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Round Table

Methods for assessing human exposure to and/or biological effects of genotoxic agents

Chairmen: M. Börzsönyi and A. Carere

The opening speaker, Dr. A. Carere, gave a short overview of the needs for more exact measurements of human exposure to mutagens/carcinogens for the purposes of an epidemiological evaluation of health risks. A table was shown (Table 1) in which the sequence of events between exposure and final effects (e.g., malignant tumors or inherited diseases) was outlined, together with possibilities of monitoring different stages of this cascade. Dr. Carere reminded the participants that an International Workshop, sponsored by several international agencies (e.g., EEC, IARC, WHO, ILO-UNEP-WHO), was recently held on this subject in Luxembourg (6-9 July, 1987). The workshop gave rise to an important document, Consensus Report [1], in which the validity and possible health significance of the biological methods which can be presently considered for monitoring human exposure to genotoxic chemicals was assessed.

During the Round Table the main features, including the stage of development, of various bio-monitoring methods were presented.

Dr. S. Gundy started discussing the cytogenetic observations - chromosomal aberrations (CAs), sister chromatid exchanges (SCEs) and micronuclei (MN) - in human somatic cells.

Table 1. - *Current possible methods for assessing human exposure to and/or biological effects of genotoxic agents*

Dose or effect(s)	Methods of assessment
External dose	Ambient monitoring
Internal dose	- Chemicals or metabolites in human specimens - Mutagenicity in urine - Thioethers in urine
Biological effective dose	- Protein binding - DNA (RNA) binding - DNA excision products
Early biological effects (in somatic and/or germ cells)	- Gene mutations - Structural and numerical effects chromosome aberrations - Sister chromatid exchanges - Micronuclei - Unscheduled DNA synthesis
Late effects	Tumor markers, exfoliative cytology
Clinical manifestations	Cancer, hereditary defects, malformations, spontaneous abortions, and other reproductive disturbances

These methods require expertise and rigorous study design with control groups adequately matched for age, sex, smoking, habits, other life-stile factors and medical history. In the case of chemical exposure, different from ionizing radiations, CAs in peripheral lymphocytes cannot be used as a biological dosimeter at the individual level. Indeed, only in a few cases, dose-response relationships have been obtained at a group level. The CA method is characterized by a rather low sensitivity and lack of compound specificity. The lesions leading to CAs are cumulative, reflecting exposure over a long period of time (years); this fact requires considering past exposures.

For all these reasons, only few chemicals so far have been shown to clearly increase the frequency of CAs in groups occupationally exposed to chemicals.

SCEs, which are considered indicative of DNA damage, are generally also nonspecific to compounds; when compared to CAs, they are much easier to perform and may be more sensitive. The persistence of lesions is shorter with SCEs (days to months) than with CAs; this instability may be responsible for the greater variability of the results. Induction of SCEs does not necessarily parallel that of CAs in human lymphocytes.

MN can occur by chromosome breakage or by lagging whole chromosomes at cell division; therefore, they are indicative of structural as well as numerical chromosome aberrations. The main advantages of this method are the simplicity and speed of scoring; moreover, it can be applied to lymphocytes, erythrocytes and exfoliated cells. However, this method is still in a developmental stage and its performances (sensitivity, specificity, relationships with CAs and SCEs) remain to be clarified.

Drs R. Benigni and I. Vincze discussed the usefulness of determining DNA repair and in particular UDS in peripheral lymphocytes as a biomonitoring assay. According to their experience and knowledge this approach is still in an experimental stage as compared to other assays. As a major limitation, this method seems to reflect very recent exposure immediately preceeding the assay and this fact may be responsible for the great variability of results.

Drs R. Crebelli and A. Pinter discussed the urinary mutagenicity method, focusing on the advantages and disadvantages of the assay applied to bacterial strains as indicator organisms. The main role of this method is the demonstration of the absorption of a mutagenic compound into the body by the detection of its stable or conjugated metabolites and/or of that fraction of unmodified compound that has not reacted with nucleophilic targets. Therefore, it gives a measure of the internal dose which, however, is not necessarily meaningful from a toxicological point of view (the most reactive metabolites or direct acting mutagens are not excreted or only marginally excreted in urine). Careful controls have to be made for possible confounding factors (e.g., smoking, diet and medication) as well as for the presence of endogenous growth factors in human urine. The nonspecificity, the noninvasive nature, the reproducibility and relative simplicity make this assay potentially useful in case of mixed, complex or unexpected exposure to mutagens.

Dr. E. Dogliotti gave a short overview of DNA adducts determination, currently considered as one of the most promising monitoring methods for detecting human exposure to genotoxic carcinogens. Three main ways of measuring extremely low numbers of DNA adducts in human cells have been developed: immunoassays, physico-chemical and post-labelling methods. Of the three, the immunological methods, based on specific monoclonal and polyclonal antibodies against structurally modified DNA components, are at the most advanced stage of development. Sensitivity and specificity are the main features of the immunoassays, which have already been used to screen potentially exposed individuals. Examples are detection of B(a)P-DNA adducts in lung cancer patients and foundry workers, detection of O^6-methyldeoxyguanosine adducts in Chinese patients living in an area with high risk of oesophageol cancer incidence and detection of cis-diamminedichloroplatinum (II)-DNA adducts in cancer patients treated with cis-platinum.

Very sensitive physico-chemical methods (such as gas chromatography-mass spectrometry (GC-MS) or fluorescence spectroscopy (F.S.)) have been developed for measuring specific DNA adducts. GC-MS methods have been used to study the urinary excretion of methylated DNA bases in laboratory animals as well as in man. F.S. has been used to detect aflotoxin Bl-guanine adduct in urine from people living in Kenya in an area known for high contamination of food with aflotoxin Bl and also to detect B(a)P-DNA adducts in people working in aluminium plants and coke ovens. Even if very promising, these new biomonitoring assays based on determination of DNA adducts are still in an experimental stage. Important problems, such as the quantitative relationships between induction of DNA adducts (with their removal or persistence), induction of other genetic endpoints and risk of cancer are still to be clarified.

At the end of the Round Table, Dr. M. Börzsönyi discussed briefly ethical problems connected with biomonitoring of human exposure to mutagens/carcinogens, focusing on the difficulty in handling data. In its conclusive remarks, M. Börzsönyi reminded that at present some of the methods discussed are able to identify groups exposed to genotoxic agents potentially at risk, but none of them is able to estimate the cancer risk at individual level. As final recommendation of A. Carere and M. Börzsönyi, the use of biomonitoring assays should be encouraged and further investigated before they can be used for routinary health surveillance purposes in man. This type of biomonitoring should eventually be used only in the framework of well planned studies subjected to adequate statistical design, including evaluation of confounding factors, and after information of people to be tested about the meaning of these studies for the prevention of health risks.

REFERENCES

1. COMMISSION OF THE EUROPEAN COMMUNITIES. 1988. *Indicators for assessing exposure and biological effects of genotoxic chemicals. Consensus and technical reports*. Office for Official Publications of the European Communities Luxembourg. (EUR 11642 EN).