Role of Cytogenetic Surveillance to Assess Exposure to Carcinogens

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1 INTRODUCTION

The hazards associated with exposure to carcinogenic and mutagenic chemicals are a growing source of public concern as well as being of research interest. Some two decades of active investigation have led to identification of carcinogens and mutagens and their sources in the human environment. Early detection of hazardous exposures may bring about significant reductions in the risk of adverse health effects through appropriate reductions in the presence of the chemicals in the general environment and in occupational settings.

Identification of such hazards relies primarily on experimental in vitro and in vivo methods. Human exposure is complex, and it is virtually impossible to address the interacting lifestyle and other factors and modifying responses from extrapolations of experimental data. Therefore, biologically significant levels of exposure can be identified using cytogenetic methods as an indicator in samples taken from exposed persons.

Exposure to genotoxic agents may initiate a sequence of events that leads to adverse health effects. The conceptual basis for application of cytogenetic assays (i.e., the measurement of certain types of chromosomal damage in human somatic cells) is that damage to DNA is the initial event in the pathogenesis of disease. Thus, cytogenetic surveillance can serve as an indicator enabling early detection of hazardous exposures.

Somatic chromosomal aberrations have been interpreted for some four decades as indicators of exposure to clastogenic agents. Most of the early studies of induced clastogenicity were performed in persons exposed to ionising radiation (Tough et al., 1960; Bender and Gooch, 1962; Buckton et al., 1962) and benzene (Vigliani and Saita, 1964). Since that time, several occupational exposure situations have been studied. However, many of the studies do not fulfill the necessary criteria for adequate study design.

Prior to designing human occupational monitoring studies, several factors must be considered (Carrano and Natarajan, 1988). These include the nature and duration of the exposure, possible confounding factors such as tobacco...
smoking, number of exposed individuals with appropriately matched controls, and the lifetime of the induced lesions.

The number of agents clearly shown to induce cytogenetic changes in humans is relatively limited (Ashby and Richardson, 1985; Sorsa and Yager, 1987). Cytogenetic studies have been published on approximately 30 exposure situations. Most of these studies deal with induction of structural chromosome aberrations; but more studies have been performed recently using sister chromatid exchanges (SCEs) as indices. Some studies have scored micronucleated peripheral blood lymphocytes (Sorsa, 1984).

2 TYPES OF CHROMOSOMAL CHANGES INDUCED BY EXOGENOUS AGENTS

Cytologically recognisable chromosomal damage includes both structural aberration (i.e., gross change in the morphology of the chromosome) and sister chromatid exchange (SCE) (i.e., symmetrical intrachromosomal exchanges between the sister chromatids), and analysis for micronuclei arising from acentric chromosome fragments or lagging whole chromosomes.

Knowledge of chromosomal aberrations is derived mainly from studies of ionising radiation, a classical mutagen. The aberrations are a result of breakage and/or exchange of chromosomal sub-units; they include breaks, interstitial deletions, inversions, duplications, rings, and interchanges between chromosomes. Exposure to ionising radiation at occupationally tolerated levels results in increased incidences of chromosomal aberrations in lymphocytes (Evans, 1982). The type and frequency of these events depends on more than the properties of the mutagen and on the stage of the cell cycle at the time of the insult. DNA damage induced by ionising radiation has only rarely been reported to lead to the development of SCEs (Gundy et al., 1984), so that even with relatively high doses of radiation virtually no increases in SCE frequency have been observed (Galloway, 1977; Cavaglia, 1980).

Induced chromosomal aberrations are divided into two classes: (1) chromosome-type involving both chromatids of a chromosome, and (2) chromatid-type involving only one of the two chromatids. Ionising radiation induces chromosome-type aberrations in the G₀ or G₁ stage of a cell (i.e., prior to replication), while chromatid-type aberrations are produced during the S or G₂ stages (i.e., during or after replication). In peripheral lymphocytes, most of which are in G₀ stage, ionising radiation induces mainly chromosome-type aberrations.

However, most mutagenic chemicals are S-dependent clastogens, and produce chromatid-type aberrations almost exclusively. S-Dependent compounds should have no direct effect on the chromosomes of peripheral lymphocytes in vivo, because peripheral lymphocytes do not replicate their
DNA until stimulated to do so in cell culture. “Radiomimetic” chemical clastogens (bleomycin, streptonigrin, and 8-methoxycaffeine) act like ionising radiation, and are ineffective at inducing SCEs relative to aberrations; however S-dependent agents are usually very efficient in inducing SCEs (Perry and Evans, 1975). Peripheral lymphocytes can, however, carry unrepaired/misrepaired long-lived lesions that are translated into aberrations during the replication of DNA in vitro. Table 1 lists different characteristic advantages and disadvantages of cytogenetic monitoring techniques using peripheral lymphocytes.

If chromatid-type aberrations survive the first mitosis and the chromosomes carrying them are replicated, the following metaphase may contain “derived” or “secondary” chromosome-type aberrations; it is impossible to tell the difference between a derived and “true” aberration. Derived chromosome-type aberrations may be formed in vivo from chromatid aberrations induced in hematopoietic stem cells or later in lymphocyte development.

Table 1. Advantages and disadvantages of cytogenetic surveillance methods using peripheral lymphocytes (PLs) for monitoring human exposure to clastogenic factors

<table>
<thead>
<tr>
<th>Advantage</th>
<th>Disadvantage</th>
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<tr>
<td>Allows detection of induced genetic damage in human cells</td>
<td>Prospective follow-up studies in exposed groups can seldom be carried out</td>
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<tr>
<td>Phytohaemagglutinin stimulates T lymphocytes, which comprise about 70% of</td>
<td>Interindividual variation in response may be caused by differences in lymphocyte populations</td>
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<td>total lymphocytes</td>
<td></td>
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<td>Damage induced in lymphocytes from different organs may be picked up in</td>
<td>PLs are not representative target cells for ill-health manifestations</td>
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<tr>
<td>PLs</td>
<td></td>
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<tr>
<td>Accumulated damage can be picked up: 90% of T lymphocytes have half-life of</td>
<td>Accumulation of damage may falsify interpretation of results of acute exposure studies</td>
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<td>about 3 years</td>
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<tr>
<td>Radiation-induced chromosome-type aberrations remain constant for years</td>
<td>Aberrations induced by most chemicals represent only S-dependent misreplicated lesions</td>
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<tr>
<td>Dose of ionising radiation received can be derived by extrapolation from human data on chromosomal aberrations</td>
<td>Dose-effect relationships with chemical mutagens are still unknown</td>
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<tr>
<td>Costs of establishing a cytogenetics laboratory are not very high, and the number of personnel needed is low</td>
<td>Tedious technique, requiring standardised laboratory techniques and skilled workers</td>
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Several reviews have been published recently on studies of chromosomes in industrial populations (Purchase, 1978; Dabney, 1981; Evans, 1982; Kucerova, 1982; Vainio and Sorsa, 1983; Forni, 1984; Sorsa, 1984; Ashby and Richardson, 1985; Sorsa and Yager, 1987). Over 100 studies have been published on occupational cytogenetics, but very few of the positive results reported have been confirmed by several independent findings. As examples, several occupational chemicals which are confirmed clastogens in humans are discussed below. For general recommendations concerning sample sizes and conflicting variables, the reader is referred to an ICPEMC publication (Carrano and Natarajan, 1988).

3.1 BENZENE

The largest volume of data on occupational exposures related to chromosomal aberrations involves benzene (Sarto et al., 1984). Following intraperitoneal administration, benzene is a moderately strong clastogen in bone marrow of both rat and mouse (IARC, 1987). A dose-related increase in the incidence of chromosomal aberrations was seen in bone-marrow cells of rats exposed to benzene vapours. The early findings of significant increases in chromosomal aberrations in blood and bone marrow and in lymphocytes from subjects exposed to benzene have been confirmed in many other investigations (Pollini and Colombi, 1964; Tough and Court-Brown, 1965).

Evidence from human studies indicates that chromosomal damage in peripheral lymphocytes may be quite persistent. Direct evidence for late expression of chromosomal rearrangements after exposure to chemical mutagens is obtained from benzene workers whose exposure ceased nearly two years before initiation of the study (Tough and Court-Brown, 1965). Increased levels of stable chromosomal aberrations have persisted for years in subjects who have been poisoned with benzene (Forni et al., 1971). Such changes may lead to formation of clones that may be progenitors of malignancy.

Benzene has not been reported to be associated with increased levels of SCEs. In fact, workers exposed to moderately low levels of benzene showed a decreased frequency of SCEs as compared to the unexposed control group (Watanabe et al., 1980). However, a fairly small number of subjects and cells was analysed, and the study did not control for the smoking behaviour of the subjects, a lifestyle factor known to be highly correlated with increased SCE frequency.
3.2 VINYL CHLORIDE

The second best studied industrial chemical for possible cytogenetic effects in workers is vinyl chloride. The increase in the incidence of chromosomal aberrations in blood lymphocytes has also been correlated with length of exposure to the chemical and with the history of exposure during the year prior to sampling. The greatest increases in the frequency of chromosomal abnormalities occurred in occupations with the highest exposures, such as autoclave operators and/or cleaners (Purchase et al., 1978). Autoclave operators were exposed to average levels of 300 to 400 ppm up to 1970, although the levels had been reduced to 5 ppm by 1975. The same population originally examined by Purchase et al. (1978) was re-examined a few years later by Anderson et al. (1981); although the exposure to vinyl chloride had been drastically decreased, the chromosomal aberration frequencies in exposed individuals were still somewhat higher than those in controls. However, the frequencies of SCEs were not significantly different in exposed and control individuals. The finding of normal SCE frequencies has also been reported for populations originally exposed to fairly high levels of vinyl chloride and monitored for several years (Hansteen et al., 1978; Natarajan et al., 1978).

3.3 STYRENE

Several studies during the last decade have shown that workers in the reinforced plastics industry, where relatively extensive exposure to styrene monomer occurs, may show increased frequencies of structural chromosomal aberrations in their peripheral blood lymphocytes (IPCS, 1983).

The first publication was based on limited data, and reported that, among styrene-exposed workers in plants where plastic boats were manufactured by manual lamination methods, the frequency of cells with chromosomal aberrations exceeded the mean of aberrant cells among unexposed controls by almost tenfold (Meretoja et al., 1977). The study was later expanded to include a total of 16 male workers and six controls (Meretoja et al., 1978). The results of Finnish studies were later confirmed in other countries (Meretoja et al., 1978; Sorsa et al., 1979). In Sweden, Hogstedt et al. (1979) found that the workers from a fibre-glass boat plant had a significant, almost threefold, increase in the frequency of aberrant lymphocytes with breaks, as compared to the group of unexposed controls.

Another study from Sweden also revealed an increased incidence of chromosomal aberrations in the lymphocytes of workers from a boat factory where unsaturated polyester resin was used (Andersson et al., 1980). When SCEs were analysed in 20 workers and in 21 controls, there was no difference between the high- and low-exposure groups; but, in general, the styrene workers had slightly increased frequencies of SCE. In the earlier Finnish
studies of Meretoja and Vainio (1979) and Sorsa et al. (1979), no increase in the frequency of SCE was observed.

Fleig and Thiess (1978) reported that the workers processing unsaturated polyester resins had about twice as many aberrant lymphocytes as a control group. However, workers manufacturing styrene (5 men) or polystyrene (12 men) who had low exposure to styrene (generally below 1 ppm or 4 mg/m³) showed no increase in the frequency of chromosomal aberrations.

Watanabe et al. (1981) studied nine men from a fibre-reinforced plastic boat factory and seven workers in a polyester-resin board factory. The frequency of cells with structural chromosomal aberrations was reported to be similar in the workers and in 13 control persons. The authors found no difference in SCE frequency between styrene-exposed workers and controls. However, in another study, Watanabe et al. (1983) found a marginal increase in the incidence of structural chromosomal aberrations in first-division metaphases in the styrene-exposed workers; there was no difference in SCE frequencies.

In a study from Norway, 18 workers exposed to low levels of styrene (well below 50 ppm) showed no increase in the number of chromosome breaks and SCEs as compared to controls; however, a significant increase in the incidence of gaps was found in the styrene-exposed workers (Hansteen et al., 1984).

Attempts have been made to score micronuclei (formed during cell division of lagging whole chromosomes or chromosome fragments) in blood lymphocytes from exposed persons. Meretoja et al. (1978) and Meretoja and Vainio (1979) reported an increase of micronuclei in cultured lymphocytes of 10 workers in polyester plastic product plants as compared to five controls. Hogstedt et al. (1981) recently found that micronucleated cells were increased in 38 workers exposed to styrene as compared to 20 controls. Neither structural chromosomal aberrations, nor micronuclei, nor SCEs were increased in reinforced plastics workers after a low-level (less than 20 ppm) exposure to styrene (Maki-Paakkanen, 1987).

Taken together, the cytogenetic studies on styrene-exposed populations indicate that increases in chromosomal aberrations (and micronuclei) appeared to occur beginning at exposures of about 20 to 25 ppm; whereas, SCEs appeared less sensitive, and were found only at higher exposure levels (exceeding 50 to 100 ppm).

3.4 EPICHLOROHYDRIN

Epichlorohydrin is another clastogen and carcinogenic agent that has been studied in several occupationally exposed populations. In an epichlorohydrin-producing plant in Czechoslovakia, an increased incidence of chromosomal aberrations was found in the peripheral lymphocytes of a group of 35 workers exposed for two years to concentrations of between 0.5 and 5.0
mg/m³ air. Pre-exposure values were used as control data (Kucerova et al., 1977). When the same group was re-examined two years later, using matched controls and with exposure levels below 1 mg/m³, a slight increase was found in the number of aberrant cells (bearing mostly chromatid and chromosome breaks) (Sram et al., 1980). Another group of 93 workers in the USA, probably exposed to concentrations of less than 5 ppm, showed increased incidences of chromosomal aberrations when compared with 75 individuals tested before starting employment (Picciano, 1979).

While criticising the previous studies on epichlorohydrin-exposed people on methodological grounds, Dabney (1986) reported small elevations in frequencies of chromosomal aberrations relative to the individuals' pre-employment samples of groups of epoxy resin employees potentially exposed to epichlorohydrin.

3.5 ETHYLENE OXIDE

Ethylene oxide is widely used for gas sterilisation of instruments and supplies. It is a monofunctional epoxide which has been shown to cause mutations and structural chromosomal changes in a wide variety of organisms (Ehrenberg and Hussain, 1981). Several recent cytogenetic studies of people occupationally exposed to ethylene oxide revealed significantly elevated frequencies of chromosomal aberrations and SCEs. In a recent large study, people working at sites with high and low exposures to ethylene oxide were monitored for SCE frequency in their peripheral lymphocytes (Stolley et al., 1984). A dose-related increase in the incidence of SCEs was observed in workers exposed to ethylene oxide, and these increases persisted for at least two years after cessation of exposure to ethylene oxide. Chromosomal aberrations also showed a dose-related increase, but only for workers at sites with the highest potential exposure (Galloway et al., 1985, 1986). When aberration frequencies were compared with levels of SCEs, there was only a weak overall association. The correlation was found only in exposed but not in control groups; and for any individual, one observation (aberrations or SCEs) could not be used to predict the other.

4 SIGNIFICANCE OF RESULTS FOR HUMAN HEALTH

Chromosomal abnormalities are characteristic features of malignant cells. An overwhelming amount of data exists concerning the association of chromosomal aberrations with cancer, and several correlations have been established between specific non-random chromosomal aberrations and certain malignant or premalignant disorders. At the clinical level, chromosome analysis is becoming of value in diagnosis. The malignant cells of most tumours have a specific chromosomal defect (Yunis, 1986). These
abnormalities are usually represented by a translocation or a loss of a chromosome band. The recent finding that these aberrations tend to cluster at chromosomal breakpoints identified with the location of proto-oncogenes has united the two leading theories of carcinogenesis. This finding is not dealt with in this presentation, but has been discussed widely in the literature (Sandberg, 1983; Yunis, 1983; Mitelman, 1985; Yunis, 1986; DeKlein, 1987; Heims and Mitelman, 1987).

4.1 GENETIC HOST-FACTORS IN SUSCEPTIBILITY TO CANCER

The first indication of a relationship between inherited constitutional chromosomal changes and a predisposition to malignancy came with the discovery that Down's syndrome patients, who have an additional chromosome No. 21, and an increased risk of developing leukemia (Harnden and O'Riordan, 1973). The subject has been summarised by Evans (1982, 1985).

Predisposition to cancer is known to be associated with the inherited chromosomal instability conditions, Bloom's syndrome (BS), Fanconi's anaemia (FA), and ataxia telangiectasia (AT) (German, 1983; Heddle et al., 1983). Specific constitutional chromosomal deletions or translocations have been identified in cases of inherited retinoblastoma, Wilm's tumour with aniridia and renal-cell carcinoma, in each of which there appears to be a homozygous functional loss of what appears to be a critical regulatory DNA sequence (Cavenee et al., 1983; Koufos et al., 1984).

Two of these cancer-prone syndromes, AT and FA, exhibit hypersensitivity to mutagens and carcinogens (Heddle et al., 1983). In cases of the third major chromosomal instability syndrome, BS, cells from affected individuals show an extraordinarily high frequency of SCEs and a high incidence of chromosomal aberrations (Chaganti et al., 1974). SCEs occur at normal frequencies in lymphocytes from individuals with AT and FA.

These chromosomal instability syndromes are relatively rare, their prevalences ranging from 1 per 10^6 FA to 1 per 10^5 AT; but affected individuals with any of the three syndromes are at an increased risk of developing cancer and show a greatly elevated incidence of chromosomal aberrations in lymphocytes and cultured skin cells as compared to normal individuals. In BS and FA patients, the predominant cancers are leukemias, but other tumours also occur. The risk of an AT homozygote developing cancer before the age of 30 years is about 30 percent, as compared with less than 1 percent for the general population. About 80 percent of these malignancies are lymphoid; other associated cancers include brain and stomach tumours. Whether the AT heterozygotes occurring at an estimated frequency of 1 to 2 percent in the population have an elevated risk of cancer is still unknown (Bridges et al., 1985).
4.2 IDENTIFICATION OF POPULATIONS AT RISK

Of the agents that cause chromosomal damage, benzene, vinyl chloride, epichlorohydrin, and ethylene oxide are known to cause cancer in humans and/or in animals. For styrene, there is at present only limited evidence to establish a cause-and-effect relationship for cancer in humans. For none of these compounds is it possible to show any direct connection between mutagenic and chromosome-breaking effects and malignant potential.

Cytogenetic damage to lymphocytes as such has little or no significance to the health of the individual, since most of the lymphocytes carrying genetic damage die and are replaced. The results of Hansteen et al. (1978) and Anderson et al. (1980) on vinyl chloride and of Laurent (1988) on ethylene oxide have demonstrated that the prevalence of chromosomal damage in the lymphocyte pool is reversible and that aberration frequencies may revert to control levels in two to three years after reduction of exposure. However, the finding of such damage does indicate that similar types of changes may have occurred in other tissues of the individual. If such chromosomal damage would be induced in germinal cells, the gametes would be expected to be damaged; this could be of considerable consequence to an individual, his offspring, and future generations.

Most experience with the use of cytogenetic surveillance derives from "high-exposure" occupational situations. To make use of cytogenetic surveys of populations exposed environmentally, additional problems exist to obtain statistically significant results from very low-level exposures, and to determine the health significance of such results in the general population and in individuals on which the observations were made. The presence of chromosomal aberrations can signal a potential problem in the population, but quantification of the abnormalities is extremely difficult. Thus, chromosomal changes are indicators of cellular genetic damage in a population, but they cannot be used to predict quantitatively other health injuries in a given individual. The recognition that chromosomal damage may be a sign of possible health risks in populations has been stated by several international expert groups (WHO, 1985; Carrano and Natarajan, 1988).

Increases in SCE frequency measured in occupationally exposed humans have generally been small. One striking exception is the magnitude and persistence of SCEs in people exposed to ethylene oxide, which suggest that measurement of SCEs is applicable for surveillance in certain worker situations, and provides an indication of exposure to mutagens. Special attention should be paid, however, to confounding factors such as smoking.

Although SCEs are easier to score than structural chromosomal aberrations, their relation to ill health is even more tenuous than is that of chromosomal aberrations. Nevertheless, SCEs are considered a sensitive measure of DNA damage, although the correlation of SCEs to point mutations or chromosomal aberrations differs depending on the agent and
the lesions produced (Evans and Vijayalaxmi, 1981). Increased frequencies of SCEs can signal the potential of genetic damage to human cells and serve as an indicator of exposure to genotoxic agents.

5 CONCLUSIONS

Many types of cancer are associated with specific or non-specific chromosomal aberrations. In several hereditary human diseases (i.e., AT, FA, BS), chromosome instability is associated with increased susceptibility to cancer.

Cytogenetic surveillance of people exposed to carcinogenic and/or mutagenic chemicals or radiation can bring to light effects on the genetic material of the individuals concerned. Surveillance of people exposed to ionising radiation has been carried out for many years, but the methods have yielded positive results only for a limited number of chemical clastogens, most experience having been obtained with agents such as benzene, vinyl chloride, styrene, epichlorohydrin, and some alkylating anticancer drugs. Depending on the lesions induced by genotoxic agents, damage to chromosomes falls into two categories: structural aberrations and sister chromatid exchanges. Because these endpoints differ in their responses to DNA lesions, they are complementary in the identification of potential genotoxicants. The peripheral blood lymphocytes in humans are suitable cells to be used in surveillance studies, because of their easy access and their ability to integrate exposure over a relatively long lifespan. Exposure to a variety of chemical mutagens may result in increased frequencies of chromosomal aberrations and/or SCEs in blood lymphocytes of exposed individuals. Also, the extent of damage is a function of dose, as is the case with vinyl chloride, benzene, ethylene oxide, and styrene. When cytogenetic endpoints are sufficiently sensitive to detect levels of exposure occurring in occupational settings, results of such tests should prompt implementation of hygienic controls or medical surveillance, even in the absence of direct evidence relating chromosomal damage to adverse health outcomes.

It must be emphasised, however, that even when cytogenetic tests on peripheral blood lymphocytes show that the genetic material has been damaged, the results can be used to estimate risk only at the population level. An increased frequency of chromosomal aberrations in a population should be considered an indication of increased risk for cancer, but cytogenetic tests should not be used to predict risk to an individual for any particular form of ill health.

The present cytogenetic test systems available are inadequate for use as a routine surveillance procedure. Their application needs to be done with care and confidence, considering possible confounding factors. Consequently, the methods are useful and informative under carefully selected conditions, and can point to agents that are capable of causing chromosomal damage
in humans. Positive findings indicate the need to take preventive health measures.

REFERENCES


Methods for Assessing Exposure of Human and Non-Human Biota


