

Serum Level of Antibodies (IgG, IgM) Against Benzo[a]pyrene-7,8-diol-9,10-epoxide-DNA Adducts in Children Dermatologically Exposed to Coal Tar



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ABSTRACT

Crude coal tar (CCT) contains polycyclic aromatic hydrocarbons (PAHs). Benzo[a]pyrene (BaP) is metabolized into a highly reactive metabolite benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) that is able to bind to DNA and creates BPDE-DNA adducts. Adducted DNA becomes immunogenic and induces immune response by production of antibodies against BPDE-DNA adducts (Ab-BPDE-DNA). Circulating Ab-BPDE-DNA was proposed as potential biomarker of genotoxic exposure to BaP (PAHs). Goeckerman therapy (GT) of psoriasis uses dermal application of CCT ointment (PAHs). In presented study (children with psoriasis treated by GT; n = 19) the therapy significantly increased the level of Ab-BPDE-DNA (EI = 0.29/0.19–0.34 vs. 0.31/0.25–0.40; median/lower–upper quartile; p < 0.01). The results support the idea of Ab-BPDE-DNA level as a possible tentative indicator of exposure, effects and susceptibility of the organism to the exposure of BaP (PAHs).

KEYWORDS

polycyclic aromatic hydrocarbons; coal tar; BPDE-DNA adducts; antibodies; psoriasis; goeckerman therapy; children

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INTRODUCTION

Psoriasis is a multifactorial, chronic inflammatory skin disease, which often breaks out during childhood. Although the incidence of childhood psoriasis is unknown, some studies indicate that the first sign of psoriatic symptoms occurs in infants, children, and adolescents (1, 2). It is estimated that 30–50% of adults with psoriasis develop the condition before the age of 20 years (2). Psoriasis can adversely affect the quality of life (3) and represents the top 10 skin diagnoses in children (4).

Children are more susceptible to genotoxic effects of environmental exposures and medical treatments than adults because their organism is still developing (5). Recent trends in childhood cancers in the USA and Europe seem to confirm children's increased exposures to genotoxic/carcinogenic substances (6).

Goeckerman therapy (GT) represents a treatment of plaque psoriasis in children (7). The therapy combines dermal exposure of crude coal tar (CCT) ointment and UV radiation (UVR) (7–12). It seems that fundamental mechanism of therapeutic effects of CCT is based on immunosuppression induced by polycyclic aromatic hydrocarbons in coal tar (PAHs) without signs of systemic immunotoxicity (9, 12). Retrospective studies have demonstrated that GT is effective in children and adolescents with moderate to severe psoriasis (7–12). However, the use of GT has recently decreased for several reasons, including a supposed genotoxicity of CCT/PAHs (13–14).

Several PAHs are recognized as potential environmental mutagens and carcinogens requiring bioactivation (15). Typical representative of PAHs, benzo[a]pyrene (BaP), is bioactivated (metabolized) into a highly reactive genotoxic metabolite, benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE), which is able to bind to DNA or proteins and create corresponding BPDE-DNA or BPDE-protein adducts (14). Increased levels of BPDE adducts indicate recent genotoxic exposure to BaP (5). Cigarette smoke contains PAHs (including BaP) and this way contributes to the total level of BPDE and related adducts in smokers (16).

Adducted DNA becomes immunogenic and induces immune response by production of antibodies against BPDE-DNA adducts (Ab-BPDE-DNA). Circulating Ab-BPDE-DNA have been found in the serum of persons exposed to PAHs/BaP (occupational exposures, smokers) (17, 18) and subsequently they were proposed as a potential biomarker of genotoxic exposure to BaP (PAHs) (19, 20). The number of studies dealing with this biomarker is relatively small and its practical use is still associated with uncertainties. Those are associated with individual factors, which are still largely unidentified (20). In order to contribute to reduction of the uncertainties, the presented study describes level of Ab-DNA-BPDE in the group of children dermally exposed to PAHs.

MATERIALS AND METHODS

STUDY GROUP

Basic characteristics of the study group were described in our previous work (8). Briefly, the group was formed

of children with chronic stable plaque psoriasis, treated by GT at the Clinic of Dermal and Venereal Diseases, University Hospital, Hradec Králové (Czech Republic). Over the period of two years, we collected the data of 19 children (12 girls and 7 boys; average age of 12 years; range 5–17 years). Patients' exposure history to PAHs (including smoking) was checked by the questionnaire and patients who admitted previous significant exposure were excluded from the monitored group. The study was approved by the Ethics Committee of the University Hospital in Hradec Králové, Czech Republic. Informed written consent was obtained from the parents of each patient.

GOECKERMAN THERAPY (GT)

Detailed treatment procedure and the content of 16 selected PAHs in dermatological CCT were described previously (8). The therapy was based on daily application of dermatological ointment, containing 3% of CCT. According to the extent of lesions, 17–40% of the total body surface was covered by CCT ointment. Simultaneously, the patients were daily whole-body irradiated by UVR. Duration of the treatment was modified according to its effectiveness (average duration of 18 days; range 14–22 days). The effectiveness of the therapy was expressed by PASI score (Psoriasis Area and Severity Index) (21).

SERUM LEVEL OF AB-BPDE-DNA

The samples of heparinized venous blood were collected by venipuncture of the cubital vein before the first treatment and again after the total completion of GT. The obtained serum samples were stored in under -70°C until they were analyzed. The level of Ab-BPDE-DNA (IgG, IgM) was determined by ELISA method. The results were expressed as the Evaluation Index (EI = absorbance of evaluated serum / absorbance of high positive control serum). Samples with EI less than 0.5 were termed as the serum with low level of Ab-BPDE-DNA. Analogously, the serum samples with EI greater than 0.5 were referred to as the serum with high level of Ab-BPDE-DNA (ELISA-VIDITEST anti-BPDE-DNA human, VIDIA, Jesenice, Czech Republic).

STATISTICAL ANALYSIS

The data were analyzed by using MATLAB rel. 2014b software (Mathworks, Inc., Massachusetts, USA). Because the Lilliefors test of normality had rejected the hypothesis of normal distribution, the nonparametric tests were used. Data were analyzed by the Wilcoxon signed rank test. The association between the serum level of Ab-BPDE-DNA after the therapy and the selected parameters was evaluated by Spearman rank Order Correlations.

RESULTS

In the group of children with psoriasis, dermatologically exposed to coal tar (within the GT) we evaluated the efficacy of the therapy and observed the level of Ab-BPDE-DNA. Smoking was reported by five patients. The number

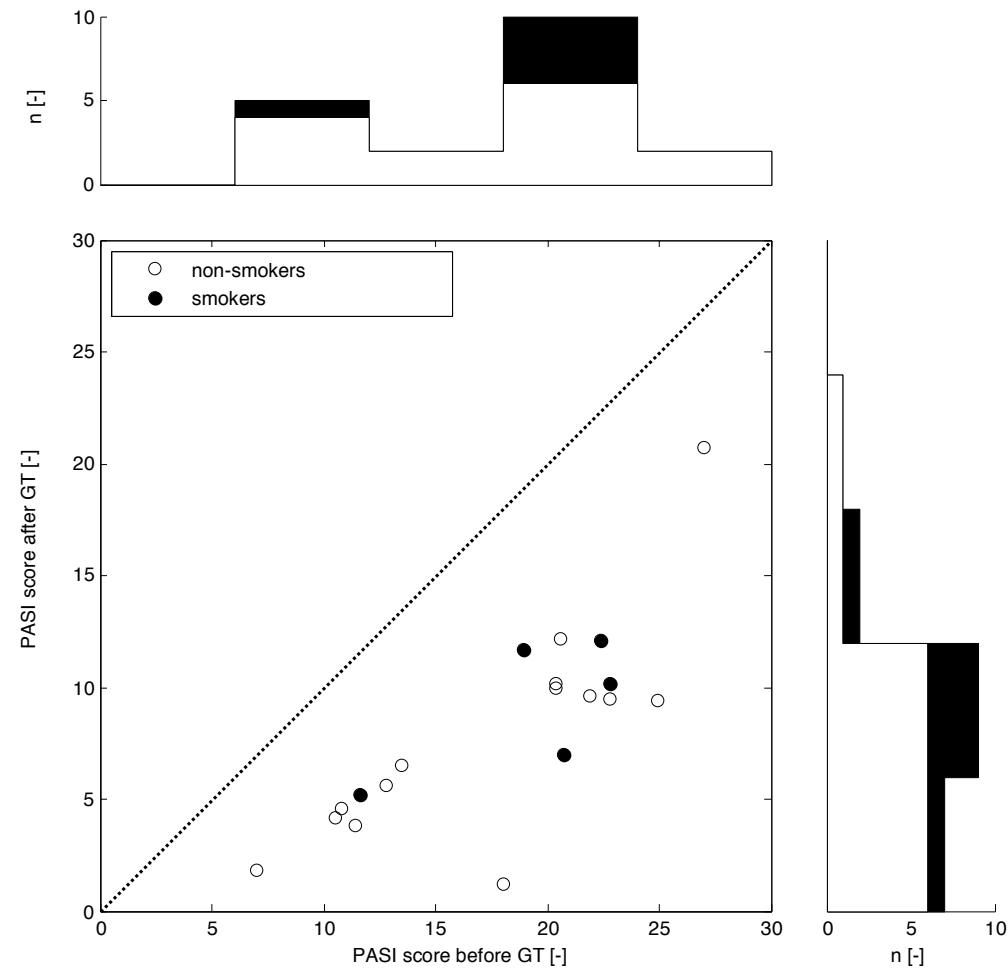


Fig. 1: The levels of PASI score. Legend: The levels are dimensionless. Scatter plot depicts PASI score before and after the GT therapy. Altogether 19 dots represent 38 measurements each dot belongs to one patient. The top histogram shows data distribution before treatment, the right side histogram corresponds to the after treatment values distribution. The white zones represent non-smokers data, and the black zones depict smokers' ones.

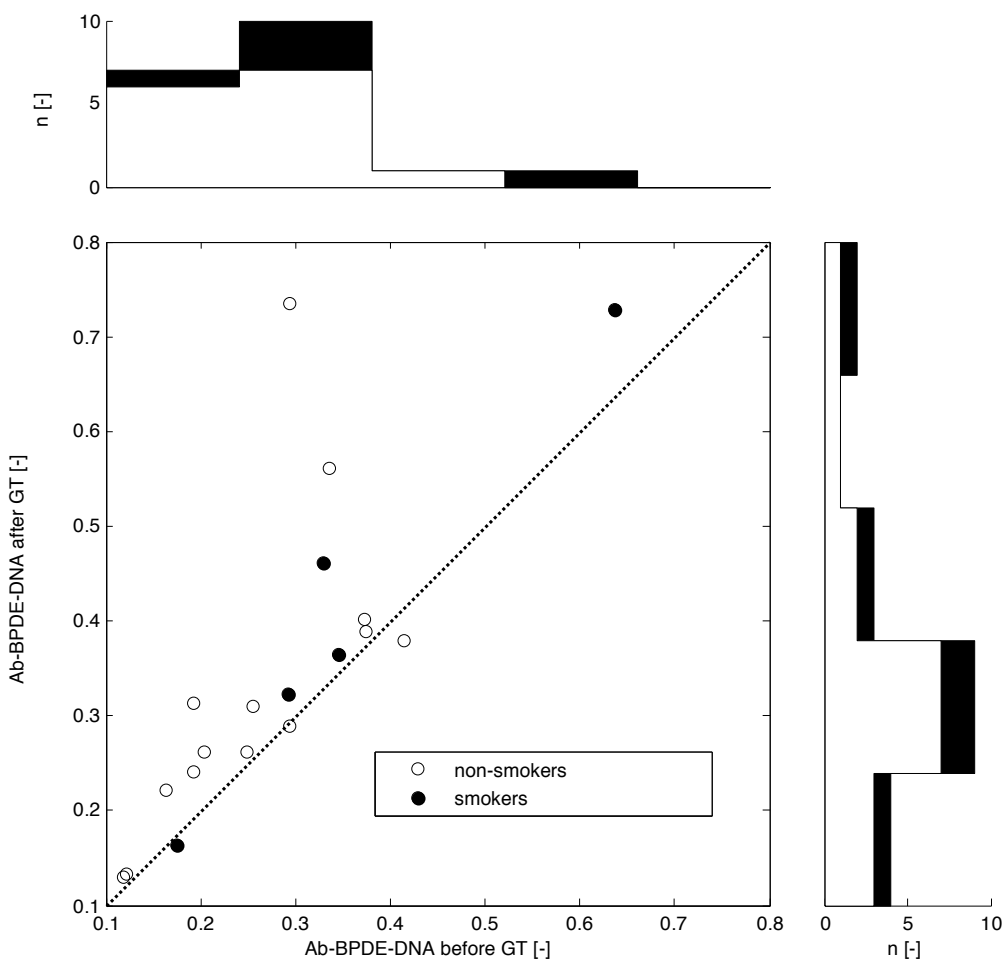


Fig. 2: The levels of Ab-BPDE-DNA. Legend: The levels are dimensionless (EI). Scatter plot depicts Ab-BPDE-DNA before and after the GT therapy. Altogether 19 dots represent 38 measurements, each dot belongs to one patient. The top histogram shows data distribution before treatment, the right side histogram corresponds to the after treatment values distribution. The white zones represent non-smokers data, and the black zones depict smokers' ones.

of smoked cigarettes was overall low, irregular and ranged from one to four cigarettes per “normal” day (outside the hospital). During hospitalization, they almost did not smoke at all. Due to the low number of patients, the group of “smokers” was not statistically evaluable. Therefore, the statistical evaluation was performed only for the whole group of respondents (see below).

The therapy was highly effective because the PASI score significantly decreased (20.4/11.9–22.3 vs. 9.4/4.8–10.2; median/lower–upper quartile; $n = 19$; $p < 0.001$) (Fig. 1).

After the therapy we found significantly increased serum level of Ab-BPDE-DNA (EI = 0.29/0.19–0.34 vs. 0.31/0.25–0.40; median/lower–upper quartile; $n = 19$; $p < 0.01$) (Fig. 2). We did not find a significant relationship between the level of Ab-BPDE-DNA (after GT) and total duration of the therapy ($r = 0.30$), body surface area covered by CCT ointment ($r = 0.02$), total time of UV radiation ($r = 0.09$) and PASI score ($r = 0.21$).

DISCUSSION

Genotoxic effects of chemical and physical factors have been studied largely in adult population, however only a limited number of studies have investigated genotoxic damage in children. It is rather surprising because children represent a population group with high sensitivity to chemical and physical factors. The studies suggest that early exposures during childhood can play an important role in the development of chronic diseases in adulthood: the earlier the exposure, the greater the risk of chronic disease, including cancer (22).

In our previous works, we studied genotoxic and immuno-toxic effects of combined exposure to PAHs and UVR on human organism. Monitored groups consisted of patients (men, women, and children) suffering from psoriasis treated by GT. In the groups of children, we found high degree of dermal absorption of PAHs (elevated levels of urinary 1-hydroxypyrene and hydroxylated phenanthrenes), elevated urinary mutagenicity (Ames test), and increased genotoxicity (chromosomal aberration in peripheral lymphocytes) (8, 10, 11). In addition, we found an elevated level of cellular stress (heat shock proteins; Hsp70) (11), oxidative stress (8-hydroxy-2'-deoxyguanosine, 8-hydroxyguanosine and 8-hydroxyguanine), and BPDE-DNA adducts (8).

In the presented study, the serum level of Ab-BPDE-DNA increased significantly ($p < 0.01$) after the therapy. However, it should be noted that the majority of Ab-BPDE-DNA values (84% of all samples) were found in the zone of low levels (EI < 0.5). Three samples ranged in high values (EI > 0.5), whilst one of them was higher even before the treatment. Similar results were obtained in our previous study focused on the level of Ab-BPDE-DNA in two groups of adult patients with psoriasis, treated by GT (3% and 5% CCT) (8). Other literary data concerning the levels of Ab-BPDE-DNA after therapeutic exposure to CCT are very limited and comparison with these data is complicated because of the use of different methodologies (23).

The term of biological monitoring includes markers of exposure, effect and susceptibility. Circulating Ab-BPDE-DNA signals the presence of genotoxic BPDE-DNA adducts in the organism. It means the level of Ab-BPDE-DNA must be influenced by the same factors as the level of BPDE-DNA adducts, i.e., by the level of exposure to PAHs, by the individual character of BaP metabolism and by the degree of adaptation and reparation processes. In addition, the serum level of Ab-BPDE-DNA also reflects the immune status and a protective capacity of the immune system against BaP induced cancer (18).

The previous paragraphs suggest that the level of circulating Ab-BPDE-DNA could serve as a marker of exposure to PAHs. Elevation of exposure to PAHs increases both the level of BPDE adducts and the related level of Ab-BPDE-DNA.

The BPDE-DNA adducts are assumed to be genotoxic and present a biological response of the organism to genotoxic exposure of PAHs. In this context, the level of Ab-BPDE-DNA can be considered a marker of genotoxic effect (marker of effect).

Finally, the Ab-BPDE-DNA level reflects individual character of metabolism (BaP), condition of adaptation and reparation processes, immune status and protective capacity of the immune system. All of these systems determine the level of individual susceptibility. For this reason, the level of Ab-BPDE-DNA can serve as a tentative marker of individual sensitivity to exposure to BaP.

Application of CCT (PAHs) increases the level of BPDE-DNA adducts and consequently increases the production of Ab-BPDE-DNA. However, PAHs have also an immunosuppressive effect and in this way they can reduce the production of Ab-BPDE-DNA. Therefore, the final level of Ab-BPDE-DNA is probably the result of these opposite processes. It seems likely that repeated or chronic exposure to PAHs (for instance, by occupational atmosphere or by smoking) reduces body immune response and presumably can reduce levels of anti-PAHs antibodies (9, 17). Described mechanisms may be one of the reasons why we found no significant association between the level of Ab-BPDE-DNA and the characteristics of exposure (particularly the duration and the extent of exposure).

We found only few epidemiological studies of PAHs exposed population to assess the impact of carcinogen-specific antibodies on the risk of tumor development and on the relation to other indicators of genotoxic exposure (17, 18, 20). Few attempts have also been made, both in vivo and in vitro, to understand the implications of an antibody response to metabolic activation of carcinogens and carcinogenesis (24, 25). Recent study provided the evidence that specific humoral immunity might modulate the genotoxic effect induced by subsequent carcinogen exposure, however, the mechanisms involved remain largely unexplored (19).

GT is an effective treatment for moderate and severe psoriasis in children (7–12). In this study, the high effectiveness of the therapy was confirmed by a significant decrease of the PASI score.

CONCLUSION

The results of presented study support the idea of Ab-BPDE-DNA level as a possible tentative indicator of exposure, effects and susceptibility of an organism to the exposure of BaP (PAHs).

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CONFLICT OF INTEREST

None declared.

APPROVAL AND INFORMED CONSENT

The study was approved by the Ethics Committee of the University Hospital in Hradec Králové, Czech Republic. Informed written consent was obtained from each patient.

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