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Alteration of hepatic ornithine decarboxylase and aniline hydroxylase activities following a single dosing of azathioprine to rats.

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Abstract

Hepatic ornithine decarboxylase and aniline hydroxylase activities increased as early as 6h after a single dosing of azathioprine to male rats and reached maximum levels at the 12th h after the treatment. However, aniline hydroxylase activity dropped to levels much lower than the controls 48 h following azathioprine treatment. The results are discussed with regard to the role of azathioprine during the promotion of hepatocarcinogenesis.

KEYWORDS: azathioprine, ornithine decarboxylase, aniline hydroxylase, hepatocarcinogenesis

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— BRIEF NOTE —

**ALTERATION OF HEPATIC ORNITHINE DECARBOXYLASE
AND ANILINE HYDROXYLASE ACTIVITIES FOLLOWING
A SINGLE DOSING OF AZATHIOPRINE TO RATS**

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Abstract. Hepatic ornithine decarboxylase and aniline hydroxylase activities increased as early as 6 h after a single dosing of azathioprine to male rats and reached maximum levels at the 12th h after the treatment. However, aniline hydroxylase activity dropped to levels much lower than the controls 48 h following azathioprine treatment. The results are discussed with regard to the role of azathioprine during the promotion of hepatocarcinogenesis.

Key words : azathioprine, ornithine decarboxylase, aniline hydroxylase, hepatocarcinogenesis.

Azathioprine (AZP), which is widely used in the treatment of various autoimmune diseases (1), inhibited the production of hyperplastic liver nodules (2) in diethylnitrosamine (DEN) - and N-2-fluorenylacetylamide (FAA) - treated rats (3). For evaluating the mechanisms of this inhibition, activities of hepatic ornithine decarboxylase (ODC, EC 4.1.1.17.), a rate-limiting enzyme of polyamine biosynthesis, and aniline hydroxylase (AH, EC 1.14.1.1.) in the mixed-function oxidase system were determined following a single dose of AZP, since Manen *et al.* (4) have already reported that elevation of AH activity and liver hypertrophy might be the result of increases in hepatic ODC activity during tumor promotion. In this study, hepatic ODC and AH activities were determined 6 to 48 h following a single intragastric administration of AZP to male rats.

Male Sprague-Dawley rats weighing 250-300 g were used in this study and were starved overnight before use. AZP (Sigma Chemical Co., St. Louis) in 0.5 % carboxymethyl cellulose (CMC, Nakarai Chemical Co., Kyoto) was administered intragastrically at a dose of 500 mg/kg body weight using gastric tubing under light ether anesthesia. Controls were treated similarly with an equal volume of CMC. All rats were given only water for 48 h after the treatment. Animals were sacrificed by a blow on the head 6 to 48 h after the AZP administration. The liver was removed rapidly and chilled immediately. Homogenates prepared in 154 mM KCl were used for determining AH activity and super-

natants obtained by centrifugation of the homogenates in 50 mM Tris-HCl buffer (pH 7.5) at 16,000 Xg were used for assaying ODC activity. ODC activity was determined using DL-[1-¹⁴C] ornithine hydrochloride (the Radiochemical Centre, Amersham, England, specific radioactivity 58 mCi/nmol) and expressed as pmoles of CO₂ per mg protein per 20 min according to the method of Fukui *et al.* (5). AH activity was measured by following the formation of p-aminophenol from aniline by the method of Imai *et al.* (6) and expressed in microunits (nmoles of product per mg protein). Protein contents in the liver homogenates and supernatants were estimated according to the method of Lowry *et al.* (7).

The number of rats examined was 3 in each experimental group. All the data was expressed as the mean \pm SD. Statistical differences between the mean values were determined by Student's *t* test after analysis of variance.

Both serum glutamate pyruvate transaminase (GPT, EC 2.6.1.2.) activity (IU/1) and percent liver weight ([liver weight/body weight] \times 100) were slightly but significantly increased only in the 48th h after a single dose of AZP administration, indicating that liver injury occurred at this time (Table 1). Histologi-

TABLE 1. CHANGES IN LIVER ODC AND AH ACTIVITIES AND SERUM GPT LEVELS FOLLOWING A SINGLE DOSE OF AZP

Time following treatment (h)	Percent liver weight	GPT	ODC	AH
0	3.00 \pm 0.01	37 \pm 1	3 \pm 0.1	117 \pm 9
6	2.93 \pm 0.05	36 \pm 1	35 \pm 7*	191 \pm 42
12	3.26 \pm 0.21	38 \pm 3	144 \pm 37*	292 \pm 36*
24	3.70 \pm 0.04***	43 \pm 7	118 \pm 33*	241 \pm 35*
48	3.83 \pm 0.06***	83 \pm 16**	32 \pm 7*	54 \pm 22**

*P < 0.01 **P < 0.02 ***P < 0.001

cal findings in the liver also revealed degenerative changes associated with small lipid deposits in the centrolobular portion. AZP administration resulted in marked increases of hepatic ODC activity as early as 6 h after the treatment, when liver injury was not apparent as indicated by no elevation of serum GPT activity; the maximal ODC activity was observed in the 12th h after AZP was given. Thereafter, ODC activity declined slowly. As ODC activity was reported to be very low during fasting (8), the increase would be indicated by AZP administration. AH activity increased similarly and reached peak levels 12 h after the AZP administration. However, the activity then dropped to levels much lower than the controls 48 h after the treatment.

The most interesting results in this study were the observations that hepatic ODC and AH activities increased early, before the appearance of AZP-induced liver injury, and that the latter enzyme activity diminished paradoxically at the time when liver damage apparently occurred. We have already reported that

liver AH activity decreases in chronically AZP-treated rats (9). The fact that diminished activity of AH can serve as one of several parameters for liver injury has also been described (10). Phenobarbital injection induced AH and other drug-metabolizing enzymes such as cytochrome P-450 in the liver microsomes, but chronic administration of the barbiturate resulted in paradoxical decrease of liver microsomal glucose-6-phosphatase (EC 3.1.3.9.) activity (11). However, there is no report of increased activity of hepatic ODC in AZP-treated rats. It has already been suggested that both liver hypertrophy and induction of microsomal mixed-function oxygenases are the results of a general series of biochemical events initiated by increases in the intracellular concentration of cyclic adenosine 3': 5'-monophosphate (4). Increase in hepatic ODC activity is mediated in these processes, and this phenotypic change may be essential for tumor promotion (4).

Furthermore, prolonged activation of cyclic adenosine 3': 5'-monophosphate-dependent protein kinase (EC 2.7.1.37.) and elevation of ODC have been suggested as being necessary for hepatocarcinogenesis (12). We have already reported that phenobarbital administration accelerated the production of hyperplastic liver nodules in DEN- and FAA-treated rats (3). Barbiturate, which is well known as a promoter of hepatocarcinogenesis (22), caused slight but significant increase in ODC activity of the liver (14), followed by liver hypertrophy and induction of microsomal drug-metabolizing enzymes (15). These events suggested that AZP and other chemicals increasing hepatic ODC, and subsequently AH activities and percent liver weight, have a promoter activity for hepatocarcinogenesis. However, AZP actually inhibited the development of hyperplastic liver nodules produced by two carcinogens, DEN and FAA (2). AZP metabolism in the liver, therefore, might be different in DEN- and FAA-treated and untreated rats. Further detailed studies are necessary to explain the discrepancy in AZP effects on the formation of hyperplastic nodules in the liver.

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