Optimizing delivery of flurbiprofen to the colon using a targeted prodrug approach

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Abstract

The carboxylic group responsible for the gastric side-effects of the propionic acid derivative, flurbiprofen, was masked temporarily to overcome these side-effects and to accomplish colon-specific delivery of the drug. An amide prodrug (FLU-GLY) was synthesized by coupling flurbiprofen with L-glycine. Confirmation and characterization of the structure of the synthesized prodrug included elemental analysis, Fourier transform (FT)-IR, FT-NMR, mass (FAB) spectroscopy, and determinations of R_f, R_t and R_M values, respectively. Aqueous solubility and lipophilicity (log P) value were determined at pH 1.2, 4.0, 6.8 and 7.4. In-vitro reversion of FLU-GLY to flurbiprofen was measured at different pHs and in a simulated colonic environment. Acute toxicity and ulceration potential were evaluated in-vivo in albino rats. Pre-formulation studies showed increased hydrophilicity but a non-significant increase in lipophilicity of the prodrug. In-vitro reversion studies suggested that the prodrug remained intact until colonic pH was attained, when the colonic microfloral enzymes (amidase) hydrolysed the FLU-GLY amide linkage, releasing the free drug. In-vivo evaluation indicated that the prodrug was much less toxic and had less ulcerogenic activity than the parent drug. Selective delivery of drugs to the colon can be useful in terms of reducing the dose administered and reducing undesirable side-effects.

Introduction

Inflammatory bowel disease (IBD), comprising Crohn's disease and ulcerative colitis, is characterized by relapsing and remitting episodes of active inflammation and chronic mucosal injury (Hugot et al 1999). The pathogenesis of IBD remains unclear. Numerous studies on the aetiology of IBD have shown that the diseases are the result of an exaggerated or insufficiently suppressed immune response, which leads to the beginning and persistence of these diseases (Helper & Rex 2001).

Currently, no drugs are available, and IBD treatment relies heavily on non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticosteroids and immunomodulators. The primary goal of drug therapy for IBD is to reduce inflammation in the colon, requiring frequent intake of high doses of NSAIDs, which may lead to gastric ulceration, bleeding and other gastric complications (Dhaneshwar et al 2007). The study of Cioli et al (1979) suggested that the direct contact of tissue with NSAIDs plays an important role in the production of gastrointestinal (GI) tract lesions. The study also confirmed that gastric side-effects were due to the presence of a carboxylic group in the parent drug. NSAIDs containing carboxylic groups are poorly absorbed from the GI tract because of unfavourable physiochemical properties. Colon-specific drug delivery (CSDD) has evolved out of the need to overcome this barrier of the GI tract, as an ideal delivery system for the topical treatment of diseases of the colon, namely IBD, colorectal cancer and amoebiosis. To achieve colonic delivery, a drug needs to be chemically and biochemically stable; it should not be absorbed in the upper intestine; and should be released abruptly into the proximal colon, which is considered to be the optimum site for CSDD (Chourasia & Jain 2003).

The prodrug approach is one of several promising tools for targeting drugs to the colon. CSDD through colon-specific prodrug activation may be accomplished by exploiting the high activity of certain enzymes at the target site compared with non-target tissues for conversion of the prodrug to active drug.

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The amino acid glycine is useful as a promoiety; it has broad-spectrum anti-inflammatory, cytoprotective and immunomodulatory properties (Habib et al 2006), and would also be expected to synergize with flurbiprofen activity. It is not only non-toxic but is also free from side-effects, being a natural component of our bodies. In order to reduce the gastric side-effects of flurbiprofen, structural modification with an amino acid was carried out to mask the carboxylic group temporarily. A strategic group attached to mask the carboxylic group would not only protect the vulnerable group and stabilize the molecule, but will also direct the drug to its target site. An increase in hydrophilicity has also been proposed due to the amino acid's polar group (Sinha & Kumria 2003).

To meet these requirements for colon specificity and reduction of the gastric side-effects of NSAIDs with a carboxylic group, a colon-specific prodrug of flurbiprofen using the amino acid glycine as a promoiety was synthesized, characterized and evaluated in pre-formulation and in-vitro kinetic studies. Its ulcerogenic activity and acute toxicity compared with those of flurbiprofen were evaluated in-vivo in albino rats.

Materials and Methods

Materials

Flurbiprofen was a gift from Elcon Drugs Pvt. Ltd (Gurgaon, Haryana, India). L-Glycine and HPLC-grade acetonitrile were purchased from Merck (India) (Bombay, India). Thionylchloride and N,N-dicyclohexylcarbodiimide were purchased from Spectrochem. Pvt. Ltd (Mumbai, India). Diethylether, methanol and N,N-dimethylformamide were obtained from Qualikems Fine Chemicals Pvt. Ltd (New Delhi, India). Triethylamine and n-butanol were purchased from Qualigens Fine Chemicals (Mumbai, India). HPLC-grade methanol and water, sodium sulfate (anhydrous), sodium bicarbonate and chloroform were purchased from Ranbaxy Fine Chemicals Ltd (New Delhi, India). Distilled water was used throughout the study. All other materials used were of analytical grade; those of synthetic grade were purified before use.

Animals

Albino rats were purchased from the Central Drug Research Institute (Lucknow, Uttar Pradesh, India) and were housed in the animal house at the Department of Pharmacology, Rajiv Academy for Pharmacy, Mathura, Uttar Pradesh, India. The in-vivo ulcerogenicity and acute toxicity studies were conducted with prior approval from the institutional animal ethical committee (IAEC/04/07/R$_1$).

Synthesis of the prodrug

The flurbiprofen–glycine prodrug (FLU-GLY) was synthesized in a two-step reaction (Figure 1). The first step was synthesis of L-glycine methyl ester (GME) hydrochloride. Freshly distilled thionylchloride (0.1 mol) was slowly added to methanol (300 mL) with cooling, after which 0.2 mol L-glycine was added. The mixture was refluxed for 10 h at 60–70°C with continuous stirring on a magnetic stirrer. The solvent was distilled at 64–65°C and the resulting product was collected and triturated with cold diethyl ether (50 mL) to remove excess dimethyl sulphite. The crude product obtained was recrystallized
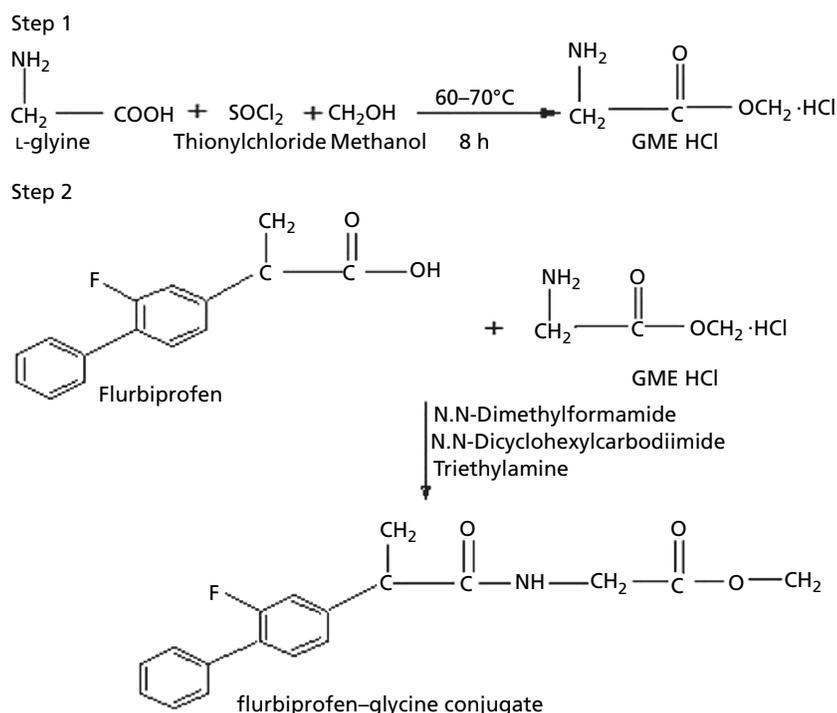
Figure 1 Reaction scheme for the synthesis of flurbiprofen–glycine prodrug. Step 1 was synthesis of L-glycine methyl ester (GME) hydrochloride. The second step was synthesis of the glycine conjugate of flurbiprofen.

with hot methanol by adding 20–25 mL diethyl ether followed by cooling at 0°C (Dhaneshwar et al 2006).

The second step was synthesis of the glycine conjugate of flurbiprofen. Twenty mmol of flurbiprofen, was dissolved in 60 mL N,N-dimethylformamide in conical flask A; 20 mmol N-N-dicyclohexylcarbodiimide was added to this with continuous stirring for 15 min. Separately, 20 mmol methyl ester hydrochloride of L-glycine was dissolved in 60 mL N,N-dimethylformamide in conical flask B, and 42 mmol triethylamine was added to it at 0°C. The contents of flask A were then added into flask B. The mixture was filtered, and an equal volume of distilled water was added to the filtrate, followed by extraction of the drug with ether. Anhydrous sodium sulfate (15 g) was added to the ether layer, and the crude product was recrystallized with methanol.

Confirmation of the prodrug structure

Elemental analysis and mass spectra of flurbiprofen (C, H, O) and FLU-GLY (C, H, N, O) were carried out on an elemental analyser (Elementar vario ELIII, Carlo Erba 1108, (Elementar Analysensysteme GmbH, Hanau, Germany) and mass spectrometer (Jeol SX 102/DA-6000, Jeol, Tokyo, Japan) at the Central Drug Research Institute (CDRI), Lucknow, Uttar Pradesh, India.

To identify the presence of organic functional groups, FT-IR spectra of flurbiprofen and FLU-GLY were recorded in potassium bromide (anhydrous grade) pellets, using a Simadzu-8400 S FTIR spectrophotometer (Simadzu, Tokyo, Japan).

To determine the nature of protons and protonated groups in flurbiprofen and FLU-GLY, the ^{1}H-NMR spectra in CDCl$_3$ were recorded on a Jeol AL 300, FT-NMR spectrometer (Jeol) at 300 MHz, using trimethylsilane as the internal standard; the chemical shifts (δ) were recorded in ppm.

Characterization of the prodrug

Thin-layer chromatography (TLC)
TLC of flurbiprofen and FLU-GLY was performed in triplicate using silica gel GF$_{254}$ as the stationary phase. Sample spots of the compound solution (5 μL) were loaded at 1.5 cm intervals. The compounds were allowed to develop by an ascending technique in a TLC jar, under conditions of equilibrium using a mobile phase of methanol:water (3:1 v/v). The plates were dried and the developed spots were localized using ultraviolet fluorescence at 254 nm. The R$_f$ values for the compound were determined as the average of three readings.

HPLC
HPLC analysis was carried out in triplicate on a Cecil 4200 system (Cecil Instruments Ltd, Cambridge, UK) using a 250×4.6 cm C$_{18}$ reverse-phase column, particle size 5 μm (Thermo Electron, Fife, Scotland, UK). The mobile phase was acetonitrile, methanol and water (ratio 2:1:2 by volume), delivered at a flow rate of 1 mL min^{-1}. Samples were injected onto the system using a 25 μL Hamilton syringe. Detection was at 247 nm (Shorbagi & El Aboul 1996). The analytical performance parameters (specificity, linearity, range, precision, accuracy, limit of detection and limit of quantification) were validated according to the International Conference on Harmonization ICH Q2B guidelines.

Deternation of R_M value
R$_M$ values were determined in triplicate by reverse-phase TLC. Silica gel GF$_{254}$ TLC plates were soaked for 5 h in acetone containing 3% v/v n-octanol and left to dry overnight (Saha et al 2002). Sample spots of 5 μL of compound solution were loaded at 1.5 cm intervals. The compounds were allowed to develop by an ascending technique in a chromatographic chamber under conditions of equilibrium using a mobile phase of methanol, water and chloroform (ratio 14:5:1 v/v/v). The plates were dried and the developed spots were localized under ultraviolet fluorescence at 254 nm. The R$_f$ values were determined for the compound as the average of three readings and the corresponding R$_M$ values were calculated using the formula $R_M = \log(1/R_f - 1)$.

Preformulation studies

Partition coefficient
Partition coefficients were determined according to the Hansch method (Avdeef et al 2000) between 10 mL n-octanol and 10 mL buffer of varying pH (1.2, 4.0, 6.8 and 7.4). Respective buffers and n-octanol were added to a separator (Hicon Ltd, New Delhi, India) mounted on an automatic shaker (Hicon). Both phases were saturated for 60 min with intermittent shaking. Weighed amounts of flurbiprofen and FLU-GLY were added to different separators, which were then shaken for 30 min to achieve drug distribution into both phases. The separators were allowed to stand for 5 min, and the aqueous and organic layers were separated, suitably diluted and analysed using the Cecil 4200 HPLC system described above, with UV detector set at 249 nm and 247 nm for FLU-GLY and flurbiprofen, respectively. Each experiment was performed six times.

Aqueous solubility
The aqueous solubilities of flurbiprofen and FLU-GLY were determined (n=6) by adding excess amounts of the solutes in HCl buffer pH 1.2, acid phthalate buffer pH 4.0 and phosphate buffer pH 6.8 and 7.4, and equilibrating them at 37°C in a water bath shaker. After 72 h of shaking, the samples were withdrawn, filtered, suitably diluted and analysed using the Cecil 4200 HPLC system described above, with UV detection at 247 nm and 217.5 nm for FLU-GLY and flurbiprofen, respectively.

Reversion studies
The reversion of the FLU-GLY to flurbiprofen was studied in HCl buffer pH 1.2, phthalate buffer pH 4.0, phosphate buffer pH 6.8, phosphate buffer pH 7.4 and phosphate buffer pH 6.8 containing fresh rat faecal content (20% w/v – to mimic the colonic environment) at 37±0.5°C. The ionic strength ($\mu=0.5$) was kept constant for each buffer by adjusting with a calculated amount of potassium chloride (Martin 1999). FLU-GLY was dissolved in sufficient volume of buffer to give a concentration of 1000 μg mL^{-1}; 1 mL of the prodrug solution was taken into glass vials and diluted to 10 mL with the buffer

to give a final concentration of $100\,\mu g\,mL^{-1}$. The vials were kept in a water bath at $37\pm0.5°C$. For analysis, 5 mL of solution was withdrawn from the vials at different time points and shaken with equal amount of n-butanol, to extract free flurbiprofen reverted from FLU-GLY. The concentration of flurbiprofen was estimated directly from the n-butanol layer using the Cecil 4200 HPLC system described above with UV detection at 315.5 nm. Each measurement was repeated six times.

In-vivo evaluation of FLU-GLY

Ulcerogenicity

Ulcerogenic activity was determined by the cold stress method (Rainsford & Whitehouse 1980), an acute model, used to determine ulcerogenic potency of a drug at 10 times the normal dose. Albino rats weighing 150–200 g were fasted overnight before administration of the compounds; water was available ad libitum. The animals were randomly distributed into control, drug and prodrug groups of six animals each. The control group received the drug vehicle – carboxymethyl cellulose (CMC, 1% w/v) by gavage. The drug (flurbiprofen) and prodrug (FLU-GLY) were administered orally, as fine particles suspended in 1% w/v CMC by continuous stirring. Following oral administration of 5 mL of the aqueous drug suspensions, the animals were stressed by exposure to cold ($-15°C$ for 1 h), with each in a separate polypropylene cage to ensure equal cold exposure. Animals were killed by cervical dislocation 2 h after drug administration.

The stomach and duodenal parts were opened along the greater curvature and the number of lesions was examined by means of a magnifying lens. The size of ulcers was measured by Microimage process software (DA1-180M v 2.01; Sunny International United Co., Ltd, Zhejiang, China), using an Olympus SP 350 camera (Olympus, Tokyo, Japan). Ulcers were scored as: 0 for normal-coloured stomach; 0.5 for red colouration; 1 for spot ulcers; 1.5 for haemorrhagic streaks; 2 for ulcers of 3 mm up to 5 mm; and 3 for ulcers of 5 mm and greater (Kulkarni 1999).

Acute toxicity study

Single-dose acute toxicity studies were carried out following OECD guidelines No. 401 (OECD 1987). Animals were fasted overnight but with free access to water. Rats were divided into three groups (control, drug and prodrug) each comprising three males and three females and were weighed just before the experiment. A dose of 2000 mg kg^{-1} of drug or prodrug, suspended in demineralized water using 1% w/v CMC as suspending agent, was administered orally by gavage. The animals in the control group received 1% w/v CMC in demineralized water. In each case, the volume administered was 5 mL. Any toxicity or mortality was recorded at 0.5, 1, 2, 4 and 6 h after dosing and twice a day thereafter for 14 days. All observations were recorded systematically, with individual records being maintained for each animal. Cage-side observations included evaluation of skin and fur; eyes; respiratory effects; autonomic effects, such as salivation, diarrhoea and urination; and central nervous system effects, including tremors and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli and altered strength. The rats were weighed again on

the 14th day, and the change in weight calculated and recorded. Rats were killed by cervical dislocation on day 14.

Gross pathological evaluation was performed, and the weights of the heart, liver, kidney, stomach and spleen were measured and recorded immediately afterwards. Relative organ weight (organ to body weight ratio) was also calculated for each organ. Histopathological examination was performed on routinely prepared sections of stomach tissues; the tissues were fixed in 10% v/v formalin immediately after removal to avoid autolysis, and weighed.

Statistical analysis

The cumulative percentage of flurbiprofen released from all the formulations ($n=6$) in the dissolution medium, with and without rat caecal contents, was compared, and the statistical significance tested using two-way analysis of variance followed by Dunnett's multiple comparison t-test. $P<0.05$ was considered significant.

Results and Discussion

Characterization of flurbiprofen and the FLU-GLY conjugate

The FLU-GLY prodrug was synthesized in two steps: the intermediate GME HCl was synthesized from glycine, and then coupled with flurbiprofen to form the FLU-GLY prodrug. Flurbiprofen and FLU-GLY were characterized from R_f, R_M, and R_t values. R_f was 0.26 ± 0.13 for GLY, 0.39 ± 0.22 for GME, 0.70 ± 0.09 for flurbiprofen and 0.75 ± 0.13 for FLU-GLY. R_M values were -0.486 ± 0.11 for flurbiprofen and -0.276 ± 0.18 for FLU-GLY. R_t values were 4.16 ± 0.16 min for flurbiprofen and 2.07 ± 0.21 min for FLU-GLY, indicating the formation of a new product.

The synthesized prodrug was further characterized by analytical techniques. The theoretically elemental analysis calculated for FLU-GLY ($C_{18}H_{18}FNO_3$) was C: 68.59%, H: 5.71%, N: 4.44%, O: 15.22%; practically, elemental analysis was C: 68.51%, H: 5.69%, N: 4.40%, O: 15.19%. FT-IR (cm^{-1}) (KBr): 2990 (N-H stretching amide), 1735 (C=O stretching ester), 1650 (C=O stretching amide), 1535 (N-H bending amide), 1250 (C-N stretching), 1090 (C-O stretching ester). ^{1}H-NMR (CDCl$_3$): 7.6 (benzene, 3H, m),7.48 (benzene, 5H, m), 3.9 (CH, 1H, q), 1.6 (CH$_3$, 3H, d), 7.27–7.42 (CONH 1H, m), 3.7 (CH$_3$ of ester 3H, s),1.48 (GH$_2$, 2H, d).

High-resolution FAB-MS theoretical calculation of M+ for $C_{18}H_{18}FNO_3$ resulted in a value of 315.15; the observed value was 315.

Thus, these analytical techniques confirmed the structure, molecular formula and molecular weight of the synthesized FLU-GLY conjugate.

Preformulation studies

Aqueous solubility and partition coefficient

The aqueous solubility and partition coefficients of flurbiprofen and FLU-GLY in different buffers (pH 1.2, 4.0, 6.8 and 7.4) are shown in Table 1. Aqueous solubilities of flurbiprofen and

Table 1 pH solubility profile and partition studies of flurbiprofen and flurbiprofen–glycine (FLU-GLY)

	Solubility (mg mL^{-1})		log P	
	FLU	FLU-GLY	FLU	FLU-GLY
HCl buffer, pH 1.2	0.22 ± 0.12	1.38 ± 0.09	3.55 ± 0.11	3.69 ± 0.12
Phthalate buffer, pH 4.0	0.28 ± 0.23	1.66 ± 0.13	2.66 ± 0.18	2.85 ± 0.15
Phosphate buffer, pH 6.8	4.87 ± 0.15	5.77 ± 0.22	2.32 ± 0.24	2.55 ± 0.19
Phosphate buffer, pH 7.4	7.35 ± 0.18	8.03 ± 0.10	2.22 ± 0.14	2.25 ± 0.11

Data are mean $\pm$ s.d. (n = 6).

FLU-GLY increased with increasing pH, which is probably due to an increase in the ionization of the compounds as the pH increased. The solubility of FLU-GLY was higher than that of flurbiprofen across this pH range. This may be due to the presence of highly polar groups, namely $-NH_2$ and $-COOH$ in glycine, which increase the polarity of the conjugate much more so than the less polar carboxylic group of flurbiprofen. This theory is supported by reverse-phase HPLC: the C_{18} column used as the stationary phase had less affinity for polar drugs (i.e. polar drug would elute first). R_t values for flurbiprofen and FLU-GLY were 4.16 and 2.07 min, respectively, confirming the higher polarity of the conjugate compared with flurbiprofen.

The enhancement of log P values of the prodrug compared with flurbiprofen was negligible at 95% confidence interval ($P > 0.3241$). The slight increase might be due to the more lipophilic character of the prodrug, which is supported by the R_M values of the conjugate and the parent compound. The higher the R_M value, the higher the lipophilicity of the compound. R_M values of FLU-GLY and flurbiprofen were −0.276 and −0.486, respectively; thus, the prodrug is slightly more lipophilic than flurbiprofen.

In-vitro reversion study

In-vitro kinetic studies confirmed negligible reversion of FLU-GLY in the gastric environment (HCl buffer pH 1.2) and caecal environment (acid phthalate buffer pH 4.0), and only 6.05% and 8.14% of the prodrug reverted to flurbiprofen in the intestinal environment (phosphate buffer pH 6.8 and 7.4, respectively) over a period of 8 h ($P = 0.3452$, t = 0.876; Figure 2). Reversion was also studied in phosphate buffer pH 6.8 in the presence of fresh rat faecal matter (20% w/v), to confirm the colonic breakdown of the prodrug. In this simulated colonic environment, 67.83% of the prodrug reverted to flurbiprofen over a period of 48 h, with first-order kinetics. The marked reversion ($P = 3.657$, t = 2.891) in the colonic environment was due to hydrolysis of the prodrug catalysed by the amidase enzyme released by colonic microflora. In-vitro reversion studies suggested that the prodrug might bypass the GI tract and would be reverted to flurbiprofen by amidase enzyme in the colon.

In-vivo evaluation of FLU-GLY

Ulcerogenic study

The ulcerogenic activity of flurbiprofen and FLU-GLY was determined by the cold stress method. Values for the ulcer
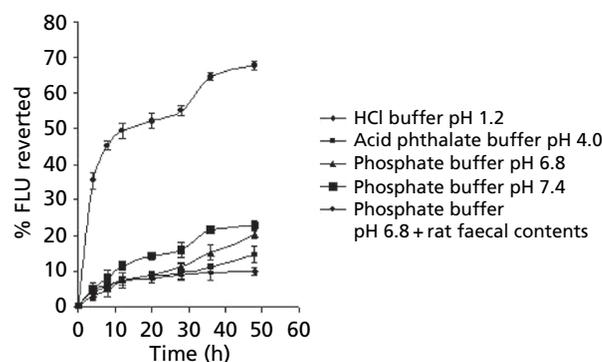
Figure 2 In-vitro reversion of flurbiprofen–glycine to flurbiprofen (FLU) at different pHs and in a colonic environment.

index (median $\pm$ range) were 2 ± 1 for control animals, 66.2 ± 4.1 for the flurbiprofen group and 17.0 ± 1.2 for the FLU-GLY group, indicating marked differences in the ulcerogenic activity of the prodrug vs flurbiprofen ($P = 3.234$). No haemorrhagic or red spots were found on the stomach walls of control animals (Figure 3A). The stomach walls of animals treated with flurbiprofen showed severe congestion, numerous haemorrhagic spots, streaks, erosion of the gastric mucosa, deep ulceration and necrotic cells (Figure 3B). Stomachs from animals treated with FLU-GLY showed haemorrhagic and red spots but no necrosis of the cells (Figure 3C). On comparing histopathology of the stomachs of control rats of (Figure 4A) and those treated with drug (Figure 4B) and prodrug (Figure 4C), more severe haemorrhage, ulcers and necrosis were evident in the drug group than the prodrug group.

Acute toxicity

Following administration of single doses of flurbiprofen and FLU-GLY, there were no overt signs and symptoms of toxicity in any of the dosed groups on the first day, except for weakness. One rat in the drug group was found dead on the first day of study. The anomalies waned over time and all surviving animals became overtly normal until termination of the study. There was no significant difference in the activities observed and no significant difference (one-way ANOVA, $P < 0.05$) in body weight gain (Table 2) or organ weight at the end of the study ($P = 0.3345$, t = 1.23).
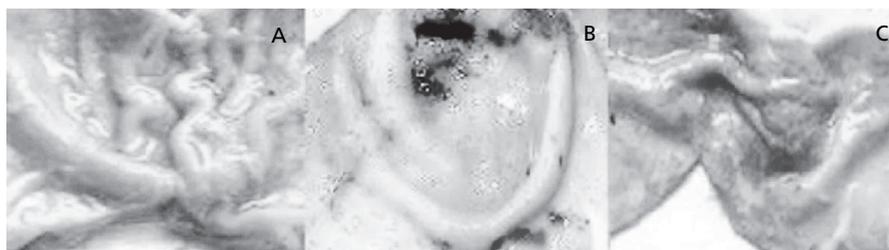
Figure 3   Evaluation of ulcerogenic activity in the stomachs of albino rats treated with drug vehicle (1% w/v CMC (A); flurbiprofen (1000 mgkg^{-1}) (B) and flurbiprofen–glycine (1000 mgkg^{-1}) (C).
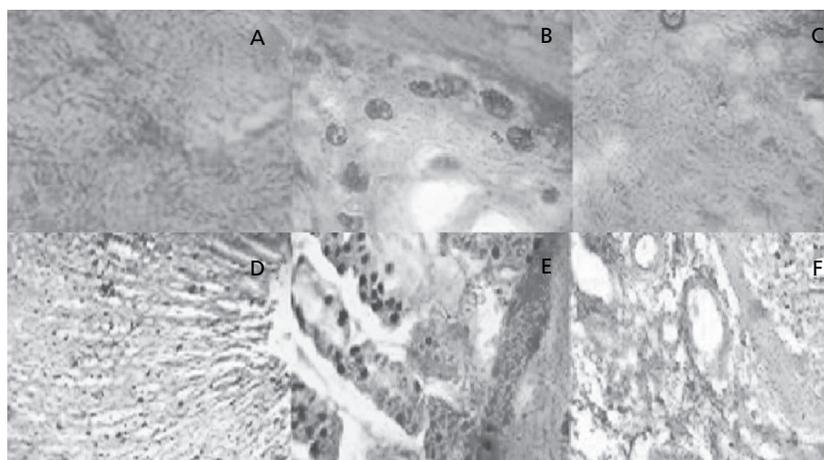
Figure 4   Histopathology of stomachs from albino rats treated with drug vehicle (1% w/v CMC (A); flurbiprofen (1000 mgkg^{-1}) (B) and flurbiprofen–glycine (1000 mgkg^{-1}) (C), and in stomachs from rats on the 14th day of the acute toxicity study, treated with drug vehicle (2% w/v CMC (D); flurbiprofen (2000 mgkg^{-1}) (E) and flurbiprofen–glycine (2000 mgkg^{-1}) (F).

Table 2   Weight (in grams) of the albino rats during the toxicity study

Treatment	day 0	day 14	Weight gain	% increase in weight
Control	161 ± 11.31	173 ± 12.72	12 ± 1.41	7.45
Drug	173 ± 16.26	184 ± 18.38	10 ± 2.12	6.05
Prodrug	171 ± 14.84	183 ± 16.97	11 ± 2.13	6.70

Values are mean ± s.d. (n = 6).

Gross pathological examination at autopsy showed cysts in the stomach and haemorrhagic abdomen in rats that had received a single dose of flurbiprofen. No changes were observed in the other organs. Histopathological evaluation of tissues from the stomachs of the drug group (Figure 4E) revealed a number of lesions, such as eosinophilic reaction at the junction of the stomach and oesophagus, severe haemorrhage, glandular destruction and severe necrosis. The prodrug group (Figure 3F) showed oedema, haemorrhage and a small amount of glandular destruction. Normal glandular arrangement was observed in the control group (Figure 3A).

The microscopic appearance of liver, kidney, heart, spleen, stomach and intestine was essentially similar in all the animals.

Result obtained in albino rats showed that FLU-GLY exhibited moderately less acute toxicity than flurbiprofen. The 50% lethal dose (LD50) was estimated to exceed 2000 mgkg^{-1} for both FLU-GLY and flurbiprofen. The marked reduction in the ulcerogenic activity and less toxic effects of the prodrug (FLU-GLY) compared with flurbiprofen (one-way ANOVA, $P < 0.05$) may be due to temporary masking of the carboxylic group of flurbiprofen, making the prodrug safe for oral use. These results, coupled with the estimation of the higher LD50, indicate that the prodrug us safe, even at higher doses.

Conclusions

The synthesized prodrug of flurbiprofen had increased solubility, lower toxicity and less ulcerogenic activity than the parent drug flurbiprofen. Thus, this prodrug approach solves not only the formulation problem of flurbiprofen (lower aqueous solubility, BCS class II drug) and reduces adverse effects, but also enables targeted delivery of the drug to the colon. Selective delivery to the colon would be more

effective in therapy in terms of reduced dose and fewer side-effects.

References

Avdeef, A., Berger, C. M., Brownell C. (2000) pH metric solubility: correlation between the acid-base titration and the saturation shake flask solubility-pH methods. *Pharm. Res*. **17**: 85–89

Chourasia, M. K., Jain, S. K. (2003) Pharmaceutical approaches to colon targeted drug delivery systems. *J. Pharm. Sci*. **6**: 33–66

Cioli, V., Putzolu, S., Rossi, V., Carrandino, C. (1979) The role of direct tissue contact in the production of gastrointestinal ulcers by anti-inflammatory drugs in rats. *Toxicol. Appl. Pharmacol*. **50**: 283–289

Dhaneshwar, S. S., Nagpal, D., Singh, R., Gairola, N., Bodhankar, S. (2006) Mutual azo prodrug of 5-aminosalicylic acid for colon targeted drug delivery: synthesis, kinetic studies and pharmacological evaluation. *Ind. J. Pharm. Sci*. **68**: 171–178

Dhaneshwar, S. S., Gairola, N., Kandpal, M., Vadnerkar, G., Bhatt, L. (2007) Colon-specific, mutual azo prodrug of 5-aminosalicylic acid with L-tryptophan: synthesis, kinetic studies and evaluation of its mitigating effect in trinitrobenzenesulfonic acid-induced colitis in rats. *Bioorg. Med. Chem*. **15**: 4903–4909

Habib, M. M., Hodgson, H. J., Davidson, B. R. (2006) The role of glycine in hepatic ischemia-reperfusion injury. *Curr. Pharm. Design* **12**: 2953–2967

Helper, D. J., Rex, D. K. (2001) Inflammatory bowel disease. *Endoscopy* **33**: 140–146

Hugot, J. P., Zouali, H., Lesage, S., Thomas, G. (1999) Etiology of the inflammatory bowel diseases. *Int. J. Colorectal Dis*. **14**: 2–9

Kulkarni, S. K. (1999) Pharmacology of gastro-intestinal tract (GIT). In: Kulkarni, S. K. (ed) *Handbook of experimental pharmacology*, 3rd edn. New Delhi: Vallabh Prakashan, pp. 148–150

Martin, A. (1999) Ionic equilibria. In: Martin, A., Bustamante, P., Chun, A. H. C. (eds) *Physical pharmacy*, 4th edn. New Delhi: B. I. Waverly Pvt Ltd, pp. 143–168

OECD (1987) Acute oral toxicity test method. In: *OECD Guidelines for testing of chemicals*, No. 404. Organization for Economic Cooperation and Development, Paris, France

Rainsford, K. D., Whitehouse, M. W. (1980) Anti-inflammatory/anti-pyretic salicylic esters with low gastric ulcerogenic activity. *Agents Actions* **10**: 451–456

Saha, R. N., Ravishankar, D., Jadhav, P. R., Sanjeev, C. (1992) Determination of flurbiprofen in pharmaceutical formulations by UV spectrophotometry and liquid chromatography. *Analytica Chimica Acta* **463**: 207–217

Shorbagi, A., El Aboul, F. T. (1996) New prodrug approach for amino acids and amino-acid like drugs. *Eur. J. Med. Chem*. **31**: 165–169

Sinha, V. R., Kumria, R. (2003) Microbially triggered drug delivery to colon: review. *Eur. J. Med. Chem*. **18**: 3–18