Allopurinol metabolism in a patient with xanthine oxidase deficiency

HISASHI YAMANAKA, KUSUKI NISHIOKA, TAKEHIKO SUZUKI,*
AND KEIICHI KOHNO*

From the Rheumatology Department, Clinical Research Centre, Tokyo Women's Medical College, 2-4-1, NS BLD, Nishishinjuku, Shinjuku, Tokyo, Japan

SUMMARY A patient with complete deficiency of xanthine oxidase would not be expected to oxidise allopurinol to oxipurinol if allopurinol did not have any alternative metabolic pathway. 400 mg of allopurinol was administered to a patient with xanthine oxidase deficiency, and plasma allopurinol, oxipurinol, hypoxanthine, and xanthine levels were determined serially by the use of high-performance liquid chromatography (HPLC). Plasma oxipurinol as well as allopurinol was increased after the administration of allopurinol, and oxipurinol reached a maximum level of 13.1 $\mu\text{g/ml}$ at 6 hours after the administration. This was the same pattern as that of normal controls. This result demonstrated the existence of some other oxidising enzyme of allopurinol than xanthine oxidase.

Allopurinol (4-hydroxypyrazolo[3,4-d]pyrimidine) is known as a potent inhibitor of xanthine oxidase (EC 1.2.3.2). In addition allopurinol itself is metabolised *in vivo* by xanthine oxidase to oxipurinol (4,6-dihydroxypyrazolo[3,4-d]pyrimidine, which also inhibits xanthine oxidase. A patient with complete deficiency of xanthine oxidase would not be expected to oxidise allopurinol to oxipurinol *in vivo*, if allopurinol did not have any alternative metabolic pathway. In this paper, we report the conversion of allopurinol to oxipurinol in a patient with deficiency of xanthine oxidase and discuss the possible metabolic pathway.

Materials and methods

The patient¹ was a 54-year-old female. She had been suffering from Graves's disease since she was 36 years old and had received methimazole continuously, but there was no history of haematuria, abdominal colicky pain, urinary calculi, or administration of allopurinol. Her parents were consanguineous and her sister had chronic thyroiditis. At the age of 49 hypouricaemia was revealed by chance. Her serum uric acid level was only 0.4 mg/dl (0.02 mmol/l). On the other hand her urinary excretion of oxypurine, determined by the method of Jørgensen and Poulsen,² was as high as 320 mg/day on a purine-

free diet. Creatinine clearance was 73.3 ml/min, and no calculus was found in her urinary tract by abdominal plain x-ray film and drip infusion pyelography.

The xanthine oxidase activity of the duodenal mucosa, which was obtained by gastrofibroscopy, was determined by the following procedures. The tissue specimens, weighing 5 to 10 mg, were homogenised in sodium pyrophosphate buffer solution (pH 8.0, 0.067 M) and centrifuged at 10 000 g for 40 minutes. The supernatants were concentrated and then reacted with a reaction mixture (pH 8.0) containing 0.5 mM of hypoxanthine at 37°C for 30 minutes, and the uric acid product was determined by the use of a spectrophotometer at 292 nm. Protein concentrations were determined by the method of Lowry *et al.*³

No activity of xanthine oxidase was found in this patient compared with that of a normal control, which was 15.4 nmol/hour/mg protein. She was diagnosed as having complete deficiency of xanthine oxidase.

400 mg of allopurinol was administered orally in one dose to this patient. At the same time 150 mg of allopurinol was administered to 2 healthy men whose serum uric acid levels were within normal limits as the controls. Plasma samples of this patient and controls were obtained serially by heparinised venous puncture until 25 hours after the administration of allopurinol.

Plasma allopurinol, oxipurinol, hypoxanthine and xanthine were determined by the following methods. Allopurinol was commercially obtained from Tanabe Seiyaku (Osaka, Japan). Oxipurinol was supplied

Accepted for publication 7 January 1983.

*Products Formulation Research Laboratory, Tanabe Seiyaku, Osaka, Japan.

Correspondence to Dr K. Nishioka.

from the same company. Xanthine was obtained from Sigma (St Louis, Missouri, USA), and hypoxanthine from Kohjin (Tokyo, Japan). Chelex-100 resin (200-400 mesh) was obtained from Bio-Rad Laboratories (Richmond, California, USA). All other chemicals were of analytical reagent grade commercially available.

Plasma concentrations of allopurinol, oxipurinol, hypoxanthine, and xanthine were determined by the method of Suzuki *et al.* (paper in preparation) by a modified procedure of Brown and Bye.⁴ Chelex-100Cu⁺⁺ resins were prepared according to the method of Brown *et al.*,⁴ packed into glass columns (0.6 × 25 cm), and washed with water followed by 0.02 M carbonate buffer, pH 11.0. Aliquots (0.2 ml) of plasma were applied to the columns. The columns were washed with carbonate buffer, pH 11.0, followed by water, and eluted with 1 M ammonium solution. The eluate was collected in a volumetric flask, which contained 0.2 ml of internal standard solution (sodium salicylate), and filled up to 10 ml. The resultant mixture was lyophilised to dryness, and the residue was then dissolved in 400 µl of water. A 100 µl aliquot of the solution was injected into the high-performance liquid chromatography (HPLC) system. Chromatography was performed with a 0.39 × 30 cm µBondapak C18 reversed-phase column and 0.01 M ammonium phosphate (pH 6.0) as the mobile phase. A detector wavelength of 254 nm was used. This method was suitable for analysing oxypurines in the range of 0.05–20 µg in 0.2 ml of plasma. Recoveries of the 4 compounds of interest were 85–95%.

Results

The plasma allopurinol and oxipurinol of this patient and healthy controls are shown in Fig. 1. In the control group the concentration of plasma allopurinol was highest at 2 hours after the oral administration of allopurinol and then rapidly decreased. The concentration of plasma oxipurinol was highest at 8 hours after administration of the allopurinol and then decreased gradually. In the patient the maximum plasma allopurinol level was observed at 1½ hours after its administration, after which it rapidly decreased. Oxipurinol increased in the same pattern as that of the controls, reaching the value of 13.1 µg/ml at 6 hours after administration, of the allopurinol.

Plasma hypoxanthine and xanthine concentrations are shown in Fig. 2. In the controls almost no significant change was seen, but in the patient hypoxanthine was markedly decreased between 6 and 8 hours after the administration of allopurinol, simultaneously with the peak level of plasma oxipurinol.

Fig. 1 Allopurinol and oxipurinol after the oral administration of allopurinol.

Fig. 2 Hypoxanthine and xanthine after the oral administration of allopurinol.

Discussion

In connection with the metabolism of allopurinol in the patient with xanthinuria it was of interest to analyse whether or not some enzyme other than xanthine oxidase might be present. There have been previous reports on the urinary excretion of allopurinol and oxipurinol when allopurinol was administered to patients with xanthinuria, but the results conflicted greatly. Various authors⁵⁻⁸ reported that allopurinol was excreted unchanged and that oxipurinol was absent in the urine of their cases. Others⁹⁻¹¹ observed the conversion of allopurinol to oxipurinol in the urine of their xanthinuric patients. In view of this finding some enzyme that oxidises allopurinol even in the absence of xanthine oxidase might be present. Chalmers *et al.*¹⁰ suggested inosinic acid dehydrogenase (EC 1. 2. 1. 14) as this alternative enzyme, but later Nelson *et al.*¹² took exception to this because allopurinol ribotide could not be a substrate for this enzyme. Then Johns *et al.*¹³ and others suggested that aldehyde oxidase (EC 1. 2. 3. 1.) had the capacity to oxidise allopurinol to oxipurinol. Yamamoto *et al.*¹⁴ noted that acute intoxication by allopurinol was observed to be greater in female than in male mice and concluded that some testosterone-dependent enzyme to oxidise allopurinol was present. Finally to support this conclusion they reported that it was aldehyde oxidase.

In this report we attempted to prove the existence of some enzyme other than xanthine oxidase in allopurinol metabolism. We analysed allopurinol and oxipurinol in the plasma of a patient with complete deficiency of xanthine oxidase by the use of HPLC and investigated the possibility of an alternative enzyme other than xanthine oxidase taking part in allopurinol metabolism. The plasma concentration of oxipurinol of this patient increased in the same pattern as that of the normal controls, and the biological half lives of allopurinol and oxipurinol showed almost the same values in these 2 groups.

This fact indirectly suggested the existence of another enzyme than xanthine oxidase. Furthermore, we must accept the possibility that

allopurinol is oxidised by this other enzyme even in normal subjects

Patients with xanthinuria may differ in their enzyme patterns. Further investigation by direct methods will be needed to clarify this.

References

- 1 Katsuki T, Shimizu T, Nishina T, Fujihira K, Nishioka K, Ohsawa T. A case of asymptomatic xanthine oxidase deficiency. *Nippon Naika Gakkai Zasshi* 1981; 70: 1263-6.
- 2 Jørgensen S, Poulsen H E. Enzymatic determination of hypoxanthine and xanthine in human plasma and urine. *Acta Pharmacol* 1955; 11: 223-43.
- 3 Lowry O H, Rosebrough N J, Farr A L, Randall R J. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; 193: 265-75.
- 4 Brown M, Bye A. The determination of allopurinol and oxipurinol in human plasma and urine. *J Chromatogr* 1977; 142: 195-202.
- 5 Engelman K, Watts R W E, Klinenberg J R, Sjoerdsma A, Seegmiller J E. Clinical physiological and biochemical studies of a patient with xanthinuria and pheochromocytoma. *Am J Med* 1964 37: 839-61.
- 6 Elion G B, Kovensky A, Hitchings G H, Metz E, Rundles R W. Metabolic studies of allopurinol, an inhibitor of xanthine oxidase. *Biochem Pharmacol* 1966; 15: 863-80.
- 7 Simmonds H A, Levin B, Cameron J S. Variations in allopurinol metabolism by xanthinuric subjects. *Clin Sci Mol Med* 1974; 47: 173-8.
- 8 Holmes E W, Mason D H, Goldstein L I, Blount R E, Kelley W N. Xanthine oxidase deficiency: studies of a previously unreported case. *Clin Chem* 1974; 20: 1076-9.
- 9 Simmonds H A. Urinary excretion of purines, pyrimidines and pyrazolopyrimidines in patients treated with allopurinol or oxipurinol. *Clin Chim Acta* 1969; 23: 353-64.
- 10 Chalmers R A, Parker R, Simmonds H A, Sneddon W, Watts R W E. The conversion of 4-hydroxypyrazolo[3,4-d]pyrimidine (allopurinol) into 4,6-dihydroxypyrazolo[3,4-d]pyrimidine (oxipurinol) in vivo in the absence of xanthine-oxygen oxidore ductase. *Biochem J* 1969; 112: 527-32.
- 11 Auscher C, Pasquier C, Mercier N, Delbarre F. Oxidation of pyrazolo[3,4-d]pyrimidine in a xanthinuric man. *Isr J Med Sci* 1973; 9: 1082.
- 12 Nelson D J, Bugge C J L, Krasny H C, Elion G B. Formation of nucleotides of [6-¹⁴C]allopurinol and [6-¹⁴C]oxipurinol in rat tissues and effects in uridine nucleotide pools. *Biochem Pharmacol* 1973; 22: 2003-22.
- 13 Johns D G. Human liver aldehyde oxidase: Differential inhibition of oxidation of charged and uncharged substrates. *J Clin Invest* 1967; 46: 1492-505.
- 14 Yamamoto I, Kyo K, Iwata H. A study of the enzyme metabolizing allopurinol (author's translation) *Uric Acid Research* 1973; 1: 21-33.