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RESEARCH PAPER

Synthesis and Evaluation of a Nonsteroidal Anti-inflammatory Polymeric Prodrug for Sustained and Site-Specific Delivery

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ABSTRACT

A new polymerizable drug derivative of diclofenac sodium was synthesized and characterized in terms of melting point, elemental analysis, and infrared spectroscopy. It was then polymerized to obtain a new polymeric prodrug. The prodrug was evaluated for its viscosity, drug content, and in vitro drug release behavior at pH 1.2 and 7.2. The in vitro studies showed that the drug release takes place predominantly at the higher pH and in a sustained manner, as hypothesized. Stability at room temperature, bioavailability, and ulcer-inducing effect of the polymeric prodrug were also studied. The investigations showed complete drug absorption from the polymeric prodrug with a statistically significant decrease in ulcer scoring effect, thus showing its potential for site-specific and sustained drug delivery.

Key Words: *Diclofenac; Prodrug; Site specificity; Sustained release*

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs), although generally effective in the management of pain and inflammation, are also associated with the development of several gastrointestinal (GI) complications, particularly damage to the upper gastrointestinal tract (namely, the stomach and the duodenum). Direct damage to the lower GI tract, however, is unusual (1). Our understanding of the

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pathogenesis of GI damage is relatively rudimentary. It appears, however, to have both local and systemic components.

Several approaches have been made in the past to overcome the GI complications associated with NSAIDs. These approaches include liquid formulations, parenteral and rectal administration, enteric coating of oral formulations, prodrugs, and microparticulate systems. Cotherapies with sucralfate (2), H_2 antagonists (3), proton pump inhibitors (4), and prostaglandin inhibitors (5) have been tried. In recent years, NSAIDs based on COX-2 selective compounds have also been used (6,7). All these approaches, however, have not been able to provide the required complete protection against GI complications, although it is believed that NSAIDs based on cyclooxygenase-2 (COX-2) inhibitors are comparatively safer.

In recent years, polymeric drug derivatives, in which the drug molecules are linked to polymeric matrices through covalent bonding of limited stability in the physiological environment, are receiving considerable attention (8–14). This is believed to be one of the most promising ways to modify the pharmacokinetics of the drugs and to achieve preferential localization to target sites.

It was proposed, therefore, to look at the problems associated with NSAIDs by adopting a polymeric prodrug approach by which the drug was covalently attached to poly(hydroxy ethyl methacrylate) [poly(HEMA)] through an ester linkage. Such a system is expected to cleave preferentially and release the drug in the alkaline environment of the lower GI tract in a site-specific manner rather than the acidic environment of the upper GI tract. Poly(HEMA) was chosen because it is known to exhibit low interfacial free energies with aqueous solutions and has a weak tendency to absorb biological species such as blood cells and proteins. It has, therefore, good biocompatibility (8). Further, poly(HEMA) that is released to the GI tract is expected to be excreted unchanged as mucosal surfaces are known not to absorb polymeric compounds. Such a site-specific delivery system should not only avoid the drug coming into contact with the gastroduodenal mucosa (in the upper GI tract) and thus prevent the side effects due to local irritation, but also be able to prolong the pharmacological activity of the drug, leading to a good sustained-release system capable of avoiding the systemic side effects. What is more, dose dumping associated with conventional reservoir sustained delivery systems can also be avoided.

We report here on the synthesis and characterization of a polymerizable NSAID drug derivative of diclofenac and polymerization of the drug derivative to obtain a new polymeric prodrug. This procedure was preferred rather than attempting to link the drug to the polymer because this should lead to polymeric or oligomeric prodrug with a 100% degree of substitution, which is required for higher yields of drug release. Even oligomeric prodrugs, when orally administered, are also known to convey the drugs across the physiological barriers, thus permitting drug absorption and increased bioavailability (8). The in vitro drug release behavior at different pH, stability, bioavailability, and ulcer-inducing effects of the polymeric prodrug are described.

EXPERIMENTAL

Materials

2-Hydroxy ethyl methacrylate (HEMA) was obtained from M/s Fluka (Buchs, Switzerland). Benzoyl peroxide was obtained from Wilson Laboratory; thionyl chloride was obtained from S. D. Fine Chemicals; dimethyl sulfoxide (DMSO) was obtained from Ranbaxy Fine Chemicals; all were used as received. Diclofenac sodium was obtained from tablets India, Chennai, as a gift sample and was used as received.

Preparation of the Polymeric Prodrug

HEMA (26.4 g) was placed in a 250-ml, three-neck, round-bottom flask fitted with a stirrer and condenser. The flask was then heated to 40°C in an oil bath. Thionyl chloride (26.18 g) was then added dropwise to the flask using a dropping funnel. When the addition was complete, the temperature of the flask was increased to 70°C, and the flask was maintained at this temperature for 3 h. The excess thionyl chloride was removed by distillation. The chloro ethyl methacrylate ([I] in Fig. 1) obtained as a liquid was purified by distillation and utilized for further experiments after confirming the replacement of hydroxyl by chlorine.

Diclofenac sodium (10.6 g) was placed in a three-neck, round-bottom flask fitted with a condenser and a stirrer. DMSO (50 ml) was added to the

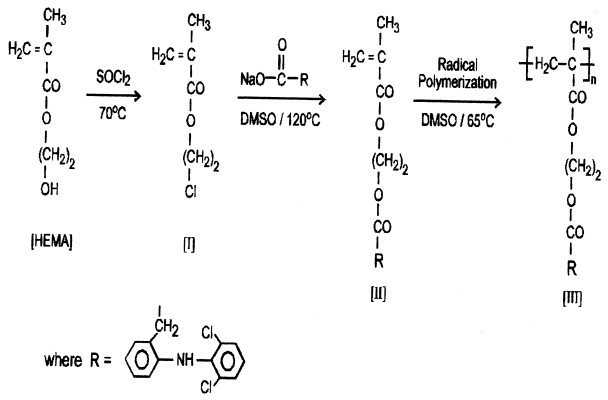

Figure 1. Synthesis of the polymeric prodrug.

flask, and the drug was allowed to dissolve. Chloro ethyl methacrylate (4.5 g) was then added, and the reaction flask was heated to 120°C. The flask was maintained at this temperature for 8 h. The contents of the flask were then poured into 500 ml of distilled water with stirring when a brown precipitate was formed. It was allowed to settle overnight. The supernatant liquid was decanted, and the precipitate obtained was filtered and dried. The monomeric drug derivative [II] thus obtained was purified by dissolving in 20 ml of acetone and reprecipitating it by pouring it into 200 ml of distilled water. It was further purified by crystallization in acetone. Elemental analysis of the drug derivative was carried out using a Perkin Elmer 240C elemental analyzer; and its infrared (IR) spectrum was recorded using a Perkin Elmer 1600 series Fourier transform infrared (FTIR) spectrophotometer.

The monomeric drug derivative (5 g) was placed in a 100-ml, three-neck, round-bottom flask fitted with a stirrer and condenser. DMSO (50 ml) was added to dissolve the drug derivative. The reaction flask was heated in a water bath to 65°C. Nitrogen gas was allowed to bubble through the reaction mixture throughout. Benzoyl peroxide (0.1 g) was then added to initiate polymerization. The polymerization was carried out for 6 h. The flask was then cooled to room temperature, and the contents were poured into 500 ml of distilled water. The dark brown precipitate of polymeric prodrug [III] obtained was filtered and dried. It was then dissolved in 20 ml of DMSO, reprecipitated from distilled water, and dried under reduced pressure to constant weight. It was then passed through a British standard 100 sieve and stored in a desiccator.

Estimation of Drug Content

A calibration curve for the pure drug in 0.1 M sodium hydroxide solution was developed between 10 and 50 μg/ml at 273 nm prior to drug estimation. The polymeric prodrug (100 mg) synthesized was dispersed in 100 ml 0.1 M sodium hydroxide solution placed in a volumetric flask. It was kept overnight with constant stirring for the complete release of the drug from the polymeric backbone by hydrolysis. The contents were then filtered to remove the particulate matters through a nylon 66 membrane pad (0.45 μ), and appropriate dilutions were made. The drug content was estimated by measuring the absorbance of the filtrate at 273 nm in a Shimadzu UV 160 A double-beam spectrophotometer.

In Vitro Drug Release Studies

The in vitro drug release studies of the polymeric prodrug were carried out in a USP 24 dissolution test apparatus (type 2). The prodrug equivalent to 100 mg of diclofenac was taken, and study of its in vitro behavior in 900 ml of acetate buffer of pH 1.2 and phosphate buffer of pH 7.2 was carried out separately over a period of 24 h at 100 rpm. Samples were withdrawn at different time intervals, filtered, and analyzed spectrophotometrically by monitoring the absorbance at 273 nm. The study was conducted in triplicate to calculate the drug release.

Stability Studies

The stability of the prodrug at room temperature (20°C–25°C) was carried out over a period of 3 months. Samples were withdrawn at the end of 30, 60, and 90 days and analyzed for physical appearance, IR spectra, in vitro drug release, and drug content by adopting a reversed-phase high-performance liquid chromatographic (HPLC) method using a Waters® HPLC system and a reversed-phase C_8 column (Kromasil C_8, 10 μ, 25 × 4 mm ID).

Bioavailability Studies

A comparative bioavailability study following a crossover design was carried out in New Zealand albino rabbits (1.5–2.0 kg) of either sex. Free drug (7 mg/kg) and prodrug containing an equivalent quantity of diclofenac were administered orally as

a suspension in 0.5% w/v carboxymethylcellulose. Blood samples (1 ml) were withdrawn from the marginal ear vein at different time intervals over a period of 24 h and transferred into heparinized tubes. The drug from the plasma was extracted, and a reversed-phase HPLC method (15) was adopted to quantitate the drug-plasma levels using a Waters HPLC system.

Ulcer-Inducing Effect

Albino rats of either sex receiving a normal daily diet and weighing between 180 and 200 g were placed in three groups of 10 animals each. Group I served as the solvent control; animals in it received 1 ml/kg of 0.5% w/v carboxymethylcellulose suspension. Group II received 18 mg/kg of the free drug, and group III received prodrug containing an equivalent quantity of drug as a suspension. The free drug and the prodrug were administered orally twice daily for 7 days as a fine microsuspension in 0.5% w/v carboxymethylcellulose. On the eighth day, the rats were sacrificed under excess ether anesthesia, and the stomachs were cut open and the mucosal lining was examined for the severity of the ulceration. The ulcer score was done as per Laurence and coworkers (16). Statistical analyses were carried out using the Student unpaired t test, and P values less than .05 were considered significant.

RESULTS AND DISCUSSION

The synthesis scheme for the polymeric prodrug is represented in Fig. 1. The IR spectra of the chloro derivative of HEMA shows the absence of signals in the region 3000–35000 cm^{-1} and the presence of characteristic signals at 1744 cm^{-1} (C=O), 1637 cm^{-1} (C=C), and 760 cm^{-1} (C–Cl), thus confirming the formation of the chloro derivative of HEMA.

A dark brown color monomeric drug derivative (yield 68%) with a melting point of 80°C was analyzed for $C_{20}H_{19}O_4NCl_2$ elemental analysis; calculated percentages were 58.96% for carbon and 4.66% for hydrogen; percentages found were 58.90% for carbon and 4.62% for hydrogen. The compound answered for both nitrogen and chlorine in Lassinge's test. The IR spectrum showed a short band at 3300 cm^{-1} (along with a broad band due to hydrogen bonding, indicating the presence of the NH function), 1735 cm^{-1} (C=O) and 1636 cm^{-1} (C=C). The polymeric prodrug so obtained was also dark brown in color and had a melting point of 99°C. The absence of monomer was confirmed by a single-spot thin-layer chromatography by adopting 9 : 1 benzene-methanol solvent system. The drug content was estimated to be 0.712 g/g of the polymeric prodrug. The low relative viscosity obtained (η_{rel} value of 1.02) suggests that the prodrug obtained had a low molecular weight. It should be mentioned here that such low molecular weight species are also capable of carrying the active agents across the mucosal barrier after oral administration, leading to increased bioavailability (8).

The in vitro drug release profile of the prodrug synthesized is given in Fig. 2. The polymeric prodrug exhibited sustained and pH-dependent drug release behavior over a period of 24 h. At pH 7.2, a burst release of 26.58% was observed within 1 h, followed by a sustained release over a period of 12 h. A maximum of 98.24% of the drug was released from the prodrug, and the time taken for 50% drug release T_{50} was found to be 4.0 h. Although there was a sustained drug release at pH 1.2, the amount of drug released at various time intervals was comparatively lower than that at pH 7.2, as hypothesized. The drug release in a 2-h period at pH 1.2 was only 7.38%, which is approximately five times less than the drug released at pH 7.2 (34.93%), and a maximum of 43.83% was released after a period of 12 h.

The slow drug release at the lower pH of 1.2 can be explained on the basis of hydrolysis of the ester group in the polymeric prodrug. Hydrolysis of the ester group adjacent to the polymeric backbone is not facile because of steric reasons. The drug release, therefore, should depend only on the rate of hydrolysis of the ester group adjacent to the drug moiety. An acid or a base must always be present to attain

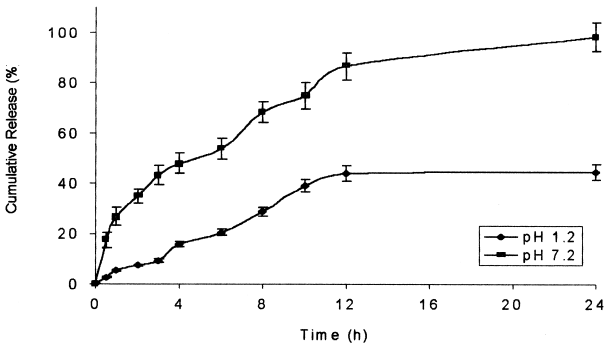

Figure 2. In vitro drug release profile of the prodrug.

suitable rates of hydrolysis. In an acidic environment, hydrolysis takes place by an acid-catalyzed mechanism, namely, protonation of the oxygen atom of the ester group with the concomitant attack on the hydroxyl group on the carbonyl carbon. Here, the acid, which is a catalyst, is regenerated. Further, acid-catalyzed hydrolysis reactions are reversible reactions tending toward equilibrium with an equilibrium constant of unity. In other words, the reaction does not proceed to completion (17). In an alkaline environment, however, hydrolysis takes place by a different mechanism, namely, a nucleophilic attack of the hydroxyl group on the electron-deficient carbonyl atom. Here, the base acts as a reactant (1 mole of the base is consumed per 1 mole of the ester hydrolyzed), and base hydrolysis reactions are irreversible reactions because the formation of the carboxylate anion is energetically more favorable. In other words, hydrolysis of esters in the alkaline environment is expected to proceed to completion. The polymeric prodrug synthesized thus released the drug predominantly in the alkaline environment. A slower release drug behavior of the prodrug at pH 1.2, when considered along with the residence time in the upper GI tract (which is less than 2 h), should mean that drug release should take place predominantly in the alkaline environment of the lower GI tract in a site-specific manner. What is more, in the present system in which the drug was covalently linked to poly(HEMA), drug release took place in a sustained manner, leading to a longer duration of drug activity.

The stability studies showed no significant change in the physical appearance of the prodrug or its IR pattern, drug content, and drug release behavior, thus indicating no chemical or physical changes occurred during storage.

The plasma concentration-time curves of the free drug and the prodrug are shown in Fig. 3. Detectable concentrations of the drug were observed for 18 h in the case of prodrug, but only for 10 h in the case of the free drug. The peak plasma concentration C_{max} for the free drug was 181.67 ± 14.22 ng/ml within 2 h T_{max}, whereas for the prodrug, it was 105.50 ± 12.95 ng/ml after a period of 4 h. The delay in T_{max} was thus due to the slower rate of drug release from the prodrug and its consequent absorption. There was no significant change in the extent of drug absorption between the free drug and the prodrug as the area under the plasma concentration-time curve (AUC) is almost the same, namely, 564.61 and 605.50 ng h/ml, respectively. This confirms the complete drug release from the polymeric prodrug and its potential in sustaining the drug release under in vivo conditions.

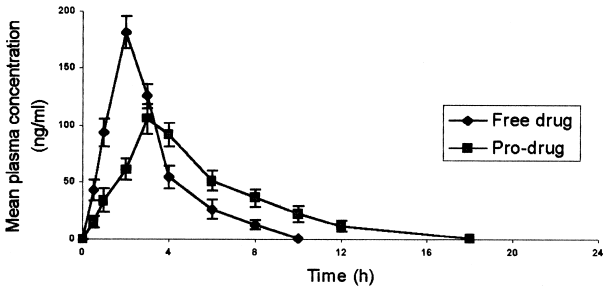

Figure 3. Plasma levels of diclofenac after free drug and prodrug administration.

The results of the ulcer-inducing study show that the solvent had no ulcer-inducing effect. The free drug was capable of inducing ulceration at the end of the 7th day of treatment, with an ulcer score of 1.33 ± 0.33, whereas the prodrug showed a statistically significant decrease ($P < .05$) in the ulcer score (0.33 ± 0.03). The low ulcer scores support our hypothesis of site-specific release and thus the capability of the polymeric prodrug in reducing the GI adverse effects of diclofenac sodium.

CONCLUSION

We conclude that the covalent linkage of diclofenac to a biocompatible polymer, poly(HEMA), through an ester group leads to a delivery system capable of releasing the drug in a sustained and site-specific manner to the lower gastrointestinal tract. The prodrug, therefore, is expected to reduce the frequency of administration and avoid or minimize the gastrointestinal adverse effects associated with diclofenac sodium. Toxicological and stability studies, however, are needed to establish its potential.

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