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INHIBITION OF FLUOROPYRIMIDINE CATABOLISM BY BENZYLOXYBENZYLURACIL

POSSIBLE RELEVANCE TO REGIONAL CHEMOTHERAPY

GEORGE C. DAHER,* FARDOS N. M. NAGUIB,† MAHMOUD H. EL KOUNI,†
RUIWEN ZHANG,* SENG J. SOONG‡ and ROBERT B. DIASIO*§

*Division of Clinical Pharmacology and ‡Division of Biostatistics, Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, AL 35294; and †Division of Biology and Medicine, Brown University, Providence RI 02912, U.S.A.

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Abstract—Regional infusion with fluoropyrimidines is useful in the treatment of hepatic metastases. However, the effectiveness of regional infusion is minimized by rapid degradation of 5-fluorouracil (FUra) and 5-fluoro-2′-deoxyuridine (FdUrd) by the liver which limits the availability of drug for anabolism to active metabolites. 5-Benzyloxybenzyluracil (BBU) is a potent inhibitor of dihydropyrimidine dehydrogenase (DPD), the initial enzyme in FUra catabolism (Naguib FMN, el Kouni MH and Cha S, *Biochem Pharmacol* **38**: 1471–1480, 1989). The effect of BBU on fluoropyrimidine catabolism in the liver was evaluated using the isolated perfused rat liver (IPRL). BBU infused at 0.35 μM over the course of 1 hr demonstrated no hepatotoxicity as measured by bile flow, O_2 uptake and lactate dehydrogenase leakage. The effect of BBU (0.35 μM) on the catabolism of FUra (10 μM) or FdUrd (10 μM) was quantitated by HPLC at 5- or 10-min intervals over a 1-hr period. BBU maximally inhibited FUra catabolism by approximately 83%. Further studies utilizing short-term (20 min) infusion of BBU prior to administration of FUra suggested that the inhibition of DPD was reversible. While FdUrd phosphorolysis was not affected, subsequent catabolism of FUra decreased by 70%. Studies on isolated hepatocytes indicated that the increased FUra level in perfusate resulted from inhibition of FUra catabolism and not from inhibition of FUra transport. The significant inhibition of FUra catabolism suggests that BBU may be useful in modulating regional chemotherapy by these fluoropyrimidines.

5-Fluoro-2′-deoxyuridine (FdUrd‖) is a pyrimidine nucleoside analogue utilized primarily in the management of hepatic metastases of advanced gastrointestinal adenocarcinoma [1] and, more recently, in systemic treatment of renal cell carcinoma [2]. 5-Fluorouracil (FUra), the pyrimidine base of FdUrd, was first introduced as a rationally synthesized anticancer drug in 1957 [3, 4] and continues to be widely utilized in the treatment of human malignancies including cancer of the breast, colon, and skin [5]. Over the past three decades, considerable attention has been focused on the biochemistry and toxicity of these fluoropyrimidines. It is now clear that the chemotherapeutic action of these prodrugs is mediated by anabolism to fluorinated nucleotide analogues in actively proliferating cells.

Although the availability of fluoropyrimidines *in vivo* is primarily regulated by catabolism [6, 7], few studies have addressed the significance of the pyrimidine catabolic pathway in chemotherapy. Fluoropyrimidine degradation in mammals is carried out by the reductive degradation pathway of the naturally occurring pyrimidines, uracil and thymine. These bases are degraded to their corresponding dihydropyrimidines by dihydropyrimidine dehydrogenase (DPD; EC 1.3.1.2), the initial enzyme in pyrimidine base degradation. DPD has been suggested to be the rate-limiting step in pyrimidine catabolism in the liver [8–10]. It is also believed that this enzyme is a target for cancer therapy since it breaks down FUra and the radiosensitizing drugs 5-iodo- and 5-bromouracil more efficiently than the natural substrates uracil and thymine [6, 10–12]. Thus, inhibition of DPD activity may be useful in enhancing the chemotherapeutic efficacy of fluoropyrimidines.

The importance of DPD in cancer chemotherapy has been demonstrated by *in vivo* and *in vitro* studies in which inhibitors of DPD were shown to enhance the chemotherapeutic index of FUra [12–15]. Some of these compounds, however, have been shown to be irreversible inhibitors of DPD (e.g. bromovinyluracil). Thus, the prolonged use of these drugs can lead to severe hematologic and gastrointestinal toxicity as a result of FUra "spillover" into the systemic circulation. Recently, 5-benzyloxybenzyluracil (BBU; Fig. 1) was identified as the most potent inhibitor of this enzyme with an apparent K_i of 0.2 μM [16]. The apparent K_m of the

§ To whom reprint requests should be addressed: Box 600 Volker Hall, Division of Clinical Pharmacology, University of Alabama at Birmingham, Birmingham, AL 35294.

‖ Abbreviations: FdUrd, 5-fluoro-2′-deoxyuridine; BBU, 5-benzyloxybenzyluracil or 5-(3′-benzyloxy)benzyluracil; FUra, 5-fluorouracil; FUrd, 5-fluorouridine; dThdPase, thymidine phosphorylase; DPD, dihydropyrimidine dehydrogenase; FUH_2, dihydrofluorouracil; FUPA, fluoroureidopropionate; FBAL, α-fluoro-β-alanine; and LDH, lactate dehydrogenase.

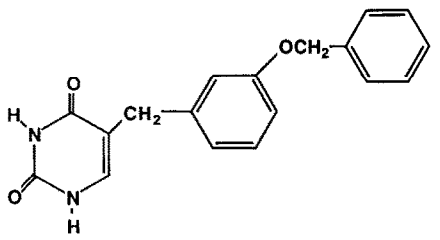

Fig. 1. Chemical structure of 5-benzyloxybenzyluracil (BBU).

naturally occurring substrate uracil was shown to be 9.3 μM [16]. It should also be noted that BBU appears to increase the therapeutic index of FUra approximately 3-fold in mice bearing DLD1 human colon carcinoma (Naguib FNM, unpublished data).

Since the liver is believed to be the primary site of pyrimidine catabolism [17] and intrahepatic infusion of FdUrd has been shown to be an effective route of drug delivery [18], this study examined the effect of BBU on fluoropyrimidine catabolism in the isolated perfused rat liver (IPRL). This model is particularly useful for evaluating the catabolism of fluoropyrimidines, since anabolism via thymidine and uridine kinases is essentially absent in mature liver. To further clarify the modulating effect of BBU on the cellular metabolism of FUra, the intracellular and extracellular concentrations of FUra and its metabolites were examined in parenchymal liver cells in the presence or absence of BBU.

MATERIALS AND METHODS

Chemicals. FdUrd and FUra were obtained from the Sigma Chemical Co. (St. Louis, MO). [6-^{3}H]FdUrd (20 Ci/mmol) and [6-^{3}H]FUra (25 Ci/mmol) were obtained from Moravek Biochemicals (Brea, CA). [*carboxy*-^{14}C]Inulin (2.7 mCi/g) was obtained from New England Nuclear (Boston, MA). The purity of the radiolabeled compounds was confirmed by HPLC to be ≥98% for [6-^{3}H]FdUrd and ≥99% for [6-^{3}H]FUra. BBU was synthesized by Dr. Shih-Hsi Chu, Brown University, Providence, RI. All solvents were HPLC grade, and all chemicals were of the highest grade available.

Isolated perfused rat liver. Male Sprague–Dawley rats weighing 200–250 g were purchased from Harlam Laboratories (Indianapolis, IN). All animals were housed in the University of Alabama at Birmingham animal facilities for at least 2 days prior to their use. The facility is maintained at 21 ± 1° with 12-hr light/dark cycles. Food (Purina rodent chow) and water were provided to the animals *ad lib*. Prior to liver isolation, rats were anesthetized with 35 mg/kg pentobarbital (i.p. injection), following an institutional review board approved protocol. The common bile duct was cannulated with polyethylene tubing (PE-10, Clay Adams). The liver was cannulated via the hepatic portal vein, and perfused in a non-recirculating system with Krebs–Henseleit bicarbonate buffer (pH 7.4), saturated with a gas mixture of oxygen (95%) and carbon dioxide (5%) [19, 20]. An outflow cannula was placed into the superior vena cava. The liver was removed from the animal and was placed on a perfusion block. The affluent perfusate was warmed to 37° and the flow rate was set to 40 mL/min to maintain an adequate supply of oxygen to the liver. The effluent perfusate was allowed to flow past an oxygen electrode to monitor the relative uptake of oxygen. Bile was collected into tared glass vials over 10-min intervals and the volume calculated by determining its weight assuming the density of bile to be 1 g/mL [21].

Infusion of BBU with FUra and FdUrd. Following an initial 20-min perfusion period, a non-hepatotoxic (assessed by bile flow, LDH leakage and O_2 consumption) concentration of BBU (0.35 μM final concentration) was infused into the affluent perfusate buffer for an additional 15-min period prior to the infusion of the fluoropyrimidine. FUra or FdUrd (10 μM final concentration) was infused along with BBU and [6-^{3}H]FUra or [6-^{3}H]FdUrd at a rate of 1 μCi/min. Affluent perfusate (2 mL) was collected every 10 min, and effluent samples (2 mL) were collected every 5 min to determine the rate (nmol/min/g liver) of [6-^{3}H]FdUrd or [6-^{3}H]FUra infusion into and out of the liver. The specific activity of the [6-^{3}H]FUra and [6-^{3}H]FdUrd during each of their infusion was 0.51 μCi/mmol.

Determination of the fluoropyrimidine metabolite profile in perfusate. An aliquot (200 μL) of each collected sample was analyzed by HPLC for FdUrd, FUra and catabolites [i.e. dihydrofluorouracil (FUH_2), fluoroureidopropionate (FUPA) and α-fluoro-β-alanine (FBAL)] as described previously [22]. Briefly, these compounds were separated using two 5 μm C_{18} reversed-phase columns (25 × 0.45 cm) connected in tandem and packed with 5 μm Hypersil ODS. Elution was carried out isocratically at 1 mL/min with a mobile phase of 5 mM tetrabutylammonium hydrogen sulfate and 1.5 mM potassium dihydrogen phosphate (pH 8.0).

Uptake and catabolism of FUra by isolated hepatocytes. Hepatocytes in suspension were isolated from male Sprague–Dawley rats (250–300 g) by modification of the collagenase perfusion technique [23], and as previously described [24]. Only cells with a viability of ≥90%, as determined by trypan blue exclusion, were utilized. Viable hepatocytes were suspended to a final cytocrit of 4–5% and were incubated at 37° in Krebs–Henseleit buffer containing 0.25% gelatin and 10 mM glucose. pH was maintained at 7.4 by passing warmed and humidified 95% O_2 and 5% CO_2 over the cell suspension. The suspension was stirred continuously throughout the experiment with a teflon paddle in specifically designed flasks as described earlier [25]. The experiment was initiated with the addition of [6-^{3}H]FdUrd or [6-^{3}H]FUra (30 μCi/μmol) to achieve a final concentration of 25 μM. BBU was added to the cell suspension, 15 min prior to the initiation of the experiment, to achieve a final concentration of 10 μM. An aliquot of 500 μL from the cell suspension was layered onto 400 μL of inert silicone oil of density 1.2 [26] in 1.5-mL plastic microcentrifuge tubes. The incubation was terminated by centrifugation at 15,000 *g* in an Eppendorf Centrifuge model 5415 C for 15 sec to

sediment all the cells. Times of incubation in the text represent the time interval between introduction of [6-^{3}H]FUra or [6-^{3}H]FdUrd into the cell suspension and starting of the microcentrifuge. The cell pellet (intracellular fraction) was immediately frozen in a dry ice/acetone bath. The oil layer contained no radioactivity, and the remaining upper fraction contained the extracellular medium. Fifty microliters of the extracellular fraction was analyzed without further processing using the same liquid chromatographic methodology described earlier [22]. The frozen cell pellet was transferred to a plastic tube immersed in ice and subjected to sonic oscillation in 1 mL of 2 mM potassium phosphate (pH 7.4) with a sonicator (Arteck 300, Farmingdale, NY) for 30 sec. The released intracellular ^{3}H-labeled sonicate was transferred to pyrex tubes and centrifuged at 25,000 *g* and 0° for 15 min to pellet cellular debris. The protein concentration was measured with 15-μL aliquots (before centrifugation), using bovine serum albumin as the standard [27]. One hundred microliters of the cell supernate was analyzed by HPLC as described above.

Determination of extracellular and intracellular spaces. To provide a measure and corrections for FdUrd, FUra and the catabolites present in the extracellular space that accompanies the intracellular radiolabeled substances into the cell pellet, a portion of the hepatocyte suspension was incubated under the same conditions described above and was exposed to 0.4 μCi (approximately 104,000 cpm/mL) of [*carboxyl*-^{14}C]inulin for 10 min. An aliquot (500 μL) of cell suspension was layered onto oil and centrifuged at 15,000 *g* to provide a measure of the extracellular space that accompanies cells in the pellet. This value was obtaiend from the ratio of the [^{14}C]inulin content in the dry pellet to the inulin concentration in the supernatant. To determine the theoretical intracellular water volume, samples of the cell suspension were exposed to [^{14}C]inulin and spun in empty microcentrifuge tubes. Intracellular water is the difference between the wet and dry weights of the cell pellet less the [^{14}C]inulin space. This technique has been described in detail previously [25, 28, 29].

Statistical analysis. The significance ($P \leq 0.05$) of the difference between the experimental and control groups was tested using two-factor analysis of variance (ANOVA) with repeated measures on one factor. Duncan's multiple range test [30] was also used as appropriate.

RESULTS

Effect of BBU on continuous FUra infusion in the IPRL. Figure 2 illustrates the metabolic profile in the perfusate of the IPRL following infusion of 10 μM FUra in the absence (Fig. 2A) or presence of 0.35 μM BBU (Fig. 2, B and C). The level of significance of the difference in FUra catabolism in the presence and absence of BBU was analyzed by ANOVA with repeated measures on one factor. Figure 2A shows that in the absence of BBU over 50% of FUra was degraded to FUra catabolites (i.e. FUH_2, FUPA, FBAL). Figure 2B illustrates the rate of elimination of FUra and FUra catabolites in the perfusate following the infusion of 0.35 μM BBU, 15 min prior to and during the infusion of 10 μM FUra. When the profile of FUra catabolites between 5 and 60 min in Fig. 2B was compared to that of Fig. 2A, there was a significant decrease ($P = 0.004$) in the rate of elimination of FUra catabolites over time. Figure 2C illustrates the level of FUra and its catabolite in the effluent perfusate following conditions in which 0.35 μM BBU was infused for only 20 min prior to the infusion of FUra. When the FUra catabolite profile in Fig. 2C was compared over time to that of Fig. 2A, a statistically significant difference was observed ($P = 0.03$). It should be noted that in Fig. 2C, the rate of elimination of FUra catabolites gradually increased throughout the experiment. The rate of elimination of FUra catabolites in Fig. 2C was increased significantly ($P = 0.02$) compared to that observed in Fig. 2B.

Effect of BBU on continuous FdUrd infusion in the IPRL. Figure 3A shows the pattern of appearance of FdUrd, FUra, and FUra catabolites in the effluent perfusate after infusion of 10 μM FdUrd. The rate of elimination of FUra catabolites was higher than that of FUra. The infusion of 0.35 μM BBU 15 min prior to and during the infusion of 10 μM FdUrd caused no significant decrease ($P = 0.8$) in the rate of elimination of FdUrd in the effluent perfusate (Fig. 3B). However, BBU significantly inhibited ($P = 0.004$) the rate of elimination of FUra catabolites and increased ($P = 0.0007$) the rate of elimination of unmetabolized FUra in the effluent perfusate.

Effect of BBU on the hepatic extraction ratio of FUra and FdUrd. Table 1 shows that the infusion of BBU (0.35 μM) for 20 min followed by the infusion of 10 μM FUra caused a significant decrease ($P \leq 0.05$) in the FUra hepatic extraction ratio (0.39 ± 0.02). When the infusion of BBU was continued during the infusion of 10 μM FUra, the heptic extraction ratio decreased further to 0.15 ± 0.03. In contrast, administration of BBU (0.35 μM) prior to and during the infusion of FdUrd did not decrease significantly the FdUrd hepatic extraction ratio.

Effect of BBU on fluoropyrimidine catabolism in isolated hepatocytes. To delineate whether BBU is inhibiting the metabolism or influx/efflux of fluoropyrimidines, the effect of BBU on their transport and metabolism was studied in isolated hepatocytes in suspension. Addition of BBU (0.35 μM) to cell suspension containing 10 μM FUra increased the intracellular concentration of FUra and reduced the intracellular concentration of its catabolites (data not shown). These studies, however, did not discern whether this increase in the intracellular concentration of FUra by BBU was due to the inhibition of FUra catabolism or to inhibition of FUra efflux. Incubation of the cells with BBU and FdUrd would allow measurement of the intracellular and extracellular concentration of FUra present only as a result of the intracellular phosphorolysis of FdUrd. Figure 4 illustrates that upon incubation with 10 μM BBU 15 min prior to and during incubation with 25 μM FdUrd, the intracellular concentration of FUra did not increase significantly ($P = 0.07$) while the intracellular

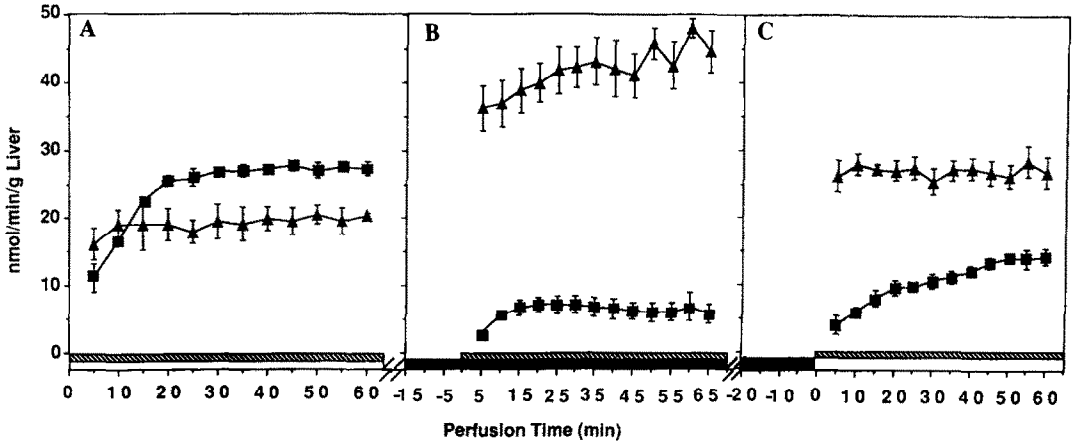

Fig. 2. Metabolic profile in the perfusate of the isolated perfused rat liver following infusion of 10 μM FUra in the absence (A) or presence (B and C) of 0.35 μM BBU. Panel (B) depicts the effect of continuous exposure to BBU starting at 15 min prior to the infusion of FUra and continuing for the duration of the experiment. Panel (C) depicts the effect of short-term exposure to BBU (i.e. 20 min) prior to the infusion of FUra. Values are means ± SEM (N = 3). Key: (▲) FUra; (■) FUra catabolites; (hatched bars) duration of FUra infusion; (solid white bars) no infusion of BBU; and (solid black bars) duration of BBU infusion.

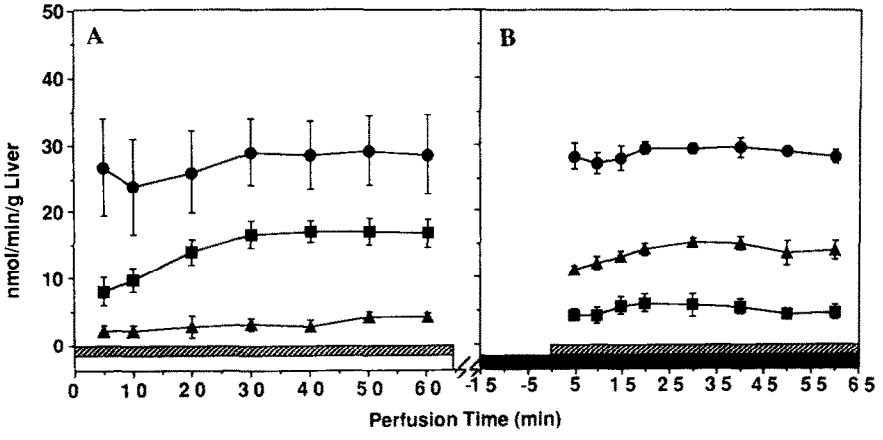

Fig. 3. Metabolic profile in the perfusate of the isolated perfused rat liver following infusion of 10 μM FdUrd in the absence (A) or presence (B) of 0.35 μM BBU. Panel (B) depicts the effect of continuous exposure to BBU starting at 15 min prior to the infusion of FdUrd and continuing for the duration of the experiment. Values are means ± SEM (N = 3). Key: (●) FdUrd; (▲) FUra; (■) FUra catabolites; (hatched bars) duration of FdUrd infusion; (solid white bars) no infusion of BBU; and (solid black bars) duration of BBU infusion.

concentration of FUra catabolites decreased significantly (P = 0.02). These results indicate that BBU is primarily inhibiting the catabolism of FUra rather than the phosphorolysis of FdUrd. Moreover, in these same experiments (Fig. 5) when the extracellular concentration of FUra was measured in the presence or absence of BBU, there was approximately a 7-fold increase (P = 0.004) in the concentration of FUra. In contrast, the extracellular concentration of FUra catabolites decreased approximately 3-fold (P = 0.03). These results support the contention that the increased level of FUra by BBU in the IPRL is caused by the inhibition of FUra catabolism rather than its transport. Moreover, the insignificant decrease (P = 0.8) in the extracellular level of FdUrd in the presence of BBU (0.35 μM) is a further indication that BBU at this concentration did not inhibit the phosphorolysis of FdUrd.

The analysis of the intracellular and extracellular metabolites by HPLC demonstrated that all of the radioactivity injected on the columns was recovered. In the control study, all of the injected intracellular radioactive metabolites were recovered as FUra, FUH_2, FUPA, and FBAL. However, in the presence

Table 1. Effect of BBU on liver extraction* of FUra and FdUrd in the IPRL

Treatment	FUra	FdUrd
Control	0.58 ± 0.05	0.44 ± 0.05
BBU → Fluoropyrimidines†	0.39 ± 0.2‡	
BBU + Fluoropyrimidines†	0.15 ± 0.03‡	0.34 ± 0.03

* $([FUra_{in}] - [FUra_{out}])/[FUra_{in}]$.

† Fluoropyrimidines (FdUrd or FUra); (→) liver is infused with BBU (0.35 μM) for 20 min prior to the infusion of 10 μM FUra; and (+) liver is infused with BBU for 15 min prior to and continuing for the duration of the experiment.

‡ $P \leq 0.05$ vs control.

of BBU, another metabolite was identified as fluorouridine (FUrd) by retention time and coelution with authentic standard. The intracellular concentration of this nucleoside accounted for 3% of the total eluted radioactivity. Nonetheless, its extacellular concentration, both in the presence or absence of BBU, accounted for 0.6% of the total injected radioactivity (data not shown).

DISCUSSION

BBU, the most potent inhibitor of DPD known to date [16], was utilized in the present study with the rationale that the liver could be exposed to less drug while still achieving optimal DPD inhibition. The results of this study clearly demonstrate that BBU significantly reduced the catabolism of FUra

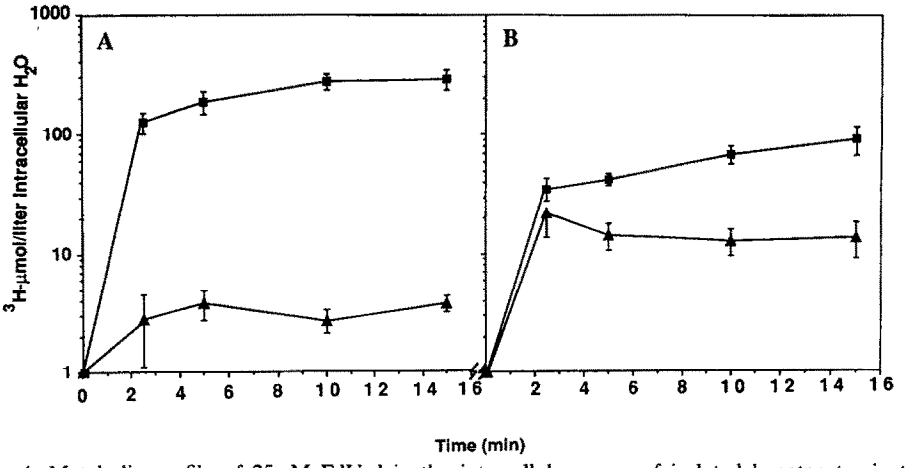

Fig. 4. Metabolic profile of 25 μM FdUrd in the intracellular space of isolated hepatocytes in the absence (A) or presence (B) of 10 μM BBU. BBU was added to the cell suspension 15 min prior to and during the addition of FdUrd. Values are means ± SEM (N = 3). Key: (▲) FUra; and (■) FUra catabolites.

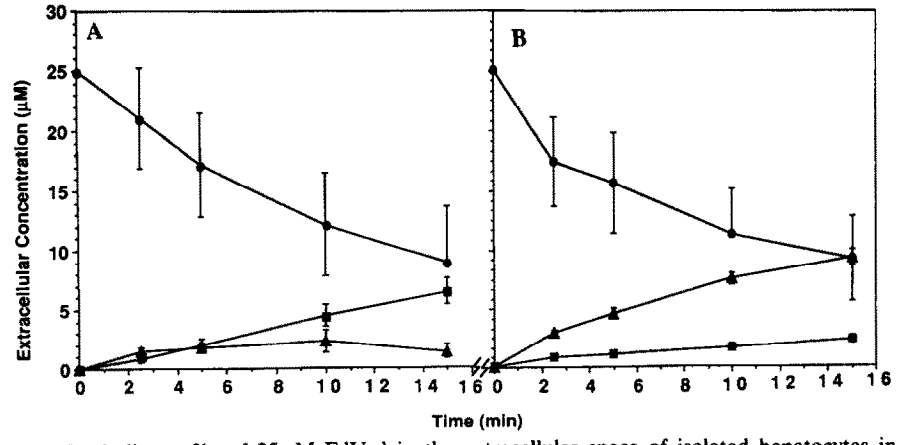

Fig. 5. Metabolic profile of 25 μM FdUrd in the extracellular space of isolated hepatocytes in the absence (A) or presence (B) of 10 μM BBU. Values are means ± SEM (N = 3). Key: (●) FdUrd; (▲) FUra; and (■) FUra catabolites.

and the rate of elimination of its catabolites in the IPRL (Fig. 2, A and B). The advantage of inhibiting the catabolism of FUra alone is that it permits the accumulation of FUra to a higher intracellular concentration. Indeed, in the presence of BBU, the level of FUra generated from the phosphorolysis of FdUrd increased in the effluent perfusate from approximately 7% in the control to 34%. However, BBU at this concentration did not affect the phosphorolysis of FdUrd (Fig. 3, A and B) while significantly inhibiting FUra catabolism. In an earlier study [31] in which 3-deazauracil (i.e. 2,4-dihydroxypyridine) was utilized to block DPD activity, inhibition of FdUrd to FUra conversion was observed following the increase of unmetabolized FUra. In the present study when FUra catabolism was inhibited in the IPRL, no significant inhibition in the phosphorolysis of FdUrd to FUra was observed. This discrepancy is to be expected since we utilized a non-recirculating perfusion system, and the liver was infused with a constant level of FdUrd. Thus, under these conditions, it is impossible to measure the inhibition of FdUrd to FUra conversion over time.

The IPRL results in the present study as well as in an earlier biochemical study [16] do not differentiate whether BBU is an inhibitor of fluoropyrimidine transport or catabolism. To better understand the effect of BBU on the cellular metabolism of FUra, isolated parenchymal liver cells were initially exposed to BBU prior to exposure to fluoropyrimidines. As noted in the results, FdUrd (in contrast to FUra) permitted the measurement of the intracellular and extracellular concentration of FUra present as a result of intracellular phosphorolysis of FdUrd. With this approach the measurement of the intracellular and extracellular concentrations of the fluoropyrimidines and their respective catabolites could also be measured. In the presence of BBU (Fig. 4), the intracellular concentration of FUra did not increase significantly ($P = 0.06$), whereas that of FUra catabolites decreased by approximately 3- to 4-fold. When the extracellular concentrations of generated FUra and FUra catabolites were examined in the presence of BBU, a proportional decrease in the level of extracellular catabolites was observed. Under these conditions, the extracellular FUra concentration was increased by approximately 7-fold. These results demonstrate that following inhibition of DPD in normal parenchymal cells, FUra is rapidly effluxed from cells. Furthermore, unmetabolized FdUrd was not detected intracellularly even when catabolism of the generated FUra was inhibited by BBU. This suggests that the transport of FdUrd was rate-limiting to its phosphorolysis by the pyrimidine nucleoside phosphorylases in the presence or absence of BBU.

In summary, the results with liver parenchymal cells strongly suggest that the concomitant increase in FUra concentration and decrease in the concentration of its catabolites results primarily from the inhibition of DPD. In this context it should be noted that the use of parenchymal liver cells in this study was to confirm whether the changes in the profile of FUra and its catabolites in the IPRL system were caused by an effect of BBU on DPD or on efflux/influx of these fluoropyrimidines. Quantitative modulation of the bidirectional fluxes and net transport of the fluoropyrimidines across the cell membrane are beyond the scope of the present study and will be addressed at a later date.

In the presence of BBU, [6-^{3}H]FUrd was detected intracellularly. This metabolite, however, was not detected in the absence of BBU. An earlier study demonstrated that the apparent K_m values of FUra and ribose-1-phosphate for uridine phosphorylase in rat liver were 36 and 20 μM, respectively. In contrast, the K_m value of the naturally occurring substrate, uracil, was reported to be 485 μM, indicating that uridine phosphorylase would have a higher affinity for FUra than for uracil. Furthermore, the concentration of ribose-1-phosphate in rat liver (120 μM) was reported to be well above its K_m value [32]. Hence, it is conceivable that once FUra catabolism is blocked, FUra concentration may rise to a level that would permit activation by the phosphorylase pathway to form FUrd.

In the present study, there was no evidence of anabolism to nucleotides. This is in agreement with previous studies from this laboratory [33] in which no nucleotides were found following exposure of hepatocytes to 300 μM FUra for 2 hr and in which DPD activity was inhibited by 2 mM thymine.

Further studies are required to clarify the biological and chemotherapeutic importance of BBU in modulating the fluoropyrimidine metabolism in the liver. Of particular interest is the increased concentration of fluoropyrimidine available for tumor cells metastatic to the liver. It could be argued, however, that using DPD inhibitors could mimic the toxicity observed in DPD-deficient patients [34]. This is especially true since inhibition of DPD activity over time will increase the availability of FUra and FdUrd in the liver resulting in a "spillover" into the systemic circulation, causing hematologic and gastrointestinal toxicity. It is reassuring that the effect of BBU was transient and reversible with time (Fig. 2C). Even if such toxicity occurs, it can be potentially overcome by the use of a carbon hemofilter. This technique has been demonstrated recently to efficiently extract drug in the hepatic venous outflow in the pig model [35]. Such an approach may prove to be clinically useful for limiting systemic exposure when modulating fluoropyrimidine chemotherapy in the liver with inhibitors such as BBU.

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