Biochemical Pharmacology, Vol. 29, pp. 2077-2080.
Pergamon Press Ltd. 1980. Printed in Great Britain.

PRELIMINARY COMMUNICATIONS

5-FLUOROURACIL IN COMBINATION WITH HYPOXANTHINE AND ALLOPURINOL: TOXICITY AND METABOLISM IN XENOGRAFTS OF HUMAN COLONIC CARCINOMAS IN MICE

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(Received 14 April 1980; accepted 12 May 1980)

Experimentally, allopurinol (Hpp) antagonizes the cytotoxicity of 5-fluorouracil (FUra) in L5178Y, L1210, P388 and Sarcoma 180 cells, but not in Walker 256 or HeLa cells in culture [1,2]. A similar antagonism was observed in L5178Y cells in vitro when FUra was incubated simultaneously with hypoxanthine (Hx) [3]; in addition, Hpp has been reported to reduce the toxicity of FUra in mice and rats [1,2,4]. The preferential utilization of 5-phosphoribosyl-1-pyrophosphate (PRPP) for the conversion of FUra to FUMP by orotate phosphoribosyltransferase (OPRTase) is thought to occur in murine leukemias, while other cell lines, such as the Walker 256, appear to activate FUra by the sequential action of uridine phosphorylase (UP) and uridine kinase (UK). In tissues utilizing PRPP preferentially for FUra conversion, the accumulation of orotic acid (OA) from the inhibition of OMP decarboxylase or of Hx from the inhibition of xanthine oxidase, by Hpp or metabolites of Hpp [5-7], may lead to competition between OA or Hx and FUra for PRPP in the conversion of OA to OMP and of Hx to IMP by OPRTase and Hx-guanine phosphoribosyltransferase (HGPRTase) respectively.

This study was designed to examine the possibilities for increasing the therapeutic index of FUra in the treatment of adenocarcinoma of the colon by using FUra in varying combinations with Hx and Hpp. Toxicity induced in the normal limiting tissues of 8- to 12-week-old CBA/CAJ mice, and the metabolism of FUra in two human colon tumors maintained in immune-deprived CBA/CAJ mice were examined.

The tumor lines, designated $HxGC_3$ (FUra-insensitive) and $HxELC_2$ (FUra-sensitive), immune-deprivation of the mice, and tumor implantation have been described previously [8]. Hx and Hpp were dissolved initially in 1 M NaOH; solutions were subsequently back-titrated with 1 M HCl to approximately pH 8.9. Incorporation of FUra into RNA has previously been shown to correlate with gastrointestinal toxicity of FUra in CBA/J mice (9). Preliminary investigations on the scheduling of Hx and Hpp by i.p. injection to tumor-bearing mice 30 minutes before, simultaneously or 30 minutes after the i.p. administration of FUra demonstrated that the simultaneous schedule offered the greatest therapeutic advantage between the incorporation of drug into the RNA of gastrointestinal tissues and uptake by the tumors. The determination of intratumor concentrations of fluorinated metabolites between 1 hr and 7 days after i.p. injection of FUra, with or without the simultaneous administration of Hx and Hpp, using T.L.C. procedures, has been reported [8,9]. The toxicity of a single dose of FUra (400 mg/kg) given i.p., either with or without the simultaneous i.p. administration of various doses of Hx (50, 100 or 200 mg/kg) or Hpp (10, 20 or 40 mg/kg) either alone or in combination, was assessed from the weight loss of normal individual male mice during a period of 20 days, as described previously [9].

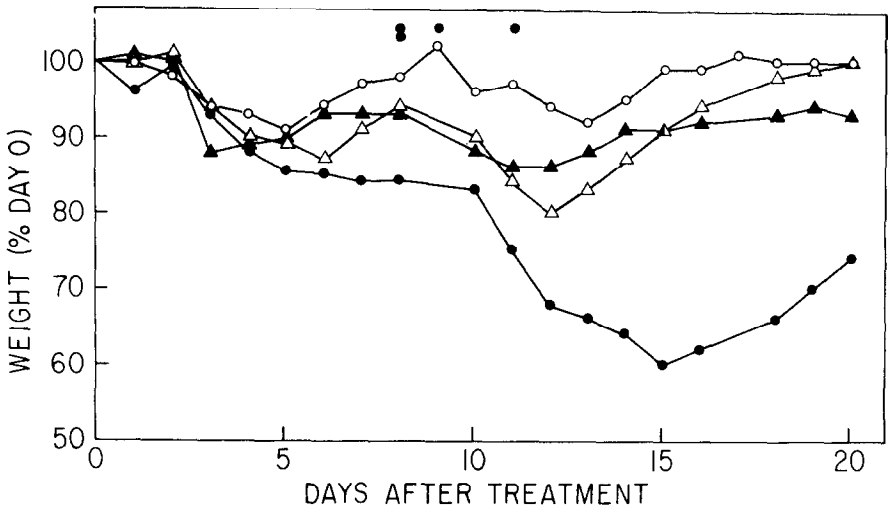

Fig. 1. Toxicity of a single i.p. administration of FUra (400 mg/kg) alone (●), or in combination with Hx (50 mg/kg) (△), Hpp (10 mg/kg) (▲), or Hx (50 mg/kg) and Hpp (10 mg/kg) (○) in normal male CBA/CAJ mice. Deaths are indicated by the solitary symbols representing the appropriate dose-group.

Treatment with FUra alone resulted in 80 percent lethality between days 8 and 11 after drug administration (Fig. 1). Protection from both gastrointestinal toxicity (days 1-8) and bone-marrow toxicity (days 8-20) after FUra treatment was obtained with both Hx and Hpp, either alone or in combination. The optimal doses that offered the greatest protection with the highest survival rate (100 percent) were 50 mg/kg for Hx, 10 mg/kg for Hpp, and these same dosage levels for the combination of Hx with Hpp. It is evident that the purine combination afforded superior protection from FUra toxicity over either Hx or Hpp alone.

Tumor-bearing mice received [6-^{3}H]FUra (100 mg/kg) either alone, or simultaneously with Hx (50 mg/kg), or Hpp (10 mg/kg), or both, by i.p. administration. Intratumor concentrations of total fluorinated anabolites consisting of FUra, FUrd, FdUrd and FUra ribo- and deoxyribonucleotides were determined between 1 and 168 hr after treatment in $HxELC_2$ and $HxGC_3$ tumors (Fig. 2). In the FUra-sensitive line, $HxELC_2$, either Hx or Hpp administered singly with FUra produced little effect on the total FUra anabolites formed, although slight potentiation was observed at 4 hr after Hpp administration; in contrast, fluorinated metabolite concentrations were elevated between 4 and 48 hr after administration of the three-agent combination and remained slightly elevated above the FUra control for up to 7 days. Similarly in $HxGC_3$ tumors, Hpp had a slight effect in potentiating FUra metabolite formation at 1 and 4 hr after treatment; Hx, however, yielded elevated FUra-metabolite concentrations greater than those observed with Hpp, during the first 24 hr after treatment, and Hx and Hpp in combination with FUra further induced elevated metabolite concentrations between 1 and 48 hr after drug administration. It is apparent that the effect of Hx and Hpp, in combination, on the metabolism of FUra was greater than after administration of either purine alone in the 2 xenograft lines studied. This suggests that the potentiation of elevated levels of Hx by Hpp is responsible for the increased formation of FUra anabolites in the tumors. It is possible that, by increasing the levels of endogenous purines, and subsequently of ribose-1-phosphate from the breakdown of IMP, that FUra may be converted more readily to its nucleoside.

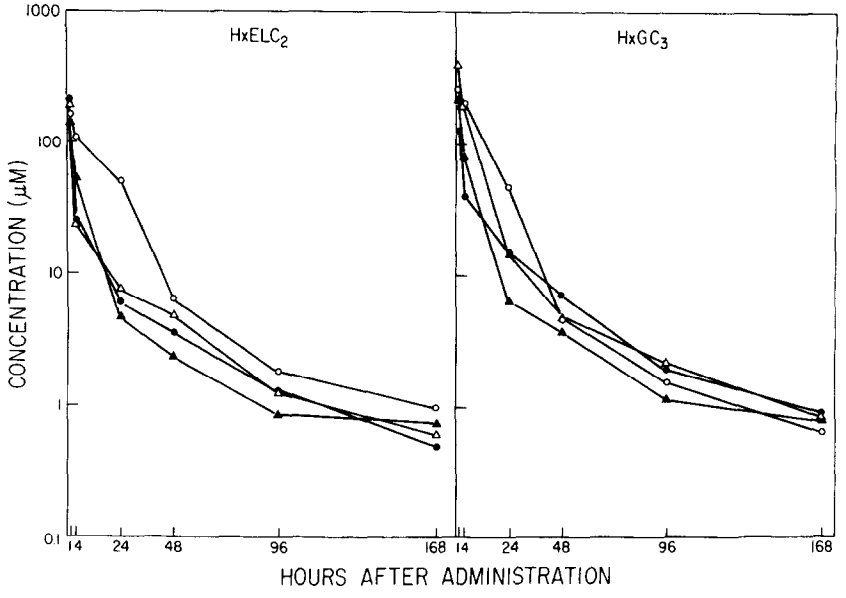

Fig. 2. Intratumor concentrations of total FUra anabolites in $HxELC_2$ and $HxGC_3$ human colon xenografts between 1 and 168 hr after i.p. administration to tumor-bearing mice of [6-^{3}H] FUra (100 mg/kg) alone (●) or in combination with Hx (50 mg/kg) (△), Hpp (10 mg/kg) (▲), or Hx (50 mg/kg) and Hpp (10 mg/kg) (○).

In normal tissues, the superior protection from FUra toxicity afforded by the simultaneous administration of Hx and Hpp may be due to rapid depletion of PRPP by the conversion of these purines, and the metabolite of Hpp, oxipurinol, to their nucleoside monophosphates by HGPRTase (or OPRTase in the case of oxipurinol), thus reducing the availability of PRPP for FUra conversion. Further, the inhibition of xanthine oxidase would potentiate levels of Hx for extended periods of time, and may ultimately lead to a prolonged depletion of PRPP; in gastrointestinal tissues, since incorporation of FUra into RNA is largely complete within 4 hr after drug administration, then this could account for the increased protection observed.

Concentrations of FUMP, FUDP, FUTP, FdUMP, FUrd and FUra (pmoles/mg DNA) measured at various times after the administration of [6-^{3}H]FUra with or without Hx and Hpp in $HxELC_2$ and $HxGC_3$ tumors are shown in Table 1. In $HxGC_3$, the elevation of metabolite concentrations during the first 48 hr after treatment was associated with increases in FdUMP, FUMP and FUrd; FUra was increased during the first 4 hr whereas the formation of FUTP and FUDP was decreased for up to 48 hr. In $HxELC_2$, FUra was the only metabolite that was not present at increased concentrations after administration of the combination. FUrd concentrations were elevated after administration of the three-agent combination to a greater extent than the other FUra anabolites in both tumor lines. It is possible that in the presence of high concentrations of FUrd, uridine kinase becomes rate limiting in conversion of the agent to ribonucleotides.

The data suggest that the possibility for increasing the therapeutic index of FUra in xenografts of human colorectal carcinomas in mice is worthy of further study; thus, our studies are currently being expanded to encompass an additional four lines of such xenografted human neoplasms. Such a study could result in the elucidation of the relative importance of OPRTase and UP/UK in the anabolism of FUra by human colon xenografts.

Table 1. Metabolism of FUra.*

Metabolite	Treatment	Concentration of FUra anabolites (pmoles/mg DNA) 1 hr	4 hr	1 day	2 days	4 days	7 days
					$HxELC_2$ colon xenografts		
FUTP	Combination	112	102	102	80	89	88
	FUra alone	182	28	35	40	34	11
FUDP	Combination	118	102	97	102	85	75
	FUra alone	159	46	21	23	39	10
FUMP	Combination	541	335	162	97	75	56
	FUra alone	526	161	266	169	48	17
FdUMP	Combination	1,330	675	525	293	155	82
	FUra alone	1,660	153	128	101	26	31
FUrd	Combination	53,800	48,800	22,500	1,990	290	100
	FUra alone	74,100	9,400	1,440	834	342	132
FUra	Combination	21,200	2,080	765	404	133	14
	FUra alone	24,200	2,120	1,030	514	58	31
				$HxGC_3$ colon xenografts			
FUTP	Combination	78	125	42	26	13	11
	FUra alone	218	184	79	56	15	11
FUDP	Combination	80	122	72	14	12	12
	FUra alone	195	189	67	61	15	6
FUMP	Combination	287	236	68	42	15	13
	FUra alone	207	76	47	28	12	9
FdUMP	Combination	1,470	882	383	210	124	45
	FUra alone	672	129	209	216	140	75
FUrd	Combination	49,800	50,400	11,700	491	121	68
	FUra alone	20,900	7,640	1,930	834	194	89
FUra	Combination	20,800	5,530	1,170	514	123	29
	FUra alone	13,300	3,150	1,800	756	151	48

*The concentrations of FUra anabolites were determined in $HxELC_2$ and $HxGC_3$ human colon xenografts between 1 hr and 7 days after the i.p. administration of [6-^{3}H]FUra (100 mg/kg) with or without the simultaneous i.p. administration of Hx (50 mg/kg) and Hpp (10 mg/kg) to tumor-bearing mice.

ACKNOWLEDGEMENTS--This work was supported by grants ACS IN99F, CA21677 and by ALSAC. The authors wish to acknowledge the technical assistance of Mr. John Phillips.

REFERENCES

1. P. M. Schwartz and R. E. Handschumacher, Cancer Res. 39, 3095 (1979).
2. P. M. Schwartz and R. E. Handschumacher, Proc. Am. Ass. Cancer Res. 20, 133 (1979).
3. M. Yoshida, A. Hoshi and K. Kuretani, Biochem. Pharmac. 27, 2979 (1978).
4. P. M. Schwartz and R. E. Handschumacher, Proc. Am. Ass. Cancer Res. 19, 111 (1978).
5. R. M. Fox, M. H. Wood and W. J. O'Sullivan, J. Clin. Invest. 50, 1050 (1971).
6. G. H. Hitchings, Adv. Enzyme Regulat. 12, 121 (1974).
7. G. K. Brown and W. J. O'Sullivan, Biochem. Pharmac. 26, 1947 (1977).
8. J. A. Houghton and P. J. Houghton, Cancer 45, 1159 (1980).
9. J. A. Houghton, P. J. Houghton and R. S. Wooten, Cancer Res. 39, 2406 (1979).