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Removal of DNA adducts

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ABSTRACT

DNA adducts are associated with a number of diseases, including cancer. Based on a recent report by our group, the aim of this study was to test the hypothesis that DNA adducts can be removed by means of one or more of the following three intervention programmes: intermittent whole-body hyperthermia; detoxification; and cell repair. The number of DNA adducts and total DNA adduct concentrations were measured in 104 patients who underwent one or more of the three intervention programmes. DNA adduct assessments were carried out on extracted genomic DNA by gas-liquid chromatography, with any DNA adducts found being localised using DNA microarrays. The baseline median number of DNA adducts was 2. The follow-up median number of adducts was highly significantly lower at 0 ($p < 0.000000000000003$). The mean total DNA adduct concentration at baseline was 9.308 ng/mL, and highly significantly lower at follow-up at 1.553 ng/mL ($p < 0.000000000000006$). Of the three intervention programmes, only the intermittent whole-body hyperthermia was associated with a significant reduction in DNA adducts. This study offers support for the hypothesis that DNA adducts can be removed by intermittent whole-body hyperthermia. The intermittent hyperthermia used involved infrared-A (wavelength 700–1400 nm, or, equivalently, a frequency of 215–430 THz) being preferentially delivered to the whole body, apart from the head, for up to one hour per session, with gradual core body temperature elevation usually occurring during the first 20–30 min. These results may offer an explanation at the molecular level for other reported clinical benefits of intermittent whole-body hyperthermia.

Introduction

A DNA adduct consists of the covalent binding of a substance to DNA [1]. In the early 1960s, it was shown that DNA adduct formation could be associated with carcinogenesis [2]. While many carcinogens are too lipophilic to be able to form covalent bonds with DNA, their metabolism by the body (in order to facilitate their excretion) may render them reactive electrophiles which can form adducts; examples of substances which require such metabolic activation include aflatoxins, arylamines, polycyclic aromatic hydrocarbons, and vinyl chloride [3]. In general, the effect of a DNA adduct may be considered to depend on the position of the adduct in the genome; at a gene location, gene expression may be suppressed, while at a gene promotor region, overexpression may occur [1]. Thus, besides carcinogenesis, there are numerous other potential clinically deleterious consequences of DNA adducts.

Background

We recently reported the association between an epoxy-fatty acid at the locus of the *N*-formyl peptide receptors and parkinsonian symptomology in a male patient [1]. Furthermore, following a combination of three interventions, the patient was found to be free of DNA adducts after one year. These interventions were: intermittent whole-body (excluding the head) hyperthermia sessions; the administration of detoxification and supportive nutrients; and participation in a cell-repair programme involving the intravenous administration of fatty acids and phospholipids. The change in DNA adduct status was associated with the patient becoming free of parkinsonian symptoms, including a complete remission of cogwheel rigidity and akinesia. He also showed improvement in his olfactory function [1].

The hypothesis

We therefore hypothesised that DNA adducts can be removed by means of one or more of the following three intervention programmes: intermittent whole-body hyperthermia; detoxification; and cell repair. The aim of this study was to test this hypothesis by assessing the level of DNA adducts of patients before and after receiving either none or one or

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Table 1
Treatment interventions received by each of the patients. + denotes that the patient underwent the corresponding intervention, while the symbol − denotes that the patient did not undergo this intervention.

Patient number	Hyperthermia	Detoxification	Cell repair
1	−	−	+
2	−	+	−
3	−	+	−
4	−	−	+
5	+	+	+
6	+	+	−
7	−	+	+
8	+	+	+
9	+	+	+
10	+	+	−
11	+	+	−
12	+	−	+
13	+	+	+
14	+	+	−
15	−	+	+
16	−	+	−
17	−	−	+
18	+	+	+
19	+	+	+
20	+	−	−
21	−	+	−
22	+	+	+
23	+	+	−
24	−	+	+
25	+	+	+
26	+	+	−
27	+	+	−
28	+	+	+
29	−	+	−
30	+	+	+
31	−	+	−
32	+	−	+
33	−	+	+
34	+	+	+
35	+	+	−
36	−	+	−
37	−	+	−
38	+	+	+
39	+	+	+
40	−	+	+
41	+	+	+
42	+	+	−
43	+	+	+
44	−	+	+
45	−	+	−
46	−	+	−
47	+	+	+
48	+	+	+
49	−	+	+
50	+	+	+
51	−	+	+
52	+	+	−
53	−	+	+
54	+	+	+
55	−	+	+
56	−	−	+
57	−	+	−
58	−	+	+
59	−	+	+
60	+	+	+
61	+	+	+
62	+	+	−
63	−	+	−
64	+	+	−
65	+	+	−
66	+	+	−
67	+	+	+
68	−	+	+
69	−	+	+
70	−	−	+
71	+	+	−
72	+	+	+
73	+	+	+
74	−	+	+
75	−	+	+
76	−	+	−
77	−	+	−
78	−	+	+
79	+	+	−
80	+	+	+
81	+	+	+
82	+	+	−
83	−	+	−
84	+	+	−
85	−	+	−
86	−	−	+
87	+	+	+
88	+	+	+
89	+	+	+
90	−	+	+
91	−	+	−
92	−	+	+
93	+	+	+
94	+	+	−
95	−	+	+
96	−	+	−
97	+	+	−
98	+	+	+
99	+	+	−
100	−	+	+
101	+	+	−
102	+	+	−
103	−	−	+
104	−	+	−

more of these three interventions.

Evaluation of the hypothesis

This hypothesis was tested by carrying out an audit of 104 patients who were assessed for DNA adducts and who were then re-tested after undergoing one or more of the following three intervention programmes: hyperthermia; detoxification; and cell repair. The intervention programme for each patient is shown in Table 1, while the upper bounds of the costs of each programme are shown in Table 2. The majority of the patients were suffering from chronic fatigue syndrome (myalgic encephalomyelitis or systemic exertion intolerance disease); additional and/or alternative diagnoses included Lyme disease (Lyme borreliosis), Epstein-Barr viral (human gammaherpesvirus 4) infection or infectious mononucleosis, multiple chemical sensitivity, dysautonomia including autonomic and peripheral neuropathy, mycotoxicity, mastocystosis, gastrointestinal tract dysbiosis, pancreatic insufficiency, Hashimoto's thyroiditis, yeast infection, Ehlers-Danlos syndrome, Gilbert's syndrome, osteoporosis, candidiasis, psoriasis, photosensitivity, Crohn's disease, gastro-oesophageal reflux disease and seasonal affective disorder. The audit was carried out with research ethics committee approval and was carried out in accordance with the Declaration of Helsinki.

The date of birth and sex of each subject were recorded. From venous blood samples taken at baseline, leucocytes were separated. These were analysed at Acumen (Tiverton, Devon, UK), a laboratory which

Table 2
Maximum total cost for each treatment intervention.

Treatment programme	Cost
Hyperthermia	≤ £600
Detoxification	≤ £1000
Cell repair	≤ £1000

has full United Kingdom Care Quality Commission (CQC) approval, for DNA adducts by analysis of extracted genomic DNA by gas-liquid chromatography. Any DNA adducts found were localised using DNA microarrays.

The hyperthermia intervention consisted of a programme of one-hour high-temperature sessions in the IRATHERM® 1000 [4,5], followed by rehydration. The detoxification intervention consisted of a programme of intravenous and oral nutritional supplementation, including glutathione, alpha-lipoic acid, selenium, methylcobalamin and ascorbic acid. The cell repair intervention consisted of a programme of intravenous supplementation with fatty acids, including linoleic acid, oleic acid, palmitic acid, α-linolenic acid, and stearic acid, and with phospholipids, including, in particular, phosphatidylcholine, as well as some oral nutritional supplementation.

Following the interventions, a further venous blood sample was taken from each patient and the above DNA adduct assay was repeated. These assays were carried out blind to the nature of any intervention programme.

Non-parametric repeated measures tests were carried out comparing the number of DNA adducts at follow-up with that at baseline. Further similar analyses were carried out in respect of each of the three possible interventions. The change in mean total DNA adduct concentration was assessed using the paired *t*-test after checking normality assumptions. All tests were two-tailed. The software package used for the statistical analyses was R version 3.0.1, running on an x86_64-w64-mingw32/x64 (64-bit) platform [6].

The mean (standard error) age at baseline was 50.9 (1.6) years and 66 (63%) of the patients were female. The mean time between baseline and follow-up testing was 18.6 (1.6) months, and for each intervention programme was as follows: hyperthermia 18.2 (2.2) months; detoxification 18.5 (1.6) months; and cell-repair 19.2 (2.1) months. The baseline median number of DNA adducts was 2 (interquartile range (IQR) = 2). The follow-up median number of adducts was highly significantly lower at 0 (IQR = 1; Wilcoxon signed rank test, $V = 4379$, $p < 3 \times 10^{-15}$); see Fig. 1. The mean total DNA adduct concentration at baseline was 9.308 (0.867) ng mL^{-1}. This was much higher than the follow-up mean total DNA adduct concentration of 1.553 (0.241) ng mL^{-1} ($t = 9.1651$, $df = 103$, $p < 6 \times 10^{-15}$).

The DNA adducts which were lost or gained during the study period are shown in Table 3.

Of the 104 patients, 56 participated in the hyperthermia programme, 95 in the detoxification programme, and 61 in the cell repair programme (see Table 1). In the hyperthermia subgroup, five patients experienced no change in the number of DNA adducts, two showed an increase, while 49 showed a decrease. Overall, the decrease in the number of DNA adducts for this subgroup was statistically significant (Wilcoxon rank sum test, $W = 1050.5$, $p = 0.04778$). In the detoxification subgroup, five showed an increase in adducts, eight no change,

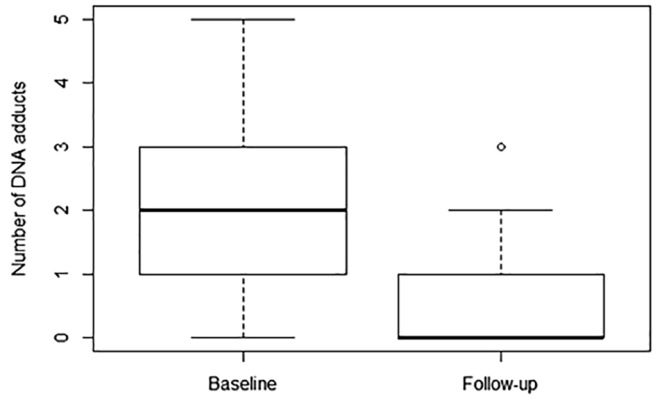

Fig. 1. Boxplots showing the number of DNA adducts at baseline and at follow-up.

Table 3
Changes in DNA adducts.

Adducts removed	New adducts acquired
Acetaldehyde complex	Copper complex
Antimony	Dimethicone
Azo dye	Hippuric acid
Benzoate	Mercury
Benzoquinone	Methylbenzoquinone
Bisphenol A	Methylparaben
Butadiene complex	Nickel complex
Butanol complex	Nitrosopyrrolidine
Butylhydroanisole	Pentachlorophenol
Cadmium complex	Sulfite complex
Calcium complex	1,3,5-Trioxane
Cetrimide	Trioxymethylene
Chloromandelic acid	Tryptamine
Chromium complex	
Cinnamyl anthranilate	
Copper complex	
Crotonaldehyde	
Cyclopentasiloxane	
Diaminoanisone	
2,4-Diaminophenol	
Dianisidine	
Diazo/diamino compound	
Dibutylphthalate	
p-Dichlorobenzene	
Dichlorodiphenyltrichloroethane	
Dichlorophenol	
Dichloroxylenol	
Dichloroxylenol	
Dieldrin	
Dimethicone	
Dimethylphthalate	
Diolein	
Diolein	
Epoxy-oleic acid	
Estradiol	
Ethylene glycol derivative	
Fenazaquin	
Fluoride complex	
Fructose-1-phosphate	
Fructose-6-phosphate	
Fungisterol	
Glycidol	
Glyco-protein	
GSH-peptide conjugate	
Hippuric acid	
3-Hydroxybutyrate	
Indole	
Juglone	
Kynurenic acid	
Lead complex	
Lindane	
Malondialdehyde	
Mandelic acid derivatives	
Mercury complex	
Mevinphos	
α-Naphthol	
β-Naphthol	
Naphthoquinone	
Nickel	
Nitrosamine	
Nitroso compound	
N-Nitrosodiethylamine	
Nitrosopyrrolidine	
Octoxynol	
Organophosphate	
Patulin	
Pentachlorophenol	
Peptide complex	
Phenoxyethanol	
Phenoxyethylene	
p-Phenylenediamine	
O-Phenylphenol	
Phosphoglycerate	
Phthalate	

(*continued on next page*)

Table 3 (*continued*)

Adducts removed	New adducts acquired
α-Pinine	
Plant sterol	
Polyethyl alcohol	
1,4-Polyisoprene	
Pyrazole-3-carboxylase	
Resorcinol	
Strontium complex	
Tartrazine	
Toluene derivatives	
Trichlorodimethyl phosphate	
2,4,6-Trichlorophenol	
2,4,5-Trichlorophenyl compound	

and 82 a decrease; the overall decrease was not significant (Wilcoxon rank sum test, $W = 402$, $p = 0.7604$). For the cell repair programme subgroup, four showed an increase, six no change, and 51 a decrease in the number of adducts; this overall decrease was again not significant (Wilcoxon rank sum test, $W = 1360.5$, $p = 0.738$).

Discussion

The results of this study support our hypothesis that human DNA adducts can be removed. Furthermore, this study has indicated that it is hyperthermia, rather than detoxification or cell repair, which is able significantly to reduce DNA adducts. As seen in Table 3, many of these adducts are of clinical importance. For example, nitrosamine is associated with malignancies [7–9], while, as mentioned above, epoxy-fatty acids may be associated with parkinsonism [1].

In the late 1950s and early 1960s, it was reported that intermittent hyperthermia enhanced the susceptibility of tumours and other tissues to ionising radiation [10,11]. This intervention appears to have other beneficial clinical applications besides the treatment of cancer. For example, murine studies have shown that transient hyperthermia has a protective action against future ischaemic damage in the forebrain [12]. It has been postulated that an important molecular-biological mechanism of the therapeutic action of hyperthermia in cancer patients could involve modulation of the expression of genes such as those of the family of heat-shock proteins (HSP) and multiple drug resistance (MDR) [13–15]. Our results may offer an explanation for this mechanism of action. Removal of DNA adducts may change the expression of the previously affected genes, in a manner related to the location of these adducts; if a given adduct had been located at an exon, this might have suppressed gene expression, while if it had been located at a promoter region, this might have caused overexpression.

It is germane to consider HSP and hyperthermia further. Following its discovery in *Drosophila* in the early 1960s by Ritossa [16,17], the heat shock response was found to occur in a wide variety of cells and living organisms and to result from the induction of HSP [18]. HSP contain highly conserved amino acid sequences across prokaryotes and eukaryotes, have important housekeeping functions, and have been implicated in many human diseases; it should be noted, however, that they are not damage-associated molecular pattern molecules [18–20]. The housekeeping functions of HSP include roles in detoxification and cell repair. Importantly, Lipsker and colleagues have shown that monocyte-derived dendritic cells express receptors which bind HSP 70 and 60 (hsp70 and hsp60, respectively) [21]. Both hsp70 and hsp60 can be internalised via receptor-mediated endocytosis, together with human leukocyte antigen (HLA) class I molecules, thereby reaching endosomal cellular compartments, which include compartments enriched in the major histocompatibility complex (MHC) class II cell surface HLA-DR receptors [21]. In turn, this paves the way for the existence of an internalisation pathway whereby hsp70 and hsp60, together with antigens bound to them, may undergo processing and loading onto HLA class I and II molecules [21].

The type of intermittent hyperthermia used in the present study, namely IRATHERM® whole-body hyperthermia, is based on the work of the late Professor Manfred von Ardenne [22]. Infrared-A (IR-A, defined as having a wavelength in the range 700 − 1400 nm, or, equivalently, a frequency in the range 215–430 THz) is preferentially delivered to the whole body (apart from the head) for up to one hour per session. During each session, gradual elevation of the core body temperature usually occurs during the first 20–30 min. Use of this intervention was claimed by von Ardenne to be clinically beneficial for a wide variety of illnesses [22]; again, our results may offer an explanation for this at the molecular level.

Grant support

Nil.

Conflicts of interest

None.

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