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Synthesis and hydrolytic behaviour of glycerol-1,2-diibuprofenate-3-nitrate, a putative pro-drug of ibuprofen and glycerol-1-nitrate

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Abstract

Nitroxylated derivatives of non-steroidal anti-inflammatory drugs appear to offer protection against the gastrotoxicity normally associated with non-steroidal anti-inflammatory drugs, ostensibly via local production of nitric oxide. A diester of ibuprofen and glycerol-1-mononitrate has been prepared via the condensation of ibuprofen with 3-bromopropan-1,2-diol, followed by silver-(I)-nitrate-mediated nitroxylation. The release of ibuprofen from this diester has been studied in a simulated gastric fluid model with direct analysis by reverse-phase HPLC, using an acetonitrile–water (80 % : 20 %) mobile phase containing trifluoroacetic acid (0.005 %). n-Propyl ibuprofen was found to undergo pH-dependent hydrolysis, ranging from negligible hydrolysis at pH 5 to 52 % hydrolysis at pH 3, over a 2-h period in this model. The ibuprofen-glycerol mononitrate diester was subjected to the most vigorous model hydrolytic conditions and was found to undergo 50 % hydrolysis during the study period. This study shows that pro-drugs of ibuprofen and glycerol mononitrate can be obtained, and can undergo degradation to the parent drugs under conditions simulating those likely to be encountered in the stomach.

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Introduction

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) associated with gastrointestinal side effects, in particular stomach ulceration, bleeding and perforation (Guslandi 1997). Use of NSAIDs also increases the risk of bleeding from existing ulcers (Soll et al 1991). The action of NSAIDs is thought to involve the inhibition of cyclooxygenases, responsible for prostaglandin synthesis, which exist in two isoforms: cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) (Xie et al 1991). Prostaglandins produced via COX-2 are involved in the inflammatory response, whereas those produced via COX-1 are believed to be responsible for maintaining mucosal integrity in the stomach. Inhibition of COX-2 is thought to be responsible for the anti-inflammatory action of NSAIDs, while the associated side effects have been broadly attributed to inhibition of COX-1 (Vane & Botting 1995; Xie et al 1992; Vane et al 1998).

One approach to counteracting the gastric side effects of NSAIDs has been to attach a nitric oxide (NO)-releasing moiety to standard NSAIDs (Wallace et al 1994), the rationale being that nitric oxide plays a similar role to prostaglandins in gastric mucosal defence (i.e. maintaining gastric mucosal blood flow and preventing

leucocyte adherence within the gastric circulation). Hence, local nitric oxide release could counteract the side effects of suppression of the cyclooxygenase enzymes so that mucosal injury does not occur (Moncada et al 1991, 2000). This hypothesis has been demonstrated by a number of NO-releasing NSAID derivatives, so called NO-NSAIDs (Wallace et al 1994).

Previously reported NO-NSAIDs include the nitroxybutyl esters of flurbiprofen (Arena & Del Soldato 1997) and naproxen (del Soldato 1998), the nitroxy groups providing NO by metabolic transformation (Artz et al 1996). S-Nitrosothiol derivatives of NSAIDs have also been reported (Garvey et al 2000) in which NO is released directly by homolytic S–N bond cleavage. An ibuprofen derivative linked via a piperazine unit to an NO-releasing diazeniumdiolate group has been reported (Saavedra et al 1999). We have investigated the use of glycerol as a molecular linking unit in the design of NO-NSAIDs, the resulting derivatives being formally pro-drugs of NSAIDs and glycerol nitrates (Ingram et al 1998). This approach allows for variation in the relative number of NSAID and nitroxy residues incorporated into the NO-NSAID pro-drug, and permits an optimum balance between local vasodilatory and systemic anti-inflammatory effects to be found. In this study we have prepared a diester of ibuprofen and glycerol-1-nitrate, and have investigated the hydrolysis of this diester in a physiologically based model that mimics the chemical conditions of the stomach.

Materials and Methods

Materials

Ibuprofen was supplied as a donation from Knoll Pharmaceutical (Nottingham, UK). n-Propyl ibuprofenate was prepared by literature methods (Hedstrom et al 1993; Tai et al 1995). 3-Bromopropan-1,2-diol (98 %) and 4-toluenesulfonic acid monohydrate were purchased from Sigma-Aldrich Ltd. Silver nitrate (99 % +) was purchased from Lancaster Synthesis (Morecambe, UK). GPR-grade toluene, petroleum and ethyl acetate were purchased from Merck Ltd and were used as supplied. Silica gel (Kieselgel 100; 70–230 mesh) for preparative column chromatography, aluminium backed plates pre-coated with silica gel (Z19,327-5), potassium permanganate reagent (Macek 1972) and diphenylamine reagent (Coldwell 1954) for thin-layer chromatography (TLC) were obtained from Merck Ltd. 99.9 % d^6-Acetone (Cambridge Isotope Laboratories, MA) and 99.9 % tetramethylsilane (Sigma-Aldrich Ltd) were used for NMR spectroscopy. HiPerSolv-grade acetonitrile (Merck Ltd), de-ionized water and spectroscopic-grade trifluoroacetic acid (Sigma-Aldrich Ltd) were used for HPLC. Pepsin (1 : 10 000; from porcine stomach mucosa) and trisodium citrate dihydrate were purchased from Sigma-Aldrich Ltd. Sodium malate hydrate, lactic acid (> 88 %) and glacial acetic acid were purchased from Merck Ltd.

Compound characterization

Infrared spectra were recorded on a Perkin-Elmer 1600 FTIR as liquid films. ^{1}H NMR spectra were recorded upon a JEOL FX90Q FTNMR spectrometer in d^6-acetone. Chemical shifts were reported in ppm values relative to tetramethylsilane. Low-resolution CI mass-spectral data measurements used ammonia as reagent gas and were performed on a VG Biotech Quattro II triple quadrupole instrument. Accurate mass measurements were performed on a VG ZAB-E instrument by manual peak matching. Low-resolution FAB mass spectra were obtained on a Micromass Autospec mass spectrometer using caesium-ion bombardment.

Preparation of glycerol-2-bromo-1,3-diibuprofenate

A solution of ibuprofen (2.00 g, 9.7 mmol), 3-bromopropan-1,2-diol (0.75 g, 4.8 mmol) and 4-toluene sulphonic acid (0.01 g, 1.1 mmol) in toluene (30 mL) was heated to reflux in a Dean-Stark apparatus for approximately 120 h. The mixture was monitored by TLC for the disappearance of ibuprofen (light petroleum (40/60) : ethyl acetate, 50 % : 50 %; detection using potassium permanganate reagent). Upon completion, the reaction mixture was washed with saturated aqueous sodium bicarbonate solution (3 × 30 mL), dried with magnesium sulfate, filtered, the solvent evaporated under reduced pressure and the residue subjected to silica-gel column chromatography (light petroleum (40/60) : ethyl acetate, 90 % : 10 %) to yield glycerol-2-bromo-1,3-diibuprofenate as an oil which was found, by NMR, to consist of a 2 : 1 mixture of diastereomers; 1.40 g (54 %); υ_{max}/cm^{-1} 2955 (CH), 1742 (C = O), 1178; δ_H0.96 (12H, d, $(CH_3)_2CH \times 2$), 1.56 (6H, d, $CH_3CH \times 2$, minor diastereomer), 1.67 (6H, d, $CH_3CH \times 2$, major diastereomer), 2.01–2.15 (2H, m, $(CH_3)_2CH \times 2$), 2.73 (4H, d, $CH_2Ar \times 2$), 3.66 (4H, d, OCH_2CHCH_2O, minor diastereomer) 3.68–4.25 (2H, m, $CH_3CH \times 2$), 4.02 (4H, d, OCH_2CHCH_2O, major diastereomer), 4.87–5.12 (2H, m, $CHBr \times 2$), 7.7–8.1 (8H, m, $ArH \times 8$); m/z (CI) 550 ($(M + 2 + NH_4)^+$, 84 %), 548 ($(M + NH_4)^+$, 84 %), 486 (100 %), 161 ($((CH_3)_2CHC_6H_4CH(CH_3)^+$), 18 %) Found ($M^+ + NH_4$), 548.2366. $C_{29}H_{43}BrNO_4$ requires 548.2375.

Preparation of glycerol-1,2-diibuprofenate-3-nitrate

Silver nitrate (0.32 g, 1.95 mmol) was added to a solution of glycerol-2-bromo-1,3-diibuprofenate (0.2 g, 0.65 mmol) in acetonitrile (15 mL) and the mixture stirred at ambient temperature. The progress of the reaction was followed by TLC (light petroleum (40/60):ethyl acetate, 50%:50%; detection using potassium permanganate and diphenylamine reagents). Upon completion of the reaction (approximately 120 h), the precipitated solid was removed by filtration and the residual solution was concentrated by rotary evaporation. The residue was subjected to silica-gel column chromatography (light petroleum (40/60):ethyl acetate, 90%:10%) to give glycerol-1,2-diibuprofenate-3-nitrate as an oil which was found, by NMR and HPLC (*vide infra*), to consist of a 4:1 mixture of diastereomers; 20 mg (10%); υ_{max}/cm^{-1} 2955 (CH), 1742 (C=O), 1642 (ONO_2 asymmetric stretch), 1233 (ONO_2 symmetric stretch). δ_H0.96 (12H, d, $(CH_3)_2CH \times 2$), 1.43 (6H, d, $CH_3CH \times 2$, minor diastereomer), 1.62 (6H, d, $CH_3CH \times 2$, major diastereomer), 2.10 (2H, septet, $(CH_3)_2CH \times 2$), 2.73 (4H, d, $CH_2Ar \times 2$), 3.66–4.37 (4H, m, $CH_3CH \times 2$, CH_2O), 4.83–5.20 (3H, m, OCH_2CH), 7.72 (4H, d, Ar*H*), 7.97 (4H, d, Ar*H*); m/z (FAB) 451 ($(M - NO_3)^+$, 15%), 161 ($((CH_3)_2CHC_6H_4CH(CH_3)^+$), 100%).

Hydrolysis studies

The simulated gastric solution (Ruby et al 1996) was prepared by adjusting 100 mL of de-ionized water to the selected pH with 5 M HCl and adding 0.125 g of pepsin, 0.050 g of sodium citrate, 0.050 g of sodium malate, 42 μL of lactic acid and 50 μL of acetic acid. The mixture was equilibrated at 37°C for 1 h. To the mixture, 0.10 g of the ester was added and the mixture agitated by an overhead stirrer. At selected time intervals of 5, 30, 60, 90 and 120 min, 10 mL of the solution was removed and diluted with 90 mL of mobile phase for direct analysis by HPLC. Each assay was performed four times.

HPLC

HPLC was performed using a Beckman ultrasphere ODS-2 5-μm 4.6 mm × 250 mm column kept at ambient temperature, a Perkin Elmer Series 410 LC pump and a Perkin-Elmer LC90UV spectrophotometric detector. The volume of samples injected was 20 μL and isocratic elutions were performed using a mobile phase comprising 80% acetonitrile and 20% water containing 0.005% trifluoroacetic acid. The flow rate was 1.2 mL min^{-1}. Suitable wavelengths for the detection of ibuprofen/n-propyl ibuprofen and ibuprofen/glycerol-1,2-diibuprofenate-3-nitrate mixtures were determined using a Hewlett Packard HP8452A Diode Array Spectrophotometer. A wavelength of 218 nm was selected for the detection of ibuprofen/n-propyl ibuprofen mixtures, and 234 nm for the detection of ibuprofen/glycerol-1,2-diibuprofenate-3-nitrate mixtures. Responses were collected and analysed via a computer integrator. These wavelengths showed excellent linear responses for esters and parent drug from the synthetic gastric mixture and mobile phase. Concentrations of ibuprofen, n-propyl ibuprofen and glycerol-1,2-diibuprofenate-3-nitrate were calculated from duplicate 5-point calibration curves constructed using peak areas obtained from analysis of the pure compounds under identical chemical conditions. A typical calibration curve had a slope of 891862, an intercept of 4.68749×10^6 and an R^2 value of 0.99204. The retention times were 3.6 min for ibuprofen and 9.0 min for n-propyl ibuprofen. Glycerol-1,2-diibuprofenate-3-nitrate was found to be a mixture of diastereomers (4:1) with retention times of 12.4 min (major diastereomer) and 13.5 min (minor diastereomer). The limit of detection, defined as the analyte concentration giving a signal equal to the blank signal plus three standard deviations of the blank, was calculated for each compound from the corresponding calibration curve (Miller & Miller 1993), giving 5.27 μg mL^{-1} for ibuprofen, 10.50 μg mL^{-1} for n-propyl ibuprofen and 10.75 μg mL^{-1} for glycerol-1,2-diibuprofenate-3-nitrate.

Results

Synthesis of glycerol-1,2-diibuprofenate-3-nitrate

Glycerol-1,2-diibuprofenate-3-nitrate was prepared by condensation of ibuprofen and 3-bromopropan-1,2-diol, followed by silver-(I)-nitrate-mediated nitroxylation (Figure 1). Migration of the bromine atom in the first step, and an acyl group migration in the second, were observed by ^1H NMR. Such rearrangements are common in glyceride chemistry, and result from migrations towards incipient or full carbonium ions generated on the glycerol backbone under strongly acidic or Lewis acidic conditions. Instances of glyceride rearrangements under the conditions used in this study have been reported (in strong acid (Daubert & Lutton 1947; Rose 1947), and as a consequence of silver-(I)-ion-induced abstraction of halides (Renshaw 1914; Fischer 1920)). Both the intermediate and final products were obtained

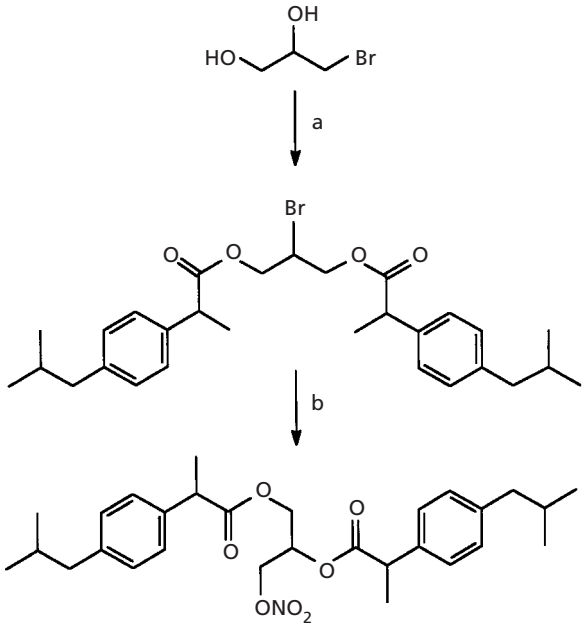

Figure 1 Synthesis of glycerol-1,2-diibuprofenate-3-nitrate. a, ibuprofen (2 equiv.), 4-$CH_3C_6H_4SO_3H$ $_{(cat.)}$, toluene, △; b, $AgNO_3$, CH_3CN, rt.

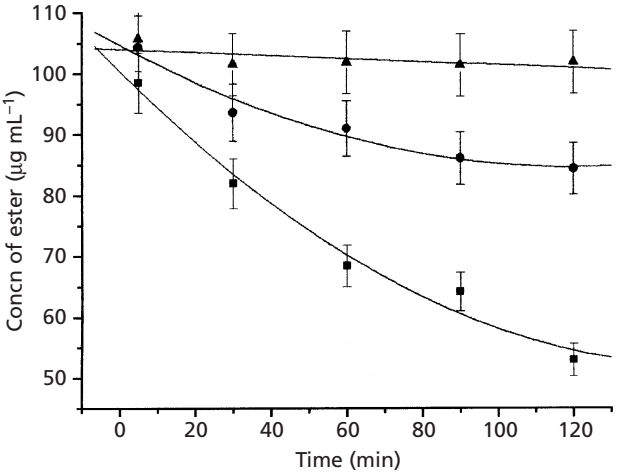

Figure 2 Concentration of n-propyl ibuprofen in simulated gastric fluid as a function of time at pH 3 (■), 4 (●) and 5 (▲). Each point represents the average of four experiments.

as, respectively, 2:1 and 4:1 mixtures of diastereomers, as observed by both ^{1}H NMR and HPLC. Diastereomer formation would be expected from the use of racemic ibuprofen. The formation of unequal amounts of diastereomers may be a consequence of differing ease of formation or degradation of diastereomers, or, in the case of the nitroxylated derivative, of the influence of the asymmmetric centre at the glycerol carbon-2. The low yield (10%) quoted for the nitroxylation reaction is an isolated yield following preparative silica-gel chromatography during which substantial loss of material was observed. The crude material contained too many impurities for use. A molecular ion could not be observed in the mass spectra of the nitroxy derivative, although this is not unusual for such compounds. The presence of the nitroxy group was confirmed by a positive reaction with diphenylamine, which forms a yellow product in the presence of nitrates (Coldwell 1959).

Hydrolysis of n-propyl ibuprofen and glycerol-1,2-diibuprofenate-3-nitrate in a simulated gastric fluid model

To examine the degradation of glycerol-1,2-diibuprofenate-3-nitrate under conditions comparable to those encountered in the stomach, we adapted a physiologically based model of the stomach (Ruby et al 1996) which contained variable parameters that allowed the examination of different physiological states. n-Propyl ibuprofen was used as substrate for studies to select model parameters. The mean fasting stomach pH of adults is approximately 2 and increases to 4–5 following the ingestion of a meal. NSAIDs are not recommended to be taken in a fasting state, consequently pHs of 3, 4 and 5 were selected to mimic the appropriate clinical range. An assay time of 2 h was selected, after which time stomach emptying would normally be effectively complete (Hunt & Spurrell 1951). Each experiment was carried out in quadruplicate.

Our findings on the degradation of n-propyl ibuprofen under these conditions are shown graphically in Figure 2. As expected, more extensive hydrolysis was observed at lower pH. However, even at the lowest pH, hydrolysis was incomplete after 2 h, with approximately 52% of the n-propyl ibuprofen remaining. At pH 5, corresponding to a fed state, very little hydrolysis was observed. The degradation of glycerol-1,2-diibuprofenate-3-nitrate in the simulated gastric fluid model was examined at pH 3 only (Figure 3), that being the pH at which greatest hydrolysis was expected. As with n-propyl ibuprofen, approximately 50% of the ibuprofen ester residues were hydrolysed after 2 h. Additional compounds were observed by HPLC as additional peaks. These may have been intermediate degradation products, such as ibuprofen glycerol mononitrates or diibuprofen glycerols. However, we have no firm evidence of the identity of these compounds, and cannot quantify them.

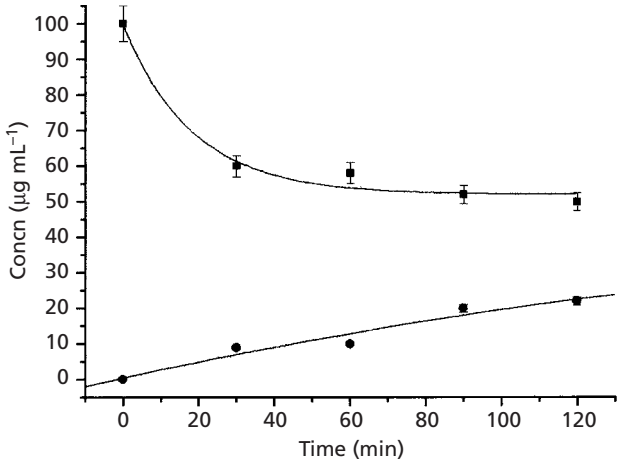

Figure 3 Concentrations of glycerol-1,2-diibuprofenate-3-nitrate (■) and ibuprofen (●) in simulated gastric fluid at pH 3 as a function of time. Each point represents the average of four experiments.

Discussion

The principle of NO-releasing NSAIDs as a new generation of NSAIDs with reduced gastric toxicity has been established by the work of Wallace et al (1994). More recent work has examined the use of molecular linker units in the design of NO-NSAIDs (Saavedra et al 1999), and the use of alternatives to nitrate esters as NO-releasing moieties (Garvey et al 2000). In this context, NO-NSAIDs based on glycerol nitrates are attractive candidates for investigation, given the widespread use of glycerol nitrates as nitrovasodilators and the scope for chemical variation offered by the glycerol unit. The synthesis of glycerol nitrates, triglycerides and several other types of glycerol derivatives has been well investigated (Bhati et al 1980) and, in principle, these methods are applicable to the preparation of glycerol-based NO-NSAIDs. However, synthetic options are restricted by the need to avoid electrophilic nitration conditions, which invariably result in nitration of the ibuprofen aromatic ring (Ingram et al 1998). To date, the only method we have found of successfully introducing the required nitrate ester functionality is the silver-(I)-nitrate-mediated nitroxylation of a precursor alkyl halide. This method is far from satisfactory as the yields are poor and many decomposition products are formed. We have also found that the precursor bromo-diesters are very difficult to obtain for NSAIDs other than ibuprofen, possibly for reasons of steric crowding. We are currently investigating alternative nitroxylation methods, and approaches to the incorporation of NSAIDs other than ibuprofen.

The mechanism of NO-NSAID degradation has not been thoroughly examined in the literature, although some researchers have asserted that in-vivo hydrolysis is a feature of their action (del Soldato et al 1999). Our findings, using a simple gastric model system, suggest that hydrolysis may be only partial over a normal 2-h gastric emptying period. However, in the case of many long-term NSAID users, 2 h may be an unrealistically short modelling period. The model does not examine the metabolism of the nitrate ester functionality to, it is assumed, NO, a process which is thought to involve microbial action (Wallace et al 1994). Future work in our laboratories will examine the effectiveness of glycerol-1,2-diibuprofenate-3-nitrate and similar compounds at protecting against NSAID-induced gastric damage in a rat stomach model.

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