ANALYSIS OF SOME BIOMARKERS TO EVALUATE THE OXIDATIVE STRESS POTENTIALLY INDUCED BY TOLUENE EXPOSURE

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Introduction

Oxidative stress is a condition of unbalance between the pro-oxidant status and the antioxidant protection. Toluene is known as a toxic substance able to cause clinical signs of central nervous system or hepatic and renal changes.

This study evaluates the oxidative stress induced by toluene working exposure, using specific biomarkers.

Methods

We studied the oxidative stress in 187 workers of a firm producing gummed texture exposed to toluene and in a control group of 150 workers of the same firm, not exposed to the toxic substance.

We evaluated environmental and personal exposure levels to toluene and the following oxidative stress biomarkers:

- level of plasmatic hydroperoxides ;
- amount of the total plasmatic antioxidant barrier to determine the capacity of the single patient to control the production of free radicals;
- amount of plasmatic SH groups;
- level of 8hydroxy-2deoxiguanosine, which is a biomarker of DNA damage detectable in urine
- ELISA-measured isoprostanes, producted from oxidation of arachidonic acid, which are considered a reliable marker of oxidative stress.

We also evaluated the same oxidative stress biomarkers in all the workers of the control group.

Results and discussion

The results showed a relevant exposure to toluene, even if under the T.L.V. value, in personal and environmental samples.

Biomarkers of oxidative stress were altered in 67 workers (35.8%) exposed to toluene with a statistically significant difference with the control group ($p<0,01$).

The different markers of oxidative stress didn't show a univocal behaviour in every worker, and this fact forced us to plan in our next researches a screening of the complete set of biomarkers tested in this first study, to avoid false negative results.

We think that, even if our results have to be confirmed in larger and more structured studies, the evaluation of biomarkers of oxidative stress could be a useful research field to investigate a possible precocious work damage from toxic substances.

NON-INVASIVE ASSESSMENT OF CHROMIUM EFFECTS ON RESPIRATORY TRACT IN ELECTROPLATING WORKERS

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Occupational exposure to chromium compounds, which are widely used in industry, may cause airway inflammation and bronchial asthma.

In this study we investigated pulmonary function, chromium in urine and in induced sputum, induced sputum cellularity and markers of inflammation in exhaled breath condensate (EBC) and in nasal lavage fluid of 16 electroplating workers exposed to chromium at lower concentration than current TLV and in 9 non-exposed workers.

All study participants were non smokers without active lung disease. Urinary chromium was 8,64 mg/g creat (SD 6,19).

Lung function values were normal for both groups. Chromium in induced sputum was higher in exposed workers (7.90 mg/L, SD 3.4 vs 1.78 mg/L, SD 0.25). Total leukocyte and neutrophils counts in induced sputum were not significantly higher in exposed subjects (82.98±49.00x104cell/ml vs 68.89±22.71x104cell/ml; 53.08± 34.79x104cell/ml vs 40.45±12.52x104 cell/ml).

In EBC median Nitrite concentration was significantly increased in exposed subjects (4.35 mmol/L, 5°-95° percentile:1.88-10.13 mmol/L vs 0.11 mmol/L, 5°-95° percentile: 0-0.72 mmol/L) ($p<0.001$). IL-6 and TNF-a were not detectable.

Median IL-6 concentration in nasal lavage fluid was higher in exposed workers (5.72 pg/ml, 5°-95° percentile: 0-65.25 pg/ml vs 0.28 pg/ml, 5°-95° percentile: 0-1.7 pg/ml) ($p<0.01$). No differences in Eosinophil Cationic Protein concentration were found. TNF-α was not detectable. In exposed workers urinary chromium levels where not correlated with any marker of inflammation and with chromium in induced sputum.

For the first time this study uses all this 3 non invasive methods to assess early changes in respiratory tract in workers exposed to chromium. These results are suggesting an inflammatory/irritative action of chromium on upper and lower airways which appears not correlated with chromium absorption as indicated by urinary concentration of this metal.

BIOLOGICAL MONITORING OF OCCUPATIONAL EXPOSURE TO STYRENE AND STYRENE-(7,8)-OXIDE

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This study investigated the capability of some urinary and haematic biomarkers to discriminate among different levels of occupational exposure to styrene (Sty) and styrene-(7,8)-oxide (StyOX) and evaluated the influence of smoking habit and genetic polymorphism of metabolic enzymes GSTM1 and GSTT1 on these biomarkers. With this aim, we recruited workers of the reinforced plastic industry (n=8), of the paint and dye industry (n=13), and a group of controls (n=22). Median personal exposure to airborne Sty and StyOX in the different working activities was 14.8, 3.1 and 0.3 mg/m3, and 126, 13 and <5 µg/m3, respectively, as evaluated by repeated measurements. These chemicals were strictly correlated with each other (Pearson r = 0.826), the ratio between Sty and StyOX being about 1000:5. Personal exposure was significantly higher in exposed workers than in controls and, among workers, in subjects of the reinforced plastic industry. Urinary biomarkers, namely unchanged styrene (StyU), mandelic acid (MA), phenylglyoxylic acid (PGA), phenylglycine (PHG), 4-vinylphenol (4-VP), and mercapturic acids (M1 and M2) were higher in end- than in pre-shift samples and significantly correlated with both airborne Sty and StyOX. The best correlations were observed between end-shift MA or MA + PGA and airborne Sty (r = 0.890 or 0.886, respectively). The excretion of mercapturic acids was 6-fold higher in subjects with GSTM1 active genotype in comparison with those with null genotype. Cysteinyl albumin and hemglobin adducts of StyOX could not distinguish the different exposure categories investigated. In conclusion, in both reinforced plastic and paint and dye industry there was co-exposure to airborne Sty and StyOX. Among the different biomarkers urinary MA and PGA and their sum showed the best capability to discriminate different exposures and are recommended for Sty exposure assessment starting from a level of 1 mg/m3.

COMPARATIVE EVALUATION OF URINARY MTBE AND BENZENE AS BIOMARKERS OF EXPOSURE TO URBAN TRAFFIC

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Benzene and methyl tert-butyl ether (MTBE) are found in urban environments as a consequence of autovehicular traffic; in fact both these compounds are added to fuels to increase octane ratings and/or reduce carbon monoxide emissions. Aim of the present study was to evaluate the possibility of using excretion of urinary MTBE (U-MTBE) and benzene (U-BENZ) as biomarkers of exposure to traffic. With this aim 127 Milan urban policemen, working as traffic wardens, were investigated. Spot urine samples were obtained prior to and at the end of the workshift (7.30-13.30 or 13.30-19.30), in different seasons. Analysis was performed by headspace-solid phase microextraction GC-MS. Median levels of airborne benzene was 9.6 µg/m3 (range 4.0-90.2 µg/m3). Urinary levels in the different seasons varied from 74 to 164 ng/L (50-657 ng/L) and from 85 to 277 ng/L (21-5065 ng/L) for U-MTBE and U-BENZ, respectively. U-MTBE increased of about 14% during the workshift, independently from the moment of the shift (morning or afternoon). U-BENZ increased of 27% in the afternoon, but decreased of 15% in the morning. An influence of the different

seasons was observed, with lower values in spring and higher in winter. Smoking increased the excretion of U-BENZ but not affected that of U-MTBE. The results of this study suggest that U-MTBE is a reliable marker for the assessment of exposure to urban traffic, while U-BENZ is influenced both from the moment of the day and smoking habit.

ANALYSES OF INFLAMMATION MARKERS IN EXHALED BREATH CONDENSATE IN SMOKERS AND NONSMOKERS BY LC-MS/MS

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Biological effect monitoring has become important for the detection of diseases of the respiratory system in early stages. In the last decade the use of exhaled breath condensate (EBC) increased rapidly because its sampling is non-invasive. Prostaglandines, leukotrienes and 3-nitrotyrosine (3-NT) were assumed to be markers of inflammation effects. In view of the accurate determination of the basal concentrations of these markers in EBC we developed some analytical procedures using LC-MS/MS and applied them on healthy smoking and non smoking subjects.
Exhaled breath condensate was collected using the ECOSCREEN sampling system. External and online solid phase extraction were performed for clean up and preconcentration. The analytes were determined by high performance liquid chromatography and tandem masss spectrometry using electron spray ionisation and selected reaction monitoring. The procedure was applied to EBC samples of 20 healthy smokers ond non smokers.
The validation of the new LC-MS/MS procedures resulted in data of high precision (2 – 8 %) and accuracy (91 – 115 %). The limits of quantitation were found to be between 5 and 10 pg/ml. In 94 % of the EBC samples 3-NT was found to be over the limit of quantitation whereas prostaglandines and leukotrienes were generally below the quantification limits. The values of 3-NT ranged between the determination limit and 184 pg/ml. 3-NT concentrations were not significantly different in EBC of smokers and non smokers. The values were distinctly lower than in studies with the application of immunochemical analytical technique (EIA) on EBC of healthy subjects.
These new procedures for determining prostaglandines, leukotrienes and 3-nitrotyrosine in exhaled breath condensate have proved accurate and reliable. Due to the fact that prostaglandine and leukotriene concentrations were below the detection limits in real EBC samples of healthy subjects, a further improvement of sensitivity is required.

PHENOL, CATECHOL AND HYDROQUINONE IN BLOOD AMONG WORKERS EXPOSED TO BENZENE

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Background: We have developed a reliable GC/MS method of determination of benzene metabolites(phenol,catechol and hydroquinone) in blood. However, blood metabolites as biomarkers of benzene exposure have not been validated.
Aim: To validate correlations between benzene exposure and metabolites in blood among workers.
Methods: After signing consent forms, 88workers provided blood samples for phenol(PH),catechol(CAT) and hydroquinone(HQ) measurements by GC/MS. One hundred and four workers provided urine samples and sPMA were measured using ELISA kits. Twenty non-exposed workers were recruited as controls. Area and personal samples were taken from each workplace for one week to calculate daily average ,weekly average and long-term average exposure levels. Blood levels of PH,CAT and HQ and urinary sPMA were compared between controls and workers. Correlations between blood metablotes,urinary sPMA and external exposure metrics were tested.
Results: Levels of free metabolites(PH,CAT and HQ) in blood significantly increased among benzene exposed workers,0.107ug/ml,0.022ug/ml,0.427ug/ml respectively. Both free and total(free+conjugated) metabolites in blood increased with the increases of exposure. When exposure increased, proportion of PH among total amount of three metabolites decreased. Free/conjugated ratios of PH and CAT other than HQ decreased when exposure increased. Urinary sPMA level increased significantly after workshift, 9.382 umol/mol Cr vs 87.329 umol/mol Cr. Urinary PMA was significantly associated with exposure metrics.
Conclusions: Metabolites(PH,CAT and HQ) in blood might be biomarkers of benzene exposure. This study also confirmed that urinary sPMA is sensitive biomarker of benzene exposure.

BIOLOGICAL MONITORING OF LOW EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS

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Aim of the work was the assessment of low exposure to polycyclic aromatic hydrocarbons (PAHs) by biological monitoring. Italian asphalt workers (AW, n=100) and roadside construction workers (CW, n=47) were investigated by measurement of hydroxylated metabolites and unmetabolized PAHs in urine spot samples collected respectively after two days of vacation (baseline), before and at the end of the monitored workshift, in the second part of the workweek. Biomarkers were determined by HPLC-fluorimetry and GC-MS.
Median airborne levels during the workshift of 15 PAHs (both vapour and particulate phases), from naphthalene to indeno(1,2,3-cd)pyrene, ranged from below 0.03 to 426 ng/m3. Median excretion values of 1-hydroxypyrene (OH-Py) in baseline, before- and end-shift samples were 1.04, 1.84 and 3.16 nmol/L for AW and 1.19, 1.39 and 1.73 nmol/L for CW; lower values were found in non-smokers compared to smokers. In all subjects a weak correlation between personal exposure to the sum of airborne 15 PAHs and OH-Py was observed. Urinary naphthalene, phenanthrene, pyrene and fluorene were detected in the majority of the samples in the range below 0.01 to 2.54 nmol/L. Significant differences in the levels of the unmetabolized compounds were found between AW and CW. Moreover in AW samples the urinary excretion of most analytes increased during the work shift. The results of this study show that AW experienced a moderate occupational exposure to airborne PAHs, resulting in a significant increase of urinary biomarkers during the workday and the workweek. Both hydroxylated metabolites and unmetabolized PAHs in urine may be suggested as biomarkers of low exposure to PAHs.

A RE-APPRAISAL ON CLINICAL MANAGEMENT OF ACUTE METHANOL POISONING

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Objective: To elucidate the re-cognition on diagnosis and treatment of methanol toxicosis after summarized data of 42 poisoning cases. Methods: The data of 42 cases with methanol poison that occurred in Guangzhou, P. R. China in May 11, 2004 was summarized. The methanol concentration in wine was detected by Guangzhou Quality Supervise Control Bureau. The epidemiological information was investigated by municipal and district CDCs (Central Disease Control). The methanol concentration of blood was measured with gas chromatogram in Occupational & Environmental Hygiene Monitor Center in our hospital. The diagnosis was according to the National Occupational hygiene Criterion (GBZ53-2002) and was consisted of three stages, including observation, slight, and severe poison. The patients were cured with general therapies. Results: The methanol concentration in wine was 16% - 46%. The average age of patients (40 men and 2 women) was 46.1 year (22 – 80 year). The average consumption of the wine was 588.1ml (50 -2000 ml) per patient. The average methanol concentration in blood was 1.61 mmol/L (0.03 – 23.60 mmol/L). Among them, 17 observation cases, 9 slight toxicosis, and 16 severe toxicosis, 35 patients were cured (83.3%), 2 with blind (4.8%), 4 with neuropsychotic sequelae (9.5%). And, one was death (2.4%). Conclusion: Success of this salvage was based on the recognition of local government, the counterplan for emergency of public health events, special therapies in poison control center (PCC), and cooperation of departments and specialist groups.