.)] and diclofenac-(*p*-hydroxybenzoate)-2-monoglyceride (DCF-PHB-MG, **3c**) as typical monomeric building blocks for the synthesis of drug–polymer conjugates (DPCs).[1] Two products arose from the hydrolysis of both the alkyl and aryl esters, diclofenac (DCF, **1**) and diclofenac lac-tam (**4**). The relative rates of hydrolysis (to liberate the parent drug) are anticipated to be significantly different, as the aryl ester is an activated ester with a good leaving group. It was hoped that from an understanding of the monomer hydrolysis kinetics the rates of drug release from a DPCs could be tailored. The structure of DCF and the DCF esters suggested the possibility of lactam formation by an intramolecular condensation (dehydration) reaction. Despite this possibility, as far as we are aware, there have been no previous reports of lactam formation directly from the hydrolysis of esters of DCF,[2] though there have been reports of lactam formation during synthesis of diclofenac analogues, particularly activated esters of diclofenac. Along with diclofenac (**1**) and diclofenac lactam (**4**), the ester's stability and degradation/hydrolytic behavior were studied in solutions of isotonic buffers with apparent pH values in the range 1-8, using sufficient acetonitrile (ACN) cosolvent to give adequate solubility for uncomplicated analysis.

Diclofenac is a potent nonsteroidal anti-inflammatory drug (NSAID) that is widely used for the treatment of pain and inflammation.[3,4] In addition to this primary activity, studies have shown that DCF is a broad spectrum antimicrobial agent.[5,6] NSAIDs like

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* *Correspondence to:* Richard Prankerd (Telephone: +61-3-99039520; Fax: +61-3-96570777).
E-mail address: richard.prankerd@monash.edu (R. Prankerd).

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DCF are also known to exert an antiproliferative effect on fibroblasts and keratinocytes,[7] which may counteract their anti-inflammatory effect in the early stages of the wound healing cycle. Further studies in acute wounds have demonstrated that even though DCF reduced fibroblast numbers in an incisional acute wound, DCF did not impair normal healing.[8] The observation that DCF has potential therapeutic activities important to the management of chronic wounds led to the intriguing possibility that it would be clinically efficacious to deliver therapeutic levels of DCF into chronic wounds.[9]

We sought to build DPCs where DCF was attached pendent to the polymer backbone through an ester linkage, which is labile in a physiological environment and provides a mechanism for controlled release. The polymer could be processed into fiber form and incorporated into wound care dressings with a key performance criterion that they must release therapeutic amounts of DCF in a controlled manner when exposed to wound exudate.

Synthesis of the diclofenac–polymer conjugate involves reaction of diclofenac conjugated via an ester linkage to a diol, as a drug incorporating monomer, with a comonomer (i.e., a diisocyanate) to produce a polyurethane-based DPC. The drugincorporating monomer is a prodrug, the hydrolytic behavior of which can be studied independently to confirm the lability of the ester linkage and to better understand diclofenac release from the final DPC product.

The purpose of this study was to (1) confirm that hydrolysis of an aryl ester construct was faster than an alkyl ester, (2) show that DCF was the predominant product of hydrolysis, (3) examine the stability of DCF and its lactam alone, (4) understand the relationships between rates of hydrolysis and pH, and (5) explore the utility of the constructs for design of a wound-responsive product.

Preparation of DPCs of DCF has previously been attempted. Two groups have described the use of standard ester linkages with nonbiodegradable polymethacrylate polymer backbones.[10,11] In both cases, the drug release profile was measured with a direct UV method that would not differentiate between DCF and its lactam degradation product.

A number of other diclofenac alkyl ester prodrugs with ester linkages have been made.[10–15] Bonina et al.[13] reported chemical hydrolysis half-lives in the range 400–500 h (pH 7.4 buffer, 32°C) for an homologs series of polyoxyethylene ester prodrugs. Tammara et al.[15] produced a series of morpholinoalkyl esters of DCF with reported chemical hydrolysis half-lives in the range 3–34 h (pH 7.4 buffer, 37°C). Both the disappearance of the prodrug and the formation of DCF were measured, with mass balance achieved. Jilani et al.[14] made some hydroxyethyl esters of DCF and reported a chemical hydrolysis half-life of about 36 h (pH 7.4, 37°C). Bonina et al.,[13] Tammara et al.,[15] and Jilani et al.[14] all used HPLC methods to quantify the prodrug separately from DCF and none reported any observations regarding the formation of lactam (**4**).

Other NSAID–polymer conjugates[16] have been made to compare hydrolysis rates for alkyl esters with aryl esters, with faster drug release observed with the aryl ester. Also, some evidence suggesting release rates were greater in more strongly basic media was found. This has important implications for wound care, as it offers the potential to develop a wound-responsive product.[17] The pH of normal skin is usually approximately 5.5, with a normal range of 4.0-7.0[18] and the pH of serum (or acute wound exudate) is 7.35-7.45[19]; chronic wound exudate is marked by an elevated pH, often in the range 8.0-8.9.[20,21] A wound bed microenvironment-responsive product would release more drug during the periods of active wound exudation, driven by the high pH of the wound, and less drug as pH of the wound microenvironment decreases because of wound healing. That is, ideally the DCF release rate profile is pH dependent.

Experimental

Materials

Diclofenac was purchased from Beta Pharm Company Ltd. (batch number: 201006280; assay: 99.12%; Shanghai, China). All other solvents or reagents were HPLC or analytical reagent grade. Supplementary Information contains further information for HPLC method validation, preparation of lactam (**4**), and additional kinetic data.

Synthesis of Diclofenac Esters

Preparation of 1,3-Dihydroxypropan-2-yl 2-(2-((2,6-Dichlorophenyl) Amino)Phenyl)Acetate (DCF-2-MG)(3b)

To a mixture of diclofenac (11.47 g, 38.7 mmol), DMAP (0.25, 2.0 mmol), and cis-1,3-benzylidene glycerol (6.99 g, 38.8 mmol), a solution of DCC (10.01 g, 48.5 mmol) in anhydrous DCM (500 mL) was added drop wise at 0°C over a period of 30 min. The reaction was stirred at 0°C for 2 h. The crude material was purified via column chromatography on silica gel (20% ethyl acetate/hexanes as eluent) to give 2-phenyl-1,3-dioxan-5-yl 2-{2-[(2,6-dichlorophenyl)amino] phenyl}acetate in 68% yield as an off-white solid. ^{1}H NMR (200 MHz, $CDCl_3$) * 7.71-7.27 (*m*, 8H), 7.27-7.09 (*m*, 1H), 7.09-6.88 (*m*, 3H), 6.63 (d, $J = 7.9$ Hz, 1H), 5.59 (s, 1H), 4.78 (s, 1H), 4.26 (dd, $J = 40.1, 12.6$ Hz, 4H), 4.01 (s, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) * 172.65, 142.88, 137.91, 137.86, 131.09, 129.52, 129.12, 128.90, 128.33, 128.14, 126.10, 124.42, 124.07, 122.19, 118.45, 101.17, 68.92, 66.91, 38.60. ESI-MS: *m/z* 460 (3%, M^+, $C_{24}H_{21}^{37}Cl_2NO_4$), 459 (13%, M^+, $C_{24}H_{21}^{37}Cl^{35}ClNO_4$), 457 (15%, M^+, $C_{24}H_{21}^{35}Cl_2NO_4$), 242 (11), 214 (100), 103 (15). IR ν_{max} (cm^{-1}): 3320, 2855, 1717, 1504, 1451, 1142, 1080, 908, 728, 697.

1-(2,6-Dichlorophenyl)indolin-2-one (diclofenac lactam) (**4**) was also isolated as a by-product in 30% yield. Spectral data were consistent both with that reported and an authentic sample (see Supporting Information).

2-Phenyl-1,3-dioxan-5-yl 2-{2-[(2,6-dichlorophenyl)amino] phenyl}acetate (3.13 g, 6.8 mmol), 10% (w/w) palladium on carbon (0.31 g) in ethyl acetate (60 mL) was hydrogenated under one atmosphere of hydrogen (balloon) for 16 h at room temperature. The catalyst was removed by filtration through Celite. The crude material was purified by reslurrying and filtration from 30% ethyl acetate/hexanes. The title compound (**3b**) was obtained in 71% yield as an off-white solid. ^{1}H NMR (200 MHz, DMSO) * 7.68-7.42 (m, 2H), 7.29-6.91 (m, 4H), 6.91-6.69 (m, 1H), 6.25 (d, $J = 7.8$ Hz, 1H), 4.90-4.62 (m, 3H), 3.79 (s, 2H), 3.65-3.39 (m, 4H). ^{13}C NMR (50 MHz, DMSO) * 171.93, 143.27, 137.57, 131.29, 131.21, 131.05, 129.58, 128.12, 126.32, 123.89, 121.12, 116.33, 76.87, 60.15, 37.72. ESI-MS: *m/z* 373 (2%, M^+, $C_{17}H_{17}^{37}Cl_2NO_4$), 371 (12%, M^+, $C_{17}H_{17}^{37}Cl^{35}ClNO_4$), 369 (18%, M^+, $C_{20}H_{21}^{35}Cl_2NO_4$), 295 (4), 279 (11), 277, (16), 242 (29), 216 (37) 214 (100), 180 (13). IR ν_{max} (cm^{-1}): 3285, 2943, 1708, 1579, 1509, 1450, 1289, 1046, 770, 743.

Preparation of 1,3-Dihydroxypropan-2-yl 4-(2-(2-((2,6-Dichlorophenyl) Amino)Phenyl)Acetoxy) Benzoate (3c)

Using the procedure described above, 1,3-dihydroxypropan-2-yl 4-(2-(2-((2,6-dichlorophenyl)amino)phenyl)acetoxy) benzoate (**3c**) was prepared in two steps from diclofenac in 62% and 67%, respectively. Diclofenac lactam (**4**) was also isolated as a by-product from the first step in 37% yield. ^{1}H NMR (200 MHz, $CDCl_3$) * 8.26-7.86 (m, 2H), 7.34 (d, $J = 8.0$ Hz, 3H), 7.23-7.09 (m, 3H), 7.07-6.88 (m, 2H), 6.70-6.48 (m, 2H), 5.14 (p, $J = 4.7$ Hz, 1H), 4.07 (s, 2H), 3.94 (d, $J = 4.7$ Hz, 4H), 2.50 (bs, 2H). ^{13}C NMR (50 MHz, $CDCl_3$) * 170.10, 154.43, 142.66, 137.63, 131.38, 131.03, 129.44, 128.85, 128.41, 127.54, 124.21, 123.48, 122.37, 121.71, 118.59, 75.81, 62.43, 38.53. ESI-MS: *m/z* 493 (2%, M^+, $C_{24}H_{21}^{37}Cl_2NO_6$), 491 (6%, M^+, $C_{24}H_{21}^{37}Cl^{35}ClNO_6$), 489 (8%, M^+,