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CHROMOSOMAL ABERRATIONS IN HUMANS INDUCED BY BENZENE

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Abstract: Adverse effects associated with occupational exposure to benzene have often been reported in humans. It has been shown, that benzene causes chromosomal aberrations, sister chromatid exchanges and micronuclei in lymphocytes of exposed workers. In addition to evidence by conventional cytogenetic methods, the genotoxic effect of benzene has also been proved by a more specific approach based on fluorescence *in situ* hybridization with DNA probes. In the present paper, the nature of benzene-induced chromosomal aberrations and supposed consequence on human health is reviewed. The new possibilities in chromosomal alterations identification by molecular cytogenetic methods are also presented.

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INTRODUCTION

Benzene is an important pollutant compound, present in both occupational and general environment. Chronic exposure to high concentrations of benzene in humans is associated with an increased incidence of myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML) [48, 43, 39].

It is well known that individuals occupationally exposed to benzene are at a much higher risk of developing leukaemia than the normal population. Therefore, many studies have been focused on occupationally benzene-exposed workers [53, 67, 58, 3, 26, 35, 56, 17, 2]. Potential for workers exposure to benzene can be higher in certain industries, such as the plants for the production of organic chemicals, shoe factories and leather manufacturing [20, 55, 57], printing companies [51], elevator manufacturing [22], petrol stations [4] and the petrochemical industry [46]. In a recent study, Glass *et al.* [11] found in occupationally exposed persons from the petroleum industry cohort an excess risk of leukaemia associated with cumulative benzene exposures, and benzene exposure intensities that were considerably lower than reported in previous studies.

Benzene has been also implicated as an environmental risk factor in leukaemia and other haematological diseases. The main sources of environmental exposure to benzene are road traffic exhaust [7] and volatile organic compounds [1]; this means urban air pollution in general [6, 44, 45, 12]. Lifestyle factors, such as cigarette smoking, can contribute to exposure [32, 29]. The soil obtained from oil production facilities and coastal refineries is also highly contaminated by benzene [33, 16].

Benzene exposure in humans and animals has been shown to result in structural and numerical chromosomal aberrations in lymphocytes and bone marrow cells, indicating that benzene is genotoxic [52]. According to Snyder *et al.* [42], benzene and its metabolites do not function well as mutagens but are highly clastogenic, producing chromosomal aberrations, sister chromatid exchanges and micronuclei. In several studies, increased levels of chromosomal aberrations in peripheral blood lymphocytes were correlated with a heightened risk of cancer, especially haematological malignancies. Thus,

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chromosomal aberrations may be a predictor of future leukaemia risk [66].

In the present review, the nature of benzene-induced chromosomal aberrations in humans is reported and new trends in chromosomal alterations identification are briefly described.

THE NATURE OF BENZENE-INDUCED CHROMOSOMAL ABERRATIONS IN HUMANS

Several studies have suggested that induction of chromosomal aberrations may play a role in benzene-induced carcinogenesis, and the aberrations detection may serve as a marker of benzene's early effects. Therefore, the nature of benzene-induced chromosomal aberrations was predominantly investigated in workers occupationally exposed to benzene. Moreover, it has been widely documented that benzene requires metabolic activation for its genotoxic effect. The requirement for metabolic activation and the complexity of benzene's metabolic pathways must be taken into account, particularly in relation to *in vitro* experiments.

In general, the genetic alterations induced by benzene mostly include aneuploidy, deletions and translocations.

Benzene and its metabolite 1, 2, 4-benzenetriol (BT) is known to cause cytogenetic changes in specific chromosomes, especially in the C-group chromosomes and the X chromosome in humans [30, 31, 8]. According to Sasiadek [36] the distribution of breakpoints in the karyotypes of examined workers exposed to benzene was significantly non-random and the breakpoints accumulated mainly on chromosomes 2, 4 and 7.

Aneuploidy induction by benzene and its metabolites has been experimentally demonstrated both *in vivo* in benzene-exposed workers and in experiments *in vitro*. The data of Chen *et al.* [13] demonstrated that both aneuploidy and chromosomal breakage are early genotoxic events induced by benzene or its metabolites *in vivo* and that the nature of chromosomal alternations might vary depending on the target organ or cell type. Trisomy accounted for the majority of the hyperdiploidy induced by BT *in vitro* in the C-group chromosomes 7 and 9 [61]. Trisomy 9 was also the major form of benzene-induced hyperdiploidy in blood cells of nondiseased workers exposed to benzene [62]. As described Eastmond *et al.* [9], the benzene metabolite hydroquinone (HQ) may contribute significantly to the numerical and structural aberrations observed in benzene exposed workers. Later *in vitro* studies of Stillman *et al.* [47] documented that HQ induces specific chromosome loss (monosomy 5, 7 and 8) in the human lymphoblast cell line. The same author also concluded that CD34+ bone marrow cells are more susceptible to HQ and show different pattern of cytogenetic aberrations compared to lymphocytes [49].

Monosomy of chromosomes 5 and 7 and long arm deletions del5(q) and del7(q) was found in human lymphocytes treated with benzene metabolites 1, 2, 4-benzenetriol (BT) and hydroquinone (HQ) *in vitro* [63]. In addition, leukaemia-specific changes such as the loss and long (q) arm deletion of chromosomes 5 and 7 have been found in the peripheral blood of otherwise healthy benzene-exposed workers [64]. Smith *et al.* [40] observed increased translocations t(8;21) and hyperdiploidy in chromosomes 8 and 21. In the same year, Carere *et al.* [5] detected hyperploidy X and 18 in peripheral lymphocytes of gasoline station attendants. Zhang *et al.* [65] detected trisomy of chromosomes 7 and 8 among workers exposed to benzene. The study of Stillmann *et al.* [48] described for the first time that benzene metabolites catechol and HQ act in synergy to induce specific chromosome del(5)(q31) found in secondary MDS/AML.

Modest but significantly increased frequencies of breakage affecting both chromosomes 1 and 9 were observed by Marcon *et al.* [27] in the cultured lymphocytes of benzene exposed workers. The incidence of dicentric chromosomes in the exposed group employed in the shoe industry was significantly higher than in the control group [20].

As far as sex chromosomes are concerned, the higher benzene concentration may induce an increase in aneuploidy frequency of sperm sex chromosome in exposed workers [25]. In sperm of exposed employees, the increase in aneuploidy frequency of 9 and 18 chromosomes have been proved, also [24]. The experiments of Liu *et al.* [26] revealed increases in frequencies not only of numerical aberrations for chromosome 1 and 18, but also of structural aberrations for chromosome 1 of sperms in exposed workers.

Chung and Kim [15] indicated that treatment with benzene metabolites resulted in the induction of monosomy 5, 7, 8 and 21 in human lymphocytes in a concentration-dependent manner.

Chung *et al.* [14] found that the proportion of micronuclei (MN) exhibiting centromeric signals for chromosome 8 was higher than that for chromosome 7 among the total induced micronuclei (MN) in BT-treated lymphocytes, suggesting that chromosome 8 is more frequently involved in the formation of MN. This result is consistent with observation that benzene cause non-random chromosome aberrations in C-group chromosomes. Several investigators reported in previous works aneuploidy of some C-group chromosomes induced by benzene and its metabolites [8, 62, 63, 64, 65]. Among C-group chromosomes, numerical changes in chromosomes 7, 8, 9 were commonly studied due their possible association with haematological disorders including leukaemia. Recently, the use of fluorescent *in situ* hybridization has also revealed that industrial benzene exposure can induce aneuploidy of specific chromosomes (7, 8, 9) in the cells of exposed subjects [12]. Trisomy 7 and 9 were induced in the human promyelocytic cell line, HL-60, when exposed *in vitro* to BT, but relative sensitivity of these chromosomes to aneuploidy induction was not compared [61]. The reason for the selective sensitivity of specific chromosomes to benzene is still unclear. One possible explanation for the preferential effect of benzene metabolite

on specific chromosomes may be suggested by the fact that only cells with non-lethal chromosome aberration could survive to be detected. The elimination of telomere on particular chromosome has been reported to lead to selective chromosome gain and loss [65]. If aberration of C-group chromosomes, especially chromosome 8, at telomere locus may not be lethal enough, then cells with aberration in these chromosomes will be detected more selectively.

According to the analysis of Marcon *et al.* [28], a significant association between cytogenetic findings and intensity of benzene exposure suggests that signal displacement in 1cen-1q12 regions of human chromosome 1 may be a marker of chemical exposure. The recent work of Kim *et al.* [21] has shown that low-level benzene exposure was associated with significant increases in both monosomy and trisomy of chromosomes 8 and 21. Translocations between chromosomes 8 and 21- t(8;21) - were 8 fold more frequent in the high-level exposure group compared to the control group.

Zhang *et al.* [66] declared that in leukaemia cases associated with benzene exposure, there is no evidence of a unique pattern of benzene-induced chromosomal aberrations in humans. On the contrary, Shen *et al.* [38] concluded that exposure to benzene may be the cause for Chinese myelodysplastic syndrome and acute myeloid leukaemia patients with t(1;7) translocation.

The findings of Lebailly *et al.* [23] suggest that AML cases with defined chromosomal abnormalities could be related to specific carcinogen exposures. Smoking and genetic polymorphism in microsomal epoxide hydrolase gene could be risk factors for AML with del(7q) or t(8;21). Polymorphisms in the genes for benzene metabolising enzymes influence the susceptibility of individuals to chromosomal aberrations in relation to benzene exposure [21]. The specific chromosomal changes found in acute myeloid leukaemia might serve as useful biomarkers of early effect in chemoteraphy and benzene induced causal models [41].

DNA-reactive benzene metabolites forming adducts or cross-links oxidative DNA damage clastogenesis due to topoisomerase II inhibition and aneugenesis due to damage to components of the mitotic apparatus are 4 specific mechanisms most frequently used to explain benzene genotoxicity [59]. Studies of the chromosomal translocations found in BZ-exposed persons and secondary human leukaemia produced by topoisomerase II inhibitors provide some additional support for this mechanism being potentially operative in BZ-induced leukaemia [60].

CHROMOSOMAL ABERRATION ANALYSIS BY MOLECULAR CYTOGENETIC METHODS

Tests for chromosomal aberrations (CA) are usually included in cytogenetic assays to determine the clastogenic properties of xenobiotics. There are 3 main methods of chromosome visualisation applied in CA analysis: Giemsa staining, banding techniques and fluorescence *in situ* hybridisation (FISH) with chromosome painting [37].

The analysis of Giemsa-stained chromosomes is limited to the quantification of chromosome aberrations (breaks, gaps). The technique of G-banded chromosomes is more informative and allows finding the preferential breakage sites in the chromosomes. Fluorescent *in situ* hybridization (FISH) is the molecular cytogenetic method, using typically 3 different kinds of DNA probes which recognize repetitive DNA sequencies (satellite, telomeric), single copy DNA sequences as well as unique sequences spanning the length of a particular chromosome.

DNA probes complementary to the whole sequences of a specific chromosome are called whole chromosome paints (WCP) or chromosome specific probes. Aberration detection is based upon visualising colour changes in metaphase chromosomes, in comparison to classical methods, which involve detection of aberrations by banding or alterations in chromosome length [54]. At metaphase, both chromosome homologues are “painted” or brightly fluorescent. Chromosome painting offers a new suitable method to study radiation and chemically induced chromosomal aberrations. It is particularly useful for detecting stable aberrations (especially translocations and inzertions), which are difficult to quantify with classical methods. Stable aberrations are a subset of chromosome aberrations, which, by their nature, are compatible with cell division and, as such, can be transmitted from one cell generation to the next and eventually become the hallmark of a clone with its own specific karyotype [50].

FISH with probes targeting centromeric or pericentromeric satellite sequences is being increasingly used to detect numerical chromosome aberrations induced by chemical and physical agents *in vitro* and *in vivo*. It has been recently shown that centromeric heterochromatin of human chromosome 1 (1 cen-1q12 region) is one of the important regions prone to breakage. Two different chromosome 1- specific DNA probes were used in the study of Rupa *et al.* [34]: a classical-satellite probe specific for the pericentric heterochromatin of chromosome 1 and alpha-satellite probe, specific for a small centromeric region adjacent to the pericentric heterochromatin region. Using these different labelled probes, hyperdiploidy of chromosome 1 could be successfully distinguished from breakage within the heterochromatic region, or between the two-labelled regions. Small increases in hyperdiploidy and in chromosomal breakage in 1cen-1q12 and 9cen-9q12 regions were detected in Estonian benzene factory workers compared with control; the frequency of breakage in the 9cen-9q12n region was higher then that observed in the 1cen-1q12 region [10]. Centromeric DNA probes have been also shown to be a valuable tool for the identification of aneuploidy occurring in interphase nuclei.

A novel chromosome banding technique - spectral colour banding (SCAN) - has been developed recently [18]. This technique is based on spectral karyotyping (SKY) combined with simultaneous hybridisation of

labelled chromosome band-specific painting probes. SCAN analysis simultaneously identifies the origin of chromosome bands by a unique spectrum for each band. SCAN analysis can identify a particular region of a chromosome such as a translocated or deleted region, so that it can be directly assigned to the corresponding band number in G-banding. SCAN is useful for full characterization of chromosomal abnormality that could not be identified by G-banding or SKY analysis. This technique can therefore be expected to become a powerful tool for cancer cytogenetic research [19].

CONCLUSIONS

Since benzene is a relatively common environmental and occupational contaminant, genotoxic effect on human health status is still a matter of interest. The frequencies of chromosomal aberrations as well as micronuclei can be used as biomarkers of effects. It seems almost certain that chromosome-specific aneuploidy and translocation play key roles in the development and progression of leukaemia as in many other cancers. Therefore, chromosome-specific aneuploidy with higher sensitivity to benzene exposure would be a useful biomarker for leukaemia risk of benzene.

More precise identification of the metabolites and metabolic pathways contributing to benzene's genotoxic effects, as well as the specific chromosomes and chromosome regions involved in the observed alternations, should be continuously important areas for present-day and future research.

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