Changes in Amine Oxidase in Plasma of Rats Treated with Hepatotoxins

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Abstract—When allyl formate (AF) was administered to rats, the marked elevation of B-form MAO activity in plasma was found with β-PEA as a substrate. In contrast, in the case of carbon tetrachloride (CCl₄), A-form MAO activity elevated predominantly. The deaminations of 5-HT and β-PEA in these plasma treated with AF or CCl₄ were not inhibited completely by a high concentration of MAO inhibitor, deprenyl or clorgyline. These results indicate the there may be two or more distinct amine oxidases released from the liver and other organs in response to CCl₄ or AF.

It is well-known that leaking enzymes such as glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and lactic dehydrogenase (LDH) are induced in the course of acute or chronic liver injury with a consequent rise in their levels in the plasma (1-3). It is also known that there is a marked elevation of GOT, GPT and LDH activity in the plasma of rats treated with agents which cause wide spread damage to liver cells (2-4). Although abnormally elevated levels of the monoamine oxidase (MAO) in plasma have been reported with postnecrotic cirrhosis and massive neoplastic replacement of the liver with fibrosis (5), the mechanism of this elevation of MAO activity in the plasma in these hepatic diseases is not yet clear. Moreover, it is difficult to believe that MAO in the plasma results from damage of hepatic mitochondria. In this study, we have determined whether amine oxidase activity in the plasma elevates after pretreatment with the centrilobular hepatotoxin, carbon tetrachloride (CCl₄), and the perilobular hepatotoxin, allyl formate (AF) (1-3).

Male and female Wistar rats were intraperitoneally administered with 1.2 ml/kg CCl₄ or 0.1 ml/kg AF in olive oil. The rats were anesthetized with 50 mg/kg pentobarbital, i.p., at 12 hr for AF and at 36 hr for CCl₄ after their administrations, and blood was collected by a heparinized syringe from the inferior vena cava. The plasmas obtained by centrifugation at 3,000 rpm for 20 min were used as the enzyme preparations. The protein contents of the enzyme preparations were measured by the method of Lowry et al. (6) with bovine serum albumin as the standard and the protein concentrations were then adjusted to 4 mg/ml. MAO activity was measured by radioisotopic assays with 100 nM 5-HT (substrate for A-form MAO) and 10 μM β-phenylethylamine (β-PEA) (substrate for B-form MAO) as described previously (7). GOT and GPT activity were measured using the GOT-UV Test and GPT-UV Test (Wako), and these activities were reported in terms of Karmen units (U).

After a single administration of AF or CCl₄, amine oxidase activities in the plasma elevated gradually, and the highest amine oxidase activities in the plasma were obtained at 12 hr with AF and at 36 hr with CCl₄, respectively (data not shown). The degrees of the increase in the activity of GOT and GPT are considered to be reliable parameters reflecting the degree of CCl₄ hepatocellular toxicity. In this study, the increases of the activity of GOT and GPT in these same plasma were also observed. GOT and GPT activity were 80±29 U and 20±16 U with saline, 7400±1260 U and 3435±1020 U with AF and 278±98 U and 99±58 U with CCl₄ in male rats, respectively. In the female rats, these were 145±44 and...
29±15 with saline, 9750±2023 U and 6610±1510 U with AF and CC14, respectively. When 1.2 ml/kg of CC14 was administered to male and female rats, higher amine oxidase activity was obtained with 5-HT as a substrate. In contrast, in the case of AF, higher plasma amine oxidase activity was obtained with β-PEA as a substrate (Table 1). In general, it is known that CC14 and AF may selectively cause fetal hepatic centrilobular and periloobular necrosis, respectively (1, 2). These results may indicate that A-form MAO localizes predominantly around the central veins of the liver; and in contrast, B-form MAO localizes predominantly around the portal veins of the liver.

Using the enzyme preparations from the plasma of rats that were administered CC14 or AF, the inhibition of enzyme activity by various concentrations of clorgyline (A-form MAO inhibitor) or deprenyl (B-form MAO inhibitor) was investigated (Fig. 1). When the enzyme treated with AF was preincubated at 37°C for 40 min with clorgyline, amine oxidase activity was highly sensitive with 5-HT as a substrate, while it was less sensitive with β-PEA as a substrate. However, even the highest concentration of clorgyline (10⁻⁴ M) did not completely inhibit the amine oxidase activity. The residual enzyme activities were 30% and 35% with β-PEA and 5-HT, respectively. When corresponding experiments were performed with deprenyl, the opposite results were obtained. At 10⁻⁴ M deprenyl, the residual enzyme activities were 20% and 30% with β-PEA and 5-HT, respectively (Fig. 1, left). When the plasma of rats administered CC14 were used as the enzyme preparations, almost similar results were obtained. The complete inhibitions of these enzyme activities could not be obtained by these inhibitors, and the residual enzyme activities at 10⁻⁴ M clorgyline were 55% and

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Table 1. Specific amine oxidase activity in rat plasma after saline, AF and CC14 administration

| Substrate | Specific amine oxidase activity (pmoles/min/mg protein) |
|-----------|--------------------------------------------------------|
|           | saline 2.0 ml/kg, i.p. | AF 0.1 ml/kg, i.p. | CC14 1.2 ml/kg, i.p. |
| Male      | β-PEA | 0.027±0.001 | 1.069±0.057 | 0.375±0.049 |
| Rats      | 5-HT  | 0.039±0.004 | 0.464±0.113 | 1.074±0.054 |
| Female    | β-PEA | 0.014±0.004 | 1.149±0.036 | 0.702±0.014 |
| Rats      | 5-HT  | 0.066±0.002 | 0.532±0.103 | 2.272±0.034 |

Specific activity was expressed as pmoles/min/mg protein. Enzyme activity was determined radiochemically using 100 µM 5-HT and 10 µM β-PEA as substrates for 60 min at 37°C. Allyl formate (AF) (0.1 ml/kg, i.p.), CC14 (1.2 ml/kg, i.p.) and saline (2.0 ml/kg, i.p.) were administered to five Wistar rats, respectively. Each value represents the mean±S.E. of five rats.

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![Fig. 1. Inhibitions of amine oxidase activity in the plasmas of rats administered AF and CC14 by clorgyline and deprenyl toward 5-HT and β-PEA as substrates. Remaining amine oxidase activity is expressed as a percentage of the control activity in the absence of clorgyline and deprenyl. The enzyme preparation was preincubated with clorgyline (upper) or deprenyl (bottom) at 37°C for 40 min. Enzyme activity was assayed radiochemically by addition of substrate, 100 µM 5-HT (●) or 10 µM β-PEA (○), at 37°C for 60 min.](image-url)
20% with β-PEA and 5-HT, respectively; and at 10^{-4} M deprenyl, they were 50% and 20% with β-PEA and 5-HT, respectively (Fig. 1, right). In general, the amine oxidases are a group of enzymes differing from each other in a variety of ways such as substrate specificity, inhibitor sensitivity and cellular localization. This group includes enzymes such as connective tissue amine oxidase, plasma amine oxidase, diamine oxidase and mitochondrial MAO. Moreover, recently there have been many reports of the possible existence of other amine oxidases; that is, clorgyline-resistant amine oxidase (8, 9) or semicarbazide-sensitive amine oxidase (10).

In this study, amine oxidase activities in the plasma of rats administered CCl₄ or AF were not inhibited completely by the high concentration of clorgyline and deprenyl. Moreover, platelet MAO activity was not found in these rats. These data may also be consistent with the existence of two or more distinct amine oxidases in plasma: native mitochondrial MAO released from liver, clorgyline-resistant amine oxidase or semicarbazide sensitive amine oxidase and plasma amine oxidase from other organs by CCl₄ and AF, although the origins of these enzymes are unknown. We are now using the plasma of AF or CCl₄ treated rats for further studies on the identification and origin of these enzymes.

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