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Responsiveness of Peroxisomal Enzymes of Rat Livers during 3′-Methyl-4-Dimethylaminoazobenzene Hepatocarcinogenesis to Clofibrate

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SUMMARY

The effect of clofibrate on the activities of peroxisomal enzymes, catalase, D-amino acid oxidase, urate oxidase, and L-α-hydroxy acid oxidase were investigated in the liver of rats treated with 3′-methyl-4-dimethylaminoazobenzene at intervals of 10, 13, 15, 17 and 19 weeks. Catalase activity was increased by treatment with clofibrate in all periods of carcinogenesis examined. The highest increased activity by clofibrate was observed at 13 weeks when all peroxisomal enzymes with the exception of D-amino acid oxidase were increased, suggesting that early degenerated hepatocytes matured during this period. At 19 weeks, the degree of increase in catalase activity in the response to clofibrate was the lowest, indicating that the lesions which did not respond to clofibrate had increased in number. Activity of D-amino acid oxidase was decreased by clofibrate throughout all periods of carcinogenesis. Both urate oxidase and L-α-hydroxy acid oxidase activities were not affected by the treatment with clofibrate during carcinogenesis.

INTRODUCTION

The enzymatic characteristics of cancer cells are seen in the decreases of enzyme activities related to cell differentiation, the increased activity of enzymes related to cell growth, the changes of isozymic patterns, and the altered control mechanisms of certain adaptive enzymes. Regarding the latter subject, relatively many studies are available in hepatomas, most of which reveal the loss or impairment of enzyme induction by dietary, substrate, or hormonal means (1-19). Such investigation has been extended to the preneoplastic livers, to determine whether alterations of enzyme regulation characteristic of hepatomas were already present in preneoplastic livers (20-50).

Recent experiments in our laboratory indicate that the catalase activity of Morris hepatoma 9618 A, highly differentiated transplantable hepatoma, are not induced by clofibrate, while the activity of newborn rat livers is

markedly increased by administration of clofibrate, concomitant with the increase in number of peroxisomes which contain the enzyme catalase (51). Moreover, even primary hepatomas induced by 3′-methyl-4-dimethylaminoazobenzen (3′-Me-DAB) did not respond to the drug, either by showing an elevation of catalase activity or proliferation of peroxisomes (52). The present report deals with the effect of clofibrate on the peroxisomal enzymes in the preneoplastic livers of rats treated with 3′-Me-DAB.

MATERIALS AND METHODS

Male Wistar rats, weighing 170–200 g, were fed on a standard diet containing 0.06 per cent of 3′-Me-DAB. The rats were sacrificed by decapitation on the 10th, 13th, 15th, 17th and 19th week after the commencement of the carcinogen ingestion. Administration of clofibrate was carried out by feeding the animals on a diet containing 0.06 per cent 3′-Me-DAB and 1 per cent clofibrate for 2 weeks before sacrifice. Preliminary experiments to determine the effect of clofibrate in the presence of the carcinogen were done as follows (Fig. 1); the rats were fed on 0.06 per cent 3′-Me-DAB for 8 weeks and then maintained on the following diets for 2 weeks: 1) a diet containing both the carcinogen and clofibrate, 2) a diet containing the carcinogen alone, 3) a diet containing clofibrate alone, and 4) a diet without either the carcinogen or clofibrate. As control, 20 week-old rats were fed either on a standard diet or the same diet containing clofibrate for 2 weeks.

The livers were excised quickly from decapitated animals, and aliquots

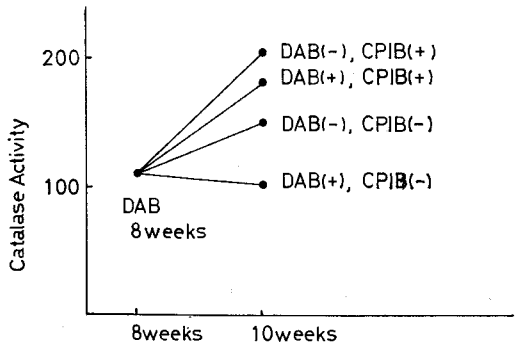

Fig. 1. The effect of clofibrate on catalase activity with or without 3′-Me-DAB.

The rats were fed on 3′-Me-DAB for 8 weeks and then fed on the following diets for 2 weeks; 1) a diet containing both the carcinogen and clofibrate [DAB(+), CPIB(+)], 2) a diet containing the carcinogen alone [DAB(+), CPIB(−)], 3) a diet containing clofibrate alone [DAB(−), CPIB(+)], and 4) a diet without either the carcinogen or clofibarte [DAB(−), CPIB(−)].

were homogenized in volumes (w/v) of cold distilled water in precooled Potter-Elvehjem homogenizer. These liver homogenates contained hyperplastic nodules at 13, 15, 17 and 19 weeks, but did not contain any tumors or portions of fibrosis. Activities of catalase, D-amino acid oxidase, and urate oxidase and L-α-hydroxy acid oxidase were measured by the method spreviously described (53), and were expressed as units/min/100 mg proteins, μg pyruvic acid produced/min/mg protein, μg uric acid oxidized/min/mg protein, and μg pyruvic acid produced/min/mg protein, respectively.

RESULTS

Fig. 1 showed the effect of clofibrate on catalase activity in the presence of the carcinogen. The rats fed on the carcinogen alone for an additional 2 weeks showed a slightly depressed activity when compared to that at 8 weeks. The activity of rats fed on both the carcinogen and clofibrate was about twice as much as that of rats fed on the carcinogen alone. An increase in activity was also seen in the rats fed on a diet without either the carcinogen or clofibrate, although the degree of increase was less than that of rats fed on a diet containing both. Although the highest activity was observed in the rats fed on a diet containing clofibrate alone, the increase was about 40 per cent when compared to the rats fed on a diet without the carcinogen or clofibrate, while the increase of the rats fed on both was 78 per cent when compared to the rats fed on the carcinogen alone. From these results, it may be said that the induction of catalase by clofibrate was not influenced by the presence of the carcinogen. Therefore, in the following experiments, the effect of clofibrate on peroxisomal enzymes was examined in the presence of the carcinogen.

Changes of catalase activity by clofibrate during carcinogenesis

Fig. 2 depicts the catalase activity of normal rat liver and the liver of 3′-Me-DAB carcinogenesis, with or without the treatment with clofibrate. The activity of normal rats was increased by the treatment with clofibrate for 2 weeks by about 50 per cent, compared with that of rats fed a diet without clofibrate. The activity of rat liver treated with 3′-Me-DAB for 10 weeks was markedly depressed. The value was 65 per cent of that of normal rat liver. However, by the treatment with clofibrate, the activity was markedly increased, showing 178 per cent of that of rats treated with the carcinogen alone for 10 weeks. The magnitude of inducibility was higher than that of normal rat liver, although the activity of rats treated with both agents was still lower than that of normal rats administered with clofibrate. At 13 weeks, the basal activity was still depressed, neverthless it was increased

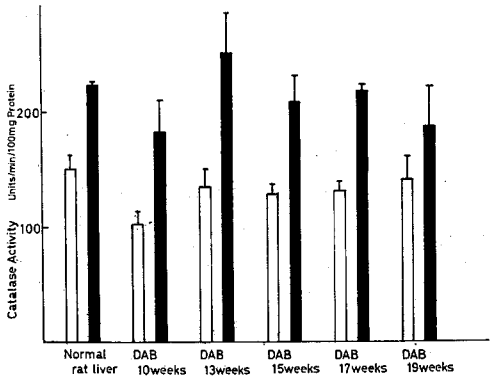

Fig. 2. The effect of clofibrate on catalase activity during carcinogenesis.

White bars show the activity of rats not treated with clofibrate. Black bars show the activity of rats treated with clofibrate. Vertical lines are standard deviations of the mean.

when compared with that of rats treated with the carcinogen for 10 weeks. The responsiveness of catalase by clofibrate was most remarkable at 13 weeks, and the activity was 186 per cent of that of rats treated with the carcinogen alone for 13 weeks. This value was higher than that of normal rats treated with clofibrate. The basal activities at 15, 17 and 19 weeks were similar to that of 13 weeks. The responsiveness to clofibrate was similar at 15 and 17 weeks, which was less than that at 10 and 13 weeks, but still was slightly higher than that of normal rats. The increase by clofibrate at 19 weeks was less than that of the normal rat.

Changes of D-amino acid oxidase activity by clofibrate during carcinogenesis

The activity of normal rat liver was depressed by the treatment with clofibrate, and was 20 per cent of that of non-treated rat liver (Fig. 3).

The activity of rats treated with the carcinogen alone was markedly depressed and then showed a gradual increase as carcinogenic course advanced. The treatment with clofibrate depressed the activity still further in all periods examined. A gradual increase in activity was not observed in the rats treated with clofibrate.

Changes of activity of urate oxidase by clofibrate during carcinogenesis

Fig. 4 showed the change of the activity of normal rat livers and that of livers during hepatocarcinogenesis with or without clofibrate administration. The activity of the normal rat liver treated with clofibrate were depressed. The activity during carcinogenesis without clofibrate was depressed at 10

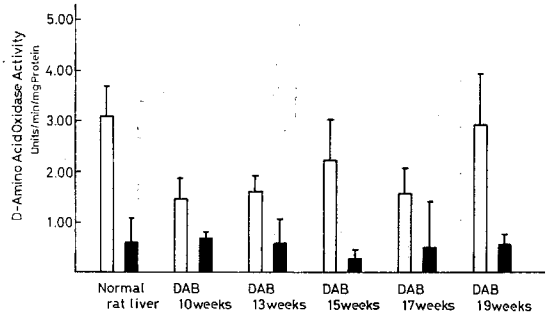

Fig. 3. The effect of clofibrate on D-amino acid oxidase activity during carcinogenesis.

White bars show the activity of rats not treated with clofibrate. Black bars show the activity of rats treated with clofibrate. Vertical lines are standard deviations of the mean.

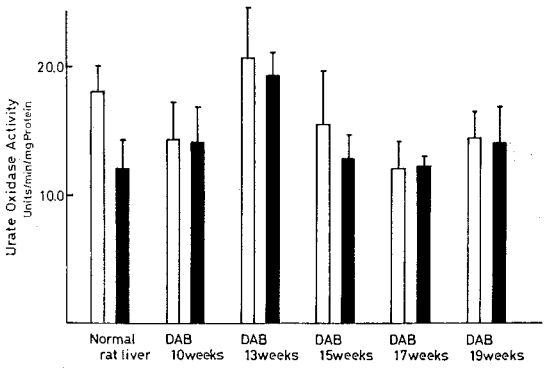

Fig. 4. The effect of clofibrate on urate oxidase activity during carcinogenesis.

White bars show the activity of rats not treated with clofibrate. Black bars show the activity of rats treated with clofibrate. Vertical lines are standard deviations of the mean.

weeks, retured to normal value at 13 weeks, and then again was depressed slightly at 15, 17 and 19 weeks. The treatment with clofibrate during carcinogenesis did not affect the change of the activity.

Changes of activity of L-α-hydroxy acid oxidase by clofibrate during carcinogenesis

The activity of L-α-hydroxy acid oxidase was markedly depressed at 10 weeks of carcinogen treatment (Fig. 5). It was, however, raised rapidly to normal values at 13 weeks. Thereafter, it was maintained at normal values. The changes of the activity during carcinogenesis were not affected by the administration of clofibrate.

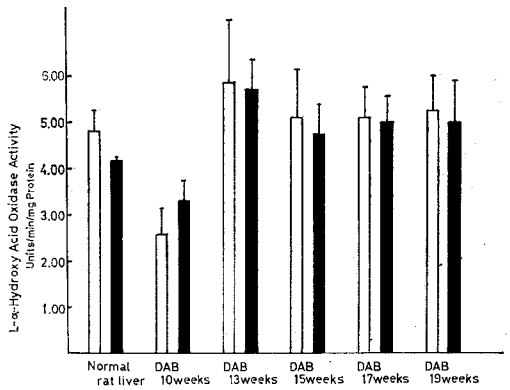

Fig. 5. The effect of clofibrate on L-α-hydroxy acid oxidase activity during carcinogenesis.

White bars show the activity of rats not treated with clofibrate. Black bars show the activity of rats treated with clofibrate. Vertical lines are standard deviations of the mean.

DISCUSSION

Data regarding regulation of enzymes during carcinogenesis have been accumlating, based on the assumption that the impaired enzyme regulation was already present in the preneoplastic cells. The majority of these studies showed impairement of enzyme regulation in the preneoplastic livers. For example, Fiala *et al* (28–30) reported a loss of the induction of tryptophan pyrrrolase by its substrate L-tryptophan. Hadjilov (31) reported that the induced increase of tryptophan pyrrolase were reduced in the 3′-Me–DAB group with both L-tryptophan and hydrocortisone as inductors. Kizer (39), Andersen (20), Kröger (40), Ruddon (46) and Poirier (41) also observed an impaired response of tryptophan pyrrolase by L-tryptophan (39), cortisone (20, 40, 46) and casein hydrolysate (41), in liver treated with 3′-Me–DAB, N-2-fluorenylacetamide and dimetylnitrosamine. The altered enzyme induction other than tryptophaan pyrrolase was reported in some enzymes of amino acid metabolism, such as tyrosine and ornitine aminotransferase, serine dehydratase and histidase (20, 41, 43), in the carbohydrate metabolism such as glucose-6-phosphatase, glucokinase, glucose-6-phosphate dehydrogenase, malic enzyme, citrate cleavage enzyme and phosphogluconic dehydrogenase (28–30, 43, 44), in the cholesterol metabolism (32–38, 47, 48), and in the cyclic AMP metabolism (21, 22, 24–27, 49).

To date, the regulation of catalase during carcinogenesis are not available in the literature except for reports from our laboratory. Catalase as well as D-amino acid oxidase, urate oxidase and L-α-hydroxy acid oxidase are

peroxisomal enzymes, and are regard as luxury enzymes of hepatocytes because none of peroxisomal enzymes are concerned with the cell growth. It is, thus, conceivable that cancerization of hepatocytes may entail profound alterations of peroxisomal enzymes. Our previous reports clearly showed the loss of the induction of catalase and proliferation of peroxisomes by clofibrate in Morris hepatoma 9618 A (51) and primary hepatomas induced by 3'-Me-DAB (52). Accordingly, we were hoping to use the loss of induction of catalase induced by clofibrate during hepatocarcinogenesis as a marker for the time periods in which critical changes were occuring within the cell which would ultimately lead to hepatoma.

The present study indicated that the loss of adaptive response of catalase to clofibrate characteristic of Morris hepatoma and primary hepatomas induced by 3'-Me-DAB was not manifested in preneoplastic livers treated with 3'-Me-DAB. Although the basal catalase activity was lower in the preneoplastic livers than in normal rat livers, the per cent induction of catalase activity was higher at 10, 13, 15 and 17 weeks than that of normal. The highest responsiveness was observed at 13 weeks when basal activities of catalase, urate oxidase, L-α-hydroxy acid oxidase were raised, suggesting that at this period hepatocytes undergo maturation. Many histological investigations have shown that the hepatocytes were degenerated at the early stages of 3'-Me-DAB carcinogensis, and then hyperplastic nodules arising from renewed hepatocytes appeared in the middle stages (54-64). The renewed hepatocytes or the hepatocytes undergiong maturation would appear to have a higher response to clofibrate than the matured hepatocytes. This consideration might be supported by the findings that more highly increased responses were observed in the newborn rats whose mothers were administered with clofibrate (51) and 5 day-old rats which were injected with clofibrate daily (unpublished data).

The responsiveness decreased gradually and fell to below normal response levels at 19 weeks. These changes of responsiveness may be due to the amount of hepatocytes which does not respond to clofibrate. Histochemical investigation of catalase revealed the hyperplastic foci, areas and nodules which were weak in catalase staining were already present at 10 weeks although limited in number. These lesions increased in number as time passed (52). When administered with clofibrate, many of these lesions showed a strong staining as well as renewed hepatocytes in the surrounding, but a few lesions showed a loss in inducibility. These non-inducible lesions increased in number or in size at 17 weeks and 19 weeks. We emphasized that these non-inducible leasions would serve as intimate precursors for the later hepatomas (52).

Thus, we must carefully evaluate the results obtained from liver homogenate, because the preneoplastic livers are a mixture of degenerative, regenerative and true neoplastic hepatocytes. Biochemical studies carried out by treating the preneoplastic livers as a homogenous mass of cells should be reevaluated on the basis of histochemical findings. Such a study was also done by Kitagawa and Pitot (65) on the induction of serine dehydratase and glucose-6-phosphatase by dietary stimuli or starvation in hyperplastic nodules of rat liver during diethylnitrosamine or 2-acethylaminofluorene feeding. They found an elevation of the level of serine dehydratase and its inducibility with time in the majority of the nodules. They emphasized that many hyperplastic nodules might have matured and become better organized within the liver tissues.

The effect of clofibrate on the activities of urate oxidase and L-α-hydroxy acid oxidase during carcinogenesis was not observed. Low basal activity of D-amino acid oxidase was further reduced by the treatment with clofibrate. A decreased activity of this enzyme was reported in normal rats treated with clofibrate by Azarnoff and Svoboda (66, 67), and Leighton *et al* (68). Kaneko *et al* (69), however, reported an increased activity by the treatment with clofibrate. We investigated the induction of D-amino acid oxidase activity under the same conditions that Kaneko *et al* used, but we found Kaneko's procedure was highly toxic to rats. From the results of Azarnoff and Svoboda, Leighton *et al*, and our present study, it would be likely that the treatment with clofibrate would decrease the activity of D-amino acid oxidase, although the mechanism is not clear.

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