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The Biphasic Change of Cytosolic Acetyl-CoA Hydrolase in Rat Liver during 3′-Methyl-4-dimethylaminoazobenzene Hepatocarcinogenesis

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When Donryu male albino rats were given diet containing 0.06% 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) for 20 weeks, the activity of cytosolic acetyl-CoA hydrolase in their livers decreased to about one-third the initial level in week 2, returned to the control level in week 7, and then decreased again to about one-tenth of the control in week 20. These changes in enzyme activity were parallel with changes in the amount of enzyme protein determined by ELISA. In 3′-Me-DAB-resistant rats, however, the enzyme activity and enzyme protein remained within the normal range during administration of 3′-Me-DAB-containing diet for 20 weeks and no tumors were detectable macroscopically. Interestingly, the biphasic change in this enzyme activity was inversely associated with the well known change of γ-glutamyltranspeptidase activity during azo-dye-induced hepatocarcinogenesis.

Key words: Acetyl-CoA hydrolase — 3′-Methyl-4-dimethylaminoazobenzene — Rat liver — Hepatocarcinogenesis

In rat liver, a novel cytosolic acetyl-CoA hydrolase (EC 3.1.2.1), which hydrolyzes acetyl-CoA to acetate and CoASH, had presumably not been detected previously because of its extreme cold lability.[1-4] At room temperature, this enzyme is usually present as an enzymatically active dimer (Mr 135,000) or tetramer (Mr 240,000),[2-4] but at low temperature, these forms dissociate into an inactive monomer.[2-4] The activity is regulated by ATP (activator) and ADP (inhibitor),[1-6] which also regulate the association-dissociation state of its subunits.[2-4] Increase in the enzyme activity has been observed in various metabolic states: namely, during enhanced fatty acid oxidation (hypolipidemic drug administration, starvation, and the early stage of diabetes),[5] during increased fatty acid synthesis (refeeding on fat-free diet with a high carbohydrate content with or without 1% thyroid powder),[5] and during the suppression of cholesterol synthesis (cholesterol feeding, oral administration of 3-hydroxy-3-methylglutaryl-CoA reductase inhibitor, and the early stage of diabetes).[7] From these results on the change in enzyme activity, we presumed that the role of this enzyme was to maintain equilibrium between the cytosolic acetyl-CoA concentration and the CoASH pool.

The enzyme is mainly localized in the liver cytosol (with about 5% as much in the kidney).[5] Thus, we consider that it should be useful clinically in diagnosis and therapy of various liver disorders. In this context, it seemed interesting to examine the change in its activity during liver carcinogenesis induced by feeding the hepatocarcinogen, 3′-Me-DAB[2] to normal Donryu albino rats. For comparison we used 3′-Me-DAB-resistant rats, which differ from the parent strain only in sensitivity to 3′-Me-DAB because they have been given diet containing 3′-Me-DAB for several generations.[8]

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[2] Abbreviations: 3′-Me-DAB, 3′-methyl-4-dimethylaminoazobenzene; γ-GTP, γ-glutamyltranspeptidase (EC 2.3.2.2).

MATERIALS AND METHODS

Materials CoASH, ATP, ADP, bovine serum albumin, phenylmethyl-sulfonyl fluoride and DL-dithiothreitol were obtained from Sigma Chemical Co. (St. Louis, MO). Acetyl-CoA was prepared from CoASH and acetic anhydride as reported by Simon and Shemin.[9] All other chemicals were of the highest grade available.[1-5]

Animals Normal male albino Donryu rats and 3′-Me-DAB-resistant male albino Donryu rats,[8] weighing 50 to 80 g, were maintained in an air-conditioned room at approximately 25°C with alternating 12 h periods of light (6:00–18:00) and dark.[5] Animals were given the experimental diet (MF; Oriental Yeast Co., Tokyo) containing 0.06% 3′-Me-DAB (Tokyo Kasei Kogyo Co., Tokyo) and water *ad libitum*. The feeding of 3′-Me-DAB continued throughout the entire experiment. Animals were sacrificed by decapitation under anesthesia with diethyl ether.

Preparation of cytosolic acetyl-CoA hydrolase The livers were perfused at 25°C with 30 ml of buffer consisting of 100 m*M* KH_2PO_4, 5 m*M* EDTA, 0.5m*M* phenylmethyl-sulfonyl fluoride, 0.02% NaN_3, and 2 m*M* DL-dithio-

threitol, adjusted to pH 7.4 with KOH at 25°C. Then the liver was homogenized in a glass homogenizer with a Teflon pestle at 25°C in two volumes of the same buffer. Enzyme activity was routinely assayed spectrophotometrically at 25°C by determining the rate of formation of CoASH from acetyl-CoA using 5,5′-dithiobis(2-nitrobenzoic acid).[1] Acetyl-CoA hydrolase activity in crude preparations was estimated by subtracting the activity with 2 m*M* ADP from that with 2 m*M* ATP.[5] The activity in homogenates was assayed in the presence of 0.025% (v/v) Triton X-100.[5]

Immunochemical techniques Antibodies against purified acetyl-CoA hydrolase from rat liver cytosol were raised in a rabbit as described previously.[5] The procedure used for enzyme-linked immunosorbent assay (ELISA) was essentially as described by Hsu *et al.*[10] and Ebisuno *et al.*[7] Double-diffusion tests were performed as described by Ouchterlony.[11]

Protein determination Protein was determined by the modification by Hartree[12] of the Lowry method with bovine serum albumin as a standard.

RESULTS

Effect of 3′-Me-DAB on cytosolic acetyl-CoA hydrolase activity Figure 1 shows the time course of changes in cytosolic acetyl-CoA hydrolase activity in rat liver during hepatocarcinogenesis induced by diet containing 3′-Me-DAB. In normal rats, the enzyme activity decreased to about one-third of the control level in week 2 of the experiment, and returned to the initial level in week 7, and then decreased progressively with the development of liver tumors. In week 20, tumor nodules occupied more than 90% of the liver. The activity in tumor nodules separated from liver in week 20 was about one-tenth the control level and the activity of apparently normal liver tissue adjacent to tumor nodules was about half the control level. In contrast, in 3′-Me-DAB-resistant rats,[8] the enzyme activity remained within the normal range (Fig. 1) and no tumors were detected macroscopically during the experiment with 3′-Me-DAB. It should be noted here that in control animals given a basal diet of the same composition but without 3′-Me-DAB for 20 weeks, there was no significant change in the activity of cytosolic acetyl-CoA hydrolase (data not shown).

Immunological comparison between the enzyme in tumor nodules and tissue adjacent to tumor nodules Next we immunologically compared the enzymes in tumor nodules and liver tissue adjacent to tumor nodules. Antiserum against rat liver acetyl-CoA hydrolase neutralized the enzyme activity in both regions, and, on plotting the inhibition rate against the antibody concentration, the two curves for these regions coincided with each other. Furthermore, an Ouchterlony double-diffusion test showed that the samples from normal rat liver, a tumor

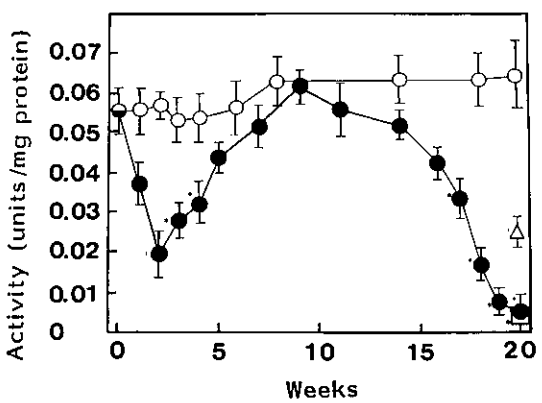

Fig. 1. Time course of change in enzyme activity during 3′-Me-DAB hepatocarcinogenesis. Normal rats (●) and 3′-Me-DAB-resistant rats (○) were given diet containing 0.06% 3′-Me-DAB *ad libitum* and were sacrificed at 11:00–11:30 a.m. in the indicated weeks. (△), activity of the liver tissue adjacent to the tumor nodule in week 20; (□), activity of tumor nodule in week 20. The closed circle in week 20 shows the activity of tumor nodule plus the adjacent tissue. Points and bars are means±SE for four animals. Different from the value for animals without 3′-Me-DAB treatment by Student's *t*-test, $^{*}P<0.05$.

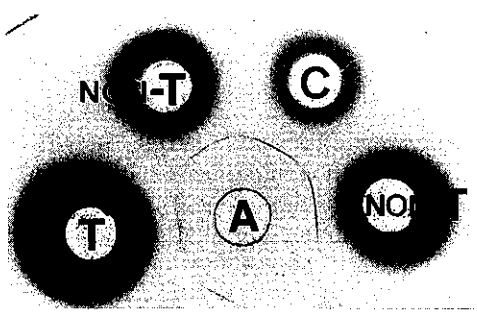

Fig. 2. Ouchterlony double-diffusion patterns. Agar gel (1%, w/v) contained 50 m*M* potassium phosphate buffer (pH 7.2). Samples with enzyme activity were concentrated by precipitation with 40% saturated ammonium sulfate by the method of Matsunaga *et al.*[5] The center well A contained 0.25 mg of IgG. Well C contained partially purified liver cytosol from rats without 3′-Me-DAB treatment (0.26 unit), well non-T contained partially purified samples from liver tissue adjacent to tumor nodules in the liver of rats given diet containing 0.06% 3′-Me-DAB for 20 weeks (0.21 unit), and well T contained partially purified samples from tumor nodules in the liver of rats given diet containing 0.06% 3′-Me-DAB for 20 weeks (0.15 unit). The photograph was taken after staining with Coomassie Brilliant Blue.

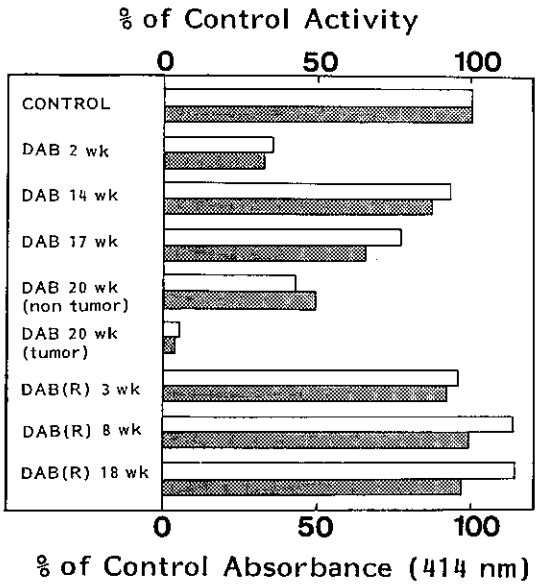

Fig. 3. Relation between enzyme activity and amount of enzyme protein determined by enzyme-linked immunosorbent assay (ELISA). Values are shown as percentages of the control activity (open columns) or of the absorbance of the control at 414 nm by ELISA (stippled columns). Control, untreated rats; DAB 2, 14, and 17 wk, rats given 0.06% 3′-Me-DAB diet for 2, 14, and 17 weeks, respectively; DAB 20 wk (non tumor), liver tissue adjacent to the tumor nodules in the liver of rats given 0.06% 3′-Me-DAB diet for 20 weeks; DAB 20 wk (tumor), tumor nodules in the liver of rats given 0.06% 3′-Me-DAB diet for 20 weeks; DAB (R) 3, 8, and 18 wk, 3′-Me-DAB resistant rats given 0.06% 3′-Me-DAB diet for 3, 8, and 18 weeks, respectively.

nodule and tissue adjacent to the tumor in week 20 each gave a single precipitin band, and that these bands fused without spur formation, although the band in the case of the tumor nodule was faint (Fig. 2). Thus these enzymes were apparently immunologically identical.

Correlation between enzyme activity and its protein concentration Next we examined whether the change in enzyme activity in animals given diet containing 3′-Me-DAB for 20 weeks was associated with a change in enzyme protein. As shown in Fig. 3, the amounts of enzyme protein in weeks 2, 14, and 17, and in tumor nodules and apparently normal liver tissue adjacent to the tumor nodules in week 20, determined by ELISA, correlated well with the enzyme activities. In resistant rats fed diet containing 3′-Me-DAB, the levels of enzyme protein were within the normal range in weeks 3, 8, and 18 and also correlated well with the enzyme activities. These results indicated that the change in enzyme activity was associated with the change in immunoreactive enzyme protein.

DISCUSSION

In the present study, we found that the change in the activity of cytosolic acetyl-CoA hydrolase during 3′-Me-DAB hepatocarcinogenesis was biphasic. The decreases in enzyme activity in the early and late stages of 3′-Me-DAB hepatocarcinogenesis seemed to be closely associated, but not coincident with the period of increase in activity of γ-GTP in the early stage (4–7 weeks) and development of hepatomas in the late stage,[13–16] although little is known about the mechanism of induction of hepatic γ-GTP during 3′-Me-DAB hepatocarcinogenesis or the change in cytosolic acetyl-CoA hydrolase activity during the carcinogenic process.

It has been reported that liver cells are injured by DAB in the first 3 weeks and that this injury is followed by regeneration of liver cells.[17, 18] Thus, decrease in acetyl-CoA hydrolase activity in the early stage may be due to injury of liver cells. This possibility is supported by our previous finding that the enzyme activity decreased to about half the control level in the period of rapid DNA synthesis in regenerating rat liver after 70% hepatectomy and then gradually returned to the normal level.[19] However, this decrease was not as great as that induced by 3′-Me-DAB in the early stage. Furthermore, in another experiment, we observed that the enzyme activity in rat liver after administration of necrotizing doses of carbon tetrachloride was almost constant for 5 days (unpublished results). These observations suggest that injury of liver cells by 3′-Me-DAB dose not result in a great decrease in enzyme activity. Thus, we consider, but have not proven, that the decrease in the enzyme activity in the early stage is partly associated with pathological changes in liver cells during or after injury. The second decrease in the late stages may be a reflection of the hepatoma growth, although it is not known whether this liver enzyme is mechanistically related to hepatocarcinogenesis.

It should be noted again that this enzyme is inducible by hypolipidemic peroxisome proliferators, such as clofibric acid and its analog,[5, 7] and that this drug induces hepatoma without induction of γ-GTP activity in putative neoplastic lesions.[20] Thus, the change in the activity of cytosolic acetyl-CoA hydrolase might be important in the process of hepatocarcinogenesis by the drug. An experiment to examine this is in progress.

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