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Lower nucleotide excision repair capacity in newborns compared to their mothers: A pilot study

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ABSTRACT

Recognition of the potential vulnerability of children and newborns and protection of their health is essential, especially regarding to genotoxic compounds. Benzo(a)pyrene B(a)P a commonly found carcinogen, and its metabolite BPDE, are known to cross the placenta. To investigate how well newborns are able to cope with BPDE-induced DNA damage, a recent developed nucleotide excision repair cell phenotype assay was applied in a pilot study of 25 newborn daughters and their mothers, using the Alkaline Comet Assay and taking demographic data into account. Newborns seemed to be less able to repair BPDE-induced DNA damage since lower repair capacity levels were calculated compared to their mothers although statistical significance was not reached. Assessment of repair capacity in combination with genotypes will provide important information to support preventive strategies in neonatal care and to define science based exposure limits for pregnant women and children.

1. Introduction

Recognition of the potential vulnerability of children and newborns and protection of their health is essential. It has already been shown that their response to genotoxic compounds, toxicants and carcinogens differs from adults [for review 1–3].

Measuring their capacity to repair DNA damage provides critical information about their resistance to genotoxic stress. Among genotoxicants to which embryos and children may be exposed *in utero* like oxidative stress, ionizing radiation, or chemical substances in mothers' diet and environment, polycyclic aromatic hydrocarbons (PAHs) are of major concern due to their ubiquitous presence particularly, in food and cigarette smoke.

Benzo(a)pyrene (B(a)P), a representative of PAHs, is a human carcinogen [4] that can be metabolically activated to BPDE. This metabolite can covalently bind DNA resulting in the formation of bulky DNA-adducts which represent a critical step in carcinogenesis. It was recently observed that B(a)P is able to cross the human placenta and enter the fetal compartments [5,6]. According to several studies, prenatal exposure to airborne PAHs results in detectable BPDE-DNA adducts in cord blood with levels comparable to or higher than maternal BPDE-DNA adducts levels [7–13]. In newborns from unemployed women significantly higher DNA adduct levels were observed in newborns living in middle and high polluted areas compared to newborns from low polluted areas in Krakow (Poland). Grouping all the newborns ($N = 70$) resulted in no effect of air pollution on DNA adduct levels [7]. In a total of 69 mother-newborn pairs of the greater Copenhagen area (Denmark), highest BPDE-adduct levels were observed in those pairs living near medium-traffic density compared to high- and low-traffic density [13]. As far as smoking is concerned, investigation of BPDE-DNA adducts in relation to prenatal smoking revealed inconclusive results. Stéphan-Blanchard et al. (2011) detected a significant decrease in B(a)P-DNA adducts in buccal cells from 25 preterm neonates with non smoking mothers (mean ± SD fmole/50 μg of DNA; 2.2 ± 4.4) compared to 18 preterm infants from mothers reporting smoking of less than 10 cigarettes a day (12.4 ± 1.0) and 21 preterm with mothers reporting more than 10 cigarettes a day (21.7 ± 4.9) [14]. Self-reported recent exposure to environmental tobacco smoke resulted in significant increase in BPDE-DNA adduct levels in both mothers and newborn ($N = 69$) [13]. BPDE-DNA adduct levels in placenta of 11 tobacco smoke exposed women

Abbreviations: APC, aphidicolin; BPDE, B(a)P-7,8-dihydrodiol-9,10-epoxide; DMSO, dimethylsulfoxide; EMS, ethylmethanesulphonate; FPG, formamidopyrimidine-DNA glycosylase; hOGG1, human 8-hydroxyguanine DNA-glycosylase; NER, nucleotide excision repair; PAHs, polycyclic aromatic hydrocarbons; PBMC, peripheral blood mononuclear cells; PBS, phosphate buffer saline; PHA, phytohemagglutinin; Pol δ, polymerase δ; Pol ε, polymerase ε; RC, repair capacity; SGA, small for gestational age; TD, tail DNA.

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(plasma cotinine levels > 3 ng/ml) were significantly higher compared to 59 unexposed women while no effect was observed on adduct levels in maternal and cord blood [15] nor on oxidative damage biomarkers [16]. The results of all these studies are difficult to interpret.

We wanted to know how well newborns are able to cope with "manmade" induced DNA damage by investigating the capacity of newborn children to repair BPDE-induced DNA adducts. Therefore, a recent developed and validated NER cell phenotype assay [17,18] was applied on 25 newborn daughters and their mothers, using the Alkaline Comet Assay and taking demographic data into account. This pilot study will allow us to assess the practical feasibility in newborns and to find out whether the NER phenotype assay is able to discriminate more or less vulnerable newborns as compared to their mothers.

2. Materials and methods

2.1. Study population

Twenty five mothers and their full term newborn daughter were enrolled in this study and recruited according to the following criteria: singleton pregnancy and uncomplicated delivery of a healthy full term girl. Only daughters were selected to rule out gender-related differences in response to the BPDE-exposure [19, for review 20, 21]. Parents were personally contacted by the neonatologist and signed an informed consent prior to participation. The study protocol was approved by the ethical committee of St-Pierre University Hospital in Brussels. Structured questionnaires and medical records were used to provide information concerning clinical and lifestyle characteristics before and during pregnancy, including maternal age, smoking and drinking behavior, vitamin supplements, nutritional habits, pregnancy complications, delivery type and type of anesthesia. Mean gestational age was 39 weeks and mean birth weight was 3303 g. The majority of the mothers took antioxidants during pregnancy (92%) and only few smoked during pregnancy (8%). 67% of the fathers smoked. Folate and vitamin B12 concentrations were measured in a subgroup and showed normal values, except for 2 mothers where folate concentration was low.

2.2. Collection and processing of blood samples

Umbilical cord blood samples from the newborns were taken immediately after delivery and venous blood samples from the mothers were collected one day after delivery together with the usual clinical tests. Umbilical and venous blood was collected in heparinised tubes with extra heparine (0.5 ml) for the umbilical cord blood samples (BD Vacutainer, Becton Dickinson, UK) and kept at 4 °C until the processing [22]. Within 24 h, PBMCs were isolated using Ficoll-Pacque (Pharmacia Biotech, Uppsala, Sweden) and were cultured in Ham's F-10 medium containing 25 mM L-glutamine (Gibco Invitrogen, Paisley, UK), supplemented with 15% fetal calf serum (FCS; Gibco) and 2% phytohaemagglutin A 16 (PHA; Murex Biotech Ltd., Dartford, UK). Samples were incubated in 5% CO_2 in a humified incubator at 37 °C.

2.3. Treatment of peripheral blood mononucleated cells (PBMCs)

After 24 h PHA stimulation, PBMCs were transferred in serum-free medium and challenged with 0.5 μM BPDE (NCI-Midwest Research Institute, MO, USA) dissolved in DMSO (Merck, Darmstadt, Germany) for 2 h, as recommended by Vande Loock et al. (2010). Control samples were exposed to 0.5% DMSO. All exposures (prepared in duplicate cultures) were processed in parallel.

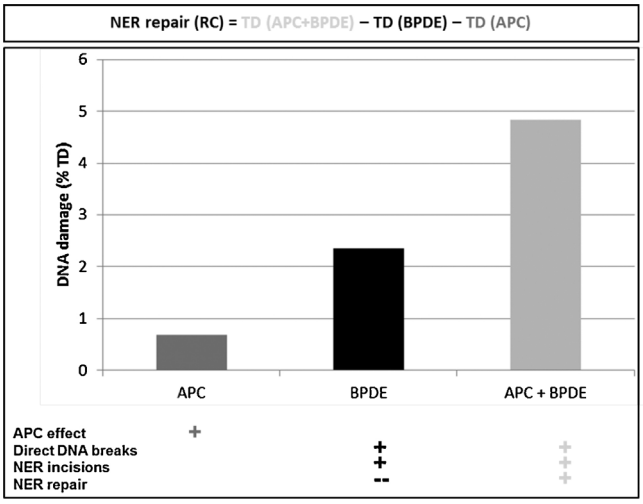

Fig. 1. Diagram depicting the contribution of the different treatments to the measured DNA strand breaks resulting in the NER capacity in the total population: (1) APC treatment alone results in a minor level of DNA damage; (2) DNA damage measured after treatment with BPDE consists of the direct DNA breaks and the NER incisions, a fraction of the induced DNA damage that is already repaired cannot be detected; (3) DNA damage measured after treatment with BPDE and APC consists of the APC effect, direct DNA breaks, the NER incisions and the remaining gaps resulting from the APC-blocked NER repair. The nucleotide excision repair capacity (RC) was defined as the amount of DNA strand breaks damage induced by BPDE in presence of APC, subtracted with the damage induced by BPDE and APC alone [17].

2.4. Alkaline Comet Assay

The comet assay was performed as described previously by Singh et al. (1988) with modifications according to De Boeck et al. (1998) [23,24]. For each electrophoresis, a positive and negative internal standard was included which consisted of human K562 erythroleukemia cell line [25] treated (%TD mean ± SD; 24.3 ± 8.14) or untreated (5.96 ± 3.17) with 2 mM EMS, respectively. The percentage of tail DNA (TD) was considered to be the most reliable DNA damage parameter [26]. Slides were coded before analysis and 100 cells per culture were scored.

2.5. Principle of nucleotide excision repair phenotype assay

Exposure to BPDE may lead to DNA strand breaks resulting from direct interaction with DNA and incisions introduced by repair enzymes. We aimed at discriminating both type of breaks by using aphidicolin (APC), a DNA polymerase inhibitor able to block the ligation step of NER. Pre incubation of the PBMCs with APC followed by BPDE treatment results in the detection of (i) the minor level APC induced DNA damage, (ii) direct DNA breaks induced by BPDE, (iii) small amount of ongoing NER repair incision and (iv) all repair incisions that are blocked by APC. Therefore the NER capacity (RC) is defined as amount of DNA strand breaks damage induced by BPDE in presence of APC, subtracted with the damage induced by BPDE and APC alone (Fig. 1).

2.6. Nucleotide excision repair phenotype assay

To measure the repair capacity, duplicate treatments were performed as previously described by Vande Loock et al. (2010); in presence or absence of 0.5 μM BPDE (2 h) and with or without 30 min pre incubation with 0.5 μg/ml aphidicolin (Sigma–Aldrich, St-Louis, USA) dissolved in DMSO. Per donor 10 PBMCs cultures