INDUCTION BY AN HEPATIC CARCINOGEN, 1-NITROSO-5,6-DIHYDROURACIL, OF SINGLE AND DOUBLE STRAND BREAKS OF LIVER DNA WITH RAPID REPAIR.[1]

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Received June 8,1973

SUMMARY: The nitrosoureas derived from 3 naturally occurring ureides were administered to rats and the velocity sedimentation of hepatic DNA in alkaline and neutral sucrose gradients determined. The potent hepatocarcinogen 1-nitroso-5,6-dihydrouracil induced apparent double strand as well as single strand breaks in liver DNA within 30 minutes. This damage seemed to be repaired within 4 hours. In contrast, 1-nitrosohydantoin and δ-nitroso-L-citrulline, neither of which are known hepatocarcinogens, did not modify the velocity sedimentation of hepatic DNA.

DNA is one of the macromolecules with which chemical carcinogens interact in target cells (1-3). Where studied, the active electrophilic forms of carcinogens react with the bases of DNA to form a variety of covalent products, including N-7 derivative of guanine (1,2).

Recently, a known method for bacteria (4) was modified (5) for analyzing the size distribution of DNA from intact liver using sucrose gradient centrifugation. The effects of several carcinogens on DNA using this approach was reported (6). Carcinogens such as dimethylnitrosamine and 2-acetylaminofluorene induce single strand but not double strand breaks within a few hours of their administration,

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[1]Supported by research grants from the National Institutes of Health (CA-10439, CA-12218, AM-14882), the American Cancer Society (BC-7N and BC-39), and by a contract (PH-43-68-959) from the National Cancer Institute to the Eppley Institute.

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followed by slow repair of the DNA which is still incomplete at 14 days. Of the many different chemicals studied, carcinogens were among the most active found to induce such damage to liver DNA. A correlation was found between carcinogenicity for liver and delayed DNA repair.

As part of a study of the possible generation of carcinogenic nitrosamines and nitrosamides in vivo from amines, ureides and nitrite (7), a variety of nitrosoureas derived from naturally occurring ureides were prepared. These included 1-nitroso-5,6-dihydrouracil (NO-DHU), which induced a 96% incidence of liver carcinomas in rats after 30 weeks of treatment with 450 mg/liter drinking water (8). A similar dose of the analogous 5-membered-ring compound 1-nitrosohydantoin has not induced liver tumors in rats after a period of exposure of 52 weeks and a further period of observation of 15 weeks[4]. Both compounds induced about a 20% incidence of kidney tumors. The open-chain nitrosourea δ-nitroso-L-citrulline is relatively non-toxic with an LD_{50} of about 2,000 mg/kg in mice (10) and has not been tested for carcinogenicity. Unlike nitrosamines, nitrosoureas probably do not require enzymic transformation before yielding an active electrophilic agent. We report here that administration to rats of a single dose of NO-DHU induced single and double strand breaks in liver DNA, as measured by alkaline and neutral sucrose gradient centrifugations (a lower sedimentation on alkaline sucrose is attributed to single or double strand breaks, and a lowered sedimentation on neutral sucrose to double strand breaks). Unlike observations with several other carcinogens (5,6), this damage was found to be rapidly repaired. Possible implications of these findings for carcinogenesis are discussed.

MATERIALS AND METHODS:

NO-DHU, m.p. 141° C, (8,9) and δ-nitroso-L-citrulline, m.p. 144° C, (10) were synthesized as previously described. 1-Nitrosohydantoin was synthesized as for NO-DHU; m.p. 114°; UV absorption (ethanol) 423 (ε, 111), 404 (122), 388

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[4]S.S. Mirvish and H. Garcia, unpublished results.

(79), and 241 (6500) nm; Elemental analysis correct within 0.1%. The products were stored under N_2 at -15° C and handled in a hood.

Male Wistar rats weighing about 100 g were used. Details of the DNA analysis in alkaline sucrose gradients have been described (5,6). In brief, rat liver DNA was radioactively labeled in vivo by partial hepatectomy and subsequent administration of ^{3}H-thymidine. After 2 weeks, the rats were injected i.p. with aqueous solutions of the nitrosoureas and velocity sedimentation patterns of the liver DNA on alkaline and neutral gradients were determined. Each experiment was repeated at least twice.

RESULTS:

The DNA from control animals was very large and in a range of molecular weights that cannot be measured accurately with current methods. It appears to be well above 1×10^9. In previous studies, maximum DNA damage with many carcinogens was observed when the rats were killed 4 hours or longer after treatment (6). At this time after injection of nitrosohydantoin, nitrosocitrulline or NO-DHU, the sedimentation profile of the DNA on alkaline gradients was similar to that of DNA from untreated controls (Fig. 1). When the rats were killed after 30 minutes, the results with nitrosocitrulline and nitrosohydantoin remained unchanged but with NO-DHU changes were observed. The peak of radioactivity was maximally displaced from the control position in both alkaline and neutral sucrose at this time. The peak shifted progressively toward that of control DNA between 1 and 4 hours (Figs. 2 and 3). Displacement of the DNA peak from the control position increased with increasing doses of NO-DHU and was detectable with doses as low as 1 mg/kg (Fig. 4). Since NO-DHU appeared to affect rat liver DNA more rapidly than most compounds previously tested, it seemed possible that the other 2 compounds might produce the same effect at a slower rate. However, none of the 3 compounds administered at 100 mg/kg had any effect on the velocity sedimentation of liver DNA from rats killed up to 24 hours. One hour after injection of dihydrouracil, dihydrouridine or uracil (each 100 mg/kg), there

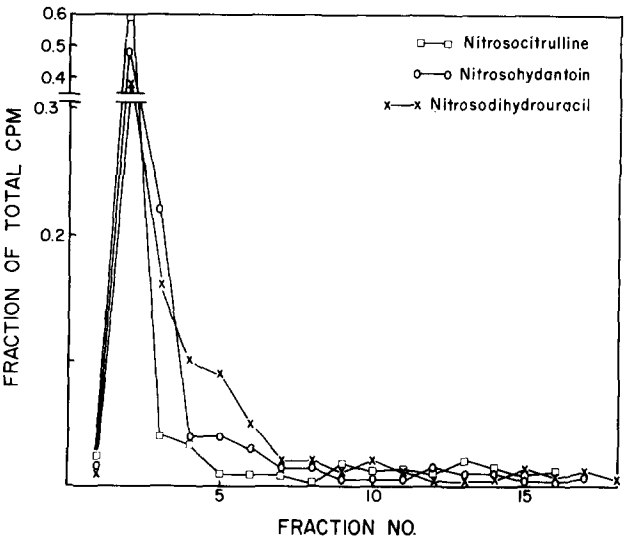

Figure 1. Alkaline sucrose gradient analysis (5) of rat liver DNA (previously labeled with ^{3}H-thymidine) 4 hours after intraperitoneal administration of 100 mg/kg body wt. NO-DHU, nitrosohydantoin and nitrosocitrulline in aqueous solution. Sedimentation is from right to left. Each experiment described in this communication included a gradient analysis of DNA from an untreated rat. The above results for nitrosohydantoin and nitrosocitrulline could not be distinguished from this control. The shoulder, fractions 4-6, observed after administration of NO-DHU was not present when the DNA was analyzed 6 hours after NO-DHU treatment (2 experiments).

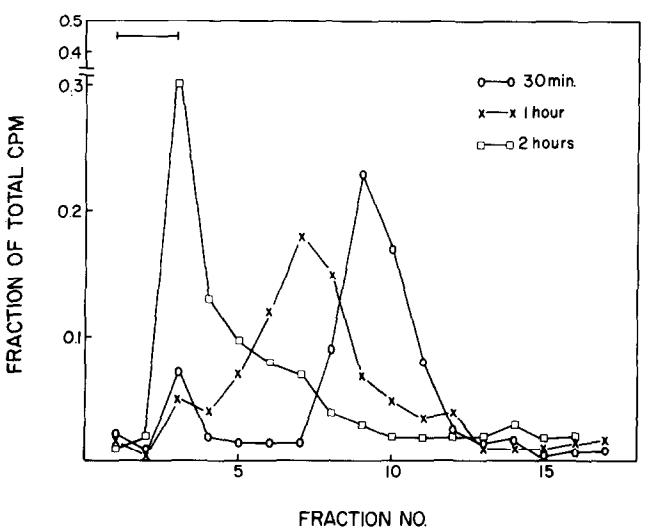

Figure 2. Alkaline sucrose gradient analysis of rat liver DNA at various times after injection of 100 mg/kg NO-DHU. In this Figure and in Figure 3 the position of the peak fraction for the untreated control (containing 0.72-0.53 of the total radioactivity detected in the particular gradient) is indicated by the horizontal line (top left).

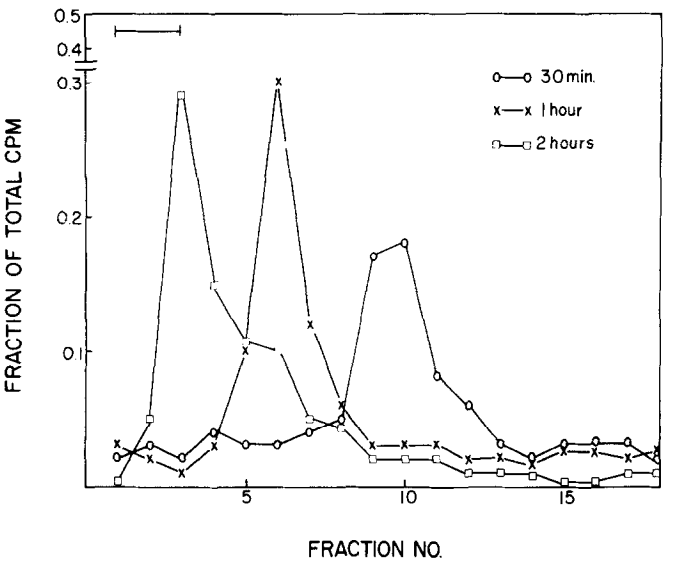

Figure 3. Neutral sucrose gradient analysis of the same DNA preparations used in Figure 2, which were from animals killed at various times after administration of 100 mg/kg NO-DHU. For neutral gradients, the method previously described for alkaline gradients (5) was modified as follows: the detergent solution (0.3 M NaCl, 0.03 M EDTA, 0.1 M Tris-HCl and 0.5% sodium dodecyl sulfate) in which the liver cells were lysed, was adjusted to pH 7.0 and layered over a 5-20% linear sucrose gradient containing 0.9 M NaCl (11). All other details were as previously described (5).

was no detectable change in the velocity sedimentation of liver DNA on alkaline sucrose.

DISCUSSION:

It is evident that the potent liver carcinogen NO-DHU induced both single and double strand breaks to liver DNA and that both types of damage seemed to be rapidly repaired in vivo within 4 hours. In contrast, nitrosocitrulline and nitrosohydantoin, the latter not being a known liver carcinogen, were without effect. The measurement of DNA damage appears to be a sensitive index of cytotoxicity and possible carcinogenicity, since the LD_{50} of NO-DHU is 850 mg/kg (8) but a dose of only 1 mg/kg induced obvious breaks in liver DNA (Fig. 4).

The finding of double strand breaks with NO-DHU and their apparent repair in vivo (Fig. 3) are particularly interesting. If at least some of the breaks observed under neutral conditions are truly double strand and not

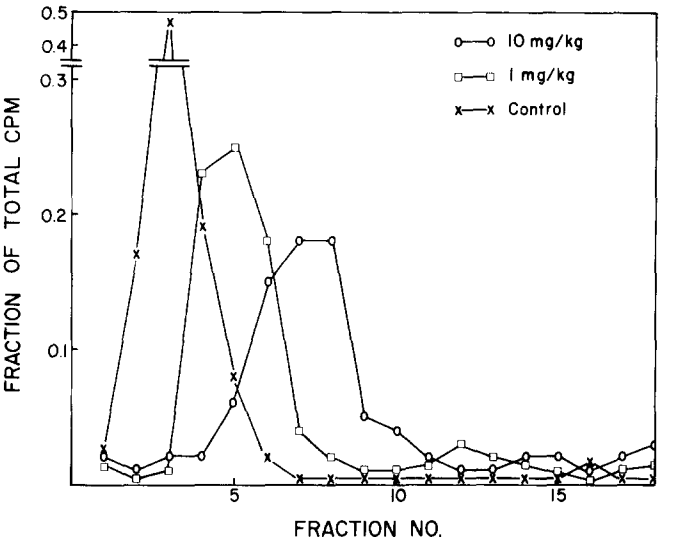

Figure 4. Dependence on dose of the change in sedimentation of DNA on neutral sucrose gradients 30 minutes after administration of NO-DHU. A similar relationship between sedimentation and dose was observed when the DNA was analyzed on alkaline sucrose gradients.

merely due to single strand breaks on complementary strands of DNA separated by too few bases to hold the strands intact, then it is difficult to envisage a mechanism for repair in a population of non-proliferating cells such as liver that does not introduce a high probability of error. Double as well as single strand breaks in DNA were observed in rats given 2 active forms of the liver carcinogen 2-acetylaminofluorene, the N-hydroxy- and N-acetoxy derivatives[5], as well as the hepatocarcinogen N-nitrosomorpholine[6]. These findings and those with NO-DHU are consistent with an hypothesis that the initiation process in chemical carcinogenesis, at least with some carcinogens, might be the induction of error-prone breaks[7]. The relatively long delay in the repair of single strand damage induced by several carcinogens may then serve only to enhance the probability of occurrence of double strand

[5]R. Michael and D.S.R. Sarma, personal communication.

[6]B.W. Stewart and E. Farber, unpublished results.

[7]D.S.R. Sarma, personal communication.

breaks in DNA such as by increasing the opportunity for cell proliferation. This phenomenon could be facilitated by the DNA replication that accompanies the cell proliferation in response to cell damage so often induced by chemical carcinogens.

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