Biomonitoring Human Exposure to VOCs : Using Individual Susceptibility Markers

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Abstract

In this study, biomonitoring methods were developed to measure BTEXs exposure level in the air, metabolites of benzene and toluene in human urine, individual susceptibility markers in human blood for evaluation of the health effects about environmental pollution. We have also performed a small-scaled molecular epidemiology study on residents in Chuncheon and workers in workplace for these method applications.

The workers in workplace were surveyed as study areas, and the residents in Chuncheon which is in the suburban area were surveyed as comparative areas in this study. Actually, 31 workers in as target group and 33 residences in as control group this epidemiological study. The results obtained from this study were as follows:

1. Benzene is a well-known carcinogen, it's median concentrations were 0.00024~0.02057ppm at suburban area and 0.002~00.654ppm at work place. These benzene concentrations were not exceed the OSHA(Occupational Safety and Health Administration) threshold benzene level of 1ppm in the states.
2. Metabolites product of benzene(*t,t*-Muconic Acid) and toluene(Hippuric Acid) were not significant both in suburban and workplace area. The median concentration of *t,t*-MA and HA were 0.0122, 1.44277g/g creatinine, respectively.
3. In the case of individual susceptibility markers as CYP1A1, 41.8% of them has homozygous wild type(W) and who has heterozygous variant type(H) was 35.4% and 22.8% of homozygous variant type(M) genetic type. In the case of CYP2E1, 62.82% of them has homozygous wild type(D) type, 34.62% of each has heterozygous variant type (DC) and 2.56% of them has homozygous variant type (CC). Who doesn't have GSTM1 gene was 46.25% and who has GSTM1 gene was 53.75%. Who doesn't have GSTT1 gene was 40.0% in study groups and who has GSTT1 gene was 60.0%. Who has W genetic type, which is homozygous wild type of GSTP1, was 69.18% and H genetic type, which is heterozygous variant type was 28.4%. M genetic type which is homozygous variant type was 2.4%.
4. Concentration differences of metabolites such as *t,t*-MA and HA in urine, which is generated by individual susceptibility marker of GSTM1, GSTT1, GSTP1 gene of Phase I and CYP1A1, CYP2E1 gene of Phase II, was examined. As a result, GSTP1 and GSTM1 indicate slight differences depend on the amount of metabolites in urine, it was not statistically significant.

Introduction

Volatile organic compounds (VOC) are important ambient pollutants because of their detrimental effects on human health and their role in the chemistry of atomosphere precursors of ozone, peroxyaceylnitrate(PAN), and other oxidants. The profile of VOC concentrations differs from one country to another due to several factors such as legislative control for VOC, composition of vehicle fuel, differences in heating pattern in residential area and meteorological conditions. (Kwangsam Na, 2001). The aims of this study were to evaluate the concentration measured the actual personal exposure measured on workers and controls. We included measurements of toluene, ethylbenzene, *m,p*-xylene and o-xylene because they probably have important source in common with benzene.

Moreover, genetic susceptibility markers can be determined by genotyping assay such as PCR (Gonzalez and Idle, 1994). Individuals lacking of having low levers of carcinogen-activating P450 or a carcinogen-inactivating phase II conjugating enzyme would be expected to be at increased risk for cancer development. Evidence has also been found about the modulation genetic damage accumulation different individuals by susceptibility markers in genes involved in the activation or detoxification of VOCs, especially of polymorphisms GSTM1, GSTT1, GSTP1, CYP1A1 and CYP2E1 genes.

Our objective was to evaluate the distribution of genotypes of CYP1A1, CYP2E1, GSTM1, GSTT1 and GSTP1 in participants as Korean population and assessed how the urinary excretion of *t,t*-MA and HA is modified by the individual susceptibility markers.

Materials and Method

Study population. The study was performed in a workplace where the case-control study is being used. Workers were recruited exclusively from the work place where the highest concentration of VOC was expected. As a control group, dwellers of the Chuncheon City were recruited. The study group consisted of 31 workers and 33 controls.

Sample collection. Sampling of airborne VOCs, urine and blood took place during 4 weeks in August 2002, 2 weeks in July. The first urine samples were collected in the next morning of sampling day. Blood samples were obtained from all workers and controls.

VOC measurements. The measurements were carried out during 4 weeks in August 2002, 2 weeks in July. We made measurements at work place workers and at suburban dwellings. For each group, we included measurements on the worker and dweller with Active VOC samplers were flow rate fixed in 20 mL/min. In the work place and suburban area, they were typically attached sampling tube with their collar outside of their clothes. When the dwellers were bathing or doing sports, the samplers were placed as close to them as possible,

at night, the samplers were placed as close to them as possible; at night, the samplers were placed beside pillow.

GC analysis of airborne BTEXs. The VOC determinations were done by the VOC were sampled by Supelco diffusive steel tubes containing 200mg Tenax TA 60/80 per tube as an adsorbent. Analyses were performed on a DS 6200A automatic thermal desorption system coupled to GC system : one with 30-m DB-624 column and a flame ionization detector.

HPLC analysis of BTEXs metabolites in urine. Urinary *t,t*-muconic acid was determined at 260nm for the estimation of benzene exposure, while urinary hippuric acid was determined at 205nm for the estimation of toluene exposure. Urine was filtered through 0.45 μm membrane filter after mixing with the same volume of methanol to that of urine and 5 μL of filtered urine was injected into the column automatically for the assay. The column used was Supelcosil C18 of 2.1mm in inner diameter and 7.5cm long.

DNA isolation and PCR analyses. Venous blood samples(5mL), drawn in EDTA as anticoagulant, were obtained from workers and controls, and DNA isolated from leukocytes by QIAgene DNA extraction kit. PCR-RFLP analyses were performed by hot start method (Brockmoller,1994).

Results and Discussion

BTEXs exposure levels

Individual exposure level to BTEXs, collected by active sampler method, appeared very higher in workplace than suburban area, these differences are significant. The median benzene, toluene, ethylbenzene, *m,p*-xylene and *o*-xylene concentration at the work place was 40, 30, 50, 380 and 70 times higher than each chemical's concentration at suburban area. Among these chemicals, benzene is a well-known carcinogen, it's median concentrations were 0.00024～0.02057ppm at suburban area and 0.002～0.654ppm at work place. These benzene concentrations were not exceed the OSHA(Occupational Safety and Health Administration) threshold benzene level of 1ppm in the states.

t,t-MA analysis of BTEXs metabolites

Metabolites product of benzene(*t,t*-MA) was not significant both in suburban and workplace area. The median concentration of *t,t*-MA was 0.01224g/g creatinine. This result is comparable to the previous study done in Ulsan 2001 that was 0.01219g/g creatinine.

HA analysis of BTEXs metabolites

Metabolites product of toluene(HA) was not significant both in suburban and workplace area. The median concentration of HA was 1.44277g/g creatinine. This result was slightly lower than American conference of governmental industrial hygienists' BEI lever that was 1.6g/g creatinine.

Analysis of susceptibility markers as CYP1A1 and CYP2E1 in Phase I system

In the case of CYP1A1, 41.8% of them has homozygous wild type(W) and who has heterozygous variant type(H) was 35.4% and 22.8% of homozygous variant type(M) genetic type. In the case of CYP2E1, 62.82% of them has homozygous wild type(D) type, 34.62% of each has heterozygous variant type (D) and 2.56% of them has homozygous variant type (CC).

Analysis of susceptibility markers as GSTM1, GSTT1, and GSTP1 in Phase II system

Who doesn't have GSTM1 gene was 46.25% and who has GSTM1 gene was 53.75%. Who doesn't have GSTT1 gene was 40.0% in study groups and who has GSTT1 gene was 60.0%. Who has W genetic type, which is homozygous wild type of GSTP1, was 69.18% and H genetic type, which is heterozygous variant type was 28.4%. M genetic type which is homozygous variant type was 2.4%.

Metabolites and genetic susceptibility markers

Concentration differences of metabolites such as *t,t*-MA and HA in urine, which is generated by genetic polymorphism of CYP1A1, CYP2E1 gene of Phase I and GSTM1, GSTT1, GSTP1 gene of Phase II, was examined. As a result, GSTP1 and GSTM1 indicate slight differences depend on the amount of metabolites in urine, it was not statistically significant.

Conclusion

As a result, even though tendency of VOCs exposure level was very higher in work place area than suburban area. The suburban exposure level was as same as general city area. This study could not clearly present health effect by environmental pollutants. Health effect by environmental pollutants, however, could happen at various level of health depend on pollutant type, level, and time duration. Thus, susceptibility markers such as GSTT1, GSTM1, GSTP1, CYP1A1, and CYP2E1, could be applied to molecular biological indicator, which is useful for vital metabolite analysis of environmental exposure, and also further study is required.

Generally, health effect of environmental pollution can be occurred in various forms and levels in the health spectrum according to types, amounts, and frequency of exposed pollutants. In spite of negative finding of health effects from this study, it is necessary to improve environmental quality continuously for preventing and protecting human health.

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