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Synthesis and evaluation of amide prodrugs of mefenamic acid for colon targeting

Shweta Gogate * and Vishal Gupta

Department of Pharmaceutical chemistry, Mansarovar Global University, Bilkisganj, Sehore, M.P.

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Abstract

Prodrug approach is one of the important approaches for targeting drugs to colon. Prodrug design has paved a way to overcome the undesirable properties associated with the existing drug and successful site-specific drug delivery to varied organs and tissues. Colon-specific drug delivery through colon-specific prodrug activation may be accomplished by the utilization of high activity of certain enzymes at the target site relative to non-target tissues for prodrug to drug conversion. For the present studies mefenamic acid was selected because of being curative agents for most prevalent colon disease namely intestinal bowel disease due to any reason. Anti-inflammatory therapy, at present, involves use of corticosteroids, as all NSAIDs are absorbed in the stomach and they do not reach to colon. Most of the NSAIDs have free carboxylic acid groups, although, it is important for their activity but they can be targeted to colon via formation of mutual prodrugs (amide). Hydrolytic enzymes of stomach to ileum do not hydrolyze such mutual prodrugs. Absorption of the NSAIDs primarily takes place in the stomach and followed with jejunum due to lipophilicity of the unionized form. Thus, they do not reach to the colon and also ulcerogenic which can also be avoided by formation of their mutual prodrugs. Ordinary treatment of IBD requires frequent intake of anti-inflammatory drugs at higher doses. Most of these drugs are rapidly absorbed from small intestine with very small fraction actually reaching the site of action i.e. colon. Interaction with non-targeted sites leads to significant adverse effects. Therefore, out of the need to overcome this formidable barrier of GIT, colon-targeted delivery has evolved as an ideal drug delivery system for the topical treatment of local diseases of colon like inflammatory bowel disease. Minimizing drug-induced side effects and mortality are the main challenges during management of IBD.

Keywords: Colon-specific drug delivery; Ulcerative colitis; Mefenamic acid; Prodrug; Anti-inflammatory activity

1. Introduction

The oral route is considered to be most convenient for administration of drugs to patients. Oral administration of conventional dosage forms normally dissolves in the stomach fluid or intestinal fluid and absorb from these regions of the GIT depends upon the physicochemical properties of the drug. It is a serious drawback in conditions where localized delivery of the drugs in the colon is required or in conditions where a drug needs to be protected from the hostile environment of upper GIT. Dosage forms that deliver drugs into the colon rather than upper GIT proffers number of advantages. Oral delivery of drugs to the colon is valuable in the treatment of diseases of colon (ulcerative colitis, crohn's disease, carcinomas and infections) whereby high local concentration can be achieved while minimizing side effects that occur because of release of drugs in the upper GIT. The colon is attracting interest as a site where poorly absorbed drug molecule may have an improved bioavailability. Also, the colon has a longer retention time and appears highly responsive to agents that enhance the absorption of poorly absorbed drugs. Apart from retarding and targeting dosage forms, a reliable colonic drug delivery could also be an important starting position for the colonic absorption of perorally applied, undigested, unchanged and fully active peptide drugs. The presence of colonic microflora (enterobacteria) that is responsible for specific enzymatic activity. The colonic bacteria are predominately anaerobic in nature and secrete

* Corresponding author: Shweta Gogate

enzymes that are capable of metabolizing substances such as carbohydrates and proteins that escape the digestion in the upper GI tract.1,2

Earlier colon was considered as a black-box acting as a site for production and temporary storage of excreta and responsible for absorption of electrolytes and water. But, because of challenging issue of treating local pathologies of colon, it has emerged as an organ of significance for target-specific delivery of drugs. There are many attributes of colon that can be explored and exploited for site-specific delivery of drugs such as :Less hostile environment, near neutral pH, less diversity and intensity of enzymatic activities than stomach and small intestine, long colonic transit (20-30 h) for extended absorption window, highly responsive to absorption enhancers, unique microbial flora and enzymes, minimized systemic exposure of drugs, reduced risk of first-pass metabolism, more chances of drug being available in its effective concentration, lower dosing and prevalence of systemic side effects and Attractive site for drugs which are hydrophilic or poorly absorbed from upper GIT.3,4

2. Colonic microflora and secreted enzymes:

Out of the 100 trillion microorganisms residing in the GIT, maximum anaerobic population is found in large intestine which is involved in fermentation of carbohydrates/proteins that escape digestion in the upper GIT. Drugs and xenobiotics are extensively metabolized by diverse array of enzymes secreted by colonic microbiota. As we travel from stomach to large intestine, the population of bacteria goes on increasing from 102-104 cfu/ml in stomach and 105-107 cfu/ml in lower small intestine to the maximum of 1011-1012 cfu/ml in the colon where, 102-104 times more anaerobic bacteria than aerobes are found. That is the reason why the occurrence of oxidative metabolism is comparatively rare in the colon as compared to liver. The reductive and hydrolytic pathways dominate the metabolic scene in the colon. The concentration of proteolytic enzymes is also low in colon that makes it a promising site for delivery of proteins and peptides. Sudden increase in the population of bacteria and related rise in the concentration of secreted enzymes in the colon is considered as a non-continuous event which is not dependent on GI transit time. This is a preferable setup that can be utilized as a triggering mechanism for the activation of a colon-specific drug delivery system. The various enzymes catalyzing reduction reactions in colon are azoreductases (azo compounds), nitroreductases (aromatic and heterocyclic nitro compounds), sulfoxide reductases (sulfoxides), hydrogenases (aliphatic double bonds and carbonyl groups) and N-oxide reductase (N-oxides). Glycosidases, glucuronidases, N-acyl amidases, sulfatases and esterases secreted by the colonic microflora are involved in hydrolysis of β-glycosides and glucuronides, amides with amino acids, sulfates and sulfamates and esters of carboxylic acids with polysaccharides respectively.5-7

The colon targeted drug delivery is developed for the effective management of IBD (Ulcerative colitis, Crohn's disease), local pathologies, chronotherapy (asthma, hypertension, cardiac arrhythmias, arthritis or inflammation), greater responsiveness to the absorption enhancers, less enzymatic activity, site for delivery of delicate drugs (Proteins and Peptides), oral delivery of vaccines as it is rich in lymphoid tissues.8

2.1. Prodrug formation for colon targeting

This approach involves the formation of prodrug for targeting drugs to colon. It involves the formation of a covalent linkage between drug and carrier in such a way that upon oral administration the moiety remains intact in the stomach and small intestine. The problem of stability of certain drugs from the adverse environment of the upper GIT can be eliminated by prodrug formation, which is converted into parent drug molecule once it reaches into the colon. Site specific drug delivery through site specific prodrug activation may be accomplished by the utilization of some specific property at the target site, such as altered pH or high activity of certain enzymes relative to the non-target tissues for the prodrug-drug conversion.9,10

2.2. Evaluation Techniques for CDDS

In-vitro evaluation, no any standardized estimate method is accessible for assessment of CDDS because an ideal in-vitro model should acquire the in-vivo environment of GIT such as pH, stirring, bacteria, enzymes, volume, enzyme activity, and other components of food. Generally, these circumstances are inclined by the diet, physical stress, and these factors make it hard to plan a standardin-vitro model. In vitro models used for CDDS are:

2.2.1. In vitro dissolution test

Dissolution of controlled-release formulations employed for colon-specific drug delivery are mainly hard, and the dissolution techniques described in the USP cannot fully imitate in-vivo situation such as those relating to bacterial environment, pH and mixing forces. Dissolution tests describing to CDDS may be carried out using the conservative basket method. Parallel dissolution studies in diverse buffers may be undertaken to distinguish the behaviour of

formulations at different pH levels. Dissolution tests of a colon- specific formulation in different media simulating pH circumstances and times likely to be stumble upon at different locations in the gastrointestinal tract have been studied. The media chosen were examined to simulate gastric fluid, pH 6.8 to simulate the jejunal region of the small intestine, and Ph 7.2 to simulate the ileum segment. Enteric- coated capsules for CDDS have been examined in a gradient dissolution study in three buffers.11-14

2.2.2. In vitro enzymatic tests

Incubation of carrier drug system in fermenter holding appropriate medium for bacteria (B. ovatus and Strectococcus faccium). The quantity of drug produced at dissimilar time intervals are determined. Drug release study is completed in buffermedium containing enzymes (dextranase, ezypectinase), or rat or guinea pig or rabbit cecal contents. The quantity of drug produced in a particular time is done, which is proportional to rate of deprivation of polymer.15

2.2.3. In vivo evaluation

A number of animals such as guinea pigs, rats, dogs, and pigs are used for screening the delivery of drug to colon because they look like the anatomic and physiological circumstances as well as the microflora of human GIT. While deciding a model for testing the CDDS, comparative model for the colonic diseases should also be measured. Guinea pigs are mainely used for experimental IBD model. The distribution of azoreductase and glucouronidase potential in the GIT of rat and rabbit is fairly equivalent to that in the human.16,17

3. Methodology

3.1. Physicochemical Characterization of drug and prodrugs

3.1.1. Melting Point Determination

The melting points of the drug and the synthesized conjugates were determined by open capillary tube using Toshniwal Melting Point Apparatus and errors are uncorrected.18

3.1.2. Thin Layer Chromatography

The purity of the synthesized derivatives was ensured by subjecting to thin layer chromatography. It was carried out on silica gel precoated plates of Merck with acetone: chloroform: acetic acid (3:2:1). as solvent system used for prodrugs and iodine vapours and UV light were used as detecting agent for visualization. All synthesized derivatives gave brown spot. Rf values were calculated from the TLC plates.19

3.1.3. Partition coefficient determination

Partition coefficient was determined in octanol/ phosphate buffer (pH 7.4) at 37± 1ºC. n-Octanol and water were mutually saturated with each other prior to use. A prodrug (10 mg) was dissolved in n-octanol (10 mL) and 10 mL distilled water was slowly added to it and the octanol- water mixture was shaken for 24 h on a wrist shaker to reach distribution equilibrium. The two layers were separated by separating funnel and aqueous layer was estimated by JascoV-530, UV- Visible double beam spectrophotometer at pre-determined λmax.20

3.2. Synthesis of mutual amide prodrugs of mefenamic acid with amino acids

3.2.1. Synthesis of amino acid methyl ester hydrochloride:

Freshly distilled thionyl chloride (0.05mol + 30% excess: 5 mL) was slowly added to methanol (100 mL) with cooling and amino acid (0.1mol) was added to it. The mixture was refluxed for 7-8 h at 60-70°C with continuous stirring on Radley's six station parallel synthesizers. Excess of thionyl chloride and solvent was removed under reduced pressure on a rotary evaporator giving crude ester which was triturated with 20 mL portions of cold ether at 0°C, until dimethyl sulphite was completely removed and dried under high vacuum. It was recrystallized from hot methanol by slow addition of 15-20 mL of ether followed by cooling at 0°C. Crystals were collected on the next day, washed twice with ether: methanol mixture (5:1; v/v) followed by pure ether and dried under vacuum to give pure product.21

3.2.2. Conversion of AAME. HCl to amino-acid methyl ester (AAME)

To suspension of (0.025mol) in 30 mL chloroform, TEA (0.05mol) was added with stirring at 0ºC for 30min. The solvent was distilled off under vacuum and the dry residue of ester was used as such for coupling step.22

3.2.3. Synthesis of mutual amide prodrugs of mefenamic acid with amino acids by CDI coupling

Mefenamic acid (0.001mol) was dissolved in DCM (10 mL) and to this solution CDI (0.0015 mol) was added at room temperature with stirring for 2-4 h. AAME (0.001 mol) in DCM (10 mL) was then added to the above solution and refluxed at 45ºC for 16-20 h. The completion of reaction was monitored by TLC using DCM: n-hexane: TEA (0.8.0.2:0.05; v/v/v). The reaction mixture was washed with distilled water (3 x 10 mL) and saturated solution of sodium bicarbonate (2 x 10 mL). The organic layer was separated and dried over anhydrous sodium sulphate. The residue obtained upon evaporation of organic layer was recrystallized with ethanol. Purification of prodrugs of mefenamic acid with amino acids was achieved by column chromatography using ethyl acetate: hexane (80:20; v/v).23,24

3.3. *In vitro* release studies of synthesized mutual prodrugs

The weighed amount of prodrug (10 mg) was transferred to 100 ml volumetric flask and volume was made up to 100 ml with PBS (pH 7.4) to obtain a stock solution of 100 µg/ml. From this 1 ml was withdrawn each time and taken in 10 ml volumetric flask. Volume was made up to 10 ml separately with SGF, SJF, SIF and SCF, respectively. These solutions were scanned between 220-380 nm on Shimadzu 1700 UV double beam spectrophotometer. Same procedure was followed for SIF and rat fecal matter.25,26

3.4. Pharmacological screening of drugs and prodrugs

3.4.1. Anti Inflammatory Activity

In the present study, the anti-inflammatory activity of drugs and prodrugs were determined by hind paw oedema method using carrageenan (0.1 ml, 1 % w/v) as phlogistic agent. Wistar albino rats (150-200 g) were divided into different groups, each comprising of six animals, including a control and a standard group. The initial volume of right hind paw of rat was measured by plethysmometer without administration of drug. A 1 % sodium carboxy methyl cellulose (CMC) suspension containing drug (100 mg) was prepared and a volume of this suspension containing an equivalent dose (Mefenamic acid-50 mg/kg/body wt) was administered orally to the standard groups. Similarly equivalent quantity of each prodrug was administered to the test groups. After 30 min of administration of the drug or prodrugs, carrageenan solution in normal saline was injected into the planter surface of right hind paw of each animal. The volume of swelling of right hind paw of each rat was measured after 0.5, 1, 2, 4 and 6 h. The mean increase in the volume of the right hind paw of rats was compared with control and standard. The percent inhibition of paw oedema was calculated as

$$\text{Percentage inhibition} = (1-Vt/Vc) \times 100$$

where Vt - mean relative change in paw oedema volume in test group, Vc – mean relative change in paw oedema volume in control group.27,28

3.4.2. Ulcerogenic Activity

Gastrointestinal toxicity of the drugs and prodrugs was measured and compared with the parent drug by measuring mean ulcer index. Wistar albino rats were divided into different groups, each comprising six animals, including a control and standard group. The control group was administered orally by 2 % acacia suspension. Test compounds and standard were administered orally (at 10 times higher dose) as a suspension with 2 % acacia daily for 5 days. The rats were fasted after the administration of last dose, thereafter they were sacrificed by decapitation and the stomach was removed, opened and washed with distilled water. The lesions on the gastric mucosa were counted by visual examination using a binocular magnifier. Ulcers greater than 0.5 mm were recorded. The mean ulcer index (UI) was calculated by severity of gastric mucosal lesions which are graded as grade 1: less than1 mm erosions, grade 2: 1-2 mm erosions and grade 3: more than 2 mm erosions. The UI was calculated as.29,30

$$UI = [1\times (\text{number of lesions of grade 1}) + 2\times (\text{number of lesions of grade 2}) + 3\times (\text{number of lesions of grade 3})]/10$$

3.5. TNBS induced experimental colitis model

3.5.1. Induction of Colitis

Rats were fasted for 24 h before experimentation. Rats were lightly anesthetized with ketamine and xylazine (20mg/kg and 5mg/kg, i.m.). A polyethylene catheter with 2 mm diameter was inserted through the rectum into the colon to a distance of 8 cm. For ulcerative colitis induction, TNBS dose was 150 mg/kg of body weight of TNBS in ethanol, 50% solution) was infused into the colon of all rats (except the normal control group) through the catheter, held in place for 30 sec. The catheter was left in place for few seconds then gently removed. For 3 days the rats were housed without

treatment to maintain the development of a full inflammatory bowel disease model with full access of food and water ad libitum. The animals of standard and test groups received orally sulfasalazine and prodrugs respectively, once daily for five continuous days. The normal control and colitis control groups received only 1% carboxy methylcellulose instead of free drug or prodrug.31

3.5.2. Assessment of colonic damage by clinical activity score:

The animals of all groups were examined for weight loss, stool consistency and rectal bleeding throughout the 11 days study. The clinical activity score was determined by calculating the average of the above three parameters for each day, for each group and was ranging from 0 (healthy) to 4 (maximal activity of colitis).32

3.6. Measurement of MPO activity in TNBS-induced colitis

The activity of intestinal MPO, was measured using the method of Krawisz , with minor modifications. Briefly, intestinal tissue samples (approximately 50-100 mg) were homogenized on ice using a polytron (13, 500 rpm, one minute) in a solution of 0.5% HTAB in 50 mM potassium phosphate buffer (HTAB, pH 6.0, 1 mL per 50 mg tissue). The resulting homogenate was subjected to three rapid freezing (70°C) and (immersion in warm water, 37°C) cycles. The samples were then centrifuged (4000 rpm, 15 minutes, 4°C) to remove insoluble material. The MPO containing supernatant (0.1 mL) was assayed spectrophotometrically after addition of 2.88 mL phosphate buffer (50 mM, pH 6.0) containing 0.167 mg/mL o-dianisidine hydrochloride and 10 µL 0.0005% hydrogen peroxide. The kinetics of absorbance changes at 470 nm was measured. Sample enzyme activity was calculated with a standard curve of known MPO unit activity. One unit of MPO activity, defined as the quantity of enzyme able to convert 1 µmol of hydrogen peroxide to water in one minute at room temperature, was expressed in mU/100 mg of tissue.33

4. Results:

4.1. Characterization of mefenamic acid prodrugs

Table 1 Physicochemical characterization of amide prodrugs

Prodrug	Colour	Melting point (^{o}C)	Yield (%)	Rf value	Log P
MA1	Yellow	155-157	67	0.54	1.86
MA2	Yellow	166-168	57	0.56	1.06
MA3	Yellow	171-172	74	0.46	0.96
MA4	Yellow	178-179	68	0.49	0.73

Spectral data of amide prodrug of mefenamic acid

- **Spectral data of amide prodrug of mefenamic acid-Isoleucine (MA1)**

IR (KBr, cm-1): 3420 (NH str), 2920 and 2857 (CH str.), 1670 (CO str. of ester), 1620, 1586, 1470 and 1407(C=C of aromatic ring), 1260 (OCH3), 756 (1,2-ortho, disubstituted);

1H NMR (δ, ppm) (DMSO): 8.37-8.20 (m, 4H, aromatic ring), 7.91-7.73 (d, 3H, CH in ring), 4.35 (d, 1H, CONH), 3.82 (1H, NH in ring), 3.30 (s, 3H of OCH3), 2.176(s, 3H, of CH3), 1.21 (d, 2H of CH2).

- **Spectral data of amide prodrug of mefenamic acid-Cysteine (MA2)**

IR (KBr, cm-1): 3439 (NH str.), 2945 and 2821 (CH str.), 2552(SH str.), 1686 (CO str. of ester), 1610, 1576, 1480 and 1405(C=C of aromatic ring), 1263 (OCH3), 750 (1,2-ortho, disubstituted);

1H NMR (δ, ppm) (DMSO): 8.38-8.22 (m, 4H, aromatic ring), 7.89-7.75 (t, 3H, CH in ring), 3.33 (d, 1H, CONH), 2.89 (1H, NH in ring), 2.81 (s, 3H of OCH3),2.54(t, 1H, SH), 2.49(s, 3H, of CH3), 2.42 (d, 2H of CH2).

- **Spectral data of amide prodrug of mefenamic acid-Glutamic acid (MA3)**

IR (KBr, cm-1): 3446 (NH str. of amide), 2952 and 2832 (CH str.), 1689 (CO str. of ester), 1617, 1566, 1476 and 1401(C=C of aromatic ring), 1254 (OCH3), 757 (1,2-ortho, disubstituted);

1H NMR (δ, ppm) (DMSO): 8.35-8.18 (m, 4H, aromatic ring), 7.90-7.87 (t, 3H, CH in ring), 4.35 (d, 1H, CONH), 3.68 (1H, NH in ring), 3.35 (s, 3H of OCH3), 2.19(s, 3H, of CH3), 1.19 (d, 2H of CH2).

- **Spectral data of amide prodrug of mefenamic acid-Aspartic acid (MA4)**

IR (KBr, cm-1): 3486 (NH str.), 2971 and 2812 (CH str.), 1697 (CO str. of ester), 1602, 1561, 1435 and 1409(C=C of aromatic ring), 1243 (OCH3), 754 (1,2-ortho , disubstituted);

1H NMR (δ, ppm) (DMSO): 8.32-8.16 (m, 4H, aromatic ring), 7.99-7.74 (t, 3H, CH in ring), 3.44 (d, 1H, CONH), 2.76 (1H, NH in ring), 2.73 (s, 3H of OCH3), 2.33(s, 3H, of CH3), 2.22 (d, 2H of CH2).

4.2. Hydrolysis studies of mefenamic acid prodrugs

Table 2 Percentage release of drugs on hydrolysis in SIF

Time (min)	**Prodrug Hydrolyzed in SIF (%)**			
	MA1	MA2	MA3	MA4
15	0.00	0.00	0.00	0.00
30	1.01	1.09	1.10	1.02
45	2.12	2.20	2.40	2.33
60	3.11	3.12	3.19	3.17
75	4.21	4.22	4.31	4.11
90	5.34	5.71	5.40	5.72
105	7.00	7.43	7.37	7.31
120	7.89	7.91	7.93	7.88
240	9.48	8.97	9.98	9.96
360	10.12	10.18	10.11	10.01

Table 3 Percentage of drug released in rat fecal matter

Time (min)	**MA1**	**MA2**	**MA3**	**MA4**
15	0.00	0.00	0.00	0.00
30	13.1	11.2	14.2	16.8
45	24.2	22.2	25.5	27.6
60	33.1	35.1	39.7	40.3
75	38.8	37.8	46.2	48.8
90	44.3	42.3	53.3	58.2
105	53.3	53.3	68.2	69.2
120	63.8	61.8	73.1	75.4
240	76.6	75.6	79.9	83.6
360	86.3	87.2	88.2	91.6

The minimum reversion was observed at gastric pH (SGF, pH 1.2) suggesting the stability of synthesized prodrugs in gastric pH, both in fasted and fed state. However, at higher pH values i.e. in SIF representing intestine, the percentage reversion was significantly higher, thereby making the free drug available for absorption in the intestine. A much higher value was observed in rat fecal matter due to the enzyme dependant hydrolysis taking place in colon.

4.3. Pharmacological study of amide prodrugs:

4.3.1. Anti Inflammatory Activity

The percentage anti inflammatory activity of mefenamic acid and its prodrugs were determined.

Table 4 Anti inflammatory activity of amide prodrugs

Group	Treatment	Percentage anti-inflammatory activity				
		0.5 hr	**1 hr**	**2 hr**	**4 hr**	**6 hr**
I	Normal % CMC	nil	nil	nil	nil	nil
II	Mefenamic acid	48.0 ± 1.1	62.0 ± 1.2	60.6 ± 2.1	56.1 ± 1.2	42.3 ± 1.5
III	Sulfasalazine	42.0 ± 1.2	50.1 ± 1.0	59.0 ± 1.3	68.1 ± 2.3	72.4 ± 1.2
IV	MA1	45.0 ± 1.1	62.5 ± 1.7	67.4 ± 1.1	70.7 ± 1.1	71.3 ± 2.2
V	MA2	42.0 ± 1.5	50.1 ± 1.8	54.4 ± 1.7	58.3 ± 1.0	66.5 ± 1.3
VI	MA3	41.0 ± 1.0	54.3 ± 1.3	61.4 ± 1.4	65.9 ± 1.4	70.8 ± 1.3
VII	MA4	40.0 ± 1.0	52.3 ± 1.3	59.4 ± 1.4	63.9 ± 1.4	71.8 ± 1.3

Values were expressed as mean ± SD of 6 observations. Comparison between Group II Vs Test Groups $P < 0.05$,

4.3.2. Ulcerogenic Activity

The ulcer index of the prodrugs was recorded to observe the extent of gastrointestinal side effects and the mean ulcer index was determined

Table 5 Results of ulcerogenic activity

Groups	Ulcer index ± S.D.
Normal Control	0.6 ± 0.12
Diseases Control	28.4 ± 1.6
Standard (Sulfasalazine)	5.4 ± 0.15
Mefenamic acid	45.6 ± 1.8
MA1	5.8 ±0.13
MA2	5..2 ± 0.2
MA3	5.8 ±0.97
MA4	5.7 ± 0.17

The mean ulcer index of standard drug mefenamic acid was found to be more than prodrugs. The minimized side effect obtained in the prodrugs might be due to the inhibition of direct contact of carboxylic acid group of the drug to the gastric mucosa which is mainly responsible for the damage.

4.3.3. Determination of Clinical activity score rate

Table 6 Clinical activity score rate

GROUPS	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11
HC	0±00	0±00	0±00	0±00	0±00	0±00	0±00	0±00	0±00	0±00	0±00
DC	0±00	0.7±1.3	1±1.73	1.6±1.5	1.6±1.5	1.8±1.7	3.1±1.0	3.2±1.0	3.3±1.1	3.3±1.1	3.33±1.15
Mefenamic acid	0±00	0.6±1.1	1.0±1.7	1.6±1.5	2±1.73	2.7±1.3	3.0±1.0	2.3±0.5	1.9±0.6	1.3±1.1	0.99±1.1
SLZ	0±00	0.3±0.6	0.8±0.9	1.8±0.8	2.7±0.6	2.8±0.8	2.4±0.5	1.6±1.13	1.1±1.0	0.7±0.75	0.38±0.6
MA1	0±00	0.6±1.1	1.5±1.5	2.1±0.8	2.7±1.0	3±1	2.6±0.5	2.1±0.50	1.5±1.0	1.1±0.83	0.7±1.1
MA2	0±00	0.7±1.3	1±1.73	1.5±1.5	2.1±1.0	2.6±0.8	2.5±0.7	1.8±1.01	1.2±1.1	0.8±1.07	0.66±0.7
MA3	0±00	0.7±1.2	1.0±1.8	2.1±0.8	2.3±0.6	2.3±0.6	2.1±0.9	1.4±1.1	0.9±1.1	0.5±0.86	0.43±0.5
MA4	0±00	0.6±1.1	1.4±1.5	2±0.88	2.7±0.6	3.0±1.0	2.8±1.0	2±1.20	1.6±1.2	1.2±1.17	0.53±0.9

Average of six readings; Two-way ANOVA followed by Bonferroni's test, statistical significance considered at P<0.01; comparing to disease control.

4.3.4. Determination of myeloperoxidse (MPO) activity

The histologic feature of IBD is marked by the presence of inflammatory cells; neutrophils, lymphocytes and histiocytes. The more acute the illness, the prominent the neutrophil component of the inflammatory infiltrate. At the present time, intestinal and colonic inflammation is evaluated either quantitatively or qualitatively by histological examination. The determination of myeloperoxidase activity in the intestine is a simple biochemical assay that can be used to quantitate inflammation.

Table 7 MPO activity of prodrugs

S. No.	Groups	MPO activity
1	HC	18.453 ± 1.659
2	DC	122.735 ± 1.982
3	Mefenamic acid	60.714 ± 1.681
4	SLZ	36.215 ± 1.611
5	MA1	47.059 ± 1.993
6	MA2	39.744 ± 1.400
7	MA3	37.490 ± 1.967
8	MA4	53.933 ± 1.251

Average of six readings is presented; statistical significance was considered at P<0.01; vs disease control.

5. Conclusion

Prodrug design concept is a part of drug discovery process which was initiated for improving drug therapy, in which a unique substance is created to have desirable pharmacokinetic characters in order to optimize pharmacologically potent structures which ultimately lead to the design of better drugs. The literature has revealed a lot of successful work on prodrugs for decreasing the toxicity and also targeting the drug to various parts of the body. Literature available in the field on colon targeting indicates the difficulty in the treatment of inflammatory bowel diseases (IBDs) which is a common symptom for all the diseases of colon, viz., ulcerative colitis, crohn's disease, irritable bowel syndrome and colon cancer due to failure of the drug to reach at the site of action i.e. colon in appropriate concentration. As most of the drugs are absorbed in the upper gastro-intestinal tract like NSAIDs which are primarily absorbed in the stomach, the treatment of IBD had ever been a great problem due to non availability of these drugs in the distal intestinal region. In the present research, it was envisaged to synthesize mutual prodrugs of mefenamic acid with amino acids to deliver

them effectively to colon without their absorption at upper part of GIT. This concept will not only target the drugs to colon but also avoid gastric irritation and will maximize the therapeutic availability that will ultimately result in lowering of the doses.

Release studies suggest that drugs start releasing from prodrug in the distal intestinal region and an appreciable release was observed in colon. Thus, prodrugs are not absorbed due to their higher molecular weight from upper GIT. As a result, the prodrug and released drugs remain in the GIT only. This showed the prodrug (MA4) are better than drug. Abolition of unwanted absorption of drugs will lower the doses, thus increasing the therapeutic utilization of drugs.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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