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Benzo[a]pyrene-induced DNA damage associated with mutagenesis in primary human activated T lymphocytes

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene (B[a]P), are widely distributed environmental contaminants exerting toxic effects such as genotoxicity and carcinogenicity, mainly associated with aryl hydrocarbon receptor (AhR) activation and the subsequent induction of cytochromes P-450 (CYP) 1-metabolizing enzymes. We previously reported an up-regulation of AhR expression and activity in primary cultures of human T lymphocyte by a physiological activation. Despite the suggested link between exposure to PAHs and the risk of lymphoma, the potential of activated human T lymphocytes to metabolize AhR exogenous ligands such as B[a]P and produce DNA damage has not been investigated. In the present study, we characterized the genotoxic response of primary activated T lymphocytes to B[a]P. We demonstrated that, following T lymphocyte activation, B[a]P treatment triggers a marked increase in CYP1 expression and activity generating, upon metabolic activation, DNA adducts and double-strand breaks (DSBs) after a 48-h treatment. At this time point, B[a]P also induces a DNA damage response with ataxia telangiectasia mutated kinase activation, thus producing a p53-dependent response and T lymphocyte survival. B[a]P activates DSB repair by mobilizing homologous recombination machinery but also induces gene mutations in activated human T lymphocytes which could consequently drive a cancer process. In conclusion, primary cultures of activated human T lymphocytes represent a good model for studying genotoxic effects of environmental contaminants such as PAHs, and predicting human health issues.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a major class of environmental contaminants to which humans are commonly exposed. They are formed by incomplete combustion of organic materials and are notably found in large amounts in diet, air pollution, cigarette smoke and some occupational atmospheres [1]. PAHs exert various toxic effects towards human health, including carcinogenic and immunosuppressive effects, and contribute to smoking-related diseases [2]. Most of these effects have been linked to the activation of the aryl hydrocarbon receptor (AhR) and subsequent transcription of AhR-regulated genes. Among PAHs, benzo[a]pyrene (B[a]P) is the most widely used model compound for studying the effects of carcinogenic PAHs. B[a]P is not only agonist for AhR, but also substrate for the induced xenobiotic metabolizing enzymes from the cytochromes P-450 (CYPs) 1 family [3]. CYP1 isoforms, metabolize B[a]P to epoxide derivatives that can covalently bind DNA to form DNA adducts; the predominant form results from addition of B[a]P diol epoxide (BPDE) to the N^2 exocyclic amino group of guanine to form a dG-N^2-BPDE adduct [4]. DNA damage has been regarded as an important factor in chemical carcinogenesis, and DNA adducts derived from B[a]P are believed to be a critical event in tumour formation by producing

Abbreviations: AhR, aryl hydrocarbon receptor; AMC, 7-amino-4-methylcoumarin; α-NF, alpha-naphthoflavone; ATM, ataxia telangiectasia mutated; B[a]P, benzo[a]pyrene; BPDE, B[a]P diol epoxide; CYPs, cytochromes P-450; DMSO, dimethyl sulfoxide; DDR, DNA damage response; DSBs, double-strand breaks; EROD, ethoxyresorufin deethylase; FPG, formamido pyrimidine glycosylase; HR, homologous recombination; PAHs, polycyclic aromatic hydrocarbons.

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mutations in critical genes such as the tumour suppressor p53 [5]. Thus, emphasis has been placed on the DNA-damaging properties of B[*a*]P, the only one PAH classified as carcinogenic to humans for lung, skin and bladder tumours by the International Agency of Research on Cancer [6]. In addition, B[*a*]P induces cancer development in lymphoid tissue and hematopoietic compartment in animals [7], and some epidemiological studies have suggested a potential role of B[*a*]P in the development of lymphomas in humans [8].

Human blood peripheral lymphocytes, in particular T lymphocytes, have been reported to possess CYP1A1 and 1B1-metabolizing systems [9], suggesting thus that it could be a useful model for studying the potential of PAHs such as B[*a*]P to be metabolized, bind DNA, and form different types of DNA adducts linked to the susceptibility seen in the induction of human cancers [10]. Whereas a significant association has been found between the level of BPDE-induced DNA adducts in T lymphocyte-enriched peripheral blood mononuclear cell cultures and risk for cancer [11], studies on the relationship between B[*a*]P metabolism and its DNA-damaging potential linked to cancer risk have provided equivocal results in these T lymphocytes-enriched cultures [12]. This is in part due to the observation that such cultured T lymphocytes are poor metabolizers of PAHs such as B[*a*]P, exhibiting significant CYP1-mediated activity only upon activation by mitogenic lectins such as phytohemagglutinin [13]. As compared with phytohemagglutinin promoting polyclonal T cell proliferation, T lymphocyte activation by anti-CD3 and anti-CD28 antibodies represents a more physiologically relevant T cell stimulation [14], and we have recently reported that AhR is present and functional only in CD3/CD28-activated T lymphocytes and not in resting counterparts [15]. Altogether, these data suggest an increased ability of the activated T lymphocytes to metabolize AhR exogenous ligands such as B[*a*]P and then to produce DNA damage. The present study was designed to explore the genotoxic response to B[*a*]P in these primary CD3/CD28-activated human T lymphocytes, a relevant model to human health issues. We report that B[*a*]P, upon metabolic activation, generates dG-N^2-BPDE adducts and DNA double-strand breaks (DSBs). In human T lymphocytes, this elicits a DNA damage response (DDR) via an ataxia telangiectasia mutated (ATM)-dependent pathway, supporting DNA repair and cell survival to B[*a*]P treatment but leading to gene mutations.

2. Materials and methods

2.1. Chemicals and reagents

B[*a*]P, alpha-naphthoflavone (α-NF), 2-morpholin-4-*yl*-6-thianthren-1-*yl*-pyran-4-one (KU-55933), dimethyl sulfoxide (DMSO), potassium bromate, DAPI, ethoxyresorufin, Asp-Glu-Asp-7-amino-4-methylcoumarin (AMC), and agarose were obtained from Sigma-Aldrich (St. Louis, MO, USA). SYBR Gold nucleic acid stain and carboxy-fluorescein succinimidyl ester (CFSE) were provided by Thermofischer Scientific (Braunschweig, Germany), whereas [^{3}H]-methyl-thymidine was purchased from Amersham Biosciences (Buck, UK). Antibodies against CYP1B1, p21, RAD51 and Hsc70, and against CYP1A1 were supplied from Abcam (Paris, France), and Santa Cruz Technology (Heidelberg, Germany), respectively. Antibodies against p53, phospho-p53 (Ser15), phospho-CHK1 (Ser317) and phospho-CHK2 (Thr68) were obtained from Cell Signalling Technology (Saint Quentin, France), while anti-phospho-ATM (Ser1981) and anti-phospho-Histone H2AX (Ser139) were from Merck Millipore (Molsheim, France). The 53BP1 antibody was purchased from Novus Biological (Abingdon, UK).

2.2. Cell culture and treatment

Peripheral blood mononuclear cells were isolated from blood donor buffy coats (written consent for the use of blood samples for the research protocol obtained according to the regulation for blood transfusion of the French blood organization Etablissement Français du Sang, Rennes (France)) by Ficoll (Thermofischer Scientific) gradient centrifugation. After separation of monocytes by a 1-h adhesion step, T lymphocytes were purified from nonadherent cells by negative selection using Dynabeads® Untouched™ Human T Cells Kit (Thermofischer Scientific). T lymphocytes were cultured in RPMI medium (Eurobio, Les Ulis, France) supplemented with 20 IU/mL penicillin, 20 μg/mL streptomycin, and 10% decomplemented fetal calf serum (Thermofischer Scientific), and stimulated with Dynabeads® T-Expander beads coated with anti-CD3 and anti-CD28 antibodies (Thermofischer Scientific) as previously reported [15]. Chemicals were used as stock solutions in DMSO. The final concentration of DMSO in culture medium was always <0.2% v/v and control cultures received vehicle containing the same dose of DMSO as treated cultures.

2.3. RT-qPCR

Total RNA was isolated from T lymphocytes using the TRIzol method (Thermofischer Scientific) and then reverse-transcribed into cDNA using the RT Applied Biosystems kit (Foster City, CA, USA). qPCR assays were performed using gene-specific primers from Qiagen for CYP1A1, CYP1B1 and growth arrest and DNA damage inducible, alpha (gadd45A) (Courtaboeuf, France) or Eurogentec for the others (Seraing, Belgium) (Table 1). The amplification curves of the PCR products were analyzed with the ABI Prism SDS software using the comparative cycle threshold method. Relative quantification of the steady-state target mRNA levels was calculated after normalization of the total amount of cDNA tested to a 18S mRNA endogenous reference as previously described [15].

2.4. RT^2 profiler™ PCR arrays

The Human DNA Repair RT2 Profiler™ PCR Array was used to profile the expression of 84 key genes encoding the enzymes that repair damaged DNA according to the manufacturer's instructions (SABiosciences, Qiagen). For each condition, we pooled equal amounts of RNAs isolated from DMSO or B[*a*]P-treated cultures of T lymphocytes established from 9 blood donors. Pooling small samples of cells for array analysis is considered advantageous in situations where the level of biological variation could be high compared to technical variation on the array [16,17]. The amplification curves of the PCR products were analyzed with the ABI Prism SDS software as reported for RT-qPCR. The expression levels of target genes were normalized relative to the expression of β2 microglobulin as housekeeping gene and were given as fold change compared with control with vehicle.

Table 1
Primer sequences for RT-qPCR.

Gene Symbol	Forward Sequence	Reverse Sequence
mdm2	GCAGTGAATCTACAGGGACGC	ATCCTGATCCAACCAATCACC
CDKN1A	GGCAGACCAGCATGACAGATT	GGCGGCCAGGGTATGTA
BBC3	GACCTCAACGCACAGTACGA	GAGATTGTACAGGACCCTCCA
PMAIP1	GGAGATGCCTGGGAAGAAG	CCTGAGTTGAGTAGCACACTCG
18S	CGCCGCTAGAGGTGAAATTC	TTGGCAAATGCTTTCGCTC