

RESEARCH COMMUNICATION

Cessation of Long-term Alcohol Administration and Two-day Cycling of Exposure Respectively Promote and Inhibit Hepatocarcinogenesis in Rats

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Abstract

The effects of different patterns of alcohol administration on hepatocarcinogenesis induced by diethylnitrosamine (DEN) in male Wistar rats were assessed using a modified Ito's medium-term bioassay system. Carcinogenic potential was scored by comparing numbers and areas of glutathione S transferase placental form (GST-P)-positive foci. The activity of ornithine decarboxylase (ODC), the rate-limiting enzyme for polyamine synthesis, was also measured as a parameter of cell proliferation. Rats were given a single i.p. injection of DEN (200 mg/kg body weight), maintained on basal solid diet for two weeks, then divided into five groups: group A maintained on liquid diet in which 36% of total calories were provided by ethanol (diet A1) for 24 weeks; group B maintained on diet A1 for 12 weeks and subsequently on control diet (diet C) for 12 weeks; group C maintained on diet C for 24 weeks; group D maintained on a cycle of two days on diet A1 followed by two days on diet C; group E maintained on another liquid diet in which 18% of total calories were provided by ethanol for 24 weeks. The numbers and areas per cm² of GST-P positive foci in group B were highest and in group D were the lowest among the five groups. ODC activities in groups A and E were significantly lower than in group C, that for group B was intermediate. These results suggest that the intermittent intake of alcohol exerts preventive potential on hepatocellular lesion development, and that interruption of long-term alcohol administration enhances hepatocarcinogenesis.

Key words: alcohol - hepatocarcinogenesis - glutathione S transferase placental form - ornithine decarboxylase

Asian Pacific J Cancer Prev, 1, 325-328

Introduction

Recently the prevalence of hepatocellular carcinoma and associated mortality have increased in Japan (Takada A et al., 1994). So that the preventive measures are urgently required. It is clear that most non-viral cases of hepatocellular carcinoma arise with a background of alcoholic liver cirrhosis and that the incidence of hepatocellular carcinoma is higher in heavy drinkers than in non-drinkers even in those with viral liver cirrhosis (Tuyns, 1979; Lieber et al., 1979; Yu et

al., 1983; Tsukuma et al., 1990; Tsutsumi et al., 1996). Thus alcohol drinking is positively linked with carcinogenesis in the liver. On the other hand, there has been a report of acceleration due to abstinence from alcohol drinking (Nishiuchi et al., 1990).

In the present study, using a modified Ito's medium-term bioassay system for hepatocarcinogenesis in rats (Ito et al., 1988), we studied the relationship between drinking pattern and numbers and areas of cell foci positive for the glutathione S transferase placental form (GST-P), early indicators for

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hepatocarcinogenesis. Further, ornithine decarboxylase (ODC) activity was measured as a marker for cell proliferation.

Materials and Methods

Chemicals

Diethylnitrosamine (DEN) was obtained from Tokyo Chemical Industry Co., Ltd., Tokyo, and [^{14}C]Ornithine from Moravек Biochemicals, Inc., Brea, CA. USA.

Animals and Treatment

The protocol of the experiment was approved by the Animal Research Committee, Osaka City University, and care of the animals was according to the standards of this institution (Guide for Animal Experimentation, Osaka City University). Seventy five male Wistar rats (Clea Japan, Inc., Tokyo) purchased at 6 weeks of age were housed in an air-conditioned room with a 12-h light, 12-h dark cycle, and given a pellet diet (Oriental Yeast Co., Tokyo) and tap water ad libitum.

The experimental design is shown in Figure 1. Seventy five rats were given a single i.p. injection of DEN (200 mg/kg body weight) and maintained on pellet diets for two weeks. They were then divided into five groups: group A maintained on liquid diet in which 5% (W/V) ethanol was included (5% Al diet) for 24 weeks; group B maintained on 5% Al diet for 12 weeks and subsequently on control liquid diet (C diet) for 12 weeks; group C maintained on C diet for 24 weeks; group D maintained on a cycle of two days with 5% Al diet and another two days with C diet for 24 weeks, and group E maintained on another liquid diet in which 2.5% (W/V) ethanol was included (2.5% Al diet) for 24 weeks. All these liquid diets were purchased from Oriental Yeast Co., Tokyo. All rats were subjected to partial hepatectomy 3 weeks after DEN injection using the method of Higgins and Anderson (1931).

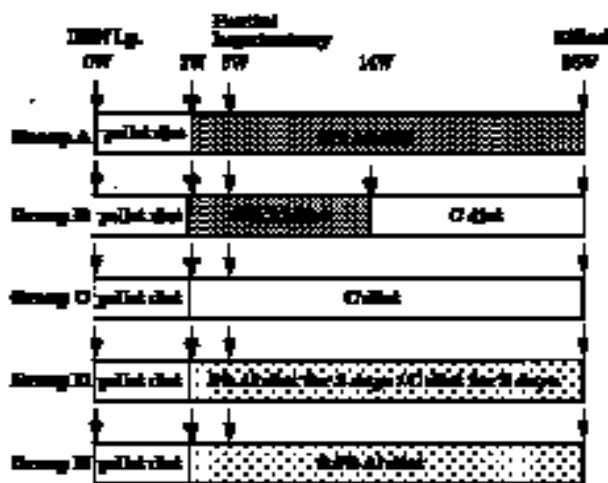


Figure 1. Schematic Illustration of the Experimental Protocol

At autopsy (26 weeks after DEN injection), livers were excised and slices 2-3 mm thick were cut with a razor blade. These were fixed in ice cold acetone and routinely processed for immunohistochemical examination of putative preneoplastic lesions, GST-P-positive foci. For measurement of ODC activity, samples of rat livers were frozen in liquid nitrogen immediately after resection.

Immunohistochemical Staining of GST-P

The avidin-biotin-peroxidase complex (ABC) method described by Hsu et al. (1981) was used to demonstrate GST-P-positive foci. After deparaffinization, liver sections were treated sequentially with normal goat serum, anti-rabbit GST-P antibody, biotin-labeled goat anti-rabbit IgG and ABC (Elite Vectastain, Vector Labs, Burlingame, CA). The sites of peroxidase binding were demonstrated by the diaminobenzidine method. Sections were then counterstained with hematoxylin for microscopic examination. The numbers and the areas of GST-P-positive foci >0.1 mm in diameter and the total areas of the liver sections examined were measured using a color video image processor (Olympus-IKEGAMI VIP-21CH, Tokyo).

Measurement of ODC Activity

ODC activities were measured by the method of Otani et al. (1985). Frozen samples of rat liver were suspended in 0.5 ml of 50 mM Tris (pH 7.5) containing 0.25 M sucrose and disrupted by homogenization for a few minutes. The homogenized suspensions were centrifuged at 100,000g for 30 min, and the supernatants assayed for ODC activities by measurement of the amount of radioactive CO_2 produced from [^{14}C] ornithine.

Statistical Analysis

Data were expressed as means \pm SD, and statistical analysis was performed using the Student's t test.

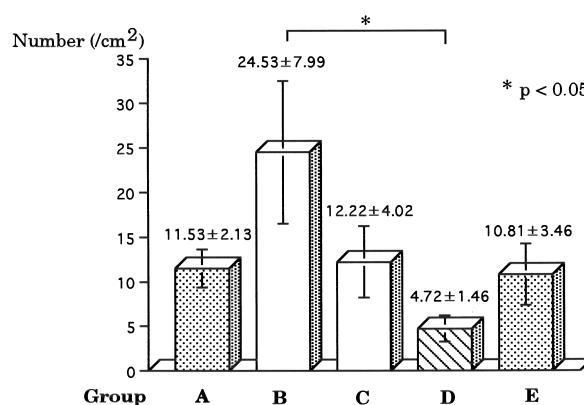


Figure 2. Numbers of Glutathione S transferase-P (GST)-positive Foci per cm². Groups A~E see Fig.1 for further details. Values are means \pm SD.

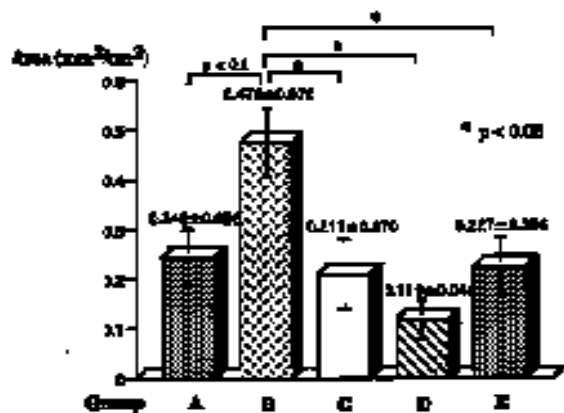


Figure 3. Areas of Glutathione S transferase-P (GST)-positive Foci per cm²

Groups A~E see Fig.1 for further details. Values are means±SD.

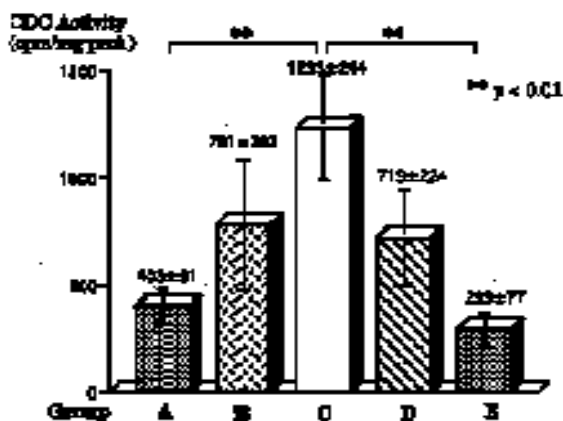


Figure 4. Hepatic ornithine decarboxylase (ODC) activity at week 26.

Groups A~E see Fig.1 for further details. Values are means±SD.

Results

There were no significant differences in final body weights and relative liver weights among the five groups.

On immunohistochemical staining, GST-P-positive foci were stained dark brown. With hematoxylin staining, the regions in serial sections had no obvious structural changes, in terms of nuclear density or atypia, and differentiation from background parenchyma was difficult. Fibrosis was not apparent in any group, but fatty change was remarkable in the groups (A and E) administered alcohol continuously.

The number of GST-P-positive foci per unit area of liver was highest in Group B and lowest in Group D. Significant difference was found between these two ($p < 0.05$), but not the other groups (Fig. 2).

The foci area was also largest in group B and smallest in group D. Significant group differences were observed between group B and C, D, E ($p < 0.05$) (Fig. 3).

Measurement of ODC activity in the livers demonstrated a significantly higher level in group C than in groups A and E ($p < 0.01$). That for group B also showed a tendency for increase as compared to group A.

Discussion

The process of chemical carcinogenesis can be divided into initiation, promotion and progression phases. In Ito's medium-term model of hepatocarcinogenesis (Ito et al., 1988), a carcinogenic substance (DEN) is administered once for initiation; from 2 weeks after, a test substance is then administered for 6 weeks; at the 3rd week, partial hepatectomy is performed as a stimulation to proliferation; and after 8 weeks, the animals are killed and carcinogenic activity is assessed. To determine the effect of alcohol on hepatocarcinogenesis, longer-term exposure was considered necessary and therefore we prolonged the period to 24 weeks. As a result, alternate two days on and off the alcohol supplement was found to cause significant inhibition of lesion development whereas promotion was evident in group B.

The numbers and areas of GST-P-positive foci and hepatic ODC activities did not show any parallelism, possibly because the enzyme assay was conducted on whole tissue, and it was impossible to measure precancerous foci separately, now intend to determine relative levels of cell proliferation in foci as compared to background parenchyma by PCNA immunohistochemistry.

Substances are considered to generally exert carcinogenic effects after metabolism in the organism. This takes place mainly in microsomes in the liver, and one of the contributing enzymes, cytochrome P450, is induced by long-term alcohol administration (Lieber et al., 1978). However, this is not immediate and increase is reported to start from the 3rd day of administration and reach a plateau at the 10th day with chronic alcohol administration in rats. In this study, it is possible that cytochrome P450 was not induced appreciably in the intermittent administration group (group D), in which alcohol administration was given for two days and then suspended for another two days. These findings suggest that the intermittent intake of alcohol was preventive to hepatocellular lesion development.

There is no doubt that alcohol intake affects hepatocarcinogenesis. The fact that there was no difference in the number or area of GST-P-positive foci between groups A and C might be related to the fact that chronic alcohol administration can suppress liver regeneration (Tanaka et al., 1996; Kurai, 1993). Since partial hepatectomy was included in the experimental design, alcohol could have affected cell kinetics, although this also would have been expected for group B.

When the hepatic ODC activities were measured, no statistically significant difference was seen between group A and B, probably because of the 12 week period after cessation of alcohol.

The present model using rats indicates that withdrawal from

long-term alcohol intake may promote liver lesion development. Clinically, hepatocarcinogenesis may be accelerated by abstinence from alcohol drinking in patients with liver cirrhosis (Nishiuchi et al., 1990). Interestingly, it has also been reported that α -fetoprotein increases temporarily when patients consuming alcohol abstain from drinking. Further studies are needed as to the changes in foci with observations over a longer period of time after interruption of alcohol and differences in hepatic function and morphology in comparison with the continuous drinking state.

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Personal Profile: Takashi Tanaka

Dr. Takashi Tanaka graduated from Osaka City University Medical School in 1984 and received his D.M.Sc. degree in 1990. From 1984 to 1986, he was a resident, 3rd Department of Internal Medicine, Osaka City University Hospital, and in 1990 he became Research Associate, Department of Public Health, Osaka City University Medical School. Since 1995 he has been Lecturer with the same title.

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