Correlation between Time of Partial Hepatectomy after a Single Treatment with Diethylnitrosamine and Induction of Adenosinetriphosphatase-deficient Islands in Rat Liver

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ABSTRACT

Full-grown Sprague-Dawley rats were given a single i.p. injection of diethylnitrosamine (80 mg/kg) and subjected to partial hepatectomy at various times from 4 hr to 7 days later to induce semisynchronized liver cell proliferation. Then, they were maintained on basal diet containing 0.05% phenobarbital, which is known to promote hepatocarcinogenesis, for 16 weeks. By this method, significant numbers of adenosinetriphosphatase-deficient islands were induced in the liver. These islands are considered to be formed by immediate progeny of "initiated cells" or cell precursors in hepatocarcinogenesis, and they can be used as a marker of carcinogenic activity. Results showed that the number of enzyme-altered islands induced was inversely proportional to the time between carcinogen treatment and subsequent partial hepatectomy. The incidence of enzyme-altered islands was greatest when the two treatments were separated by 4 hr and decreased when they were separated by 7 days. These data suggest that carcinogen-induced DNA damage, if not repaired before cell proliferation, is intimately related to the initiation-fixation process of carcinogenesis.

INTRODUCTION

It is generally accepted that cell proliferation plays an important role in initiation of chemical carcinogenesis (2, 9, 24, 29). The liver undergoing restorative hyperplasia after PH is a good system for investigations on this phenomenon. Craddock (3, 4, 7) reported that a single treatment of adult rats with various carcinogens induced liver cell cancer in high incidence when given during the period of increased DNA synthesis following PH but very rarely if at all when given in the prereplicative period. She suggested that the variation in susceptibility during the cell cycle is due to a change in the physical state or functional activity of the genetic material or of some other cell component (5). She further postulated that DNA damage induced by a carcinogen given early in the prereplicative period might be repaired before the cell enters S phase (6, 7). However, it was shown that liver DNA synthesis begins about 16 hr after PH and reaches a plateau in 24 hr (7). In addition, metabolism of carcinogen takes some time, especially in hepatectomized animals. Therefore, it does not seem possible to conclude from the above results that a key event in the cell cycle dependence of carcinogenesis is repair of damaged DNA.

There have been no previous reports in which PH is performed at various times after a single treatment of carcinogen and the difference in carcinogenic activity is compared in a time sequence basis.

In this work, adult rats were given a single i.p. injection of DENA, subjected to PH at various times after treatment with DENA, and maintained on a diet containing phenobarbital. This paper shows that significant numbers of enzyme-altered islands could be induced in the liver by this new method. Studies on the relation of the time between treatment with DENA and PH and the numbers of enzyme-altered islands are reported, and the relation between repair of carcinogen-induced DNA damage and carcinogenesis is discussed.

Recently, extensive detailed studies on the alkylation of DNA by nitrosamines and removal of O6-alkylguanine from DNA have been conducted with rat liver (11, 18, 21, 27). Thus, in combination with biochemical data obtained from these studies, our morphological approach to pulse carcinogenesis should be of value for the understanding of the relation between repair of carcinogen-induced DNA damage and the initiation-fixation process (2).

MATERIALS AND METHODS

Male Sprague-Dawley rats (8 weeks old) weighing 250 g were obtained from Charles River Japan, Inc. (Atsugi-shi, Japan). The animals were housed in plastic cages and fed on CE-2 diet (CLEA Japan Inc., Tokyo, Japan). For this study, full-grown animals were obtained in this laboratory as follows. The animals were weighed weekly, and the average body weight curve was recorded. Animals 21 weeks old were used for experiments, when they weighed an average of 450 g and their weight curve was gradually reaching a plateau. They were divided into 6 groups (A to F) of 5 to 6 animals each. The protocol used for induction of enzyme-altered islands is illustrated in Chart 1. The animals in Groups A to E received a single i.p. injection of DENA (80 mg/kg; Tokyo Kasei, Co., Tokyo, Japan) dissolved in 0.9% NaCl solution (20 mg/ml) between 10 a.m. and 11:30 a.m. PH of animals under ether anesthesia was performed as described by Higgins and Anderson (12) at various times after a single injection of DENA between 10 a.m. and 11:30 a.m. except for Group A. Partial hepatectomy was performed 4 hr later in Group A, 24 hr later in Group B, 3 days later in Group C, and 7 days later in Group D. Sham operation without partial hepatectomy was carried out in group E 24 hr after DENA treatment. To detect the effect of PH and 0.9% NaCl solution injection, as one representative control group, the animals in Group F were subjected to PH 24...
The largest number of enzyme-altered islands was observed in Group A, in which PH was performed 4 hr after injection of DENA. Considerable numbers of enzyme-altered islands were also found in Groups B and C, in which PH was performed 24 hr and 3 days, respectively, after DENA treatment; but only a few islands developed in Group D, in which partial hepatectomy was performed 7 days after DENA injection. Very few enzyme-altered islands developed in Group E, which received DENA only. One can assume that PH was carried out at infinite time in this group when one compares the numbers of islands against the time (Table 1). No enzyme-altered islands developed in Control Group F, in which only partial hepatectomy was performed without previous DENA injection. In general, the number of enzyme-altered islands induced tended to be inversely proportional to the time between treatment with DENA and PH. The incidence of enzyme-altered islands was greatest when the 2 treatments were separated by 4 hr and decreased when they were separated by 7 days.

DISCUSSION

In the present study, significant numbers of enzyme-altered islands were induced in the liver by the method described above. Since these islands are considered to be formed by immediate progeny of “initiated cells” and cell precursors in hepatocarcinogenesis, they can be used as potential indicators for putative preneoplastic hepatocytes (1, 8, 14, 17, 20, 25, 26, 30, 31). The promotive effect of phenobarbital on enzyme-altered islands and liver tumors has been investigated (14, 16, 17, 22, 23). The data indicate that phenobarbital would be a useful tool in pulse carcinogenesis study as an “amplifier” in picking up weak carcinogenic activity. We used full-grown animals, in which liver cells are considered to be in minimum replicative activity. The carcinogen-induced cell proliferation was probably negligible compared with the semisynchronized cell proliferation induced by PH since carcinogen treatment alone was found to induce very few enzyme-altered islands in rat liver. We have not yet determined whether a single injection of DENA, followed after various times by PH and administration of diet containing phenobarbital as in the present experiments, induces liver cancer. However, there is considerable circumstantial evidence in support of this possibility (8, 17, 23).

Resting cell phase (intervening cell cycles), calculated as the time between treatment with DENA and PH plus the time lag before entering the S phase after PH (estimated as 24 hr), is also introduced here for the sake of convenience (Table 1). The results demonstrate that the number of enzyme-altered islands was inversely proportional to the time between carcinogen treatment and PH, and to resting cell phase. These data suggest that some cellular processes that attenuate the carcinogenic effect of DENA might proceed in proportion to the duration of the resting cell phase. One could assume that, by prolonging the interval between the administration of the carcinogen and PH, the intervening cell cycles tend to repair the probable DNA-damaging effect of the substance administered.

Previous work suggested that, among the alkylating products, O6-alkylguanine was essential for mutagenic and carcinogenic effect (19, 20). Extensive studies on the formation of O6-alkylguanine and its removal from DNA have been conducted. Goth and Rajewsky (11) reported that the elimination of O6-ethylguanine produced by N-ethyl-N-nitrosourea from
Carcinogen-induced DNA damage is proportional to the length of the resting phase. (b) Untimely induced cell proliferation facilitates the initiation-fixation process. Carcinogen-induced DNA damage, if repaired efficiently during the resting phase before cell proliferation, may be innocuous.

It is relevant to note that carcinogen-induced transformation frequency in vitro was reduced when cultures were treated with hydroxyurea (13) or maintained in a density-dependent nongrowing state (15) for some time after carcinogen treatment.

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REFERENCES

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