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Short communication

Oxidative DNA damage in diabetes mellitus: its association with diabetic complications

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Abstract

Aims/hypothesis. Augmented oxidative stress induced by hyperglycaemia possibly contributes to the pathogenesis of diabetic complications. Oxidative stress is known to increase the conversion of deoxyguanosine to 8-oxo, 2′-deoxyguanosine in DNA. To investigate the possible contribution of oxidative DNA damage to the pathogenesis of diabetic complications, we measured the content of 8-oxo, 2′-deoxyguanosine in the urine and the blood mononuclear cells of Type II (non-insulin-dependent) diabetic patients.
Methods. We studied 53 Type II diabetic patients and 39 age-matched healthy control subjects. We assayed 8-oxo, 2′-deoxyguanosine by HPLC-electrochemical detection method.
Results. The content of 8-oxo, 2′-deoxyguanosine in the urine and the mononuclear cells of the Type II diabetic patients was much higher than that of the control subjects. Urinary 8-oxo, 2′-deoxyguanosine excretion and the 8-oxo, 2′-deoxyguanosine content in the mononuclear cells from the diabetic patients with complications were higher than those from the diabetic patients without complications. Urinary excretion of 8-oxo, 2′-deoxyguanosine was significantly correlated with the 8-oxo, 2′-deoxyguanosine content in the mononuclear cells. The 8-oxo, 2′-deoxyguanosine content in the urine and mononuclear cells was correlated with the haemoglobin A_{1c} value.
Conclusion/interpretation. This is the first report of a direct association between oxidative DNA damage and the complications of diabetes. The augmented oxidative DNA damage in diabetes is speculated to contribute to the pathogenesis of diabetic complications. [Diabetologia (1999) 42: 995–998]

Keywords Oxidative stress, 8-oxo-2′-deoxyguanosine, diabetic complication, smoking.

Hyperglycaemia, a key clinical manifestation of diabetes mellitus, not only generates reactive oxygen species (ROS), but also attenuates anti-oxidative mechanisms by scavenging enzymes and antioxidant substances [1]. As ROS cause strand breaks in DNA and base modifications including the oxidation of guanine residues to 8-oxo, 2′-deoxyguanosine (8-oxodG), 8-oxodG can serve as a sensitive biomarker of oxidative DNA damage [2]. 8-OxodG was increased in the kidneys of diabetic rats, and insulin treatment reduced both urinary albumin excretion and 8-oxodG formation in the kidney [3]. A recent study reported an increase in the 8-oxodG content in mononuclear cells and ROS level in Type I (insulin-dependent) and Type II (non-insulin-dependent) diabetic patients when compared with control subjects [4]. Another study reported that urinary 8-oxodG excretion was higher in Type II diabetic patients than in the control subjects [5]. Urinary 8-oxodG excretion correlated with glycated haemoglobin [5]. We speculated that diabetes-associated modifications of DNA by ROS might contribute to the diabetic complications.

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Abbreviations: 8-oxodG, 8-Oxo, 2′-deoxyguanosine; ROS, reactive oxygen species.

Table 1. The clinical characteristics of the non-obese Type II diabetic patients and the control subjects

Group	Control subjects	Type II diabetic patients	
		without complications	with complications
n	39	21	32
Sex (f/m)	21/18	10/11	16/16
Smoking (non-smoker/smoker)	29/10	14/7	20/12
Age (years)	57.7 ± 6.1	58.1 ± 6.5	58.9 ± 7.8
Duration of diabetes (years)	–	10.16 ± 7.21	11.55 ± 8.29
Treatment (diet/SU/insulin)	–	6/7/8	9/9/14
Average of HbA_{1c} for past 2 years	–	7.08 ± 0.61	7.41 ± 0.83
Nephropathy (norm/micro/macro)	–	21/0/0/0	0/14/18
Retinopathy (NDR/SDR/PDR)	–	21/0/0	0/25/7

Abbreviations used are: SU, Sulphonylurea; norm, normoalbuminuria; micro, microalbuminuria; macro, macroalbuminuria; PDR, diabetic proliferative retinopathy; SDR, diabetic simple retinopathy, and NDR; without diabetic retinopathy. Mean ± SD

In this study, we investigate the oxidative DNA damage in Type II diabetic patients by measuring the 8-oxodG in the urine and mononuclear cells. We discuss the significance of oxidative DNA damage in diabetic complications.

Subjects and methods

Protocol. The Tohoku University Institutional Review Board approved the study protocol. We studied 53 Type II diabetic patients and 39 age-matched healthy control subjects. Informed consent was obtained from each subject. The clinical characteristics of the Type II diabetic patients and the control subjects are shown in Table 1.

Measurement of 8-oxodG in the urine and mononuclear cells. The urinary 8-oxodG samples were prepared by the method of Loft et al. [2]. The mononuclear cells were separated in Mono-poly resolving medium (Dainippon Seiyaku, Osaka, Japan). DNA was extracted from mononuclear cells using WB DNA extractor kits (Wako Chemical, Tokyo, Japan) [6]. This chaotrophic DNA isolation method was reported to produce the lowest and least variable 8-oxodG value [7]. After DNA digestion, the samples were analysed by HPLC-ECD [8].

Statistical analysis. Statistical difference was assessed by repeated measure ANOVA. Wilcoxon's test was used for paired comparison, and the Mann-Whitney U-test for unpaired comparison.

Results

We found that 24-h urinary excretion of 8-oxodG was different according to gender, presence or absence of diabetes and smoking status (Fig. 1A). In the non-smoking, non-diabetic subjects, men had a considerably higher urinary 8-oxodG excretion than women (218 ± 81 vs 161 ± 56 pmol · kg^{-1} · day^{-1}, $p < 0.05$). The smokers had a significantly higher urinary 8-oxodG excretion than the non-smokers (260 ± 95 vs 161 ± 56 pmol · kg^{-1} · day^{-1}, $p < 0.02$, in female control subjects; 363 ± 195 vs 218 ± 81, $p < 0.05$, in male control subjects; 588 ± 250 vs 352 ± 183, $p < 0.02$, in female diabetic subjects; 785 ± 338 vs 486 ± 201, $p < 0.05$, in male diabetic subjects). Urinary 8-oxodG excretion was significantly higher in the diabetic patients than in the control subjects (352 ± 183 vs 161 ± 56 pmol · kg^{-1} · day^{-1}, $p < 0.001$, in female non-smokers; 486 ± 201 vs 218 ± 81, $p < 0.001$, in male non-smokers; 588 ± 250 vs 260 ± 95, $p < 0.05$, in female smokers; 785 ± 338 vs 363 ± 194, $p < 0.02$, in male smokers). We also noted that 24-h urinary 8-oxodG excretion in non-smoking male diabetic patients with complications was significantly higher than in the non-smoking male diabetic subjects without complications (573 ± 237 vs 361 ± 122, $p < 0.05$) (Fig. 1A).

The diabetic patients had a significantly higher content of 8-oxodG in the mononuclear cells than the non-smokers (78.7 ± 43.1 vs 17.9 ± 11.2 fmol/μg DNA, $p < 0.0001$, in female non-smokers; 84.0 ± 31.4 vs 23.7 ± 11.2, $p < 0.0001$, in male non-smokers; 82.1 ± 42.2 vs 23.7 ± 9.8, $p < 0.05$, in female smokers; 96.6 ± 47.8 vs 26.1 ± 13.6, $p < 0.02$, in male smokers, Fig. 1B). There was no significant difference in the 8-oxodG content in mononuclear cells according to gender or smoking habits. The non-smoking female patients with complications had higher 8-oxodG content in the mononuclear cells than the patients without complications (92.1 ± 31.6 vs 60.3 ± 22.4, $p < 0.05$) (Fig. 1B). The 8-oxodG content in the mononuclear cells from non-smoking male diabetic patients with complications (94.5 ± 30.2) were significantly higher than those from similar patients but without complications (62.2 ± 21.7, $p < 0.05$).

We confirmed that there was a significant correlation between urinary 8-oxodG excretion and the mononuclear cell content ($r = 0.855$, $p < 0.0001$, data not shown). There was a significant correlation between the duration of diabetes and urinary 8-oxodG excretion ($r = 0.684$, $p < 0.002$) as well as the 8-oxodG content in mononuclear cells ($r = 0.718$, $p < 0.001$) in the non-smoking subjects. There was a significant correlation between HbA_{1c} and urinary 8-oxodG excretion ($r = 0.783$, $p < 0.0001$). We also found signifi-

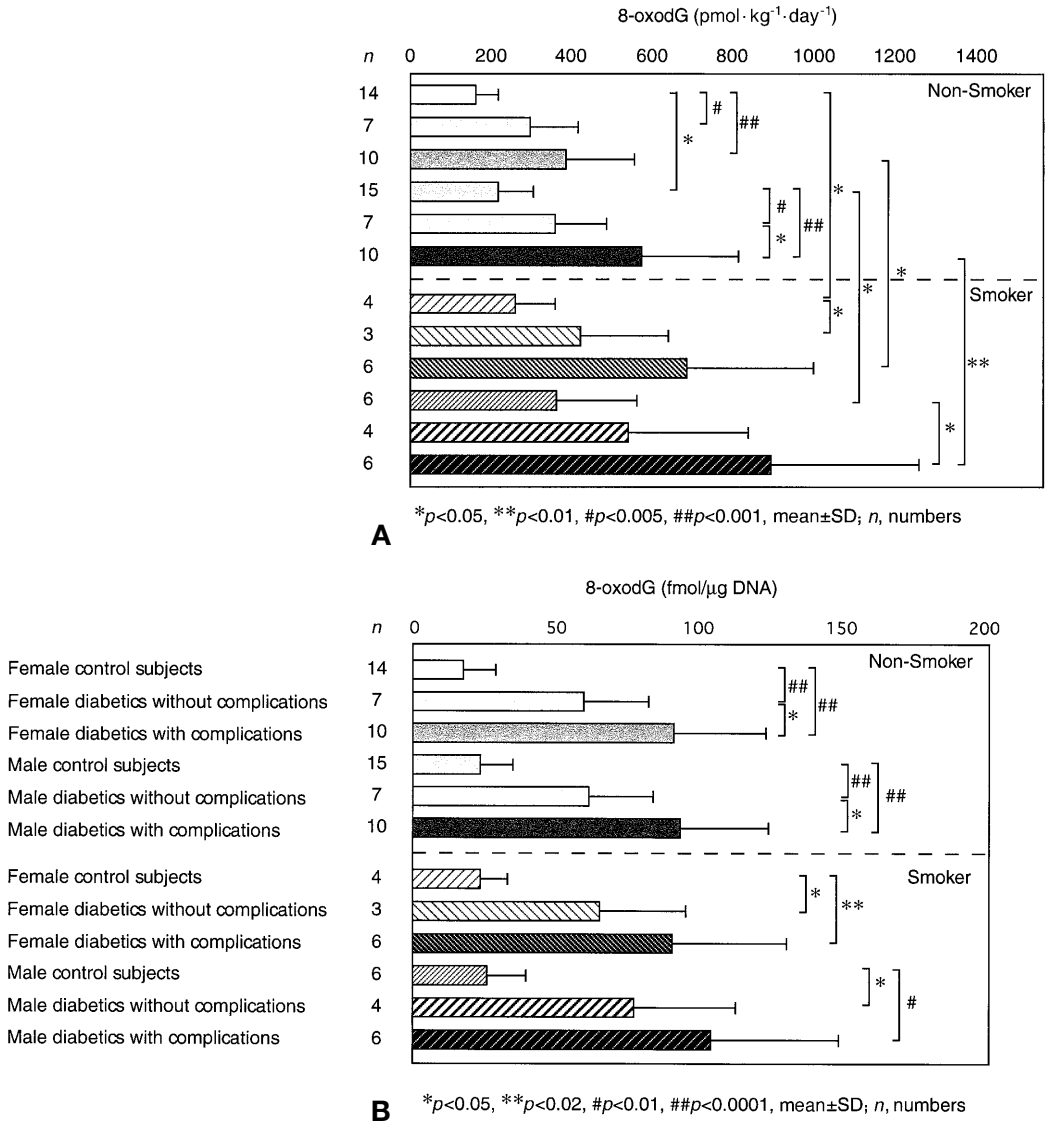

Fig. 1. **A** 24-h urinary 8-oxodG excretion in the Type II diabetic patients and the control subjects. **B** The content of 8-oxodG in the mononuclear cells of the Type II diabetic patients and the control subjects

cant correlation between HbA_{1c} and 8-oxodG content in the mononuclear cells ($r = 0.796$, $p < 0.0001$).

Discussion

The present study shows increased oxidative DNA damage in diabetic patients compared with control subjects. Several reports have shown that diabetes increases the oxidative damage to DNA [3, 4]. We confirmed a significant positive correlation between urinary 8-oxodG excretion and the content of 8-oxodG in mononuclear cells. These data suggest that urinary 8-oxodG excretion possibly is a useful marker of oxidative DNA damage in diabetic patients. An association between poor glycaemic control in Type I and Type II diabetic patients and oxidative stress has been established [1]. It has been reported that poor glycaemic control was associated with high urinary 8-oxodG excretion [5]. This study indicates an association between HbA_{1c} and urinary 8-oxodG excretion as well as the 8-oxodG concentrations in mononuclear cells. Hyperglycaemia might lead to increased 8-oxodG in urine and mononuclear cells through the overproduction of ROS. We found increased oxidative DNA damage in diabetic patients with complications compared with the diabetic subjects without complications. These findings provide evidence that the increased oxidative stress in diabetes might contribute to the pathogenesis of diabetic complications. Other investigators reported that insulin treatment reduced both urinary albumin excretion and papillary 8-oxodG formation in the kidneys of streptozotocin-diabetic rats [3].

Smoking is known to increase the formation of 8-oxodG in vivo [9]. Our study showed increased con-

centrations of 8-oxodG in urine but not in mononuclear cells of smoking subjects. Smoking has been identified as a risk factor for diabetic nephropathy, retinopathy, neuropathy and cardiovascular disease [10]. Thus, we speculate that the increased oxidative stress caused by smoking in diabetes might contribute to the pathogenesis of diabetic complications.

This is the first report of a direct association between oxidative DNA damage and the complications of diabetes. We established that 8-oxodG in urine and mononuclear cells serves as a useful biomarker for the evaluation of oxidative stress in diabetic patients.

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