THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 61:183–187, 1973

Vol. 61, No. 3
Printed in U.S.A.

URINE OROTIC ACID–OROTIDINE LEVELS IN AZARIBINE-TREATED PATIENTS WITH PSORIASIS*

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ABSTRACT

Azaribine, as a pyrimidine analog, blocks the decarboxylase conversion of orotidylic acid to uridine monophosphate with a resultant excretion of accumulated orotic acid and orotidine in the urine. Patients treated with azaribine may develop transitory, severe central nervous system symptoms of depression, lethargy, and ataxia. These side effects are not predictable from oral dosage, and blood levels of the drug are very difficult to determine. All of our psoriatic patients treated with azaribine excreted large but variable amounts of orotic acid–orotidine in the urine. Spot urine ratios of orotic acid–orotidine:creatinine correlated very well with measured 24-hr urine output of orotic acid–orotidine.

Patients with central nervous system symptoms were found to have very high urine levels of orotic acid–orotidine. These symptoms can be prevented by monitoring the urinary orotic acid–orotidine:creatinine ratio levels and keeping them within a range which is still compatible with successful management of the psoriatic lesions.

Triazure® (azaribine) the triacetylated oral form of 6-azauridine is an effective systemic drug for controlling moderate to severe psoriasis [1]. In a double-blind crossover study [2] conducted by us, the effect of azaribine in 27 patients with psoriasis was evaluated at a dose of 125 mg/kg/day. Clinical response was considered to be good to excellent in 18 out of 21 patients on the drug, whereas 19 of the 20 patients on the placebo showed no improvement or deterioration of their skin lesions. Toxic side effects include a mild reversible anemia and central nervous system (CNS) symptoms of depression, and lethargy which may progress to ataxia. These symptoms are not predictable from the oral dosage of azaribine administered. Blood and urine levels of azaribine and its breakdown products including 6-azauridine are difficult to measure, requiring the use of complicated chromatographic techniques.

Azaribine is a nucleoside structural analog of uridine [3]. When 6-azauridine was given orally [3] it was poorly absorbed and the small amount that was absorbed was rapidly excreted in the urine, predominantly as 6-azauracil following cleavage of the ribose group. Although 6-azauridine is not believed to pass the blood brain barrier [3], 6-azauracil passes the blood brain barrier easily and is very toxic to the central nervous system [4–6]. In contrast to oral 6-azauridine, repeated oral dosage of azaribine results in significantly prolonged blood levels of 6-azauridine with minimal formation of 6-azauracil [3, 7].

Manuscript received February 28, 1973; in revised form May 8, 1973; accepted for publication May 14, 1973.

This work was supported in part by Research Grant No. AM 11649 from the National Institutes of Health.

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The de novo pyrimidine pathway [3, 7, 8] for the conversion of orotic acid to uridylic acid is reviewed in Figure 1. 6-azauridine is converted to the monophosphorylated form, 6-azauridylic acid, by uridine kinase [3]. This derivative competitively inhibits the decarboxylase conversion of orotidylic acid (OMP) to uridylic acid, resulting in the accumulation of OMP and orotic acid and a decreased production of RNA [9] and DNA. OMP is excreted in the urine as orotidine. Orotic acid is not detected in normal tissue and only 1–2 mg are excreted in the urine daily in a normal subject. Approximately 2.5 mg of orotidine are excreted in a 24-hr urine [10]. Orotic acid has only two known pathways of metabolism in humans. By a reversal of reactions in Figure 1, OMP can be reduced to dihydro-orotic acid and subsequently hydrolyzed to carbamyl aspartate. Most of the orotic acid is converted in the de novo pyrimidine pathway to uridylic acid [11].

The following report describes the procedures used by us for following psoriatic patients on azaribine with urine orotic acid–orotidine (OA + O)/creatinine ratios as a guideline to prevent CNS side effects.

MATERIALS AND METHODS

Selection of Patients

Thirty-eight patients with untreated moderate to severe psoriasis were selected for study. The patients received no systemic or topical treatment to their skin for one month prior to the study. The patients selected had no generalized systemic illness such as anemia, renal or liver disease which would contraindicate use of an antipyrimidine. Initial examination included history and physical examination, urinalysis, chest x-ray, determination of CBC, SGOT, alkaline phosphatase, creatinine, and blood sugar. Photographs were obtained. Women of child-bearing age received a pregnancy test before therapy and during treatment at regular intervals. Most of the subjects were followed as out-patients.

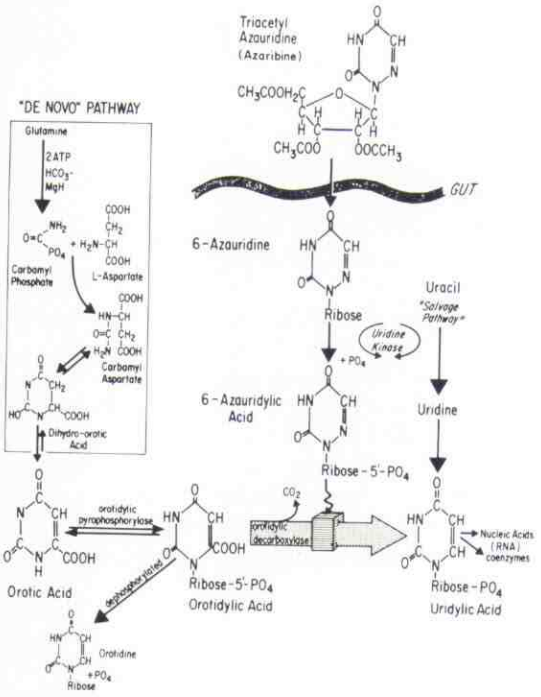

FIG. 1: De novo pyrimidine pathway for the synthesis of uridylic acid from orotic acid. The decarboxylase block by 6-azauridylic acid is shown.

Plan of Study

The patients were initially begun on 125 mg azaribine/kg/day in four equally divided doses taken after meals and at bedtime. The patients were seen weekly and the time of treatment for each varied from approximately 4–10 weeks depending on response to therapy. It was possible to obtain good clinical results (80–90 percent resolution of psoriatic plaques) within 4–10 weeks after beginning therapy in most patients. At each clinic visit the skin response was evaluated on a grading scale as follows: Patients with 80–100 percent clearing of the skin were graded as excellent; those with 60–80 percent improvement as good; 40–60 percent resolution of the lesions was graded as fair; 40 percent improvement to no change was graded as no improvement; and progression of the disease was graded as worse. The patients were also evaluated for the CNS side effects of depression, fatigue, lethargy, and ataxia. Symptoms of nausea were also documented.

The patients were told to void and discard their urine at home before coming to the clinic in the a.m. Urine samples were collected at each visit; 1 cc of urine was sent to the laboratory for spot urine creatinine determination and 1 cc was frozen for determination of OA + O levels.

Orotic Acid–Orotidine Determinations

OA + O determination has been reviewed in detail in several publications [10, 12] and we have previously shown [2] that OA + O can be measured in the urine of psoriatic patients by a modification of Rogers and Porter's [12] spectrophotometric method for determining urine orotic acid levels. The assay as originally described by Adachi [13] is briefly reviewed here as it applies to azaribine therapy. It is based upon the principal that orotic acid and/or orotidine react with bromine water to form 5,5'-dibromobarbituric acid. This compound is reduced by ascorbic acid to barbituric acid. Barbituric acid reacts with p-dimethylaminobenzaldehyde (Ehrlich's reagent) forming a yellow color which has a maximum optical density at 480 mμ. Pure OA + O produce an optical density proportional to concentration. In the urine of an individual secreting large amounts of OA + O, the concentration of OA + O is high, allowing the use of very small volumes of urine.

Before assay, all precipitated OA + O was redissolved by warming the sample, and the analysis was carried out at room temperature. A .05-ml aliquot was mixed with 2.95 ml of 0.2 M potassium citrate buffer, pH 2.5. A blank was prepared from 3 ml of the buffer. 0.5 ml of saturated bromine water was added to the sample. After 1 min 1.0 ml of 5% ascorbic acid in the buffer was added. After 2 min 2 ml of 2.5% p-dimethylaminobenzaldehyde in N-propanol were added, and after 90 min the optical density at 480 mμ was determined in a Beckman spectrophotometer. A standard curve was determined for known values of orotic acid added to control urines and the concentrations of orotic acid in micrograms/milliliter for specimens under consideration were taken from the

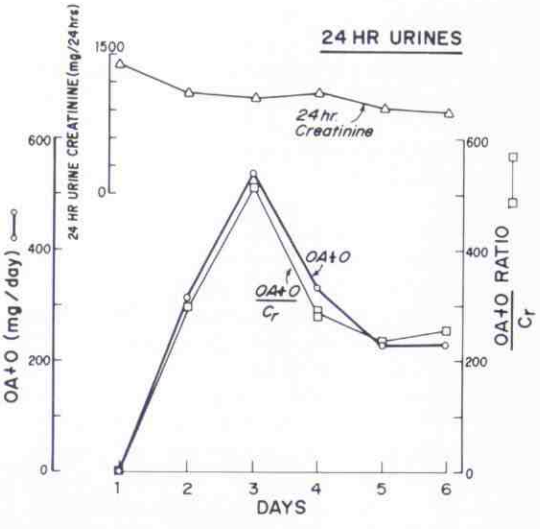

FIG. 2: OA + O/creatinine ratio correlations.

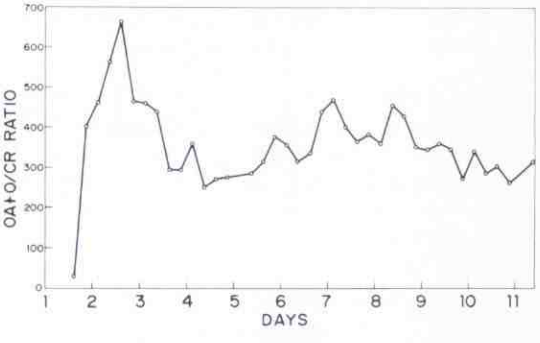

FIG. 3: Daily ratio response curve. This is a representation of the type of curve observed in 6 patients who had daily urine ratios determined for at least 10 days. Two patients were hospitalized and had daily ratios for 4 weeks. All of these patients showed the initial 48-hr peak.

standard curve. The concentration of OA + O in the urine sample (μg/ml) was divided by the urine creatinine (mg/ml) resulting in a ratio expressed as μg/mg. Azaribine (4–8 mg), 6-azauridine (3 mg), 6-azauridylic acid (3 mg) and uridine (5 mg) did not give color in the assay.

RESULTS

Since it was difficult and tedious for out-patients to collect and save 24-hr urine specimens, we determined that a random urine determination for OA + O expressed as a ratio over creatinine on the same specimen correlated well with the amount of OA + O excreted in the urine in 24 hr. This correlation is shown in Figure 2.

The daily response curve for excretion of OA + O in any individual patient on azaribine is shown in Figure 3. The OA + O ratios from most of our patients peaked in the first 48 hr after beginning azaribine and then fluctuated at a lower level over the following weeks of treatment. This excretion

TABLE I

Azaribine therapy for psoriasis (orotic acid + orotidine/creatinine ratios)

Patient	Weeks treatment	Dosage gm/day	Ratio range	Number of determinations	Ratios >600 symptoms*
1	6	8	321–450	6	
2	13	6	167–545	10	
3	8	8	250–350	4	
4	8	10	305–438	3	
5	5	8	305–808	4	2†—808, 706 Depression, very fatigued
6	7	6	240–1135	7	2—628, 1135 Extreme fatigue
7	7	12	227–364	6	
8	6	8	290–577	6	
9	14	8	275–500	6	
10	10	6–7	110–1300	8	3—1000, 1200, 1300 Very severe depression, nausea
11	5	8	277–448	4	
12	11	9.5	160–666	9	2—650, 666 None
13	5	8	457–689	4	1—689 Marked fatigue
14	8	7	441–530	6	
15	6	6	284–610	3	1—610 Depression
16	8	4.5–6	210–612	5	1—612 Depressed, fatigue
17	6	8	397–633	6	1—633 None
18	6	6	486–606	3	1—606 None
19	7	6	180–669	7	2—618, 669 None
20	6	8	212–500	10	
21	7	8	222–508	5	
22	6	6	383–472	4	
23	6	8	247–324	3	
24	8	8	250–400	5	
25	4	8	222–368	4	
26	5	6	220–340	4	
27	13	6–8	500–1040	9	6—1040, 1006, 765, 979, 943, 677 Very depressed, nausea, lethargic
28	7	8	550–924	6	4—616, 924, 859, 918 Very fatigued, ataxia and nausea
29	2	8	400–700	4	3—600, 650, 700 Marked depression
30	1	6	500–600	2	1—600 Depression, fatigue and nausea
31	6	8	220–450	6	
32	6	8	250–400	4	
33	4	6	1000–1300	3	3—1000, 1100, 1300 Very depressed
34	6	6	200–300	4	
35	12	9	340–550	8	
36	4	4.5	100–110	2	Unreliable, not taking full dose
37	4	9	110–180	3	Unreliable, not taking full dose
38	5	12	0–43	4	Unreliable, not taking full dose

* Severe CNS symptoms were at time of elevated ratio only as noted.

† The number and level of all ratios over 600 are noted.

Note: Patients unable to keep weekly clinic visits or failure to return weekly accounts for absent urine values for some of the weeks on the drug.

pattern in psoriatics agrees with results reported by Falon et al [14] on leukemic patients who were given 6-azauridine intravenously.

The subsequent results of the urine OA + O determinations in this study were all from samples taken at least 5 days after a patient was begun on azaribine therapy. Therefore, the OA + O ratios analayzed would not be influenced by the early 48-hr peak. The results of the OA + O creatinine ratios for all of our patients treated with azaribine are summarized in Tables I, II, and Figure 4. In Table I are listed all of the patients, number of weeks on the drug, dosage, ratio range, number of urine OA + O creatinine determinations, the number and value of each ratio over 600, and symptoms. The severe CNS symptoms as noted were at the times of the elevated ratios only although the dosage range for both the symptomatic and asymptomatic patients was similar. Table II summarizes the number of urine determinations, ratio ranges, and their correlation with definite severe CNS symptoms. Figure 4 represents a histogram summary of the urine ratio determinations and their relationship to severe CNS symptoms.

Analysis of these data has shown a statistically significant difference in the incidence of CNS symptoms between the patients with ratios above or below 600. Using Fisher's Exact Test for tables, the p value is <0.001. Patients who had OA + O creatinine ratios that ranged between 250 and 600 had minimal to no CNS symptoms with good clearing of their psoriatic plaques. However when a patient's OA + O ratio began to go above 600, he would begin to experience marked mental symptoms of depression, fatigue and/or lethargy. Three patients who had ratios over 600 also had symptoms of nausea and/or emesis. We observed that if the daily dosage of azaribine was lowered by 25–40 percent in a patient who began to develop CNS symptoms, there was a rapid decrease to absence of the CNS symptoms within 24–48 hr and a corresponding drop in the OA + O ratio. Three patients from this group and three from another study who were followed with OA + O ratios below 200 had poor clinical responses. The ratio was also an aid in determining whether a patient was actually taking his full prescribed daily dosage of azaribine. A patient who misses doses of the drug will have very low ratios (Table I). Most of our patients had 80–90 percent resolution of their psoriatic lesions at a dosage level of azaribine that would keep their average ratio between 200 and 600 during the course of treatment. This ratio level corresponded to a dosage range of 3.5–12.5 gm of azaribine per day in our patient group. The average dosage for satisfactory therapy with minimal CNS symptoms was 7.5 gm/day. Five of our patients who had very severe depression and/or ataxia had very high ratios.

Without the use of the OA + O/creatinine ratio we found it difficult to adjust and monitor the proper dosage of azaribine in treating our psoriatic patients. The ratio was helpful in determining when a patient was at a safe dosage level with regard to preventing CNS symptoms.

TABLE II

Spot urine ratio (OA + O/creatinine) determinations: summary of 197 urines evaluated

Ratio	CNS symptoms at time of ratio*	
	Negative	Positive
<600	164	0
>600 < 700	6	9
>700	0	18†

* All CNS symptoms noted here were moderate to severe (lethargy, fatigue and/or depression).

† All severe CNS symptoms as noted in Table 1.

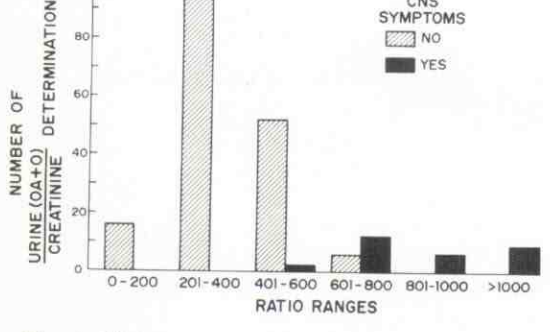

FIG. 4: Histogram: summary of 197 urine determinations for OA + O and relationship to severe CNS symptoms.

COMMENTS

There is a rare genetic disorder of pyrimidine metabolism in humans characterized by retarded growth and development, hypochromic anemia, and excessive urinary excretion of orotic acid [11]. The anemia is unresponsive to the usual forms of therapy. This genetic disease is known as hereditary orotic aciduria [8] and less than 10 cases have been reported. It represents the only specific genetic disorder of pyrimidine nucleotide synthesis so far elucidated in man. Studies of hemic cells, liver homogenates, and fibroblasts from these children have demonstrated reduced activities of both orotidylic pyrophosphorylase and orotidylic decarboxylase. These enzymes catalyze the conversion of orotic acid to uridylic acid. Reduced levels or inhibition of these enzymes result in excretion of high levels of orotic acid in the urine. Administration of 6-azauridine in man produces a somewhat analogous situation. All of our patients on azaribine (125 mg/kg/day) excreted large amounts (0.2–1.3 gm) of orotic acid–orotidine (OA + O) in the urine in 24 hr.

Azaribine is an effective method for controlling severe psoriasis, but while under treatment with

this drug most psoriatic patients will experience moderate to severe CNS symptoms if their daily dosage is too high. From our experience we believe that patients should be started on a maximum initial dose of 6–8 gm of azaribine per day. Most patients in our experience do not tolerate a higher initial dosage without symptoms of depression, fatigue, nausea and/or emesis. However, it can be difficult to predict from the calculated daily dosage of azaribine whether or not a patient will develop CNS side effects. We have found that the spot urine OA + O creatinine ratio is an excellent guide to follow psoriatic patients on azaribine. By following the spot urine ratios we have been able to adjust the dosage of azaribine in our severe psoriatic patients in order to keep their ratios within a therapeutic range while at the same time preventing them from developing CNS side effects.

Azaribine was kindly supplied by the Calbiochem Company, San Diego, California.

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