[S-8]

Biomarkers available in workplaces

eung-Hee MAENG

Center for Occupational Toxicology, Occupational Safety & Health Research Institute,

Korea Occupational Safety & Health Agency

The monitoring of genotoxic effect or oxidative DNA damage in workers exposed to hazardous materials is increasingly applied for hazard identification or risk assessment purposes in workplaces. The current generation of biomarkers has the potential to allow for the earlier detection of occupational disease, for the reduction of misclassification of exposure and outcome.

Exposure to hexavalent chromium (Cr[VI]) compounds has been of concern to industries such as those involved in the primary production of chromate, chromium plating, chromate pigment manufacture and the welding of stainless steel. Occupational exposure to Cr(VI) has been known to be strongly associated with increased incidence of human lung cancer (IARC, 1982, 1990). And also, a health hazard to welders is development of inflammation, pneumoconiosis, lung cancer. It is believed that this is likely due, in part, to the presence in welding fumes of several Cr(VI) species, whose solubility depends primarily on which process (i.e. manaual metal arc versus metal-inert gas) is used. Futhermore hazardous agents associated with welding processes include gases (O_3, NO_x, SO_x, CO), other metals (Fe, Mn, Zn, Ni, F, Hg, Pb, Sn, Cd), chemicals (phosgens, aldehyde, isocyanate), particles (asbestos, SiO_2), physical factors (ultraviolet light, ionizing radiation, electric current, electro magnetic field)(Beckett, 1996; Hewit, 2001; Korezynski, 2000; Recette et al., 2001). While various genotoxic biomarkers such as

chromosome aberrations, sister-chromatid exchanges, DNA-DNA cross links and Cr-DNA adduct have been employed in order to monitor the early biological effectsof Cr(VI) or welding fume, intracellular reduction of metals by cellular antioxidants to reactive intermediates and possibly reactive oxygen species (ROS) including HOo, Oo, O2o- and H_2O_2 is believed to be important in their pathogenesis including carcinogenesis.

In Cr(VI)-exposed workers, blood Cr concentration is statistically correlated with the frequency of chromatid exchange as well as the total frequency of chromosome/chromatid breaks and exchanges detected byGiemsa staining although significant increase is not noticed when compared to controls. The frequency of translocation detected by the fluorescence in situ hybridization (FISH) technique in chromium-exposed workers was significantly higher than that in the controls, and it is correlated with the blood Cr concentration.

Although exposure to Cr(VI) doses not increase 8-OH-dG levels in Korean chromate pigment workers (Kim et al., 1999), possible involvement of oxidative DNA damage from ROS in carcinogenesis of Cr(VI) is need to be clarify. However in the above Cr(VI)-exposed workers of chrome plating plants, the concentration of malondialdehyde (MDA), the metabolite of lipid peroxidation in the exposed workers is found to be higher than that in the controls, although no statistically significant correlation between MDA level and blood or urine Cr levels is observed.

In the experiment to clarify whether reactive oxygen species are involved in its mechanism of Cr(VI) induced carcinogenesis, the levels of 8-hydroxydeoxyguanine (8-OH-dG), which is measured by HPLC-ECD, increase significantly in the lung tissues of the rats exposed for one week at the low concentration (0.18 mg/m3, $p<0.05$), as compared with the controls. However, there is no difference in the 8-OH-dG levels at the higher concentration or with more than two weeks of exposure. The 8-OH-dG repair activities decrease in a dose-dependent manner during two weeks of exposure, on the contrary they recover at three weeks of repeated exposure when these repair activities are measured by base excision repair

activity assay with fluorescently labeled synthetic oligonucleotide containing an 8-OHGua residue in its sequence (5-GGTGGCCTGAC8-OH-GCATTCCCCAA-3). These results suggest that the DNA damage caused by hexavalent Cr inhalation is induced by the generation of reactive oxygen species and by inhibition of base excision repair activity, possibly by 8-oxo-guanine glycosylase 1 (OGG1) during the earlier phase of exposure. However, the 8-OH-dG levels and its repairactivities recovered to the level of the controls in the latter inhalation exposure period.

The clastogenic effects of welding fume depends on the material and process parameter : conventional chromosome analysis (CA) of 101 manual metal arc welding on mild steel (MMA/MS) welders show negative incidence of CA while a significant increase in chromatid breaks and for cells with aberration is found in the metal arc welding on 42 stainless steel (MMA/SS) welders. As for welding, many of the epidemiology studiesperformed are difficult to compare because of differences in worker populations, industrial settings, welding techniques, duration of exposure, and other occupational exposures besides welding fumes. Therefore, to study both the toxicity and behavior of welding fumes in respiratory system, OSHRI developed a welding fume exposure system for experimental animals that include a generator, an exposure chamber and a fume collector. The exposure system generates fumes through a flux-covered manual metal arc welding process that uses a stainless steel consumable electrode (MMA/SS).

Alveolar macrophages have a potential for expressing and excreting many factors that are immunologically active and active on cytokines (monokines and lymphokines) and are thus indirectly involved in the genesis of pulmonary fibrosis directing pneumoconiosis and carcinogenesis. One of these factors is the tumor necrosis factor alpha (TNF-). Statistically significant increases of TNF- are observed in the bronchoalveolar lavage (BAL)of the rats exposed to MMA/SS for over 15 days at the concentration of TWA 107 mg/m^3(high dose) and for over 30 days at the concentrations of TWA 64 mg/ m^3 (low dose) or 107 mg/ m3 (high dose). In addition, a significant elevation of interleukin-1 (IL-1) is observed in the lavage fluid of the 30 days (high dose) group.

In contrast to the tendency in Cr(VI), welding fume-induced levels of 8-hydroxydeoxyguanine (8-OH-dG) in rat lung, which are measured by immunohistochemistry using anti-8-hydroxyguanine antibody (Travigen, Inc. USA), increased significantly in the lung tissues of the rats exposed to welding fume (MMA/SS) as compared to controls, in dose-dependent and duration-dependent manner. On the other hand, the level of 8-oxo-guanine glycosylase 1(OGG1) mRNA expression is inhibited in a dose-dependent manner during one day, 15 days and 30 days of exposure respectively when OGG1 mRNA expression is analyzed by RT-PCR from the rat lung mRNA and the primers for rat OGG1 are designed from the concensus squence of the human and mouse OGG1 genes. However, this inhibition effect is much greater during the first day of exposure, and it almost recovers to control level as time passing. This pattern is similar to that of Cr(VI). These results suggest that the DNA damage by welding fume exposure is induced by the generation of reactive oxygen species and by inhibition of its repair activity, mainly by 8-oxo-guanine glycosylase 1 (OGG1) expecially during the earlier phase of exposure. However, the 8-OH-dG induced by welding fume has comparably longer retention time.

In conclusion, conventional CA has limitations to monitor the genetic effects of Cr(VI) and welding fume in exposed workers even though a few results show increased incidence in MMA/SS welders. Translocation analyzed by FISH technique can be a good marker of genetic effects by Cr(VI). 8-OHdG and OGG1 mRNA expression may possibly good markers of cellular oxidative DNA damage for welding fume if validated in exposed workers, but they have limitation as biomarkers for Cr(VI). TNF- and IL- can be promised biomarkers to welding fume exposure in early pathogenesis coupled with oxidative damage markers.