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Increased oxidative damage to all DNA bases in patients with type II diabetes mellitus

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Abstract Gas chromatography-mass spectrometry was used to measure the oxidative DNA damage in diabetic subjects and controls. Levels of multiple DNA base oxidation products, but not DNA base de-amination or chlorination products, were found to be elevated in white blood cell DNA from patients with type II diabetes as compared with age-matched controls. The chemical pattern of base damage is characteristic of that caused by an attack on DNA by hydroxyl radical. An increased formation of the highly reactive hydroxyl radical could account for many of the reports of oxidative stress in diabetic subjects. There was no evidence of an increased DNA damage by reactive nitrogen or chlorine species.

Key words: Diabetes; Oxidative stress; DNA damage; Hydroxyl radical

1. Introduction

The vascular and other complications of diabetes mellitus are frequently suggested to involve oxidative damage resulting from the hyperglycaemia and/or hyperlipidaemia. For example, decreased levels of antioxidants (especially ascorbate) and increased levels of lipid peroxidation products, such as F_2-isoprostanes, have been reported in diabetic patients (prior to the onset of complications) by several groups [1–4]. Free radicals and other reactive oxygen species can also often damage DNA [5–8]. Guanine is the most oxidisable base in DNA and increases in the levels of its oxidation product 8-hydroxy-2′-deoxyguanosine (8OHdG) have been reported in mononuclear cells from patients with both insulin- and non-insulin-dependent diabetes [9]. This was paralleled by an increased DNA strand breakage in these cells [10]. An increased urinary excretion of 8OHdG has also been reported [11] in diabetic subjects. Urinary 8OHdG excretion is believed to result from the repair of oxidative damage to DNA and its nucleotide precursor pool [11].

Rises in 8OHdG in DNA can be produced by the attack of several different reactive oxygen species, including hydroxyl radical (OH•), singlet oxygen, certain peroxyl radicals and (to a limited extent) peroxynitrite, $ONOO^-$ [6,12,13]. Peroxynitrite is a cytotoxic species generated when superoxide radicals ($O_2^{\bullet -}$) combine with nitric oxide and its formation has been implicated in both the origin [14] and in the progression [15] of diabetes. However, an attack of these different species on DNA can be distinguished by the pattern of damage that is caused to the other DNA bases. For example, OH• produces a multiplicity of products from all four DNA bases, whereas singlet oxygen is selective for guanine [6,8,12,16]. Peroxynitrite generates mainly base de-amination and nitration products (e.g. xanthine from guanine, hypoxanthine from adenine) and 8OHdG is only a minor product [13,17]. Hypochlorous acid (HOCl), a reactive species produced by the enzyme myeloperoxidase in isolated neutrophils, produces pyrimidine oxidation and chlorination products in DNA rather than 8OHdG [18].

In the present paper, we have identified the species most likely to be responsible for the increased levels of oxidative DNA damage in patients with type II diabetes by examining the molecular pattern of damage to all four DNA bases using gas chromatography-mass spectrometry (GC-MS) [8,19]. The pattern of damage observed is exactly that expected from OH•.

2. Materials and methods

2.1. Patients

Non-insulin-dependent diabetic patients (17 males, 21 females, 55.4 ± 6.6 years) were recruited from well-characterised patients attending the Coimbra University Hospital's Ophthalmology and Endocrinology services. Healthy age-matched controls (9 males, 27 females, 50.7 ± 15.2 years) were recruited from the Institute staff and from the University for the elderly, Coimbra. None of the subjects was taking antioxidant supplements. This study was approved by the Ethics Committee of Coimbra University Hospitals.

2.2. Sample collection

Blood was collected by venipuncture into sampling vials (10 ml) containing lithium-heparin. The samples were centrifuged at $2000 \times g$ for 10 min at room temperature. Blood cells including the buffy layer were transferred into new containers and were stored at −70°C until analysis.

2.3. Reagents

Unless otherwise stated, chemicals were of the highest quality available from Sigma Chemical (Poole, Dorset, UK), BDH Chemical (Gillingham, Dorset, UK) or Aldrich (Milwaukee, WI, USA). Ribonucleases A (bovine pancreas, molecular biology grade) and T_1 (*Aspergillus oryzae*) were from Sigma. 8-Hydroxyadenine, thymine glycol and FAPy-guanine were synthesised as described in [20]. 2-Hydroxyadenine, 5-hydroxycytosine and 5-hydroxymethyl hydantoin were gifts from Dr. Miral Dizdaroglu (NIST, Gaithersburg, USA). Other reagents needed for GC-MS were obtained as described in [19].

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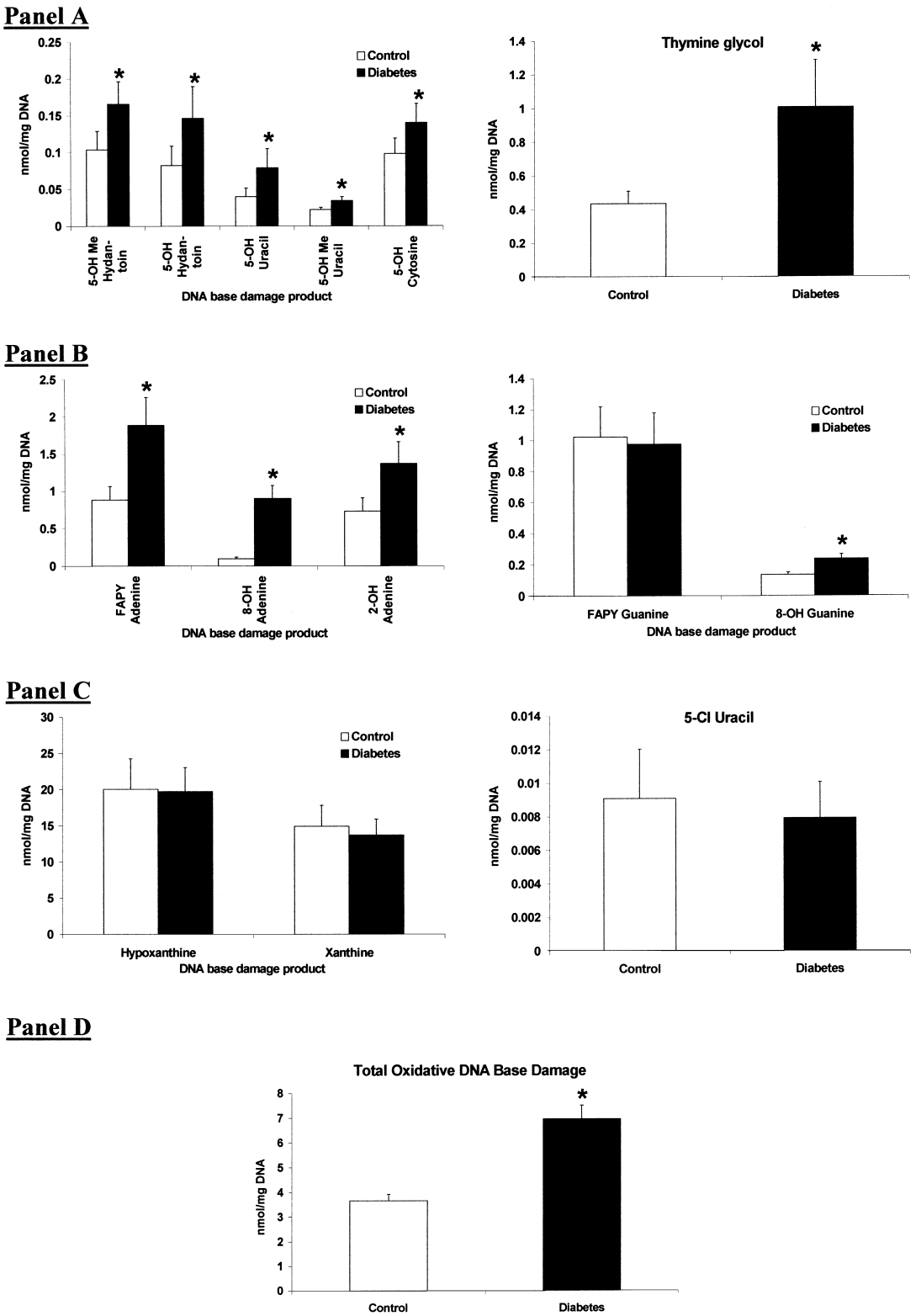

Fig. 1. Levels of DNA base damage products in diabetic and control DNA. A: Pyrimidine oxidation products, B: purine oxidation products, C: base deamination (xanthine, hypoxanthine) and chlorination (5-chlorouracil) products, D: total oxidative DNA damage. Results are mean ± S.D. *Significant difference, unpaired *t*-test at 99% confidence level.

2.4. DNA isolation

DNA isolation was carried out as described in John et al. [21] with the modifications described in [22]. The DNA was treated with a mixture of RNases A and T_1 to remove the RNA contamination [22].

2.5. Analysis of DNA base damage

This was performed essentially as described in [23] with the modifications described in [19]. Derivatisation was carried out at room temperature in the presence of ethanethiol, a protocol which prevents

artifactual further oxidation of DNA bases during sample processing [19].

3. Results

DNA was isolated from blood samples taken from type II diabetic patients and age-matched controls. The DNA was freed of RNA, hydrolyzed in acid, the base products converted to volatile derivatives, separated by GC and the products analysed by MS. Precautions were taken throughout to prevent any artifactual oxidation of undamaged DNA bases [19,22,24]. The advantage of GC-MS analysis of oxidative DNA damage is that it allows the simultaneous quantitation of a wide range of DNA base damage products, including 8-hydroxyguanine [8]

Fig. 1 summarises the data obtained. There was no significant rise in 5-chlorouracil (C), a marker of the attack of HOCl on cytosine residues in DNA [18]. Similarly, levels of the base de-amination products hypoxanthine and xanthine did not increase (C). The levels of 8-hydroxyguanine were elevated (B), as expected from previous reports [9,11]. However, levels of 5-OH uracil, 5-OH methyluracil, thymine glycol, 5-OH methylhydantoin, 5-OH hydantoin, 5-OH cytosine, 2-OH adenine, 8-OH adenine and FAPy-adenine were also elevated. Only FAPy-guanine remained unchanged. The total of DNA base oxidative damage products was approximately doubled (Fig. 1D).

4. Discussion

Our data show that the levels of products generated by oxidative damage to all four DNA bases (cytosine, adenine, guanine and thymine) are markedly elevated in total blood cell DNA from patients with type II diabetes mellitus. This is exactly the pattern one would expect from an attack of OH• upon DNA [6,8,12]. This highly reactive free radical species can cause damage to many biomolecules (reviewed in [25]) and its apparent generation in excess in diabetes could account for much of the oxidative damage to various biomolecules that has been described in previous papers [1–4]. Since many DNA base oxidation products are mutagenic and/or block the DNA replication [5,7], our data have implications when considering the possibility of an elevated risk of cancer development in diabetic subjects [26–28]. We obtained no evidence for an increased attack upon DNA by HOCl or by $ONOO^-$ or other nitrogen-related species, since levels of chlorouracil, xanthine or hypoxanthine were not increased. Of course, these species might still be formed in excess in diabetes, but attack other molecular targets.

Our data show one anomalous feature, in that levels of FAPy-guanine were not increased (Fig. 1). When OH• attacks guanine in DNA, both 8-hydroxyguanine and FAPy-guanine are normally produced. The initial product of addition, 8-hydroxyguanine radical, has to undergo one electron oxidation to generate 8-hydroxyguanine or reduction processes to generate FAPy-guanine [29]. The fact that only the former product is elevated suggests that the micro-environment of the DNA is more oxidising than usual [30], in keeping with many other observations on diabetic subjects [1–4,31]. In agreement with our data, the salicylate hydroxylation method [32,33] has been used to trap OH•s in diabetic patients [34] and our data show that such radicals are causing considerable damage, to DNA at least.

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References

[1] Baynes, J.W. (1991) Diabetes 40, 102.
[2] Wolff, S.P. (1993) Br. Med. Bull. 49, 642–652.
[3] Patrono, C. and Fitzgerald, G.A. (1997) Arterioscler. Thromb. Vasc. Biol. 17, 2309–2315.
[4] Gopaul, N.K., Anggard, E.E., Mallet, A.I., Betteridge, D.J., Wolff, S.P. and Nourooz-Zadeh, J. (1995) FEBS Lett. 368, 225–229.
[5] Kasai, H. (1997) Mutat. Res. 387, 147–163.
[6] Halliwell, B. and Aruoma, O.I. (1991) FEBS Lett. 281, 9–19.
[7] Floyd, R.A. (1990) Carcinogenesis 11, 1447–1450.
[8] Dizdaroglu, M. (1990) Methods Enzymol. 193, 842–857.
[9] Dandona, P., Thusu, K., Cook, S., Snyder, B., Makowski, J., Armstrong, D. and Nicotera, T. (1996) Lancet 347, 444–445.
[10] Collins, A.R., Raslova, K., Somorovska, M., Petrovska, H., Ondrusova, A., Vohnout, B., Fabry, R. and Dusinska, M. (1998) Free Radic. Biol. Med. 25, 373–377.
[11] Leinonen, J., Lehtimaki, T., Toyokuni, S., Okada, K., Tanaka, T., Hiai, H., Ochi, H., Laippala, P., Rantalaiho, V., Wirta, O., Pasternack, A. and Alho, H. (1997) FEBS Lett. 417, 150–152.
[12] Halliwell, B. (1998) Free Radic. Res. 29, 469–486.
[13] Douki, H. and Cadet, J. (1996) Free Radic. Res. 24, 369–380.
[14] Suarez-Pinzon, W.L., Szabo, C. and Rabinovitch, A. (1997) Diabetes 46, 907–911.
[15] Szabo, C. (1996) Free Radic. Biol. Med. 21, 855–869.
[16] Mori, T., Tanko, K., Takimoto, K. and Utsumi, H. (1998) Biochem. Biophys. Res. Commun. 242, 98–101.
[17] Spencer, J.P.E., Wong, J., Jenner, A., Aruoma, O.I., Cross, C.E. and Halliwell, B. (1995) Chem. Res. Toxicol. 9, 1152–1158.
[18] Whiteman, M., Jenner, A. and Halliwell, B. (1997) Chem. Res. Toxicol. 10, 1240–1246.
[19] Jenner, A., England, T.G., Aruoma, O.I. and Halliwell, B. (1998) Biochem. J. 331, 365–369.
[20] Dizdaroglu, M. (1994) Methods Enzymol. 234, 3–16.
[21] John, S.W.M., Weitzner, G., Rozen, R. and Scriver, C.R. (1991) Nucleic Acids Res. 19, 408.
[22] Rehman, A. and Halliwell, B. (1999) Free Radic. Res. (in press).
[23] Spencer, J., Jenner, A., Chimel, K., Aruoma, O.I., Cross, C.E., Wu, R. and Halliwell, B. (1995) FEBS Lett. 375, 179–182.
[24] England, T.G., Jenner, A., Aruoma, O.I. and Halliwell, B. (1998) Free Radic. Res. 29, 321–330.
[25] Halliwell, B., Gutteridge, J.M.C. (1999) Free Radicals in Biology and Medicine, Chapters 2–4, 3rd edn., Oxford University Press, Oxford, UK.
[26] Shoff, S.M. and Newcomb, P.A. (1998) Am. J. Epidemiol. 148, 234–240.
[27] Will, J.C., Galuska, D.A., Vinicor, F. and Calle, E.E. (1998) Am. J. Epidemiol. 147, 816–825.
[28] Mori, M., Nishida, T., Sugiyama, T., Komai, K., Yakushiji, M., Fukuda, K., Tanaka, T., Yokoyama, M. and Sugimori, H. (1998) Jpn. J. Cancer Res. 89, 246–253.
[29] Fuciarelli, A.F., Wegher, B.J., Blakeley, W.F. and Dizdaroglu, M. (1990) Int. J. Radiat. Biol. 58, 397–415.
[30] Alam, Z.I., Jenner, A., Daniel, S.E., Lees, A.J., Cairns, N., Marsden, C.D., Jenner, P. and Halliwell, B. (1997) J. Neurochem. 69, 1196–1203.
[31] Nourooz-Zadeh, J., Rahimi, A., Tajaddini-Sarmadi, J., Tritschler, H., Rosen, P., Halliwell, B. and Betteridge, D.J. (1997) Diabetologia 40, 647–653.
[32] Grootveld, M. and Halliwell, B. (1986) Biochem. J. 237, 499–504.
[33] Powell, S.R. (1994) Free Radic. Res. 21, 355–370.
[34] Ghiselli, A., Laurenti, O., De Mattia, G., Maiani, G. and Ferro-Luzzi, A. (1992) Free Radic. Biol. Med. 13, 621–626.