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Exposure and Effect Biomarkers for Environmental Pollution and Cigarette Smoking: Statistical Analysis of Data from Volunteers

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Abstract

The HPLC-MS/MS results of benzene and nicotine metabolites as exposure biomarkers obtained on urine samples of 336 non or ex -smokers and 110 smokers living in the same area of Italy, and of oxidized DNA and RNA bases as oxidative stress (effect) biomarkers in a subsample of 133 subjects, are analyzed by means of statistical tools. There is a positive Pearson's correlation (0.65) between cotinine and S-phenyl-mercapturic acid (SPMA) concentrations in smokers, and the intercept for cotinine=0 corresponds to the mean SPMA value of the non-smokers of 0.2 μg/g of creatinine. Oxidative stress biomarkers in smokers (n=31) positively correlate with SPMA and better with cotinine, indicating that DNA/RNA damage is partly produced by benzene but also by other smoke constituents; the non-smokers mean values are comparable to the smoker's, indicating that environmental pollution significantly contributes to DNA and RNA damage, and suggesting that the nucleic acids repair mechanism is saturated by smoking.

Introduction

The environmental exposure to benzene, resulting from traffic and smoking, causes oxidative damage to DNA and RNA [1, 2]. This produces a tissue inflammatory damage, cell aging, diabetes, neurodegenerative, cardiovascular, and other age-related diseases, and even the development of some cancers. Oxidative stress is an imbalance between the production of ROS (Reactive Oxygen Species) and the ability of the biological system to repair the damage. The most sensitive dose biomarkers used for the assessment of benzene exposure are urinary SPMA and t,t-muconic acid (t,t-MA), while cotinine, the urinary metabolite of nicotine is the biomarker for smoking exposure. The oxidative damage/repair markers of DNA and RNA, 8-hydroxy-guanine (8oxoGua), 8-hydroxy-2'deoxyguanosine (8oxoGuo) and 8-oxo-7,8-dihydroguanosine (8oxodGuo) determined in the same samples show a correlation between exposure to benzene and oxidative stress.

Materials & Methods

SPMA, t,t-MA and cotinine: samples were hydrolyzed with 6N HCl at pH 2 and added with internal standards before being subjected to SPE: in order to retain and elute the acidic and basic metabolite using the same cartridge, the pH of the washing and eluting solvents was varied obtaining two fractions that are separately injected into the HPLC system. Separation was performed on reverse phase C18 column using a gradient of acetonitrile and acetic acid 1% v/v in water. The precursor --> product ionic transitions monitored are in the negative ion mode for the acids, i.e. 141.0 -->97.00 for t,t-MA, 145.0 --> 100.0 for t,t-MAd_4, 240.1 --> 109.1 for SPMA and 238.1 --> 109.1 for SPMAd_2, and in the positive ion mode for cotinine, 177.3 --> 80.10, and for cotinine-$d_3$180.3 --> 80.10.

The biomarkers of oxidative stress have been analyzed injecting the centrifuged urine added with the internal standards 8oxodGuo ($^{13}C^{15}N_2$) and 8oxoGuo ($^{13}C^{15}N_2$) directly into the HPLC system. They are separated on the same column using a gradient of 10% methanol in acetonitrile and 0.5% acetic acid (v/v). The m/z transitions are all in the positive ion mode: for 8oxoGua 168.0 --> 140.0, for 8oxodGuo 284.3 --> 168.0, 8oxoGuo 300.24 --> 168.2, 8oxodGuo ($^{13}C^{15}N_2$) 287.16 --> 171.1 and for 8oxoGuo ($^{13}C^{15}N_2$) 302.23--> 171.0.

The normality of the distribution of the concentrations of the analytes was evaluated using the one-sample Kolmogorov–Smirnov test. Statistical analyses were always performed using parametric

methods (Pearson's correlation, t-test for independent variables). When transformation of the data to attain a normal distribution was necessary, parametric tests were applied on the log-values.

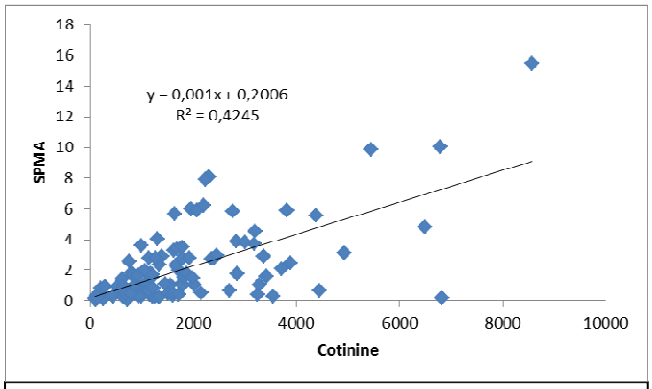

Figure 1: SPMA and cotinine regression in smokers

Results

The cutoff value for the definition of smoker is urinary cotinine > 100 μg/g of creatinine. The mean SPMA value in smokers is about ten times that of non-smokers (0.23 vs 2.08 μg/g of creat.). If SPMA of smokers is plotted against cotinine the intercept for cotinine=0 corresponds to the mean SPMA value of the non-smokers (figure 1). The mean values for the oxidative stress markers are reported in table 1.

Table 1: oxidative stress markers expressed as nmol/millimol creat.

All n=133			Smokers n= 24			Non-Smokers n= 80			Ex- Smokers n=29		
8oxo Gua	8oxo dGuo	8oxo Guo	8oxo Gua	8oxo dGuo	8oxo Guo	8oxo Gua	8oxo dGuo	8oxo Guo	8oxo Gua	8oxo dGuo	8oxoG uo
24.56	3.13	4.61	24.17	3.33	4.52	28.12	2.83	4.57	21.73	3.25	4.65

The Pearson's correlation among all the analytes are reported in table 2 for smokers, while again, there are no correlations for non-and ex-smokers.

Table 2: Pearson's r for urinary biomarkers in smokers

Log_	t,t-MA	Cotinine	8oxoGua	8oxodGuo	8oxoGuo
SPMA	0.434	0.652	0.203	0.677	0.394
t,t-MA		0.406	0.058	0.259	0.247
Cotinine			0.386	0.748	0.502
8oxoGua				0.384	0.099
8oxodGuo					0.561

Conclusions

Results indicate that cigarette smoke is the main source of benzene exposure in smokers, for which the environmental benzene is a small additional risk factor, even if it cannot be defined a negligible one, due to its carcinogenic properties. The positive correlation of SPMA with 8oxodGuo (0.677) confirms the DNA damage/repair activity is due to the benzene exposure, and that of cotinine (0.748) also to smoke as a complex mixture of toxicants. The non-and ex-smokers mean values are not statistically different from the smoker's, indicating that environmental pollution significantly contributes to DNA and RNA damage; this result, together with the correlation of the oxidative stress markers with cotinine suggests that in smokers the nucleic acids repair mechanism is activated and also saturated by smoking, and it is not able to further increase in response to environmental exposures to oxidative stress.

References

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