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EPIGENETIC CHANGES UPON MULTI-RESIDUE EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS

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Introduction

Besides genetic mechanisms, rapidly growing evidences have linked environmental pollutants (e.g. polycyclic aromatic hydrocarbons, PAH), with epigenetic variations, including changes in DNA methylation among others [1]. Whether these changes are a response to DNA damage and/or a consequence of environment exposure needs to be further explored. To date, studies concerning the effect of PAH exposure on the global DNA methylation are limited and contradictory results have been observed [2-5]. The reported differences might be partially explained by the differences between single compound exposures, generally used for in vitro studies [2;3], and humans' environmental multi-residues exposure [4;5].

In this context, the present study was intended to study the epigenetic modification induced by multi-residue exposure to PAH, based on an animal model. Rats sub-chronically exposed to a mixture of 16 US-EPA priority PAH were used to assess the (hydroxyl)methylation status of genomic DNA and RNA, together with reduced and oxidized forms of glutathione.

Materials and Methods

Animal Experimentation.

Sixty-four Long Evans female rats were randomly allocated to each experimental groups receiving from 0.01 to 0.8 mg/kg body weight of the 16 PAH pointed out by US-Environmental Protection Agency, by oral administration, 3 times per week over a 90-day period. The exposure levels were far below the LD50 for all the molecules tested. After the 90-day experiment, livers were dissected and weighed. The base of the left lateral liver lobe was divided into 5 pieces of 100mg each, which were placed in cryogenic tubes, frozen in liquid nitrogen and stored at -80°C before analysis.

Liver Samples Analysis

Glutathione determination. Glutathione (GSH) and glutathione disulfide (GSSG) were analysed in liver tissue by UPLC-MS/MS, according to a protocol recently published [6].

Epigenetic markers analysis. Genomic DNA/RNA was isolated from frozen tissue using the AllPrep DNA/RNA kit (Qiagen) following manufacturer´s protocol. Isolated DNA/RNA (1 µg) was further enzymatically hydrolyzed to individual deoxyribonucleosides [7]. 2'-deoxycytidine(2-DC), 5-methyl-2'-deoxycytidine (5-mDC), 5-hydroxymethyl-2´-deoxycytidine (5-hmDC) were quantified using a HILIC-UPLC-MS/MS method.

Results

Glutathione modulation. GSH/GSSG ratio significantly increased in the rats treated with lower PAH concentration (i.e. 0.01, 0.02, and 0.04 mg/kg bw) (fig. 1). Furthermore, the synthesis of reduced glutathione seems to reach a threshold at PAHs treatment 0.04 mg/kg bw, followed by a decrease in GSH/GSSG ratio at the higher PAH treatments.

Global DNA (hydroxy)methylation modulation. Decreased 5-mDC and 5-hmDC in DNA were observed for rats exposed to lower doses (i.e. 0.01, 0.02, and 0.04 mg/Kg bw) (figure 2, left side). In contrast, increased levels of 5-mDC and 5-hmDC in DNA were observed at highest treatment levels (i.e. 0.4 and 0.8 mg/Kg bw).

Global RNA (hydroxy)methylation modulation. The RNA 5-mDC decreased in the first 3 treatment groups (i.e. 0.01, 0.02, and 0.04 mg/Kg bw) until reaching a plateau between treatments 0.04 and 0.2 mg/

kg bw (fig. 2, right side). The RNA 5-mDC at 0.4 and 0.8 mg/kg bw is slightly higher than for control. Unlike 5-mDC, 5-hmDC increases in the first 3 treatment groups, and decreases to a lower value than the control group in the highest level (fig. 2, right side).

Discussion

In the present study, to be as close as possible to the environmental exposure, liver samples from rats treated systemically with a mixture of PAH have been analysed for glutathione and epigenetic alterations. Liver was chosen because of its essential role in PAH metabolism. In fact, during phase I metabolism, PAH molecules are oxidized into several epoxide and diol metabolites that are biologically more active forms then the mother compounds. These metabolites are further conjugated to glutathione during phase II metabolism or may induce direct lesions to DNA and/or RNA.

It has been hypothesized that an increased need of GSH, upon environmental exposure to persistent pollutants, shunt homocysteine into GSH synthesis and further reduces methionine and S-adenosylmethionine (SAM), which are key methyl donors for the methylation of DNA, RNA and other proteins [8]. Our results confirm this hypothesis. We observed an increase of GSH after chronical exposure to relatively low amounts of 16 US-EPA PAHs due to a stimulation of phase II detoxification mechanism though GSH conjugation in response to unbalance redox environment. This increased need of GSH leads to global DNA and RNA hypomethylation. In contrast, our results indicate an increased level of DNA methylation for the highest exposure levels. This can be explained by the already described positive association between global DNA methylation levels and B(a)P DNA-adducts. The positive association is potentially due to increased methylation at sites of BaP–DNA adducts [9]. Thus, we further hypothesize that depending on the levels of exposure, PAH toxic mechanism is changing from depletion of global DNA/RNA methylation at lower levels to the formation of DNA adducts at higher levels.

Conclusion

In conclusion, these results demonstrated that a multi-residue exposure to PAHs affects glutathione status, DNA (hydroxy)methylation, and RNA (hydroxy)methylation. In addition, we found a hormetic response relationship between PAH concentration, the levels of glutathione and several epigenetic marks at environmental relevant doses. This hormetic response may give critical insights into the PAH epigenetic carcinogenicity.

References

1. Ravegnini, G., Sammarini, G., Hrelia, P., and Angelini, S. (2015) Toxicol Sci.148(1), 2-13
2. Tabish, A.M., Poels, K., Hoet, P., and Godderis, L. (2012) PLoS ONE 7(4): e34674
3. Pavanello, S., Bollati, V., Pesatori, A.C., Kapka, L., Bolognesi, C., Bertazzi, P.A., and Baccarelli, A. (2009) Int J Cancer 125, 1692–1697
4. Yauk, C.L., Polyzos, A., Rowan-Carroll A., Kortubash, I., Williams, A., and Kovalchuk, O. (2008) Environ Mol Mutagen 49(1), 26-35.
5. Janssen, B., Godderis, L., Pieters, N., Poels, K., Kicinski, M., Cuypers, A., Fierens, F., Penders, J., Plusquin, M., Gyselaers, W., Nawrot, T. (2013). Particle and Fibre Toxicology, 10 (22), 1-11
6. Luyts, K., Smulders, S., Napierska, D., Van Kerckhoven, S., Poels, K., Scheers, H., Hemmeryckx, B., Nemery, B., Hoylaerts, M.F., and Hoet, P.H. (2014) Part Fibre Toxicol., 11:61.
7. Godderis, L., Schouteden, C., Tabish, A., Poels, K., Hoet, P., Baccarelli, A., and Van Landuyt, K. (2015) Biomed Res Int., Article ID 845041
8. Lee, D.H., Jacobs, D.R. Jr., and Porta, M. (2009) Environ Health Perspect 117(12), 1799-802.
9. Herbstman, J.B., Tang, D., Zhu, D., Qu, L., Sjödin, A., Li, Z., Camann, D., and Perera, F.P. (2012) Environ Health Perspect 120(5), 733-8.

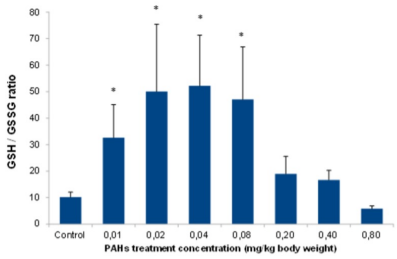

Figure 1 – Glutathione modulation in rat liver upon exposure to PAH mixture.
Upon One-way ANOVA statistical analysis, the differences among means of each group were statistically significant (P = 0.0107). Subsequently, using a Bonferroni's multiple comparisons test the statistical significances vs. control group has been analysed and graphically represented (* - P < 0.05)

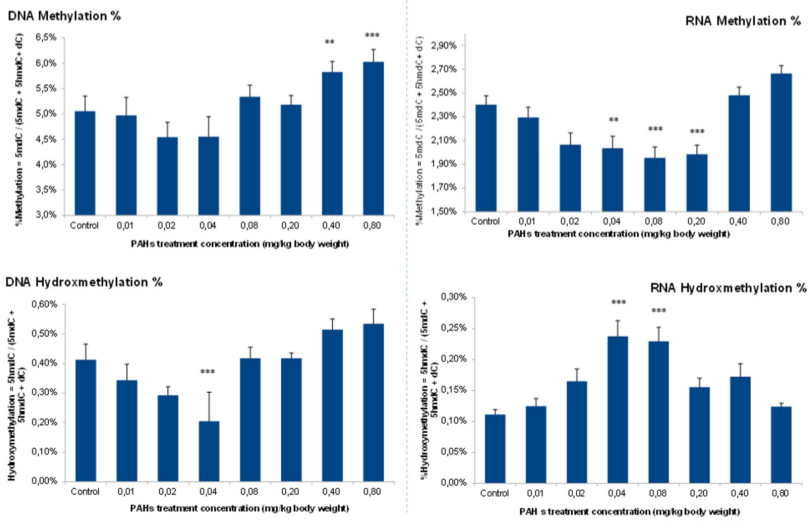

Fig. 2 - Global DNA/RNA (hydroxy)methylation modulation in rat liver upon exposure to PAH mixture.
Upon One-way ANOVA statistical analysis, the differences among means of each treatment group were statistically significant (P values < 0.001). Subsequently, using a Bonferroni's multiple comparisons test the statistical significances vs. control group has been analysed and graphically represented (** - P < 0.01; *** - P < 0.001)