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Research paper

Synthesis and properties of *N*-nicotinoyl-2-(5-fluorouracil-1-yl)-D,L-glycine ester as a prodrug of 5-fluorouracil for rectal administration

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Abstract

N-Nicotinoyl-2-(5-fluorouracil-1-yl)-D,L-glycine (NFG) methyl-(NFGM), ethyl-(NFGE) and isopropyl esters (NFGIp) were synthesized and their potential as a prodrug of 5-fluorouracil (5-FU) for rectal administration was investigated. Chemical conversion proceeded either by elimination of (5-FU) or by hydrolysis of ester group. 5-FU was released from NFGIp, NFGE and NFGM 90.5%, 71.3% and 48.5% of the dose, respectively, in 80% human plasma and 79.8%, 56.3% and 31.6%, respectively, in pH 7.4 buffer solution after 48 h of incubation at 37 °C. Release of 5-FU occurred mainly from NFG esters but very slightly from NFG, which suggested that release of 5-FU was greatly dependent on the stability of the ester group against hydrolysis. Solubility (*M*) in pH 7.4 buffer solution was 0.13, 0.09 and 0.04 and apparent partition coefficient in 1-octanol/pH 7.4 buffer solution was 0.76, 1.61 and 4.2, respectively, for NFGM, NFGE and NFGIp, which were in the ranges suitable for rectal absorption. Plasma concentration (μg/mL) of NFGM, NFGE and NFGIp at 50 min after rectal administration to rats was 1.9, 4.6 and 6.7, respectively, and that for 5-FU was below the limit of detection. Their potential as prodrugs of 5-FU for rectal administration is suggested.

Keywords: *N*-Nicotinoyl-2-(5-fluorouracil-1-yl)-D,L-glycine esters; 5-Fluorouracil prodrug; 5-Fluorouracil; Rectal administration; Rectal absorption

1. Introduction

5-Fluorouracil (5-FU) is one of the oldest antitumor agents used for gastrointestinal, pancreas, breast, ovary and colorectal cancer for several decades [1–3]. It is administered parenterally because it shows highly variable oral bioavailability due to the unpredictable and incomplete absorption after ingestion of the drug and a marked bioinactivation by dihydrouracil dehydrogenase in the liver and the mucosal membrane of the gastrointestinal tract [4–6]. Reports on the development of 5-FU prodrug have appeared aiming at enhancing oral absorption and diminishing first-pass metabolism. Burr and Bundgaard synthesized various 5-FU prodrugs which possessed higher lipophilicity than 5-FU and suggested them as potential prodrugs of 5-FU for rectal or oral delivery [7,8]. Wang et al. [9] reported that various 1-alkoxycarbonyl, 1-acyloxymethyl and 3-acyl prodrug derivatives of 5-FU with improved lipophilicity increased the corneal penetration dramatically and protected 5-FU from metabolic bioinactivation compared with the parent drug. Taylor and Sloan [10] synthesized

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1-alkylcarbonyloxymethyl prodrugs of 5-FU with improved lipophilicity and reported that the ability of the prodrugs to deliver total 5-FU species into skin was greater than the delivery of 5-FU by the parent drug. Shimma et al. [11] synthesized various *N*-alkoxycarbonyl-5′-deoxy-5-fluorocytidine derivatives as an orally available 5-FU prodrug, which were sequentially converted to 5-FU by enzymes that are highly expressed in the human liver and then in tumors. To improve bioavailability and avoid first-pass metabolism in the liver, *N*-nicotinoyl-2-(5-fluorouracil-1-yl)-D,L-glycine methyl-, ethyl- and isopropyl esters were prepared and their potential as a prodrug of 5-FU for rectal administration was investigated in the present study.

2. Materials and methods

2.1. Chemicals and instruments

Nicotinamide, L-(+)-dimethyl tartarate, L-(−)-diethyl tartarate and solvents for NMR were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and used as received. 5-Fluorouracil and L-(+)-diisopropyl tartarate were purchased from Tokyo Kasei (Tokyo, Japan) and periodic acid was the product of Wako Chemical Co. (Osaka, Japan). Solvents for HPLC were obtained from Merck Inc. (Darmstadt, Germany). All other chemicals were reagent grade, commercially available products. IR spectra were recorded on a Bomem MB 100 FT-IR spectrophotometer (Bomem). ^{1}H NMR spectra were taken on a Brucker AC-200 spectrometer (Brucker), and the chemical shifts are in ppm downfield from tetramethylsilane. Elemental analysis was carried out by an Elemental Analyzer System (Profile HV-3). A Shimadzu UV-2101 PC spectrophotometer (Shimadzu; Tokyo, Japan) was used for UV spectrophotometry of the drugs. A Polytron PT 3100 homogenizer was used for homogenization of the tissue of rats and an Eppendorf Centrifuge 5415C (Eppendorf, Hamburg, Germany) was used for centrifugation. An Orion 320 pH meter with a combined pH electrode (Orion; Boston, MA, USA) was used for pH measurements. Melting points were taken on a Mel Tem II (Laboratory Devices, Holliston, MA, USA) and are uncorrected. TLCs were performed on Merck silica gel 60 Kieselgel F_{254}. The HPLC system consisted of Model 305 and 306 pumps, a 117 variable UV detector, a Model 234 autoinjector, a Model 805 manometric module, and a Model 811C dynamic mixer from Gilson (Middleton, WI, USA). A Gilson 712 software was employed for the data analysis. Calibration curves were constructed from the peak area versus the concentration of standard solutions of 5-FU and NFG esters (1–10 ppm).

2.2. Preparation of methyl-, ethyl- and isopropyl glyoxalate

Glyoxalate esters were prepared by the reaction of tartaric acid ester and periodic acid according to the method by Kelly et al. [12]. Briefly, L-(+)-dimethyl tartarate (17.8 gm, 100 mmol) was placed in a flask containing 180 mL of anhydrous ethyl ether, and added periodic acid (22.8 gm, 100 mmol) in small portions while stirring mechanically under nitrogen atmosphere at 10 °C. After the addition was completed, the reaction mixture was stirred for 1 h and the resulting precipitates were removed by filtration under reduced pressure. The filtrate was dried over anhydrous Na_2SO_4 and the solvent was removed by flash evaporation. The resulting residue was distilled under reduced pressure (bp. 47–54 °C/25 mm Hg) and 9.14 g of methyl glyoxalate was obtained (yield; 52%). Preparation of ethyl glyoxalate (yield; 81%; bp. 40–45 °C/22 mm Hg) and isopropyl glyoxalate (yield; 62%; bp. 64–65 °C/25 mm Hg) was achieved by the same procedure using L-(−)-diethyl tartarate and L-(+)-diisopropyl tartarate, respectively.

2.3. Preparation of *N*-nicotinoyl-2-hydroxy-D,L-glycine (NHG) methyl-, ethyl- and isopropyl esters

Nicotinamide (6.6 gm, 54.0 mmol) and methyl glyoxalate (9.1 gm, 104.0 mmol) were placed in a 500 mL flask containing 180 mL of methylene chloride and stirred at room temperature for 10 days. The resulting precipitates were filtered and purified by fractional crystallization in methanol (yield 60%). MP 138–140 °C. IR (Nujol): ester carbonyl (1742 cm^{-1}), amide carbonyl (1668 cm^{-1}), amide NH (1600 cm^{-1}). ^{1}H NMR (D_2O): 4.07 (s, 3H, methyl), 6.05 (d, 1H, C*H*-FU), 7.40 (m, 1H, pyr), 8.21 (m, 1H, pyr), 8.90 (m, 1H, pyr), 9.04 (m, 1H, amide), 9.21 (br s, 1H, pyr). Preparation of NHG ethyl- and isopropyl ester was achieved by the same procedure using ethyl- and isopropyl glyoxalate, respectively.

2.4. Preparation of *N*-nicotinoyl-2-acetoxy-D,L-glycine (NAG) methyl-, ethyl- and isopropyl esters

NHG methyl ester (3.7 gm, 14.6 mmol) was suspended into a solution of acetic anhydride (30 mL) and acetic acid (30 mL) at 0 °C and stirred for 24 h at room temperature. The reaction mixture was concentrated by flash evaporation. The resulting residue was dissolved in 120 mL of ethyl acetate, washed with 10 mL of 10% $NaHCO_3$and then 10 mL of distilled water, dried over anhydrous Na_2SO_4, removed the solvent by flash evaporation and obtained an oily residue (Yield 99%). IR (Nujol): ester carbonyl (1755 cm^{-1}), amide carbonyl (1678 cm^{-1}), amide NH (1590 cm^{-1}). ^{1}H NMR ($CDCl_3$): 2.75 (acetyl; s, 3H), 3.86 (s, 3H, methyl ester), 6.45 (d, 1H, C*H*-FU), 7.47 (m, 1H, pyr), 8.22 (m, 1H, pyr), 8.89 (m, 1H, pyr), 9.20 (br s, 1H, pyr). Preparation of NAG ethyl- and isopropyl ester was achieved by the same procedure using NHG ethyl- or isopropyl ester, respectively.

2.5. Preparation of *N*-nicotinoyl-2-(5-fluorouracil-1-yl)-D,L-glycine (NFG) methyl-, ethyl- and isopropyl ester

NAG methyl ester (3.7 gm, 14.6 mmol), 5-FU (1.9 gm, 14.6 mmol) and triethylamine (2.1 mL, 14.6 mmol) were

dissolved in 30 mL of anhydrous dimethylformamide. After stirring for 24 h at room temperature, the solvent was removed by flash evaporation. To the residue was added 10 mL of distilled water, and extracted with 60 mL of ethyl acetate five times. The combined extract was dried over anhydrous Na_2SO_4 and the solvent was removed by flash evaporation. The resulting residue was dissolved in cold solution of ethyl ether and chloroform, and 5-FU was removed by fractional crystallization. The crude product (yield 58%) contained about 70% of *N*-nicotinoyl-2-(5-fluorouracil-1-yl)-D,L-glycine methyl ester (NFGM) and 30% of *N*-nicotinoyl-2-(5-fluorouracil-3-yl)-D,L-glycine methyl ester (NFGM-3), which were separated by medium pressure liquid chromatography (MPLC) as described in the next section. In a separate run, anhydrous acetonitrile was adopted as solvent. The reaction mixture was stirred for 10 days at room temperature. NFGM was formed exclusively as precipitates, which were collected and recrystallized from methanol. Preparation of NFG ethyl- and isopropyl ester was achieved using NAG ethyl- or isopropyl ester, respectively. Overall yield for NFG methyl ester, ethyl ester and isopropyl ester was 54%, 61% and 33%, respectively. The physical data of NFGM, NFGE and NFGIp are listed in Table 1.

2.6. Separation of NFGM and NFGM-3 by MPLC

MPLC system consisted of a VSP-2000 Ceramic pump, UV-D_2 UV monitor and a DC-1200 fraction collector from Eyela (Tokyo Rikaki Kai; Tokyo, Japan). A portion of the sample (500 mg) containing NFGM and NFGM-3 was dissolved in 2 mL of methanol and injected on a Yamazen column (500 × 40 mm) packed with Lichroprep RP-18 (25–40 μm). They were eluted with a mobile phase of 40% methanol in water at a flow rate of 4 mL/min, in which NFGM eluted ahead of NFGM-3.

2.7. Calibration of 5-FU and NFG esters in 80% human plasma

A 0.2 mL portion of a solution of 5-FU and NFG ester in 0.1 M, pH 7.4 phosphate buffer and 0.8 mL of human plasma were mixed to make the final concentration of 6.25, 12.50, 25.00 and 50.00 μg/mL. A 0.1 mL portion of this solution was placed in a microtube, added 0.4 mL of 0.1 M $ZnCl_2$ solution, vortexed for 2 min, and centrifuged for 10 min at 14,000 rpm. The supernatant was diluted with 0.05 M, pH 4.1 acetate buffer to half concentration, and filtered through 0.45 μm membrane filter (Acrodisc LC13 PVDF). A 20 μL portion of the supernatant was analyzed by HPLC as described previously [14]. Calibration curves were constructed from the peak area versus the concentration of standard solutions of 5-FU and NFG esters.

Table 1
Physical data of *N*-nicotinoyl-2-(5-fluorouracil-1-yl)-D,L-glycine methyl-(NFGM), ethyl-(NFGE) and isopropyl ester (NFGIp)

NFGM: mp: 168–170 °C	IR (cm^{-1}): 1761; 1726; 1705; 1665
^{1}H NMR: $CDCl_3$	NMR: 3.80 (s, 3H), 6.21 (d, 1H, C*H*-FU), 7.40 (m, 1H, pyrid), 7.90 (d, 1H, pyrim C^6H), 8.20 (m, 1H, pyrid), 8.89 (m, 1H, pyrid), 9.03 (m, 1H, amide), 9.20 (s, 1H, pyrid)
EA: found (calcd)	EA: C, 48.66 (48.45); N, 17.32 (17.40); H, 3.63 (3.54)
NFGE: mp: 168–170 °C	IR (cm^{-1}): 1761; 1724; 1704; 1664
^{1}H NMR: $CDCl_3$	NMR: 1.25 (t, 3H), 4.30 (q, 2H), 6.10 (d, 1H, C*H*-FU), 7.40 (m, 1H, pyrid), 7.87 (d, 1H, pyrim C^6H), 8.20 (m, 1H, pyrid), 8.91 (m, 1H, pyrid), 9.2 (s, 1H, pyrid)
EA: found (calcd)	EA: C, 49.62 (50.0); N, 16.59 (16.74); H, 3.92 (3.87)
NFGIp: mp: 168–170 °C	IR (cm^{-1}): 1752; 1726; 1700; 1662
^{1}H NMR: $CDCl_3$	NMR: 1.25 (d, 6H), 5.10 (septet, 1H), 6.50 (d, 1H, C*H*-FU), 7.40 (m, 1H, pyrid), 8.01 (d, 1H, pyrim C^6H), 8.20 (m, 1H, pyrid), 8.90 (m, 1H, pyrid), 9.20 (s, 1H, pyrid)
EA: found (calcd)	EA: C, 51.74 (51.60); N, 16.28 (16.00); H, 4.32 (4.28)

2.8. Chemical conversion of NFG esters in 80% human plasma and in pH 7.4 phosphate buffer solution

A solution of NFG ester (1.0 mM) in 0.1 M, pH 7.4 phosphate buffer was incubated with stirring at 37 °C. At an appropriate time interval, a 0.1 mL portion of the solution was removed and diluted 20-fold with 0.05 M, pH 4.1 acetate buffer. It was centrifuged at 14,000 rpm for 10 min and the concentration of NFG ester, NFG, and 5-FU in a 20 μL portion of the supernatant was analyzed by HPLC as described in the previous section. To determine the stability in 80% human plasma, 0.2 mL of NFG ester solution (5.0 mM) in 0.1 M, pH 7.4 phosphate buffer and 0.8 mL human plasma were mixed and incubated at 37 °C with stirring. At an appropriate time interval, a 0.1 mL portion of the sample was removed and mixed with 0.1 mL of 0.1 M $ZnCl_2$ solution to precipitate protein. It was vortexed for 2 min and centrifuged at 14,000 rpm for 10 min. A 0.1 mL portion of the supernatant was diluted 10-fold with 0.05 M, pH 4.1 acetate buffer and filtered through 0.45 μm membrane filter. The concentration of NFG ester, NFG, and 5-FU in a 20 μL portion of the filtrate was analyzed by HPLC as described in the previous section.

2.9. Solubility

NFG ester (50 mg) was placed in a microtube containing 1 mL of 0.1 M, pH 7.4 phosphate buffer solution and was shaken at 25 °C for 1 h to ensure the solubility equilibrium. After centrifugation, a 20 μL portion of the supernatant was analyzed by HPLC as described in the previous section.

2.10. Apparent partition coefficient

To a 20 mL portion of NFG ester solution (1.0 mM) in 0.1 M, pH 7.4 phosphate buffer saturated with 1-octanol was added 20 mL of 1-octanol saturated with 0.1 M, pH 7.4 phosphate buffer solution. The mixture was shaken at 37 °C for 1 h to ensure equilibrium and centrifuged. The concentration of NFG ester in aqueous phase was analyzed by HPLC as described previously. Apparent partition coefficient was calculated by employing the equation $(C_o - C_w)/C_w$, where C_o and C_w represent the initial and equilibrium concentration of the drug in aqueous phase, respectively.

2.11. Plasma concentration of 5-FU and NFG esters after rectal administration

The animal experiment was conducted in accordance with the *Guide for the Care and Use of Laboratory Animal* as adopted and promulgated by the National Institutes of Health. Male Sprague–Dawley rats weighing 200–250 g were fasted for 24 h prior to drug administration except water. They were anesthetized with ether and cannulated with polyethylene tubing SP 45 (0.96 mm o.d. Natsum, Japan) through femoral artery. The enemas (0.5 mL), which were prepared by dissolving 5-FU and NFG esters (9 mg equivalent of 5-FU/kg) in pH 7.4 isotonic phosphate buffer containing 5% dimethylformamide, were administered about 2 cm within the rectum using a rectal polyethylene tube. Blood samples of about 0.4 mL were collected from the femoral artery with a heparinized syringe at appropriate time intervals. Concentration of 5-FU and NFG esters in each sample was analyzed by HPLC as described in the previous section.

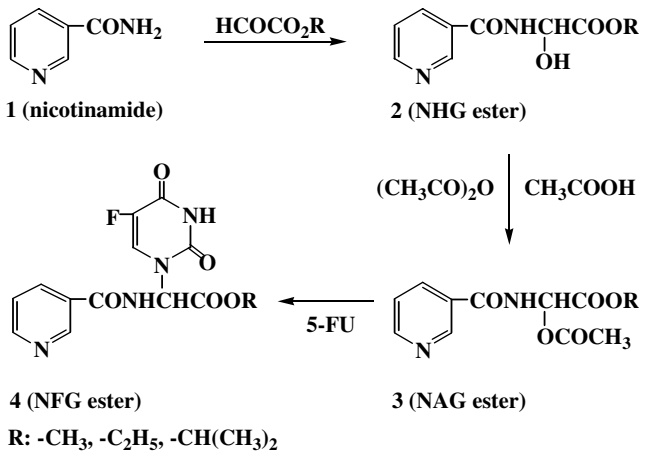
2.12. Plasma concentration and % recovery of NFGIp from colonic tissue and contents after rectal administration to rats

The enema of NFGIp was administered to rats as described previously. At a predetermined time, the rats were anesthetized by diethyl ether and blood was collected by intracardiac puncture through a heparinized syringe. After blood collection, the animal was sacrificed and tissue and contents of the distal colon were obtained. Concentration of 5-FU and NFG esters in the blood was analyzed by HPLC according to the same protocol as described previously. The distal colonic contents were diluted 10-fold with pH 4.5 isotonic phosphate buffer. The sample was vortexed and centrifuged at 5000 rpm for 3 min. To the distal colon segment, pH 4.5 isotonic phosphate buffer solution was added and homogenized using a polytron homogenizer. The homogenate was centrifuged at 5000 rpm for 5 min. To a 0.1 mL portion of the supernatant, 0.9 mL of methanol was added to precipitate protein in the sample, vortexed for 2 min and centrifuged for 5 min at 14,000 rpm. A 20 μL portion of the supernatant was analyzed by HPLC as described in the previous section.

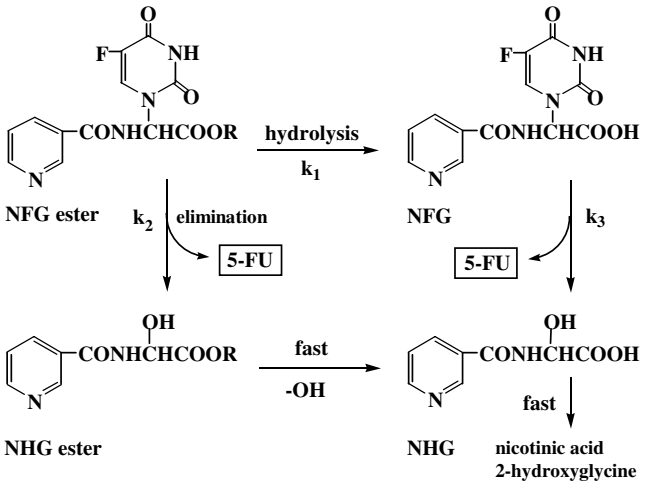
3. Results

3.1. Preparation of NFG esters

NFG esters were prepared by modifying the methods of Nichifor and Schacht [13] and Lee et al. [14]. The processes are shown in Scheme 1. *N*-Nicotinoyl-2-hydroxy-D,L-glycine (NHG) ester was prepared by the reaction of nicotinamide and glyoxalate ester. The reaction proceeded very slowly, and side products were produced at elevated temperature. When the reaction was carried out at room temperature for 10 days with the mole ratio of nicotinamide and glyoxalate ester 1:2, it resulted in good yield with no appreciable side products. Acetylation of NHG ester proceeded with acetic anhydride and acetic acid at 0 °C. Reaction of NAG ester and 5-FU in dimethylformamide produced a mixture of *N*-1 and *N*-3 substituted derivatives, which were separated by MPLC. Running the reaction in acetonitrile, nicotinoyl-2-(5-fluorouracil-1-yl)-D,L-glycine ester, the *N*-1 substituted derivative, was formed exclusively. Structures of NFG esters were identified by the data from IR and ^1H NMR spectra and elemental analysis as listed in Table 1.



Scheme 1. Synthesis of *N*-nicotinoyl-2-(5-fluorouracil-1-yl)-D,L-glycine (NFG) ester.



Scheme 2. Proposed pathway for the chemical conversion of *N*-nicotinoyl-2-(5-fluorouracil-1-yl)-D,D-glycine (NFG) ester.

3.2. Physicochemical properties of NFG esters

NFG esters were incubated in pH 7.4 phosphate buffer solution and in 80% human plasma at 37 °C to investigate the extent of 5-FU release at such conditions. Chemical conversion of NFG esters took place either by hydrolysis of the ester group to produce NFG (k_1) or by release of 5-FU (k_2) as shown in Scheme 2. Time courses for the formation of 5-FU and NFG from NFG esters in 80% human plasma and in pH 7.4 phosphate buffer solutions are shown in Fig. 1 and Fig. 2, respectively. In 80% human plasma, degradation of NFG esters progressed 100%, 92% and 90% in 24 h for NFGM, NFGE and NFGIp, respectively. The extent of 5-FU formation was 52%, 62% and 82% and that of NFG was 48%, 30% and 8% in 24 h for NFGM, NFGE and NFGIp, respectively. In pH 7.4 phosphate buffer solution, degradation of NFG esters progressed 98%, 92% and 79% in 24 h for NFGM, NFGE and NFGIp, respectively, which was slightly slower than that in 80% human plasma. The extent of 5-FU formation was 29%, 50% and 66% and that of NFG was 69%, 42% and 13% in 24 h for NFGM, NFGE and NFGIp, respectively. Degradation of NFGIp proceeded mainly by the release of 5-FU with limited ester hydrolysis to show very low level of NFG ($k_2 > k_1$). On the other hand, degradation of NFGM, which was slightly faster than that of NFGIp, produced comparable amount of 5-FU and NFG ($k_1 \geqslant k_2$). Formation of 5-FU was in the order of NFGIp > NFGE > NFGM and was more favored in 80% human plasma. As usually expected, formation of NFG by ester hydrolysis was in the order of NFGM > NFGE > NFGIp, which took place more readily in pH 7.4 phosphate buffer solution. Apparent rate constants and half-lives of the degradation of NFG esters for the first 8 h in 80% human plasma and pH 7.4 buffer solutions at 37 °C were obtained from semi-logarithmic plot of the residual NFG ester (%) versus time,

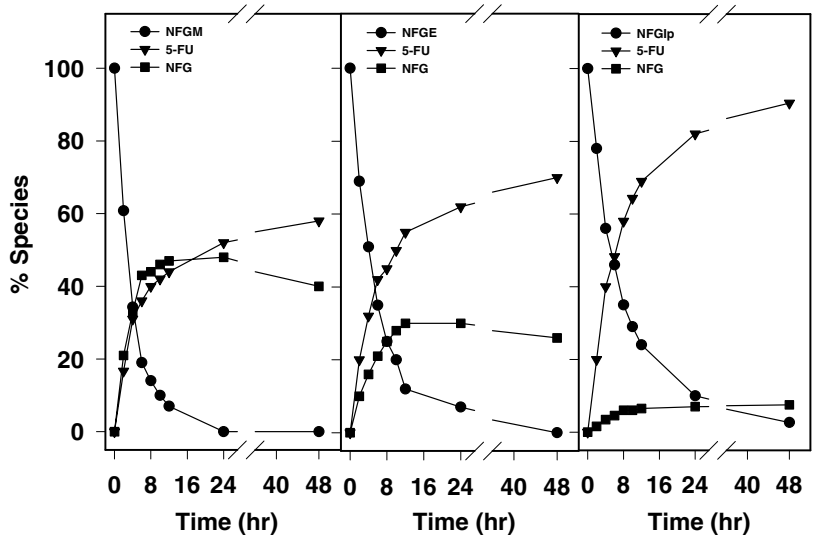


Figure 1. Degradation of NFG ester and formation of 5-FU and NGF during incubation in 80% human plasma at 37 °C.

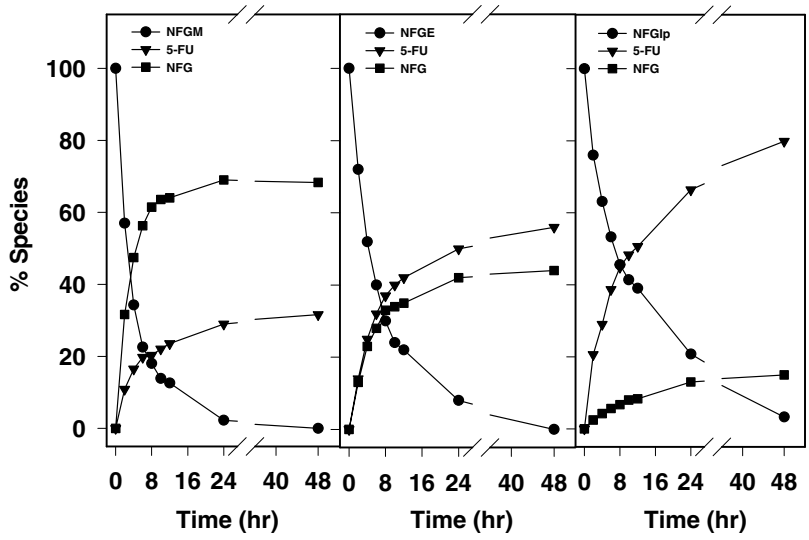


Figure 2. Degradation of NFG ester and formation of 5-FU and NGF during incubation in pH 7.4 phosphate buffer solution at 37 °C.

Table 2
Apparent rate constants and half-lives of the degradation of NFG esters for the first 8 h during incubation in pH 7.4 phosphate buffer and 80% human plasma at 37 °C

	pH 7.4 buffer		80% plasma	
	k (h^{-1})	$t_{1/2}$ (h)	k (h^{-1})	$t_{1/2}$ (h)
NFGM	0.219	3.16	0.279	2.48
NFGE	0.148	4.67	0.189	3.67
NFGIp	0.098	7.06	0.147	4.72

and the results are summarized in Table 2. Apparent rate constants for the degradation of NFG esters were lower in pH 7.4 phosphate buffer solution than 80% human plasma, and they were in the order of NFGM > NFGE > NFGIp. As listed in Table 3, solubility of 5-FU, NFGM, NFGE or NFGIp determined in pH 7.4 phosphate buffer solution at 25 °C was 0.10, 0.13, 0.09 or 0.04 M, respectively. Apparent partition coefficient in 1-octanol/pH 7.4 phosphate buffer solvent system at 37 °C was 0.15, 0.76, 1.61 or 4.2, for 5-FU, NFGM, NFGE or NFGIp, respectively. Compared with 5-FU, apparent partition coefficient of NFG ester increased substantially without significant decrease in aqueous solubility.

Table 3
Physical properties of *N*-nicotinoyl-2-(5-fluorouracil-1-yl)-D,L-glycine methyl-(NFGM), ethyl-(NFGE) and isopropyl ester (NFGIp)

	5-FU	NFGM	NFGE	NFGIp
S[a]	0.10	0.13	0.09	0.04
PC[b]	0.15	0.76	1.61	4.20
RT[c]	4.9	10.0	15.0	14.0

[a] Solubility (M) in pH 7.4 buffer, 20 °C.
[b] Apparent partition coefficient at 37 °C, *n*-octanol/pH 7.4 buffer.
[c] Retention time (min); column: C_{18}, 10 μm 3.9 × 300 mm; mobile phase: pH 4.5 isotonic phosphate buffer and methanol (8/2 for 5-FU, NFGM and NFGE, 7/3 for NFGIp).

3.3. Plasma concentration of 5-FU and NFG esters after rectal administration

Plasma concentration of 5-FU, NFGM, NFGE and NFGIp was determined after rectal administration to rats. As shown in Fig. 3, plasma concentration (μg/mL) of NFGM, NFGE and NFGIp at 50 min after rectal administration to rats was 1.9, 4.6 and 6.7, respectively, which are in parallel order as the apparent partition coefficient, and that for 5-FU was below the limit of detection (1.0 μg/mL) in all cases. Fig. 4 shows plasma concentration and % recovery from the distal colonic tissue and contents at 1 and 3 h after rectal administration of NFGIp to rats. Plasma concentration of NFGIp was 8.4 μg/mL and the fraction recovered from distal colonic tissue and contents

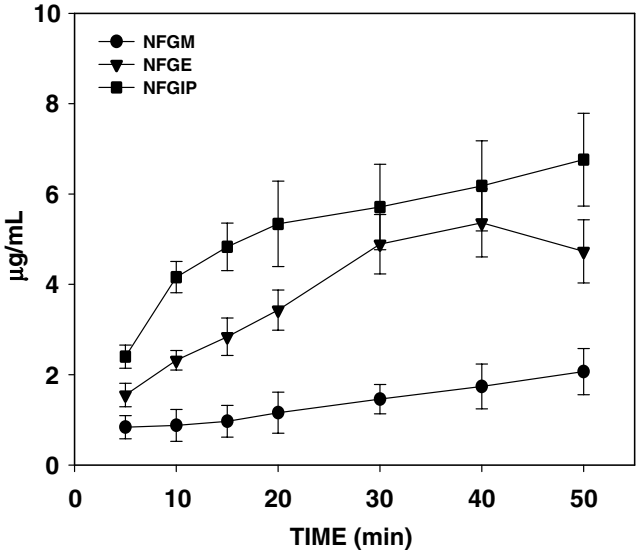


Figure 3. Plasma concentration of NFG ester after rectal administration to rats (9 mg equivalent of 5-FU per kg). Each data point represents mean ± SE ($n = 5$).

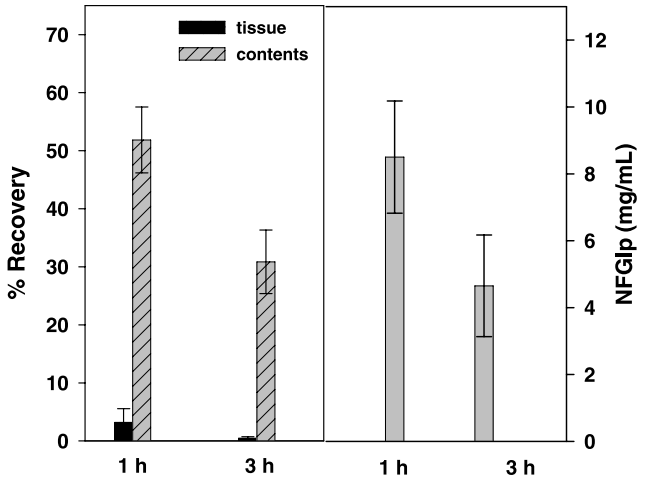


Figure 4. Recovery (%) from distal colonic tissue and contents (left) and plasma concentration (right) of NFGIp after rectal administration to rats (9 mg equivalent of 5-FU per kg). Each data point represents mean ± SE ($n = 5$).

was 4% and 52% of the dose, respectively, at 1 h after the drug administration. At 3 h after the drug administration, plasma concentration of NFGIp was 4.7 μg/mL and % recovery was 0.5 and 32 from distal colonic tissue and contents, respectively. In all cases 5-FU was not detected either from the plasma or from the colonic tissue and contents. At 6 h after the drug administration, NFGIp was not detected from the plasma, colonic tissue and contents, implying that absorption as well as elimination of NFGIp might be completed within 6 h after rectal administration.

4. Discussion

Reaction of NAG ester and 5-FU in dimethylformamide produced a mixture of *N*-nicotinoyl-2-(5-fluorouracil-1-yl)-D,L-glycine (NFG) ester, a *N*-1 substituted uracil derivative, and *N*-nicotinoyl-2-(5-fluorouracil-3-yl)-D,L-glycine ester, a *N*-3 substituted uracil derivative. Buur and Bundgarrd [15] reported that *N*-3 substituted uracil derivatives showed a shift of λ_{max} from 265 to 299 nm by the change of media from neutral to alkaline whereas *N*-1 substituted ones did not change. Judging from this property, the first fraction, which was the major product, was *N*-1 derivatives, whereas the second fraction was *N*-3 derivatives. Adoption of acetonitrile as a reaction solvent afforded exclusively one product, which showed no change in λ_{max} by the change of media from neutral to alkaline, suggesting that it was *N*-nicotinoyl-2-(5-fluorouracil-1-yl)-D,L-glycine (NFG) ester. Suda et al. [16] reported that *N*-1 substituted uracil derivatives are stable against dihydrouracil dehydrogenase, which is a desirable characteristic for 5-FU prodrugs.

NFG is produced by hydrolysis of NFG esters (k_1), whereas 5-FU is formed by elimination of 5-FU from NFG ester (k_2) and NFG (k_3) as suggested in Scheme 2. Formation of 5-FU was in the order of NFGIp > NFGE > NFGM and was more favored in 80% human plasma. Formation of NFG by ester hydrolysis was in the order of NFGM > NFGE > NFGIp, which agrees with general preference for ester hydrolysis. In earlier period of incubation, the rate of NFG formation (k_1) is much greater than the rate of NFG disintegration (k_3), resulting in an increase of NFG in the medium. As the incubation time extends, the concentration of NFG ester as well as the rate of NFG formation decreases to a point that the rate of NFG formation falls behind the rate of NFG disintegration, which should result in a net decrease of NFG to exhibit a decline in the level of NFG in the medium. It was observed in 80% human plasma that the level of NFG increased rapidly in earlier period of incubation to give a maximum and slowly decreased in the order of NFGM > NFGE > NFGIp, which was less noticeable in pH 7.4 phosphate buffer solution. The level of NFG may not decline appreciably as far as the rate of NFG formation is greater than the rate of NFG disintegration during most of the incubation period as observed in pH 7.4 phosphate buffer solution. If the incubation period had been extended further, the level of NFG would have declined after certain point in the order of NFGM > NFGE > NFGIp as observed in 80% human plasma. On incubation of NFGM in 80% human plasma (left portion in Fig. 1), it was noticed that the amount of NFGM became nil after 24 h, when the level of NFG began to decrease slightly and the level of 5-FU increase accordingly. The slope of 5-FU formation *vs* time at this point might represent the rate of NFG disintegration to release 5-FU, which was very slow ($k_1, k_2 \gg k_3$). Because the release of 5-FU took place mostly from NFG ester, the stability of ester against hydrolysis is an important factor for NFG esters to be a promising prodrug of 5-FU. To summarize, 5-FU was produced most readily from NFGIp in 80% human plasma ($k_2 > k_1 \gg k_3$), whereas NFG was produced most readily from NFGM in pH 7.4 phosphate buffer solution (k_1, k_2, k_3).

Absorption of drugs from various parts of the gastrointestinal tract is mainly achieved by passive diffusion and so the solubility and partitioning properties of the drugs are considered of great importance. For rectal absorption, a greater water solubility is required as compared with oral absorption because the fluid volume available for dissolution is small in the rectum compared with that in peroral route. An adequate lipophilicity, which is required for permeation through the rectal membrane, might be expressed in terms of partition coefficient between 1-octanol and aqueous buffer solution at pH 7 or 8, the reported pH value of the rectal fluid [17]. Since aqueous solubility and lipophilicity show a reverse relationship, an optimal balance between these properties is required for optimal absorption. Bundgaard et al. [18] reported that the rectal absorption of allopurinol was less than 3%, whereas various N_1-acyl and N_1-acyloxymethyl prodrugs of allopurinol derivatives with high aqueous solubility and adequate lipophilicity at physiological pH showed bioavailability ranging from 11% to 94%. Burr and Bundgaard [7,8] studied oral and rectal absorption characteristics of various

3-acyl, 1-alkyloxycarbonyl and 3-acyloxymethyl prodrug derivatives of 5-FU and established a structure-rectal absorption relationship to obtain some generally useful information on the physicochemical properties required for optimal rectal absorption. They reported that 5-FU was not absorbed rectally whereas the prodrugs showed bioavailability of 5-FU ranging from 0% to 100%. They stated that partition coefficient between 1-octanol and aqueous phase should be greater than 0.5 and the solubility in water greater than 0.05 M in order to achieve an extent of absorption to be more than 50% through the rectal route. Solubility and apparent partition coefficient of NFGM, NFGE or NFGIp were in the ranges which might be suitable for the rectal absorption. In addition, because the promoiety of the prodrug is composed of nicotinic acid and glycine, safe endogenous molecules, there is no concern of toxicity of the promoiety after conversion of the prodrug to 5-FU. Moreover, nicotinic acid, which is generated as a metabolite of the promoiety, may reduce an adverse effect of 5-FU, pellagra [19].

Plasma concentration of 5-FU, which represents the difference between the amount of 5-FU released by absorbed NFG ester and the amount of 5-FU eliminated by excretion and metabolism, was below the limit of detection for NFGM, NFGE and NFGIp. Considering very short biological half-life of 5-FU, it might be very unlikely to expect relatively high level of 5-FU in the plasma from the prodrug administration.

5. Conclusion

NFGM, NFGE and NFGIp were synthesized and their potential as a prodrug of 5-FU was investigated. Solubility and apparent partition coefficient of NFGM, NFGE and NFGIp were in the ranges suitable for rectal absorption. Plasma concentration of NFG ester after rectal administration to rats was in the order of NFGIp > NFGE > NFGM and that of 5-FU was below the limit of detection. Release of 5-FU occurred mainly from NFG esters but very slightly from NFG, which suggested that release of 5-FU was greatly dependent on the stability of the ester group against hydrolysis. In this regard, structural modifications which would allow the stability against hydrolysis, enhanced solubility and optimum lipophilicity are under study.

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