Dose Response of Hepatocyte Replication in Rats following Continuous Exposure to Diethylnitrosamine

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ABSTRACT

Chronic exposure to the hepatocellular carcinogen diethylnitrosamine (DEN) causes a dose-dependent accumulation of the promutagenic DNA adduct O⁴-etethyldeoxythymidine in hepatocytes and increases in the number of initiated hepatocytes as indicated by γ-glutamyl transpeptidase positive foci. Initiation is thought to be dependent on the quantity of promutagenic DNA adducts, their efficiency for causing base pair mismatch, and the extent of replication in the target tissue. If the extent of replication is also dose dependent, then this dependence could alter the number of promutagenic DNA adducts that mispair prior to repair and enhance the clonal expansion of initiated cells.

We have examined the effect of DEN on hepatocellular proliferation over a wide range of doses. Six-week-old male F-344 rats were exposed to drinking water containing 0.4, 1, 4, 10, 40, or 100 ppm DEN for 1, 4, or 10 weeks. Following exposures to DEN, rats were injected i.p. with [³H]thymidine, sections from the left, right median and anterior right lobes of the liver were processed for autoradiography and the labeling index of hepatocytes determined. A progressive increase in hepatocyte replication was induced by exposure to 40 and 100 ppm DEN. This was especially marked in the left lobe where 40 and 100 ppm DEN induced increases of 800 and 1500%, respectively, over controls after 10 weeks of exposure. Exposure to 4 and 10 ppm DEN resulted in a 300 to 400% increase in hepatocyte replication in all lobes, whereas 1 and 0.4 ppm DEN did not significantly increase cell proliferation compared to unexposed controls.

INTRODUCTION

Cell replication is an essential step in the carcinogenic process (1-3). It is necessary for converting promutagenic lesions in DNA to mutations, for clonally expanding the number of initiated cells (4), and for fixing additional mutagenic events in initiated cells (5). While there have been numerous studies on dose and time relationships for chemical carcinogens and cell proliferation, there have been few reports examining dose responses covering orders of magnitude and using extended periods of carcinogen exposure (6). If carcinogen-induced cell proliferation is elevated with increasing dose, the quantitative relationship between exposure and carcinogenesis would be expected to vary nonlinearly (7).

The alkylating agent DEN² is a well studied liver carcinogen which, when administered continuously to rats, produces a well-characterized dose-response relationship between concentration in the drinking water and tumor incidence (8-10). Peto et al. (8) demonstrated that the induction of liver tumors at low concentrations of DEN in the drinking water could not be predicted by simple extrapolation downwards of the dose-response relationships found at higher DEN concentrations. Although studies have demonstrated an increase in labeling indices after continuous administration of DEN, these studies used only single high concentrations of DEN (11, 12).

Previous work in this laboratory demonstrated that O⁴EtdT, a promutagenic adduct, accumulates in the hepatocytes of rats exposed to DEN in a dose-dependent manner (13). O⁴EtdT reached apparent steady state concentrations after 7-week exposure to DEN and remained unchanged after 10-week exposure. The DNA adduct concentrations were linearly related to DEN concentrations in the animals drinking water containing from 0.4 to 40 ppm DEN, but was less than linear at 100 ppm. The initiation of hepatocytes, as assessed by growth-selected GGT⁺ focus induction, across the left, right median and anterior right lobes of the liver during continuous exposure to similar concentrations of DEN also occurred in a dose- and time-dependent manner (14). These studies also demonstrated that accumulation of O⁴EtdT and induction of foci correlated with hepatocellular tumor induction in a lobe-specific manner (15).

Since the initiating and promoting capability of a chemical carcinogen can be influenced by the rate of cell proliferation, studies which determine the effects of continuous administration of different concentrations of a chemical carcinogen on cell replication are needed in order to better establish the quantitative relationships between DNA adducts, cell proliferation, and tumor induction. Therefore, we have examined differences in labeling indices of hepatocytes in the left, right median and anterior right lobes of rat livers during 10 weeks of continuous administration of drinking water containing DEN in concentrations ranging from 0.4 to 100 ppm.

MATERIALS AND METHODS

Male Fischer-344 rats (4 weeks old, 90-120 g) from Charles River Breeding Laboratories (Kingston, NY) were housed in polypropylene shoe box type cages on hardwood chip bedding. The animals were maintained on a 12-h light-dark cycle at a temperature of 22 ± 1°C and 55 ± 5% humidity in a room with a filtered air supply. Following a 2-week quarantine, rats were randomized into groups of seven (three or four per cage). Animals received NIH-07 rat chow and distilled deionized water containing 0, 0.4, 1, 4, 10, 40, or 100 ppm DEN (Eastman Kodak, Rochester, NY) for 1, 4, or 10 weeks ad libitum. DEN consumption was monitored at regular intervals throughout the experiment. At the end of the exposure period, animals were injected i.p. with [³H]thymidine (NEN, Boston, MA, specific activity, 6.7 Ci/mmol) at a concentration of 1 μCi/g body weight. Two h after injection, rats were killed by CO₂ asphyxiation, livers were weighed and sections were taken from the left, right median and anterior right lobes of the liver. Liver sections were fixed in formalin, embedded in paraffin, sectioned at 5 μm and fixed to glass slides. The slides were then dipped in NBT2 emulsion (Eastman Kodak, Rochester, NY), exposed for 3 weeks, developed, and counterstained with hematoxylin & eosin. The percentage of labeled hepatocytes in each section was determined by dividing the number of labeled hepatocytes by the total number of hepatocytes in 10–20 random fields. A minimum of 1000 hepatocytes per liver lobe were counted to determine percentage labelling. A Neuman-Keuls multicomparison test (P < 0.05) was used to analyze differences between dose groups within each time point, and Student's t test was used to analyze differences within dose groups.

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2 The abbreviations used are: DEN, diethylnitrosamine; GGT⁺, γ-glutamyl transpeptidase positive; HCCA, hepatocellular carcinoma; O⁴EtdT, O⁴-etethyldeoxythymidine.

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RESULTS

The rate of water consumption per kilogram of body weight and thus DEN consumption, decreased with time for all dose groups (Fig. 1). Throughout the experiment the 100 ppm dose groups consumed proportionately less DEN than the lower dose groups. The 40 ppm dose groups also consumed proportionately less DEN during the final 3–4 weeks. Consumption curves were generated using the function $\text{DEN}(t) = ae^{-kt} + b$, where $t$ is time in days, $a$ is the difference between the initial and asymptotic rates of DEN consumption during long periods of exposure, and $k$ is the rate constant governing the decrease in the rate of DEN intake.

A dose-dependent increase in the labeling indices was evident after 1 week of administration in all three lobes examined (Table 1). In the 100-ppm group, labeling indices in the left and right median lobes continued to increase throughout the experiment. A progressive increase in labeling index was seen only in the left lobe of rats exposed to 40 ppm. Labeling indices in lower dose groups decreased from week 1 to week 4, after which they remained constant until termination of the experiment. Labeling indices in the 0.4- and 1-ppm dose groups were not significantly different from controls. The 4-ppm dose group showed significant increases compared to control, which varied with dose range that was previously used in tumor (8–10), DNA adduct (11), and hepatocyte initiation studies (14). Results indicate that rates of replication can be heterogeneous between lobes and are time- and concentration-dependent. Since relative liver weights did not increase during treatment, except for those of the exposures to 100 ppm DEN, for 10 weeks, it is assumed that the increase in the rate of replication relative to time and concentration was due to compensatory hyperplasia that occurs in response to the cytolethal effects of DEN (16–18). The increase in liver weight in rats exposed to 100 ppm DEN for 10 weeks may also represent proliferation associated with nodular hyperplasia and early neoplasia since this is a fully carcinogenic exposure regimen (18). Histopathological observations were comparable to previous studies employing necrogenic and subnecrogenic doses of DEN (19, 20).

DISCUSSION

Hepatocellular replication, as indicated by incorporation of tritiated thymidine during S phase, was examined over a DEN dose range that was previously used in tumor (8–10), DNA adduct (11), and hepatocyte initiation studies (14). Results indicate that rates of replication can be heterogeneous between lobes and are time- and concentration-dependent. Since relative liver weights did not increase during treatment, except for those of the exposures to 100 ppm DEN, for 10 weeks, it is assumed that the increase in the rate of replication relative to time and concentration was due to compensatory hyperplasia that occurs in response to the cytolethal effects of DEN (16–18). The increase in liver weight in rats exposed to 100 ppm DEN for 10 weeks may also represent proliferation associated with nodular hyperplasia and early neoplasia since this is a fully carcinogenic exposure regimen (18). Histopathological observations were comparable to previous studies employing necrogenic and subnecrogenic doses of DEN (19, 20).

Rates of hepatocyte replication were time- and concentration-dependent at the higher doses, reached a plateau in the intermediate doses and were not significantly greater than controls in the lower doses. Results suggest that a dose of 4 ppm is very effective.
observed interlobular differences in HCCA formation. Greater incidences of HCCA have been observed in the left lobe, followed by the right median and anterior right lobes during chronic administration of DEN (15). The greater labeling indices in the left lobe correlate with the preferential distribution to and/or metabolism of DEN in the left lobe, followed by the right median and anterior right lobes (14). Studies have also shown that OEtEtdT accumulation and GGT+ focus induction, relative to number and volume, show a similar lobe distribution (14).

This study indicates that DEN-induced changes in rates of replication are nonlinear with respect to administered dose and time, and correlate with incidence of hepatocellular carcinomas observed in previous studies. This nonlinearity has implications for the extrapolation of risks to low-dose exposure for environmental agents. Initiation, promotion, and progression are major factors involved in the carcinogenic process and cell replication is an essential determinant in each of these factors. We have demonstrated that the extent of cell proliferation can vary greatly between high and low exposures and over time. Risk assessments which use high- to low-dose extrapolations to predict tumor incidence depend on an agent’s effect on rates of cell initiation and/or cell proliferation (7). Therefore, extrapolation from high to low exposure should include information on how agents effect rates of replication in order to make predictions of tumor incidence more accurate.

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