Separation of 7-methyl- and 7-(2-hydroxyethyl)-guanine adducts in human DNA samples using a combination of TLC and HPLC

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We have used a combination of thin-layer chromatography (TLC) and high pressure liquid chromatography (HPLC) to achieve separation of $^{32}$P-postlabelled 7-methylguanine and 7-(2-hydroxyethyl)-guanine adducts. The level of these two adducts was determined in human total white blood cells (mean values 0.7 to 1.5 adducts per $10^7$ normal nucleotides) and isolated lymphocytes (mean values 1.1 to 12 adducts per $10^7$ normal nucleotides). The separation of these two adducts revealed that the level of 7-(2-hydroxyethyl)-guanine was twice the level of 7-methylguanine adducts in total white blood cells, whereas, in isolated lymphocytes it was at least four times more than the 7-methylguanine adduct. The combined level of these two adducts in the lymphocytes of non-smokers was 1.1 to 8.4 adducts per $10^7$ normal nucleotides and in the lymphocytes of smokers, the level was 5.6 to 12 adducts per $10^7$ normal nucleotides. We also report detection of three unidentified adducts in the samples analysed, and at least one of these adducts seemed to be related to smoking. The chromatographic behaviour and depurination at neutral pH indicated the probable 7-alkylguanine or 3-alkyladenine nature of these unidentified adducts.

Introduction

DNA is subject to in vivo modifications by various exogenous and endogenous agents (1-4). The formation of adducted DNA bases is a consequence of covalent linkage of reactive electrophilic species with ring and exocyclic nitrogens and exocyclic oxygens. Various alkylating species react with N-7, N-3 and O$^6$ position of guanine, N-3 position of adenine and O$^4$-position of thymine (3). N-7 position of the guanine being the most reactive, 7-alkylguanine is the major lesion induced in DNA by endogenous and exogenous alkylating agents (4-6). Due to non-involvement of the N-7 position of guanine in the stabilization of double helical DNA through hydrogen bonding, small 7-alkylguanines have been shown to be non-mutagenic per se (3). However, the destabilization of the glycosyl bond due to N-7 substitution on guanine results in the presence of additional unidentified adducts in these samples.

Chemicals and instruments

Salmon testis DNA and micrococcal nuclease were obtained from Sigma Chemical Co., USA; nuclease P1 and spleen phosphodiesterase from Boehringer Mannheim, Germany. [$^{32}$P]ATP (sp. act. 3000 Ci/mmol) was purchased from Amersham, UK and cloned T-4 polynucleotide kinase from US Biochemical Corporation, USA. Polyethyleneimine TLC plates were from Macherey Nagel, Germany. Strong anion exchange cartridges were purchased from J.T.Baker, Holland.

Semi-preparative HPLC was performed on a Beckman Gold system coupled with a module 168 diode array detector. The analysis of radiolabelled samples was done on a Beckman model coupled in series with 166 UV and 171 radioisotope detector using a 50 $\mu$l Teflon tube as a flow cell.

Synthesis of standards

Deoxyguanosine-5'-monophosphate (5'-dGMP; 5 mg) was treated with 100 mM dimethyl sulphate (DMS) in 0.5 M Tris-HCl buffer (1 ml), pH 7.4 at room temperature for 1 h. The reaction mixture was extracted with ethylacetate (1 volume) twice and pre-purified on a strong anion-exchange cartridge prior to HPLC separation of 7-methyl-deoxyguanosine-5'-monophosphate. The adduct was purified by semi-preparative HPLC on a reverse phase (Nucleosil 120-5 C18, 10×250 mm) column using a linear gradient of

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methanol in 50 mM ammonium formate, pH 4.6. The concentration of methanol was maintained initially at 10% for 12 min and then increased to 25% in the next 25 min. Unmodified and modified nucleotides were eluted at retention times of 15.9 and 19.2 min respectively. The identity of the adduct was confirmed by running TLC plates under UV light at neutral, alkaline and acidic pH.

7-(2-Hydroxyethyl)-deoxyguanosine-5'-monophosphate adduct was synthesized by treating 5'-GMP with ethylene oxide (100 mM) in 50 mM Tris-HCl (1 ml), pH 8.0. The reaction mixture was incubated at 37°C for 4 h. HPLC separation was achieved as described above. DNA standards for these adducts were obtained by treating salmon testin DNA with DMS and ethylene oxide and the level of 7-alkylguanine and adducts was determined by depurination at neutral pH as described earlier (20, 24).

Isolation of DNA from total white blood cells and isolated lymphocytes

DNA was isolated from total white blood cells from four middle aged non-smoker males (age 40–60 years). Lymphocytes were separated from the blood drawn from eight female donors (age 37–81 years, four smokers and four non-smokers). Twenty ml samples of total blood were mixed with equal volumes of 10 mM Tris-HCl, pH 7.4, 1 mM MgCl₂ and 1 mM Triton-X 100 (10%). The samples were gently vortexed and centrifuged at 4000 r.p.m. for 5 min. The total white cells pellet was washed thrice and finally suspended in 5 ml of the same buffer. The cell suspension was mixed with Triton-X 100 (10%, 100 µl) and kept on ice for 5 min. The nuclear pellet obtained by centrifugation at 4000 r.p.m. (5 min) was suspended in 50 mM Tris-HCl, pH 8.0 (2 ml) and treated with RNase A and T1 followed by proteinase K treatment. Proteins were removed by extraction with pre-treated phenol (1 volume) and chloroform/isoamyl alcohol (1 volume). DNA was precipitated with ethanol and 0.1 M (final concentration) sodium acetate and washed twice with 70% ethanol. The concentration and purity of DNA, reconstituted in water, was measured by measuring absorption at 260, 280 and 320 nm on a Beckman DU 640 spectrophotometer. RNA contamination was determined by reverse phase HPLC after enzymatic hydrolysis of DNA to deoxyribonucleotides.

For the isolation of DNA from lymphocytes, isolated lymphocytes were treated as described above.

³²P-postlabelling

The aliquots of DNA (5 µg) from white blood cells and lymphocytes, as well as DNA reacted in vitro with DMS and EIO, were incubated at 37°C with micrococcal nuclease (80 nM/µg DNA, 2 µl) in 3 mM Bicine (0.5 µl), pH 9.0 and 0.2 mM CaCl₂ (0.5 µl) for 2 h followed by addition of spin column phosphodiesterase (1.6 µM/µg DNA, 20 µl) and 20 mM ammonium acetate (1.0 µl), pH 5.0. The incubation was continued for a further 2 h. The DNA digests were applied to strong anion exchange cartridges which were equilibrated with 5 mM ammonium formate, pH 5.2. The eluents (4 ml) were freeze dried and taken for postlabelling. DNA samples reacted in vitro with DMS and EIO were diluted with appropriate proportions of unmodified ST DNA to obtain uniform adduct levels.

³²P-postlabelling was carried out in a total volume of 2.0 ml containing 2 ml of [³²P] ATP, 40 mM Bicine buffer, pH 9.6, 20 mM MgCl₂, 2 mM spermidine and 6U T4 polynucleotide kinase. The mixture was incubated at 37°C followed by incubation with nuclease PI (2.5 µg, 0.5 µl) for 10 min. The pH of the reaction mixture was adjusted to 5.5 with 1% formic acid (1.0 µl). The mixture was heated to 65°C to convert bisphosphate nucleotides to 5'-monophosphate nucleotides.

³²P-postlabelled mixtures were applied to pre-washed 10X20 cm PEI TLC plates and developed with 0.1 M ammonium formate, pH 5.2 in the first dimension. The plates were developed in the second dimension with 0.5 mM ammonium formate, pH 5.3 and 30% n-propanol (solvent system A). Alternatively, the second dimension was developed with the proportion of n-propanol increased to 50% (solvent system B). The plates were exposed to X-ray films (Kodak XAR-5) overnight. The areas of the plates corresponding to adduct spots on autoradiographs were cut and counted in a Cerenkov counter. For the background subtraction, the corresponding area of plates on which samples depurinated prior to labelling were applied, were cut and counted.

The areas of plates corresponding to 7-methyl and 7-(2-hydroxyethyl)-deoxyguanosine-5'-monophosphate adducts after counting were eluted with 10 mM ammonium formate (1 ml), pH 5.3 by sonication in an ultrasound bath for 30 s. The extract was filtered through 0.2 µm filter, freeze dried and reconstituted with water. Aliquots of these extracts were analysed on HPLC coupled with monitoring UV and radioisotope detectors in series. The retention times of the adducts were confirmed by analysing the aliquots of TLC extracts spiked with synthesized 7-methyl and 7-(2-hydroxyethyl)-deoxyguanosine-5'-monophosphate adducts which were used as UV markers. The separation was carried out on Kromasil C18, 150X2.0 mm, 5 µm column protected from particulate material with a 0.5 µm pre-column filter. The elution was carried with 10% 0.2 M ammonium formate buffer, pH 4.6, initially for 10 min, followed by a linear gradient over the next 10 min to 10% methanol, which was maintained for 5 min. The methanol concentration in the solvent was increased to 30% in 10 min and then to 100% in the next 10 min. 7-Methyldeoxyguanosine-5'-monophosphate and 7-(2-hydroxyethyl)-deoxyguanosine-5'-monophosphate eluted separately at the retention times of 7.1 and 5.9 min, respectively.

The recovery of adducts was determined from the levels of adducts detected by ³²P-postlabelling in DNA modified in vitro with DMS and EIO separately in each experiment. These values were used to calculate levels of adducts in human DNA samples.

Results

Recovery of 7-methylguanine and 7-(2-hydroxyethyl)-guanine adducts by ³²P-postlabelling

The recovery of 7-methylguanine and 7-(2-hydroxyethyl)-guanine adducts from the DNA reacted with DMS and ethylene oxide, respectively, was determined as described earlier, except the concentration of T4 polynucleotide kinase in the phosphorylation reaction was increased from 3 to 6 U (20). This resulted in substantial increase in the recovery of 7-(2-hydroxyethyl)-guanine adducts. The overall recovery of 7-methylguanine and 7-(2-hydroxyethyl)-guanine adduct was 49.7 ± 17.5 (n = 5) and 23.9 ± 7.9 (n = 5) percent respectively. The recoveries of 7-methyl and 7-(2-hydroxyethyl)guanine adducts in DNA samples reacted in vitro with DMS and EIO were determined in each experiment in order to calculate the level of these adducts in human DNA samples.

Combined levels of 7-methyl and 7-(2-hydroxyethyl)-guanine adducts in total white blood cells and isolated DNA

TLC analysis of ³²P-postlabelled DNA digests were carried out in two solvent systems (A and B) in parallel experiments. In the solvent system A with 30% n-propanol in D2 the adduct spot in which 7-methylguanine and 7-(2-hydroxyethyl)-guanine adducts co-migrated was well resolved from other adduct spots (Figure 1). In the same solvent system lymphocyte DNA samples, in addition to the spot corresponding to 7-methylguanine and 7-(2-hydroxyethyl)-guanine adducts, showed two additional spots (X and Y). Most of these putative adduct spots did not appear when DNA digests were depurinated at neutral pH prior to postlabelling, thereby, leading to the inference about the 7-alkylguanine/3-alkyladenine nature of these adducts. The combined level of 7-methylguanine and 7-(2-hydroxyethyl)-guanine adducts in total white blood cells and isolated lymphocytes is given in Table I. The level of these adducts in total white blood cells range from 0.7 to 1.5 adducts per 10⁷ normal nucleotides, which is lower than the level of adducts in isolated lymphocytes (1.1 to 12.0 adducts per 10⁷ normal nucleotides). The level of adducts in the lymphocytes of smokers (5.6 to 12.0 adducts per 10⁷ normal nucleotides) was higher than that in non-smokers (1.1 to 8.4 adducts per 10⁷ normal nucleotides).

The radioactivity in the spots X and Y in DNA from lymphocytes was equivalent to 2.8 ± 2.2 and 3.2 ± 1.8 adducts per 10⁷ normal nucleotides (values not corrected for recovery). The spot Y was more prominent in the lymphocyte DNA of smokers (3.9 ± 0.28 adducts per 10⁷ normal nucleotides) than non-smokers (2.1 ± 0.3 adducts per 10⁷ normal nucleotides, detectable only in two samples). The solvent system B with 50% n-propanol in D2, resolved other unidentified adduct spots, which moved farther than 7-methylguanine and 7-(2-hydroxyethyl)-guanine adducts (Figure 2). The uppermost spot in this solvent system corresponded to 0.2 ± 0.1 adducts per 10⁷ normal nucleotides (values not corrected for recovery).
Fig. 1. Autoradiographs of TLC analysis of human DNA. (A) Total white blood cells; (B) depurinated control; (C) lymphocytes from smoker; and (D) lymphocytes from non-smoker. In all chromatographs spots corresponding to 7-methyl-5'-dGMP and 7-(2-hydroxyethyl)-5'-dGMP adducts are indicated with arrows. ‘B’ is a background spot in these chromatographs. In (C) and (D) two additional spots are labelled as X and Y. D1 and D2 are the directions of chromatography and ‘O’ is the origin.
Separation of 7-methylguanine and 7-(2-hydroxyethyl)-guanine adducts by HPLC

The co-migration of 7-methylguanine and 7-(2-hydroxyethyl)-guanine adducts on TLC in different solvent systems necessitated their separation by HPLC. However, one of the problems encountered earlier was that the injection of postlabelled samples directly into HPLC resulted in the migration of both these adducts along with the residual normal nucleotides. In order to completely remove the residual normal nucleotides, the first separation was carried out on TLC. The areas of TLC plates corresponding to 7-methyl and 7-(2-hydroxyethyl)-guanine adducts were sonicated in 10 mM ammonium formate buffer for 30 s. This procedure ensured the extraction of more than 90% of radioactivity. The subsequent HPLC analysis of these extracts resulted in the separation of 7-methyl and 7-(2-hydroxyethyl)-guanine adducts whose identities were established by their co-migration with synthetic standards used as UV markers. Figure 3A shows the autoradiograph of TLC analysis of DMS and ethylene oxide modified DNA. The adduct spot corresponding to 7-methylguanine and 7-(2-hydroxyethyl)-guanine were resolved on HPLC following their extraction from the TLC plate (Figure 3B).

Similarly, the adduct spots corresponding to 7-methyl-dGMP and 7-(2-hydroxyethyl)-dGMP adducts in human samples were analysed on HPLC after their extraction from TLC plates. Figure 4A and 4B shows HPLC resolution of 7-methylguanine and 7-(2-hydroxyethyl)-guanine adducts in human total white blood cells. The peak area corresponding to two adducts being in equal ratio. However, the recovery of 7-methyl-dGMP and 7-(2-hydroxyethyl)-dGMP as calculated from the recovery experiments was an average of 49.7 and 23.9%, respectively. Based on this recovery data, the level of 7-(2-hydroxyethyl)-guanine adduct is twice the adduct level of 7-methylguanine in the human total white blood samples analysed in the present study. The HPLC analysis of 7-methylguanine/7-(2-hydroxyethyl)-guanine adduct spots from TLC analysis of lymphocyte DNA (Figure 4C, D) shows the peak area corresponding to 7-(2-hydroxyethyl)-guanine adduct two to six times the peak area of 7-methylguanine adduct. This was the general

### Table 1. Level of 7-methylguanine and 7-(2-hydroxyethyl)-guanine adducts in human total white blood cells and lymphocytes

<table>
<thead>
<tr>
<th>Sample</th>
<th>Adducts $10^7$ normal nucleotides</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) White blood cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>1.5 ± 0.63 (n = 4)</td>
<td>ns</td>
</tr>
<tr>
<td>2.</td>
<td>1.0 ± 0.27 (n = 4)</td>
<td>ns</td>
</tr>
<tr>
<td>3.</td>
<td>0.9 ± 0.29 (n = 4)</td>
<td>ns</td>
</tr>
<tr>
<td>4.</td>
<td>0.7 ± 0.12 (n = 4)</td>
<td>ns</td>
</tr>
<tr>
<td>(b) Lymphocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>12.0 ± 1.13 (n = 3)</td>
<td>s</td>
</tr>
<tr>
<td>2.</td>
<td>11.8 ± 1.97 (n = 4)</td>
<td>s</td>
</tr>
<tr>
<td>3.</td>
<td>10.0 ± 0.56 (n = 4)</td>
<td>s</td>
</tr>
<tr>
<td>4.</td>
<td>8.40 ± 1.69 (n = 4)</td>
<td>ns</td>
</tr>
<tr>
<td>5.</td>
<td>5.60 ± 1.27 (n = 4)</td>
<td>s</td>
</tr>
<tr>
<td>6.</td>
<td>3.90 ± 0.45 (n = 4)</td>
<td>ns</td>
</tr>
<tr>
<td>7.</td>
<td>2.70 ± 0.96 (n = 4)</td>
<td>ns</td>
</tr>
<tr>
<td>8.</td>
<td>1.10 ± 0.30 (n = 4)</td>
<td>ns</td>
</tr>
</tbody>
</table>

s, Smoker; ns, non-smoker.

Fig. 2. Autoradiographs of TLC analysis of human DNA in solvent system B (see Materials and methods). (A) Total white blood cells; (B) depurinated control; and (C) lymphocytes. An unidentified adduct is marked with an arrow. 'A' is the position of 7-methyl-5'-dGMP and 7-(2-hydroxyethyl)-5-dGMP adducts. Directions of the chromatography are the same as in Figure 1.
Fig. 3. (A) Autoradiograph of TLC resolution of 7-methyl-5'-dGMP and 7-(2-hydroxyethyl)-5'-dGMP adducts from in vitro reacted DNA with DMS and EtO. The spot corresponding to these two adducts has been marked. (B) HPLC analysis of the two adducts extracted from the above TLC plate. The synthesized standards are used as UV markers. Peak 'a' is 7-(2-hydroxyethyl)-5'-dGMP and peak 'b' is 7-methyl-5'-dGMP.
Fig. 4. Co-migration of UV markers 7-(2-hydroxyethyl)-5'-dGMP (peak 'a') and 7-methyl-5'-dGMP (peak 'b') with radioactive peaks obtained after excision of corresponding TLC spots from DNA analysis of total white blood cells (panels A, B) and isolated lymphocytes (panels C, D). The migration of radioactive peaks from spots X and Y (Figure 1) is shown in panels (E) and (F), respectively.

pattern in all lymphocyte DNA samples analysed in the present study.

The areas of TLC plates corresponding to the adduct spots other than 7-methylguanine and 7-(2-hydroxyethyl)-guanine adducts were also extracted and analysed by HPLC. Figure 4e shows the relative migration of peaks from adduct spot X on HPLC. The adduct spot Y on HPLC analysis migrated with the retention time of 16.7 min (Figure 4f). Both these peaks corresponding to spots X and Y disappeared on depurination at neutral pH. These adduct peaks did not co-migrate with any of the known 7-alkylguanine adduct standards available in this laboratory. The standards used in spiking experiments included, 7-(2-hydroxyethyl)-guanine through 7-(2-hydroxyoctyl)-guanine adducts (20).

Discussion

The levels of 7-alkylguanine adducts in DNA from target and surrogate tissues are indicative of relative exposure to alkylating agents. 7-methylguanine adducts are formed by exogenous exposure to methylating agents like nitrosamines (25) or by endogenous methylating agents like betaine, choline and S-adenosylmethionine (26-28), whereas 7-(2-hydroxyethyl)-guanine adducts are formed by exogenous exposure to ethylene oxide (22), which besides being an industrial chemical is also a component of cigarette smoke (23,29). This adduct is also formed as a result of exogenous exposure to ethene which is metabolically converted to ethylene oxide (30). Besides, 7-(2-hydroxyethyl)-guanine adduct is also formed as a consequence of reaction of DNA with endogenously formed lipid peroxidation products (31,32). In this study we report the relative adduct levels of 7-methylguanine and 7-(2-hydroxyethyl)-guanine, analysed as 5'-monophosphate nucleotides following nuclease P1 treatment, in human total white blood cells and isolated lymphocytes. The combined adduct level of 7-methylguanine and 2-(hydroxyethyl)-guanine which could not be separated individually on TLC, was higher in isolated lymphocytes of non-smokers' (mean values 1.1 to 8.4 adducts/10$^7$ normal nucleotides) than in total white blood cells (mean values 0.7 to 1.5 adducts/10$^7$ normal nucleotides). This is in conformity with earlier studies, with the possible explanation being that lymphocytes have a half-life of several months compared to the short half-life of granulocytes which comprise more than half of the total white blood cells (19). The adduct levels in the lymphocytes of smokers (mean values 5.6 to 12.0 adducts/10$^7$ normal nucleotides) were higher than in non-smokers (mean values 1.1 to 8.4 adducts/10$^7$ normal nucleotides) and are likely to be due to the presence of methylating and hydroxyethylating agents like tobacco-specific nitrosamines and ethylene oxide in tobacco smoke. In earlier studies, similar higher levels of adducts in DNA from different blood cells from smokers compared to non-smokers have been reported (19, 33).

In order to separate 7-methylguanine and 7-(2-hydroxy-
ethyl)-guanine adducts and to find out their relative levels in the samples analysed, the excised spots on TLC were extracted and analysed on HPLC using synthesised 7-methyl- and 7-(2-hydroxyethyl)-guanosine-5' monophosphates as UV markers. This procedure resulted in the separation of these two adducts (Figure 3) which revealed the level of 7-(2-hydroxyethyl)-guanine adduct in total white blood cells was twice that of 7-methylguanine. Moreover, in case of lymphocytes the level of the former adduct was 4 to 12 times higher than 7-methylguanine. Though to our knowledge there is no earlier study where both these adducts in DNA have been analysed together, the 7-(2-hydroxyethyl)-guanine adduct levels are comparable to those reported by van Delft et al. (34) in white blood cells using immunochromatographic techniques, yet, in that study no significant difference was observed in the adduct levels in smokers and non-smokers. A related study on hydroxyethylvaline adducts in haemoglobin has shown a higher level of adducts in smoking mothers than non-smoking mothers as well as the placental transfer of alkylating species (29). In earlier studies involving mass spectrometric techniques, 1–2 adducts per 10⁹ normal nucleotides in humans and animals have been reported (22,35). The results on relative levels of 7-methylguanine and 7-(2-hydroxyethyl)-guanine are interesting when compared to levels of methyl and 2-hydroxyethyl adducts of haemoglobin where a higher background level of former adduct has been reported (36,37).

In this study at least three additional adducts were located on TLC. Two of these spots, which we called spots X and Y (Figure 1C and 1D) were specific to lymphocytes. At least one of these spots (spot Y) seemed to be related to smoking. These spots on HPLC analysis following the excision from TLC plates, eluted later than 7-methylguanine adducts. Though spot X showed two peaks on HPLC at the retention times of 7.4 and 9.7 min with the latter peak being the more prominent, both of these spots disappeared following the depurination at neutral pH. There was at least one additional adduct spot visible when TLC was carried out in solvent system B, and like spots X and Y disappeared on depurination (Figure 2). However, this adduct spot was found in both total white blood cells and lymphocytes alike and was at the same level in both smokers and non-smokers. The adduct enrichment in this study has been carried using strong anion exchange cartridge eluted with a weak buffer (5 mM ammonium formate). This method ensures the elution of only zwitterionic species. In addition the solvent system for the development of thin layer chromatography also allows the movement of simple alkylated species with no net charge at the pH of chromatographic solvent. Under these conditions of exclusion, the inference drawn about the nature of the unidentified adduct spots visible is that these could be either 7-alkylguanine or 3-alkyladenine adducts with the nature of alkyl side chains not known. This inference is based on the observation that alkylolation at the N-7 position of guanine and N-3 position of adenine induces positive charge on these atoms which besides making the adducted nucleotides zwitterionic, also make glycosidic bonds labile. However, considering the extreme labile nature of 3-alkyladenine nucleotides the probability of these adducts being 7-alkylguanine adducts is high. Though the nature of the HPB releasing DNA adducts formed by the tobacco-specific nitrosamines NNK or NNK is not known (38), the possibility of the smoking related unknown adduct being tobacco-specific nitrosamines induced is not ruled out.

In our earlier research (20,21) we studied different types of 7-alkylguanine adducts by a postlabelling technique, yet none of the unidentified adduct spots matched with the known standards. In addition to methyl and 2-hydroxyethyl protein adducts another common background protein adduct detected in humans and animals is 2-carboxyethyl adduct (37). The corresponding 7-(2-carboxyethyl)-guanine adduct standard, however, was not available in this study.

Thus in this study we have shown the separation of 7-methylguanine and 7-(2-hydroxyethyl)-guanine adducts by a combination of TLC and HPLC techniques. The results of this investigation show that the levels of 7-(2-hydroxyethyl)-guanine adducts in both total white blood cells and lymphocytes is higher than 7-methylguanine adducts, with the level of these adducts being higher in smokers than non-smokers. The presence of some unidentified adducts has also been shown and further work is being carried out to identify these adducts.

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References
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