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The Effects of 5-benzylacyclouridine on the Cytotoxicities of Fluorinated Pyrimidine Antimetabolic Agents in L5178Y Cells[1]

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ABSTRACT

The benzylacycoluridines (BAU and BBAU) are potent and specific inhibitors of uridine phosphorylase (UrdPase). In contrast to the report that benzylacyclouridines potentiated 5-fluoro-2'-deoxyuridine (FdUrd) cytotoxicity against human solid tumor cells (Cancer Res., 44:1852, 1984), continuous exposure of mouse lymphoma L5178Y cells, to FdURd, 5-fluorouridine (FUrd), 5'-deoxy-5-fluorouridine (5'-dFUrd), or 5-fluorouracil (FUra) showed no potentiation of cytotoxicity by benzylacyclouridines. In fact, under the conditions employed, benzylacyclouridines protected the cells from the cytotoxicity of FdUrd, FUrd, or 5'-dFUrd, but not FUra in a dose dependent manner. Intraperitoneal coadministration of BAU or BBAU and a 5-fluorinated pyrimidine (*i.e.*, FdUrd, FUrd, or FUra), to mice bearing L5178Y cells also did not significantly increase the life span compared to those treated with the antimetabolites alone. Anabolism of these nucleosides through the sequential action of UrdPase and orotate phosphoribosyltransferase (OPRTase), inhibition of nucleoside transport by benzylacyclouridines, or both could be responsible for the ineffectiveness of UrdPase inhibitors to potentiate the antineoplastic activity of fluoropyrimidines in L5178Y cells.

Key Words: Benzylacyclouridine, Uridine phosphorylase inhibitor

Abbreviation: BAU (5-benzylacyclouridine), 5-benzyl-1-(2-hydroxymethyl) uracil; BBAU (benzyloxybenzylacyclouridine), 5-(*m*-benzyloxybenzyl)- 1-(2'-hydroxytheoxymethyl) uracil; 5'-dFUrd, 5'-deoxy-5-fluorouridine; dThdPase, thymidine phosphorylase; dUTPase, 2'-deoxyuridine-5'-triphosphate pyrophosphatase; FdUrd, 5-fluoro-2'-deoxyuridine; FUra, 5-fluorouracil; FUrd, 5-fluorouridine; NBMPR (nitrobenzylthioinosine), 6-[(4-nitrobenzyl) thio]-9-β-D-ribofuranosylpurine; OPRTase, orotate phosphoribosyltransferase; Rib-1-P, ribose-1-phosphate; PRibPP, phosphoribosylpyrophosphate; UrdPase, uridine phosphorylase.

INTRODUCTION

Since the 1950's when its antitumor activity was first shown (Heidelberger *et al.*, 1975), 5-fluorouracil (FUra) has remained as one of the few drugs effective against solid tumors in man. There are four pathways by which FUra may be activated. Firstly, in the presence of an appropriate deoxyribose donor, FUra can be converted to 5-fluoro-2'-deoxyuridine (FdURd) by thymidine phosphorylase (dThdPase) then to 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP) by dThd kinase (Birnie *et al.*, 1963). Secondly, the formation of FdUrd from FUra is also possible *via* deoxyribosyl-transfer due to a dThdPase-bound 2-deoxyribose-1-phosphate (dRib-1-P) intermediate (Iltzsch *et al.*, 1985). Thirdly, FUra may undergo direct conversion to 5-fluorouridine-5'-monophosphate (FUMP) by the action of orotate

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phosphoribosyltransferase (OPRTase) (Reyes, 1959). Finally, it can be salvaged to FUMP by the consecutive actions of uridine phosphorylase (UrdPase) and uridine-cytidine kinase (Heidelberger, 1975; Zimmerman and Seidenberg, 1964). FUMP may then be phosphorylated to produce its cytotoxic effects by: 1) conversion to 5-fluorouridine-5'-triphosphate (FUTP) with subsequent incorporation into RNA (Reyes and Heidelberger, 1965; Wilkinson and Pitot, 1973; Heidelberger, 1975); 2) conversion to FdUMP *via* FUMP → 5-fluorouridine-5'-disphosphate (FUDP) → 5-fluoro-2'-deoxyuridine-5'-disphosphate (FdUDP) → FdUMP, involving ribonuleoside diphosphate reductase, and the covalent binding of FdUMP to thymidylate synthetase which causes *thymineless death* (Reyes and Heidelberger, 1965; Heidelberger, 1975); and 3) conversion to 5-fluoro-2'-deoxyuridine-5'-triphosphate (FdUTP) which may have two fates: incorporation into DNA (Cooper *et al.*, 1972; Caradonna and Cheng, 1980; Kufe *et al.*, 1981), or degradation to FdUMP by 2'-deoxyuridine-5'-triphosphate pyrophosphatase (dUPTase). The incorporation of FUra into DNA (Schuetz *et al.*, 1984) is kept minimal by the high activities of dUTPase and the repair mechanism of uracil-DNA glycosylase which removes FUra moieties from DNA (Cheng and Nakayama, 1982).

Birnie *et al.*, (1963) proposed that pyrimidine phosphorylases are responsible for the cleavage of FdUrd to the less effective FUra, and thus inhibitors of these enzymes may potentiate the antitumor activity of FdUrd. On the basis of the speculation of Birnie *et al.*, (1963) and the observations that certain tumors have little or no dThdPase (Krenitsky *et al.*, 1964; Zimmerman and Seidenberg, 1963; Marsh and Perry, 1963; Lazarus *et al.*, 1974; Zielke, 1979; Woodman and Sariff, 1980; Niedzwicki *et al.*, 1981), Niedzwicki *et al.* (1984a; 1984b) observed such potentiation of FdUrd cytotoxicity by BAU and BBAU against human pancreatic and lung carcinoma cells. A recent review article dealt with various aspects of benzylacyclouridines (Cha, 1989).

In the present study, we have tested the effects of UrdPase inhibitors, 5-benzyl-1-(2'-hydroxyethoxymethyl) uracil (BAU, 5-benzylacyclouridine) and 5-(3-benzyloxybenzyl)-1-(2'hydroxyethoxymethyl) uracil (BBAU, 5-benzyloxybenzylacyclouridine) (Niedzwicki *et al.*, 1982) on the cytotoxicity of FUra, FUrd, or FdUrd against the mouse lymphoma L5178Y cells *in vitro* and *in vivo*.

MATERIALS AND METHODS

Chemicals

BAU and BBAU were obtained from Dr. Shih-Hsi Chu, Brown University, Providence, RI. Fischer's media, Puck's saline G, horse serum, antibiotics, and trypan blue stain (4%) were obtained from Grand Island Biological Co., Grand Island, NY; [5-^{3}H]Urd, [2-^{14}C]FUrd and [2-^{14}C]Urd from Moravek Biochemical Inc., Brea, CA; silica gel G/UV_{254} polygram thin layer chromatography (tlc) plates from Brinkmann Instruments, Inc., Westbury, NJ; ACS scintillant from Amersham/Searle Crop., Arlington Heights, IL; Omnifluor from New England Nuclear Corp., Boston, MA. 6-[(4-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine (nitrobenzylthioinosine NBMPR), and other unlabelled pyrimidine compounds were purchased from Sigma Chemical Co., St. Louis, MO.

Cell culture

L5178Y mouse lymphoma cells were routinely grown in suspension cultures in Fischer's medium supplemented with horse serum (10%), penicillin (100 units/ml) and streptomycin (100 μg/ml) in a humidified incubator maintained at 95% air: 5% CO_2 at 37° (Fischer and Sartorelli, 1964). Doubling times ranged from 9.5 to 12 hr.

Combination treatments of cells in culture by 5-benzylacyclouridines and fluoropyrimidines

For the cytotoxic studies, 4.8 ml aliquots of cell suspensions of cells in the exponential phase of growth (3 to 10×10^3 cells/ml) were preincubated for 5 min with 0.1 ml of BAU (10 to 100 μM) or its congeners before the addition of 0.1 ml of an antimetabolite preparation. Control tubes received 0.2 ml of media and 4.8 ml of cells. After 3 days of incubation at 37°, cell numbers were determined using a Model B Coulter Counter (Hialeah, FL), and percentage of the control growth rate (number of doublings during the incubation period) was calculated.

The effect of BAU on incorporation of [5-^{3}H]Urd or [2-^{14}C]FUrd into acid soluble and insoluble fractions of L5178Y cells in culture

[5-^{3}H]Urd or [2-^{14}C]Furd incorporation studies were carried out with a slight modification of the

previously described method of Chu *et al.* (1968) L5178Y cells grown in 100 ml culture bottles were harvested by centrifugation at 550 × g for 10 min. The cells were washed with growth medium (Fischer's medium supplemented with 10% horse serum) twice. Then, 4.8 ml aliquots of cell suspension in growth medium (5×10^4 cells/ml) were pre-warmed to 37°, and incubated with or without 0.1 mM BAU. After 5 min 0.1 ml of [5-^{3}H]Urd (2 μCi/tube) was added and the cells were further incubated for 15 hr. For the [2-^{14}C]FUrd incorporation studies, 4.8 ml aliquots of cell suspension of 2 to 3×10^5 cells/ml and [2-^{14}C]FUrd (0.003 μCi/tube) were incubated for periods of 2 to 22 hr were used. For periods of less than 1 hr incubation, 4.8 ml aliquots of 2×10^6 cells/ml cell suspension and [2-^{14}C]FUrd (0.01 μCi/tube) were used. The incorporation was stopped by adding 1 ml of cold 10 μM nitrobenzylthioinosine (NBMPR) in growth medium immediately followed by centrifugation at 550 × g for 2 min. Pellets were rinsed twice with the NBMPR medium and finally lysed in 1 ml of 0.2 N perchloric acid (PCA), followed by cooling in ince-water for 30 min, then centrifugation. Pellets were rinsed with 1 ml of the PCA solution twice and the washings were combined with the original supernatant. The combined supernatants (3 ml) were, then, neutralized with KOH. After centrifugation to precipitate the salt formed, 1 ml aliquots of the supernatant were counted in 9 ml of ACS scintillant fluid to determine the radioactivity. Pellets were dissolved in 0.4 ml of 1 N NaOH to hydrolyze RNA at 90° for 15 min. Proteins and cell debris were removed by centrifugation and 0.3 ml aliquots of the supernatants were counted in 9.7 ml of ACS scintillant fluid for radioactivities.

The effects of 5-benzylacyclouridines on the uptake of [2-^{14}C]Urd by L5178Y cells in culture

The effects of 5-benzylacyclouridines on the uptake of Urd by cultured L5178Y cells were determined by measuring the total radioactivities of cell pellets after 5 min incubation of the cells with [2-^{14}C]Urd and various concentrations of BAU or BBAU. Cells were prepared as described above for the incorporation studies. Uptake was started by mixing 0.25 ml of cell suspension (4×10^6 cells/ml) and 0.25 ml of Fischer's medium containing [2-^{14}C]Urd (0.02 μCi/tube, 58 mCi/mmole) and various concentrations of BAU (1 to 500 μM) or BBAU (1 to 10 μM) in a tube containing 0.15 ml of oil mixture (DC 550 silicone fluid 84 parts and paraffin oil 16 parts). After 5 min incubation at room temperature, the reaction was stopped by centrifugation through the oil layer; then the supernatant was removed. The tubes were rinsed twice with water which was removed along with the oil mixture. To the cell pellets, 0.5 ml aliquots of 0.4 N KOH were added; then incubated for 15 to 18 hr at 37° to hydrolyze RNA. After centrifugation to precipitate the denatured proteins, 0.5 ml of the supernatant was counted in 9.5 ml of ACS scintillant. Rates of uptake (cpm/min/10^6 cells) is expressed as percent of the control.

Combination treatments of tumor cells *in vivo* by 5-benzylacyclouridines and pyrimidine antimetabolites

In these experiments, male mice [B6D2F$_1$ (C57BL/6 × DBA/2) obtained from Cumberland Farm, Clinton, TN] were used. L5178Y cells were maintained in abdominal cavities of mice by intraperitoneal transplantation of 5×10^6 cells/mouse for every 7 to 10 days. At Day 0, mice weighing 20 to 22 g were inoculated with about 10^7 L5178Y cells/0.1 ml/mouse. Twenty-four hr after transplantation (Day 1), 0.1 ml of one of the following drug preparations were injected intraperitoneally once per day from Day 1 to Day 5: experiment 1 (8 mice per group), Puck's saline G, BBAU (10 mg/kg/day), FdUrd, or FdUrd + BBAU; experiment 2 (9 mice per group), Puck's saline G, BAU (30 mg/kg/day), FUrd (2 mg/kg/day), or FUrd + BAU; and experiment 3 (10 mice per group), Puck's saline G, BAU (30 mg/kg/day), FUra (25 mg/kg/day), or FUra + BAU. BAU and BBAU dissolved in 50% DMSO were used. Thereafter, the survival times of the mice were recorded. Antitumor activity of the drugs was evaluated by the increase in life-span (ILS) over controls. ILS (%) was calculated by the following formula:

$$\text{ILS}\ (\%) = \left[\frac{\dfrac{\text{Individual survival date in the treated group}}{\text{No. of mice in the treated group}}}{\dfrac{\text{EIndividual survival date in the control group}}{\text{No. of mice in the control group}}} - 1\right] \times 100$$

RESULTS

Combination treatments of cells in culture by 5-benzylacyclouridines and pyrimidine antimetabolites

To test the hypothesis that UrdPase inhibitors

Table 1. The effects of 5-benzylacyclouridines on the cytotoxicity of various pyrimidine antimetabolites

Antimetabolite [μm]		BAU or BBAU [μm]		% Control growth: Antimetabolite alone	% Control growth: Antimetabolite & BAU or BBAU	n	p[a]
FUra	0.1	BAU	100	71.7±0.7[b]	70.4±0.4	2	0.151
	0.2			24.9±7.4	24.2±7.7	2	0.933
FUrd	0.001	BAU	100	57.7±4.9	74.2±4.9	3	0.045
	0.0015			27.1±9.0	41.8±9.0	3	0.050
FdUrd	0.001	BAU	100	65.8	75.8	1	
5'-dFUrd	5.0	BAU	50	49.0	61.8	1	
FUra	0.1	BBAU	10	79.1	78.6	1	
	0.2			29.4	27.2	1	
FdUrd	0.0001	BBAU	10	74.7±11.8	89.3±5.2	3	0.121
	0.00015			47.8±16.1	79.5±9.8	3	0.044

[a]: Probability of null hypothesis between antimetabolite alone and antimetabolite plus 5-benzylacyclouridines.
[b]: Average±S.D.

Table 2. The effects of 0.1mM BAU on the incorporation of radiolabeled Urd and FUrd in L5178Y cells in culture

Substrate [μM]		Labeling time [hr]	% Control: Acid sol.	Acid. insol.	Total
Urd	0.02	15	71.4 (64.2)[a]	81.1 (571.3)	
FUrd	0.2	0.3			76.9 (1.8)
	0.2	0.5			76.2 (3.5)
	0.2	0.5			74.8 (3.5)
	0.2	0.6			74.6 (2.9)
	0.035	2	83.5 (0.9)	78.8 (1.2)	
	0.01	20	58.6 (1.2)	76.7 (4.2)	
	0.01	22	62.6 (1.5)	71.3 (4.7)	

[a]: cpm/10^3 cells

may potentiate the cytotoxicity of FdUrd in cells with little or no dThdPase activity (Niedzwicki *et al.*, 1981 and 1982), cytotoxic effects of FdUrd, FUra, FUrd, and 5'-dFUrd in combination with a UrdPase inhibitor (BAU or BBAU) were evaluated by measuring the rates of growth (number of doublings during the 3-day culture period) of L5178Y cells as shown in Table 1. This cell line was shown to have no dThdPase activity (Niedzwicki *et al.*, 1981). 5-Benzylacyclouridines alone did not affect the growth rate of this cell line. Under these conditions, there was no potentiation of the antimetabolite cytotoxicities by the 5-benzylacyclouridines. There were significant dose dependent ($P<0.05$) protections of cells from the cytotoxic effects of FUrd, FdUrd, and 5-'dFUrd, by 5-benzylacyclouridines. The degree of protection from the cytotoxicity of 5'-dFUrd is similar to those of other combinations (BAU + FUrd or BAU + FdUrd).

The effect of BAU on incorporation of [5-^{3}H]Urd and [2-^{14}C]FUrd into acid soluble and insoluble fractions of L5178Y cells in culture

To study whether or not there is a correlation between the cytotoxicity and the incorporation of a pyrimidine nucleoside into acid soluble and insoluble fractions of L5178Y, the cells were incubated with labeled Urd or FUrd in the presence or absence of 0.1 mM BAU. This BAU concentration is similar to that used in most of the cytotoxicity studies. Table 2 shows that BAU inhibited the incorporation of

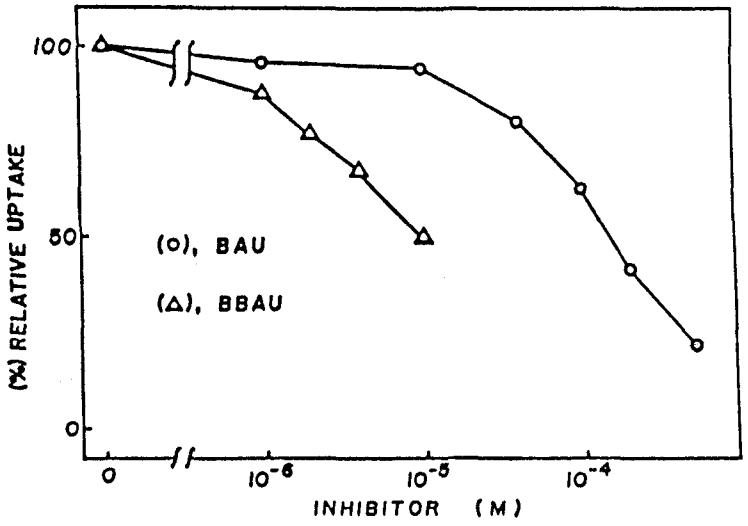

Fig. 1. Effects of BAU and BBAU on [2-^{14}C]Urd uptake in cultured L5178Y cells. 10^6 cells were incubated with 0.02μCi Urd (58 mCi/mmol) for a 5 min period at room temperature. Rates of uptake are expressed as percent of the control.

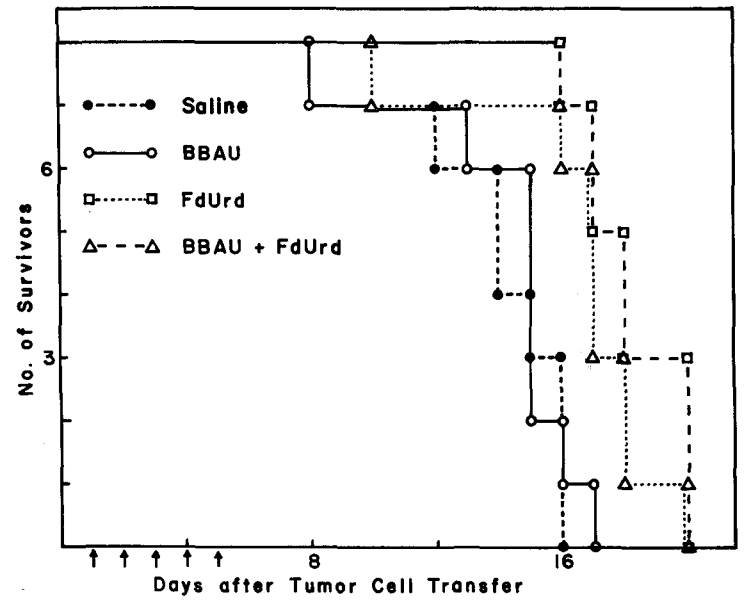

Fig. 2. The effect of combination treatment of FdUrd with BBAU on the survival of mice bearing L5178Y cells. 1.02×10^7 cells were transplanted at Day 0 and the drugs were administered i.p. once per day from Day 1 to Day 5. The concentrations of drugs are: saline (●), 0.1ml of Puck's saline G; BBAU (○), 10 mg/kg/day; FdUrd (□), 50 mg/kg/day; and BBAU + FdUrd (△), 10 mg/kg/day + 50 mg/kg/day.

either [5-^{3}H]Urd or [2-^{14}C]FUrd into both the acid soluble and acid insoluble fractions of L5178Y cells. The degree of inhibition of incorporation, 20 to 30% (71.4 and 81.1% of control) (Table 2), by BAU is comparable to that of protection of L5178Y cells from the cytotoxicity of FdUrd (15% for 0.1nM) or FUrd (23% for 1 nM) (Table 1).

[2-^{14}C]Urd uptake by L5178Y cells in culture

The rates of uptake for 5 min incubation periods at room temperature were determined and expressed as percent of the control as shown in Fig. 1. Both BAU and BBAU inhibited the uptake of [2-^{14}C]-Urd and the inhibition was dose dependent. IC_{50}, inhibitor concentration that gives 50% of Urd uptake, for both BAU and BBAU were estimated from this graph. IC_{50} for inhibition of Urd uptake by L5178Y cells was 10 μM for BBAU which is approximately 10-fold more potent than that for BAU ($\sim$ 100 μM).
fluorinated

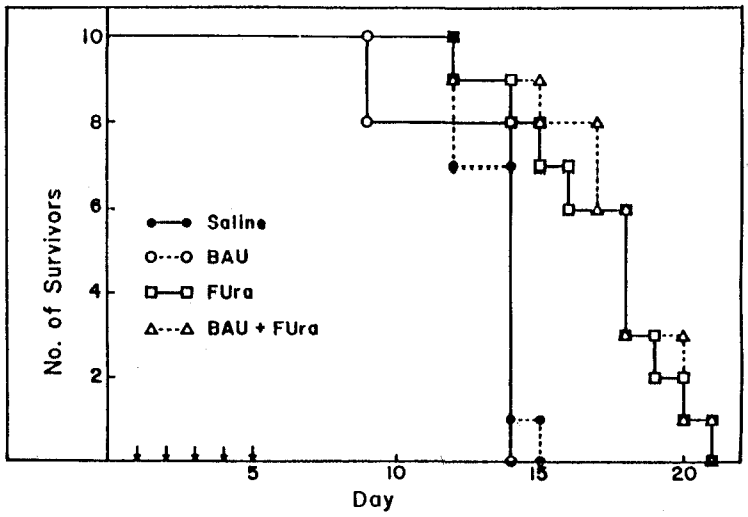

Fig. 3. The effect of combination treatment of FUra with BAU on the survival of mice bearing L5178Y cells. 1.23×10^7 cells were transplanted at Day 0 and the drugs were administered i.p. once per day from Day 1 to Day 5. The concentrations of drugs are: saline (●), 0.1ml of Puck's saline G; BAU (O), 30 mg/kg/day; FUra (□), 25 mg/kg/day; and BAU + FUra (△), 30 mg/kg/day + 25 mg/kg/day.

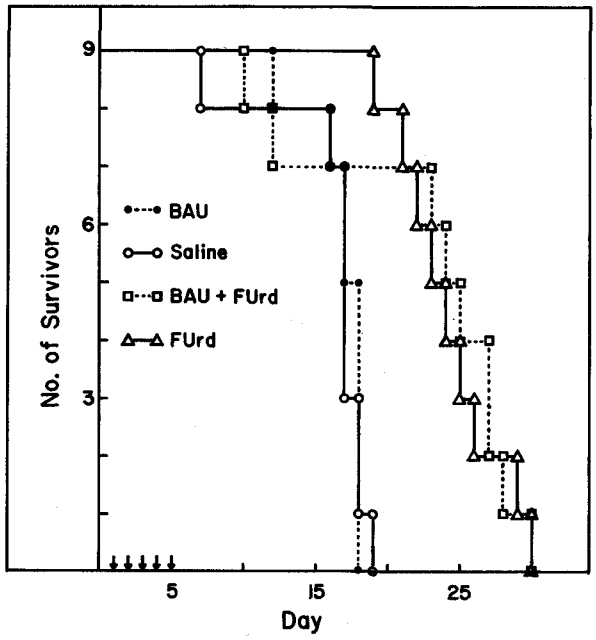

Fig. 4. The effect of combination treatment of FUrd with BAU on the survival of mice bearing L5178Y cells. 0.95×10^7 cells were transplanted at Day 0 and the drugs were administered i.p. once per day from Day 1 to Day 5. The concentrations of drugs are: BAU (●) 30 mg/kg/day; saline (O), 0.1ml Puck's saline G; BAU + FUrd (□), 30 mg/kg/day + 2 mg/kg/day; and FUrd (△), 2 mg/kg/day.

Combination treatments of tumor cells *in vivo* by 5-benzylacyclouridines and pyrimidine antimetabolites

The effects of UrdPase inhibitors, BAU (30 mg/kg/day) and BBAU (10 mg/kg/day), on the antitumor activities of FUra (25 mg/kg/day), FUrd (2 mg/kg/day), and FdUrd (50 mg/kg/day) against ascitic L5178Y cells were tested. Mice were given drugs by daily intraperitoneal injection for 5 consecutive days, while control groups received saline only. Fig. 2 shows that the combination treatment of BBAU and FdUrd produced less antitumor activity than FdUrd alone. Similar results (Fig. 3) were obtained with the combination of FUrd and BAU. The combination treatment of FUra and BAU also did

Table 3. Increase in life span (ILS) and anlaysis of variance by 2-way classification *in vivo* experiments

	Between control & BAU or BBAU			Between control & FUra			Interaction		
	ILS (%)	F[a]	P[a]	ILS (%)	F	P	ILS (%)	F	P
BAU & FUrd	4.1	0.44	0.52	50.0	22.46	0.00	41.8	0.05	0.82
BBAU & FdUrd	−3.2	0.54	0.54	24.0	11.69	0.00	12.1	1.71	0.20
BAU & FUra	−0.1	0.99	0.67	23.0	30.73	0.00	26.6	0.08	0.77

[a]F: F-value; P: P-value

not produce any better effect than FUra alone (Fig. 4). The ILS values are listed in Table 3.

The analysis of variances of two-way classification using data obtained from the experiments depicted in Fig. 2 to 4, showed that there are highly significant differences between the control groups and the fluoropyrimidine treated groups (Table 3), and no significant difference between the control groups and the BAU (or BBAU) treated groups. Furthermore, there are also no significant interaction between the fluoropyrimidine treated groups and the combination groups (fluoropyrimidine plus BAU or BBAU treated groups) as indicated by P values in Table 3.

DISCUSSION

The present study on cytotoxicity studies in L5178Y cells *in vitro* (Table 1) showed that, contrary to expectations (Birnie *et al.*, 1963; Niedzwicki *et al.*, 1981 and 1982), the UrdPase inhibitors, BAU and BBAU did not potentiate the cytotoxicity of fluorinated pyrimidines under the conditions employed. In fact, they slightly but significantly protect (or antagonize) the action of FdUrd, FUrd, or 5'-dFUrd in cultured L5178Y cells. However, BAU or BBAU have no effect toward the action of FUra. The lack of potentiation of FdUrd by benzylacyclouridines is in contrast to the studies of Chu *et al.*, (1984a; 1984b) who demonstrated that BAU and BBAU potentiated FdUrd cytotoxicity against human pancreatic carcinoma (DAN) *in vivo* as well as *in vitro* and, to a lesser extent, human lung cancer (LX-1) *in vitro*. Because of a strong correlation between the ratio of dThdPase to UrdPase activities and the degree of potentiation of FdUrd cytotoxicity by BBAU, they concluded that the ratio of dThdPase to UrdPase activities may determine the effects of BBAU on the cytotoxicity of FdUrd. The observed lack of BBAU and BAU to potentiate and their antagonizing the cytotoxicity of FdUrd in 1518Y cells cannot be explained by the ratio of dThdPase to UrdPase, because this cell line is known to have no dThdPase activity (Niedzwicki *et al.*, 1981).

In accordance with the speculation that the cytotoxicity of 5'-dFUrd *in vitro* would be significantly decreased when blocking the formation of its active metabolite (FUra) by the UrdPase inhibitor (50 μM BAU), the data in Table 1 show that BAU protects L5178Y cells from the cytotoxicity of 5'-dFUrd. The other combinations, BAU + FUrd or BAU + FdUrd afforded the same degree of protection as the combination, 5'-dFUrd and BAU. This indicates that the normal pathway to metabolism of FdUrd, FUrd and 5'-dFUrd in this cell line is the release of FUra from these fluorinated pyrimidine nucleosides by UrdPase. Since L5178Y cells do not have dThdPase activity (Niedzwicki *et al.*, 1981), the degradation of these compounds to FUra must be carried out by UrdPase.

Important factors to be considered in relation to the lack of potentiation by benzylacyclouridines of the cytotoxicity of FdUrd in the present study are the ratios of UrdPase to OPRTase and Rib-1-P to PRibPP as suggested by Houghton and Houghton (1983). Since UrdPase in cells with the high ratios of Rib-1-P to PRibPP and UrdPase to OPRTase would predominantly catalyze the reaction toward a nucleoside formation, in which FdUrd cleavage would be less likely, benzylacyclouridines will not have any effect in this case. However, if cells have low ratios of UrdPase to OPRTase, and Rib-1-P to PRibPP, the salvage of FUra to FUMP mainly occurs by OPRTase, thereby blocking FdUrd cleavage by UrdPase may indeed decrease the cytotoxicity of FdUrd, while it will have no effect on the cytotoxicity of FUra.

The *in vitro* protection by benzylacyclouridine of L5178Y cells born by mice which were treated with

fluorinated uridines could also be caused by the inhibition of the transport of these fluorinated nucleosides (Lee *et al.*, 1984) as well as preventing the formation of FUra then which cannot be converted to the toxic FUMP. Alternatively, one must also consider the prevention of degradation products of FdUrd or FUrd, such as fluorocarbamyl-β-alanine or fluoro-β-alanine, which may have toxic effects to the cell as discussed by Hull *et al.* (1988).

BAU inhibited the incorporation of Urd or FUrd into acid soluble and insoluble fractions of L5178Y cells (Table 2). The uptake of Urd in L5178Y cells was also inhibited by BAU and BBAU (Fig. 1). In the latter study, the inhibition was dose dependent and BBAU was about 10-fold more potent. These results are in agreement with the results of the cytotoxicity study in the *in vitro* system, in which benzylacyclouridines did not potentiate the cytotoxicity of pyrimidine nucleoside antimetabolites including FdUrd and FUrd. The inhibition of Urd uptake, or incorporation of Urd or FUrd into acid soluble and insoluble fractions of L5178Y cells by BAU or BBAU could have been caused *via* inhibition of the nucleoside transport (Lee *et al.*, 1984).

From the results of *in vivo* studies (Figs. 2-4) and the analysis of Table 3, it is concluded that BAU or BBAU does not potentiate the antitumor activity of FUra, FUrd, or FdUrd against L5178Y cells *in vivo,* thereby no increase in the life-span of mouse bearing these cells was observed. Since it has been known that many tumor cell lines lack dThdPase and that FdUrd can be cleaved by UrdPase, a combination of a UrdPase inhibitor and FdUrd *in vivo* was proposed to increase the therapeutic efficacy of FdUrd. (Niedzwicki *et al.*, 1981 and 1982). In contrast to this speculation and to the results of Chu *et al.* (1984a), all three combination did not increase the life span of mice bearing L5178Y leukemia cells which do not posses dThdPase (Niedzwicki *et al.*, 1981).

Martin *et al.*, (1982) reported that Urd injection increased the therapeutic index of FUra in mice bearing colon cancer by expanding the pool of Urd nucleotides which increased the clearance of the FUra moiety from RNA and caused a much faster recovery of DNA synthesis in normal cells than in tumor cells. It has also been reported that BAU increased the plasma level of Urd in rats (Karle *et al.*, 1981) and mice (Levy *et al.*, 1982; Monks *et al.*, 1983; Darnowski and Handschumacher, 1985). LaCreta *et al.* (1989) also observed increased plasma half life of FdUrd when rats were pretreated with inhibitors of UrdPase or dThdPase. From these studies, it is obvious that plasma uridine concentrations of mice bearing L5178Y cells when treated with benzylacyclouridines must increase. Therefore, the phosphorolysis of FdUrd and FUrd should be prevented by this double action, *i.e.*, the inhibition by benzylacyclouridine and the product inhibition by uridine, leading to accumulation hence potentiation of FdUrd, particularly in L5178Y cells which do not have dThdPase. This potentiating effect *in vivo* and the protecting effect shown *in vitro* may act in opposite directions and cancel each other to show no pyrimidine acyclouridine effect (Fig. 2-4).

In conclusion, 5-benzylacyclouridines did not show potentiation of fluorinated pyrimidines *in vivo* as well as *in vitro* in L5178Y cell line. Instead, *in vitro,* FdUrd, FUrd, 5'-dFUrd but no FUra protected L5178Y cells in the presence of BAU or BBAU and fluorinated pyrimidines. The protection of 5-benzylacyclouridines could be due to the anabolism of FdUrd that occurs mainly through the sequential action of UrdPase and OPRTase in this cell line, or that the 5-benzylacyclouridines inhibit nucleoside transport, or both. The prevention of degradation of FUra by limiting its supply is suggested as a possibility.

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REFERENCES

Birnie GD, Kroeger H and Heidelberger C: *Studies of fluorinated pyrimidines. XVIII. The degradation of 5-fluoro-2'-deoxyuridine and related compounds by nucleoside phosphorylase. Biochemistry 2:566-571, 1963*

Cha S: *Development of inhibitors of pyrimidine metabolism. Yonsei Med J 30:315-326, 1989*

Caradonna SJ and Cheng Y: *The role of deoxyuridine triphosphate nucleotide hydrolase, uracil-DNA glycosylase, and DNA polymerase alpha in the metabolism of FUdR in human tumor cells. Mol Pharmacol 18:513-520, 1980*

Cheng YC and Nakayama K: *Effects of 5-fluoro-2'-deoxyuridine on DNA metabolism in HeLa cells. Mol Pharmacol 23:171-174, 1982*

Chu M-Y, Naguib FNM, Iltzsch MH, el Kouni MH, Chu S-H, Cha S and Calabresi P: *Potentiation of 5-fluoro 2'-deoxyuridine antineoplastic activity by the uridine phosphorylase inhibitors benzylacyclouridine and ben-*

zyloxybenzylacyclouridine. Cancer Res 44:1852-1856, 1984a

Chu S-H, Chen Z-H, Rowe EC, Naguib FNM, el Kouni MH and Chu M-Y: *Synthesis and biological activity of hydroxymethyl analogs of 5-benzylacyclouridine and 5-benzyloxybenzylacyclouridine. Nucleosides and Nucleotides 3:303-311, 1984b*

Cooper GM, Dunning WF and Greer S: *Role of catabolism in pyrimidine utilization for nucleic acid synthesis in vivo. Cancer Res 32:390-397, 1972*

Darnowski JW and Handschumacher RE: *Tissue-specific enhancement of uridine utilization and 5-fluorouracil therapy in mice by benzylacyclouridine. Cancer Res 45:5364-5368, 1985*

Fischer GA and Sartorelli AC: *Development maintenance and assay of drug resistance. Methods Med Res 10:247-262, 1964*

Heidelberger C: *Fluorinated pyrimidines and their nucleosides (eds. by AC Sartorelli and DG Johns, Handbook of Experimental Pharmacology) Vol. 38, Part 2, pp 193-231. New York: Springer-Verlag 1975*

Heidelberger C, Chaudhuri NK, Dannenberg P, Mooren D, Griesbach L, Duschinsky R, Schnitzer RJ, Plever E and Scheiner J: *Fluorinated pyrimidines, a new class of tumor-inhibited compounds. Nature 179:663-667, 1957*

Houghton JA and Houghton PJ: *Elucidation of pathways of 5-fluorouracil metabolism in xenografts of human colorectal adenocarcinoma. Eur J Cancer Clin Oncol 19:807-815, 1983*

Hull WE, Port RE, Herrmann R, Britsch B and Kunz W: *Metabolites of 5-fluorouracil in plasma and urine, as monitored by ^{19}F nuclear magnetic resonance spectroscopy, for patents receiving chemotherapy with or without methotrexate pretreatment. Cancer Res 48:1680-1688, 1988*

Iltzsch MH, el Kouni MH and Cha S: *Kinetic studies of thymidine phosphorylase from mouse liver. Biochemistry 24:6799-6807, 1985*

Karle JM, Anderson LW, Dietrick DD and Cysyk RL: *Effect of inhibitors of the de novo pyrimidine biosynthetic pathway on serum uridine levels in mice. Cancer Res 41:4952-4955, 1981*

Krenitsky TA, Barclay M and Jacquez JA: *Specificity of mouse uridine phosphorylase. J Biol Chem 239:805-812, 1964*

Kufe DW, Major PP, Egan EM and Loh E: *5-fluoro-2'-deoxyuridine incorporation in L1210 DNA. J Biol Chem 256:8885-8888, 1981*

LaCreta FP, Barbour S, Williams W and Williams WM: *Effects of pyrimidine nucleoside phosphorylase inhibitors on hepatic fluoropyrimidine elimination in the rat. Cancer Res 49:2567-2573, 1989*

Lazarus H, Barell EF, Oppenheim S and Krishan A: *Divergent properties of two human lymphocytic cell lines isolated from a single specimen of peripheral blood. In Vitro 5:303-310, 1974*

Lee KH, el Kouni MH, Chu S-H and Cha S: *Inhibition of nucleoside transport in murine lymphoma L5178Y cells and human erythrocytes by the uridine phosphorylase inhibitors 5-benzylacyclouridine and 5-benzyloxybenzylacyclouridine. Cancer Res 44:3744-3748, 1984*

Levy EJ, Glasser T, Dreyer RN and Handschumacher RE: *Control of the turnover and utilization of circulating uridine by liver bypass, nitrobenzylthioinosine-PO_4 or benzyl acyclouridine. Proc Am Assoc Cancer Res 23:212, 1982*

Marsh JC and Perry S: *Thymidine catabolism by normal and leukemic human leukocytes. J Clin Invest 43: 267-278, 1964*

Martin DS, Stolfi RL, Sawyer BC, Spiegelman S and Young CW: *High-dose 5-fluorouracil with delayed uridine "rescue" in mice. Cancer Res 42:3964-3970, 1982*

Monks A, Ayers O and Cysyk RL: *Effect of 5-benzylacyclouridine, a potent inhibitor of uridine phosphorylase, on the metabolism of circulation uridine by the isolated rat liver. Biochem. Pharmacol. 32:2003-2009, 1983*

Niedzwicki JG, Chu S-H, el Kouni MH, Rowe EC and Cha S: *5-Benzylacyclouridine and 5-benzyloxybenzylacyclouridine, potent inhibitors of uridine phosphorylase. Biochem Pharmacol 31:1857-1861, 1982*

Niedzwicki JG, el Kouni MH, Chu S-H and Cha S: *Pyrimidine acyclonucleosides, inhibitors of uridine phosphorylase inhibition of uridine phosphorylase. Biochem Pharmacol 30:2097-2101, 1981*

Reyes P: *The synthesis of of 5-fluorouridine-5'-phosphate by pyrimidine phosphoribosyltransferase of mammalian origin. I. Some properties of the enzyme from P-1534J mouse leukemia cells. Biochemistry 8:2057-2063, 1959*

Reyes P and Heidelberger C: *Fluroinated pyrimidines. XXVI. Mammalian thymidylate: Its mechanism of action and inhibition by fluorinated nucleotides. Mol Pharmacol 1:14-30, 1965*

Schuetz JD, Wallace HJ and Diasio RB: *5-Fluoracil incorporation into DNA of CF-1 mouse bone marrow cells as a possible mechanism of toxicity. Cancer Res 44:1358-1363, 1983*

Wilkinson DS and Pitot HC: *Inhibition of ribosomal ribonucleic acid maturation in Novikoff hepatoma cells by 5-fluorouracil and 5-fluorouridine. J Biol Chem 248:63-68, 1973*

Woodman PW, Sariff AM and Heidelberger C: *Specificity of pyrimidine nucleoside phosphorylase and the phosphorolysis of 5-fluoro-2'-deoxyuridine. Cancer Res 40:507-511, 1980*

Zielke HR: *Isolation of thymidine-resistant cells from a thymidine sensitive acute lymphoblastic leukemia cell*

line. Cancer Res 39:3373-3376, 1979
Zimmerman M and Seidenberg J: *Deoxyribosyl transfer. I. Thymidine phosphorylase and nucleoside deoxyribosyltransferase in normal and malignant tissue. J Biol Chem 239:2618-2621, 1964*

=국문초록=

L5187Y 세포에 대한 불화피리미딘 대사억제제 독성에 관한 Benzylacyclouridine의 영향

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Benzylacyclouridines (BAU and BBAU)는 uridine phosphorylase (UrdPase)의 선택적이고 강력한 상경억제제이다. 보고된 바에 의하면 (Cancer Res., 44: 1852, 1984) Benzylacyclouridines가 5-fluoro-2'-deoxyuridine (FdUrd)의 인체 암세포에 대한 독성을 증가시켜 준다고 하였지만, L5187Y 세포를 사용한 본 실험에서는 Benzylacyclouridines가 FdUrd를 포함하여 5-fluorouridine (FUra) 모두에 대해 조금도 세포 독성을 증가시키지 못하였을 뿐만아니라, 오히려 세포를 그들 독성으로부터 투여량에 비례하여 보호하였다. 복강내 주사에 의한 생체 실험에서도 Benzylacyclouridines는 5-fluorinated pyrimidine에 의한 L5187Y를 지닌 쥐 (mouse)의 life-span을 연장시켜 주지 못하였다. 본 실험에서 Benzylacyclouridines가 기대했던 fluorinated pyrimidine 항암제의 효과를 증진시키지 못한 이유는 nucleosides의 anabolism이 UrdPase와 orotate phosphoribosyltransferase이 의한 sequential 작용에 의하던가 또는 Benzylacyclouridines에 의한 nucleosides의 수송억제에 의하던가, 아니면 두가지 다 복합적으로 작용한 결과로 생각된다.