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UNSCHEDULED DNA SYNTHESIS IN ISOLATED HEPATIC NUCLEI AFTER TREATMENT OF RATS WITH METHYLNITROSOUREA IN VIVO

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SUMMARY: Administration of the carcinogenic methylating agent, methylnitrosourea, to rats caused a significant increase in endogenous DNA synthesis assayed subsequently in isolated hepatic nuclei in vitro. DNA synthesis was related directly to the dose of carcinogen and inversely to the interval between treatment and isolation of nuclei. This synthesis appears to represent the continuation in vitro of unscheduled, reparative DNA synthesis initiated in damaged cells in vivo.

The ability to repair damaged DNA may represent a major cellular defense against carcinogenesis. Reduction of this reparative capacity characterizes some human diseases associated with a high risk of developing cancer (1) and also distinguishes certain tissues in rats which are targets for carcinogenesis by exposure to N-nitroso compounds (2,3). Conversely, the rat liver, which is comparatively resistant to carcinogenesis by single doses of carcinogenic alkylating agents, is distinguished by its high capacity to repair DNA that has been damaged by such agents (2-7). Evidence that hepatic cells perform excision repair on DNA damaged by carcinogenic alkylating agents has come from detection of: 1) transient increases and subsequent decreases in the number of single strand breaks in DNA (3,4); 2) rapid loss of certain alkylated bases from DNA (6,7); and 3) the unscheduled synthesis of DNA (8,9,10). Previous studies have shown that nuclei

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Abbreviation used: MNU, methylnitrosourea.

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isolated from S phase cells of regenerating rat liver maintain the capacity to perform scheduled DNA replication *in vitro* (11,12). In this study we demonstrate that unscheduled DNA synthesis also can be demonstrated *in vitro* in nuclei isolated from non-replicating cells of intact livers after damaging DNA by exposure to a chemical carcinogen *in vivo*.

MATERIALS AND METHODS

While under light ether anesthesia, F344 male rats (150-200 g, Charles River) received by injection into the portal vein a weight adjusted volume (5.0 ml per kg) of 5 mM sodium acetate, pH 5.0, containing 0.15 M NaCl, alone or including methylnitrosourea (Ash Stevens) in various concentrations. Following treatment the surgical incisions were sutured and animals were returned to holding cages. After various intervals livers were removed, and nuclei isolated (11). Endogenous DNA synthetic activity was assayed in triplicate as follows: Nuclei were suspended in 0.3 M sucrose (about 600 µg DNA per ml) and 50 µl of this suspension was added to tubes at 0°C containing 50 µl of incubation buffer (0.3 M Tris-HCl, pH 7.8, with 12 mM $MgCl_2$ and 6 mM ATP) and 50 µl of deoxyribonucleoside-5'-triphosphates (60 µM dATP and dGTP, 6 µM dCTP with 2.4 µM [^{3}H]dTTP at 17-20 Ci per mmole). Nuclei were incubated at 37°C for 15 min and then returned to 0°C. After addition of bovine serum albumin as carrier, macromolecules were precipitated with perchloric acid. Nuclear DNA was washed free of unincorporated radioactivity and selectively extracted according to a previously described technique (13). The nuclear DNA synthetic activity was determined as µmoles [^{3}H]dTMP incorporated per mole deoxyribonucleoside monophosphate during 15 min at 37°C. Incorporation of precursor was linear over this period.

RESULTS

Methylnitrosourea (MNU), a spontaneously active, carcinogenic methylating agent, was administered to adult rats by injection via the portal vein. After various intervals, livers were removed and cell nuclei isolated (11). Our initial studies indicated that the rate of endogenous DNA synthesis in hepatic nuclei isolated 30 to 60 min after intraportal injection of 0.5 mmole MNU per kg of body weight was significantly greater ($P<0.01$) than that observed in nuclei isolated from the livers of control animals after intraportal injection of acetate-buffered saline alone (data not shown). Based on these observations experiments were performed to examine the

kinetics for stimulation of nuclear DNA synthesis and so to determine the optimal interval between carcinogen treatment and removal of liver for detection of MNU-activated synthesis. Fig. 1 compares the rates of endogenous DNA synthesis in nuclei from livers removed 10 min to 24 hr after administration of 0.25 mmole MNU per kg. Maximum rates of DNA synthesis were observed in nuclei isolated from livers 10 and 45 min after the intraportal injection of MNU. After 45 min, DNA synthesis in hepatic nuclei diminished as the interval between treatment with carcinogen and removal of livers increased. In nuclei isolated from livers 12 hr after treatment, the rate of endogenous DNA synthesis had nearly returned to control levels.

The effect of the dose of carcinogen was examined in nuclei isolated from livers 45 min after administration of MNU (Fig. 2). Substantially (4- to 5-fold) increased rates of endogenous DNA synthesis were observed in nuclei isolated after treating rats with the relatively low dose of 0.05 mmole MNU per kg. After treatment with 0.50 mmole MNU per kg, the endogenous DNA synthesis in liver nuclei was about 10 times that observed in nuclei from the untreated livers of control animals. The nuclear response was dose-dependent, but there appeared to be a plateau in effect of the carcinogen at doses above 0.10 mmole MNU per kg.

The nuclear DNA synthesis activated by MNU was further characterized with respect to requirements for and utilization of dNTP precursors. Incorporation of [^{3}H]dTMP into the DNA of MNU-treated nuclei was inhibited by more than 80% when one or all three unlabeled dNTPs was omitted. When each of the other three dNTPs was substituted for [^{3}H]dTTP as the labeled dNTP in complete assay mixtures, all four dNTPs were found to be incorporated into DNA at nearly equal frequency. These results indicate that the MNU-activated nuclear DNA synthesis was a template-directed, gap-filling process.

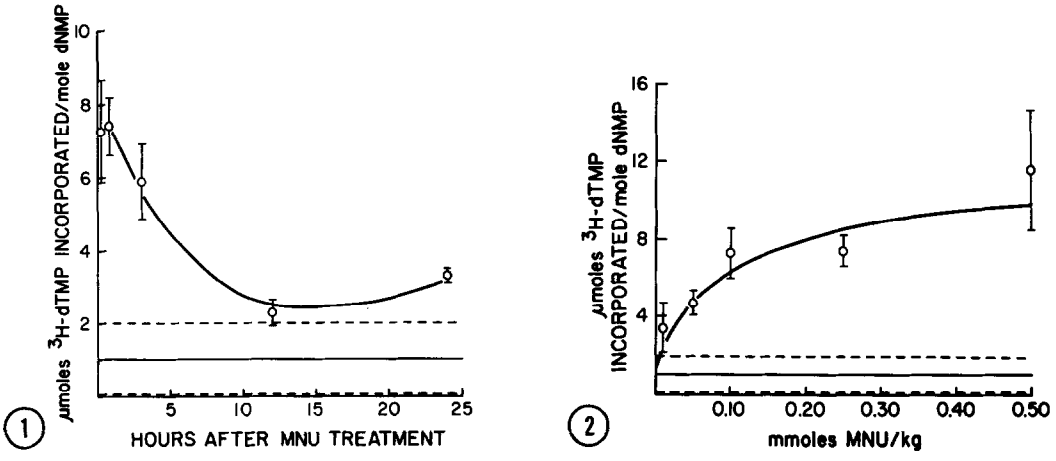

Fig. 1. Kinetics of Activation of Nuclear DNA Synthesis by MNU Treatment In Vivo. Rats received a single injection of MNU at 0.25 mmole per kg. At time intervals between 10 min and 24 hr later, livers were removed and nuclei isolated from them. Nuclear DNA synthetic activity was assayed in the isolated nuclei as described in MATERIALS AND METHODS. Times of carcinogen treatment were adjusted so that nuclei were isolated and assayed at one time. The mean values (O) for DNA synthetic activity assayed in two separate experiments are illustrated (vertical lines denote the range). The solid horizontal line represents the control value which is the average (N=7) DNA synthetic activity assayed in nuclei isolated from livers 45 min after administration of acetate-buffered saline alone. Horizontal dashed lines enclose 2 standard deviations of the mean control activity.

Fig. 2. Dose Response for Activation of Nuclear DNA Synthesis by MNU Treatment In Vivo. Rats were administered various doses of MNU as described in MATERIALS AND METHODS. Forty five min after treatment, nuclei were isolated from livers and nuclear DNA synthetic activity assayed. The average values of at least two separate experiments are illustrated (O) with vertical lines enclosing the range of values or one standard deviation of the mean. The horizontal lines are as described in the legend to Fig. 1.

DISCUSSION

Our results demonstrate that administration of MNU to rats by portal vein injection stimulates the capacity of nuclei, isolated from non-replicating liver cells, to incorporate dNTP precursors into nuclear DNA in vitro. The degree of stimulation was related directly to carcinogen dose and related inversely to the interval between treatment and removal of livers for isolation of nuclei. We believe that the endogenous DNA synthesis which we assayed in vitro in nuclei isolated from carcinogen-damaged livers largely represents the gap-filling component of DNA excision repair initiated in whole cells

in vivo. The immediate activation of this unscheduled DNA synthesis is consistent with studies in vivo which show an initial rapid loss of N^3-methyladenine and O^6-methylguanine from hepatic DNA of rats treated with MNU (6,7). Furthermore, reduced DNA synthetic activity was observed in nuclei isolated at times when the rates of loss of these lesions have been shown to be diminished in liver in vivo (7) (i.e., at 12 hr). The nuclear response also appeared to plateau at doses of MNU (0.25 and 0.50 mmole per kg) at which saturation or inhibition of removal of O^6-methylguanine from hepatic DNA has been observed in vivo (14).

To our knowledge this represent the first report of unscheduled reparative DNA synthesis analyzed in a subcellular system in vitro following treatment of experimental animals with carcinogen in vivo. These results complement and extend previous studies of reparative DNA synthesis in subcellular systems derived from human cells in culture (15,16,17), or isolated Ehrlich ascites tumor cells (18) after exposure to chemical (15,17) or physical (15-18) carcinogens. The combined in vivo - in vitro approach, utilizing nuclei isolated from liver or other target tissues for carcinogenesis, may prove useful for determining the biochemical features of DNA excision repair following damage to DNA in various tissues by chemical carcinogens.

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