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DETERMINATION OF TETRAHYDROXYLATED METABOLITES IN HAIR AND DNA OF RATS UNDER CONTROLLED EXPOSURE TO A MIXTURE OF POLYCYCLIC AROMATIC HYDROCARBONS: NEW BIOMARKERS FOR ASSESSING HUMAN EXPOSURE

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1. Introduction

Although the analysis of polycyclic aromatic hydrocarbon (PAHs) metabolites in urine has long been considered a reference biomarker of exposure to PAHs, hair analysis was also more recently demonstrated to enable the biomonitoring of PAH exposure with the advantage of wider windows of detection [1]. In addition to the "classically-analyzed" monohydroxylated metabolites, recent studies have shown the interest of the analysis of tetrahydrotetrols in urine for the assessment of human exposure to PAHs [2]. To obtain more comprehensive information on exposure, the range of OH-PAHs used as biomarkers was widened to include tetra-OH-PAH isomers in hair. This study hypothesized that these metabolites could constitute suitable biomarkers for the assessment of PAH exposure as they allow quantitative evaluation of the internal dose, appear more relevant than mono-hydroxylated forms for the measurement of heavy compounds and supply information about the toxicity of the parent compound linked to individual's specific metabolism.

The present work therefore aims to develop a method for the determination of tetra-OH-HAPs in hair as new biomarkers of chronic PAH exposure. The suitability of the latter was evaluated by comparing the concentration measured in hair and the levels of DNA adduct found in a rat model exposed to a PAH mixture.

2. Materials and methods

Animal treatment. Adult female Long Evans rats (n=64) were randomly allocated to experimental groups receiving low doses of 0.01 to 0.8 mg/kg of body weight of a mixture of 16 PAHs solubilized in oil, by oral administration, 3 times per week over 90 days. Control rats received the vehicle only. Hair were shaved prior to the beginning of the experiment in order to ensure that the hair collected at the end of the experiment only represented the period of exposure and stored at -20°C. Blood was collected after the last PAH administration and DNA isolation was performed as previously described [3].

Method for the determination of tetra-OH-PAHs in hair. A method based on gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) allowing the quantitative analysis of 10 tetra-OH-PAHs representative of 4 parent PAHs (benz[a]anthracene-r-8,t-9,c-10,t-11-tetrahydrotetrol (B[a]A-RTCT), benz[a]anthracene-r-8,c-9,c-10,t-11-tetrahydrotetrol (B[a]A-RCCT), benz[a]anthracene-r-8,c-9,t-10,t-11-tetrahydrotetrol (B[a]A-RCTT), benzo[a]pyrene-r-7,t-8,t-9,c-10-tetrahydrotetrol (B[a]P-RTTC), benzo[a]pyrene-r-7,t-8,t-9,t-10-tetrahydrotetrol (B[a]P-RTTT), benzo[a]pyrene-r-7,t-8,c-9,c-10-tetrahydrotetrol (B[a]P-RTCC) and benzo[a]pyrene-r-7,t-8,c-9,t-10-tetrahydrotetrol (B[a]P-RTCT) and phenanthrene-tetrahydrotetrol (tetra-OH-Phe) and chrysene-tetrahydrotetrols (tetra-OH-Chry I and II) obtained by hydrolysis of their respective diol-epoxydes) was designed [4]. Hair powder extract was submitted to liquid-solid extraction, followed by C_{18} solid-phase purification. The analytes were derivatized using N-Methyl-N-(trimethylsilyl)trifluoroacetamide and analyzed by GC-MS/MS in negative ionization mode. The calibration curve performed on 10 concentration levels was linear from the LOQ up to 40 pg/mg for all the isomers of tetra-OH-Phe, tetra-OH-Chry, tetra-OH-B[a]A and tetra-OH-B[a]P tested. The coefficients of determination were above 0.970 and the recoveries stood between 55.0 % and 82.6 % for each compound. This method allows reaching limits of quantifications ranging from 0.05 to 1 pg/mg in hair depending on compound.

3. Results and discussion

The applicability of the tetra-OH-PAH analysis in hair was firstly evaluated on the dose-response study conducted on rats under repeated exposure to PAHs. With the exception of B[a]A-RTCT, B[a]A-RCTT and B[a]P-RTCC, which were only detected in hair at the two highest levels of exposure, all the investigated tetra-OH-PAHs were measured in both the controls and the treated rats, regardless of the levels of exposure (Fig 1). These results therefore pointed out the high sensitivity of the method for

monitoring environmental exposures. As to the appearance profiles of tetra-OH-PAHs in hair, tetra-OH-chry appear as the most abundant metabolites followed by tetra-OH-B[a]A and tetra-OH-B[a]P (Fig 1). The results obtained for tetra-OH-B[a]Ps in hair were consistent with those observed in the previous study on B[a]P only, with B[a]P-RTTC as preponderant compound in this matrix [4]. The analysis of tetra-OH-PAHs with more than 4 aromatic rings allows detecting levels of exposure 2 to 10 times below those detected by the analysis of their respective mono-hydroxylated forms (data not shown). For instance, contrary to B[a]P-RTTC which was already detected in control rats, 3-OH-B[a]P was only detected from the intermediary levels (0.08 mg/kg). Strong linear relationships (R^2 ranging from 0.805 to 0.964, $p<0.001$) were observed between the administered dose and the tetra-OH-PAH concentration in hair for 7 out of the 10 analytes confirming the relevance of tetra-OH-PAH analysis in hair as biomarkers of exposure to PAHs.
Finally, the analytical parameters determined for the tetra-OH-PAHs in hair's method have been transposed to the DNA one to determine whether the isomers identified in hair could be released after DNA hydrolysis [3]. The upgraded DNA method allowed detecting the presence of tetra-OH-Phe, tetra-OH-B[a]A and tetra-OH-chry and tetra-OH-B[a]P metabolites in DNA hydrolysates. Statistical analysis conducted on B[a]P-RTTC isomer demonstrated linear relationships between the dose of PAHs administered and the number of DNA adducts formed ($p<0.05$) ; or the concentration detected in hair ($p<0.001$; Fig 2A) as well as a significant correlation between B[a]P-RTTC concentration in hair and the number of DNA adducts ($p<0.05$; Fig 2B) showing its ability to predict DNA-alterations.

4. Conclusion
By widening the range of PAH metabolites used as biomarkers of exposure so as to include tetra-OH-PAH isomers (especially those exhibiting over 4 aromatic rings), the analysis of tetra-OH-PAH in hair will enable multi-exposure assessments which are more accurately representative of actual situations of exposure to PAHs. Since the various types of exposure to PAH's are highly suspected to be associated with certain chronic diseases, one is naturally led to wonder if these biomarkers could not also be predictive of effects induced by these compounds at the level of biological tissue.

5. Acknowlegment
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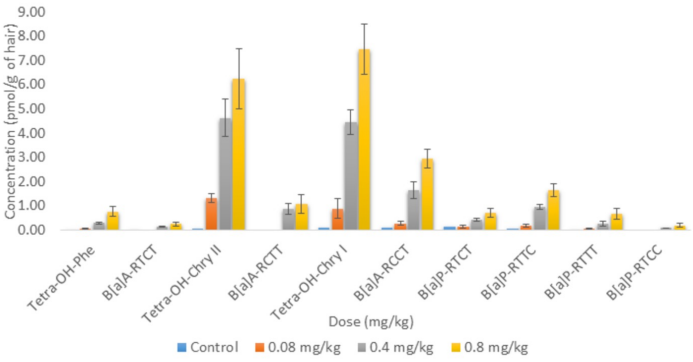

Fig 1: Concentration of tetra-OH-PAHs in hair of rats exposed to a mixture of PAHs (0.08, 0.4 and 0.8 mg/kg body weight) for 90 days .

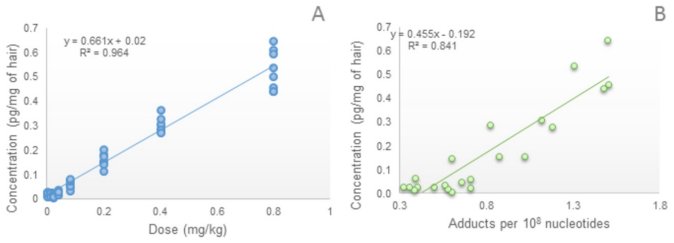

Fig 2: B[a]P-RTTC concentration in hair of rats exposed to PAHs C) Correlation between B[a]P-RTTC concentration in hair and the quantity of DNA adducts per 10^8 nucleotides for B[a]P-RTTC.