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Original Article

Aggrandizing Delivery of Nalidixic Acid to Colon Employing A Targeted Prodrug Approach: Synthesis and *in vivo* Evaluation

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ABSTRACT

Objective: The project was aimed at synthesizing and characterizing amino acid conjugate of Nalidixic acid (NA) that is expected to enhance solubility without affecting permeability and is capable of delivering NA to colon without significant reversion of prodrug in gastrointestinal conditions. **Methods:** Thus, nalidixic acid-L-tryptophan conjugate (NA-TRYPh) was prepared by conventional coupling method and the prodrug was characterized by FTIR, HPLC, NMR, FAB mass and elemental analysis. The conjugate was then subjected to selected pharmaceutical preformulation studies like aqueous solubility analysis and pH partition studies. **Results:** These studies established 1.24 folds higher solubility of the NA-TRYPh over NA in phosphate buffer pH 7.4 without compromising its partitioning ability. The *in vitro* stability studies suggested its potential of safe transit to colon where the moiety is capable of reverting to 90.52% NA after 48 hrs of the experiment. *In vivo* evaluation of NA-TRYPh in an experimentally induced colitis established its efficacy an anti-inflammatory prodrug moiety that was supported by histological studies. In addition to its ability to control colonic ulcers NA-TRYPh demonstrated insignificant (P >0.05) gastric ulcerogenic potential. Colonic MPO activity for NA-TRYPh in mU/100 mg tissue was found to be 44.97 which were much less than plain NA (75.5). **Conclusion:** Conclusively, the conjugate when suitably formulated can be considered as therapeutically efficacious drug delivery system with fewer pharmaceutical limitations

Key words: Colon Specific Pro drug, Nalidixic Acid, L- Tryptophan, Inflammatory Bowel Disease, *in vivo* toxicity studies.

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INTRODUCTION

Drug targeting may be defined as the delivery of a drug to a specific organ, tissue, or cell population. Chemical drug targeting involves the deliberate modification of a drug's structure (usually bioreversibly), causing it to accumulate in a target tissue: site-specific release may be triggered by a chemical or enzymatic condition not present elsewhere in the body. The colon is an important challenge to the validity of the drug targeting approach, as conditions there are largely similar to those prevailing elsewhere in the gastrointestinal (GI) system, and the luminal pH gradient through the GI tract is too gradual for effective local drug release on strictly chemical grounds. On the other hand, the colon is an important drug target for the treatment of pathologies of the colon itself, for the relief of the chronic constipation that accompanies opioid drug treatment, and as a potential portal site for peptide and protein drugs that are not absorbed from other regions of the gastrointestinal tract.[1]

During the last few decades, the pharmaceutical research has focused its attention on the development of formulations that can release the drug in the body in a controlled and specific manner to decrease the number of daily dosing, improve patient compliance and minimize adverse reactions. These formulations, defined as controlled release systems or Drug Delivery System outweigh the disadvantages associated with conventional treatment systems. The creation of pharmaceutical forms able of selective colon release is gaining particular interest, above all, for the treatment of local diseases such as ulcerative colitis, Crohn's disease, rectal cancer, and irritable bowel syndrome.[2-4] A prodrug should be sufficiently hydrophilic and bulky to minimize the absorption in the stomach and small intestine, but must become more lipophilic in the colon to be absorbed. Due to the wide variety of enzymes produced by intestinal flora at this level, generally anaerobic bacteria responsible of different enzymes production such as esterases, amidase, nitroreductase, azoreductase, deaminase, urea dehydroxylase, it has been possible to prepare prodrugs subject to enzymatic cleavage.[5] Particular interest in recent years has been focused on the activity of azoreductase that catalyzes the reduction of a diazo bond with regeneration of two primary amino groups and glycosidases that catalyzes the hydrolysis of glycosidic bonds. The colon-specific prodrug for excellence, presenting a diazo bond, is sulfasalazine; however, other prodrugs were developed by coupling, through a -glycosidic bond, an hydrophilizing residue (glucose or galactose) to steroids such as dexamethasone, Prednisolone, hydrocortisone and fluorocortisone.[6] The sugar presence makes the therapeutic agent sufficiently polar to suffer minimal absorption in the small intestine, while in the colon, thanks to the bacterial glycosidases activity, the polar fraction releases the steroid, which can carry out its action.[7-9] The prodrugs based on amino acids are very effective in the treatment of IBD (inflammatory bowel disease) for the presence of very chemically reactive groups that are, at the same time, sites of various enzymes action (esterase, amidase, azoreductase); in particular, tryptophan possesses one amino group and a carboxyl one. It is an essential amino acid that is necessary for normal growth in infants and for nitrogen balance in adults. It is a precursor of indole alkaloids in plants. It is a precursor of serotonin (hence its use as an antidepressant and sleep aid). It can be a precursor to niacin, albeit inefficiently, in mammals.

To be colon-specific, a prodrug should be chemically and biochemically stable and nonabsorbable in the upper intestine so that it could be delivered to the colon in intact form and the linkage between the drug and the promoiety should be dissociated to liberate the active drug in the colon.[10,11] According to these requirements for being colon-specific, we have designed and synthesized colon-specific L-Tryptophan (TRYPh) based prodrug of NA, a drug used against both gram-positive and gram-negative bacteria. Functionalization occurs at the level of the amino group in position and the amino group directly linked to the chiral car-

bon with the formation of amide or diazo bonds.

Its ulcerogenic and acute toxicity activity compared with those of NA were evaluated *in-vivo* in albino rats.

MATERIALS AND METHODS

Materials

Nalidixic acid (NA) was a gift from Elcon Drugs Pvt. Ltd (Gurgaon, Haryana, INDIA). L-Tryptophan (TRYPh) 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, Pranveer Singh institute of technology Kanpur, Uttar Pradesh, INDIA.

Synthesis of the prodrug

The NA–tryptophan prodrug (NA-TRYPh) was synthesized in a two-step reaction (Figure 1). The first step was the synthesis of L-tryptophan methyl ester (TME) hydrochloride. Freshly distilled thionylchloride (0.1mol) was slowly added to methanol (300 ml) with cooling, after which 0.2 mol L-tryptophan was added. The mixture was refluxed for 10 h at 60-70^{0}C with continuous stirring on a magnetic stirrer. The solvent was distilled at 64- 65^{0}C and the resulting product was collected and triturated with cold diethyl ether (50ml) to remove excess dimethyl sulphite. The crude product obtained was recrystallized with hot methanol by adding 20- 25 ml diethyl ether followed by cooling at 0°C.[12] Crystals were collected on the next day and washed twice with ether: methanol mixture (5:1) followed by pure ether and dried under vacuum to give pure TME. HCl.

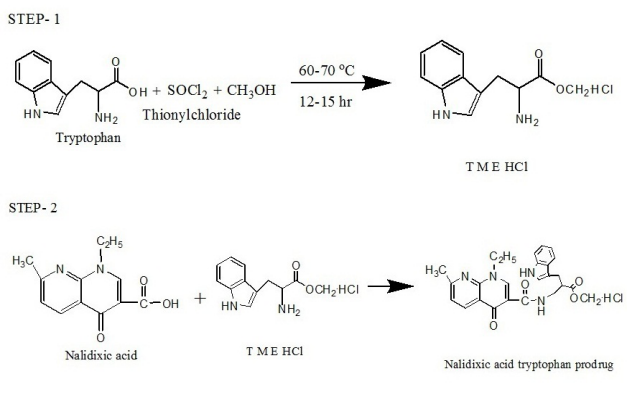

Figure 1 : Reaction scheme for the synthesis of NA-Tryptophan prodrug (NA-TRYPh). Step 1 was synthesis of L-Tryptophan methyl ester (TME) hydrochloride. The second step was synthesis of NA-TRYPh.

TME. HCl

m.p.: 159^{0}C (uncorrected), R_f = 0.37 in chloroform:methanol (2:1), % yield: 73, IR: 3590 cm^{-1} indole N-H stretching, 1735 cm^{-1} C=O saturated ester stretching, 1470 cm^{-1} C-H bending CH_2, 1430 cm^{-1} and 1370 cm^{-1} C-H bending CH_3, 1240 cm^{-1} C-O saturated ester stretching, ^{1}H NMR (DMSO-d6): δ 10.65 [d, 1H] NH-indole, δ 7.10- 7.16 [m, 4H] and δ 6.9 [d, 1H] CH-indole, δ 4.15 [s, 3H] CH3-methyl, δ 2.5 [t, 1H] CH-methine, δ 2.6 [d, 2H] CH2-methylene.

The second step was synthesis of the tryptophan conjugate of NA (NA-TRYPh). 20 mmol of NA, 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 15min. Separately, 20mmol methyl ester hydrochloride of L-tryptophan was dissolved in 60ml N, Ndimethylformamide 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.

Characterization of Prodrug structure

Mass spectra of NA (C, H, O) and NA-TRYPh (C, H, N, O) were carried out on an elemental analyzer (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 NA and NA-TRYPh were recorded using a Spectrum Two IR Spectrometer, Perkin-Elmer.

To determine the nature of protons and protonated groups in NA and NA-TRYPh, 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 (d) were recorded in ppm.

High-performance liquid chromatography analysis (HPLC) was carried out in triplicate on a Cecil 4200 system (Cecil Instruments Ltd, Cambridge, UK) using a 250 × 4.6 cm C18 reverse-phase column, particle size 5 mm (Thermo Electron, Fife, Scotland, UK). Samples were injected and eluted with the mobile phase at a flow rate of 1 ml/min. Detection was at 247 nm. The analytical performance parameters (specificity, linearity, range, precision, and accuracy, limit of detection and limit of quantification) were validated according to the International Conference on Harmonization ICH Q2B guidelines.

Qualitative powder X-ray diffraction of NA and its NA-TRYPh was performed using Bruker Axs D8 Advance X-ray diffractometer (Germany) at IIT (Roorkee, INDIA). A voltage of 40 kV and a current of 30 mA were used for the generator, with copper as the tube anode material. The samples were exposed to copper potassium alpha radiation over a range of two theta angles from 3º to 40º, and the % crystallinity index was calculated using the following formula:

Percentage crystallinity index = $(I_020 - I_{am}/I_020) \times 100$, where I_020 is intensity at 22.5º and I_{am} is lowest 2Θvalue near 18º.

Preformulation studies

Aqueous solubility

The aqueous solubilities of NA and NA-TRYPh 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 analyzed using the Cecil 4200 HPLC system described above, with UV detection at 249 nm and 247 nm for NA-TRYPh and NA, respectively.

Partition coefficient

Partition coefficients were determined according to the Hansch method[13] between 10ml 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 NA and NA-TRYPh 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 analyzed using the Cecil 4200 HPLC system, with UV detector set at 249 nm and 247 nm for NA-TRYPh and NA, respectively. Each experiment was performed six times.

In vitro stability studies

The absorbance maxima (λ_{max}) of synthesized compounds were determined on UV-1700, Pharma Spec, UV-Vis spectrophotometer in HCl buffer (pH 1.2), acid phthalate buffer pH 4.0, phosphate buffer (pH 7.4) and distilled water (pH 6.8). All chemicals used in the preparation of buffer were of analytical grade. *In vitro* stability studies were carried out in HCl buffer (pH 1.2), acid phthalate buffer pH 4.0, phosphate buffer (pH 7.4) and distilled water (pH 6.8). The total buffer concentration was 0.05 M and a constant ionic strength (m) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.[14] The feasibility of hydrolysis of the amide linkage by amidases secreted by intestinal microflora was tested with the help of release study in the rat fecal matter at 37±1^{0}C. All the kinetic studies were carried out in triplicate. The K values from the plots were calculated separately and average K and SD values were determined. The half-lives were calculated using software 'PCP Disso' developed by Department of Pharmaceutics, Poona College of Pharmacy, Pune. The process was validated as per U.S.P. XXIV edition using different parameters like accuracy, selectivity, sensitivity and reproducibility. NA-TRYPh (10 mg each) was introduced in 900 ml of HCl buffer taken in a basket and kept in a constant temperature bath at 37±1^{0}C. The solution was occasionally stirred and 5 ml aliquot portions were withdrawn at various time intervals. The aliquots were directly estimated on UV spectrophotometer at 249 nm for the amount of NA-TRYPh remaining. NA, which was supposed to be released by the synthesized prodrug, did not interfere with absorption of NA-TRYPh because its λ_{max} was found to be 247 nm, which was substantially different from NA-TRYPh. Therefore estimation of prodrug in presence of released NA was carried out over a period of 10 h. The same procedure as described earlier was followed; except that the HCl buffer was replaced by phosphate buffer. The kinetics were monitored by the decrease in prodrug concentration with time over a period of 10 h. The aliquots were directly estimated on a UV spectrophotometer at 249 nm for the amount of NA-TRYPh remaining. To study the release of NA from NA-TRYPh in rat fecal matter,[15] prodrug was dissolved in a sufficient volume of phosphate buffer (pH 7.4) so that the final concentration of the solution was 250 µg/ml. Fresh fecal material of rats was weighed (1 g) and placed in different sets of test tubes. To each test tube containing weighed amount of rat fecal matter, 1 ml of the prodrug solution was added and diluted to 5 ml with phosphate buffer (50 µg/ml). The test tubes were incubated at 37^{0}C under anaerobic conditions in a CO_2 incubator for different intervals of time. For analysis, the aliquots of NA-TRYPh were removed from the test tubes at different time intervals and estimated directly on a UV spectrophotometer at 249 nm. The concentration of prodrug remaining was determined from the calibration curve of NA-TRYPh in different buffers.

Pharmacological evaluations

Pharmacological screening of the synthesized compounds was carried out in the Department of Pharmacology, Pranveer Singh Institute of Technology. The experimental protocols for the same were approved by the Institutional Animal Ethical Committee Approval No. (IAEC/PSIT/1273/ac/09).

Induction of inflammation

Inflammation was induced by the method of.[16,17] Briefly, before induction of colitis, rats were starved for 24h but had free access to water. The rats were lightly anesthetized with ether. A rubber cannula (o.d., 2mm) was inserted rectally into the colon such that the tip was 8 cm proximal to the anus, approximately at the splenic flexture. 2,4,6-trinitrobenzene sulphonic acid (TNBS) dissolved in 50% (v/v) aqueous ethanol was instilled into the colon via the rubber cannula (15 mg/0.3 ml per rat) [16-18].

Evaluation of TNBS-induced colitis

Rats of either sex weighing between 150 and 175 g were divided into 13 groups of 4 each including control and standard group. They were starved 48 h (water *ad libitum*) prior to drug administration. NA-TRYPh were administered orally (10-30 mg/kg) as aqueous solution or suspension. Doses were chosen in accordance with their antiinflammatory activity showed in a rat model. The animals were sacrificed 7 h post drug. Stomach specimens were removed and placed on saline-soaked filter paper. The specimens were inverted over the index finger and the presence or absence of gastric irritation was determined. For each stomach specimen, the mucosal damage was assessed and the number of ulcers is noted and the severity recorded with the following scores: 0.0= no ulcer, 1.0= superficial ulcers, 2.0= severe ulcers and 3.0= perforation. The mean score of each treated group minus the mean score of the control group was considered as the 'severity index' of gastric damage (level of significance is $P< 0.01$ with respect to control).

Using the distal colon (4 cm), myeloperoxidase (MPO) activity was measured as described previously.[19] One unit of myeloperoxidase activity is defined as that degrading 1µmol of peroxide per minute at 25^{o}C.

Data analysis

Results on the colonic damage score (CDS), the MPO and pharmacokinetic values were expressed as mean ± S.E. The statistical differences among the results of the various groups were compared by the Student's *t*-test. A value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Synthesis of TME

HCl of tryptophan was carried out by adding thionyl chloride in methanol followed by refluxing with tryptophan at 60-70^{0}C for 7 hrs. A Carboxylic group of tryptophan was protected by treating it with thionyl chloride. TME. HCl was conjugated with NA and amide linkage was found between carboxylic group of NA and amino group of tryptophan.

Elemental analysis calculated for Nalidixic acid, $C_{15}H_{13}O_2F$ C: 73.79, H: 5.324, O: 13.09 found to be C: 73.61, H: 5.31, O: 13.14. Elemental analysis calculated for Nalidixic acid-Tryptophan conjugate $C_{18}H_{18}FNO_3$, C: 68.59, H: 5.71, N: 4.44, O: 15.22, found to be C: 68.51, H: 5.69, N: 4.40, O: 15.19. This confirms the purity and molecular formula of the synthesized prodrug NA-TRYPh and NA.

NA

m.p.: 229.5^{0}C, R_f= 0.602 ± 0.084 in methanol: water (3:1 v/v), Mass Spectroscopy (M+): 233.0927, IR: 3300-2500 cm^{-1} (O-H stretching of

COOH group), 1440 cm^{-1} (O-H bending of COOH group), 1350 cm^{-1} (C-O bending of COOH group), ^{1}H NMR (Figure 2): 7.85 ppm (Benzene, 3H, m), 8.85 ppm (Benzene, 5H, m), 5.12 ppm (CH, 1H, q) and 1.6 ppm (CH_3, 3H, d), , R_M: -0.462 ± 0.081, R_t (Figure 3): 4.26.

NA-TRYPh

m.p.: 257^{0}C, R_f= 0.754±0.0079, Mass spectroscopy (M+): 487.1609, IR: 2990 cm^{-1} (N-H stretching of amide), 1709.9 cm^{-1} (C=O stretching of ester), 1601 cm^{-1} (C=O stretching of amide), 1430 cm^{-1} (N-H bending of amide), 1127 cm^{-1} (C- N stretching), 1051 cm^{-1} (C-O stretching of ester), ^{1}H NMR (Figure 2): 6.8647- 8.459 ppm (CONH of amide 1H, m), 2.0341ppm (CH_3 of ester 3H, s), 1.424ppm (GH_2, 2H, d), R_M: -0.18±.0064, R_t (Figure 3): 3.32.

X-ray diffraction of NA showed the crystalline structure while NA-TRYPh showed amorphous structure (Figure 4). The % crystallinity index calculated for NA and NA-TRYPh was 95% and 87.5%, respectively.

The aqueous solubility and partition coefficients of NA and NA-TRYPh in different buffers (pH 1.2, 4.0, 6.8 and 7.4) were shown in Figure 5. Aqueous solubilities of NA and NA-TRYPh increased with increasing pH, which is probably due to an increase in the ionization of the compounds as the pH increased. The solubility of NA-TRYPh was higher than that of NA across this pH range. This may be due to the presence of highly polar groups, namely –NH2 and –COOH in TRYPh, which increase the polarity of the conjugate much more so than the less polar carboxylic group of NA. This theory is supported by reverse-phase HPLC: the C18 column used as the stationary phase had less affinity for polar drugs (i.e. polar drug would elute first). R_t values for NA and NA-TRYPh were 4.26 and 3.32 min, respectively, confirming the higher polarity of the conjugate compared with NA.

The enhancement of log P values of the prodrug compared with NA was negligible at 95% confidence interval (P> 0.5311). The slight increase might be due to the more lipophilic character of the prodrug, which is supported by the RM values of the conjugate and the parent compound. The higher the RM value, the higher the lipophilicity of the compound.

Kinetic Study Results

In order to check the stability of prodrug at acidic and alkaline pH, their release kinetics were studied by monitoring the decrease in concentration of NA-TRYPh with time, in HCl (pH 1.2) and in phosphate buffer (pH 7.4). Kinetic studies confirmed that these prodrugs were stable and did not release NA in aqueous buffers of pH 1.2 and 7.4. Thus, the objective of bypassing the upper GIT without any free drug release was achieved. The hydrolysis kinetics was further studied in rat fecal matter[20] to confirm the colonic hydrolysis of amide prodrug, over a period of 48

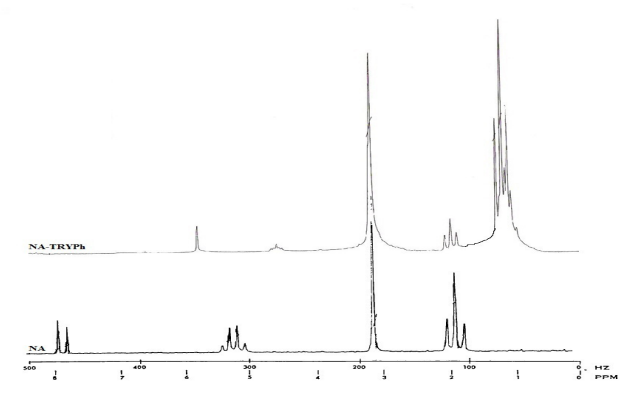

Figure 2 : NMR spectra of Pure drug NA and Prodrug NA-TRYPh.

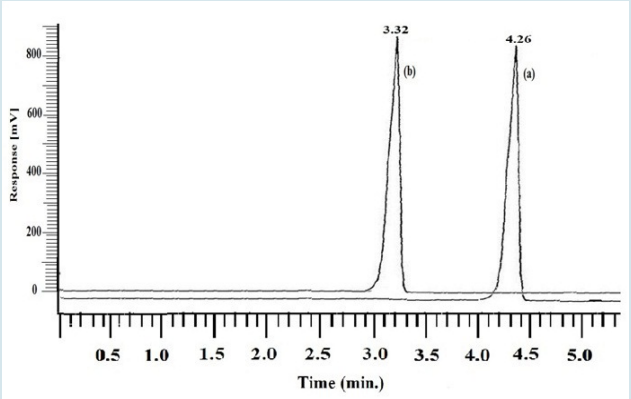

Figure 3: HPLC of a) Pure drug NA and b) Prodrug NA-TRYPh.

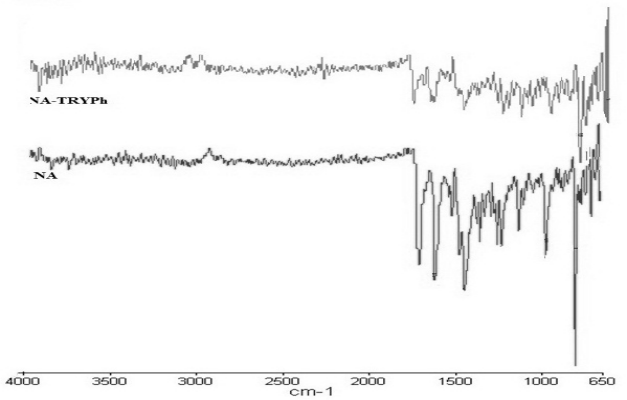

Figure 4 : XRD spectra of Pure drug NA and Prodrug NA-TRYPh.

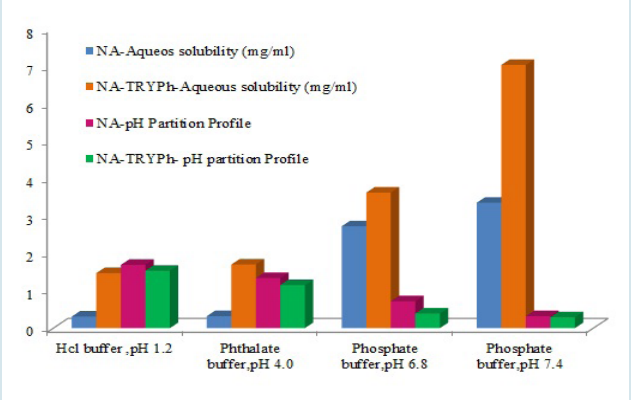

Figure 5 : Aqueous solubility and pH Partition profile of Pure drug NA and Prodrug NA-TRYPh at different pH values.

h. The cumulative percent release of NA from the prodrug followed first order kinetics (Figure 6) with 90.52% release from NA-TRYPh. *In vitro* kinetic studies confirmed that the release of NA from synthesized conjugate in the rat fecal matter was almost complete over a period of 48 h.

Biological results

In order to evaluate the feasibility of orally administered amide prodrug of NA for targeted drug delivery to the inflamed colon in IBD, 2,4,6-trinitrobenzene sulphonic acid (TNBS)- induced experimental colitis model was chosen. Severity of inflammation was quantified by determining the clinical activity score, colon to body weight ratio and myeloperoxidase activity. There is a direct correlation between the severity of inflammation and these parameters, i.e. more severe the inflammation, higher are the values for these parameters and lower the mitigating effect of the prodrugs. After induction of experimental colitis, the animals were housed without treatment for the next 3 days to maintain the development of a full IBD model. During these 3 days, the clinical activity score for all groups increased consistently. After a lag time of 24-48 h, all drug-receiving groups showed a decrease in severity of inflammation. On the 7th day, a significant difference between the drugs treated groups and a colitis control group was observed. Values for the ulcer index (median ±range) were 2 ± 1 for control animals, 66.2 ± 4.1 for the NA group and 17.0 ± 1.2 for the NA-TRYPh group, indicating marked differences in the ulcerogenic activity of the prodrug vs NA (P< 3.234). No haemorrhagic or red spots were found on the stomach walls of control animals (Figure 7A). Stomach from animals treated with NA-TRYPh showed haemorrhagic and red spots but no necrosis of the cells (Figure 7B). The stomach walls of animals treated with NA showed severe congestion, numerous haemorrhagic spots, streaks, erosion of the gastric mucosa, deep ulceration and necrotic cells (Figure 7C).On comparing histopathology of the stomachs of control rats, (Figure 8A) those treated with prodrug (Figure 8B) and drug (Figure 8C), more severe haemorrhage, ulcers and necrosis were evident in the drug group than the prodrug group. On day 11 (24 h after the drug administration), the animals were sacrificed and colon/body weight ratio was determined to quantify inflammation. NA-TRYPh (0.0074 ± 0.00043) treated group showed a distinct decrease in the colon/body weight ratio compared to colitis control group (Table 1). The results of Myeloperoxidase (MPO) activity were expressed as MPO units per gram of wet tissue and one unit of MPO activity was defined as that degrading 1 mmol min^{-1} of hydrogen peroxide at 25 ^{0}C.[21] Colonic MPO activity for NA-TRYPh in mU/100 mg tissue was found to be 44.97 which was much less than plain NA (75.5) (Table 1) suggesting a lower neutrophil infiltrate in the inflamed colon. Macroscopic damage of colon segments in colitis control was characterized by severe mucosal necrosis and ulceration with thickening of bowel wall accompanied by hyperemia, edema of the submucosa, epithelial disruption, mucosal erosions with goblet cell depletion and a mixed inflammatory infiltrate containing polymorphonuclear leukocytes and lymphocytes. *In vivo* treatment with NA-TRYPh resulted in the significant decrease in the extent and severity of colonic damage. Its histopathological features clearly indicated that the morphological disturbances associated with TNBS administration were corrected by treatment with NA-TRYPh. The histopathological sections of prodrug treated rat livers and pancreas showed no adverse effects on liver or any signs of pancreatitis. From these particular findings, it can be concluded that the synthesized prodrugs have improved safety profile than NA. Statistical differences between the groups were calculated by One-way ANOVA followed by Dunnett's post hoc test. Differences were considered at a P value of <0.05 in relation to control.

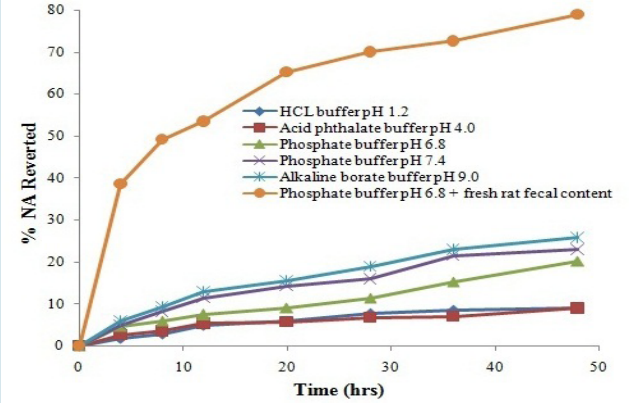

Figure 6 : *In vitro* reversion study of Nalidixic acid-Tryptophan conjugate at different pH and in presence of fresh rat fecal content at pH 6.8.

Table 1: Comparative biological activity of Pro drug.

Compound	Ulcer Index* ± SD	MPO activity*	Colon / body weight ratio*
HC	2 ± 1	-	-
NA	66.2 ± 4.1	75.5	0.091 ± 1.001
NA-TRYPh	17.0 ± 1.2	44.97	0.0074 ± 0.00043

HC= Healthy Control, NA= Nalidixic acid, NA-TRYPh= Prodrug of Nalidixic acid with L-Tryptophan *= Average of six readings

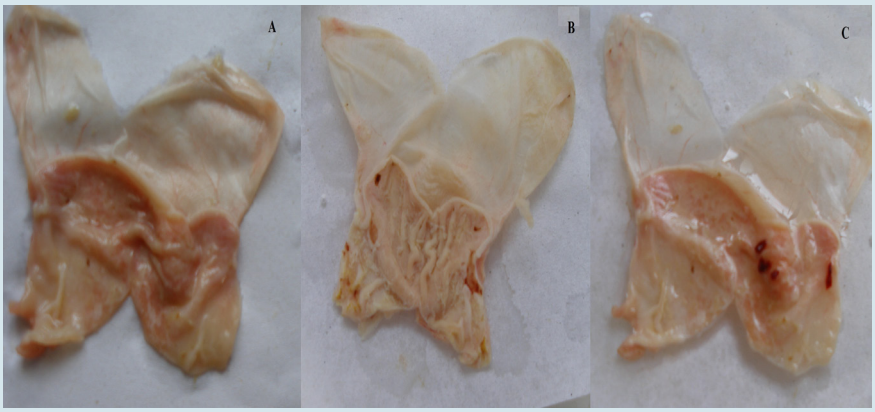

Figure 7 : Evaluation of ulcerogenic activity in the stomachs of albino rats treated with drug vehicle (1% w/v CMC) (A); NA-TRYPh (1000mgkg^{-1}) (B) and NA (1000 mgkg^{-1}) (C).

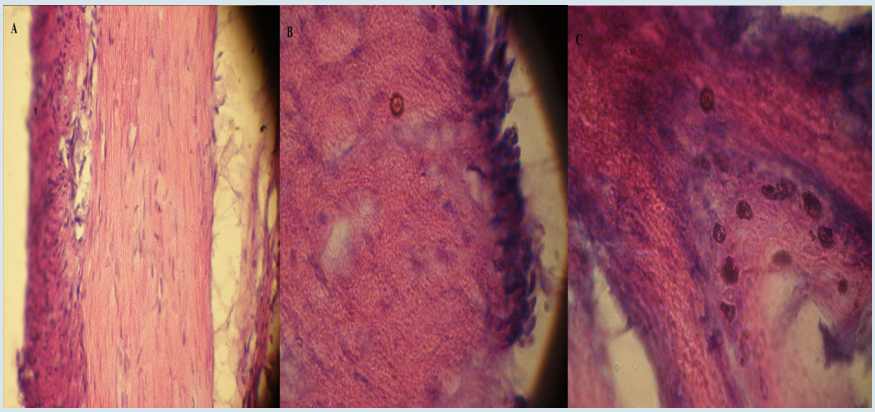

Figure 8 : Histopathology of stomachs from albino rats treated with drug vehicle (1% w/v CMC) (A); NA-TRYPh (1000mgkg^{-1}) (B) and NA (1000 mgkg^{-1}) (C).

CONCLUSION

The data generated as an outcome of this work demonstrates that this new prodrug has a remarkable ameliorating effect on the disruption of colonic architecture and suppresses the course of TNBS-induced colitis effectively. The criteria for the selection of tryptophan as the carrier has also proven correct, as it has effectively delivered NA to the colon.

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CONFLICT OF INTEREST

None

ABBREVIATION USED

NA:Nalidixic acid; NA-TRYPh: Nalidixic acid-L-tryptophan conjugate; MPO: Myeloperoxidase; TRYPh: L-Tryptophan; TME. HCl: L-tryptophan methyl ester hydrochloride ().

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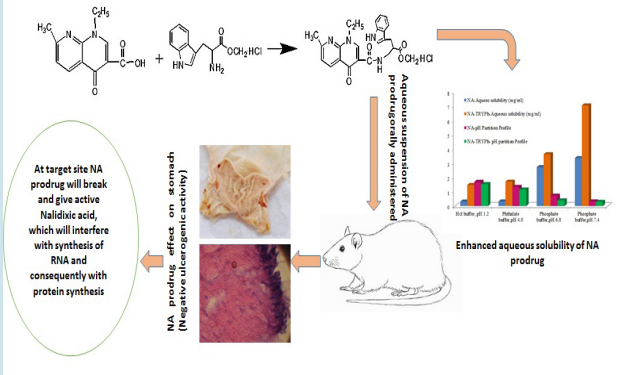
PICTORIAL ABSTRACT



SUMMARY

- An attempt has been made to develop amino acid conjugate of Nalidixic acid (NA) that is expected to enhance solubility without affecting permeability and is capable of delivering NA to colon without significant reversion of prodrug in gastrointestinal conditions.
- Thus, nalidixic acid-L-tryptophan conjugate was prepared by conventional coupling method and the prodrug was characterized by elemental analysis.
- The conjugate was then subjected to selected pharmaceutical preformulation studies like aqueous solubility analysis and pH partition studies.
- It was concluded that the conjugated prodrug can be considered as therapeutically efficacious drug delivery system with fewer pharmaceutical limitations.

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