

Redox balance of Thiols in the exhaled breath condensate (BEC) in two populations with different exposure to traffic related pollutants.

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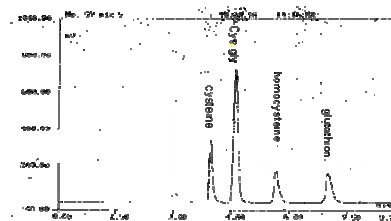
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Thiols such as cysteine (Cys), cysteinylglycine (Cysgly), homocysteine (Hcy) and glutathion (GSH) are widely distributed in humans and play an important role in biological systems. Normal levels of thiols in physiologic fluids are considered as markers of good homeostatic equilibrium (Bloemen, 2007). On the contrary, their alteration has been associated with various diseases (asthma, COPB), usually related with increased production of reactive oxygen species (ROS). Therefore, levels of reduced and oxidized forms of thiols in plasma, in red cells and, more in general in all biological fluids - including exhaled breath condensate (EBC) - is considered a good marker of ROS activation and an early marker of related diseases (Kharitonov, 2006). Two groups of patients (older than 65 years), living in "hospices for retired peoples" have been recruited and compared, (n= 38, age 82 ± 9y) with the aim of evaluating cardiovascular and respiratory adverse-effects of environmental pollution, namely of pollutants related to urban traffic. In particular, one Hospice was located within 200m from high traffic crossroads, whereas the other was sited in a park, far from the main crossroads, and this group of subjects was compared with 35 subjects (age 70±8y) living in Aprica, a remote alpine site (1118 m. a.s.l.). EBC samples were collected and HPLC analysis was performed for the evaluation of thiol levels. In addition, oxidative stress state was also evaluated by isoprostane (8-iso-PGF2alfa), a lipid peroxidation index, by LC-MS/MS method. No differences in 8-iso-PGF2alfa were found between the two groups as the levels were under the detection limits (150µM). In the EBC of 35 subjects from Aprica the presence of various molecules, showing SH groups, was found, in particular in the oxidized form (fig.). Retention time of eluted peaks did not correspond to known thiols. Therefore, an accurate qualitative and quantitative analysis of the various thiols was not possible, only using HPLC. However, there was a striking individual variability of the various chromatographic peaks, which corresponded to fluorescent adducts detected by fluorimetric detector. Since the method is highly specific for S containing groups, it is presumed that observed peaks mainly correspond to low molecular

weight molecules with active SH groups (fig.). Since no signal concerning reduced species was detected, two possible hypotheses can be envisaged : 1) reduced species could be present in small amounts below the lowest threshold for detection; 2) the absence of reduced species could be due to the pro-oxidant environment of the breath exhaled condensate. In the latter evenience, total thiols, obtained by reduction of all thiols by a specific compound, such as tris (2carboxiethyl) phosphine, only included oxidized thiols



The number of samples from Milan, due to the very old age of patients living in public hospices, and then the difficulty of obtaining good quality samples, was not sufficient to permit a significant comparison between the 2 groups of subjects. More sensitive and specific detection instruments, such as mass LC, will provide a more precise detection of observed components with SH groups. However, present findings provide for the first time a method to analyze thiols in the exhaled breath condensate and then the possibility of using EBC thiols as an early marker of altered redox balance in the respiratory tract, in order to evaluate initial local damage from environmental pollution.

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