Cancer Chemother Pharmacol (1981) 5:151–155

Cancer Chemotherapy and Pharmacology

© Springer-Verlag 1981

Allopurinol Modulation of Fluorouracil Toxicity

Richard M. Fox[1], Robert L. Woods[1], Martin H. N. Tattersall[1], Anita A. Piper[1], and Danny Sampson[2]

[1] Ludwig Institute for Cancer Research, University of Sydney, Sydney, N.S.W. 2006
[2] Department of Biochemistry, Royal Prince Alfred Hospital, Sydney, N.S.W. 2050, Australia

Summary. *Considerable interest has developed in the modulation of fluorouracil activity by nucleosides. The toxicity of fluorouracil in mice is known to be reduced by concurrent administration of allopurinol, presumably because biochemical pathways activating fluorouracil in normal tissues are blocked.*

We have given allopurinol (300 mg t.d.s. PO) concurrently with continuous infusions of fluorouracil (2.0–2.25 g/m²/day × 5) to 34 patients with colorectal cancer and 11 patients with various adenocarcinomas. There were 41 patients assessable for toxicity. Stomatitis was the predominant dose-limiting toxicity (22% grade 1, 19% grade 2, and 27% grade 3 toxicity). Neutropenia (< 1,000/μl) occurred in 17% patients. Among 26 colorectal cancer patients assessable for response there was a 15.4% response rate.

We conclude that allopurinol modulates fluorouracil toxicity in man, allowing a two-fold increase in dose. However, at least in colorectal cancer no greater frequency of tumour response is seen than with lower doses of fluorouracil given by standard schedules of administration without allopurinol.

Introduction

Fluorouracil (FU) is widely used, either as a single agent or with other drugs, for treating gastrointestinal, breast, and other carcinomas. A variety of administration schedules have been devised for FU but superiority of any one schedule is not apparent [13]. Considerable interest has recently developed in the possibility of modulating fluorouracil therapy by nucleosides. This approach is based on the complexity of the biochemical pathways of fluorouracil activation, and the possibility that FU may interfere with the synthesis of DNA and/or RNA in different tissues [6]. For instance, the addition of thymidine to FU infusions reduces the minimal toxic dose of FU to about one-third and changes the dose-limiting toxicity from gastrointestinal to myelosuppression [14].

FU can be activated to fluorodeoxyuridine monophosphate (FdUMP), one of its active metabolites, by three (potential) enzymatic pathways: (I) orotate phosphoribosyl transferase (OPRTase); (II) uridine phosphorylase; or (III) thymidine phosphorylase (Fig. 1). The relative importance of these three pathways in normal and malignant tumour tissues has not been clearly established [6]. Schwarz et al. have shown that allopurinol can prevent toxicity of FU in mice [11]. One rationale is that both allopurinol and its in vivo metabolite oxipurinol are converted to ribonucleotides by OPRTase and hypoxanthineguanine phosphoribosyltransferase (HGPRTase), both of which are strong inhibitors of orotate decarboxylase (ODCase) [2]. The subsequent accumulation of orotidine monophosphate (OMP) and elevation of orotic acid compete with FU activation by OPRTase [11]. Changes in purine levels in the serum may also modulate the activation to FU ribonucleosides.

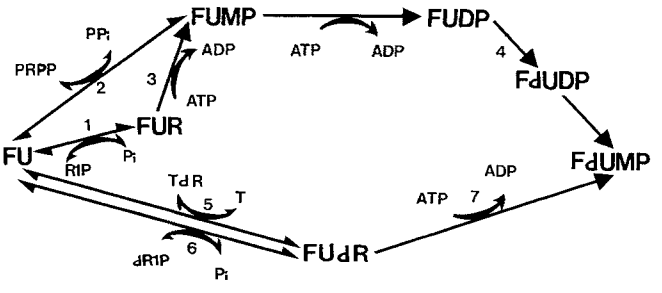

Fig. 1. Fluorouracil: potential anabolic pathways. 1, uridine phosphorylase; 2, orotate phosphoribosyltransferase; 3, uridine kinase; 4, ribonucleotide reductase; 5, pyrimidine deoxyribosyltransferase; 6, thymidine phosphorylase; 7, thymidine kinase

Reprint requests should be addressed to: R. M. Fox

0344-5704/81/0005/0151/$ 01.00

Prevention of FU toxicity in mice by allopurinol presumably reflects inhibition of FU activation in normal tissues [11]. In a preliminary communication, we reported that allopurinol modulated fluorouracil toxicity in man [4]. We now extend these initial observations, showing that allopurinol can modulate FU toxicity in man so that an approximately two-fold increase in dose is possible when FU is given by 5-day IV infusion. However, this approach does not appear to increase response rates to FU, at least in colorectal cancer.

Materials and Methods

1. Patients

Forty-five patients with advanced recurrent or metastatic carcinoma were studied. The primary site was colorectal in most cases (34 pts.). The other sites are indicated in Table 1. This study includes the patients described in our preliminary report [4]. Patients with renal insufficiency (serum creatinine > 120 μmole/l) or jaundice (serum bilirubin > 2 mg%) were excluded.

2. Investigations

Pretreatment investigations included full blood count, biochemical profile, chest X-ray, and where appropriate, organ imaging procedures to document disease extent (isotope liver scans, abdominal grey-scale ultrasound and CAT scan).

In a smaller group of patients additional biochemical and pharmacological studies were carried out. These included:

a) Erythrocyte orotidylate decarboxylase (ODCase) activity determination. This was carried out as previously described by us [2], on red cells collected prior to therapy.

b) Plasma thymidine was measured by radioimmunoassay before and during treatment, as previously described by us [8].

c) Plasma fluorouracil levels during therapy were measured by HPLC (D.C. Sampson et al., unpublished work).

d) Plasma oxipurinol and allopurinol and allopurinol levels were assayed during therapy by HPLC (A. A. Piper and R. M. Fox, unpublished work).

3. Treatment Plan

Patients were given allopurinol 300 mg t.d.s. PO on days 1–6 of the therapy schedule and 2 l of normal saline was infused daily. On days 2–6 FU was given as a 24 h infusion in the normal saline. Thirty-four patients received 2.0 g FU/m^2/day × 5. Nine patients received 2.25 g FU/m^2/day × 5 and one patient each received 2.5 and 2.9 g/m^2/day × 5.

Several patients in this study were unable to take allopurinol orally. They were given allopurinol prepared as the sodium salt, as described by Kann et al. [9], added to the IV infusion. HPLC studies demonstrated that there was no incompatibility between FU and sodium allopurinol.

Table 1. Primary tumour sites of patients treated with fluorouracil-allopurinol

Colorectum	34
Stomach	4
Ovary	3
Adenocarcinoma, unknown primary site	1
Mucoepidermoid carcinoma of anus	1
Breast	1
Prostate	1

Table 2. Toxicity grading

Grade	Stomatitis	Leukopenia (WCC × 10^9/μl)
0	None	> 4
1	Sore mouth	2.5–4
2	Ulcers, able to eat	1.0–2.5
3	Ulcers, unable to eat	< 1.0

4. Posttreatment Follow-up

Patients were seen daily during therapy and then at weekly intervals following therapy. Any toxicity was documented and full blood counts and biochemical profiles obtained. Toxicity (stomatitis, neutropenia, diarrhoea etc.) was documented (Table 2). Objective assessment of response was by standard ECOG criteria.

Some patients received two or more courses of therapy, depending on response and toxicity.

Results

1. Toxicity

There were 32 patients who received at least one course of FU at 2.0 g/m^2/day × 5 and nine receiving 2.25 g/m^2/day × 5, who were assessable for toxicity. The resultant toxicity in these groups was similar, and for concise presentation, both groups will be discussed together.

a) Stomatitis. Thirteen patients (32%) did not develop any subjective or objective manifestations of stomatitis; nine patients (22%) developed grade 1 stomatitis, a further eight (19%) grade 2, and 11 patients (27%) had grade 3 stomatitis. With further courses of therapy stomatitis was averted in these patients by reducing the duration of infusion to 4 days (Table 2).

b) Skin Rash. Nine patients developed an erythematous rash. This commenced on day 3–5 of therapy

and resolved spontaneously over several days by desquamation. The rash was predominantly on the face and/or upper torso. The rash was either less intense or did not occur with subsequent courses of therapy. It was more pronounced in patients developing stomatitis.

c) Haematological. The majority of patients (73%) had no fall in either WCC or platelet count. Two patients (5%) developed grade 1 or 2 neutropenia. Seven patients (17%) developed grade 3 neutropenia, and in six of these patients this was associated with thrombocytopenia (platelet count $< 100{,}000/\mu l$). All these seven patients had grade 3 stomatitis as well (Table 2).

d) Septicaemia. The complication of septicaemia occurred in four of the seven patients with grade 3 neutropenia. It was fatal in three patients and was uniformly associated with thrombocytopenia, grade 3 stomatitis, and diarrhoea. Apart from these three patients diarrhoea, nausea, and abdominal pain were minimal, but mild diarrhoea was seen in three patients with grade 3 stomatitis.

e) Neurotoxicity. One patient developed nocturnal hallucinations and a further patient developed extrapyramidal signs. It was not possible to correlate this complication unequivocally with FU-allopurinol therapy, as the patient concerned was also receiving phenothiazine medication. A third patient had a grand mal fit. One patient, who had a pretreatment ECOG performance status of 4 with advanced pulmonary metastases, died on the second day of therapy of respiratory failure. He was also receiving morphine.

No changes in biochemical parameters of liver or renal function were associated with the therapy, apart from the patients who developed septicaemia.

2. Tumour Response

There were 26 patients with colorectal carcinoma who were assessable for response. Two of these patients had a complete response (CR) (one patient with supraclavicular fossa nodes, mediastinal and intrababdominal mass, another with perineal nodules). Two patients had a partial response (PR) (one patient with metastatic liver disease, another with umbilical mass). There was thus a 15.4% response rate. There was no change (NC) in a further 19 patients, including four with responses of less than 50% decrease in size. Three patients had progressive disease. The response duration ranged from 2–11 months. Of the four patients with gastric cancer, one had a CR and another a PR (both patients had metastatic liver disease).

The remaining patients with other primary sites did not respond. All these patients had had intensive prior chemotherapy.

3. Clinical Pharmacology

a) Pretreatment Erythrocyte Orotidylate Decarboxylase. ODCase was assayed in 16 patients. The bimodal distribution of ODCase previously reported by us [3] was apparent in this group of patients. The mean ODCase activity was 0.92 ± 0.57 μmoles/CO_2 formed per hour per milligram of erythrocyte protein. No association could be made between toxicity (either stomatitis and/or neutropenia) and enzyme activity (Table 3).

b) Plasma Fluorouracil Levels During Therapy. These were determined in blood samples collected daily from 13 patients during their 5-day infusions. The mean fluorouracil level was 591 ± 428 ng/ml. Of five patients with mean plasma levels below 400 ng/ml, two had no stomatitis and three had grade 1 stomatitis and no neutropenia. Of the seven patients with mean serum levels above 400 ng/ml, three had no stomatitis, one had grade 2 stomatitis, and three had grade 3 stomatitis, two of whom had neutropenia associated with septicaemia. The level of FU in any individual patient fluctuated from day to day, with variations of 1.5- to 64-fold. The ranges were wider in patients receiving gravity infusions than in those receiving infusions by pump (Table 3).

c) Plasma Oxipurinol and Allopurinol Levels During Therapy. The plasma level of oxipurinol and allopurinol was determined daily during the 5-day fluorouracil therapy courses in eight patients. Six of these patients received allopurinol orally while two received the same dose of allopurinol by continuous IV infusions. In the patients receiving oral allopurinol, the mean plasma oxipurinol level was 8.7 (range 4.6–12.8) $\times 10^{-5}$ *M*. The mean plasma oxipurinol levels in the patients receiving IV allopurinol was 8.9 and 10.4×10^{-5} *M*. There was little change in the daily serum oxipurinol between day 1 and day 5 of the therapy course. The levels rose on the second day and remained constant, indicating subsequent lack of inhibition of conversion of allopurinol to oxipurinol. Plasma allopurinol levels were an order of magnitude lower with a mean level of 7.5×10^{-6} *M* (range 2.4–22.0), indicating over 90% conversion to oxipurinol. In this group of patients, no correlation

Table 3. Pharmacological data

Patient	Erythrocyte ODCase (μmole/CO_2/h/mg erythrocyte protein)	Plasma fluorouracil (ng/ml)	Plasma thymidine ($\times 10^{-7}$ *M*)	Plasma oxipurinol ($\times 10^{-5}$ *M*)	Plasma allopurinol ($\times 10^{-6}$ *M*)
1	–	2,522 ± 2,991	–	–	–
2	0.71	1,818 ± 1,451	4.6 ± 1.5	8.9 ± 1.9	4.8 ± 3.0
3	–	879 ± 431	–	9.3 ± 3.1	8.7 ± 5.2
4	0.66	659 ± 661	5.5 ± 2.3	–	–
5	2.26	574 ± 135	8.2 ± 0.6	10.4 ± 4.5	2.4 ± 0.9
6	0.50	550 ± 335	–	7.0 ± 3.7	6.5 ± 5.9
7	–	540 ± 540	–	10.8 ± 3.1	3.8 ± 3.3
8	1.87	431 ± 119	–	7.6 ± 1.3	22.0 ± 3.8
9	0.41	375	3.6 ± 0.9	12.4	< 2.0
10	–	373 ± 214	11.4 ± 1.1	–	–
11	–	338 ± 264	–	–	–
12	0.47	307 ± 220	–	12.8 ± 2.2	6.2 ± 4.6
13	0.73	198 ± 188	–	–	–
14	0.69	–	–	4.7 ± 1.2	6.0 ± 5.1
15	0.28	–	8.62 ± 2.72	–	–
16	1.64	–	5.33 ± 1.33	–	–
17	–	–	3.1 ± 1.09	–	–
18	1.03	–	–	–	–
19	0.46	–	–	–	–
20	0.70	–	–	–	–
21	1.37	–	–	–	–
22	0.94	–	–	–	–

Erythrocyte ODCase was measured prior to commencement of therapy and other parameters daily during therapy (figures give mean ± standard deviation during course of therapy)

between toxicity and plasma oxipurinol levels could be made (Table 3).

d) Plasma Thymidine. This was measured daily throughout the 5-day fluorouracil therapy in eight patients. The pretreatment plasma thymidine levels ranged from 4.4×10^{-7} to 1×10^{-6} *M*. There was no consistent change in the plasma thymidine levels during treatment with allopurinol and fluorouracil (Table 3).

Discussion

The only reported investigation of the dose-toxicity relationship of fluorouracil given by 5-day continuous infusion is that by Seifert et al. [12]. They found that a daily dose of 30 mg FU/kg ($\approx 1.1-1.2$ g/m^2/day $\times$ 5) was the maximal tolerable dose, and at this dosage five of eight patients developed mild stomatitis and three severe stomatitis. At a higher dose of 35 mg/kg/day (1.3–1.4 g/m^2/day) seven of eight patients developed stomatitis.

We have shown that when allopurinol is given with continuous FU infusions, it is possible to administer 2–2.25 g/m^2/day $\times$ 5. At this dosage level stomatitis is clearly the limiting toxicity, with 27% of patients developing severe stomatitis, but diarrhoea and abdominal pain were unusual. The majority of patients receiving this therapy did not develop myelosuppression; however, a 17% incidence of severe leukopenia (fatal in three patients) was seen.

A common toxic effect encountered was a self-limiting erythematous rash. It is not clear whether this was induced by allopurinol or fluorouracil. Neurotoxicity, a recognised complication of FU therapy, was minimal.

Concomitant allopurinol administration with fluorouracil does not appear to have reduced tumour response. However, it is apparent that at least in colorectal carcinoma, there was not a greater frequency of tumour responses seen than with FU given by standard administration schedules [13]. There were insufficient patients with other tumour types treated to comment on response.

It is intriguing to compare the dose-toxicity patterns in patients receiving fluorouracil infusions with either thymidine or allopurinol. Vogel et al. administered thymidine at a dose of 8 g/m^2/day for 5 days, beginning at the same time as a 5-day infusion of FU at doses of 5–20 mg/kg/day. Myelosuppression was the dose-limiting toxicity and the minimal toxic dose of FU was 7.5 mg/kg/day (≈ 0.3 g/m^2/day) [14].

The pharmacokinetics of this form of therapy has been studied by Kirkwood et al. [10]. They showed that patients receiving FU by IV bolus injection with concurrent infusions of TdR had prolongation of the $T_{1/2}$ of FU. They assumed this increase in $T_{1/2}$ reflected competition by thymidine for FU degradation. By contrast, the limiting toxicity with concomitant allopurinol administration is stomatitis, with fewer patients developing myelosuppression. The plasma fluorouracil levels at 2.0 g/m^2/day were twice those reported by Hillcoat et al., who administered 1 g/m^2/day (not > 2 g) by continous infusion over 5 days [12].

In our study myelosuppression appeared to correlate with higher plasma flourouracil levels. It is not clear why the dose-limiting toxicity of FU when given by IV push injection or by infusion in conjunction with thymidine is myelosuppression, as against gastrointestinal toxicity when FU is given by infusion alone or with allopurinol. A definitive answer to this problem will require understanding of the activation pathways of FU in gastrointestinal mucosa and bone marrow cells, and the relative role of FUTP inhibition of RNA synthesis versus FdUMP inhibition of thymidylate synthetase.

We have shown that concomitant allopurinol and fluorouracil therapy does not significantly alter the pretreatment plasma thymidine level. Thus, an increase or decrease in plasma thymidine levels does not explain the modulation of FU toxicity with allopurinol. It is also apparent that plasma levels of oxipurinol are similar to those seen in patients receiving equivalent doses of allopurinol [13]. This, and the finding that allopurinol administerd in IV infusions produces similar oxipurinol levels, suggest that FU therapy does not inhibity absorption of allopurinol.

Future attempts at modulating FU tumour response and/or toxicity will clearly require an exact understanding of the relative importance of the biochemical pathways in normal and malignant tissues capable of anabolising FU to its active phosphorylated metabolites, and of levels of purine bases and nucleotides.

Acknowledgements. We thank Ms. M. Dyne and Ms. W. Deveski for their expert technical assistance.

References

1. Chabner BA (1979) Antimetabolites. In: Cancer chemotherapy 1979. Excerpta Medica, Amsterdam, p 1
2. Fox RM, Wood MH, O'Sullivan WJ (1971) Studies on the coordinate activity and lability of orotodylate phosphoribosyl-transferase and decarboxylase in human erythrocytes, and the effects of allopurinol administration. J Clin Invest 50: 1050
3. Fox RM, Wood MH, Royse-Smith D, O'Sullivan WJ (1973) Hereditary orotic aciduria types I and II. Am J Med 55: 791
4. Fox RM, Woods RL, Tattersall MHN, Brodie GW (1979) Allopurinol modulation of high dose fluorouracil therapy. Cancer Treat Rev 6: 143
5. Elion GB, Yii TF, Gutman AB, Hitchings GH (1968) Renal clearance of oxipurinol, the chief metabolite of allopurinol. Am J Med 45: 69
6. Heidelberger C (1973) Pyrimidine and pyrimidine nucleoside antimetabolites. In: Cancer medicine. Lea and Febiger, Philadelphia, p 768
7. Hillcoat BL, McCulloch PB, Figuerodo AT, Ebsan MH, Rosenfeld JM (1978) Clinical response and plasma levels of 5-Fluorouracil in patients with colonic cancer treated by drug infusion. Br J Cancer 38: 719
8. Holden L, Hoffbrand AV, Tattersall MHN (1980) Thymidine concentrations in human sera: variations in patients with leukaemia and megaloblastic anaemia. Eur J Cancer 16: 115
9. Kann HE, Wells JH, Goldie JF, Schein PS, Cooney DA, Smith ER, Seegmiller JE, Carbone PP (1968) The development and use of an intravenous preparation of allopurinol. Am J Med Sci 256: 53
10. Kirkwood JM, Ensminger W, Rosowsky A, Papathanasopoulos N, Frei E III (1980) Comparison of pharmacokinetics of 5-fluorouracil and 5-fluorouracil with concurrent thymidine infusions in a phase I trial. Cancer Res 40: 107
11. Schwartz PR, Handschumacher RE (1979) Selective antagonism of 5-fluorouracil cytotoxicity by 4-hydroxypyrazolopyrimidine (allopurinol) in vitro. Cancer Res 39: 3095
12. Seifert P, Baker LH, Reed ML, Vaitkevicius VK (1975) Comparison of continuously infused 5-fluorouracil with bolus injection in treatment of patients with colorectal adenocarcinoma. Cancer 36: 123
13. Smith FP, Byrne PJ, Cambareri RC, Schein PS (1979) Gastrointestinal cancer. In: Cancer chemotherapy 1979. Excerpta Medica, Amsterdam, p 292
14. Vogel SJ, Presant CA, Ratkin GA, Klahr C (1979) Phase I study of thymidine plus 5-fluorouracil infusions in advanced colorectal carcinoma. Cancer Treat 63: 1

Received September 23/Accepted November 27, 1980