Identification and validation of sensitive markers for the biomonitoring of workers occupationally exposed to potential mutagens/carcinogens. Application to workers exposed to epoxide-producing carcinogens and cobalt-containing dust.

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Summary

Biomonitoring of occupational exposures relies on surveillance of exposure and its biological consequences. Extensive monitoring would include a follow-up of all steps of this sequential process, namely measurement of the extemal dose, intemal dose, and biologically active dose, of early biological effects, structural or functional changes, and illness. In practice an objective analysis must define which parameters are the most relevant for given exposure conditions. As far as cancer prevention is concerned, DNA adducts, haemoglobin adducts, and sister chromatid exchanges can be used as measures of biological interactions. Mutations, which are fixed genetic changes, can be used as measures of early biological effects.

With this research project a federal network of five university teams with recognised and complementary expertise in (geno-)toxicity and occupational medicine identified and validated sensitive biomarkers for the assessment of cancer risk in workers occupationally exposed either to epoxide-forming carcinogens or to cobalt or mixtures containing it.

For epoxide-forming carcinogens, both DNA adducts and haemoglobin adducts were evaluated as parameters for biomonitoring. Different experimental and biomonitoring approaches were used: comparative in vitro studies of the adducts formed by ethylene oxide and propylene oxide in human lymphocytes, animal experiments after intraperitoneal injection of 7,8-styrene oxide into mice or rats, field studies where N-terminal valine adducts of ethylene oxide and propylene oxide were determined in a group of 18 workers in a plant where medical equipment was sterilised, in 36 propylene workers, and in 337 workers genotyped for glutathione S-transferase (GST). The conclusions are quite clear. The modified Edman degradation method for the assessing N-terminal valine adducts is a sensitive and specific method allowing quantification of increased adduct concentrations resulting from relatively low (occupational) exposures to ethylene and propylene oxide (circa 0.01 ppm). With this

methodology, adduct formation in animals can be studied very accurately for exposures to propylene and styrene. The use of the HPLC-UV technique for DNA adduct measurements allows assessment of these adducts under experimental conditions and in (highly) exposed animals. Regarding the relationship between detoxification enzymes and N-terminal valine adducts, there are some indications in favour of GST - of which some isoenzymes are present in erythrocytes - and none in favour of microsomal epoxide hydrolase.

A second line of technological research was carried out on the most sensitive and most specific method for quantifying DNA adducts, using ethylene oxide as a reference. The results clearly demonstrated the advantages (high sensitivity and selectivity) of HPLC/ES MS-MS as a technological approach to detecting adducts.

With the assessment of the carcinogenic potential of cobalt and mixtures containing it, a very challenging question was addressed to three teams. These collaborated very closely to study the general toxic and more specific genotoxic effects of these compounds. At the start of the project it was essentially known that workers exposed to cobalt in association with tungsten carbide are more at risk of lung cancer than those exposed to cobalt alone, and that the physico-chemical characteristics of tungsten carbide facilitate the transport of electrons from metallic cobalt to electrophilic acceptors. Preference was given on the one hand to a systematic approach from mechanisms to biomonitoring, and on the other hand to serial evaluation of the major *in vivo* and *in vitro* markers of genotoxicity in animals.

The DNA breaking potential (DNA breaks, alkali-labile sites, and micronuclei) of the cobalt/tungsten carbide mixture *in vitro* is always more pronounced than that of cobalt alone, in both primary human lymphocytes and rat alveolar cells (macrophages and type II epithelial cells). We observed no capacity to induce gene mutations *in vivo* in transgenic mice (Big Blue); we were unable to determine whether this was due ascribed to a too-low sensitivity of the test method or to the fact that DNA lesions induced by cobalt and its alloys on DNA are not expressed as gene mutations.

The co-mutagenic capacity of cobalt as specific inhibitor of one of the steps of nucleotide excision repair was very accurately demonstrated *in vitro* in human lymphocytes. The implications of the potentially double action of cobalt - directly on DNA and indirectly through inhibition of repair enzymes - must not be overlooked, in particular when one considers mixed exposures, quite frequent at the workplace. On the basis of our better understanding of the genotoxic effects of cobalt and its alloys, biomonitoring was started. 40 workers exposed to cobalt alone, 40 workers from the hard metal industry, and 40 controls were studied; these three groups were matched as well as possible for age, socio-economical factors, and essentially smoking habits, well known for their effects on biomarkers for genotoxicity. All the results conceming the major biomarkers for exposure and genotoxic effects are available but the final statistical analysis has yet to be performed.

The scientific research of the network has led to an accurate assessment of biomarkers for biomonitoring two types of occupational exposure. The results are now summarised with other complementary data from the literature and will be integrated into an interactive user-friendly web site available to authorities and all persons concerned by occupational medicine.