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BIOLOGICAL MONITORING OF RISK OF BLADDER CANCER IN PERSONS OCCUPATIONALLY EXPOSED TO AROMATIC AMINES

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Abstract: Recent advances in molecular biology and toxicology have greatly contributed to the early diagnosis of biological changes which may evoke neoplasms. This paper reviews the issues regarding the screening of persons occupationally exposed to carcinogenic aromatic amines. The screening was designed for an early detection of bladder cancer by means of biochemical tests. The applied tests facilitated the estimation of the level of aromatic amines which penetrate an organism (biomarkers of exposure), early diagnosis of the biochemical disorders which may influence cancer development (biomarkers of early effects) and the detection of genetic predispositions which enhance risk of such disorders (biomarkers of susceptibility).

INTRODUCTION

Epidemiological studies have confirmed very definitely that the occupational exposure component plays an important role in the development of bladder cancer. Various estimates attribute 18–35 per cent of all cases to occupation (30). An opinion exists that aromatic amines are particulary responsible for causing bladder cancer (22, 29). According to the literature data bladder cancer is diagnosed via cystocopy in 4.7%–11% of workers exposed to aromatic amines (38). Such a high percentage of incidence necessitates a regular screening of the exposed.

The detection of bladder cancer so far has been based on cytoscopy and confirmed by biopsy. The presence of bladder cancer can also be diagnosed indirectly from the results of such laboratory tests as haematuria or cytology of urine deposit according to Papanicolaou. Neverthelles, various data indicate that cancer develops long before it can be diagnosed by means of currently used laboratory and cystoscopic tests (12, 31, 43).

Long-term observations on the screening of persons exposed to chemical carcinogens, including carcinogenic aromatic amines, question conventional thinking concerning the efficacy of screening for bladder cancer (43, 44). Screening neither

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decreased the mortality nor extended the survival of persons with bladder cancer. Therefore, other criteria than those in present use (lower mortality, longer survival) should be taken into consideration in evaluating the screening efficacy.

Bearing in mind the data presented during the International Conference on Bladder Cancer Screening in High-Risk Groups (Cincinatti, Ohio, USA, 1989), it has been suggested that screening efficacy can be assessed by selecting out from the population persons with a particularly high risk of bladder cancer in order to help them with special preventive programs. It has also been proposed that only those persons who meet the following conditions be classified as a group with a particularly high risk for bladder cancer:

1. they should be significantly exposed to carcinogenic substances for a relatively long period of time;
2. they should be at the age at which the occurrence of bladder cancer is most likely;
3. they should be smokers;
4. they should show the genetic predispositions which reveal a high risk of bladder cancer (e.g. free acetylator); and
5. they should manifest biochemical disorders which might influence cancer development.

Now a days it is well known that the transformation of normal cells into neoplasm cells is a multi-phase process which takes time (37, 49). Precancer changes result from various disorders in cellular mechanisms which regulate metabolic and dividing activities: so-called early biochemical changes. These changes may, but do not have to, influence the transformation of normal cells into neoplasmic cells (35). The transformation may occur most likely in persons who already manifest such changes while being still exposed to carcinogenic substances (mutagenic and promoting). Hence, the detection of precancer changes and the withdrawal of persons with such changes from further exposure to chemical carcinogens is of extreme importance. This would particularly refer to persons who, besides the biochemical characteristics corresponding with precancer changes, meet the orther conditions listed above.

Screening for selecting out persons with a high risk for cancer would limit the number of bladder cancer patients. Such a procedure seems to be far more advantageous than the lengthening of survival with early detection of bladder cancer. In order to accomplish this goal, relevant biochemical testing should be applied. They would allow us to assess the volume of exposure to chemical carcinogens (exposure markers), to reveal biochemical disorders influencing cancer development (biomarkers of early biological effects), and to detect genetic predispositions of enhanced risk (biomarkers of susceptibility).

Thus, biomarkers (biological markers) could become laboratory tests performed on biological fluids (urine) or cells (cells of exfoliative bladder epithelium) which, by means of biochemical and cellular terms could define the level of carcinogens penetrating an organism, assess harmful biological effects and indicate individual predispositions to revealing such effects.

EXPOSURE BIOMARKERS

At present there are a lot of methods at our disposal which maybe useful in assessing the volume of exposure to carcinogenic aromatic amines. Among them there are methods which directly measure the level of carcinogenic amines or their metabolites in biological fluids and facilitate direct assessment of the exposure volume on the basis of cytogenic changes (chromosome aberations, exchange of sister chromatids) (5, 24).

Among biomarkers which maybe useful in assessing the exposure to carcinogenic aromatic amines, tests for molecular dosimetry, based on the quantitative measurement of adducts produced by aromatic amines or their metabolites with DNA or proteins, seem to be the most promising (3, 28, 36). Tests which can be considered as both exposure biomarkers and biomarkers of early biological effects belong to this group. Whether they should be placed in the first or second group of biomarkes depends on the kind of desired information: if we are interested in the volume of exposure, or in the detection of early effects evoked in a organism by the exposure.

The fact that carcinogenic amines are electrophilic compounds and/or that they undergo biotransformation into metabolites showing such properties is the basis for quantitative measurement of adducts produced by those amines with DNA. They may covalently bind to nucleophilic sites present in DNA bases, producing relevant adducts. If adducts are not removed from DNA through repair processes, or if certain errors occur during the repair process, they are able to cause point mutations of genes. The majority of DNA adducts of carcinogenic aromatic amines are a product of covalent binding to C8 deoxyguanosine carbon. Adducts can be producecd also (but in much smaller quantities) by covalent binding to exocyclic nitrogen and deoxyguanosine oxygen (N^2 and 0^6 and also deoxyadenosine (N^6) (1, 2, 28).

At present, three groups of methods are used in the detection and quantitative determination of DNA adducts of carcinogenic aromatic amines: immunological, physico-chemical and radioisotopic. The sensitivity of these methods is relatively high and it allows the detection of one adduct per $10^6 - 10^{10}$ DNA nucleotides (13, 18, 36, 41).

The development of immunological methods is most dynamic. They can be easily applied if the kind of DNA adduct produced by aromatic amine is known (41). Other methods, especially a radioisotopic technique (^{32}p postlabelling method) can be used even if the kind of DNA adduct is unknown (11, 40).

Usually DNA adducts produced by carcinogenic amines are assessed from the material derived from lymphocytes of the peripheral blood (4). There are, however, findings which provide evidence that application of the isotopic technique (^{32}p-ATP of high specific activity) allows the detection of adducts such as 4-aminobiphenyl, for example, products with DNA of bladder epithelium cells, obtained from individual urine samples (45).

Great difficulties faced during the preparation of DNA samples from cells and their analysis for the presence of adducts have encouraged attempts to use, for the purpose of molecular dosimetry, assays of adducts produced by carcinogenic aromatic amines with proteins such as haemoglobin and serum albumin (7, 26, 36).

It seems that the assessment of DNA and protein adducts of carcinogenic aromatic amines maybe very useful in analysing risk of bladder cancer. It has

produced direct evidence concerning not only past exposure to genotoxic aromatic amines but it also allows us to indicate in the genetic material potential carcinogenic impairments.

An evident increase in the level of DNA and protein adducts of carcinogenic amines can be considered as an enhanced risk of bladder cancer. It results, among others, from specific ability of aromatic amines to induce in man cancer only in the bladder.

BIOMARKERS OF EARLY BIOLOGICAL EFFECTS

Discovery of cellular oncogenes and suppressor genes (so called antioncogenes) which control the processess of cellular profileration and diferentation has created new diagnostic measures to detect changes in the cellular genome caused by carcinogenic substances including aromatic amines. It is already known that changes in the structure of genes controlling cellular proliferation and differentiation are the initial biological effects of carcinogenic aromatic amines which may transform normal cells into neoplasmic cells (21, 37).

A great number of methods are being currently employed in testing for the detection of changes in the chemical structure and expression of oncogenes and antioncogenes, as well as for the detection of the presence of oncogene and antioncogene protein products. There also are methods employed in the quantitative determination of the proteins in urine or in epithelial cells present in urine. Quite a lot of those methods can be now used in detecting early biochemical changes which precede the transformation of normal bladder cells into neoplasm cells (6, 18).

A number of genes which are apparently linked with neoplastic transformation of bladder cells have been identified. The analysis of the Harvey gene rat sarcoma (H-ras gene) has provided most information. It seems to be one of the main targets of the mutagenic effect of carcinogenic aromatic amines. Mutation or activation of the H-ras gene produces the mutated protein 21kD (p21) which usually participates in transmitting the cellular growth messenger from membrane into nucleus. When the mutated protein p21 appears in the cell or its level increases, then an activating signal is constantly, without any control, transmitted into a cellular nucleus and the cellular division is stimulated (14, 51).

The erb gene is another gene which can be the subject of the mutagenic effect of aromatic amines. A mutated erb gene contributes to the overproduction or activation of the membrane receptor for the epidermal growth factor (EGF) and the tumoric growth factor (TGF-α) controlling proliferation of bladder epithelium cells. The investigation of neoplasm cells from bladder cancer has revealed not only an increased number of receptors but also their relationship with EGF and TGF-α (33).

The level of protein p21 can be determind in the exfoliative cells of bladder epithelium, present in urine or bladder wash specimens, using various methods (19, 20). The radioimmunological method with antibody Y-13-259 according to De Biasi et al. (10) and the enzymeimmunological method (enzyme-linked immunoblot assay) according to Meyers et al. (32) deserve special mention.

The level of EGF receptors (EGFr) can be assessed in epithelial cells present in urine or obtained from bladder wash specimens. EGFr can be identified according to the cell ability to bind ^{125}J or by means of immunofluoroscence (34).

Transformation of bladder epithelium normal cells into neoplasm cells is linked not only with changes in the mechanisms controlling proliferation and differentiation but also with critical changes in the structure of these cells, especially in the plasmatic membrane. Simple proteins or protein-sugar-lipid complexes, not found in normal cells, emerge in the changed structure. These new proteins are known as tumor-associated antigens. Their presence can be detected using specific (monoclonal) antibodies. Existing immunohistochemical techniques facilitate both the detection of tumor-associated antigens and their intracellular location (17).

The appearance of new proteins in bladder epithelium cells undergoing a neoplastic transformation is not the only effect: the altered differentiation causes also quantitative changes in the level of some proteins, especially those which form important structural elements of the cytoskeleton.

F-action, a polymer of C-actin globular protein, which produces microfilament has become a subject of great interest among researh workers who are searching for molecular markers for the neoplastic process. An increased level of F-actin in cells is linked with high cellular differentiation whereas a decreased F-actin content marks cells that are less differentiated (39). Recent studies using bladder wash specimens collected from patients stratified by risk factors of a positive QFIA cytology and haematuria showed a close correlation between low F-actin and risk of bladder cancer (39). In these studies, F-actin was determined by its binding to fluorochrome combined with phalloidin characterised by a strong relationship with this protein.

BIOMARKERS OF SUSCEPTIBILITY

There is no doubt that the human population manifests different susceptibilities to the carcinogenic effect of xenobiotics. The differentiation results partly from the specifity of genetic predispositions and partly from the prequel of past illnesses or exposures. The acetylation phenotype has proved to be of great significance in the analysis of genetic predispositions to bladder cancer, especially in persons exposed to carcinogenic aromatic amines. The rate of acetylation is, according to Mendelian laws, an inherited feature and it is linked with one of the atosomal allele pairs.

Slow acetylators are recessive homozygotes, while fast acetylators are predominative homozygotes, and intermediate acetylators form the group of heterozygotes (47).

Lower et al. were the first researchers to call attention to the relationship between the slow acetylation phenotype and an enhanced risk of bladder cancer (25). They revealed an excess of slow acetylators (67%) among bladder cancer patients living in urban areas in comparison with a control group free from bladder cancer (51%). Although, the relationship between the acetylation phenotype and the incidence of cancer linked with exposure to chemical carcinogens has not yet been analysed, such a hypothesis has been already formulated.

In 1982, Cartwright et al. (8) showed that the slow acetylation phenotype greatly affects the occurrence of bladder cancer in persons occupationally exposed to carcinogenic aromatic amines. The responsibility of the slow acetylation phenotype for the occurrence of bladder cancer in conditions of occupational exposure to aromatic amines has also been confirmed by Hanssen et al. (16), as well as by Hanke and Krajewska (15).

Marker drugs are used in defining acetylation phenotypes (among others isoniazid, sulphometasine, procainamid). They are metabolised with the participation of N-acetylotransferase – an enzyme whose activity is determined according to whether it belongs to fast or slow acetylators. The technique depends on determinig either the acetylated derivative of a marker drug excreted with urine or its free form. The acetylation index is obtained from the ratio between the acetylated and free form of the marker drug. Recently it has become popular to define the acetylation phenotype via a quantitative determination of caffeine metabolites 5-acetyloamino-6-formylamino-3-methyluracil (AFMU) and -methylxanthine (1X) after drinking, prior to examination, one or two cups of coffee. The ratio between AFMU and 1X defines the acetylation rate (46).

It is assumed that the oxidation phenotype also plays an important role in defining individual susceptibility to the carcinogenic effect of aromatic amines on the bladder. A higher susceptibility to the carcinogenic effect of aromatic amines is linked with the high activity of certain forms of cytochrome P-450.

The determination of the debrisoquine oxidation rate is crucial in the analysis of xenobiotic oxidation polymorphism and the interrelationship between oxidation and bladder cancer. Debrisoquine is oxidated in the liver with cytochrome P-450IID1 to 4-hydroxy derivative. Until now, at least four gene mutations have been discovered which may produce the slow phenotype of debrisoquine oxidation. It is presumed that slow oxidisers of debrisoquine constitute 5–10% of the human population and this index varies in different ethnic groups (50).

A potential relationship between the oxidation phenotype and bladder cancer has not yet been formulated in any hypothesis, nevertheless it has been analysed within an extended research project covering various aspects of polymorphism of cytochrome P-450s.

Several papers have appeared (Cartwright et al.)(9), Kaisary et al. (23) and Roots et al. (42) which confirm that the slow phenotype of debrisoquine oxidations is less susceptible to bladder cancer.

Testing with an application of debrisoquine was relatively simple. The examinee was given *per os* 10 mg of debrisoquine (1 tablet) then the concentration of debrisoquine (D) and its 4-hydroxy derivative 74-DH were determined. Individuals with the fast oxidation phenotype displayed a D/4-HD ratio below 1.9; in persons with the slow oxidation phenotype the ratio was over 20.8 and those with intermediate oxidation phenotype displayed a ratio between 1.9 and 20.8.

Manifold casual factors should be searched for in the epidemiological studies due to the multifarious nature of bladder cancer. Apart from genetic determinations such as acetylation and oxidation phenotypes, exposure to aromatic amines and a person's life-style, especially smoking and nutrition habits (drinking much coffee, consumption of fried meat) also play a very important role. Co-occurence of all the above mentioned factors in one person creates an especially high risk of bladder cancer.

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