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8-Hydroxydeoxyguanosine

A marker of oxidative DNA damage in systemic lupus erythematosus

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Abstract

8-Hydroxydeoxyguanosine (8OHDG) is a specific marker of oxidative damage to DNA. We have observed that patients with SLE (systemic lupus erythematosus), have undetectable levels of urinary 8OHDG by HPLC. Further analysis by GC-MS confirmed that levels of 8OHDG in SLE urine were 10^3-fold lower than in an age- and sex-matched control group. Experiments utilising cultures of SLE and normal lymphocytes exposed to H_2O_2 confirmed the impaired ability of SLE lymphocytes to repair 8OHDG. We subsequently observed in SLE patients that 8OHDG had accumulated in low molecular weight DNA associated with circulating immune complexes. We suggest that oxygen radicals may induce pathology in SLE by maintaining the presence of an antigenic form of DNA in the circulation.

Key words: Systemic lupus erythematosus (SLE); Rheumatoid arthritis

1. Introduction

Chronic inflammation is associated with considerable tissue injury. It has been postulated that this may result from perpetual stimulation of phagocytic cells by bacterial and immunological stimuli activating the membrane-bound NADPH oxidase and releasing reactive oxygen species (ROS) [1]. ROS are formed from oxygen in one-electron reduction reactions and include superoxide anion, hydrogen peroxide and hydroxyl radical. Of these, the hydroxyl radical has a very short half-life, reacting at diffusion controlled rates with many cellular biomolecules.

In some disease states, the inflammatory process and thus the production of ROS may be amplified in the confined space of a certain tissue. In rheumatoid arthritis (RA), inflammation is usually confined to the joint and is thought to contribute to the joint deformities commonly associated with the disease [2]. Although several research groups believe that the production of ROS in inflammatory joint disease is relevant to the disease process [3,4], there has been no direct demonstration that the highly reactive hydroxyl radical is formed in vivo in an inflamed joint. The reasons for this are twofold. Firstly, the hydroxyl radical is very reactive, diffusing only 1.5 nm before reacting with a target molecule [5]. Secondly, previous attempts to measure interactions between hydroxyl radicals and biomolecules, predominantly lipids, have relied on semi-quantitative assays such as (i) the thiobarbituric acid (TBA) assay, which measures lipid peroxidation, but is non-specific and subject to interference from prostaglandins (a major problem in inflammation research [6]; and (ii) the diene conjugation assay, another non-specific measure of lipid peroxidation [7]. In spite of this, ROS have been shown to readily damage not only lipids but also proteins [2,7,8], and DNA intracellularly [9,10]. Damage to DNA induces strand breaks [11], sister chromatid exchanges and the subsequent production of clastogenic factors [12], and alterations in base structure [13]. Monitoring DNA damage induced specifically by ROS has been the strategy used in this present study to provide evidence of oxidative damage to DNA in vivo.

Formation of the altered base, 8OHDG, has been shown to be a very sensitive marker of ROS-induced damage [14,15]. Its production has been affirmed in vitro following exposure of DNA to γ-radiation [16], UV light [17], hydrogen peroxide [18] and PMA-stimulated neutrophils [19]. It is believed to be formed by the addition of a hydroxyl radical to C-8 of deoxyguanosine, and can be measured using high performance liquid chromatography (HPLC) with electrochemical detection following enzymic digestion of DNA [20].

This paper describes the use of HPLC and gas chromatography mass spectroscopy with selected ion monitoring (GC-MS/SIM) assays to measure 8OHDG as a sensitive and specific marker of ROS-induced damage to DNA, and discusses the relationship between this oxidative change and inflammatory autoimmune pathology.

2. Materials and methods

2.1. Patient groups

Early morning urine samples and plasma samples (venous blood collected into heparin and centrifuged for 30 min at $300 \times g$) were

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collected from 33 SLE patients with normal renal function (all satisfied the preliminary criteria of the ARA for diagnosis of SLE) (29 female, 4 male, age range 20–64, median = 39), 10 RA patients diagnosed according to standard ARA criteria (9 female, 1 male, age range 57–80, median = 67) and two groups of 10 normal controls: young normal controls (8 female, 2 male, age range 20–47, median = 26) not differing in age or sex from the SLE group, and elderly normal controls (9 female, 2 male, age range 62–82, median = 70) not differing in age or sex from the RA patients. RA patients were prospectively selected to have an active inflammatory status based on an elevated level of the acute phase protein, C-reactive protein (CRP). In this group CRP values ranged from 42–123 mg/l, median = 64 mg/l (normal range < 6 mg/l). The 33 SLE patients studied had a range of inflammatory disease activity as determined by levels of erythrocyte sedimentation rate (ESR) (5–92 mm/h, median = 26 mm/h, normal range < 20 mm/h).

Two different indicators of inflammatory status were used because CRP is not a good indicator of inflammatory activity in SLE, since disease-associated anaemia can effect ESR but not CRP values in RA. All samples were stored at −20°C prior to analysis.

2.2. Preparation of urine samples

Early morning urine sample (0.5 ml) was added to a 1 ml C-18 SEP-PAK column (Waters, Watford, UK) preconditioned with 10 ml methanol and 10 ml water. The eluant was discarded and the column washed with 5 × 1 ml of 5% aqueous methanol. The fraction containing 80HDG as the nucleoside in the highest concentration was then eluted with 3 × 1 ml of 15% aqueous methanol. The eluant was evaporated to dryness and the sample resuspended in 200 μl HPLC mobile phase buffer. Recoveries using spiked urine samples were consistently 65–70%. This separation procedure using reverse-phase material selectively removed many electrochemically active compounds present in high concentration in urine that would otherwise have masked the presence of picomolar quantities of the nucleoside, 80HDG. All urine measurements of 80HDG were expressed relative to creatinine to correct for differences in urine dilution [20].

2.3. Isolation of DNA from cryoprecipitates

Plasma samples were incubated at 4°C for 7 days to induce cryoprecipitation. The pellet formed was washed in PBS and the DNA extracted using proteinase K (100 μg/ml; Sigma, Poole, Dorset) and 1% SDS (Sigma) in a 1 ml volume of 20 mM EDTA, pH 8.0, added to the 1 ml volume (PBS) of resolubilised immune complex. The mixture was incubated at 65°C for 30 min and protein removed by extracting three times with ½ volume of phenol and ½ vol. of chloroform (British Drug Houses, Poole, Dorset). To precipitate the DNA, 2 vols. of cold ethanol in the presence of 0.3 M sodium acetate were added to the aqueous phase which was then left at −20°C for 24 h. The DNA was washed with 70% ethanol, lyophilised and resuspended in 100 μl Tris EDTA, pH 8.0. Prior to analysis by HPLC, the DNA was digested as described by Beland [21].

2.4. Analysis of immune complexed DNA

Agarose gel electrophoresis was used to determine the purity of DNA isolated from immune complexes and also to estimate its size. Using a mini gel apparatus 0.2 g of agarose was dissolved in 20 ml 89 mM Tris-borate buffer (TBE), pH 8.0, to give a gel concentration of 1%. Ethidium bromide was added to a final concentration of 0.75 μg/ml. 20 μl of DNA mixed with 5 μl loading buffer (0.25% Bromphenol blue, 0.25% xylene cyanol and 40% w/v sucrose in water) and loaded onto the gel and the electrophoresis was carried out at 70 V for 30 min in TBE.

2.5. Lymphocyte preparations subjected to oxidative stress

Peripheral blood monocytes (PBMC) were isolated from the peripheral blood of patients with SLE and normal controls and re-suspended to 1 × 10^6/ml and cultured in 24-well plates in a volume of 2 ml complete culture medium.

These PBMC derived from normal and SLE cells were incubated with or without 200 μM H_2O_2 for 7 days. At intervals during this period cell viability was determined and cells and supernatants were collected. Supernatants were replaced by fresh culture medium after sampling at each time point between 0 and 7 days. The cells were separated by centrifugation at 300 × *g* for 10 min at 4°C, washed twice in PBS and re-suspended to 10^8/ml in ice-cold TBE. DNA was isolated from the cells according to the method of Maniatis et al. [22] and digested as for immune complexed DNA before analysis by HPLC/ECD. 2 vols. of ice-cold ethanol were added to each sample of supernatant collected after centrifugation. Any DNA was allowed to precipitate at −20°C for 24 h and pelleted by centrifugation at 0°C for 10 min in an Eppendorf centrifuge. The remaining supernatant was freeze-dried and resuspended in 1 ml of sterile water. Ethanol precipitation of the supernatants was used to remove large fragments of DNA arising from cell death so that the only detectable 80HDG was from individual bases or small fragments of DNA arising from excision repair. 10 μl of supernatant digested and treated as DNA was analysed by HPLC.

2.6. Hydrolysis and derivitisation of DNA samples

Prior to analysis by GC-MS/SIM, DNA samples were hydrolysed with 1 ml of formic acid (88%; Mallinckrodt Inc., Paris, KY) in evacuated and sealed tubes at 150°C for 30 min. This removes the ribose residue and, as a result, in contrast to the HPLC method, the derivatives of deoxyguanosine (guanine) and deoxyadenosine (adenine) are measured. After hydrolysis, samples were lyophilised and trimethylsilylated with 0.25 ml of a 1.5:1 mixture of bis (trimethylsily)-trifluoroacetamide (BSTFA, containing 1% trimethylchlorosilane; Sigma) and acetonitrile, in polytetrafluoroethylene capped hypovials (both from Pierce Chemical Co., Rockford, IL) by heating at 130°C for 30 min. Urine samples were derivatised only as for DNA.

2.7. Characterisation of DNA base damage

Analysis of the prepared urine and DNA samples was either by: (i) HPLC using isocratic reverse-phase chromatography – DNA bases were analysed on an ODS 5 μm 25 cm column with a guard column of pellicular ODS material (both from Technicol, Stockport, Cheshire, UK), at a flow rate 0.8 ml/min using a mobile phase of 30 mM sodium hydroxide, 10 mM acetic acid, 41 mM sodium acetate and 12.5 mM citric acid, pH 5, containing 10% methanol. UV detection was at 254 nm and 80HDG was detected by ECD using a glassy carbon working electrode at an applied potential of +0.6 V vs. Ag/AgCl; or, (ii) gas chromatography mass spectroscopy with selected-ion monitoring (GC-MS/SIM) – hydrolysed and derivatised DNA samples and derivatised urine samples were analysed using a mass selective detector controlled by a computer work station and interfaced to a gas chromatograph (all instruments from Hewlett-Packard Co., Avondale, PA, with model numbers S970B, S970C, and S890A, respectively). The injection port and the ion source were kept at 250°C. The GC-MS interface was maintained at 270°C. Separations were carried out on a fused silica capillary column (12.5 m × 0.32 mm i.d.) coated with crosslinked 5% phenylmethyl silicone gum phase (film thickness, 0.17 μM). The column was programmed from 120°C to 250°C at 8°C/min, after 2 min at 120°C. Helium was used as the carrier gas at an inlet pressure of 10 kPa. Samples were injected using the splitless mode. Mass spectra were obtained at 70 eV. Authentic samples of isobarbituric acid (5-hydroxyuracil; Sigma), 4,6-diamino-5-formamidopyrimidine (Sigma), and 2-amino-6,8-dihydroxypurine (8-hydroxyguanine; Chemical Dynamics Corp., South Plainfield, NJ), were available commercially.

2.8. Quantitation of DNA base damage

DNA base products were quantitatively measured using 8-azaadenine (Sigma) as an internal standard. Aliquots of the internal standard were added to the DNA samples. The samples were then lyophilised, hydrolysed, trimethylsilylated and analysed by GC-MS/SIM. Ion currents of the typical ions of the DNA base products were monitored along with the *m/z* 265 ion of the trimethylsilyl derivative of 8-azaadenine.

3. Results

3.1. Urinary excretion of 80HDG

To investigate the presence of ROS-induced damage in DNA from RA and SLE patients, urinary excretion of 80HDG was measured both by HPLC and GC-MS. Early morning urine samples were collected and applied

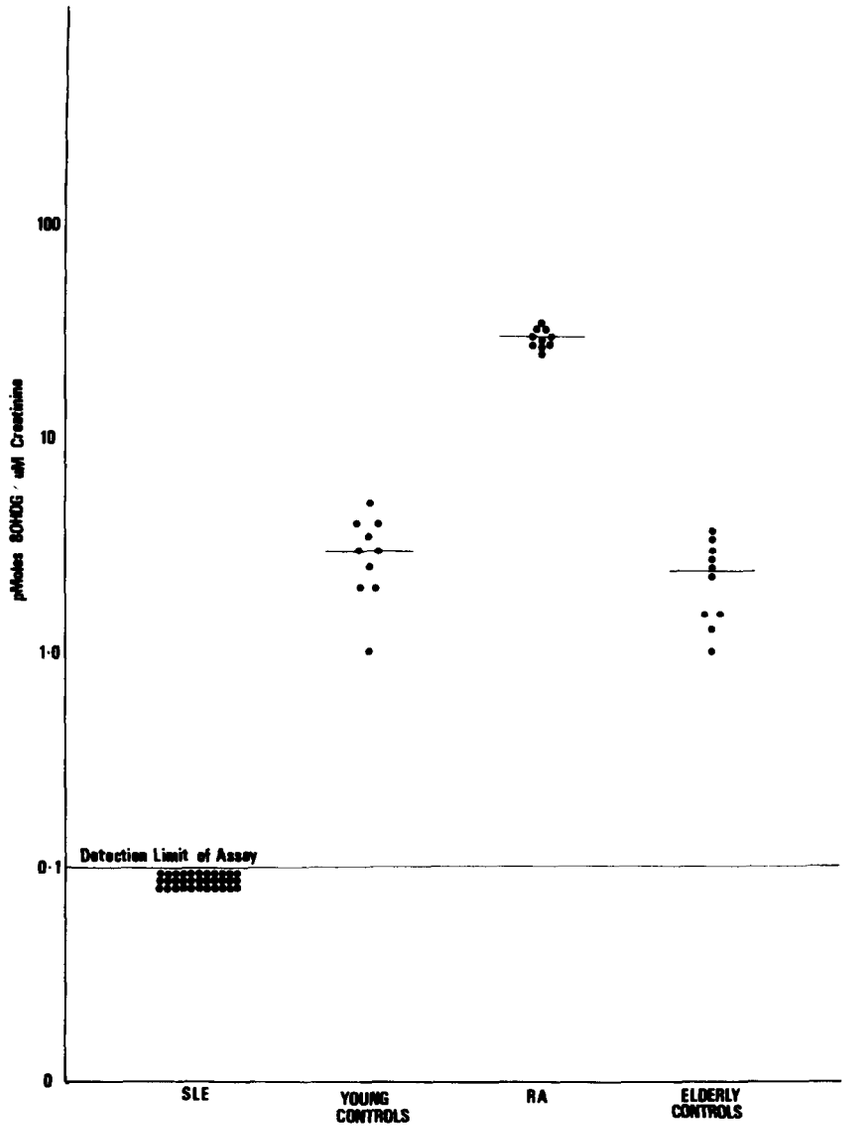

Fig. 1. Log plot of levels of 80HDG found in early morning urine samples of SLE patients (n = 33), young normal controls (n = 10), RA patients (n = 10) and elderly controls (n = 10) detected by HPLC/ECD. The figure shows the median and ranges for each of the groups. All the results for the SLE group are below the detection limit of the assay. Each point plotted represents the mean of triplicate urine samples analysed for each person studied. The retention time of 80HDG was consistently 17.2 ± 0.3 min.

to a C_{18} mini column to selectively remove compounds that interfered with the electrochemical detection and GC-MS procedures. Measurement of 80HDG was made relative to creatinine to correct for urine concentration. Of the samples from 33 SLE patients with normal renal function which were analysed, levels of 80HDG excreted were undetectable by HPLC in all samples, i.e. levels were < 0.1 pmol/μmol creatinine. The normal range established from the two groups of controls by HPLC was 1.0–5.2 pmol 80HDG/μmol creatinine for the young controls and 0.75–3.9 pmol 80HDG/μmol creatinine for the elderly controls. Results from the patient group with active RA showed that the level of urinary excretion of 80HDG was significantly elevated when compared to normal (25–32 pmol/μmol creatinine; $P < 0.001$; Fig. 1).

In order to confirm the HPLC results indicating undetectable levels of 80HDG in SLE urine samples, a random selection of specimens were also analysed by GC-MS/SIM, a well-established technique for identification of compounds on the basis of their individual molecular weights and molecular weight fragments produced during atomic bombardment. It was found that levels of 80HDG in urine samples from SLE patients were detectable in the femtomolar range by GC-MS/SIM (Fig. 2A), previously determined to be below the limits of detection of the HPLC assay.

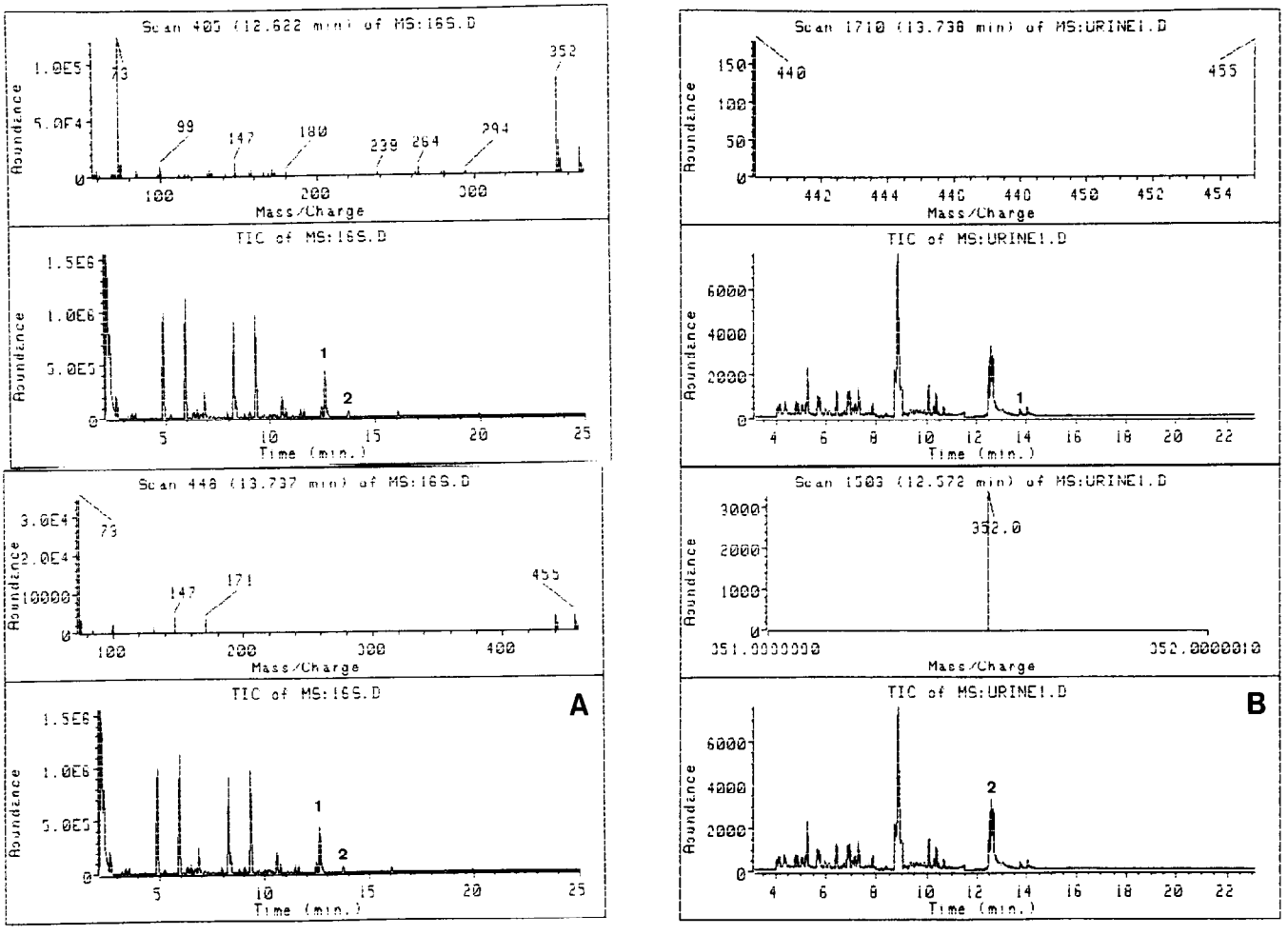

Fig. 2. (A) Identification of modified bases in SLE urine. Ion current profiles of the ions at *m/z* 440/455 and 352. Peak identification: 1, 8-hydroxyguanine (3 fmol 80HDG/μg DNA); 2, 8-hydroxyadenine (7 pmol 80HDG/μg DNA). (B) Mass spectra of modified DNA bases derived from circulating plasma complexes in a typical SLE patient. Partial mass spectra obtained from the monitored ions and their abundance at the elution positions; 1, identified as 8-hydroxyadenine and 2, identified as 8-hydroxyguanine.

3.2. *Detection of 80HDG in immune complexes*

In order to try and establish the fate of 80HDG in SLE, and based on a working hypothesis that abnormally low levels of 80HDG in SLE urine suggested a lack of repair of this adduct, DNA present in cryoprecipitates from SLE plasma was examined for the presence of 80HDG.

In contrast to the results obtained for the SLE urine samples, levels of 80HDG found in the DNA isolated from immune complexes were very high (0.38–3.6 pmol 80HDG/μg DNA) and were detectable both by HPLC and GC-MS (Table 1). These levels are of the order of 100-times greater than the range determined by Richter et al. for nuclear DNA (0.025 pmol/μg DNA) [23], considered to be a reflection of normal oxidative stress to the cell. DNA was isolated from cryoprecipitates of RA patients as a control group, as for the SLE patients; low levels of DNA were found in the complexes from rheumatoids (2–8 μg DNA compared with 100–270 μg DNA in the SLE group for the same volume of plasma, $P < 0.001$; and levels of 80HDG were also found to be significantly lower at less than 30 fmol 80HDG/μg DNA, $P < 0.001$).

GC-MS/SIM was used to confirm the identity of the DNA-derived adducts. The partial mass spectra of 8-hydroxyadenine, a major neutrophil-induced DNA damage product [19], and 8-hydroxyguanine (derived from 80HDG during hydrolysis) and their abundance at elution positions 1 and 2, respectively, are shown in Fig. 2B.

3.3. *Origin of immune complexed DNA*

In order to try and determine the origin of immune complexed DNA in SLE sera the DNA present in immune complexes isolated from SLE sera was purified, isolated from the antibody as for cellular DNA, and subjected to agarose gel electrophoresis. Approximately 200 μg of DNA could be isolated from complexes obtained from 10 ml of plasma, whereas normal cryoprecipitates contained approximately 2–7 μg and RA patients 2–8 μg. Fig. 3 shows that the immune complexed

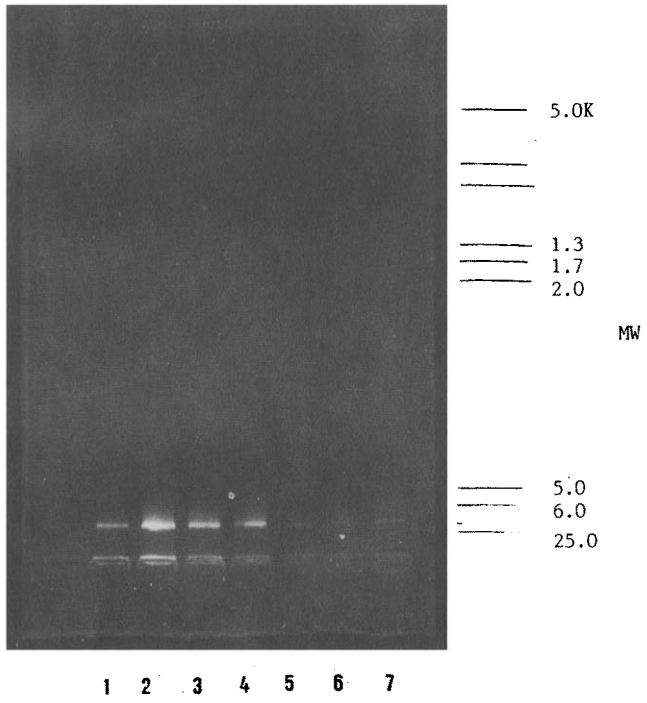

Fig. 3. A typical gel of DNA isolated from immune complexes of SLE, RA and normal control plasma. Lane 1 represents DNA from an RA patient; lanes 2–4, DNA from sera of SLE patients; and lanes 5–7 DNA from normal controls. Although the DNA recovered varies in amount, there is no significant difference in size. The predominant band of DNA corresponds to approximately 20 kb from the comparison of molecular weight markers of lambda DNA cut with the restriction enzymes *Eco*RI and *Hin*dIII which give the M_w profile shown.

DNA was consistently of the order of 20 kb based upon a comparison with M_w markers. There was no difference in size of DNA from patients with or without renal disease, or from patients with RA or the normal controls.

3.4. Removal of 80HDG during oxidative stress of PBMC

No significant difference was found between the loss of viability for SLE and normal cells incubated in the absence of H_2O_2. In the presence of H_2O_2 a significant decrease in viability was found both in SLE ($P < 0.001$) and normal ($P < 0.001$) cells when compared with the untreated cells between days 2 and 7 (Fig. 4A and B). SLE cells showed a marginally significant reduction in viability compared to normal cells under H_2O_2-induced oxidative stress ($P < 0.05$) between days 2 and 6.

Parallel extractions of DNA from these cells were analysed for the presence of 80HDG (Fig. 4C,D). Cells incubated in the absence of H_2O_2 had detectable levels of 80HDG in the DNA after 4 days. For normal cells levels of 80HDG then plateaued at a constant level for the remainder of the incubation period. In contrast the level of 80HDG in SLE cells continued to rise gradually and appeared to be still increasing at day 7. DNA isolated from cells incubated in the presence of H_2O_2 had the highest level of 80HDG at day 1 in both the SLE and control groups. In normal cells, this level reached a minimum at day 6. This was not significantly different from the level at the equivalent time point in the untreated cells. The profile of 80HDG as a component of DNA in SLE cells differed from that of the normal cells: whilst a similar peak of 80HDG was achieved at day 1, the rapid return to baseline was not seen, and instead there was a gradual reduction in the level of 80HDG. The differences in the two curves at days 2, 4 and 6 were significant at $P < 0.01$.

80HDG was not detectable in the supernatants from normal or SLE PBMC incubated in the absence of H_2O_2. However, Fig. 4E shows 80HDG was detectable in the supernatants of both SLE and normal PBMC incubated with H_2O_2, reaching a maximum at day 2 in normal cells while SLE cells produced a gradual increase over the 7 day period but reached only 10% of normal maximum values at day 2 and 40% of the normal maximum after 7 days, showing quite clearly a markedly delayed removal of 80HDG by the SLE cell.

4. Discussion

Two patient groups with the inflammatory conditions RA or SLE were used to study the role of ROS-induced DNA damage in inflammation.

Patients with SLE have diverse clinical manifestations, including arthritis, vasculitis, skin rashes and renal complications, and the disease is notable for the wide variety of antibodies found in the serum; anti-double-stranded DNA antibodies being the most specific serological find-

Table 1
Comparison of values of 80HDG found in five cryoprecipitate samples analysed by GCMS and HPLC/ECD

Cryoprecipitate	80HDG (pmol, GCMS)	80HDG (pmol, HPLC/ECD)	DNA (μg)	pmol 80HDG/μg DNA (GCMS)	pmol 80HDG/μg DNA (HPLC/ECD)
1	78 ± 6	48 ± 5	127 ± 4	0.61 ± 0.05	0.37 ± 0.04
2	346 ± 13	414 ± 11	182 ± 3	1.89 ± 0.07	2.27 ± 0.06
3	134 ± 5	106 ± 6	135 ± 6	0.98 ± 0.03	0.78 ± 0.04
4	217 ± 13	222 ± 7	118 ± 7	1.84 ± 0.09	1.88 ± 0.06
5	152 ± 8	164 ± 7	104 ± 5		

Results (means ± 1 S.D.) are expressed as pmol 80HDG/ng DNA of three separate experiments by both GCMS and HPLC/ECD.

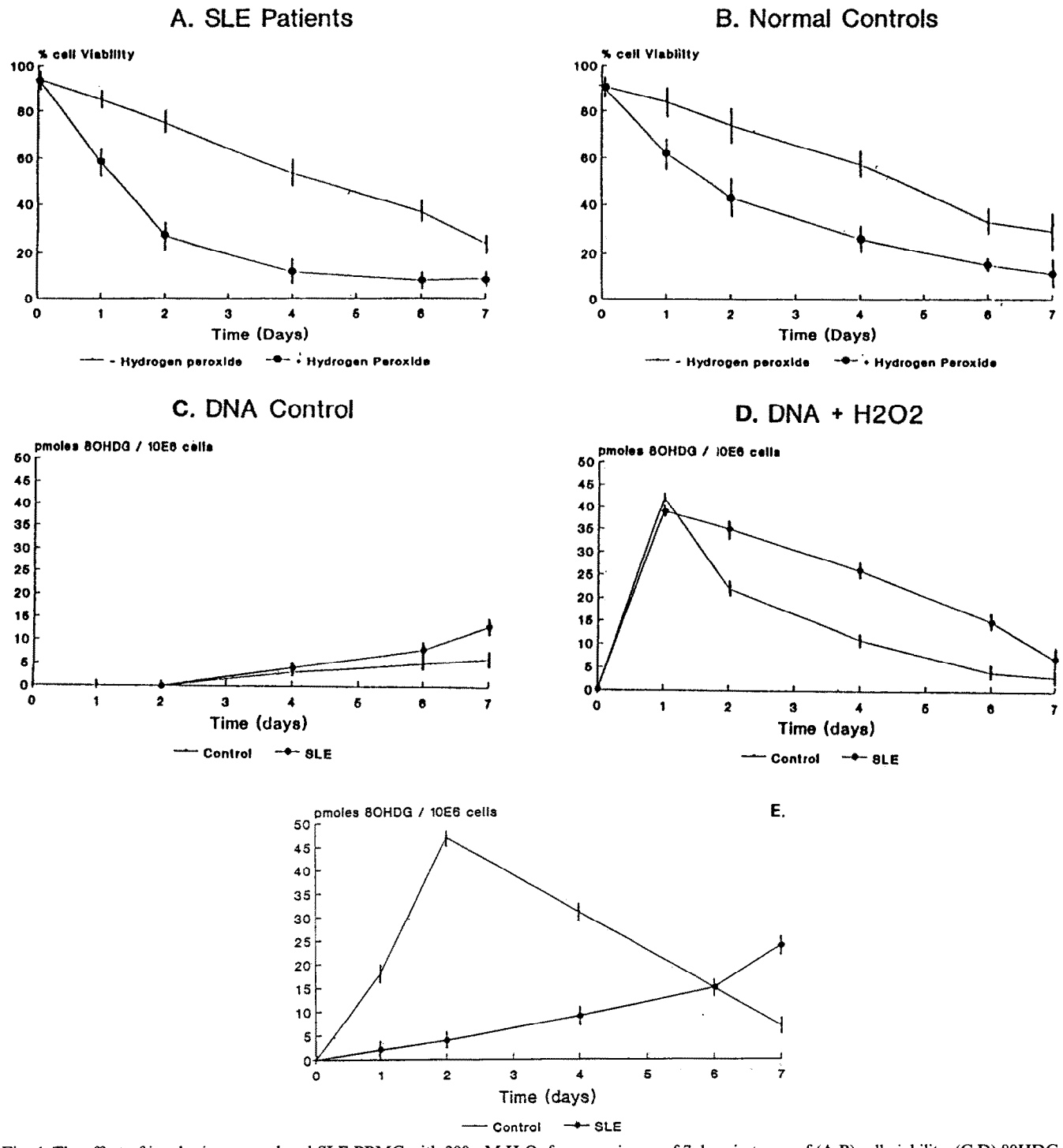

Fig. 4. The effect of incubating normal and SLE PBMC with 200 μM H_2O_2 for a maximum of 7 days in terms of (A,B) cell viability, (C,D) 8OHDG formation in cellular DNA, and (E) corresponding 8OHDG formation in supernatants of cells. The results are expressed as the mean ± S.D. of three separate experiments.

ing in SLE. A further abnormality of SLE is the presence of circulating immune complexes [24].

Rheumatoid arthritis is a chronic, systemic inflammatory disorder characteristically involving peripheral joints [25]. The aetiology is unknown but there is accumulating evidence that a factor on the D locus of the sixth chromosome (DR4/Dw4&Dw14) occurs in a significantly high number of sero-positive cases [26,27]. Evidence for an autoimmune disturbance in RA rests largely upon the presence of serum rheumatoid factors, predominantly antibodies of the IgM class, reacting with autologous immunoglobulins of the IgG class.

It has been suggested by several groups that ROS may contribute to the development of both RA and SLE and both are considered to have ROS-related aetiologies [28]. In particular, ROS modification of both IgG [29] and DNA [30] can render these macromolecules more susceptible to forming interactions with circulating autoantibodies in RA and SLE, respectively, thus promoting immune complex formation, which may be important in their pathogenesis. The results of 80HDG detection in urine and immune complex-derived DNA of SLE and RA patients showed that whilst both disease states had similar inflammatory components, as determined by standard serological markers of inflammatory activity, the patterns of DNA damage were very different. RA patients responded to increased DNA damage caused during inflammation by increasing the output of 80HDG in urine. However, in SLE, there was no elevation of 80HDG excretion with inflammatory activity but an accumulation of the altered DNA base in circulating immune complexes, resulting in a urine output 1,000-fold lower than in normal controls (as dected by GC-MS/SIM in the femtomolar range).

The results of 80HDG formation as measured by HPLC with electrochemical detection rely on the identification of the modified base by its co-elution with a known standard. It is possible that other compounds present could co-elute with the standard thus either contributing to the 80HDG peak or giving a false-positive result. Since the HPLC technique was unable to give definitive proof of 80HDG production, both the urine and immune complex samples from the SLE and control groups were also analysed by GC-MS/SIM. Using this technique, identification of modified bases depends not only on the co-elution of the compound with its known standard but also on a comparison of the mass spectrum of the compound with the mass spectrum of the standard. Using this technique it was possible to unequivocally confirm that DNA isolated from the immune complexes of SLE patients had significantly higher levels of 80HDG than the corresponding RA control group. It was also found that levels of 80HDG in urine from controls were not significantly different measured by GCMS from those measured by HPLC.

The results from the measurement of 80HDG in rheumatoid patients suggest that, during chronic inflammation, increased oxidative stress leads to ROS-mediated damage to DNA and that the damage produced (measured as 80HDG, though not exclusively) is rapidly and efficiently removed from the target cell with a subsequent increase in the excretion of the modified base in urine. The presence of 80HDG in urine of normal healthy individuals also affirms that this altered base is a by-product of normal oxidative metabolism and likely to be the product of a cellular repair mechanism [20]. The very low levels of excreted 80HDG in urine, and the accumulation of 80HDG in the circulating immune complexes of SLE patients, suggests that there is an abnormal repair of damaged DNA in these patients. However, levels of 80HDG were detected in the femtomolar range in the urine of SLE patients, which ruled out a complete defect in repair. Alternatively it may be argued that immune complexed DNA is very slowly degraded and 80HDG may be released into urine via this route. Although it has been shown that patients with SLE have defective clearance mechanisms for the altered base adduct 0–6 methylguanine [31], it remains to be determined whether or not 80HDG can be repaired by such cells. This may have implications in the increased risk of malignancy seen in patients with inflammatory disorders since 80HDG is mutagenic, causing faulty base pairing at its location [32,33].

Increased sensitivity of SLE cells to ROS-producing systems, particularly UV light has been reported previously [34,35]; this led us to design experiments to investigate the efficiency with which SLE cells could repair the 80HDG adduct induced by incubation of PBMC from SLE patients with H_2O_2. Our results using H_2O_2 confirm the increased susceptibility of SLE cells to oxidative stress on the basis of viability counts. In addition, both normal and SLE cells exposed to H_2O_2 showed a rapid conversion of dG to 80HDG suggesting that there was an equal susceptibility to damage. However, whilst normal cells showed a partial removal of the damaged mutagenic base from the DNA paralleled by an increase of the base in the supernatant, SLE cells showed a considerable decrease in this rate of removal, suggesting an impaired ability to excise 80HDG from the DNA which would in turn contribute to the increased number of cells dying. These in vitro results are entirely consistent with our in vivo data on the excretion of 80HDG in SLE urine. Since our results show that the oxidised DNA (as shown by the presence of 80HDG as well as several other oxidative products of base damage, including 8-hydroxyadenine) that accumulates within the immune complexes is of the order of 20 kb, consistent with results published by other workers [34], we would suggest that this DNA has been derived from cell death/lysis following excessive oxidative injury to the cell, rather than from a repair process which may remove several bases upstream or downstream from the altered base.

In conclusion, we have demonstrated increased ROS-mediated damage to DNA in the related chronic inflammatory disorders RA and SLE. However, the way in which this damage manifests itself is clearly different in each disease. It remains to be clarified whether or not cells from SLE patients have an inherent deficiency in the repair of 80HDG, and whether this is a phenomenon peculiar to lymphocytes or common to all cell types in SLE, but the observation of damaged DNA circulating in plasma containing high levels of 80HDG in combination with our urine and cell studies suggests that SLE patients cannot efficiently remove 80HDG from cellular

DNA which may result in cell death and release of oxidised DNA. In addition, we have previously shown that denaturation of double-stranded DNA by ROS results in an increased binding of anti-DNA antibodies present in sera from SLE patients [30] and that ROS modification of human DNA produces a more discriminating antigen for the diagnosis of SLE [36]. ROS-damaged DNA may, therefore, play a significant role in the generation of immune complexes which are of recognised importance in the pathogenesis of this disease.

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