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DNA repair deficiency and BPDE-induced chromosomal alterations in CHO cells

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Abstract

The induction of chromosomal aberrations and sister chromatid exchanges by BPDE was evaluated in parental and different DNA repair deficient Chinese hamster ovary cell lines in order to elucidate the mechanisms involved in their induction. These included the parental line (AA8), nucleotide excision repair (UV4, UV5, UV61), base excision repair (EM9), homologous recombination repair (Irs1SF) and non-homologous end joining (V3-3) deficient ones. The ranking of different cell lines for BPDE-induced chromosome aberrations was: UV4, Irs1SF, UV5, UV 61, EM9, V3-3, and AA8 in a descending order. Cells deficient in NER and HRR were found to be very sensitive, indicating the importance of these pathways in the repair of lesions induced by BPDE. For induction of SCEs, HRR and BER deficient cells were refractory, whereas the other cell lines responded with a dose-dependent increase. The possible mechanisms involved in BPDE-induced chromosomal alterations are discussed.

Keywords: BPDE; DNA repair; Chromosomal aberrations; Sister chromatid exchanges; Benzo(α)pyrene

1. Introduction

Benzo(α)pyrene (B(α)P) is a recognized environmental pollutant and is also formed during grilling of meat and inefficient combustion of fossil fuels, cigarette smoke, etc. DNA adducts formed due to exposure of B(α)P are considered to be the initiating step for its mutagenic and carcinogenic effects. B(α)P is metabolically activated by the cells to give rise to highly reactive Benzo(α)pyrene-7,8-diol-9,10 epoxide (BPDE) adducts which predominantly bind to the exocyclic amino position of guanine [1]. In a study employing human cell free extracts, nucleotide excision repair (NER) and base excision repair (BER) have been shown to be involved in the repair of BPDE-induced DNA adducts [2].

Biological effects of genotoxins are usually evaluated by their cytotoxicity and their ability to induce mutations, transformation and chromosomal alterations. Surprisingly, very few detailed studies have been carried out on the cytogenetic effects of BPDE in mammalian cells. BPDE has been shown to preferentially induce chromatid breaks localized to chromosome region 3p21,3 in lymphocytes of patients having oral premalignant lessions [3]. The present study is an attempt to understand the relative importance of different mechanisms

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involved in the repair of DNA lesions induced by BPDE employing chromosomal alterations (aberrations and sister chromatid exchanges (SCEs)) as end points. In order to achieve this, we have employed several Chinese hamster ovary (CHO) cell lines deficient in different DNA repair pathways such as, NER, BER, non-homologous end joining of DNA double strand breaks (NHEJ), homologous recombinational repair (HRR) and transcription coupled repair (TCR).

The rationale for this approach being that the defect in any particular DNA repair pathway will lead to higher frequencies of chromosomal alterations in the repair deficient cells compared to parental cells, indicating the possible involvement of that pathway in the repair of BPDE-induced damage in bestowing the sensitivity.

2. Material and methods

2.1. Cell lines

Parental CHO cells (AA8) and mutants deficient in different DNA repair pathways, such as nucleotide excision repair (UV4 and UV5), transcription coupled repair (UV61), homologous recombinational repair (Irs1SF), non-homologous end joining (V3-3), and base excision repair (EM9) were employed (Table 1). All these cell lines were originally isolated and kindly provided by L.H. Thompson.

2.2. Cell culture and treatments

Cells were cultured in Hams F10 medium with 10% foetal calf serum and antibiotics. BPDE [58917-67-2] was obtained from NCL Chemical Repository (Kansas City, MO). 5-bromo-2-deoxyuridine (BrdUrd) [59-14-3] was purchased from Sigma. For treatment, the following schedule was employed, which allows scoring both chromosomal aberrations and SCEs in the first mitosis following treatment [4,5]. Exponentially growing cells were seeded in medium containing BrdUrd (6 μg/ml) for 10 h prior to treatment. BPDE was dissolved in DMSO [67-68-5], (the final DMSO concentration did not exceed 1%) and cells were treated with three different final concentrations, namely, 0.8, 1.6, and 3.2 μM. The highest concentration employed gave about 30 to 40% reduction in mitotic indices in the parental cells in a preliminary experiment. After BPDE treatment (15 min), the cells were allowed to recover for different durations taking into consideration the treatment induced mitotic delay as determined in preliminary experiments (i.e., 14 h for AA8, UV4 and UV5, 20 h for V3-3, EM9, and UV61, 26 h for Irs1SF). Before fixation in acetic acid [64-19-7]: methanol [67-56-1] (1:3), the cells were treated with colcemid [477-30-5] (0.2 μg/ml) for 3 h and subjected to hypotonic shock. Standard air-dry preparations were made. For scoring chromosomal aberrations and SCEs, the slides were stained by fluorescence plus Giemsa technique [6]. In the case of AA8, V3-3 and UV5, 200 metaphases for each experimental point were scored for determining the frequencies of aberrations and, for UV4, Irs1SF, EM9 and UV61 cell lines, only 100 metaphases were scored due to reduced mitotic indices. For determining the frequencies of SCEs, 25 cells were scored for all the experimental points. The statistical significance of differences between the control and treated ones in all cell lines was calculated employing Student's *t*-test.

Table 1
Genotypes and characteristics of CHO cell lines used in this study

Cell line	Genotype	Defect	Human homologous
AA8	WT	WT	
UV4	ERCC1−	deficient in NER	XPF
UV5	ERCC2−	deficient in NER	XPD
V3-3	XRCC7−	deficient in NHEJ	DNA-PK
Irs1SF	XRCC3−	deficient in HR	Rad 51
EM9	XRCC1−	deficient in BER	DNA ligase III
UV61	ERCC6−	deficient in TCR	CSB

3. Results

3.1. Chromosomal aberrations

The spontaneous frequencies of chromosomal aberrations varied between cell lines, the DNA repair deficient cells having higher frequencies than the parental cells (AA8). AA8 cells had a mean of 1.5 aberrations, per 100 cells. A mean of 4.0, 4.0, 7.5, 8.0, 36 and 76 aberrations per 100 cells were observed in V3-3, UV61, UV5, UV4, EM9 and Irs1SF, respectively. Both chromatid breaks and exchanges were observed in all DNA repair deficient cells. Cells deficient in BE repair (EM9) and HR (Irs1SF) repair had very high frequencies of spontaneous aberrations.

BPDE is classified as an "S" dependent agent, i.e., requiring an intervening S phase following treatment to visualize the induced chromosomal aberrations. BPDE induced only chromatid type of aberrations, namely, breaks, iso-chromatid breaks and different types of chromatid exchanges (Table 2). In general, BPDE induced more chromatid exchanges than chromatid breaks. For comparison between different cell lines under study, the respective spontaneous frequencies of aberrations were deducted from the total and only induced ones were considered. In order to evaluate the sensitivity to induction of chromosomal aberrations by BPDE in different cell lines, a comparison was made by calculating the mean value of induced chromosomal aberrations obtained at all the different doses used and the ranking of sensitivity was carried out taking into consideration the sensitiv-