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SHORT COMMUNICATIONS

Interaction of allopurinol with human blood

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Allopurinol, 4-hydroxypyrazolo[3,4-*d*]pyrimidine, a structural analogue of hypoxanthine, is both an inhibitor and a substrate for the enzyme xanthine oxidase [1] and is oxidized to oxipurinol, 4,6-dihydroxypyrazolo[3,4-*d*]pyrimidine. The product, oxipurinol, is also an inhibitor of xanthine oxidase [2] and, for this reason, allopurinol has been effectively used for the treatment of gout and other hyperuricemic states [3–5].

In addition to the primary metabolite, oxipurinol, Nelson *et al.* [6] have reported the formation of allopurinol-1-riboside from allopurinol. An early report [7] indicates that children with Lesch–Nyhan syndrome, who lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRTase), excrete little allopurinol-1-riboside. This can be interpreted as evidence that the ribonucleoside is formed via the ribonucleotide. It has been observed [8] in patients taking allopurinol that the concentration of 5-phosphoribosyl-1-pyrophosphate (PRPP) in red blood cells is reduced significantly, due to the conversion of allopurinol to the ribonucleotide, a process that consumes PRPP. Nelson *et al.* [6] have suggested that the formation of the ribonucleoside depends on the level of HGPRTase and PRPP present in each tissue. This is in agreement with the recent report [9] on the uptake of hypoxanthine, an analogue of allopurinol, by human erythrocytes, suggesting that the conversion of hypoxanthine to inosine monophosphate (IMP) depends on the availability of PRPP.

Both allopurinol and oxipurinol are analogs of endogenous compounds, which creates problems with their assay in biological fluids. This difficulty in assaying allopurinol may account for the scarcity of pharmacological as well as pharmacokinetic studies of the drug despite its widespread use. Recently, Kramer and Feldman [10] reported a simple and efficient high-pressure liquid chromatographic (HPLC) method for the separation of allopurinol and its metabolites. In their study, Kramer and Feldman [10] observed that allopurinol when incubated in whole blood is rapidly removed from plasma, presumably by the red blood cell. The present investigation was designed to examine this process.

Allopurinol, oxipurinol, and allopurinol-1-riboside were gifts from the Burroughs Wellcome Co., Research Triangle Park, NC, and 2-[^{14}C]allopurinol (sp. act. 58.1 mCi/mmole) was obtained as a gift from the National Cancer Institute, Bethesda, MD. The chemical purity of the radioactive allopurinol was 99% as determined by isotopic dilution and by thin-layer chromatography in two solvent systems.

The *in vitro* studies were conducted using whole blood collected less than 48 hr prior to the experiment. An initial volume of 25 ml whole blood was incubated at 37° in a shaker bath. The experiment was started by adding various volumes of [^{14}C]allopurinol standard solution to whole blood to obtain the desired concentration and withdrawing 0.5 ml of the sample immediately (zero time). Thereafter, 0.5-ml samples were withdrawn at appropriate time intervals. The experiment was performed using initial concentrations of 2, 5, 10, 20, 30, and 50 μg/ml of allopurinol in whole blood. Samples were immediately centrifuged at 10,000 *g* and the plasma was removed for assay.

The assay method was a modification of the HPLC method described by Kramer and Feldman [10] which included additional washing steps. The solvent fractions obtained from 6 to 9 min and 11 to 14 min which correspond to allopurinol and the metabolite, respectively, were collected and counted by liquid scintillation spectroscopy.

To investigate the effects of structurally analogous compounds on the uptake and metabolism of allopurinol, oxipurinol, xanthine, hypoxanthine, uric acid, caffeine, allopurinol-1-riboside, and 6-mercaptopurine (each 20 μg/ml of whole blood) were incubated along with [^{14}C]allopurinol (10 μg/ml of whole blood).

To investigate the site of metabolism of allopurinol, whole blood was centrifuged and separated into the different fractions—plasma, red blood cells (RBC), and the buffy coat. The RBC and the buffy coat containing white blood cells (WBC) were washed three times with isotonic phosphate buffer. The washed RBC fraction was then resuspended in the buffer to make a 45% suspension and the WBC to make a 5% suspension. No attempt was made to check the morphological integrity of the suspended cells. The different fractions—WBC, RBC, and plasma—were incubated with [^{14}C]allopurinol at a concentration of 10 μg/ml.

To investigate the effect of aging on the capacity of red blood cells to take up and metabolize allopurinol, a 25-ml aliquot of whole blood was withdrawn from the same bag of blood (kept at 4°) over a period of several days. Each of the above blood samples was incubated with [^{14}C]allopurinol (10 μg/ml of whole blood), and the samples were collected and analyzed by the method described previously.

The changes in the concentration of allopurinol levels in whole blood are plotted semilogarithmically in Fig. 1. The initial velocities were calculated from these data and are shown in Table 1.

A plot of initial velocity versus substrate concentration resulted in a hyperbolic curve characteristic of an enzyme-catalyzed reaction. A double-reciprocal plot (Lineweaver–Burk, $1/v$ versus $1/S$) of the data is shown in Fig. 2. Evaluation of these data by linear regression resulted in an apparent K_M of 10.9 $\mu g \cdot ml^{-1}$ (8×10^{-5} M) and V_{max} of 42.6 $\mu g \cdot ml^{-1} \cdot hr^{-1}$ (3.1×10^{-4} M $\cdot$ hr^{-1}). These values represent overall constants for the loss of allopurinol from plasma and involve both uptake and metabolism of allopurinol in whole blood. Table 2 shows the effects of structurally analogous compounds on the metabolism of allopurinol by human blood. As can be seen from the table, both hypoxanthine and 6-mercaptopurine inhibited allopurinol metabolism by whole blood. The other compounds studied had no effect.

Data from experiments designed to examine different fractions of whole blood (plasm, RBC, and WBC) indicated that only the RBC fraction was capable of metabolizing allopurinol.

The effect of the age of the blood on the capacity of RBC to metabolize allopurinol is shown in Fig. 3 where initial velocity is plotted against time (in days) since collection. As can be seen from the figure, the red blood cells retained their maximum ability to metabolize allopurinol through day 2 after collection. There was a 50% reduction

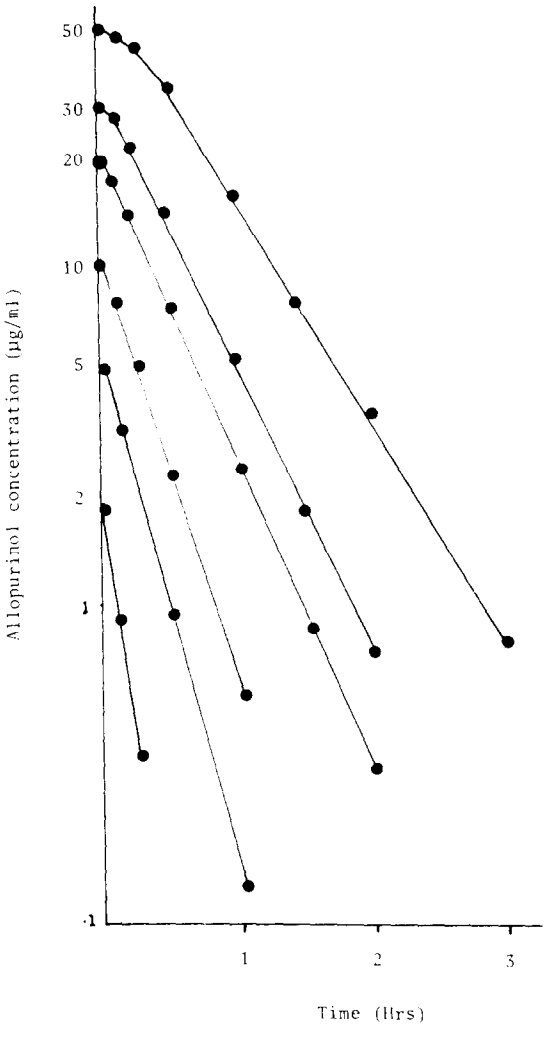

Fig. 1. Semi-logarithmic plots of allopurinol concentration versus time as a function of initial concentration. Each value is the average of two experiments.

in metabolizing capability by day 8.

Until 1970, no ribonucleotides of allopurinol and oxipurinol had been found. The nucleotides were first detected by Fox *et al.* [11] through the observation that patients taking allopurinol excreted increasing levels of orotic acid and oritidine. They found that inhibitors of orotidine 5′-phosphate decarboxylase were formed from allopurinol and oxipurinol. In the present investigation, mass spectrometric analysis showed that the metabolite isolated following incubation of allopurinol in whole blood was allopurinol-1-riboside. This finding is in agreement with the previous work of Krenitsky *et al.* [12] and Dean *et al.* [13] who reported the conversion of allopurinol to the 1-riboside in human red blood cells.

Earlier work [14] has shown that there is a substantial loss of the activities of enzymes such as catalase, glyoxalase and cholinesterase as red blood cells become older. The present study with allopurinol shows that there is a progressive decrease in the uptake of allopurinol by red blood cells with increasing age of the collected blood. Maximum activity was seen through day 2 following blood collection, and thereafter activity decreased sharply. The present

Table 1. Initial rate of allopurinol disappearance in whole blood

Initial concentration of allopurinol, $[S]$ ($\mu g \cdot ml^{-1}$)	$1/[S]$ ($ml \cdot \mu g^{-1}$)	Initial rate of loss ($\mu g \cdot ml^{-1} \cdot hr^{-1}$)	$1/v$ ($ml\text{-}hr \cdot \mu g^{-1}$)
2.00	0.50	6.80	0.147
5.00	0.20	12.80	0.078
10.00	0.10	20.80	0.048
20.00	0.05	25.60	0.039
30.00	0.033	33.70	0.029
50.00	0.020	36.20	0.027

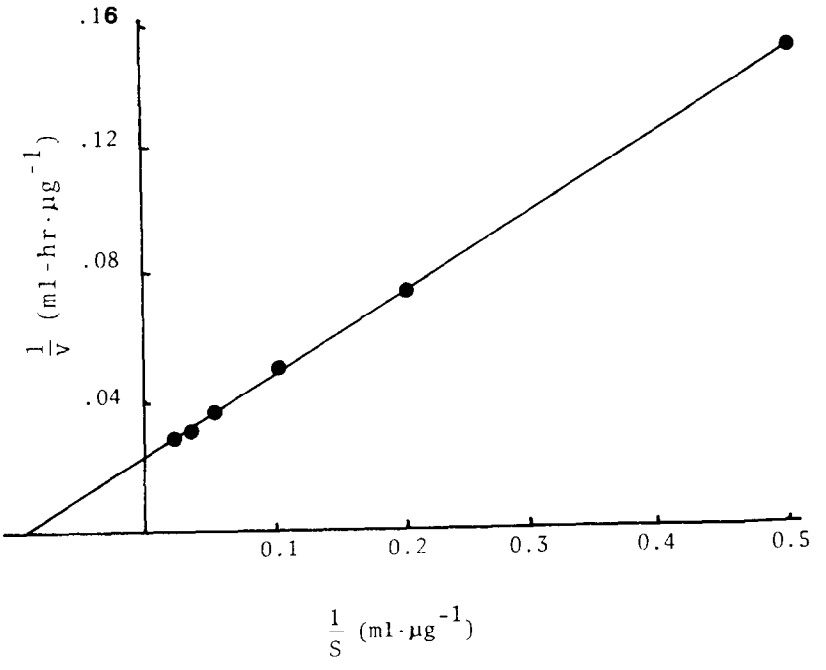

Fig. 2. Lineweaver–Burk plot for allopurinol loss from whole blood. Each point is the average of two experiments.

investigation clearly shows that the time since blood collection is an important factor in the ability of the red blood cell to take up and metabolize allopurinol. This apparent decrease in the activity of the red blood cell may have been due to many factors. With aging, there is an accumulation of ADP and a slight decrease in pH due to the accumulation of lactic acid, the end product of glycolysis. This is of great consequence, since it has been reported [15] that the synthesis of PRP which is needed for the metabolic conversion of allopurinol to its riboside is severely limited by the controlling influence of certain metabolites on the catalytic activity of PRPP synthetase. A major factor in the control of this system is the potent inhibition of the enzyme by ADP. A similar effect on PRPP synthetase by GDP and 2,3-diphosphoglycerate has been reported [15]. The level of 2,3-diphosphoglycerate varies with decline of ATP and also under conditions of impaired blycolysis such as pyruvate kinase deficiency.

The present study on the effect of red blood cell age on allopurinol uptake and metabolism revealed another important feature. A new, unidentified peak was found in the chromatograms from aged red blood cells. This peak had the same retention time (4.5 min) as that of hypoxanthine. These aged blood cells were found to be unable to take up and metabolize allopurinol. However, after washing these cells with phosphate buffer (pH 7.4) several times and resuspending them in phosphate buffer, it was observed that the uptake process took place. Adding hypoxanthine to the suspension blocked the process. These data clearly show that the level of hypoxanthine may be an important factor in the uptake and metabolism of allopurinol by blood cells. An earlier report by Pang [16] indicated that there was no metabolism of allopurinol by blood cells; this may be due to the fact that the experiment was conducted with aged blood cells or that high levels of hypoxanthine were present. During the course of the present study, it was

Table 2. Effects of structurally related compounds on allopurinol metabolism by whole blood*

	Level of allopurinol with time‡ (μg/ml)						
	Time (hr)						
Compounds†	0	0.125	0.25	0.5	1.0	1.5	12
Control§	10.00	7.42	4.82	2.40	0.46		
Oxipurinol	10.00	7.50	4.88	2.52	0.48		
Allopurinol-1-riboside	10.00	7.46	4.76	2.42	0.45		
Xanthine	10.00	7.48	4.90	2.53	0.49		
Uric acid	10.00	7.26	4.86	2.46	0.46		
Caffeine	10.00	7.29	4.78	2.52	0.50		
Hypoxanthine	10.00	7.38	4.92	4.95	4.89	4.92	4.84
6-Mercaptopurine	10.00	7.47	4.88	4.90	4.78	4.82	4.88

* All readings are means of two experiments.
† Initial concentration of potential inhibitors was 20 μg/ml for all experiments.
‡ Initial concentration of allopurinol was 10 μg/for all experiments.
§ Allopurinol alone.

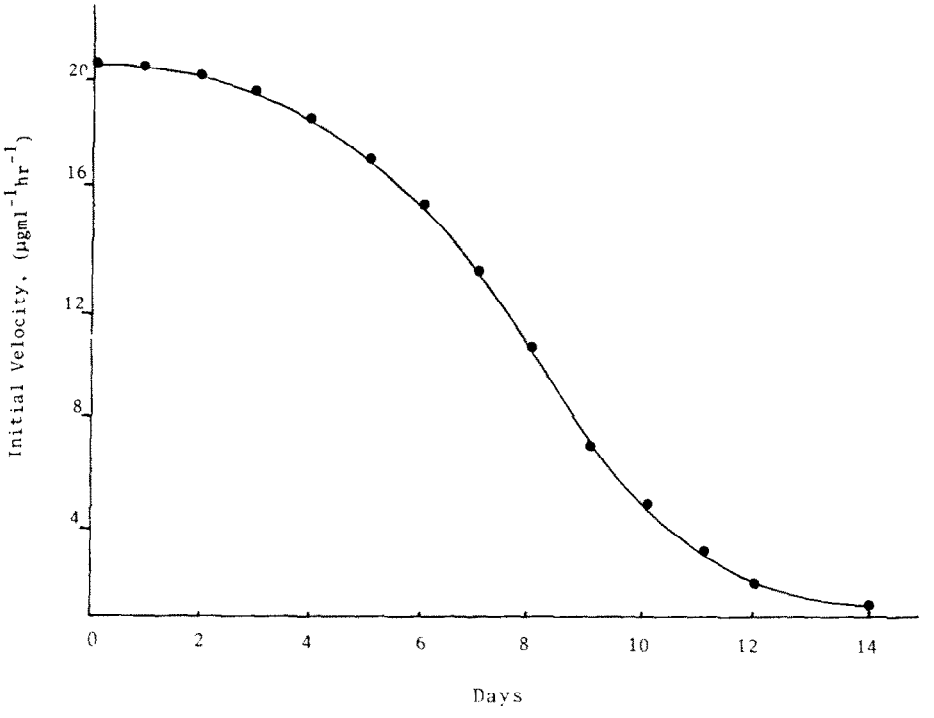

Fig. 3. Effect of aging on red blood cells activity. Each point is the average of two experiments.

found that some fresh samples of blood obtained from a blood bank and some samples collected from volunteers did not metabolize allopurinol. On analyzing these samples, it was noted that they all contained the "extra" peak with the retention time matching that of hypoxanthine. The level of hypoxanthine can vary with diet and other factors and may shed some light on a recent report by Sved and Wilson [17] in which they did not observe any uptake of allopurinol by erythrocytes. This may have been due to the fact that in their study the blood was collected immediately after a meal which may have increased the hypoxanthine blood level. Peak number I in their blank plasma (their Fig. 1) has the same retention time as that of hypoxanthine.

The present study demonstrates that allopurinol is taken up and metabolized by human red blood cells and that this process is influenced by various factors. The effect of this process on the *in vivo* pharmacokinetics of allopurinol is not known. Following oral administration of the normal maintenance dose of allopurinol (300 mg), peak plasma concentrations range up to 5.4 μg/ml [18]. At those levels, uptake metabolism *in vitro* proceeds at a rapid rate. If this process proceeds at a comparable rate *in vivo*, it may play an important role in the disposition and therapeutic effect of allopurinol in man. These aspects are currently under investigation.

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