

Effect of Allopurinol on the Toxicity of High-Dose 5-Fluorouracil Administered by Intermittent Bolus Injection

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The effect of allopurinol pretreatment on the toxicity of 5-fluorouracil (5-FU) was examined in a clinical trial. Twenty-three patients were given bolus infusions of 5-FU every two weeks in doses that produced mild toxicity (0.8–1.9 g/m²). On alternate courses patients were pretreated with allopurinol either 300 mg two hours prior to and 10 hours after 5-FU, or 300 mg every 8 hours for 4 doses starting 24 hours before 5-FU. Seventeen and 20 pairs of courses were evaluable from the 2- and 24-hour pretreatment groups, respectively. Allopurinol did not produce a significant degree of protection against 5-FU-induced myelosuppression or mucositis on either dose schedule. Neurotoxicity manifesting as both cerebellar and encephalopathic signs and symptoms was the most important toxicity encountered and was dose-limiting for 5-FU on this schedule. Mean oxipurinol serum concentrations at the time of 5-FU administration were 24 μ M and 104 μ M for the 2- and 24-hour allopurinol pretreatment schedules respectively. Allopurinol increased the T_{1/2} of 5-FU by a mean of 67% in three of the four patients studied. Pretreatment with allopurinol did not reduce the toxicity of 5-FU administered as an intravenous bolus.

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IN ORDER to damage cells, 5-fluorouracil (5-FU) must be converted intracellularly to either 5-fluorodeoxyuridine monophosphate (FdUMP) or 5-fluorouridine triphosphate (FUTP). The former is a potent inhibitor of thymidylate synthetase,^{1,2} while the latter is incorporated into RNA where it interferes with RNA stability and function.^{3,4} Three routes of activation of 5-FU to its nucleotide forms have been identified (Fig. 1): (1) 5-FU can be converted directly to FUMP by the enzyme orotidine phosphoribosyl-transferase (OPRTase) in a reaction that also requires 5-phosphoribosyltransferase (PRPP); (2) 5-FU can react sequentially with uridine phosphorylase and then uridine kinase to form FUMP; or (3) 5-FU can react with deoxyribose-1-phosphate and be converted to FdUR directly by thymidine phosphorylase. This latter pathway is probably of little significance in man because of the scarcity of deoxyribose-1-phosphate.

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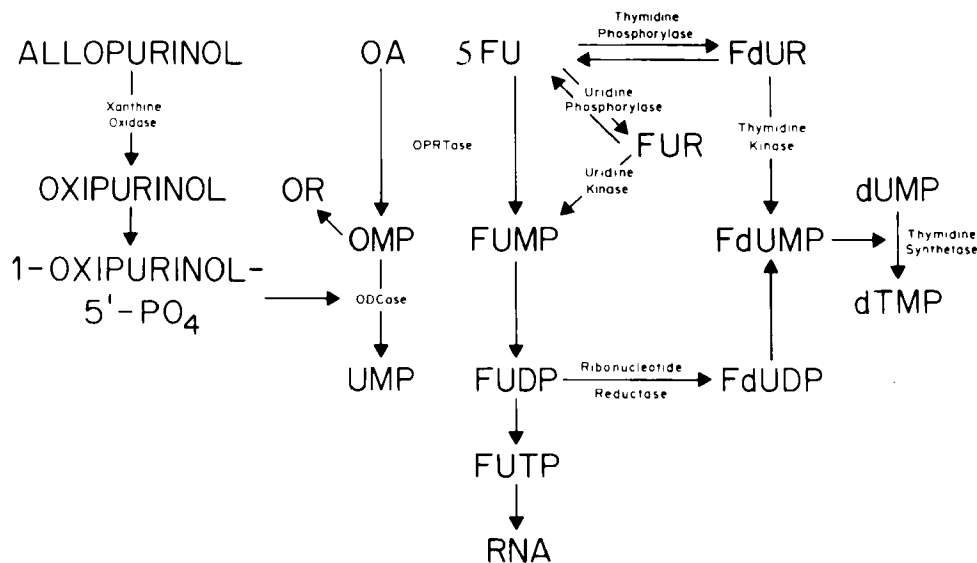
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Tissues may differ in the degree to which they depend on one or another pathway for 5-FU activation, and in the extent to which these pathways can be blocked by inhibitors. Allopurinol (HPP) has recently been identified as a modulator of 5-FU activity.⁵⁻⁸ As outlined in Figure 1, the major metabolite of HPP, oxipurinol, is metabolized to 1-oxipurinol-5'-monophosphate^{9,10} which is a competitive inhibitor of orotidylate decarboxylase.⁹⁻¹² The net effect of this inhibition is an increase in intracellular orotic acid concentration, and elevated urinary orotidine excretion.^{11,13,14} Orotic acid is a better substrate for OPRTase than is 5-FU, and thus the activation of 5-FU via OPRTase is inhibited.¹⁵ Consumption of PRPP by the conversion of oxipurinol to its nucleotide form may also contribute to the diminished activation of 5-FU by OPRTase. There are at least two conditions under which HPP might increase the selectivity of 5-FU: (1) when the major route of 5-FU activation is via OPRTase in normal tissues and via uridine phosphorylase and uridine kinase in malignant tissues; (2) when there is much greater OPRTase activity in malignant tissues, so that when just enough HPP is present to block the OPRTase activity in normal tissue, significant activity would still persist in the malignant tissue.

In vitro studies have demonstrated that HPP can modulate the toxicity of 5-FU to cells in culture, and can increase the therapeutic ratio of 5-FU against some, but not all, murine tumors *in vivo*.^{5,6} Two phase I trials

FIG. 1. Pathways of allopurinol, orotic acid, and 5-fluorouracil metabolism. OA: orotic acid; OMP: orotidine monophosphate; OR: orotidine; UMP: uridine monophosphate; 5-FU: 5-fluorouracil; FUMP: 5-fluorouridine monophosphate; FUDP: 5-fluorouridine diphosphate; FUTP: 5-fluorouridine triphosphate; FdUR: 5-fluorodeoxyuridine; FdUMP: 5-fluorodeoxyuridine monophosphate; FdUDP: 5-fluorodeoxyuridine diphosphate; dUMP: deoxyuridine monophosphate; dTMP: thymidine monophosphate.



in humans have demonstrated that when 5-FU is injected as a constant infusion for five days, concurrent administration of HPP increases the maximum tolerated dose by approximately twofold, resulting in a fourfold increase in serum 5-FU concentration \times time exposure.^{7,8,16} When given as a five-day constant infusion, the serum concentration of 5-FU is low and the dose limiting toxicity is mucositis.¹⁷ This trial endeavored to determine whether allopurinol could protect against the 5-FU toxicity associated with short term exposure to very high concentrations of 5-FU as are encountered when 5-FU is given by bolus injection, and whether allopurinol could protect against 5-FU induced myelosuppression as well as mucositis. To achieve these aims, a dose schedule utilizing bolus administration of maximum tolerated doses of 5-FU once every two weeks was selected for study.

Materials and Methods

Patient Selection

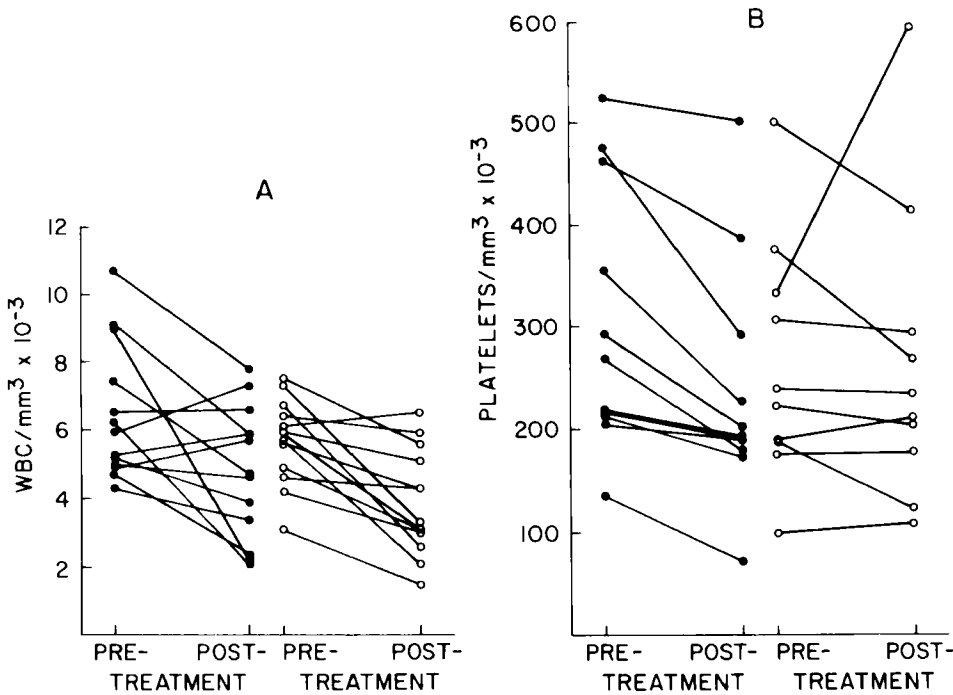
Twenty-three patients with a histopathologically proven diagnosis of cancer who had received and failed all therapies of proven merit consented to enter this study. Additional inclusion criteria were a life expectancy of at least two months, a serum creatinine level of less than 1.5 mg/dl and/or a creatinine clearance of greater than 60 ml/min, a leukocyte count of at least 4000 cells/mm³, a platelet count greater than 150,000 cells/mm³, a total serum bilirubin less than 3, an alkaline phosphatase that was less than twice the normal range, and a serum glutamic oxaloacetic transaminase that was below thrice the normal range. All patients were able to ingest oral medications and had recovered from toxicities that could be attributed to prior therapy.

Study Design and Treatment Plan

5-FU was administered as a single intravenous bolus injection. The initial dose of 5-FU was 1.1 g/m², and treatment was repeated every two weeks or as soon thereafter as toxicity had completely cleared. Subsequent doses of 5-FU were increased by 0.3 g/m² until measurable toxicity occurred. Measurable toxicity was defined as a 50% or greater fall in either leukocyte or platelet count, or a grade 2 or greater mucositis (subjective mouth soreness plus observable mucosal erythema or ulceration). Once measurable toxicity was documented, on the next course the patient received the same dose of 5-FU in conjunction with one of two HPP pretreatment programs. One consisted of 300 mg of HPP 2 hours before and 10 hours after the 5-FU bolus; the other of 300 mg of HPP every 6 hours for 4 doses, the first dose being given 24 hours prior to the 5-FU injection. Subsequent courses were given with HPP, and the 5-FU dose was increased by 0.3 g/m² until the patient again incurred toxicity.

Evaluation

Patients were evaluated for toxicity and response on days 1, 8, and 15 of each treatment course. These evaluations included complete blood counts, platelet counts, and examination of the mouth for mucositis. Liver function tests, blood urea nitrogen, serum creatinine, prothrombin time, and available tumor markers were checked at the beginning of each course of therapy. A partial response was defined as a greater than 50% reduction in the product of the perpendicular diameters of measurable lesions. Stable disease was considered to be present when measurable lesions either diminished



FIGS. 2A AND 2B. Effect of HPP on 5-FU-induced changes in (A) leukocyte (WBC) and (B) platelet counts. Patients received either 5-FU alone (● --- ●) or 5-FU in combination with HPP 300 mg 2 hours before and 10 hours after 5-FU (○ --- ○). Measurements were made prior to therapy and at weekly intervals thereafter; post-treatment data points represent the maximum changes observed during the 21-day follow-up period.

by less than 50% or increased by less than 25% in the absence of new lesions.

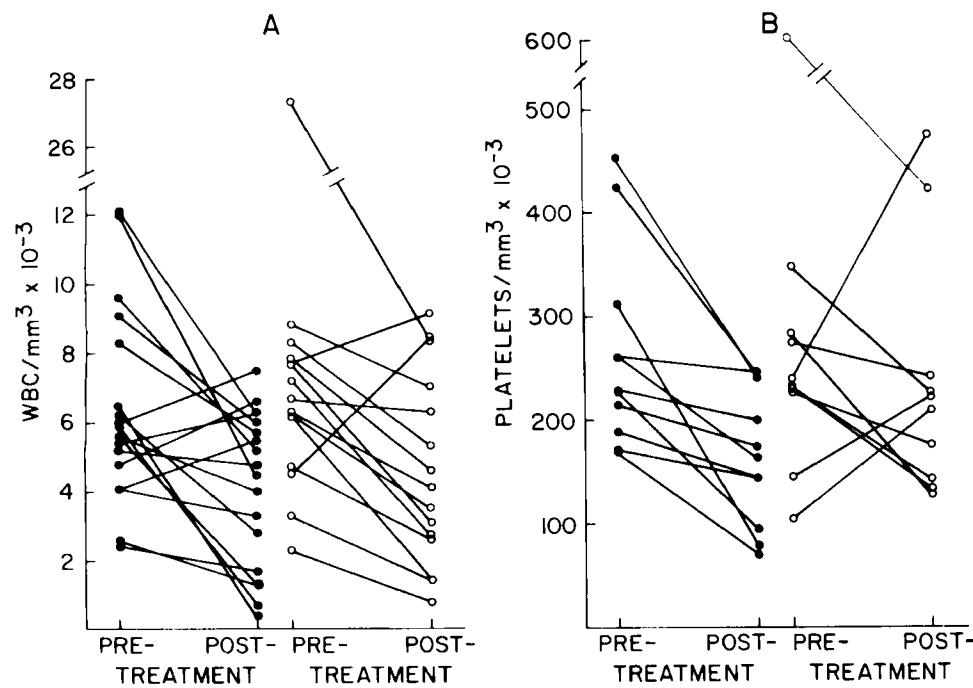
Assays

5-FU, HPP, and oxipurinol serum concentrations were measured by reversed-phase high pressure liquid chromatography using a technique developed in this laboratory to separate all three compounds.¹⁸ Pharmacokinetic parameters were calculated in selected patients using computer assisted modeling. An insufficient num-

ber of data points in the distribution phase of the serum concentration *versus* time profile precluded multicompartment analysis and therefore, the data were fit to a one compartment model.

Results

Twenty-three patients received 99 courses of therapy with 5-FU. The crossover study design yielded 59 paired courses of treatment for statistical analysis. Individual changes in leukocyte and platelet counts are shown in



FIGS. 3A AND 3B. Effect of HPP on 5-FU-induced changes in (A) leukocyte (WBC) and (B) platelet counts. Patients received either 5-FU alone (● --- ●) or 5-FU in combination with HPP 300 mg every 8 hours for 4 doses starting 24 hours prior to 5-FU (○ --- ○). Measurements were made prior to and at weekly intervals thereafter; post-treatment data points represent the maximum changes observed during the 21-day follow-up period.

TABLE 1. Toxicity Observed in Paired Treatment Courses without and with HPP 300 mg Given 2 Hours Before and 10 Hours after IV Bolus Fluorouracil

Dose (g/m ²)	No. of patients	No. of courses		Mean percent (±SD) change in leukocytes		Mean percent (±SD) change in platelets		Incidence and severity of mucositis*	
		Without HPP	With HPP	Without HPP	With HPP	Without HPP	With HPP	Without HPP	With HPP
1.1	5	11	11	-13 ± 33	-24 ± 27	-21 ± 14	-15 ± 27	1-G2	1-G1
1.5	1	1	1	-21	-52	-36	ND†	0	0
1.9	1	1	1	-49	-37	-12	-5	0	0

* Eastern Cooperative Oncology Group scale, grade (G) I-IV.

† NE: not evaluable.

Figures 2A and 2B and 3A and 3B for each of the two HPP treatment schedules. The pattern of hematologic and mucosal toxicity is summarized in Tables 1 and 2 for each different 5-FU dose level. There was no statistically significant difference ($P > 0.05$) in the incidence or severity of myelosuppression between those courses of 5-FU administered with and without HPP on either of the two HPP dose schedules (t test on paired data, and Mann-Whitney U-test).

Considering both HPP dose schedules together, mucositis occurred on 5 of 31 courses (16%) administered without HPP, and on 2 of 28 courses (7%) administered with HPP ($P = 0.29$). Evaluation of the effect of HPP on mucositis was hampered by the small number of courses on which mucositis occurred. However, in the group of patients receiving 1.5 g/m² 5-FU, 3 of 6 courses given without HPP produced mucositis, whereas mucositis did not occur on any of the 5 courses given with HPP. This trend suggests that HPP may have had a protective effect at the higher 5-FU doses.

Nausea with or without vomiting occurred on 7 courses given to five patients; three of these occurred on courses where HPP was not given, and four on courses where HPP was administered. Diarrhea occurred on 1 course in each of two patients; one episode occurred in the absence of HPP treatment, and the other with HPP treatment.

The major side effect associated with this dose schedule of 5-FU administration was neurotoxicity. Neurotoxicity occurred in 13 of 23 patients. Figure 4 shows

the incidence of the first appearance of neurotoxic symptoms as a function of the number of courses administered. It was noteworthy that while the incidence of initial neurotoxic symptoms was highest in association with the second and third courses, two patients had their first symptoms on their first course, and one patient had no symptoms until the fifth course. Neurotoxicity was manifested by varying degrees of ataxia, most notably staggering gait and dysmetria, light-headedness, dementia and organic brain symptoms including forgetfulness, lack of attention, nightmares, confusion, difficulty with short-term memory, orientation, and intellectual function. One patient suffered an unobserved episode of sudden loss of consciousness that was ascribed to a seizure. Neurologic symptoms were gradual in onset and resolution, occurring as early as day 8 after 5-FU injection in some patients. Symptoms improved during the two-month period following discontinuation of 5-FU chemotherapy.

Following the administration of HPP by mouth, oxipurinol is the major metabolite found in the plasma.¹⁹ The HPP and oxipurinol concentrations were measured at the time of 5-FU administration in four patients receiving HPP two hours before 5-FU and in four patients receiving 24 hours of HPP pretreatment. Two hours after HPP ingestion the geometric mean serum HPP concentration was 5.5 uM (range, 1.7–13.0 uM), and the mean oxipurinol concentration was 23.9 uM (range, 8.9–44.0 uM). Following 24 hours of HPP pretreatment, the geometric mean serum HPP concentration was 2.4

TABLE 2. Toxicity Observed in Paired Treatment Courses without and with HPP 300 mg Given every 8 Hours for 4 Doses Starting 24 Hours before IV Bolus Fluorouracil

Dose (g/m ²)	No. of patients	No. of courses		Mean percent (±SD) change in WBC		Mean percent (±SD) change in platelets		Incidence and severity of mucositis*	
		Without HPP	With HPP	Without HPP	With HPP	Without HPP	With HPP	Without HPP	With HPP
0.8	2	2	2	-33 ± 2	-26 ± 62	-51 ± 10	35 ± 92	0	
1.1	7	11	9	-25 ± 39	-26 ± 40	-32 ± 24	-1 ± 66	1-G2	1-G1
1.5	3	5	4	-48 ± 29	-48 ± 25	-24 ± 25	-22 ± 42	2-G2	0
								1-G4	0

* Eastern Cooperative Oncology Group scale, grade (G) I-IV.

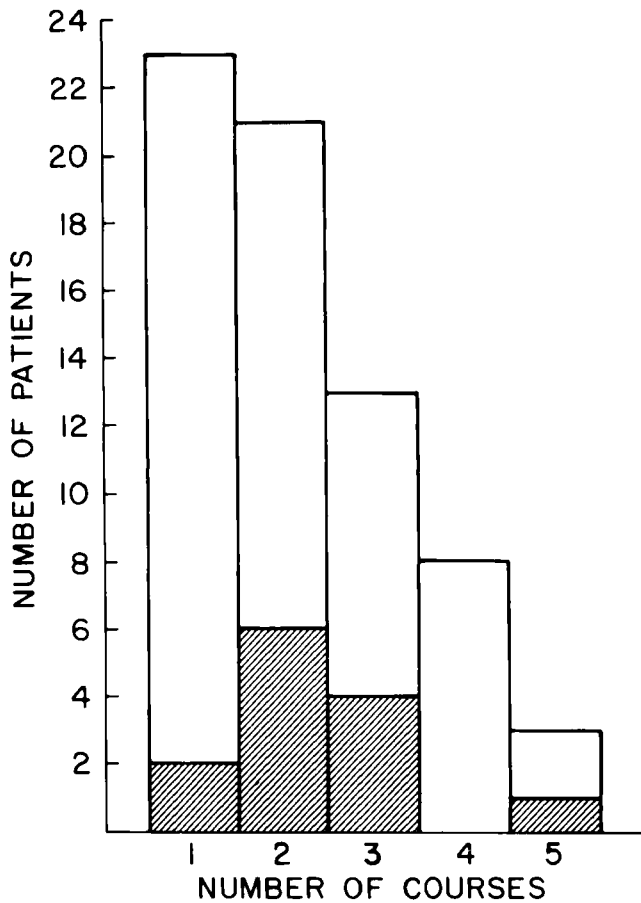


FIG. 4. Incidence of initial neurotoxic course as a function of the number of 5-FU treatment courses administered. Shaded bar, number of patients experiencing neurotoxicity for the first time on the specified course; open plus shaded bar, total number of patients without prior neurotoxicity treated on the specified course.

uM (range, 0.8–7.8 uM) and the mean oxipurinol concentration was 104 uM (range, 78–200 uM).

The effect of HPP administration on the pharmacokinetics of 5-FU was examined in four patients. Each patient served as his/her own control, receiving 5-FU

alone by rapid intravenous injection on one occasion, and then the same dose of 5-FU following 24 hours of HPP treatment two weeks later. The serum 5-FU decay curves were fitted to a one compartment model. The mean elimination half-life was 23.4 ± 0.4 minutes for 5-FU given without HPP, and 28.4 ± 1.4 minutes for 5-FU given with HPP, an increase of 21%. This difference was statistically significant ($P < 0.05$, two-sided test). There was, however, no difference in peak serum 5-FU concentrations which averaged 3800 uM (range, 1500–14,000 uM).

Seventeen of the 23 patients were evaluable for response by virtue of having measurable disease and receiving at least two courses of 5-FU. (The histologic types of tumors are presented in Table 3). Two partial responses were observed. The first was in a patient with breast carcinoma who had a partial regression of soft tissue nodules lasting six months at which time therapy with 5-FU was discontinued because of neurotoxicity. The second was in a patient with liposarcoma who had a partial regression of an 8 cm mediastinal mass that lasted three months until he too had to discontinue treatment with 5-FU because of neurotoxicity. Disease stabilization occurred in two patients with colon carcinoma, and lasted two and seven months, respectively. Since the design of this study was to treat patients with alternating courses of 5-FU and 5-FU plus HPP, no statement can be made about the effect of HPP on the response rate of 5-FU alone.

Discussion

When administered as a five-day constant infusion, HPP reduces the toxicity of 5-FU and permits approximately twice as much drug to be administered.^{7,8,16} The dose-limiting toxicity of 5-FU on a five-day constant infusion schedule is mucositis,¹⁷ and the coadministration of HPP did not change this pattern of toxicity. Thus while HPP provided some degree of protection for the gastrointestinal epithelium, no conclusion could be drawn regarding protection of marrow. However, *in vitro* studies with normal human bone marrow cells suggested that 100 uM oxipurinol could provide a four-fold degree of protection at 5-FU concentrations of less than 40 uM when the 5-FU exposure was maintained for the full period of granulocyte/macrophage colony formation in soft agar (10–14 days).¹⁶ The aim of this study was to determine whether HPP could provide significant protection for marrow *in vivo* when 5-FU was administered on a schedule that produced myelosuppression as the dose-limiting toxicity. The data presented in Tables 1 and 2 provide strong evidence that HPP did not produce any clinically useful protection of marrow despite the achievement, following 24 hours of HPP pretreatment, of HPP and oxipurinol concentrations in the serum comparable to those that were effective in pro-

TABLE 3. Clinical Responses to 5-FU/HPP Treatment

Tumor types	Total patients	Evaluable patients	Response*
Lung (adenocarcinoma)	3	2	NR
Colorectal	10	6	2 SD: 2, 7 mo
Melanoma	1	1	NR
Breast	1	1	1 PR: 6 mo
Liposarcoma	1	1	1 PR: 3 mo
Renal cell carcinoma	1	1	NR
Hypernephroma	1	1	NR
Cervix (squamous cell carcinomas)	1	1	NR
Adenocarcinoma (unknown primary)	3	2	NR
Epidermoid carcinoma (unknown primary)	1	1	1 SD: 2 mo

* PR: partial response; SD: stable disease; NR: no response.

viding protection against constant five-day infusions of 5-FU.¹⁶

There are several possible explanations for the inability of HPP to provide marrow protection in this study. Relative to gastrointestinal epithelium, marrow cells may depend less on OPRTase than upon uridine phosphorylase and uridine kinase for conversion of 5-FU to FUMP. In addition, the contribution of each of the two pathways to FUMP formation may vary with 5-FU concentration. During 5 day constant infusions of maximum tolerated doses of 5-FU the serum concentration averaged 5.2 μ M (16), whereas peak 5-FU concentrations following bolus injections of maximum tolerated doses was in the range of 3800 μ M. Additional study will be required to determine the biochemical basis for the inability of 5-FU to protect marrow against high concentrations of 5-FU. Finally, little is known about how long an exposure to HPP or oxipurinol is required to achieve maximal intracellular concentrations of orotic acid and orotidine. Fox *et al.*¹¹ reported that urinary excretion of orotic acid and orotidine did not reach steady-state for 6–8 days after the start of therapy with conventional doses of HPP, suggesting that longer periods of HPP pretreatment might be more effective in reducing 5-FU marrow toxicity.

Although the bolus infusion dose schedule was chosen for its ability to produce myelosuppression, the dose-limiting toxicity of this schedule turned out to be neurotoxicity. 5-FU has been reported to produce an acute cerebellar syndrome consisting of ataxia, dysmetria, nystagmus, and slurred speech, and the incidence of this toxic manifestation has been related to dose-rate.²⁰ However, in this study organic brain symptoms were also a prominent feature of the toxicity. The cerebellar symptoms have been ascribed to the accumulation of fluorocitrate and fluoracetate in the cerebellum.^{21,22} To our knowledge, there is no information available on the effect of HPP or oxipurinol on the metabolism of 5-FU to either of these metabolites, and because of the long time course of the symptoms relative to the courses of 5-FU given with or without HPP, there is no basis for implicating HPP as a contributor to the neurotoxicity either through a direct effect on the brain, or indirectly through an effect on metabolism of 5-FU intracellularly. The most important conclusion that can be drawn at this time is that the incidence and severity of the neurotoxicity unequivocally precludes the more extensive use of the 5-FU/HPP combination on this dose schedule.

The finding that HPP reduces the clearance of 5-FU from the serum is of interest because hepatic metabolism of 5-FU to dihydrofluorouracil and eventually to α -fluoro- β -alamine is thought to be the major route of clearance, and HPP is not known to affect this biochemical pathway. The prolongation of the 5-FU half-life, however, is in agreement with our previous finding that oxipurinol is an important determinant of 5-FU serum

concentrations during five-day constant infusion of the latter drug. Since the major effect of HPP and oxipurinol on 5-FU is thought to be inhibition of phosphoribosylation, this finding suggests that conversion of 5-FU to nucleotides may contribute significantly to its clearance. However, other mechanisms such as competition between 5-FU and orotic acid for dihydrothymine reductase may also explain the effect of HPP on 5-FU clearance.

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