DNA Repair Synthesis and Survival of Repair Deficient Human Cells Exposed to the K-Region Epoxide of Benz(a)anthracene[1] (36979)

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Cultured fibroblasts and lymphocytes of patients afflicted with xeroderma pigmentosum (XP) usually show a reduced level of DNA repair synthesis (1–5) and an increased sensitivity to the lethal effect of uV-radiation (6–9). The XP cells also have a defective unscheduled incorporation of ^{3}HTdR when exposed to several chemical carcinogens including nitroquinoline or nitropyridine oxides (9, 10) and *N*-hydroxy- or *N*-acetoxy-2-acetylaminofluorene (11, 12). These oncogenic and mutagenic compounds affect the clone-forming capacity of the XP cells to a higher degree than that of normal fibroblasts (8, 9).

In this paper we report the capacity of benz(a)anthracene (BA) and its K-region epoxide and dihydrodiol to elicit a DNA repair synthesis in normal and XP cells. Furthermore we compared the sensitivity of the repair deficient XP cells with that of normal cells following exposure to the K-region epoxide of BA. The epoxide was chosen because it seems to represent the proximate form of the precarcinogen BA. It is considerably more active than the parent hydrocarbon and other metabolites (*e.g.*, dihydrodiol) in producing oncogenic transformation of cultured cells (13–15) and in inducing mutations in mammalian cells (16).

Materials and Methods. Cell cultures of fibroblasts were obtained from skin-punch biopsies taken from the forearm of an XP patient and control persons of comparable age. At the time of the experiment the cell cultures had passed through 4–5 transfer passages. The stock cultures were kept in 10 cm diameter plates (Falcon plastic) in a CO_2-incubator and were fed Eagle's minimum essential medium (MEM) supplemented with 20% fetal calf serum and antibiotics. The level of DNA repair synthesis of the XP cells (female 18 yr old patient, M.T., Edmonton), compared to that of control cells, was about 21% following exposure to uV radiation, 4NQO, or *N*-acetoxy-AAF (9–11).

Carcinogens. Benz(a)anthracene (BA), BA-5,6-epoxide and BA-*cis*-5,6-dihydrodiol were dissolved in DMSO and diluted with tissue culture media to the desired concentrations. Monolayers of normal and XP cells, which were grown on 22 mm^2 cover slips placed into plastic dishes (35 mm in diam), were exposed to the compounds for 5 hr, rinsed repeatedly and transferred to new dishes with the regular MEM. The medium used during exposure to the chemicals contained only 5% fetal calf serum.

DNA repair synthesis. The unscheduled incorporation of ^{3}HTdR (10 μCi/ml for 90 min) in combination with autoradiography and evaluation of the number of grains in the autoradiographic preparations were used to estimate DNA repair synthesis (1, 17–19). Prior to the onset of the experiments the cultured cells were kept for 2 days in a medium without arginine and supplemented with only 5% dialized fetal calf serum. This pretreatment reduces the proliferation of mammalian cells (19, 20). Thus DNA repair synthesis can be readily distinguished from DNA replication associated with the duplication of chromosomes (19).

Clone-forming capacity. Twelve-hundred XP cells were seeded in 5 cm plastic plates (Falcon), exposed 14 hr thereafter to the vari-

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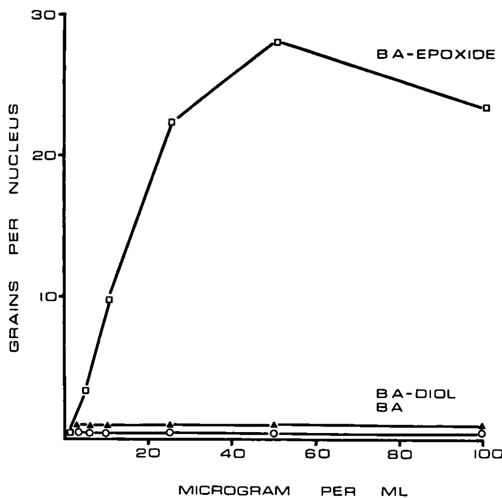

FIG. 1. DNA repair synthesis in normal human cells exposed for 5 hr to BA (○) BA-5,6-epoxide (□) or BA-*cis*-5,6-dihydrodiol (▲) at concentrations ranging from 5 to 100 μg/ml tissue culture medium.

ous compounds for 5 hr; rinsed and kept in MEM which was changed each third day. On the ninth day posttreatment the cultures were fixed (ethanol:acetic acid, 3:1) and stained with 1% aqueous toluidine blue. The clones containing about 50 cells or more were counted under a regular dissecting microscope.

Results and Discussion. An unscheduled incorporation of ^{3}HTdR into nuclear DNA was observed in nondividing cells exposed for 5 hr to BA-5,6-epoxide at concentrations ranging from 5 to 100 μg/ml (Fig. 1). At these doses the precarcinogen BA and the metabolite BA-*cis*-5,6-dihydrodiol did not trigger a detectable amount of DNA repair synthesis in normal cells.

The active BA-epoxide was used to treat cultured fibroblasts of XP patients and nonafflicted persons. The ensuing DNA repair synthesis in the two cell types was compared (Fig. 2). In normal cells the extent of unscheduled ^{3}HTdR incorporation increased at first with the concentration of the BA-epoxide and then decreased at higher concentrations. This pattern closely resembles that seen in normal human cells exposed to several quinoline and pyridine *N*-oxides (9), or *N*-acetoxy- and *N*-hydroxy-AAF (11, 21). The decrease in DNA repair synthesis at higher doses probably reflects a general toxic effect, since the higher concentrations are, in fact, lethal. In contrast to the response of normal cells, the level of DNA repair synthesis in the XP fibroblasts is low and only slightly affected by the various concentrations of the BA-epoxide (Fig. 2). The BA-epoxide can now be included with several 4NQO derivatives, 4NPO derivatives, and the *N*-acetoxy- or *N*-hydroxy-AAF, in a group of oncogenic and mutagenic compounds capable of eliciting DNA repair synthesis in normal cells, but only slightly in the cultured XP cells.

The lethal effect of single doses of BA, BA-*cis*-5,6-dihydrodiol and BA-5,6-epoxide was examined by measuring the clone-forming capacity of treated cells. BA-epoxide had a strong killing effect on normal and XP cells, whereas the corresponding concentrations of BA and BA-dihydrodiol did not reduce the clone-forming capacity (Fig. 3). These observations are in full agreement with cytotoxicity studies on Chinese hamster cells (13).

The sensitivity of normal cells was compared with that of XP cells by exposing them for 5 hr to the BA-epoxide and estimating their clone-forming capacity. The results clearly demonstrate an elevated sensitivity of

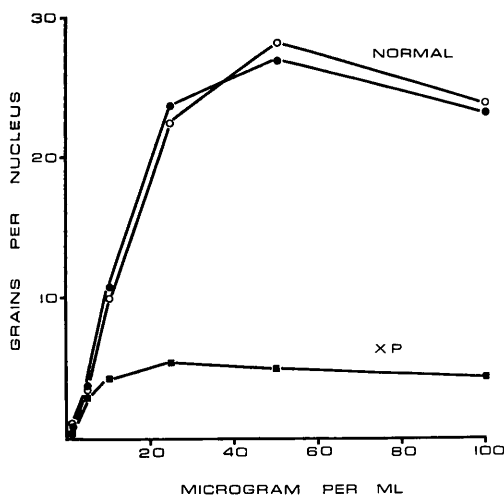

FIG. 2. DNA repair synthesis following treatment with BA-epoxide in cultured fibroblasts of one XP patient (■) and two nonafflicted persons (○, ●).

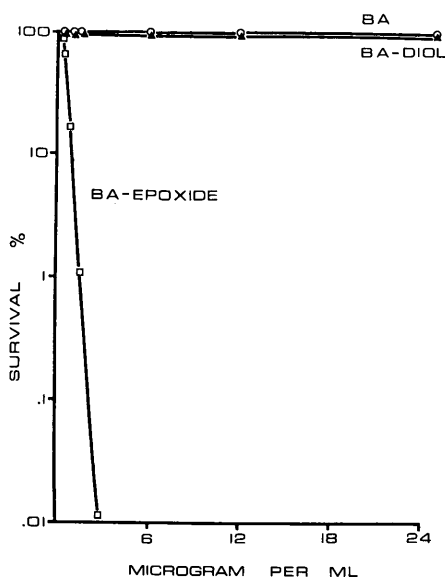

FIG. 3. The clone-forming capacity of XP fibroblasts exposed for 5 hr to BA (○) BA-5,6-epoxide (□) or BA-*cis*-5,6-dihydrodiol (▲).

the XP cells towards BA epoxides (Fig. 4). This result closely resembles the increased susceptibility of XP cells following exposure to uV (6–10) and oncogenic 4NQO derivatives (8, 10).

The high level of DNA repair synthesis following the BA-epoxide exposure is indicative of a relatively large extent of DNA alterations, whereas the lack of DNA repair synthesis in BA or BA-dihydrodiol exposed cells suggests an absence of DNA damage. These different effects at the DNA level seem to be reflected at the cellular level by a high cytotoxicity following exposure to BA-epoxide and by only slight cell killing at corresponding concentrations of the precarcinogenic or the inactive metabolite BA-*cis*-dihydrodiol. This difference may also be linked to the relatively high frequency of mutations found after BA-epoxide application to somatic mammalian cells but not observed following exposure to BA or BA-dihydrodiol (16). Copying errors could occur during DNA repair synthesis in the BA-epoxide treated cells, whereas the opportunity to produce such DNA microlesions would be absent in the nonrepairing BA or BA-dihydrodiol treated cells.

According to numerous studies on bacterial systems, strand interruptions or copying errors take place during cell divisions when DNA with unrepaired alterations replicates. Factors which reduce (*e.g.*, excision deficient mutations) or postpone repair (*e.g.*, holding in nonnutritive liquid medium) will increase lethality or mutability (*22*, *23*). If this interpretation is applicable to mammalian cells, then the elevated susceptibility of the XP cells to the BA-epoxide could be due to either an incomplete repair of DNA alterations prior to cell division or the presence of bound BA-epoxide (*24*) during DNA replication at S-phase of the mitotic cycle. The ensuing copying mistakes may be responsible for the increased lethality in the repair deficient XP cells exposed to BA-epoxide.

Summary. Cultured cells of a xeroderma pigmentosum patient (XP) and two normal persons were exposed to the precarcinogen benz(*a*)anthracene, its highly reactive K-region epoxide and its metabolite *cis*-5,6-dihydrodiol. Only the BA-epoxide induces DNA alterations which are followed by a detectable level of DNA repair synthesis. The XP cells show a reduced level of DNA repair synthesis and an increased sensitivity to the lethal ef-

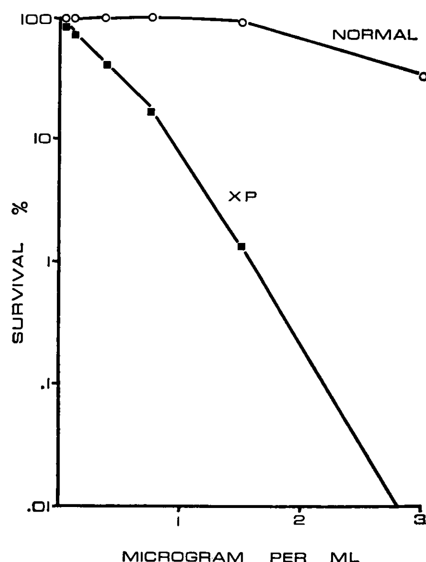

FIG. 4. The clone-forming capacity of normal cells (○) and XP cells (■) exposed for 5 hr to BA-5,6-epoxide.

fect of BA-epoxide. The response of the XP cells to BA-epoxide resembles that following uV-irradiation or treatment with the oncogenic 4'-nitroquinoline-1-oxides or *N*-acetoxy- and *N*-hydroxy-2-acetylaminofluorene.

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