JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55: 513–517

Received October 4, 2002
Accepted January 20, 2003
DOI 10.1211/002235702900
ISSN 0022-3573

Synthesis and hydrolytic behaviour of 2-mercaptoethyl ibuprofenate–polyethylene glycol conjugate as a novel transdermal prodrug

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Abstract

Thiolated derivatives of ibuprofen and its polyethylene glycol ester were synthesized via condensation of 2-mercaptoethyl ibuprofenate with carboxy-terminated polyethylene glycol. The release of ibuprofen from this polymeric prodrug has been studied under conditions simulating those encountered in the skin. The polymeric prodrug of ibuprofen was found to undergo pH-dependent hydrolysis, ranging from negligible hydrolysis at pH 4 to 23.9% hydrolysis at pH 8.5 (15% at pH 7.4) after 48 h at 37 °C. The polymer–drug conjugate was subjected to enzymatic hydrolysis in human plasma. The polymer showed considerable enzymatic hydrolysis (68% after 48 h). The results showed that the polymeric prodrug model of non-steroidal anti-inflammatory drugs (NSAIDs) described here can be used in topical formulations of NSAIDs. It is expected that the novel thiol derivative will have both enhanced transdermal penetration and stability to oxidation which make it a suitable candidate for transdermal formulations.

Introduction

It has been found that certain esters and amides containing a thio (SH) group are capable of markedly increasing the flux of drugs through the skin. In general, materials containing the SH group, or that are capable of yielding the SH group upon biochemical transportation, are of potential use in transdermal formulations. Such esters and amides are generally non-irritating, even when in contact with skin for a long time, and due to their low volatility, they are free of sulfide odour. The SH moiety is also known to act as an antioxidant, and thus assists in prolonging the shelf-life of some of the drugs which are sensitive to this type of degradation and improves their therapeutic performance (Blank & Blank 1996).

Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) associated with gastrointestinal side-effects, in particular stomach ulceration, bleeding and perforation (Ingram et al 2001). Inadequate pharmacokinetics of ibuprofen post-oral administration, and localized therapeutic targets warrant its use as a topical dosage form.

Inflammation and pain are generally localized in joints and other defined areas, but treatment with NSAIDs is generally systemic, which causes gastric irritation and other side-effects. Topical application to the inflamed area is of value, but in many cases the rate of penetration of the drug through the skin is not adequate to sustain therapeutic levels. The main barrier to the penetration of drugs, whether into the skin or all the way through it, is the stratum corneum, which consists of keratinocytes embedded in a matrix of lipid bi-layers. There are many penetration enhancers which have been used in conjunction with NSAIDs (Akhter & Barry 1985; Milosovich et al 1989; Deckner & Lombardo 1994; Park et al 2000). Keratin is the major component of dead cells of stratum corneum. A structural feature of keratin is the presence of S-S bonds derived from the sulfur-containing diamino-acid cystine. It has been demonostrated that the esters and amides of thioglycolic acid, mercaptopropionic acid and mercaptoethanol can open S-S cystine bonds, and cause enhanced penetration of various drugs including NSAIDs. The SH moiety in all of these compounds is known also to act as an antioxidant. Novel molecules thus obtained have both enhanced transdermal penetration and stability to oxidation.

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This research relates to a novel method and composition for enhancing transdermal penetration of NSAIDs. The thio derivative of ibuprofen as a model NSAID was synthesized by esterification of ibuprofen with 2-mercaptoethanol. The resulting 2-mercaptoethyl ibuprofenate was then converted to a high-molecular-weight ester derived from poly(ethylene glycol) (PEG) containing a carboxylic acid end group. This new conjugate of ibuprofen was synthesized for a number of reasons: PEG is a common constituent of topical drug formulations and has an enhancing effect on skin permeation (Bonina et al 1995); the thiol (SH) group possesses inherent skin-penetration-enhancing ability and acts as an antioxidant; PEG derivatives of ibuprofen are hydrophilic and should impart to the prodrug a suitable water solubility, in addition to an increase in lipophilicity due to esterification of the ibuprofen carboxylic group.

Materials and Methods

Materials

Ibuprofen was purchased from Sigma-Aldrich Ltd. Succinic anhydride, 1,3-dicyclohexylcarbodiimide (DCC), 1,1′-carbonyldiimidazole (CDI) and poly(ethylene glycol) monomethoxy ether (MPEG) (number average molecular weight 5000) were obtained from Fluka. Dimethylformamide (DMF) was purified overnight by stirring with anhydride $MgSO_4$ followed by distillation at a reduced pressure. All other chemicals were of reagent grade.

Compound characterization

Infrared spectra were recorded on a 4300 Shimadzu FT-IR instrument. ^{1}H NMR and ^{13}C NMR spectra were recorded upon a 400 AM and Brucker 200 AC spectrometer in $CDCl_3$. The amount of released drug was determined by a 2100 Shimadzu spectrophotometer using a 1-cm quartz cell.

Preparation of 2-mercaptoethyl ibuprofenate (MEI)

A solution of ibuprofen (6.18 g, 30.0 mmol) and CDI (6.07 g, 37.5 mmol) in tetrahydrofuran (THF) was stirred for 2 h at ambient temperature and then treated with 2-mercaptoethanol (2.8 mL, 40 mmol). The mixture was stirred for 15 h at room temperature. The mixture was monitored by TLC for the disappearance of ibuprofen (light petroleum 40/60–ethyl acetate, 50:50.

Upon completion, the reaction mixture was washed with saturated aqueous sodium bicarbonate solution, dried with magnesium sulfate, and filtered. The solvent was evaporated under reduced pressure, and the residue subjected to silica-gel column chromatography (light petroleum 40/60–ethyl acetate, 90:10) to yield the 2-mercaptoethyl ester of ibuprofen as a yellow oil.

FT-IR (ν_{max} cm^{-1}): 1000–1100 (CH_2O), 1510, 1555, 1616 (ph), 1730 (COO), 1735–1740 (COS), 650–700 (CH_2S). ^{1}H NMR ($CDCl_3$) δ (ppm) 0.88 (d, 6H, $(CH_3)_2$), 1.50 (d, 3H, $CHCH_3$), 1.78 (m, 1H, $(CH_3)_2CH$), 2.42 (d, 2H CH_2 ph), 6.5–7.8 (m, 4H, ph).

Analysis: calculated for $C_{15}H_{22}O_2S$ (MW = 266); C 67.6, H 8.27, O 12.03, S 12.03.

Found: C 67.4, H 8.30, O 12.12, S 12.18.

Preparation of polymer–drug conjugate (MEI-PEG)

Monomethoxy polyethylene glycol (MPEG) (30 g, 6 mmol) and succinic anhydride (0.69 g, 6 mmol) were dissolved in 40 mL DMF and stirred overnight at 100 °C. The resulting MPEG monosuccinate (MPEG-COOH) was purified by repeated reprecipitation into diethyl ether from the solution of a mixed solvent consisting of diethyl ether–chloroform (50:50 v/v). MPEG-COOH was obtained in 80% yield.

MPEG-COOH (7.56 g, 1.5 mmol), 4-(*N*,*N*-dimethylamino) pyridine (DMAP, 0.22 g, 1.8 mmol) and DCC (0.37 g, 1.8 mmol) were stirred at room temperature for 30 min. 2-Mercaptoethyl ibuprofenate (0.478 g, 1.8 mmol) was added to the mixture. The mixture was stirred at room temperature for 24 h. The precipitated dicyclohexyl urea was filtered and the polymer was purified by repeated precipitation with a mixed solvent of diethyl ether and methylene chloride (50:50 v/v).

FT-IR (ν_{max} cm^{-1}): 1000–1100 (CH_2O), 1510–1555, 1616 (ph), 1730 (COO), 1735–1740 (COS), 650–700 (CH_2S). ^{1}H NMR ($CDCl_3$); δ (ppm): 0.88 (d, 6H, $(CH_3)_2$), 1.50 (d, 3H, $CHCH_3$), 1.78 (m, 1H, $(CH_3)_2CH$), 1.78 (m, 1H, $(CH_3)_2CH$), 2.42 (d, 2H CH_2 ph), 6.5–7.8 (m, 4H ph). ^{13}C NMR, δ (ppm): 173 (COO), 52.5 (CH_2O), 3.88 (CH_2S), 49 (CH_2CH_2O), 145–138 (ph).

Characterization of hydrolysis products

The hydrolysis products were characterized at different conditions. Twenty milligrams of drug–polymer conjugate was dissolved in 30 mL of 0.1 M pH 10 glycine buffer solution. The reaction mixture was stirred at 60 °C. Ten milliliters of the mixture was withdrawn after 24 h, 48 h and 72 h hydrolysis. The hydrolysis solution was neutralized with 2 M HCl, and the solvent was evaporated in-vacuum. The resulting product was treated with 10 mL of diethylether and heated. The suspension was then filtered and the etheral solution was evaporated under reduced pressure. The hydrolysis products were characterized by measuring the UV absorbance using a 2100 Shimadzu spectrophotometer. The optical densities at 223 nm and 256 nm were measured to determine the concentration of MEI and free drug (ibuprofen), respectively.

The characterization of hydrolysis products was also followed in 50 mL of 0.1 M phosphate buffer solution at 37 °C (physiological conditions) for 2 days. The hydrolysis products were characterized by UV spectrophotometry and the drug content was estimate by measuring the absorbance at 223 nm.

Drug release studies

The chemical hydrolysis rate of MEI-PEG conjugate was determined in a cellophane dialysis tube (The Scientific Instrument Centre Ltd, London, UK) in 5 mL of isotonic phosphate buffer solution (pH 7.4), at 37 °C. The tube was transferred into a flask containing 25 mL of the same buffered solution. The external solution was stirred continuously and 3-mL samples were removed at timed intervals and replaced with 3 mL of buffer solution. The absorbance of the sample was determined against a reagent blank at 223 nm, and determined from the calibration curve obtained previously under the same conditions. The enzymatic hydrolysis rate of polymer–drug conjugate was determined in human plasma according to the method described for chemical hydrolysis. Plasma fractions (4 mL) were diluted with 1 mL of isotonic phosphate buffer, pH 7.4, and transferred into a cellophane dialysis tube. The tube was immersed into a flask containing 25 mL of isotonic phosphate buffer. The samples were withdrawn at intervals and deproteinized by adding 0.01 M HCl and then centrifuged. The clear supernatant was analysed by UV spectrophotometry.

In-vitro demonstration of the thiol group reaction

To a three-necked flask (100 mL) under nitrogen was added 10 mL of water (pH 7.2), 200 mg cystine and 200 mg of MEI. The mixture was stirred at room temperature for 24 h and the reaction was followed by TLC using silica-gel precoated plates. As an eluant, BuOH–H_2O–AcOH (10:2:6) was used. The plates were developed by spraying a solution of 5% ninhydrin in ethanol and heating. After about 5 h, a spot corresponding to cysteine was formed and this spot grew as a function of time.

Preparation of aqueous gel

Carbopol gels, containing mercaptoethyl ibuprofenate or its PEG conjugate, were prepared by dispersing Carbopol 934 (1.5% w/w) in distilled water with constant stirring. MEI or its polymeric prodrug were solubilized in ethanol (20%w/w) together with methyl-*p*-hydroxybenzoate (0.1%w/w). The ethanolic solution was added to the carbopol dispersion and the mixture was then neutralized, and made viscous by adding triethanolamine (2.0% w/w). The gels were stored at room temperature for 24 h.

Results and Discussion

Synthesis of polymer–drug conjugate

A new polymer–drug conjugate comprised of ibuprofen and modified polyethylene glycol carrier was prepared, and connected by an oxyethylene spacer group via a hydrolysable thioester bond. Synthesis of the polymer–drug conjugate involves structural modification of both ibuprofen and PEG before coupling. Carboxy terminated polyethylene glycol (PEG-COOH) was prepared by treating monomethoxy polyethylene glycol (MPEG) with succinic anhydride (Figure 1). The mercaptoethyl ester of ibuprofen (MEI) was prepared by a common esterification procedure. Ibuprofen was activated with CDI for 2 h at room temperature. 2-Mercaptoethyl ibuprofenate (MEI) was prepared by reaction of activated intermediate with mercaptoethanol (Figure 2). The resulting low-molecular-weight prodrug of ibuprofen was purified and esterified with PEG-monosuccinate (PEG-COOH) using DCC as activating agent to produce the polymer–drug conjugate (MEI-PEG, Figure 3). The spectral characteristics of the product showed the presence of thioester, normal ester and oxyethylene units.

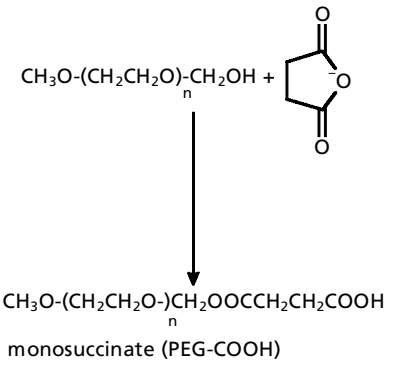

Figure 1 Preparation of carboxy-terminated polyethylene glycol.

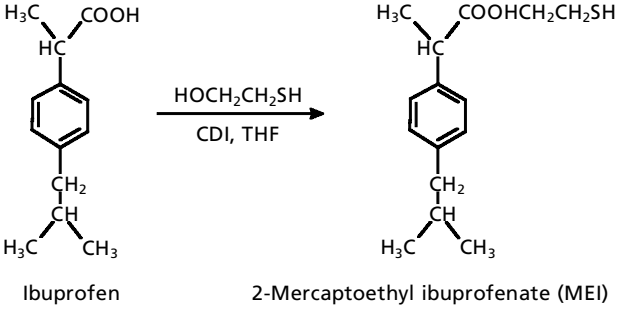

Figure 2 Preparation of the mercaptoethyl ester of ibuprofen as a low-molecular-weight prodrug containing a thiol group.

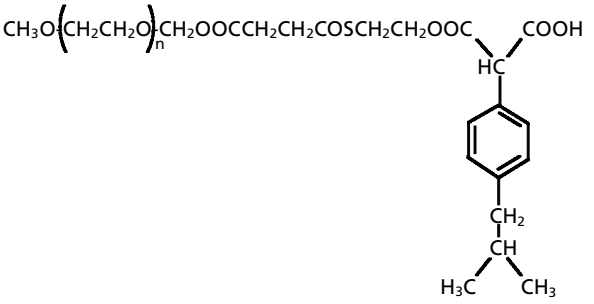

Figure 3 Chemical structure of PEG–mercaptoethyl ibuprofenate conjugate.

Chemical and enzymatic hydrolysis

The characterization of hydrolysis products under severe conditions (pH 10, 60 °C) indicated that the main product of hydrolysis was MEI after 24 h. As the reaction progressed, two other products were monitored by UV and TLC, corresponding to ibuprofen and mercaptoethanol.

At physiological conditions (pH 7.4, 37 °C), no evidence of the formation of the free drug was found after 2 days of hydrolysis. This means that the thioester group between the drug and polymer end-group could be hydrolysed easily in the presence of normal ester bond. The enhanced reactivity of the thioester over the normal ester bond is supposed to be an important factor for the release of MEI as the main hydrolysis product at physiological conditions. Ibuprofen could be formed via hydrolysis of the ester bond in MEI after about 35 h at pH 10 and 60 °C.

As shown in Figure 3, two hydrolysable bonds are present between the drug and polymer chain in MEI-PEG conjugate. The characterization of hydrolysis products by spectroscopic methods revealed that MEI can be produced during hydrolysis at pH 7.4. The release of prodrug occurs through hydrolysis of the thioester bond. The thioester bond is susceptible toward hydrolysis compared with a normal ester bond (Davaran et al 1997, 1998).

The prodrug had a notable stability in phosphate buffer at pH 7.4 (Figure 4). An essential prerequisite for success in the use of prodrugs is that prodrug reconversion into the parent drug occurs in the skin. Since the preparation of the skin homogenates may cause some problems due to the tenacious and elastic nature of the outermost layer of skin, different models have been developed to mimic skin esterase activity and to assess the susceptibility of ester prodrugs in undergoing bioconversion in the skin. Many authors reported the possibility of using human plasma to assess the hydrolysis rates of ester prodrugs for dermal delivery (Bonina et al 1995). As shown in Figure 4, the polymeric prodrug was hydrolysed significantly by human plasma.

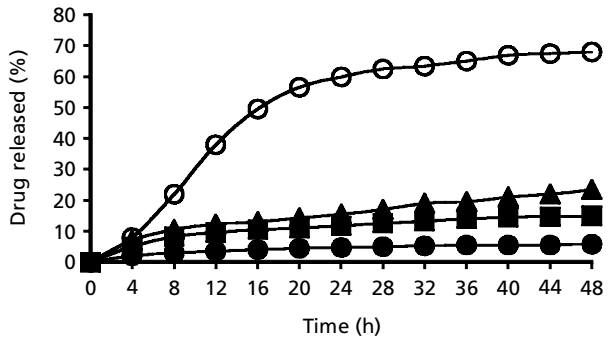

Figure 4 Percent of 2-mercaptoethyl ibuprofenate released in human plasma (○) and pH 8.5 (▲), pH 7.4 (■) and pH 4 (●) buffered solutions as a function of time. Each point represents the average of four experiments.

In-vitro demonstration of the reaction between the prodrug and skin

The hydrolysis studies indicated that the main product of hydrolysis, under physiological conditions, was mercaptoethyl ibuprofenate. However, it was demonstrated in-vitro that the mercaptoethyl derivative of ibuprofen can convert the disulfide bond of cysteine to the mercapto group of cystine.

A gel based on Carbopol 934 in distilled water was prepared with addition of ethanolic 2-mercaptoethyl ibuprofenate, or its PEG conjugate, as the active ingredients. 2-Mercaptoethyl ibuprofenate gel was irritating when applied to the skin, and also had an unpleasant sulfide odour. The PEG conjugate was non-irritating even when in contact with the skin for a long time and was free of sulfide odour due to its low volatility.

The water solubility of MEI and MEI-PEG conjugate was determined. The values were 58 μg mL^{-1} and 380 μg mL^{-1}, respectively.

As expected, the presence of PEG chains in the polymeric prodrug increased the hydrophilicity. The prepared gel was clear and highly stable. The water solubility of MEI was lower than ibuprofen. This is in agreement with the increasing hydrophobic character of MEI compared with parent drug.

Conclusion

This study shows that the polymeric prodrug of 2-mercaptoethyl ibuprofenate and polyethylene glycol monosuccinate can be prepared in reasonable yield, and can undergo chemical and enzymatic degradation to SH-containing product under conditions simulating those encountered in the skin.

Primary investigations demonstrated that drug–polymer conjugate has no irritating effects on the skin, and can convert S-S bonds to SH bonds. Therefore, it is expected that its dermal absorption would be more than that of ibuprofen.

The next aim of this work will be the evaluation of the polymeric prodrug described here in acrylic-type prolonged-release transdermal patches. The structure of the prepared patch and skin permeability studies will be reported in the future.

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