Tissue distribution and characteristics of xanthine oxidase and allopurinol oxidizing enzyme

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Abstract—Tissue distribution and levels of allopurinol oxidizing enzyme and xanthine oxidase with hypoxanthine as a substrate were compared with supernatant fractions from various tissues of mice and from liver of mice, rats, guinea pigs and rabbits. The allopurinol oxidizing enzyme activities in liver were quite different among the species and the sex difference of the enzyme activity existed only in mouse liver. In mice, the highest activity of allopurinol oxidizing enzyme was found in the liver with a trace value in lung, but the enzyme activity was not detected in brain, small intestine and kidney, while the highest activity of xanthine oxidase was detected in small intestine, lung, liver and kidney in that sequence. The allopurinol oxidizing enzyme activity in mouse liver supernatant fraction did not change after storage at -20°C or dialysis against 0.1 M Tris-HCl containing 1.15% KCl, but the activity markedly decreased after dialysis against 0.1 M Tris-HCl. On the contrary, the xanthine oxidase was activated 2 to 3 times the usual activity after storage at -20°C or dialysis of the enzyme preparation. These results indicated that allopurinol was hydroxylated to oxipurinol mainly by the enzyme which is not identical to xanthine oxidase in vivo. A possible role of aldehyde oxidase involved in the allopurinol oxidation in liver supernatant fraction was discussed.

4-Hydroxypyrazolo(3, 4-d) pyrimidine (allopurinol) is reportedly a powerful inhibitor of xanthine oxidase both in vivo (1–3) and in vitro (3, 4) in experimental animals and man. This compound is also a substrate for the enzyme in vitro; and the product of its xanthine oxidase-catalyzed oxidation, oxipurinol (4,6-dihydroxypyrazolo (3, 4-d) pyrimidine), is also an inhibitor of the enzyme (4).

There is no evidence, however, that allopurinol is oxidized to its major metabolite oxipurinol by xanthine oxidase in vivo, although the drug is rapidly converted to oxipurinol in mice, dogs and humans (5).

Recently, it has been reported that purified aldehyde oxidase, but not xanthine oxidase, readily oxidizes compounds having a substituted pyrimidine ring structure including allopurinol (6).

The present report includes experimental data showing different tissue distributions

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and some characteristics of xanthine oxidase and of allopurinol oxidizing activity in the supernatant fraction from various animals.

The enzyme responsible for the *in vivo* oxidation of allopurinol is also discussed.

**MATERIALS AND METHODS**

6-14C-allopurinol was synthesized according to the methods of Elion *et al.* (5) and hypoxanthine-8-14C was purchased from Daiichi Pure Chemical Co. Ltd. All other chemicals were reagents of analytical grade.

All animals were decapitated except for rabbits which were sacrificed by air embolism. Tissues were rapidly removed, rinsed with cold saline, blotted with filter paper and weighed.

Supernatants from liver and other organs were prepared as described by Della Corte and Stirpe (7) with minor modifications. The tissues were homogenized for 1 min with 5 volumes of 0.1 M Tris-HCl buffer containing 1.15% KCl, pH 8.5, in a glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 600 × g at 4°C for 20 min and the supernatant was again centrifuged at 105,000 × g at 4°C for 60 min. The supernatant was used as an enzyme preparation unless otherwise indicated. In some experiments, the enzyme preparation was stored at −20°C or dialyzed against 200 volumes of 0.1 M Tris-HCl buffer containing 1.15% KCl, pH 8.5, or 0.1 M Tris-HCl buffer, pH 8.5, at 4°C for 20 hr.

Allopurinol oxidizing activity and xanthine oxidase activity were determined by measuring the formation of 14C-oxypurinol from 14C-allopurinol and of 14C-xanthine and 14C-uric acid from 14C-hypoxanthine, respectively. When allopurinol was used as a substrate, the assay mixture contained 12.5 μ moles Tris-HCl buffer, pH 8.5, 0.06 μ moles 14C-allopurinol and 50 μl of enzyme preparation in a final volume of 125 μl. The reaction was stopped after 30 min incubation at 37°C by the addition of 20 μl of 20% TCA. All other procedures were according to the method of Iwata *et al.* (8). When hypoxanthine was used as a substrate, the assay mixture contained 12.5 μ moles Tris-HCl buffer, pH 8.5, 0.06 μ mole hypoxanthine-8-14C and 50 μl of enzyme preparation in a final volume of 125 μl. The reaction was stopped after 10 min incubation at 37°C by the addition of 20 μl of 20% TCA. The precipitated protein was removed by centrifugation and the supernatant was applied to Toyo No. 51 chromatographic paper. Chromatograms were developed in n-butanol-80% formic acid-isopropanol-acetone-30% TCA (40 : 25 : 20 : 25 : 12 by volume) for 14-16 hr. Regions of strips containing hypoxanthine, xanthine and uric acid were viewed under ultraviolet light and cut out. The radioactivity was counted in a liquid scintillation counter, Beckman model LS-150. Protein was determined by the method of Lowry *et al.* (9).

**RESULTS**

*A comparison between the activities of the allopurinol oxidizing enzyme and xanthine oxidase in liver supernatant fraction of various species of animals*

Allopurinol oxidizing enzyme and xanthine oxidase activity in liver extracts of various species of animals of both sexes were assayed in the presence of excess concentration of
allopurinol (0.48 mM) and hypoxanthine (0.48 mM) and results are shown in Table 1. The allopurinol oxidizing activity of mouse liver was confirmed to be quite different between male and female as previously reported (8), however significant sex differences were not seen with the other species. The activity of the enzyme was highest in male mice, followed by rabbits, guinea pigs, female mice and Sprague-Dawley strain of rats, while no activity was detected with Wistar strain of rats of either sex. With respect to xanthine oxidase, there was no sex difference in any species examined. Hypoxanthine was oxidized in Wistar rats and lowest xanthine oxidase activity in contrast with allopurinol oxidation was seen in rabbits of both sexes.

**Tissue distribution of allopurinol oxidizing and xanthine oxidase activities in mice**

Allopurinol oxidizing enzyme activity was compared with xanthine oxidase in various organs of mice. Allopurinol oxidizing enzyme was mainly present in the liver, and a certain

### Table 1. Comparison of allopurinol oxidizing enzyme and xanthine oxidase activities in liver supernatant fractions of various species of animals

| Species       | Allopurinol oxidizing* activity | Xanthine oxidase** activity |
|---------------|---------------------------------|-----------------------------|
|               | Male                           | Female                      | Male                        | Female                      |
| Mouse         | $28.7 \pm 2.0$ (14)             | $7.2 \pm 0.5$ (12)          | $9.2 \pm 1.0$ (10)          | $9.8 \pm 1.3$ (10)          |
| Rat (S.D)     | $5.8 \pm 0.4$ (5)               | $5.3 \pm 0.5$ (5)           | $8.6 \pm 1.4$ (5)           | $8.0 \pm 1.0$ (5)           |
| Rat (Wistar)  | n.d.                            | n.d.                        | $7.6 \pm 1.0$ (4)           | $6.8 \pm 0.9$ (4)           |
| Guinea pig    | $18.3 \pm 1.7$ (8)              | $17.6 \pm 1.5$ (6)          | $5.4 \pm 0.6$ (5)           | $4.6 \pm 0.5$ (5)           |
| Rabbit        | $24.6 \pm 2.5$ (5)              | $25.9 \pm 2.5$ (3)          | $1.3 \pm 0.1$ (5)           | $1.8 \pm 0.1$ (3)           |

* $\mu$ moles oxipurinol formed/100 mg protein/hr  
** $\mu$ moles xanthine + uric acid formed/100 mg protein/hr  

Each value represents the mean $\pm$ S.E. and figures in parentheses indicate number of experiments. 

S.D.: Sprague-Dawley, n.d.: not detected.

### Table 2. Tissue distribution of allopurinol oxidizing enzyme and xanthine oxidase activities in mice

| Organs       | Allopurinol oxidizing* activity | Xanthine oxidase** activity |
|--------------|---------------------------------|-----------------------------|
|              | Male                           | Female                      | Male                        | Female                      |
| Brain        | n.d.                            | n.d.                        | Trace                       | Trace                       |
| Lung         | Trace                           | Trace                       | $15.2$                      | $14.6$                      |
| Liver        | $26.8$                          | $6.7$                       | $6.8$                       | $8.2$                       |
| Small Intestine | n.d.                           | n.d.                        | $65.6$                      | $62.2$                      |
| Kidney       | n.d.                            | n.d.                        | $3.0$                       | $3.6$                       |

* $\mu$ moles oxipurinol formed/100 mg protein/hr  
** $\mu$ moles xanthine + uric acid formed/100 mg protein/hr  

Values represent the average of results obtained in two experiments. Eight animals were used for each experiment. n.d.: not detected.
amount of activity was detected in the lung of both sexes. The enzyme activity was, however, not detected in other organs under these experimental conditions. Xanthine oxidase was found to be widely distributed in all organs tested. The highest enzyme activity was seen in the small intestine and the activity in the liver was approximately one ninth of that in the small intestine.

Effect of freezing on hydroxylation activities of allopurinol and hypoxanthine

Della Corte and Stirpe (7) have already reported that xanthine oxidase activity of rat liver supernatant measured with oxygen as the electron acceptor was greatly increased by storage of the supernatant at $-20^\circ$C. In our experiment, the enzyme preparations of mouse liver supernatant were tested for hydroxylation activities of allopurinol and hypoxanthine both before and after the freezing treatment in order to characterize the differences. As shown in Fig. 1, the relative xanthine oxidase activity in mouse liver supernatant was

![Fig. 1. Effect of freezing on hydroxylation activities of allopurinol and hypoxanthine.](image)

![Fig. 2. Effect of dialysis on hydroxylation rates of allopurinol and hypoxanthine.](image)
increased about 2-fold with freezing, while such treatment had no effect on the allopurinol oxidizing activity.

Changes in enzyme activities before and after dialysis of the supernatant fraction

We reported that the activity of partially purified allopurinol oxidizing enzyme was markedly decreased after dialysis, but that the potassium ion protected the enzyme activity (10). In the present experiment with mouse liver supernatant, we confirmed these results which are shown in Fig. 2. On the contrary, xanthine oxidase activity was increased about 3-fold after dialysis of the enzyme preparation against Tris-HCl buffer with or without 1.15% KCl.

DISCUSSION

We have presented evidence that the liver supernatant fractions from various species of animals have the ability to convert allopurinol to oxipurinol and that the enzyme mainly responsible for the oxidation seems to be different from xanthine oxidase. The levels of allopurinol oxidizing enzyme in liver exhibited pronounced species and strain differences. The levels in male mice and rabbits were high as compared with rats and guinea pigs. However, the activities of xanthine oxidase in the liver were not so different among the species of animals studies except for rabbits. Allopurinol oxidizing enzyme was concentrated in the liver, while xanthine oxidase was concentrated in the small intestine, lung, liver and kidney in this order.

Our previous experiments (8) and the present study as shown in Table 1 demonstrated that allopurinol oxidizing activity in liver of male mice was 3 to 4 times higher than in females, while there was no sex difference in xanthine oxidase activity. In addition we had observed that testosterone treatments in castrated and ovariectomized mice increased the allopurinol hydroxylating enzyme but not xanthine oxidase (8). With regard to the nature of xanthine oxidase, it has been reported that the enzyme activity was increased after storage at $-20^\circ\text{C}$, prolonged dialysis and proteolytic treatment of rat liver supernatant fraction (7, 11, 12). Stirpe and Della Corte (11) formulated a hypothesis that the increase in the enzyme activity with various conditions is due to the conversion of xanthine dehydrogenase (Type D) to xanthine oxidase (Type O). We observed similar results, as above mentioned, in mouse liver xanthine oxidase activity. The hydroxylation rate of hypoxanthine is markedly elevated by storage at $-20^\circ\text{C}$ or dialysis of the supernatant, while the allopurinol hydroxylation rate did not change or even decreased with the same treatments.

As shown in Fig. 2., potassium ion was required for allopurinol oxidizing enzyme activity, but not for xanthine oxidase activity.

The in vitro studies on a comparison of the substrate specificities of the partially purified xanthine oxidase and aldehyde oxidase revealed that allopurinol was an appropriate substrate for aldehyde oxidase (6). Huff and Chaykin (13, 14) reported that aldehyde oxidase has a higher activity in male than in female mice and is induced by testosterone when N1-methyl-nicotinamide is used as substrate. In the first report from our laboratory on the study of allopurinol, we showed that there was marked sex difference in acute toxicity of the drug
in mice and that females had higher LD50 values than did the males (15). Elion et al. suggested that as a consequence of its low clearance rate, oxipurinol may play a significant role in the pharmacological and toxic effect of allopurinol (5). These findings and the present results suggest that aldehyde oxidase but not xanthine oxidase, may be the enzyme responsible for the in vivo oxidation of allopurinol, when a high dose is administered. The oxidation of allopurinol by aldehyde oxidase might be relevant to the observation that a xanthinuric patient who presumably lacked xanthine oxidase excreted appreciable amounts of oxipurinol (16).

We investigated the relationship between allopurinol oxidizing enzyme and aldehyde oxidase in mice (10, 17). The oxidation of both N1-methylnicotinamide and allopurinol appeared to be catalyzed by a single enzyme, aldehyde oxidase (17). This conclusion is based on the following evidence; the postnatal changes seen with allopurinol and N1-methylnicotinamide oxidizing activities were similar during growth and the level of both activities increased in a parallel fashion upon the attainment of sexual maturity. The rate of loss of the activities of both enzymes by heat denaturation as well as by dexamethasone administration was similar. The inhibitors of allopurinol oxidizing enzyme also suppressed N1-methylnicotinamide oxidation. Aldehyde oxidase activity with N1-methylnicotinamide as substrate was competitively inhibited by allopurinol. The rate of increase of the activities in both enzymes from mouse liver supernatant was almost parallel during each step of purification. Thus, it was confirmed that xanthine oxidase in the enzyme preparation does not influence allopurinol oxidation at least under the assay condition used herein.

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