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LABORATORY RESEARCH

The Detection of DNA Adducts (Risk Factors for DNA Damage). A Method for Genomic DNA, the Results and Some Effects of Nutritional Intervention

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Abstract

Purpose: *Detection of organic chemical and metal DNA adducts in clinical samples.*

Design: *Open comparison between controls, people having routine laboratory investigations and groups of people with known exposure to toxic chemicals/metals.*

Materials and Methods: *Using genomic DNA from peripheral blood leucocytes, organic chemical DNA adducts were group separated by gas–liquid chromatography and metal DNA adducts were identified by plasma emission spectrography and spectrophotometry. There were 12 controls, 54 people having routine laboratory investigations, 14 tobacco smokers, seven people with known pesticide exposure, five with known toxic metal exposure, one person exposed to mycotoxins and two people with known nickel sensitivity. Eight of the participants with positive findings were re-tested after nutritional intervention.*

Results: *The previous finding of nitrosamine adducts in smokers is confirmed (13 of 14 smokers). Nitrosamine adducts were also found in nine non-smokers. DNA adducts were found to halogenated phenols (10), halogenated benzenes (10), vinyl halides (1), aldehydes (1), chloroethylenes (2), aflatoxin/mycotoxins (5), malondialdehyde (22) and lindane (3). The lindane DNA adduct is a new finding. Seven unidentified adducts were found. Adducts were found to lead (9), cadmium (20), mercury (7), aluminium (1), antimony (4), arsenic (8), nickel (20), strontium (1), copper (1), manganese (4), chromium (1) and cobalt (1). Two of the controls had low-level adducts. In eight people, 3 months of nutritional intervention led to the elimination of some DNA adducts and a semi-quantitative reduction in the level of others.*

Conclusions: *Significant numbers of DNA adducts can be detected in clinically relevant blood samples. In smokers, the expected nitrosamine adducts are accompanied by cadmium DNA and nickel DNA adducts. Nickel DNA adducts were found in some non-smokers and nickel is a known carcinogen. Lindane DNA adducts are identified for the first time. The results link gene research with the nutritional and environmental approaches to medicine. Early indications are that the burden of DNA adducts may be reduced by nutritional intervention.*

Keywords: DNA, DNA adducts, DNA damage, gene expression, cancer, smoking, minerals, nickel, lindane, toxic chemicals, toxic metals, malondialdehyde, environmental contaminants, nutrition.

INTRODUCTION

A wide range of toxic chemicals form addition compounds with DNA. The work on this is extensively referenced in publications from the International Agency for Research on Cancer [1, 2]. Many DNA adducts have been characterized in animal models, and some in

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humans [3–36]. Exocyclic DNA adducts that are mutagenic and carcinogenic can also be formed by some products of lipid oxidation [37–39].

The carcinogenic nature of many environmental contaminants, industrial chemicals and some endogenous lipid peroxidation products is explained by the formation of DNA adducts [40]. They change the structure and shape of the DNA so that difficulties occur in reading the genetic code [41]. They can activate oncogenes and may block tumour-suppressor genes [42]. Some metals and metallo-organic compounds also form adducts with DNA. The carcinogenic risks from metals are well established [43–69].

Detecting DNA adducts in humans is one way of assessing cancer risk and evaluating damage from chemical exposure [70]. These techniques have been applied to tobacco smokers [71]. In trying to develop a clinically meaningful method, I have had to consider the difficulty that some adducts may be corrected or bypassed by DNA repair enzymes or they may be on an area of DNA that is of little importance in terms of cancer risk. DNA repair has received detailed study in relation to some specific carcinogens [72]. The method described here uses genomic DNA from leucocytes. That is, DNA fragments produced by restriction enzymes in the cell. These enzymes recognize short, specific, sequences within the DNA and then catalyse double-stranded cleavage of the molecule [73–76]. They can degrade DNA that has been altered in some way. An adduct that is not repaired would probably fall into this category. Genomic DNA has been used in the study of aberrant methylation patterns [77–79] and for improved identification of single base changes [80].

Specialist research methods such as ^{32}P-post-labelling [21, 31, 36], mass spectrometry [14], gas chromatography/electron capture negative ionization mass spectrometry [25] and fluorescence techniques [9, 42] have enabled the exploration of the fundamental characteristics of specific DNA adducts. My aim has been to develop a technique for the detection of adducts in routine clinical samples where precise structural information is not needed. For example, nitrosamine adducts are associated with tobacco smoking and smokers have an increased incidence of lung cancer. The detection of nitrosamine adducts as a group rather than specific structural information about individual nitrosamines might still be a valuable indication of increased risk.

In this present study, two analytical procedures were applied to the genomic DNA. The organic adducts were separated into chemical groups and identified by gas–liquid chromatography. Metals associated with the DNA were specifically identified by direct current emission spectrometry.

The results are given for a group of patients referred for routine nutritional investigations, smokers, patients with known pesticide exposure, three cases of toxic metal exposure and one case of known exposure to mycotoxins. The findings are compared with the results from 12 control subjects.

Three patients with toxic metal DNA adducts, two with pesticide-related adducts and two with endogenous lipid peroxidation DNA adducts were re-tested after nutritional intervention.

METHODS

Separation of Genomic DNA from Leucocytes in a Peripheral Blood Sample

The MagPrep™ blood genomic DNA kit produced by Novagen (catalogue number 70690–3 supplied by CN Biosciences Ltd, Beeston, Nottingham, UK) was employed for the separation of the genomic DNA. The method is based on the capture of leucocytes on magnetic silica particles followed by lysis of the cells and immobilization of the genomic DNA on the surface of the magnetic particles.

Duplicate 100 μl aliquots of freshly collected heparinized whole blood were processed according to the kit manufacturer's protocol, including the additional step that ensures that

the end product is highly purified DNA (MagPrep blood genomic DNA kit, technical bulletin 255). A Magtight™ separation stand was used for the separation of the magnetic particles (Novagen catalogue number 69964–3). The DNA was released from the particles by adding 30 μl of elution buffer, warming to 56°C and vigorous vortex mixing for 1 min.

All analyses were performed in duplicate.

The DNA yield was established using a commercially available method (Sigma-Aldrich Co. Ltd, Poole, UK; catalogue number DNA-QF) which uses the fluorescent dye bisBenzimide (Sigma technical bulletin MB-590). The yield was consistently in keeping with the findings reported in the MagPrep blood genomic DNA kit technical bulletin 255. Quadruplicate extractions of DNA were within $\pm 3\%$ of the mean.

The Detection and Identification of Organic Adducts by Chemical Group Using Gas–Liquid Chromatography

Ten microlitres of the genomic DNA yield was added to 10 μl of hexane (high-performance liquid chromatography grade; Sigma-Aldrich; catalogue number 27,050–4) containing 50 μg l^{-1} of 4-nitrosopiperazine-1-carboxylic acid ethyl ester (Sigma-Aldrich; catalogue number N 7882) as the internal standard. After vortex mixing for 2 min and standing at room temperature for a further 10 min, 10 μl of the organic phase was injected into a pre-column sample loop of a capillary column-adapted HP5710A gas chromatograph (Hewlett Packard) fitted with a 30 m $\times$ 0.32 mm capillary column (HP part number 19091G-113). The carrier gas was high-purity nitrogen (BOC, London, UK) and the capillary-sleeved flame ionization detector was supplied with electrolytic hydrogen (Whatman Hydrogen Generator, The LabSales Company, Cambridge, UK).

The analytical conditions were: carrier gas, nitrogen 1.3 ml min^{-1}; injector temperature 100°C; initial column temperature 100°C; initial time 3 min; ramp rate 5°C min^{-1}; final column temperature 180°C; detector temperature 200°C.

The detector amplifier output was recorded at a chart speed of 0.5 mm sec^{-1} and a range sensitivity setting of 10 mV (Graphic 1002, Lloyds, UK). The output was also converted to a digital signal and interfaced (Widoshi I/O Card, Maplin Electronics, Rayleigh, UK) with a personal computer. An in-house software program was used to integrate the signals and to provide a printout of the adduct peaks found. These were identified with reference to the internal standard and the results of the calibration runs (see Table 1).

Note that, while very convenient, computer processing was not essential. However, most modern gas–liquid chromatography instrument manufacturers do provide software that facilitates excellent data handling.

The results were interpreted in terms of the chemical group to which any detected adduct belonged. Within each group, individual adducts were not identified, even if they were separated on the chromatogram. When an unidentified peak was found in an individual sample, the software reviewed that region of the chromatogram for each sample in the batch. If the peak was found in all or most of the batch of samples it was considered an artefact or contaminant.

Batch-to-batch comparisons also allowed our library of detectable adducts to be extended gradually. Unfortunately, all of the adducts found so far have not been identified.

Detection and Identification of Metals in the Genomic DNA

The remaining 20 μl of the eluted DNA was placed in a small (30 $\times$ 5 mm) acid-washed glass tube and evaporated to approximately 5 μl in a heating block at 110°C. Two graphite anodes, as used in the Beckman/Spectraspan IIIA atomic emission spectrometer, were placed pointed end down into the remaining solution. The liquid was absorbed into the tips and they appeared dry within 1 min.

TABLE 1. The chemicals used as calibration standards for the organic adducts (gas–liquid chromatography method). The following substances were used to establish the chromatographic retention characteristics of the chemical groups or individual compounds

Chemical group	Calibration
Aromatic amines/nitrosamines	N-nitrodimethylamine (Sigma N 3623) N-nitrosodiethylamine (Sigma N 0258) N-nitrosodiphenylamine (Sigma N 5882) Acetal (Aldrich A90–2) Acetaldehyde (Sigma A 4081)
Halogenated phenols	2,3-dichlorophenol (Aldrich D6, 980–7) 2,4-dichlorophenol (Aldrich 10,595–3) 2,6-dichlorophenol (Aldrich D7–020–1) 2,4,5-trichlorophenol (Aldrich 15,651–5) 2,4,6-trichlorophenol (Aldrich T5,530–1) Tetrachlorophenol (Nanogen) Pentachlorophenol (Aldrich P260–4)
Halogenated benzenes	1,3-dichlorobenzene (Aldrich 11,380–8) 1,4-dichlorobenzene (Aldrich 32,933–9) 1,3,5-trichlorobenzene (Aldrich T5,460–7) 1,2,3,4-tetrachlorobenzene (Aldrich 13,184–9)
Vinyl halides	Vinyl bromide (Aldrich 43,419–1) Vinyl chloride (Sigma 4–8625)
Aldehydes/malondialdehyde	Acrolein (Sigma 4–8501) Crotonaldehyde from crotonic acid (Sigma C 4630) Methylglyoxal (Sigma M 0252) Maladialdehyde (Sigma 10–838–3)
Chloroethanes	Chloroethane (Sigma 4–0015) Volatile halocarbons std #2 (Sigma 38,600–6)
Aflatoxin/mycotoxin	Aflatoxins B1, B2, G1, G2 (Sigma kit AF-1) Sterigmatocystin (Sigma S 3255)
Pesticides	Nanogen™ individual and mixed quality control standards (Nanogen)
Calibration checks (mixed standards)	Volatile mixture for gas chromatography–mass spectroscopy—60 components (Sigma 4–7932) Semi-volatiles calibration standards kit (Sigma 4–1118) Phenol mixture—11 components (Sigma 4–8859)

For the detection of metal adducts, stock standards of individual metals intended for atomic absorption and atomic emission analyses were obtained from Sigma-Aldrich Chemical Co. (Poole, UK) and from Merck (R&L Slaughter Ltd, Upminster, UK. 'Aristar' or 'Spectrosol' standards). These were diluted in high-purity water. All metals were identified using two or more emission spectral lines. Where appropriate, both atomic and ionic spectra were identified.

Avoiding finger contact and any other source of contamination, the graphite anodes were fitted into the plasma source and the spectrometer was prepared for photographic recording. The accessory mirror was brought into position as the bellows were expanded. This allowed recording on a Polaroid or standard $5 \times 4''$ photographic emulsion. In our machine, a $5 \times 4''$ photographic adapter can also be inserted in place of the exit slit plate and this gives better focus and definition. When this adapter is used, the accessory mirror and bellows arrangement are not required.

After calibration against a mercury lamp, the grating was set to the standard reference position of 253.2 nm by alignment to the internal LED source. This allowed spectral orders 84–98 to be recorded in the ultraviolet region. An exposure covering orders 51–67 can also be used to record spectral lines from the end of the ultraviolet region into the visible region.

While very convenient for use in the visible region, Polaroid emulsions are not suitable for recording ultraviolet spectra. For this region, an X-ray film with a lathanide image intensifier screen (Kodak XAR film and Kodak X-Omatic Regular screen) was used.

The dark slide was opened and the plasma was started according to the manufacturer's recommendations. The exposure continued for 30 sec and the dark slide was closed before stopping the plasma. The photographic record was assessed on a light box using a series of overlays showing the spectra of pure metals.

Our instrument was also set up for multi-element work using a 10×10 matrix of detectors in the plane of the output slits. This was convenient but by no means essential. Using it involved pre-judging the metals likely to be found. Unexpected results could be missed.

An internal standard of 10 μg of rhodium was included.

The Reporting of Results

Initial studies demonstrated the remarkable precision of the DNA extraction. This negated any need to quantify the precise DNA yield for individual samples. However, rather than just report the presence or absence of an adduct, a semi-quantitative reporting method was devised. This was particularly useful when looking for changes in follow-up tests.

For the gas–liquid chromatography analyses, an adduct peak was reported as a 'trace' if the signal reached $3\times$ the baseline variability. Results between $3\times$ and $6\times$ the baseline variability were reported as +, while larger peaks were reported as + +, + + + or + + + + +. A + + + + peak was at or above the peak height of the internal standard peak. Standard additions all represent 1 μg of the test substance.

Note that, with the exception of pesticides, organic adducts were identified by chemical group. It would only be possible to report an exact concentration for an individually identified chemical as the molecular weight and detector sensitivity will vary for each substance within the group. Pesticides were identified using Environmental Protection Agency (EPA) methods and Nanogen^{TM} quality control materials.

A similar reporting method was employed for metal adducts when using the 10×10 matrix of detectors. Photographic records of the spectral lines may be semi-quantified using a simple densitometer but, with a little experience, it is easy to grade these by eye as the spectra are compared with those on the overlays. A + + + + peak represented approximately 3 μg of the metal.

In the system used here it would be easily possible to quantify the metal concentrations. However, as that is not necessary for clinical usefulness and may be beyond the equipment available to some workers who may wish to confirm these findings, I used the same semi-quantitative reporting method as for the organic adducts.

Groups of People Studied

Group 1. Twelve people in good health, who had never smoked, had not taken any drugs for a minimum of 12 months prior to the test and with no known workplace or hobby-related exposure to toxic chemicals. There were seven females aged 19–51 years and five males aged 26–61 years.

Group 2. Fifty-four people referred for routine nutritional investigations; 33 females aged 17–63 years and 21 males aged 24–55 years.

Group 3. Fourteen tobacco smokers; nine males aged 20–68 years and five females aged 23–49 years.

TABLE 2. Number of organic chemical adducts found in each group

	Group 1 (control) $n = 12$	Group 2 (routine) $n = 54$	Group 3 (smokers) $n = 14$	Group 4 (pesticides) $n = 7$*	Group 5 (toxic metal) $n = 5$*	Group 6 (mycotoxin) $n = 1$*	Group 7 (nickel) $n = 2$*
Aromatic amines (general)	0	2 (4%)	2 (14%)	0	0	0	0
Nitrosamines	1 (8%)	5 (9%)	13 (93%)	1	1	0	1
Halogenated phenols	1 (8%)	3 (6%)	4 (29%)	2	0	0	0
Halogenated benzenes	0	4 (7%)	3 (21%)	2	0	1	0
Vinyl halides	0	1 (2%)	0	0	0	0	0
Aldehydes	0	1 (2%)	0	0	0	0	0
Chloroethylenes	0	2 (4%)	0	0	0	0	0
Aflatoxin/mycotoxins	0	3 (6%)	1 (7%)	0	0	1	0
Malondialdehyde related	1	7 (13%)	9 (64%)	3	1	0	1
Lindane	0	1 (2%)	0	2	0	0	0
Unidentified adducts	1 (8%)	4 (7%)	2 (14%)	1	0	0	0

* Number too small to give percentage values.
The group 7 patient with the nitrosamine adduct had been a smoker.

Group 4. Seven people with known pesticide exposure; three males aged 24, 47 and 61 years and four females aged 18, 29, 34 and 65 years.

Group 5. Three males (aged 38, 41 and 59 years) and two females (aged 24 and 50) with known toxic metal exposure.

Group 6. One 32-year-old female with known exposure to mycotoxins.

Group 7. Two females, aged 18 and 37 years, with known sensitivity to nickel.

Following the initial studies, three people with toxic metal DNA adducts, two with pesticide-related DNA adducts, two with endogenous lipid peroxidation DNA adducts, and one with a nickel DNA adduct were re-tested after 3 months of treatment with nutritional intervention programmes. Their follow-up results are also reported and compared with the results from two people with toxic metal DNA adducts, one with a pesticide adduct and three with endogenous lipid peroxidation adducts who remained untreated.

RESULTS AND DISCUSSION

The findings for organic chemical adducts are given in Table 2 and those for metal adducts are given in Table 3. For clarity of presentation, the semi-quantitative assessments of the levels of adducts found have not been included. All adducts reported were assessed as at least + by the scheme mentioned above. Semi-quantitative data for the people involved in the follow-up studies after nutritional intervention can be found in Tables 4 and 5.

Organic Chemical Adducts

One person in the control group had an unidentified adduct, another had an adduct to a halogenated phenol and an adduct to nitrosamine. Among the 54 people in group 2 there was a total of 33 adducts. Seven people had more than one adduct and one person had five adducts. On further enquiry his general practitioner revealed that he had fathered two children with serious genetic abnormalities, but there was nothing in his history to suggest abnormally high levels of chemical exposure.

TABLE 3. Number of metal adducts found in each group

	Group 1 (control) $n = 12$	Group 2 (routine) $n = 54$	Group 3 (smokers) $n = 14$	Group 4 (pesticides) $n = 7$*	Group 5 (toxic metal) $n = 5$*	Group 6 (mycotoxin) $n = 1$*	Group 7 (nickel) $n = 2$*
Toxic metals							
Lead	0	2 (4%)	4 (29%)	0	2	0	1
Cadmium	1 (8%)	4 (7%)	11 (79%)	0	3	0	1
Mercury	0	3 (6%)	2 (14%)	0	2	0	0
Aluminium	0	0	1 (7%)	0	0	0	0
Antimony	0	1 (2%)	2 (14%)	1	0	0	0
Arsenic	1 (8%)	2 (4%)	3 (21%)	1	1	0	0
Nickel	1 (8%)	5 (9%)	8 (57%)	1	2	1	2
Strontium	0	1	0	0	0	0	0
Metals that also have nutrient functions							
Copper	0	0	1 (7%)	0	0	0	0
Manganese	0	1 (2%)	2 (14%)	0	1	0	0
Chromium	0	0	0	0	1	0	0
Cobalt	0	0	1 (7%)	0	0	0	0

* Number too small to give percentage values.
Zinc was found in all of the DNA samples. The group 7 patient with the lead and cadmium adducts had been a smoker.

TABLE 4. Nutritional intervention studies

Patient group	Pre-treatment adducts	Post-treatment adducts
Toxic metals		
1*	Cd + + + Pb + + Nitrosamine + + + +	Cd + + Nitrosamine + +
2	Pb + + + Mn +	Mn +
3	Cd + + + + Sb + +	Sb + +
Pesticides		
4	Lindane + + +	Lindane + +
5*	Lindane + + Nitrosamine + + +	Nitrosamine + +
Malondialdehyde		
6	Malondialdehyde + +	None
7*	Malondialdehyde + + + Acrolein + + Cd + + + Nitrosamine + + +	Acrolein + + Nitrosamine + + +
Nickel		
8	Ni + + + + p-Dichlorobenzene + +	Ni + +

* Cigarette smokers.
Cd, cadmium; Pb, lead; Mn, manganese; Sb, antimony; Ni, nickel.

The most common non-smoking-related adducts were malondialdehyde related. This may well reflect excessive oxidative stress and/or poor antioxidant status [37–40, 70]. Thirteen of the 14 smokers in group 3 had nitrosamine adducts. This is in accordance with published findings [14–17, 81, 82]. One person in group 4 and one person in group 5 were also smokers and had nitrosamine adducts. The person in group 7 with a nitrosamine adduct did not report smoking but, on subsequent enquiry, she had been a smoker until 3 years prior to these tests.

The five people in group 2 with nitrosamine adducts were non-smokers, but three of them suffered passive smoking either at home or in the workplace. The one person in the control

TABLE 5. Repeat tests on untreated people

Patient group	Adducts found on initial testing	Adducts found after 3 months (untreated)
Toxic metals		
1	Cd + + + Pb + +	Cd + + + + Pb + +
2	Cd + + As + + +	Cd + + As + + +
Pesticides		
1	Lindane + + +	Lindane + + +
Malondialdehyde		
1	Malondialdehyde + + Acrolein + + +	Malondialdehyde + + + Acrolein + + +
2	Malondialdehyde + + + +	Malondialdehyde + + (*)
3	Malondialdehyde + + Cd + + + Nitrosamine + +	Malondialdehyde + + Cd + + + Nitrosamine + + (**)

* Improved result. **Passive smoker (workplace exposure).
Cd, cadmium; Pb, lead; As, arsenic.

group with a nitrosamine adduct had never smoked tobacco and was not exposed at home or at work.

In the smokers, the incidence of other adducts was also high. Nine of them had malondialdehyde-related adducts, four had halogenated phenol adducts, three had halogenated benzene adducts and one had an aflatoxin adduct, probably related to a hobby interest in collecting fungi. The smokers also had a high incidence of metal adducts (see below).

In the pesticide-exposed group of seven people, two had adducts to halogenated phenols with which they had worked. Two others had adducts to halogenated benzenes but no specific exposure history. However, one of these lived in a house that had recently been carpeted throughout with a wool carpet moth-proofed with para-dichlorobenzene. Three of the seven had malondialdehyde-related adducts, two of them had lindane adducts and one of them had an unidentified adduct whose position within the chromatogram suggested another organochlorine compound.

One of the people with a lindane adduct was a farmer, but he had no recollection of using this pesticide. The other was a woman with a history of breast cancer who thought she had used a lindane-containing preparation on her two children for the treatment of head lice.

I believe that this is the first time that a specific lindane adduct has been demonstrated. If this is repeatable by other workers it will be a substantial contribution to the understanding of a possible connection between lindane and the incidence of breast cancer. It comes at a time when lindane has already been banned in many countries. Malondialdehyde DNA adducts are associated with breast cancer [21, 26].

The one person with known exposure to mycotoxins did have an adduct to one of these substances. She also had an adduct to a halogenated benzene. There was no obvious exposure history to account for this.

Metal Adducts

In the control group, one person had adducts to both cadmium and nickel, but there was no history of smoking and no other obvious source of these metals. Another person had an adduct to arsenic without any obvious exposure source.

In the 54 people in group 2, 19 metal adducts were found, the most common being adducts to nickel (five), closely followed by cadmium (four) and mercury (three). Only one person in this group had more than one metal adduct. He had adducts to cadmium, lead and nickel, but there was no history of smoking.

Cigarette smoke contains nickel and cadmium, but the high incidence of DNA adducts

to these metals in smokers is, as far as I know, a new finding. Nickel is known to be carcinogenic [47, 48, 50, 54, 56, 58, 65, 67]. The smokers also had a high incidence of adducts to other toxic metals.

In the seven pesticide-exposed people, one had antimony and nickel adducts without specific identifiable exposure, and another had an arsenic adduct. He had been exposed to arsenic compounds used in rodent control.

Four of the five people with known exposure to toxic metals had more than one metal adduct and in three cases these reflected the metals to which they had been exposed. Lead and cadmium in one case, lead, mercury and cadmium in another, and cadmium, mercury, manganese, chromium, nickel and arsenic in the third. The latter person was severely disabled and infertile. Soon after these investigations he underwent tests for possible bowel cancer. He subsequently died in hospital after an exploratory operation during which extensive metastatic cancer was identified. The other two members of this group had both been exposed to cadmium but they did not have cadmium adducts. One had mercury and nickel adducts and the other had a nickel adduct.

The two people in the nickel-sensitivity group both had nickel DNA adducts. This is of serious concern given the known carcinogenic properties of nickel. One of these women had given up smoking 3 years prior to these tests. In addition to the nickel adduct, she had cadmium, lead and nitrosamine adducts.

Zinc was found to be associated with DNA in every sample analysed. This is in accordance with published findings. See [83] for a review of this subject.

Nutritional Intervention Studies

Eight people from the various groups agreed to be re-tested for DNA adducts after a 3-month nutritional programme. In all cases, 30 mg of zinc (as zinc gluconate) was given last thing at night. In all other respects the recommended programmes were tailored to the needs of the individual patients and other supplements given were based on their laboratory test findings.

Six people from various groups who remained untreated for 3 months were re-tested for DNA adducts (Table 5).

The small number of participants and the variability of the treatment programmes ensure that only minimal conclusions can be drawn from the findings. However, the detection of such large numbers of adducts in the main part of this study and the apparent usefulness of nutrient intervention, even in this small number of people, justify the inclusion of the data in Table 4.

In every case, following nutritional intervention, there was a reduction in the number of adducts present and/or the level of some individual adducts. Unfortunately, only one of the participants was totally free of adducts after treatment. That person had a malondialdehyde adduct that responded to the inclusion of antioxidants in the supplement programme. In terms of known carcinogens, nitrosamine and nickel adducts were reduced in level but not eliminated during this limited study. In two people cadmium adducts were successfully removed and the level of the adduct was reduced in a third person. A lead adduct was also resolved in one participant.

The new finding of a lindane DNA adduct adds weight to the possible carcinogenic properties of this pesticide. In this limited intervention study, two participants had lindane adducts prior to the use of the supplement programme. One was removed completely and the other was detected at a slightly lower level.

Only one of the people in the untreated group showed any reduction in their DNA adduct burden (Table 5).

Clearly, only limited conclusions can be drawn from this intervention study, but I believe that it does indicate the potential usefulness of a more complete investigation using larger numbers of participants. Whether it would be ethical to divide people with laboratory-

proven nutrient deficiencies into treatment and non-treatment groups is outside the scope of this paper.

CONCLUSIONS

It is widely accepted that numerous carcinogenic and potentially carcinogenic chemicals bind to DNA. The resulting adducts lead to mutations and this is an important initiating event in many cancers.

This study demonstrates that DNA adducts are a common finding in people with known exposure to toxic organic chemicals and toxic metals. There is also a fairly high incidence of adducts in people without specifically identified exposure sources. This includes nitrosamine adducts in some non-smokers, particularly those with smoking colleagues or domestic passive smoking. The mutagenic and carcinogenic nature of many toxic chemicals is explained by the formation of DNA adducts.

This paper provides the first evidence of a specific DNA adduct with lindane and adducts with some of the toxic metals. It also identifies a potentially serious consequence of nickel sensitivity and ongoing nickel exposure. Nickel DNA adducts are formed and nickel is a known carcinogen.

The method detailed here is capable of detecting DNA adducts in clinical blood samples. DNA adducts can activate oncogenes and they may be able to block tumour-suppressor genes. However, it is very clear that much more work is needed to quantify properly the potential connections between some of these adducts and carcinogenic risk.

The results provide links between the rapidly advancing fields of molecular biology and gene research and the nutritional and environmental factors with which readers of the *Journal of Nutritional & Environmental Medicine* concern themselves. I hope these findings will stimulate discussion and further work in this area.

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