Formation of 8-nitroguanine in DNA treated with peroxynitrite in vitro and its rapid removal from DNA by depurination

Vladimir Yermilov**, Julieta Rubio***, Hiroshi Ohshima*

Unit of Endogenous Cancer Risk Factors, International Agency for Research on Cancer, 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France

Received 27 October 1995

Abstract Peroxynitrite is a strong oxidant formed by reaction of nitric oxide with superoxide in inflamed tissues. We have demonstrated that 8-nitroguanine is formed dose-dependently in calf thymus DNA incubated with low concentrations of peroxynitrite in vitro. 8-Nitroguanine in acid-hydrolyzed DNA was chemically reduced into 8-aminoguanine, which was analyzed using high performance liquid chromatography with electrochemical detection. Only peroxynitrite, but not nitrite, tetranitromethane nor NO-releasing compounds, formed 8-nitroguanine. Antioxidants and desferrioxamine inhibited the reaction. 8-Nitroguanine was depurinated from DNA incubated at pH 7.4, 37°C ($t_{1/2}$ = ~4 h). Peroxynitrite did not increase 8-oxoguanine levels in DNA.

Key words: Peroxynitrite; 8-Nitroguanine; DNA damage; 8-Oxoguanine; Nitric oxide; Superoxide

1. Introduction

Chronic infection and inflammation have been associated with an increased risk of a variety of human cancers. Reactive oxygen and nitrogen species generated by inflammatory cells have been proposed to induce DNA and tissue damage, chromosomal aberrations and mutations, which contribute to the multistage process of carcinogenesis [1–3].

Recent studies have shown that NO reacts rapidly with superoxide anion ($O_2^{\bullet-}$) to form peroxynitrite, which is a strong oxidant and can initiate reactions characteristic of hydroxyl radical ($HO^{\bullet}$), nitronium ion (NO_2^+) and nitrogen dioxide radical ($NO_2^{\bullet}$) [4–6]. It has been shown that essentially all of the NO produced by macrophages activated with phorbol 12-myristate-13-acetate is converted to peroxynitrite [7]. Increased levels of deamination and oxidation products of DNA bases have been detected in macrophages activated with lipopolysaccharides and interferon-γ [8]. Peroxynitrite formed by macrophages has been implicated as one possible mechanism for oxidative DNA damage [8].

Increasing evidence now suggests that peroxynitrite is a major agent causing tissue damage induced by inflammation in vivo [9–11]. Peroxynitrite oxidizes sulfhydryl groups and induces membrane lipid peroxidation [12–14]. It also nitrates tyrosine residues in proteins to form 3-nitrotyrosine, which is now measured as a marker of peroxynitrite-induced protein damage [15–17]. Since peroxynitrite is a relatively stable compound (the half-life is about one second at physiological pH [5]), it could penetrate the nucleus, where it might induce damage in DNA. It has been reported that peroxynitrite induces strand breaks in plasmid DNA [18] and oxidative damage in isolated DNA in vitro [19]. We recently found that guanine reacts rapidly with peroxynitrite under physiological conditions to form several substances, two of which are yellow [20]. On the basis of chromatographic and spectral evidence we identified the major compound (which accounts for about 80% of all compounds formed) as a novel adduct, 8-nitroguanine (nitro^{8}Gua) [20]. In order to study the biological significance of this new adduct, it is essential to determine whether nitro^{8}Gua is formed in DNA.

For this study, we have developed a new sensitive and specific method to analyze nitro^{8}Gua in DNA, which we have used to measure the formation and stability of nitro^{8}Gua in DNA. Various factors known to influence oxidation and nitration reactions by peroxynitrite have also been studied in relation to their effects on the formation of nitro^{8}Gua in DNA.

2. Materials and methods

2.1. Chemicals

Nitro^{8}Gua was synthesized by reaction of guanine with peroxynitrite as described previously [20]. 8-Aminoguanine was prepared by acid hydrolysis of 8-aminoguanosine (Sigma Chemical Co., St Louis, MO) [21]. Peroxynitrite was synthesized in a quenched flow reactor and excess hydrogen peroxide was destroyed by granular manganese dioxide [4,22]. 3-(4-Morpholinyl)-sydnone imine (SIN-1) was a gift from Hoechst Laboratories (Paris). All other chemicals were commercially available.

2.2. Confirmation of nitro^{8}Gua in DNA hydrolysates

In order to confirm the formation of nitro^{8}Gua in DNA, we carried out the reaction on a large scale (50 mg calf thymus DNA, 0.1 mM peroxynitrite in 50 ml 0.1 M phosphate buffer, pH 7.0). DNA was precipitated with ice-cold ethanol (2 volumes), washed twice with 100 ml of 75% ethanol and dried in a Savant Speed-Vac. DNA was hydrolyzed in 50 ml of 0.1 N HCl for 30 min at 100°C and dried under vacuum. The bases were separated by preparative high performance liquid chromatography (HPLC) (1.0 × 25-cm, Nucleosil C 18, 5 μ Société Française Chromato Colonne, Neuilly Plaisance, France) under isocratic conditions with 20 mM ammonium formate buffer, pH 6.0 at a flow rate of 2 ml/min. Fractions containing nitro^{8}Gua were collected and concentrated. The concentrated fractions were analyzed in a Spectraphysics SP 8800 HPLC system with a 0.46 × 15 cm ultrasphere ODS column (5 μ, Beckman, Berkeley, CA) under isocratic conditions with the same ammonium formate buffer as above at a flow rate of 0.8 ml/min. A Waters Lambda-Max model 481 UV/vis LC spectrophotometer was used to detect nitro^{8}Gua at 396 nm and 8-aminoguanine at 254 nm.

*Corresponding author. Fax: (33) (72) 73 85 75;
E-mail: Ohshima@IARC.FR

**Visiting Scientist at IARC from: N.N. Petrov Research Institute, Leningradskaya St. 68, Pesochny-2, 189646 St Petersburg, Russia

***Present address: Instituto de Investigaciones Biomédicas, Departamento de Genética y Toxicologia Ambiental, Apartado Postal 70228, CD Universitaria, 04510 México DF, México

Abbreviations: nitro^{8}Gua, 8-nitroguanine; oxo^{8}Gua, 8-oxoguanine; SIN-1, 3-(4-morpholinyl)-sydnone imine; EC, electrochemical detector.

SSDI 0014-5793(95)01281-8

2.3. Reaction of calf thymus DNA with peroxynitrite

The reactions were carried out at room temperature (~20°C). Peroxynitrite prepared in 1 N NaOH at various concentrations (100 μl) was added to a reaction mixture (final volume, 1 ml) containing 0.1 M sodium phosphate buffer, pH 7.0, calf thymus DNA (0.2 mg), 100 μM diethylenetriaminepentaacetic acid (DTPA), a metal chelator and an appropriate amount of HCl to neutralize the NaOH present in the peroxynitrite solution (final pH was 7.2). Control experiments were performed using the same concentrations of decomposed peroxynitrite.

2.4. Reactions of calf thymus DNA with NO-releasing compounds, sodium nitrite and tetranitromethane

The NO-releasing compounds (10 or 100 mM) were dissolved freshly in water, except for *S*-nitroso-*N*-acetyl-D,L-penicillamine which was prepared in 50% DMSO. The solution (100 μl) was added to a reaction mixture containing 0.1 M sodium phosphate buffer, pH 7.0, calf thymus DNA (1 mg/ml) and 100 μM DTPA (final volume, 1 ml). Tetranitromethane was added directly to the reaction mixture to give a final concentration of 1 or 10 mM. The reaction mixtures were incubated at 37°C for 1–18 h.

2.5. Effects of ferric and ferrous ions, hydroxyl radical scavengers, glucose, anti-oxidants and metal chelators on nitro^{8}Gua formation in DNA

The effects of ferric ion- or ferrous ion-ethylenediaminetetraacetic acid (EDTA) on the formation of nitro^{8}Gua in DNA were studied by adding $Fe^{3+}NH_4(SO_4)_2$ or $Fe^{2+}(NH_4)_2(SO_4)_2$ (final concentrations of 1, 10, 100 and 1000 μM) and a 1.1-fold excess EDTA to the reaction mixture (1 ml) containing 0.1 M sodium phosphate buffer, pH 7.0, calf thymus DNA (1 mg), 100 μM DTPA and 0.1 mM peroxynitrite. Similarly, a reaction mixture containing 0.1 M sodium phosphate buffer, pH 7.0, calf thymus DNA (1 mg/ml), 100 μM DTPA and 1 mM concentrations of scavengers and antioxidants, except for uric acid (0.1 mM), was incubated with 0.1 mM peroxynitrite. Effects of metal chelators (DTPA, EDTA and desferrioxamine) were studied in the presence of 0.1 M sodium phosphate buffer, pH 7.0, calf thymus DNA (1 mg), 0.1 mM of a chelator and 0.1 mM peroxynitrite.

2.6. Depurination of nitro^{8}Gua formed in DNA

Nitro^{8}Gua-containing calf thymus DNA (100 mg) was prepared as described in 2.2. Isolated DNA was dissolved in phosphate buffer-saline, pH 7.4 at a concentration of 500 μg/ml. The solution was incubated at 37 °C for up to 6 h.

2.7. Determination of nitro^{8}Gua in DNA

After the reaction, DNA was precipitated with cold ethanol (2 volumes), washed twice with 2.5 ml of 75% ethanol and 1 ml ethanol and dried in a Savant Speed-Vac. The dried DNA samples were hydrolyzed in 0.1 N HCl (~1 ml/mg DNA) at 100°C for 30 min. HCl was removed in a Speed-Vac and the residue was dissolved in 100 μl of 0.1 M Tris-HCl buffer, pH 8.5. To a 50 μl aliquot, a small amount of sodium hydrosulfite was added in order to reduce nitro^{8}Gua to 8-aminoguanine. The samples before and after reduction were analyzed using a Spectraphysics HPLC (model SP 8810) equipped with two reverse-phase columns in series (15 × 0.46-cm Ultrasphere ODS column, 5 μ Beckman) under isocratic conditions with a 12.5 mM citric acid 25 mM sodium acetate buffer containing 25 μM EDTA (pH 5.2) at a flow rate of 1 ml/min. The reduced nitro^{8}Gua (i.e. 8-aminoguanine) and 8-oxoguanine (oxo^{8}Gua) were detected by an electrochemical detector (EC) (Waters model M460) at a potential of +600 mV. Guanine was detected by a UV spectrophotometer (Spectra SERIES UV 100, thermoseparation) at 254 nm. Under these conditions, retention times of 8-aminoguanine, oxo^{8}Gua and guanine were 12.7, 14.6 and 12.9 min, respectively. All experiments were carried out in triplicate and statistical significance was calculated using Student's *t* test.

3. Results and discussion

In order to confirm the formation of nitro^{8}Gua in DNA, we treated a large amount of calf thymus DNA with peroxynitrite. The acid-hydrolyzed DNA was purified by preparative HPLC and the fractions containing nitro^{8}Gua were collected and evaporated to dryness. This concentrated fraction was analyzed by HPLC with UV detection at 396 nm, as previously reported [20]. A yellow peak eluted at the same retention time as a standard nitro^{8}Gua (7.8 min). Upon reduction with sodium hydrosulfite, this yellow peak disappeared and a new peak, detectable only at 254 nm, appeared at a retention time of 11.3 min. The new peak co-eluted with 8-aminoguanine standard. These results indicate that DNA hydrolysates contained nitro^{8}Gua, which was converted to 8-aminoguanine by chemical reduction. The same DNA sample did not show this yellow peak before acid-hydrolysis, excluding the possibility that nitro^{8}Gua is present as a depurinated compound in the sample. Similarly, DNA treated with decomposed peroxynitrite did not contain nitro^{8}Gua. These results indicate that nitro^{8}Gua is formed in the DNA by the treatment with peroxynitrite.

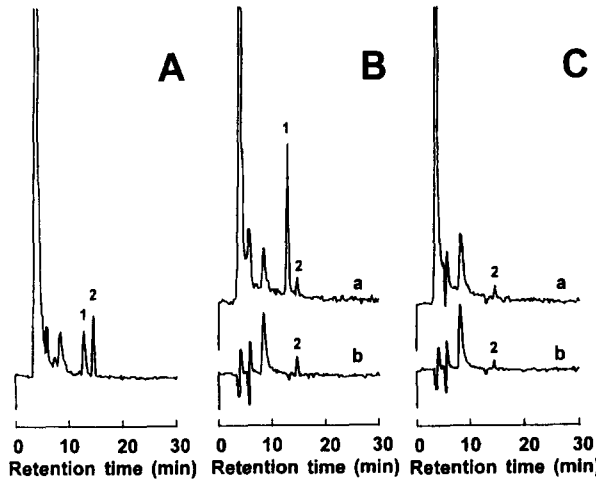

Fig. 1. Typical chromatograms obtained by HPLC-EC analyses of (A) standards, (B) acid-hydrolysates of DNA treated with peroxynitrite and (C) those of DNA treated with decomposed peroxynitrite. (a,b) after and before reduction with sodium hydrosulfite, respectively. Peak 1 is nitro^{8}Gua after reduction (i.e. 8-aminoguanine) and peak 2 is oxo^{8}Gua.

Although nitro^{8}Gua is not detectable by EC, 8-aminoguanine has been reported to be electrochemically active [21]. As shown above, nitro^{8}Gua is easily reduced by sodium hydrosulfite to 8-aminoguanine [20]. We have therefore developed a new method to analyze nitro^{8}Gua in DNA sensitively and specifically, that involves acid-hydrolysis of DNA, and chemical conversion of nitro^{8}Gua into 8-aminoguanine, which is then detected by HPLC-EC. Fig. 1 shows typical chromatograms of acid-hydrolysates of calf thymus DNA after incubation in vitro with 0.1 mM peroxynitrite (Fig. 1Ba,b) or with decomposed peroxynitrite (Fig. 1Ca,b). Both samples showed a peak corresponding to oxo^{8}Gua before reduction with sodium hydrosulfite (Fig. 1Bb,Cb). After the reduction, bases from peroxynitrite-treated DNA showed a new peak at a retention time corresponding to 8-aminoguanine (Fig. 1Ba), but such a peak was not observed in samples from DNA treated with decomposed peroxynitrite (Fig. 1Ca). The method is sensitive and specific, the limit of detection for nitro^{8}Gua being approximately 1 pmol injected. The presence of nitro^{8}Gua can be confirmed by injecting the sample before and after reduction with sodium hydrosulfite, nitro^{8}Gua being specifically detected by EC only after the reduction.

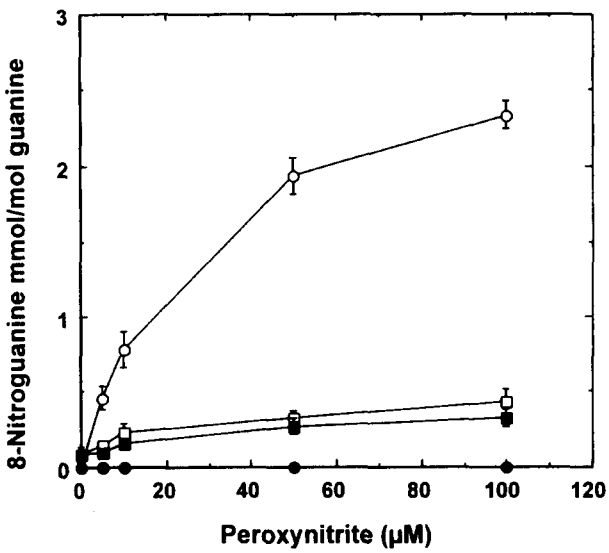

Fig. 2. Effect of peroxynitrite concentration on formation of nitro^{8}Gua and oxo^{8}Gua in DNA. One millilitre of 100 mM sodium phosphate buffer, pH 7.0, containing 0.1 mM DTPA and 0.2 mg calf thymus DNA was incubated with various concentrations of peroxynitrite or decomposed peroxynitrite at room temperature (~20°C). ○—○, nitro^{8}Gua formed with peroxynitrite; □—□, oxo^{8}Gua formed with peroxynitrite; ●—●, nitro^{8}Gua formed with decomposed peroxynitrite; ■—■, oxo^{8}Gua formed with decomposed peroxynitrite.

Fig. 2 shows that the level of nitro^{8}Gua in DNA increased dose-dependently with peroxynitrite concentration. Nitro^{8}Gua was formed even with peroxynitrite concentrations as low as 5 μM. Decomposed peroxynitrite did not generate nitro^{8}Gua. The level of oxo^{8}Gua also increased with increased concentration of peroxynitrite, but also with decomposed peroxynitrite. This suggests that trace amounts of hydrogen peroxide present in peroxynitrite solutions could be involved in the oxidation of guanine in DNA, but not peroxynitrite itself. Peroxynitrite has been reported to generate both HO$^\bullet$ and nitrogen dioxide radical ($NO_2^\bullet$) by homolytic cleavage [4,6]. Our results suggest that peroxynitrite generates a nitrating agent (or several), which effectively reacts with guanine moieties in DNA to form nitro^{8}Gua, whereas an HO$^\bullet$-like compound may not be generated from peroxynitrite, or if generated, it reacts with other components in the reaction mixture more rapidly than with guanine.

Nitro^{8}Gua was formed in DNA treated with peroxynitrite, but was not detected in DNA incubated with NO-releasing compounds (*S*-nitroso-*N*-acetyl-D,L-penicillamine, sodium nitroprusside, SIN-1), sodium nitrite or the strong nitrating agent, tetranitromethane during incubation for up to 18 h. We studied the reaction of DNA with SIN-1 more extensively, because it has been reported that SIN-1 releases both NO and $O_2^{\bullet-}$, which may react to form peroxynitrite [23,24]. SIN-1 increased dose-dependently the level of oxo^{8}Gua, but not that of nitro^{8}Gua in DNA. Incubation of DNA (1 mg/ml) with 10 mM SIN-1 for 6 h resulted in the formation of 1.19 ± 0.15 mmol oxo^{8}Gua/mol guanine, whereas a control DNA sample incubated without SIN-1 contained 0.08 ± 0.01 mmol oxo^{8}Gua/mol guanine. The formation of oxo^{8}Gua was inhibited by the addition of either superoxide dismutase (500 U/ml) alone, catalase (500 U/ml) alone or the two in combination, by 65, 60 or 70%, respectively. Thus, the formation of oxo^{8}Gua by SIN-1 could be mediated by $O_2^{\bullet-}$ or hydrogen peroxide generated from SIN-1. No nitro^{8}Gua was detected in any DNA sample incubated with SIN-1 for a short (30 min) or long period (18 h), although in each case oxo^{8}Gua was formed. One possible reason for this observation could be that peroxynitrite is not formed from SIN-1 under the present conditions. Further studies are needed to elucidate the mechanism by which oxo^{8}Gua, but no nitro^{8}Gua, is formed in DNA treated with SIN-1. We are currently studying formation of nitro^{8}Gua and oxo^{8}Gua in DNA in reaction systems where both NO and $O_2^{\bullet-}$ are generated simultaneously.

Nitration of tyrosine and phenolics by peroxynitrite has been reported to be catalyzed by ferric ion [4,25]. We have examined the effects of ferric and ferrous ion on nitro^{8}Gua formation in DNA. As we previously observed for nitration of guanine in vitro [20], Fe^{3+}-EDTA at concentrations 1, 10, 100 and 1000 μM did not affect the formation of nitro^{8}Gua in DNA (DNA and peroxynitrite concentrations were 1 mg/ml and 0.1 mM, respectively). Under the same conditions, Fe^{2+}-EDTA at concentrations of 1, 10 and 100 μM did not affect the reaction, but a high concentration (1000 μM) inhibited it by ~45%. Fe^{2+}-EDTA, but not Fe^{3+}-EDTA, dose-dependently increased the level of oxo^{8}Gua in DNA treated with either peroxynitrite or decomposed peroxynitrite, possibly by oxidation with HO$^\bullet$ generated from Fenton-type reactions.

Fig. 3 shows the effects of various scavengers of hydroxyl radical, antioxidants and glucose on the formation of nitro^{8}Gua in DNA incubated with peroxynitrite. *N*-Acetylcysteine as well as antioxidants such as ascorbic acid and uric acid significantly inhibited the reaction. Hydroxyl radical scavengers such as DMSO, D-mannitol and ethanol did not affect it. Metal ion chelators such as EDTA and DTPA also did not affect the

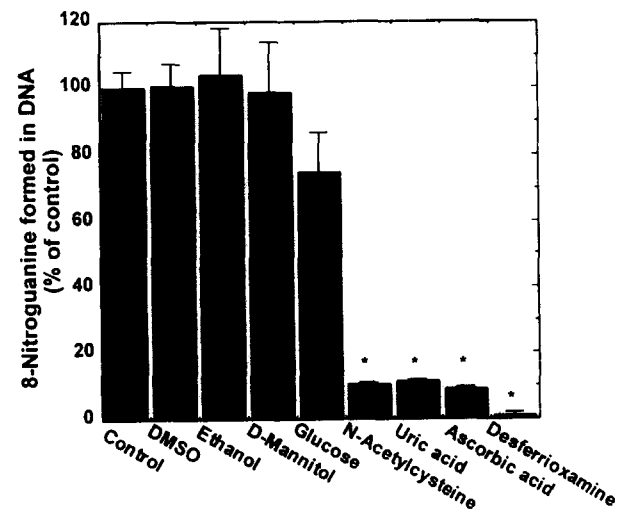

Fig. 3. Effects of hydroxyl radical scavengers, glucose, anti-oxidants and desferrioxamine on the formation of nitro^{8}Gua in DNA incubated with peroxynitrite. One millilitre of 100 mM sodium phosphate buffer, pH 7.0, containing 0.1 mM DTPA, 1 mg calf thymus DNA and a test compound was incubated with 0.1 mM peroxynitrite at room temperature (20°C). The concentrations of test compounds were 1 mM, except for uric acid and desferrioxamine, whose concentrations were 0.1 mM. The results are expressed as % of control without a test compounds which formed 2.92 ± 0.15 mmol nitro^{8}Gua/mol guanine in DNA (mean ± S.D., $n = 3$). *, significantly different from controls at $P \leq 0.005$.

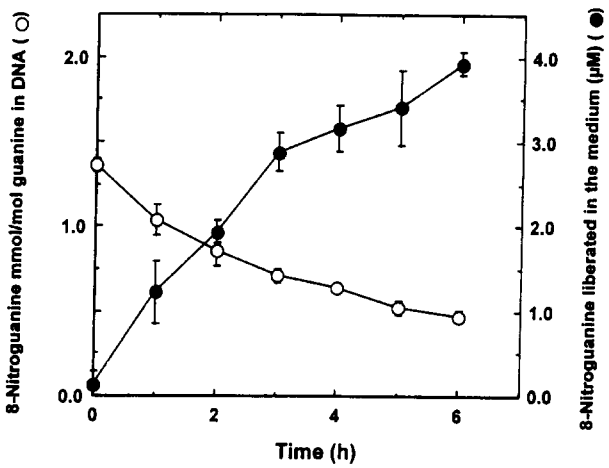

Fig. 4. Removal of nitro[8]Gua from DNA by depurination. Calf thymus DNA containing nitro[8]Gua was dissolved in phosphate buffer saline, pH 7.4 (500 μg/ml) and incubated at 37°C. At every 1 h incubation, the reaction mixture was removed and DNA was precipitated with ethanol and washed twice with 75% ethanol. The washings were combined and dried in a Speed-Vac for the analysis of free nitro[8]Gua liberated from the DNA into the medium. ○–○, nitro[8]Gua in DNA; ●–●, free nitro[8]Gua liberated in the medium.

reaction, although desferrioxamine completely inhibited the formation of nitro[8]Gua in DNA. This inhibitory effect of desferrioxamine could be due to a direct reaction between desferrioxamine and peroxynitrite, as shown previously for inhibition of the peroxynitrite-induced oxidation of deoxyribose and DMSO as well as lipid peroxidation [5,12].

Upon incubation of DNA containing nitro[8]Gua at 37°C and pH 7.4, the adduct disappeared rapidly from DNA and free nitro[8]Gua appeared in the medium (Fig. 4). The half life of nitro[8]Gua in DNA was estimated to be about 4 h. These results suggest that nitro[8]Gua formed in DNA is potentially mutagenic, because its depurination yields apurinic sites in DNA, which can induce G:C→T:A transversions [26]. Recent studies have demonstrated that peroxynitrite induced the same G:C→T:A transversions predominantly in the *supF* gene after exposure of the plasmid pSP189 to peroxynitrite in vitro followed by transfection into bacterial and mammalian cells [27].

In conclusion, we have provided evidence for the formation of nitro[8]Gua in DNA treated with peroxynitrite in vitro. This novel adduct is formed uniquely by peroxynitrite, and no other NO-releasing compound or nitrating agent forms the same adduct. Nitro[8]Gua is rapidly depurinated from DNA, yielding apurinic sites which are potentially mutagenic. Peroxynitrite could therefore play an important role in carcinogenesis by inducing DNA and tissue damage. Our method to analyze nitro[8]Gua in DNA will be useful to study whether peroxynitrite induces damage in DNA in cells and tissues. Nitro[8]Gua could serve as a specific marker of DNA damage induced by NO and peroxynitrite in inflamed tissues, like several other oxidized DNA bases, such as oxo[8]Gua, which have been measured as markers of oxidative damage.

Acknowledgements: The authors would like to thank Dr. J. Cheney for editing the manuscript. V.Y. and J.R. are recipients of a European Science Foundation fellowship and a Universidad Nacional Autonoma de México Scholarship, respectively.

References

[1] Ohshima, H. and Bartsch, H. (1994) Mutat. Res. 305, 253–264.
[2] Ames, B.N., Shigenaga, M.K. and Hagen, T.M. (1993) Proc. Natl. Acad. Sci. USA 90, 7915–7922.
[3] Gordon, L.I. and Weitzman, S.A. (1993) Cancer J. 6, 257–261.
[4] Beckman, J.S., Chen, J., Ischiropoulos, H. and Crow, J.P. (1994) Methods Enzymol. 233, 229–240.
[5] Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P.A. and Freeman, B.A. (1990) Proc. Natl. Acad. Sci. USA 87, 1620–1624.
[6] Pryor, W.A. and Squadrito, G.L. (1995) Am. J. Physiol. 268, L699–L722.
[7] Ischiropoulos, H., Zhu, L. and Beckman, J.S. (1992) Arch. Biochem. Biophys. 298, 446–451.
[8] deRojas-Walker, T., Tamir, S., Ji, H., Wishnok, J.S. and Tannenbaum, S.R. (1995) Chem. Res. Toxicol. 8, 473–477.
[9] Mulligan, M.S., Hevel, J.M., Marletta, M.A. and Ward, P.A. (1991) Proc. Natl. Acad. Sci. USA 88, 6338–6342.
[10] Cazevieille, C., Muller, A., Meynier, F. and Bonne, C. (1993) Free Radic. Biol. Med. 14, 389–395.
[11] White, C.R., Brock, T.A., Chang, L.Y., Crapo, J., Briscoe, P., Ku, D., Bradley, W.A., Gianturco, S.H., Gore, J., Freeman, B.A. and Tarpey, M.M. (1994) Proc. Natl. Acad. Sci. USA 91, 1044–1048.
[12] Radi, R., Beckman, J.S., Bush, K.M. and Freeman, B.A. (1991) Arch. Biochem. Biophys. 288, 481–487.
[13] Radi, R., Beckman, J.S., Bush, K.M. and Freeman, B.A. (1991) J. Biol. Chem. 266, 4244–4250.
[14] van der Vliet, A., Smith, D., O'Neill, C.A., Kaur, H., Darley Usmar, V., Cross, C.E. and Halliwell, B. (1994) Biochem. J. 303, 295–301.
[15] Haddad, I.Y., Pataki, G., Hu, P., Galliani, C., Beckman, J.S. and Matalon, S. (1994) J. Clin. Invest. 94, 2407–2413.
[16] Kaur, H. and Halliwell, B. (1994) FEBS Lett. 350, 9–11.
[17] Kooy, N.W., Royall, J.A., Ye, Y.Z., Kelly, D.R. and Beckman, J.S. (1995) Am. J. Respir. Crit. Care Med. 151, 1250–1254.
[18] King, P.A., Anderson, V.E., Edwards, J.O., Gustafson, G., Plumb, R.C. and Suggs, J.W. (1992) J. Am. Chem. Soc. 114, 5430–5432.
[19] Douki, T. and Cadet, J. (1995) Free Radic. Res., in press.
[20] Yermilov, V., Rubio, J., Becchi, M., Friesen, M.D., Pignatelli, B. and Ohshima, H. (1995) Carcinogenesis 16, 2045–2050.
[21] Sodum, R.S., Nie, G. and Fiala, E.S. (1993) Chem. Res. Toxicol. 6, 269–276.
[22] Reed, J.W., Ho, H.H. and Jolly, W.L. (1974) J. Am. Chem. Soc. 96, 1248–1249.
[23] Hogg, N., Darley Usmar, V.M., Wilson, M.T. and Moncada, S. (1992) Biochem. J. 281, 419–424.
[24] Haddad, I.Y., Crow, J.P., Hu, P., Ye, Y., Beckman, J. and Matalon, S. (1994) Am. J. Physiol. 267, L242–L249.
[25] Beckman, J.S., Ischiropoulos, H., Zhu, L., van der Woerd, M., Smith, C., Chen, J., Harrison, J., Martin, J.C. and Tsai, M. (1992) Arch. Biochem. Biophys. 298, 438–445.
[26] Loeb, L.A. and Preston, B.D. (1986) Annu. Rev. Genet. 20, 201–230.
[27] Juedes, M.J. and Wogan, G.N. (1995) Proc. Am. Assoc. Cancer Res. 36, 164 (Abstract).