Chromosome Aberrations in Xeroderma Pigmentosum Cells Exposed to the Carcinogens, 4-Nitroquinoline-1-oxide and *N*-Methyl-*N'*-nitro-nitrosoguanidine[1] (37194)

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Variations in sensitivity within the human population should be considered when deciding on permissible or safe levels of carcinogenic agents. At present this aspect of environmental carcinogenesis is somewhat neglected because of a scarcity of reliable information about the range of sensitivity and the response of sensitive cells towards particular chemical carcinogens. In this paper we report the frequency of chromosome aberrations in cultured cells of a patient with xeroderma pigmentosum and of control persons following exposure to the potent chemical carcinogens, 4-nitroquinoline-1-oxide (4NQO) and *N* - methyl - *N'* - nitro-nitrosoguanidine (MNNG). The level of DNA repair synthesis of the XP cells which were used in this study was about 21% of that found in control fibroblasts following uv-irradiation (1) or exposure to 4NQO (1, 2). Chromatid breaks and chromatid exchanges which can be readily quantified were employed as a sensitive indicator of an induced damage to the genome of the cultured cells. The two carcinogens which are capable of inducing chromosome aberrations (3–5) were chosen because their interaction with the cellular DNA must differ. The alkylating MNNG elicits a comparable degree of unscheduled DNA synthesis in cultured XP cells and normal cells (1, 6) whereas a 4NQO application leads to a relatively high level of DNA repair synthesis in control cells but evokes only a low level in XP cells (1, 7).

Materials and Methods. The XP cells were obtained from a small punch biopsy taken from the skin of one XP patient (female, 18 yr old). Fibroblasts from two nonafflicted persons of comparable age and sex were used as controls. The doubling time of the XP cells and control cells was 30 and 24 hr, respectively. The culture technique has been previously described (1). The experiments were performed on cells grown on cover slips (22 mm^2) which were placed in plastic plates (3.5 cm diameter), fed Eagle's minimum essential medium supplemented with 20% calf serum and kept in a CO_2 incubator. The 4NQO was dissolved in ethanol and MNNG in distilled water. These stock solutions were diluted with culture medium to the desired concentrations. Colchicine was applied for 4 hr prior to the sampling of the cells for chromosome studies. Fixing with ethanol–acetic acid (3:1), staining with aceto–orcein and scoring of metaphase plates for chromosome aberrations followed the usual cytological procedure. The presence of a dislocated or unaligned chromatid fragment was used to identify chromatid breaks. All other chromatid "gaps" were excluded from the count (8).

Results and Discussion. The frequency of metaphase plates with chromosome aberrations in populations of cultured normal and XP cells following exposure to various concentrations of 4NQO is shown in Fig. 1. The types of observed chromosome aberrations included single or multiple chromatid breaks, single or multiple chromatid exchanges and chromosome fragmentation (Figs. 2–4). In the XP cells, a striking increase in chromatid aberrations occurred at doses of 4NQO which did not significantly elevate the frequency of chromosome aberration in the control fibroblasts. About 12 to 15 times higher concentration of 4NQO must be applied to normal cells in order to obtain frequencies of chromosome aberrations which are comparable to those induced in the sensitive XP

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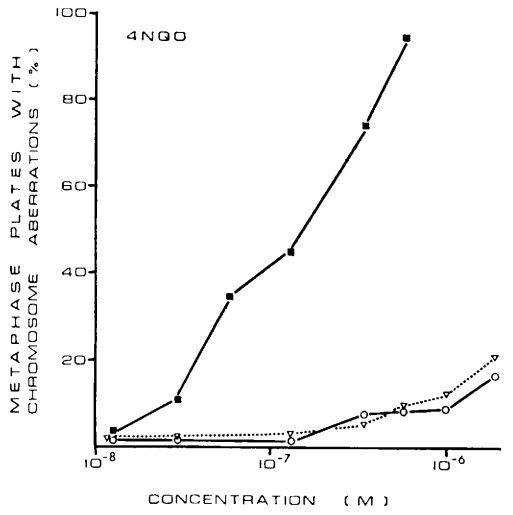


FIG. 1. Frequency of metaphase plates with chromosome aberrations of cultured XP cells (■) and cells from two normal persons (▽,○) exposed for 90 min to various concentrations of 4NQO.

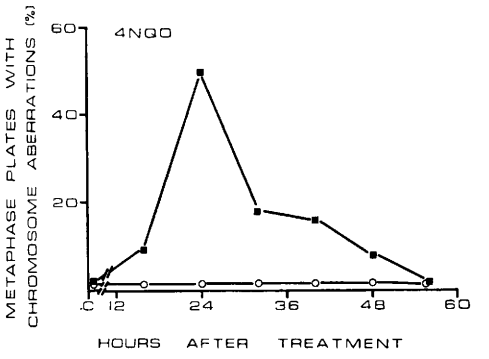
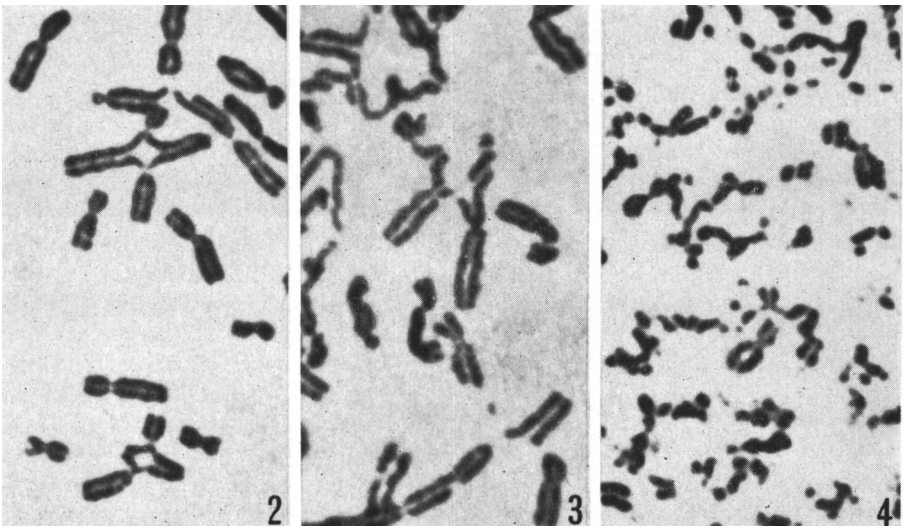


FIG. 5. Frequency of metaphase plates with chromosome aberrations in XP (■) and normal (○) cell cultures following a single dose of 4NQO (1×10^{-7} *M*, 90 min). C = the frequency of metaphase plates with chromosome aberrations in untreated cultures.

cells. Since an exclusive reliance on one sampling period could easily lead to erroneous conclusions, a time study has been performed (Fig. 5). For a 2 day period the frequency of XP metaphase plates with chromosome aberrations was above that seen in equally treated normal cells. Thereafter the level of chromosome aberrations of the 4NQO exposed normal and XP cells was in the range of untreated cells. Thus the 4NQO concentration used induced only a wave of chromosome aberrations in the XP cells.

There is no significant difference between the response of XP cells and control cells towards MNNG (Figs. 6, 7). The frequency of metaphase plates with chromatid breaks



FIGS. 2–4. Part of metaphase plates of cultured XP cells exposed for 90 min to 2.5×10^{-7} *M* 4NQO and sampled 20 hr posttreatment. Fig. 2. Two translocation figures. Fig. 3. Chromatid breaks, multiple chromatid exchanges and chromatid fragments. Fig. 4. Severe fragmentation of the entire chromosome complement.

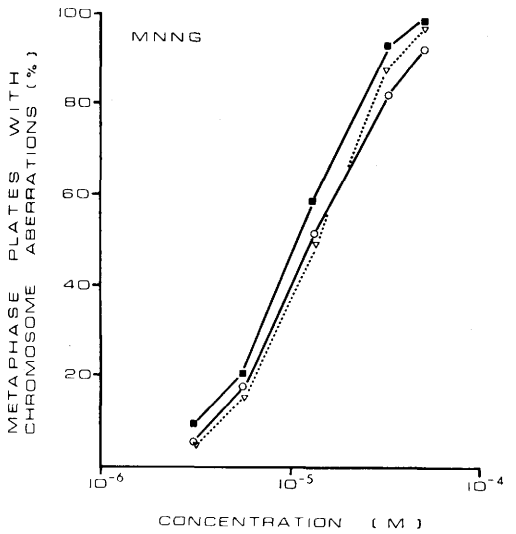


FIG. 6. Frequency of metaphase plates with chromosome aberrations of cultured XP cells (■) and cells from two normal persons (▽,○) exposed for 3 hr to MNNG.

and chromatid exchanges in the cultured XP cells resembles that found in control fibroblasts treated with equimolar concentrations of MNNG.

The XP cells do not seem to be defective in their capacity to form exchanges as shown in Table I. The ratio between chromatid breaks and exchanges varies only slightly between XP cells treated with 4NQO, which evokes a reduced DNA repair synthesis, XP

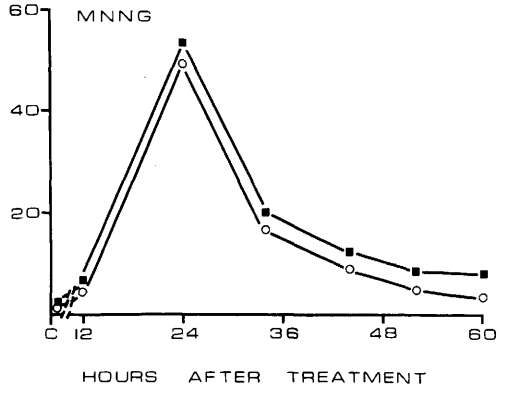


FIG. 7. Frequency of metaphase plates with chromosome aberrations in cultured XP (■) and normal (○) cell cultures following a single dose of MNNG (1.2×10^{-5} *M*, 3 hr). Ordinate: metaphase plates with chromosome aberrations (%).

TABLE I. Ratios of Chromatid Breaks and Exchanges in Normal and XP Cells.

	Type of aberrations (%)[a]			
	4NQO (8×10^{-7} *M*)		MNNG (2×10^{-5} *M*)	
	Breaks	Exchanges	Breaks	Exchanges
XP	67	33	68	32
Control	63	37	64	36

[a] Samples were taken 24 hr posttreatment; a total of 1204 chromosome aberrations were analyzed.

cells exposed to MNNG, which elicits a normal DNA repair synthesis, and normal cells treated with either of these two mutagenic agents.

It is tempting to link the elevated sensitivity of XP cells towards the chromosome damaging effect of 4NQO with their reduced capacity to repair 4NQO-induced DNA alterations and to relate the normal sensitivity of XP cells towards MNNG with a normal level of DNA repair following the application of this carcinogen. The elevated frequency of chromosome aberrations in uv-irradiated XP cells (9) and the reduced repair of uv-induced DNA changes (9, 10) seems to fit in this pattern. The different responses of XP cells towards 4NQO and uv on the one hand and MNNG on the other are also reflected in their different sensitivities to the lethal effect of these agents. It is likely that the reduced clone-forming ability of uv-irradiated (11–13) or 4NQO-treated (7, 12, 14) XP cells is mainly caused by the elevated frequency of chromosome aberrations. However, it is difficult at present to argue convincingly for or against a causal relationship between breakage of DNA strands, their repair or lack of repair, and events at the chromosomal level. One could postulate that unrepaired DNA alterations are involved in the formation of chromatid breaks and that there are more unrepaired DNA changes in XP cells than in normal cells after 4NQO exposure or uv-irradiation but comparable numbers of unrepaired lesions in XP and normal cells following a MNNG treatment. Whatever the molecular explanation of the observed phenomenon may prove to be, the results have revealed a genetic condition which causes an elevated

sensitivity towards the chromosome damaging effect of some but not all carcinogens.

Summary. The frequency of chromatid breaks and chromatid exchanges was estimated in cultured xeroderma pigmentosum (XP) cells and control cells exposed to the carcinogens 4-nitroquinoline-1-oxide (4NQO) and *N*-methyl-*N'*-nitro-nitrosoguanidine (MNNG). The level of DNA repair synthesis in the XP cells which were used in this study is about 21% of that found in cells of nonafflicted persons following exposure to 4NQO. The XP cells show an elevated sensitivity to the chromosome damaging effect of 4NQO. Doses of 4NQO, which have no effect on control cells, increased significantly the frequency of metaphase plates with chromosome aberrations in the XP cells over a 2 day period post-treatment. On the other hand the frequency of chromatid breaks and exchanges of XP cells resembled that found in control cells exposed to equimolar concentrations of MNNG. It appears likely, but unproven, that the different sensitivity of the XP cells towards 4NQO and MNNG is linked with their different capacity to repair 4NQO and MNNG induced DNA alterations.

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