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Use of DNA Adducts in the Assessment of Occupational and Environmental Exposure to Carcinogens

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INTRODUCTION

MANY CARCINOGENS react covalently with DNA and may thereby initiate the multistage process leading to cell transformation and clinical malignancy [1]. Recently, methods have become available for the determination of DNA adducts in man [2–6]. The two principal techniques are the ^{32}P-postlabelling and immunoassay (Fig. 1). In the ^{32}P-postlabelling assay DNA is degraded enzymatically to 3′-nucleotides and a high specific activity phosphate group is introduced in the 5′-position from ATP using polynucleotide kinase [7, 8]. The adducts are analysed by thin-layer chromatography. In the immunoassay an antibody is raised against carcinogen-modified DNA or nucleotide [9, 10]. The assay of samples is usually carried out as a competitive ELISA. Both techniques are sensitive enough to allow adduct detection at levels encountered in humans. The ^{32}P-postlabelling assay may optimally detect one adduct per 10^{10} normal nucleotides [2, 3].

We have applied the above assays to humans who are either occupationally or environmentally exposed to carcinogenic compounds. In each case total white blood cells were used, and were coded in order to exclude bias.

FOUNDRY WORKER STUDIES

Air in iron foundries contains a number of carcinogens including polycyclic aromatic hydrocarbons (PAHs) [11]. Epidemiological studies from several countries have shown an excess risk of lung cancer among foundry workers and the risk appears to correlate with exposure to PAHs [11, 12].

Blood samples were drawn from foundry workers and their job descriptions were used to classify their exposures. The levels of aromatic adducts in foundry workers exceeded those in the controls. A highly significant dose-response relationship was observed both by the postlabelling [13] and immunoassay [14]. The adduct levels decreased to about one third in the course of a 4 week summer vacation indicating that they were job-related. Three different laboratories have carried out the postlabelling assays and their results correlate to a high degree [15]. A correlation was also noted between the postlabelling and immunoassay data [2].

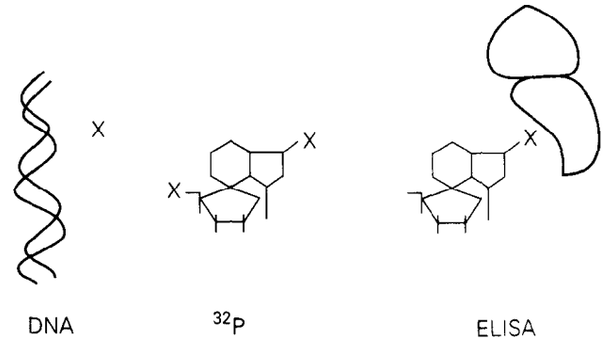

Fig. 1. Two sensitive methods, ^{32}P-postlabelling and immunoassay, for the determination of DNA adducts. X = adduct. Left, adducted DNA; middle, adducted nucleotide, digested from human DNA and postlabelled to the 5′-position with polynucleotide kinase; right, adducted nucleotide recognised by a specific antibody raised against *in vitro* adducted DNA or nucleotide.

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Received and accepted 17 Dec. 1990.

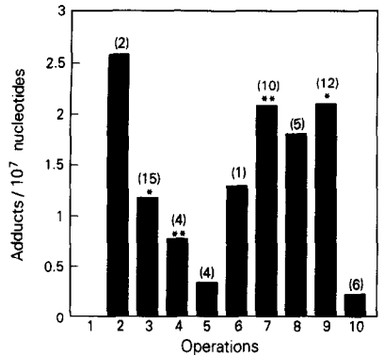

Fig. 2. DNA adducts in white blood cells of occupational groups of foundry workers as determined by postlabelling. The results are means of determinations from three laboratories (*$P < 0.05$, **$P < 0.01$, in comparison with the results of the referents (controls). (Number of individuals indicated at the top of the columns in parentheses.) From Ref. 16. Occupations: 1 = pattern making; 2 = sand preparation; 3 = moulding; 4 = melting; 5 = casting; 6 = shake-out; 7 = fettling; 8 = transport; 9 = other; 10 = control.

We have recently analysed the foundry data by occupational titles (Fig. 2). High adduct levels were found in workers whose jobs entailed high exposure to PAHs, e.g. those involved in sand preparation, moulding, shake-out, fettling and transport [16].

ADDUCTS AND AIR POLLUTION IN POLAND

Upper Silesia in south-west Poland is a main coal producing area also containing a number of coal-based industries such as coke works. The ambient air levels of pollutants such as PAHs are high in this area. Benzo(a)pyrene levels of 10–60 ng/m^3 have been measured in Silesian towns while the levels in unpolluted European and US towns are below 5 ng/m^3.

We obtained blood samples from coke workers (positive controls) in Silesia, from the local population, from those occupationally unexposed to PAHs, and from the rural population from eastern Poland. Coke workers had the highest adduct levels, as expected, but there was a surprising difference between the Silesian and rural population [17]. Both the postlabelling and immunoassay data showed 2–3 times higher levels of adducts in the Silesian population (Fig. 3). The postlabelling adduct patterns were quite similar between the Silesian residents and coke workers suggesting a common origin to the adducts. The adduct levels in the rural control population were similar to the Finnish control populations. These studies showed for the first time an association between environmental pollution and DNA

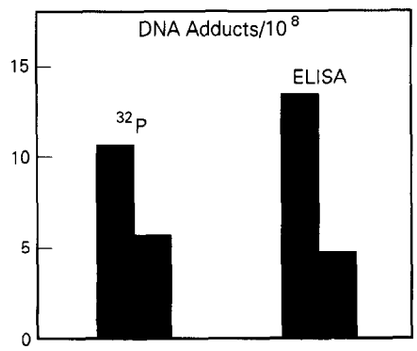

Fig. 3. Levels of aromatic white cell DNA adducts in Silesian (solid bars) and rural (shaded bars) populations by the postlabelling technique and immunoassay.

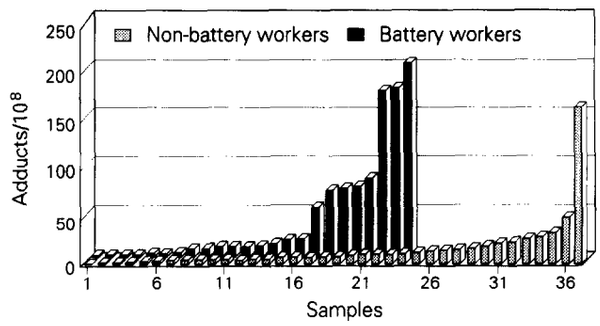

Fig. 4. White blood cell DNA adducts of individual Polish coke workers in battery (solid bars) and non-battery (shaded bars) operations.

adducts in humans. They show the power of the adduct studies when humans are exposed to complex mixtures from multiple sources.

A salient feature in DNA adduct studies is a large interindividual variation [18]. Figure 4 shows adduct levels in Polish coke workers, divided into battery (highest exposures to PAHs) and non-battery workers. The adduct levels vary by about 50-fold and are likely to reflect differences in exposure and individual kinetic/metabolic factors.

ADDUCTS IN ESTONIANS

Estonia has the world's largest refinery of shale oil, located in Kohtala-Järve (eastern Estonia). Shale is broken in open quarries and the organic material is extracted by heat distillation. Workers are exposed to a number of shale oil components including aromatic hydrocarbons and phenols; some exposure to PAHs also takes place. Shale oil workers were one study group.

The village of Saka is located 7 km from the shale oil industry and 3 km from a nitrate fertiliser factory. As pollution from both industries has caused concern in Saka, some residents were selected for the study. A control group was recruited from Tallinn.

Aromatic adduct levels in total white cells DNA of shale workers did not differ from those in the Tallinn residents (Table 1). The first sample of Saka residents showed a significantly elevated level of adducts. Five samples of each group were independently analysed by Dr K. Randerath, with results similar to ours.

The Saka residents differed from the other populations in many respects. They live mainly in single family houses, heated by stoves, while shale workers and Tallinn residents live in

Table 1. White blood cell DNA adducts in three Estonian populations

Group	No.	Adducts (S.D.) per 10^7 nucleotides
Shale oil workers	22	0.32 (0.17)
Saka residents		
Sample 1	20	1.41 (0.69)*
Sample 2	16	0.47 (0.20)
Tallinn residents	11	0.42 (0.29)

*$P < 0.001$ as compared to shale workers and Tallinn residents.

apartment houses, centrally heated. As the first sampling in Saka took place in the coldest time of the year (temperature −10° to −20°), with active wood and coal burning, a repeat sampling was taken later in milder climatic conditions (temperature +5°C). In the second sampling the Saka residents had only slightly more DNA adducts than the two other populations. Thus heating systems may have contributed to the adduct levels in the Saka residents. However, it is also known that pollution from the industry is periodical, depending on releases of pollutants and meteorological conditions. Heavy pollutant clouds hit the village some 10 times per year. It is well known that in most populations the main intake of PAHs is through food. As own gardens are common in Saka, contribution by vegetables and other garden products is also likely. Thus the origin of high levels of adducts detected in the first sampling remains unresolved.

CONCLUSIONS

The new DNA adduct detection techniques have been applied by us and others to study intense occupational exposures to complex mixtures containing PAHs. Dose-response relationships have been demonstrated between estimated exposure and level of aromatic DNA adducts. Two examples were taken from application to environmental monitoring, where in one case a clear elevation of adduct levels correlated with environmental pollution. Adduct determinations are indispensable tools in estimating complex, multisource exposures. Whether they relate to a risk of cancer at a group or individual level awaits longitudinal studies.

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