PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 189, 353–361 (1988)

Catabolism of 5-Fluoro-2′-deoxyuridine by Isolated Rat Intestinal Epithelial Cells (42818)

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Abstract. The kinetics of conversion of 5-fluoro-2′-deoxyuridine (FdUrd) to 5-fluorouracil (FUra) by isolated rat intestinal epithelial cells was investigated. Also, the effects of potential inhibitors of this reaction, which is catalyzed by uridine phosphorylase and thymidine phosphorylase, were determined. A 2.5% suspension of isolated cells was incubated with FdUrd or FUra, and at specific times cells were lysed with perchloric acid and fluoropyrimidines were determined by high-performance liquid chromatography. During a 25-min incubation with either FdUrd or FUra, the amount of drug in the incubation system (total volume 0.8 ml) fell by less than 5%. However, in the presence of FdUrd, the amount of FUra increased linearly over 25 min. The apparent V_{max} and K_m for FUra formation were 17–27 nmole/mg DNA/min and 1.6–2.5 mM, respectively. With each nucleoside phosphorylase inhibitor, the apparent K_m increased but V_{max} was unaffected. The apparent K_i values were as follows (in mM): 5-nitrouracil (an inhibitor of both uridine phosphorylase and thymidine phosphorylase), 0.12; 4-thiothymine (a uridine phosphorylase-selective inhibitor), 1.52; and 6-benzyl-2-thiouracil (a thymidine phosphorylase-selective inhibitor), 0.73. It was concluded that intestinal epithelial cells are capable of degrading FdUrd to FUra and that the cells possess both uridine phosphorylase and thymidine phosphorylase activity.

5-Fluorouracil (FUra) and its nucleoside analog 5-fluoro-2′-deoxyuridine (FdUrd) are used in the treatment of various solid tumors (1, 2). Although FdUrd is much more potent than FUra against several tumors *in vitro* (3, 4), the two drugs are approximately equipotent after bolus iv administration *in vivo* (5, 6), presumably because of rapid conversion of FdUrd to FUra (7, 8, 9). Metabolism by opposing anabolic and catabolic pathways is important in the activation and elimination of the fluoropyrimidines. Anabolic metabolism results in the formation of the principal cytotoxic nucleotides 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP) and 5-fluorouridine-5′-triphosphate (FUTP). FdUMP is a potent inhibitor of thymidylate synthase, and consequently of DNA synthesis (10). FUTP is incorporated into RNA (11), resulting in functional abnormalities (12, 13, 14). The relative contributions of the two nucleotides to the observed cytotoxic effects are controversial and may vary depending on the type of cell. Catabolic metabolism of FUra results in the formation of inactive products. FdUrd is broken down to FUra, which can undergo further anabolic or catabolic metabolism. The conversion of FdUrd to FUra is mediated by at least two distinct pyrimidine nucleoside phosphorylases (15), uridine phosphorylase (EC 2.4.2.3) and thymidine phosphorylase (EC 2.4.2.4), which are present in different proportions in various normal and tumor cells (15, 16, 17).

A major dose-limiting toxic effect of the fluoropyrimidines is gastrointestinal damage, manifested as nausea and vomiting, diarrhea, stomatitis, enteritis, and ulcerations (2). Early *in vivo* studies (18) suggested that FUra has greater gastrointestinal toxicity than FdUrd. In studies on the mechanism of gastrointestinal toxicity of fluoropyrimidines in mice, Houghton *et al.* (19) showed that single ip doses of FUra produced greater toxicity, assessed by weight loss, diarrhea, and rectal bleeding, than equimolar doses of FdUrd. The severity of toxicity was proportional to the extent of intestinal epithelial incorporation of drug into RNA, which was greater with FUra.

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0037-9727/88 $1.50

Since FdUrd must be converted to FUra before incorporation into RNA (20), its lower gastrointestinal toxicity could be due, in part, to incomplete systemic conversion. *In vivo,* the systemic conversion of FdUrd to FUra is rapid, but recent studies in the monkey (9) demonstrated that peak plasma concentrations of FUra after FdUrd administration were only about 25% of those produced by administration of an equimolar dose of FUra. Further, since FUra elimination is dose-dependent and its clearance is greater at low concentrations (9, 21), FUra elimination would be expected to occur more rapidly after FdUrd administration because of the lower plasma concentrations. As a result of both factors (lower concentration and higher clearance), the total exposure of intestinal cells to FUra would be lower after FdUrd than after FUra administration.

The lower gastrointestinal toxicity of FdUrd could also be due, in part, to less extensive or slower conversion to FUra in intestinal epithelial cells than in other tissues, such as the liver (22). To examine this possibility, the kinetics of FdUrd catabolism by isolated rat intestinal epithelial cells were investigated. The use of this preparation allowed study of FdUrd metabolism by intact intestinal cells while avoiding the confounding effects of metabolism by other tissues. The effects of pyrimidine nucleoside phosphorylase inhibitors having different enzyme selectivities were also determined in an attempt to assess the relative contributions of uridine and thymidine phosphorylase to FdUrd catabolism in intestinal epithelial cells.

Materials and Methods. *Chemicals.* FdUrd was provided by Hoffman–LaRoche, Inc. (Nutley, NJ). The uracil derivatives and pyrimidine nucleotides, as well as calf thymus DNA and eosin Y, were purchased from Sigma Chemical Co. (St. Louis, MO). [2-^{14}C]FUra (58 mCi/mmole) was purchased from Moravek Biochemicals, Inc. (Brea, CA). Na$H^{14}CO_3$ (>40 mCi/mmole) and NCS solubilizer were obtained from Amersham (Arlington Heights, IL); [*methyl*-^{3}H]-thymidine and Aquasol were obtained from New England Nuclear (Boston, MA). Ion-exchange resins were obtained from Bio-Rad Laboratories (Richmond, CA). Dulbecco's modified Eagle's medium (DME) and fetal bovine serum were from GIBCO Laboratories (Grand Island, NY).

Animals. Male Sprague–Dawley rats weighing 150–230 g (Laboratory Supply Co., Indianapolis, IN) were used. Animals received food and water *ad libitum* and were maintained on a 12-hr light, 12-hr dark cycle.

Intestinal cell isolation. The procedure of Lawson *et al.* (23) was followed. Briefly, rats were sacrified by a blow to the neck and the intestine was exposed through a midline abdominal incision. The portion of small intestine extending from 5 cm distal to the stomach to 5 cm proximal to the large intestine was excised and immersed in 50 ml of saline-dithiothreitol solution (NaCl, 9 g/liter; dithiothreitol, 0.15 g/liter) at room temperature. The tissue was cut into 4.5-cm segments (9–10 fragments were obtained from each animal) and everted with a glass rod to expose the mucosal surface and remove luminal contents. After cleaning by gentle stirring in saline-dithiothreitol solution, the intestinal segments were placed in individual test tubes containing 10 ml of pH 7.3 phosphate buffer containing 7.94 g/liter sodium citrate. The tissues were incubated for 15 min at 37°C in a water bath shaker. The intestinal mucosal epithelial cells were then released by incubation at 37°C in 10-ml aliquots of pH 7.3 phosphate buffer containing 0.08 g/liter dithiothreitol. The tissues were incubated in fresh buffer for sequential times of 8, 15, 15, and 15 min. Prior to transferring the tissue for each subsequent incubation, the tubes were vortexed for 10 sec to release cells. The cell fraction from the initial 8-min incubation was discarded and the remaining three fractions were pooled and centrifuged at 800*g* and 4°C for 5 min. The isolated cells were washed three times by centrifugation (800*g*, 4°C) for 5 min with 10 ml of pH 7.3 phosphate buffer containing 1.44 g/liter EDTA. The isolated cells were resuspended in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 5 mg/ml Hepes, and 80 μg/ml $NaHCO_3$. Approximately 0.60 to 0.80 ml of packed isolated cells was obtained from each rat. The total time of the cell isolation procedure was about 100 min.

Preliminary experiments. In preliminary experiments, viability was also assessed by the ability of isolated cells to incorporate [^{3}H]thymidine into DNA. In these experiments, a 10% cell suspension was preincubated at 37°C for 5 min. A 0.4-ml aliquot of the cell suspension was then mixed with 0.4 ml of DME medium containing 4 μM nonlabeled thymidine and tracer [^{3}H]thymidine. Parallel studies were run in the presence and absence of FdUrd and the enzyme inhibitors. The reaction mixture was incubated at 37°C and the reaction was stopped at various times by addition 5 ml of ice-cold 10% (w/v) trichloroacetic acid (TCA). As a control, TCA was added to the mixture prior to the addition of [^{3}H]thymidine. The tubes were placed in ice for 30 min and the TCA precipitate was collected by centrifugation at 1200g for 10 min at 4°C and then washed twice with 5 ml of ice-cold 5% TCA and once with 5 ml of ethanol. The pellets were combined with 0.50 ml of NCS solubilizer, and the test tubes were covered tightly and incubated at 50°C. After 30 min, the solubilized pellets were placed in glass scintillation vials to which 50 μl of glacial acetic acid and 10 ml of Aquasol were added. Cellular DNA was determined by the spectrophotofluorometric method of Puzas *et al.* (24). In subsequent experiments, cell viability was routinely determined by the dye (eosin Y) exclusion method of Hanks et al. (25).

In vitro metabolism of FdUrd and FUra. After preincubation at 37°C for 5 min, 0.40 ml of a 5% cell suspension was mixed with 0.40 ml of FdUrd-containing DME medium in the presence or absence of inhibitors. Inhibitor effects were investigated in two ways. In one, cells were incubated with a fixed concentration of inhibitor and varying concentrations of FdUrd. In the other, a fixed concentration of FdUrd and varying concentrations of inhibitor were employed. The reaction was stopped and the cells were lysed at various times by the addition of 0.10 ml of 40% perchloric acid. In control tubes, perchloric acid was added before substrate.

Fluoropyrimidine analyses. The total medium and cellular concentrations of FdUrd, and of FUra derived from FdUrd, were determined by HPLC. The procedures of sample preparation and HPLC analysis were modified slightly from previously described methods (26, 27). The perchloric acid filtrates were combined with 0.10 ml of internal standard, 5-chlorouracil (45 μg/ml), and then neutralized with 0.14 ml of 4.6 N KOH. The mixture was centrifuged at 100g at 4°C for 5 min. Pyrimidines were isolated by sequential cation-exchange and anion-exchange chromatography, as described in detail previously (26).

The HPLC system consisted of a Beckman model 110A pump (Beckman Instruments, Inc., Fullerton, CA), a Rheodyne model 7125 syringe loading sample injection valve with a 20-μl sample loop (Rheodyne, Inc., Cotati, CA), a Beckman model 160 ultraviolet detector operated at a wavelength of 254 nm, and a Hewlett–Packard model 3390A integrator (Hewlett–Packard Co., Palo Alto, CA). FdUrd, FUra, and ClUra were separated on a C_{18} analytical column (Hewlett–Packard Hypersil-DDS, 5 μm, 200 × 4.6 mm i.d.). The mobile phase consisted of 4 mM sodium acetate, pH 4.7, containing 1.5% acetonitrile and was delivered at 1.7 ml/min. The retention times of pyrimidines were as follows (in min): FUra, 2.5; ClUra, 4.7; and FdUrd, 6.9. The retention time of 5,6-dihydro-5-fluorouracil (H_2FUra), the initial product of FUra catabolism (7), was not determined due to the unavailability of the compound. However, H_2FUra is unstable in alkaline solutions and at room temperature. For example, Sommadossi *et al.* (28) reported rapid and complete breakdown of H_2FUra in solutions above pH 8. In addition, van den Bosch *et al.* (29) demonstrated extensive degradation of H_2FUra over a period of 3 hr at room temperature and a pH of 4.5. Since, in the present experiments, fluoropyrimidines were extracted with pH 10.7 buffer and the extracts were evaporated at 50°C (26, 27), it was assumed that the concentration of H_2FUra in samples injected onto the HPLC column was negligible. In the HPLC method employed, FUra was completely separated from the nucleotides 5-fluoro-2′-deoxyuridine-5′-monophosphate and uridine-5′-monophosphate, which eluted from the column earlier than FUra.

Standard curves for FUra and FdUrd were constructed by plotting the peak area ratio (fluoropyrimidine to internal standard)

against the known amount of fluoropyrimidine in each standard; the curves were linear over the concentration range encountered in these studies. The concentration of fluoropyrimidines in the samples were calculated from the peak area ratios of the samples and the slope and *Y*-axis intercept of the standard curve.

Estimation of $^{14}CO_2$ production. Aliquots (1-ml) of a 5% suspension of isolated intestinal epithelial cells were mixed with 0.10 ml of FUra (77 μM) and tracer amounts of [2-^{14}C]FUra in DME medium. The flasks were sealed with rubber stoppers (Stopper Top, Kontes, Vineland, NJ) connected to a plastic well (Center Well, Kontes) which extended into the flask. The flasks were incubated in a shaking water bath at 37°C for 60 min. At various times, 0.30 ml of 30% ethanolamine in methanol was loaded via a syringe into the plastic well just before the reaction was stopped by an injection of 0.30 ml of 6 *N* HCl into the reaction mixture. This caused lysis of the cells and release of CO_2. For controls, ethanolamine solution and HCl were added to the flasks before incubation. The flasks were incubated at room temperature for 3 hr. The ethanolamine from the plastic well was then transferred to a scintillation vial containing 1 ml of 0.1 *N* NaOH and 10 ml of Aquasol. The vials were placed in the dark for at least 24 hr before liquid scintillation counting to minimize chemiluminescence. The radioactivity was determined using a Parkard Tri-Carb 300 liquid scintillation counter (United Technologies, Packard, Downers Grove, IL). The efficiency of CO_2 trapping was determined by adding tracer $NaH^{14}CO_3$ and varying amounts of nonlabeled $NaHCO_3$ (in 1 ml) to the incubation flasks as above. Recoveries of 99 ± 0.1 and 98 ± 3.5% (mean ± SE, n = 3) were obtained at $NaHCO_3$ concentrations of 1 and 15 mM, respectively.

Calculations. The amount of FdUrd in the incubation system decreased by less than 5% over the time course of the experiments. Plots of the amount of product (FUra) versus time indicated linear production of FUra over the initial 25 min of incubation. Plots of velocity of FUra formation versus FdUrd concentration yielded right rectangular hyperbolas described by the Michaelis–Menten equation. Apparent V_{max} and K_m were obtained from Hanes–Woolf plots (S/v versus S), which have the equation $S/v = (1/V_{max})S + K_m/V_{max}$, where S is the substrate (FdUrd) concentration and v is the velocity of FUra formation. In experiments performed at a fixed concentration of inhibitor and varying concentrations of FdUrd, the apparent competitive inhibitor constant (K_i) was calculated from $K'_m = (K_m/K_i)I + K_m$, where K'_m is the apparent K_m in the presence of inhibitor and I is the inhibitor concentration (30). In experiments performed at a fixed concentration of FdUrd and varying concentrations of inhibitor, the apparent K_i was estimated from Dixon plots ($1/v$ versus I) as described by Segel (30). V_{max}, K_m, and K_i are described as apparent values, since they were derived from experiments with intact cells and could be influenced by cellular uptake as well as metabolism of the substrates and inhibitors.

Statistical analysis of the difference between two means was evaluated by Student's t test (31). The level of significance was set at $P < 0.05$.

Results. *Preliminary experiments.* The isolated intestinal epithelial cell preparation used in these experiments contained approximately 60% crypt cells and 40% columnar villus epithelial cells, as determined by light microscopy. Cell viability, determined by the dye exclusion method, was at least 95% after a 60-min period of incubation. The ability of isolated cells to incorporate [^{3}H]thymidine into macromolecules was also determined. The tritium content of the TCA precipitate from cells incubated with 4 μM [^{3}H]thymidine increased progressively over a 60-min period. FdUrd, in a 500-fold molar excess (2 mM) markedly suppressed [^{3}H]thymidine incorporation, but the pyrimidine nucleoside phosphorylase inhibitors, at concentrations close to the K_i value, had no significant effect.

Metabolism of FdUrd and FUra by isolated rat intestinal epithelial cells. In the presentation of results, concentrations and amounts of FdUrd and FUra refer to total cellular plus extracellular drug, since cells were lysed with perchloric acid before fluoropyrimidine analysis. The time course of

changes in the total amounts of FdUrd and FUra in the incubation system (0.80 ml) is shown in Fig. 1A. Over a period of 25 min, the amount of FdUrd in the system did not fall to less than 95% of the initial level. However, there was a linear increase in FUra over this period. In the experiment illustrated, the amount of FUra at 25 min was approximately 3% of the initial amount of FdUrd. To determine whether FUra was catabolized by the isolated intestinal epithelial cells, two types of experiments were performed. In the first, cells were incubated with varying concentrations of FUra (9.6, 39, and 78 μM) for 30 min. There was no significant decrease in the amount of FUra in the 0.8-ml incubation system over the range examined (Fig. 1B). Also, there was no detectable FdUrd formation. In the second type of experiment, the ability of isolated intestinal epithelial cells to convert [2-^{14}C]FUra to $^{14}CO_2$ was tested. No $^{14}CO_2$ was produced over a 1-hr period by cells incubated with 9.6 μM [2-^{14}C]FUra. The stable substrate (FdUrd) level, and the lack of product (FUra) disappearance, under the experimental conditions employed, justified the subsequent use of FUra formation rates in kinetic analyses.

Conversion of FdUrd to FUra. The time course of FUra formation from FdUrd at varying FdUrd concentrations, in the presence and absence of pyrimidine nucleoside phosphorylase inhibitors, is shown in Fig. 2. In controls, there was a linear increase in FUra over 25 min; the rate of FUra formation was proportional to the initial concentration of FdUrd, which ranged from 0.41 to 2.85 mM. Hanes–Woolf plots (S/v versus S) of the data (Fig. 3) yielded straight lines, consistent with Michaelis–Menten kinetics. The K_m was 1.60–2.50 mM and the V_{max} was 17–27 nmole/mg DNA/min (Table I).

In the presence of fixed concentrations of the inhibitors, FUra formation from FdUrd remained linear with time at each level of FdUrd tested. However, the rates were lower than those in controls (Fig. 2). Hanes–Woolf plots (Fig. 3) of these data yielded lines that were essentially parallel to those of the controls. The effects of the inhibitors on the kinetic parameters for FUra formation are shown in Table I. With each inhibitor, there was an increase in the apparent K_m but no significant change in V_{max}. The estimated K_i values ranged from 0.12 mM for 5-nitrouracil to 1.52 mM for 4-thiothymine.

The effects of varying concentrations of

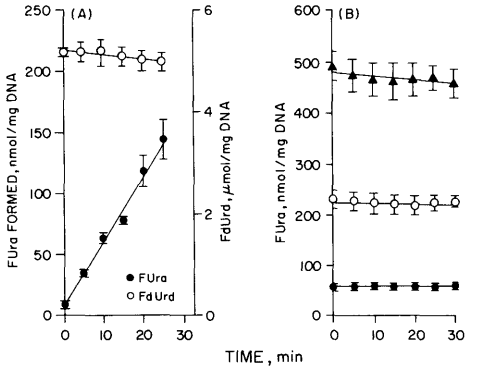

FIG. 1. Time course of fluoropyrimidine elimination by isolated rat intestinal epithelial cells. (A) Time course of formation of FUra from FdUrd. The initial concentration of FdUrd was 0.81 mM. (B) Time course of elimination of FUra. The initial concentrations were 9.6, 39, and 78 μM. Data symbols represent means ± SE (n = 3).

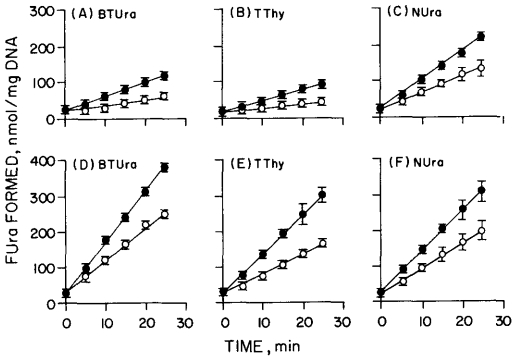

FIG. 2. Dose dependence of FUra formation from FdUrd by isolated rat intestinal epithelial cells and the effects of pyrimidine nucleoside phosphorylase inhibitors. Data symbols represent means ± SE (n = 3) of values in the absence (●) and presence (○) of inhibitors. (A) and (D) Effect of 6-benzyl-2-thiouracil (0.73 mM) on FUra formation at initial FdUrd concentrations of 0.41 and 2.85 mM, respectively. (B) and (E) Effect of 4-thiothymine (1.28 mM) at initial FdUrd concentrations of 0.41 and 2.85 mM, respectively. (C) and (F) Effect of 5-nitrouracil (0.10 mM) at initial FdUrd concentrations of 1.23 and 2.85 mM, respectively.

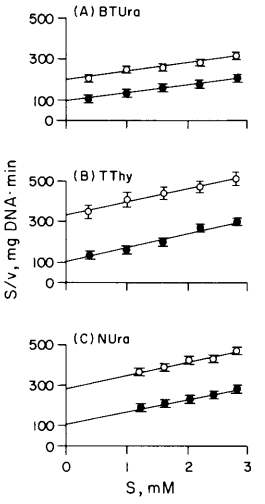

FIG. 3. Hanes–Woolf plots of the effects of pyrimidine nucleoside phosphorylase inhibitors on the formation of FUra from FdUrd by isolated rat intestinal epithelial cells. Data symbols represent means ± SE (n = 3) of values in the absence (●) and presence (○) of inhibitors. (A) Effect of 6-benzyl-2-thiouracil (0.73 mM). (B) Effect of 4-thiothymine (1.28 mM). (C) Effect of 5-nitrouracil (0.10 mM).

the inhibitors on FUra formation at a fixed initial concentration of FdUrd (0.81 mM) were also studied. Qualitatively similar results were obtained with the three inhibitors. With each inhibitor, there was a dose-dependent decrease in the rate of formation of FUra. Dixon plots ($1/v$ versus inhibitor concentration) yielded straight lines. The estimated K_i values (Table I) were in generally close agreement with those obtained from experiments with fixed concentrations of inhibitor and varying concentrations of substrate.

Discussion. *FdUrd and FUra metabolism.* The present results demonstrate that isolated rat intestinal epithelial cells are capable of converting FdUrd to FUra but do not degrade FUra to CO_2. The intestinal mucosa resembles most other tissues in its ability to catabolize FdUrd to FUra or possibly further metabolites. The reaction, catalyzed by pyrimidine nucleoside phosphorylases (20), has been reported to occur in all normal tissues examined (20) and in a large number of tumors (15, 20, 32, 33). The intestinal mucosa differs markedly from the liver in the kinetics of FdUrd breakdown; kinetic studies have not been reported for other tissues. The K_m for the conversion of FdUrd to FUra in isolated rat intestinal epithelial cells (1600–2500 μM) is about 10-fold the K_m for FdUrd elimination by the isolated perfused rat liver (161–194 μM) (22). Clarkson *et al.* (34) re-

TABLE I. KINETIC PARAMETERS FOR FdUrd CATABOLISM BY ISOLATED RAT INTESTINAL EPITHELIAL CELLS

Experiment	V_{max} (nmole/mg DNA/min)	K_m (mM)	K_i	
			Hanes–Woolf method (mM)	Dixon method (mM)
Control	26.9 ± 1.30[a]	2.50 ± 0.12		
6-Benzyl-2-thiouracil	25.7 ± 2.05	5.02 ± 0.15[b]	0.73 ± 0.06	0.73 ± 0.03
Control	22.1 ± 2.09	2.49 ± 0.09		
4-Thiothymine	17.0 ± 1.89	5.80 ± 1.13[b]	1.52 ± 0.62	0.68 ± 0.13
Control	16.9 ± 2.13	1.60 ± 0.24		
5-Nitrouracil	13.5 ± 1.08	3.18 ± 0.27[b]	0.12 ± 0.03	0.11 ± 0.01

Note. A 2.5% suspension of cells was incubated with FdUrd and nucleoside phosphorylase inhibitors of varying enzyme specificity. V_{max} and K_m values were calculated from the equation of Hanes–Woolf plots. Apparent K_i values were obtained by two methods. In experiments employing varying concentrations of FdUrd (0.41–2.85 mM) and fixed levels of inhibitors (6-benzyl-2-thiouracil, 0.73 mM; 4-thiothymine, 1.28 mM; and 5-nitrouracil, 0.10 mM), K_i was calculated from $K'_m = (K_m/K_i)I + K_m$. In experiments with a fixed concentration of FdUrd (0.81 mM) and varying concentrations of inhibitors (6-benzyl-2-thiouracil, 0.91–9.1 mM; 4-thiothymine, 0.14–14 mM; and 5-nitrouracil, 0.03–0.19 mM), K_i was estimated graphically from Dixon plots (30).

[a] Values represent means ± SE (n = 3–4).

[b] Significantly different from control ($P < 0.05$).

ported that in patients receiving 30 mg/kg FdUrd by iv bolus administration, the plasma concentration after 10 min was about 100–450 μM. In the monkey (9), iv bolus administration of FdUrd in doses of 10 and 20 mg/kg produced peak plasma concentrations of about 360 and 690 μM, respectively. The plasma concentrations during constant infusion are much lower (34). The K_m for FdUrd breakdown by isolated rat intestinal epithelial cells is thus well above the plasma concentrations achieved during clinical use of the drug.

The inability of isolated rat intestinal epithelial cells to convert FUra to CO_2 contrasts with observations of extensive conversion in the liver and kidney (7, 21, 28, 35), but is similar to findings in several other tissues. The pathways of FUra metabolism are analogous to those for uracil (7, 36). FUra catabolism involves the sequential formation of H_2FUra, α-fluoro-β-ureidopropionic acid, and ultimately CO_2, α-fluoro-β-alanine, and ammonia (7, 36). The three reactions are catalyzed by dihydrouracil dehydrogenase (EC 1.3.1.2), dihydropyrimidinase (EC 3.5.2.2) and β-ureidopropionase (EC 3.5.1.6), respectively. As previously shown for uracil (35, 37), the lack of CO_2 production from FUra could be due to absence or low activity of any of these enzymes. In the present studies with isolated rat intestinal epithelial cells, analyses for the individual intermediate metabolites were not performed, making it impossible to indicate whether FUra was partially catabolized. By analogy with the results described for uracil, however, isolated rat intestinal epithelial cells appear to most closely resemble normal extrahepatic tissues (36, 38) and several hematopoietic tumors (36) in their limited capacity for FUra catabolism.

Metabolism and gastrointestinal toxicity. The kinetic studies with isolated rat intestinal epithelial cells suggest a possible further explanation for the reports that FUra produces more severe gastrointestinal toxicity than FdUrd (18, 19). In studies on the mechanism of gastrointestinal toxicity of fluoropyrimidines in mice, Houghton *et al.* (19) obtained evidence that toxicity is due to incorporation of the drugs into RNA, rather than formation of FdUMP. Since FdUrd must be converted to FUra before incorporation into RNA (20), a high K_m for pyrimidine nucleoside phosphorylase activity, as demonstrated with the isolated rat intestinal epithelial cells, would be expected to limit the formation of FUra from FdUrd, and incorporation into RNA, possibly reducing gastrointestinal toxicity.

Inhibition of FdUrd breakdown. Most normal cells contain at least two nucleoside phosphorylases, uridine phosphorylase, and thymidine phosphorylase, for which FdUrd is a substrate (15), while many tumor cells appear to contain only one or a large predominance of one type of enzyme (15, 38, 39). The present findings that uridine phosphorylase-selective (4-thiothymine), thymidine phosphorylase-selective (6-benzyl-2-thiouracil), and nonselective (5-nitrouracil) inhibitors (17) each decreased the conversion of FdUrd to FUra indicate that rat intestinal epithelial cells contain both types of enzyme. The lack of effect of the inhibitors on [^{3}H]thymidine uptake and incorporation, as shown in the preliminary experiments, indicates that the compounds inhibited the nucleoside phosphorylases rather than the cellular uptake of FdUrd. The K_i values obtained with these intact cells were about 4- to 30-fold greater than those reported from studies with extracted uridine phosphorylase (from Sarcoma 180) and thymidine phosphorylase (from mouse liver) (17). These differences may be due in part to differences in the enzymes in various tissues, the type of enzyme preparation studied (intact cell vs cytosolic extract), or the substrates utilized (FdUrd in studies with isolated rat intestinal epithelial cells vs uridine and thymidine in others).

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Received February 8, 1988. P.S.E.B.M. 1988, Vol. 189.
Accepted September 2, 1988.