DNA Repair Synthesis Following Exposure of Guinea-Pig Pancreatic Slices to Methyl-N-Nitrosourethane in vitro

Chronic oral administration of methyl-n-nitrosourethene (NMUT) induces exocrine pancreatic cancer in the guinea-pig[1]. NMUT, like other N-nitroso compounds, alkylates DNA bases[2]. Our previous studies have shown that NMUT is taken up by the guinea-pig pancreas when administered in vivo, and that it methylates pancreatic DNA and RNA bases both in vivo and in vitro[3,4]. We report here the results of investigations on DNA repair synthesis in guinea-pig pancreatic slices following in vitro exposure to NMUT.

Male white Hartley guinea-pigs (600–800 g) (Litton Bionetics, Ft. Dietrick, Md.) were sacrificed and the pancreas was removed. 3 slices from the duodenal region of the pancreas (40–60 mg, approximately 1 mm thick) were incubated at 37 °C in triplicate sets of 25 ml Erlenmeyer flask containing 10 ml modified Eagles minimum essential medium (Flow Laboratories, Inc., Rockville, Md.), supplemented with 20% fetal calf serum, penicillin G. (100 U/ml), fungizone (0.25 µg/ml) and streptomycin (100 µg/ml; Grand Island Biological Co., Grand Island, N.Y.) and L-glutamine (3.4 m*M*; Difco Lab., Detroit, Mich.) in a 5% CO_2 atmosphere.

The slices were incubated with 10 m*M* hydroxyurea (HU; Sigma Chemical Co., St. Louis, Mo.) for 2 h, following which 20 m*M* NMUT (NSC No. 2860, Lot ET1-148-1; Starks Associates, Inc., Buffalo, N.Y.; obtained from NCI, Bethesda, Md.), was added to incubation mixture in which the slices were exposed for further 15 min. Control slices were also incubated for the same period in the presence or absence of HU, but without NMUT. Following NMUT treatment, the slices were washed (2×) with Hanks' Balanced Salt Solution and incubated for 90 min in fresh medium containing 10 m*M* HU and 10 µCi/ml ^{3}H-methyl-thymidine (^{3}H-TdR; 5 Ci/m*M*; Amersham-Searle, Arlington Heights, Ill.). Control slices were treated similarly and incubated with or without HU. Slices from each flask were then homogenized in a solution containing 0.32 *M* sucrose, 10 m*M* phosphate buffer (pH 6.8) and 1 m*M* EDTA. DNA was isolated by a previously published method[5], and the DNA content was determined colorimetrically by the diphenylamine reaction[6]. Data representing the ^{3}H-TdR incorporation into isolated DNA are expressed as DNA dpm/µg DNA. Experiments were replicated on 3 occasions.

^{3}H-TdR incorporation into DNA in the presence of HU was taken as a measure of DNA repair synthesis, as it has been shown that HU selectively suppresses the normal DNA replicative synthesis without inhibition of DNA repair synthesis[7–9]. Normal DNA synthesis in the sodium acetate control was determined by ^{3}H-TdR incorporation into DNA in the absence of HU.

Incorporation of ^{3}H-TdR into DNA pancreatic tissue slices.

Treatment	Mean radioactivity in DNA (dpm/µg DNA)
Sodium acetate (NaAc)	57.0 ± 3.7
10 m*M* HU + NaAc	13.0 ± 1.4
10 m*M* HU + 20 m*M* NMUT	94.5 ± 13.8

After 2 h of HU-preincubation, the slices were exposed to 20 m*M* NMUT for 15 min; control slices received sodium acetate alone and were incubated for the same period. The treated slices were then washed free of NMUT and incubated for 90 min in the medium containing 10 m*M* HU and 10 µCi/ml of ^{3}H-TdR; control slices were treated similarly except that incubation ^{3}H-TdR was either in the presence or absence of HU. The radioactivity values are means ± standard error of 3 replicate experiments.

The Table shows that HU suppressed the normal DNA replicative synthesis by approximately 77%. HU has induced similar effects on normal DNA replicative synthesis in various cells[7–9]. However, NMUT treatment resulted in an approximately 7-fold increase over the control (+ HU) in ^{3}H-TdR incorporation into DNA in the presence of HU. This increase in ^{3}H-TdR incorporation into DNA might be attributed to DNA repair synthesis.

On the basis of studies by CLEAVER[7], and others[8,10], it seems less likely that, in the presence of HU, a short-term (90 min) ^{3}H-TdR incorporation into DNA would be significantly influenced by the precursor deoxyribonucleoside pool size. It has been shown, however, that both the purine and pyrimidine deoxyribonucleosides can be incorporated during the repair synthesis following alkylation of DNA[11]. Additionally, HU has been successfully used in following the DNA repair synthesis of cultured peripheral lymphocytes which, like the pancreas, are largely free of S-phase cells[9,12].

Previous studies have shown that the cultured mammalian cells are capable of excising the alkylated DNA bases enzymatically[13–15]. It is quite possible that a similar excision of NMUT-methylated DNA bases followed by DNA repair synthesis occurs in guinea-pig pancreas.

Zusammenfassung. Nachweis, dass Behandlung mit Methyl-n-Nitrosouretan die DNA-Synthese in Gewebeschnitten von Meerschweinchen-Pankreas in vitro induziert.

K. HASUMI, Z. M. IQBAL, A. ALARIF and S. S. EPSTEIN[16]

Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland (Ohio 44106, USA), 25 November 1974.

[1] H. DRUCKREY, S. IVANKOVIC, J. BUCHLER, R. PREUSSMAN and C. THOMAS, Z. Krebsforsch. *72*, 167 (1968).
[2] P. D. LAWLEY, Nature, Lond. *218*, 580 (1968).
[3] A. ALARIF and S. S. EPSTEIN, International Agency for Research in Cancer, W. H. O. publication, in press.
[4] A. ALARIF, R. G. WILBER and S. S. EPSTEIN, Cancer Res., in press.
[5] W. C. SCHNEIDER, in *Methods in Enzymology* (Eds. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1957), vol. 3, p. 680.
[6] K. BURTON, Biochem. J. *62*, 315 (1962).
[7] J. E. CLEAVER, Radiation Res. *37*, 334 (1969).
[8] M. W. LIEBERMAN, S. SELL and E. FARBER, Cancer Res. *31*, 1307 (1971).
[9] W. N. BRANDT, W. G. FLAMM and N. J. BERNHEIM, Chem. biol. Interaction *5*, 327 (1972).
[10] B. Z. NORDENSKJOLD, L. SKOOG, N. C. BROWN and P. REICHARD, J. biol. Chem. *245*, 5360 (1970).
[11] M. W. LIEBERMAN and M. C. POIRIER, Cancer Res. *33*, 2097 (1973).
[12] R. G. EVANS and A. NORMAN, Nature, Lond. *217*, 455 (1968).
[13] J. J. ROBERTS, J. M. PASCOE, B. SMITH and A. R. CRATHORN, Chem. biol. Interactions *3*, 49 (1971).
[14] A. R. PETERSON, J. S. BERTRAM and C. HEIDELBERGER, Cancer Res. *34*, 1592 (1974).
[15] I. DAMJANOV, R. COX, D. S. R. SARMA and E. FARBER, Cancer Res. *33*, 2122 (1973).
[16] Environmental Health Programs, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106, USA. This work was supported by the National Cancer Institute Contract No. NOI-CP-23284.