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Lipid peroxidation in early type 1 diabetes mellitus is unassociated with oxidative damage to DNA

Robert D. Hoeldtke[a,*], Kimberly D. Bryner[a], Linda L. Corum[a], Gerald R. Hobbs[b], Knox Van Dyke[c]

[a]Department of Medicine, West Virginia University Morgantown, Morgantown WV, USA
[b]Department of Community Medicine and Statistics, West Virginia University Morgantown, Morgantown WV, USA
[c]Department of Pharmacology, West Virginia University Morgantown, Morgantown WV, USA

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Abstract

Oxidative stress damages DNA in experimental diabetes, and in vitro studies have suggested that it is linked to lipid peroxidation. The objective of the study was to determine whether lipid peroxidation, as assessed with malondialdehyde excretion in recent-onset type 1 diabetes mellitus, is associated with oxidative damage to DNA, as assessed from 8-hydroxydeoxyguanosine excretion. A 3-year longitudinal study of recent-onset type 1 diabetes mellitus was performed. Age- and sex-matched control subjects were studied once. Patients were studied as inpatients at West Virginia University Hospitals. Thirty-seven patients with recent-onset (2-22 months) type 1 diabetes mellitus (male ,10; female, 27) were enrolled in a longitudinal study of oxidative stress. The mean age of the patients was 20 years. None of the patients had hyperlipidemia or were treated with lipid-lowering drugs. Only 1 patient had hypertension and was being treated with β-adrenergic blocking therapy. Thirty-six patients completed the study; one withdrew after the second evaluation. Lipid peroxidation was assessed by measuring malondialdehyde excretion. Oxidative damage to DNA was assessed from 8-hydroxydeoxyguanosine excretion. Malondialdehyde excretion was increased in the diabetic patients at the first evaluation (2.43 ± 0.31 μmol/g creatinine), second evaluation (2.34 ± 0.24), and third evaluation (1.93 ± 0.15) compared with control subjects (1.51 ± 0.11) ($P < .005$). 8-Hydroxydeoxyguanosine excretion, however, was not increased in the diabetic patients. There was no correlation between malondialdehyde and 8-hydroxydeoxyguanosine excretion. We confirmed the presence of oxidative stress in early diabetes as assessed from malondialdehyde excretion. We were unable, however, to confirm oxidative damage to DNA in this cohort of patients; and there was no evidence of a correlation between lipid peroxidation and DNA damage.

1. Introduction

Oxidative damage to DNA, as reflected by the formation of 8-hydroxydeoxyguanosine (8-OHdG), has been documented in experimental diabetes [1] and several clinical studies [2,3]. In animal models, oxidative damage to DNA leads to deletions and gene mutations; and some [4], but not all [5], human studies have indicated that diabetes has similar effects on DNA in white blood cells. Mitochondrial DNA may be particularly susceptible [6]. The biochemical basis for oxidative damage to DNA is poorly understood. Park and Floyd [7] studied calf thymus DNA in vitro and showed that lipid peroxidation, as measured by the formation of thiobarbituric acid reactive substances, correlated with the rate of 8-OHdG formation. An association between lipid peroxidation and oxidative damage to DNA has been confirmed in multiple other studies of bacteria and experimental animals [8]. Malondialdehyde (MDA) is an abundant by-product of lipid peroxidation, and MDA–8-OHdG adducts have been characterized in the urine of diabetic animals and man [9]. For these reasons, it seemed likely that lipid peroxidation is correlated with oxidative damage to DNA in man. The purpose of the present project was to test this

* Corresponding author. Morgantown, WV 26508, USA. Tel.: +1 304 594 2490, +1 304 282 4524 (cell).
E-mail address: hoeldtkerob@comcast.net (R.D. Hoeldtke).

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hypothesis in patients with oxidative stress secondary to type 1 diabetes mellitus. We postulated that MDA excretion and 8-OHdG excretion would be increased in parallel and that correlations between these 2 parameters would be demonstrable.

2. Research designs and methods

2.1. Patients in protocol 1

Thirty-seven patients (male, 10; female, 27) with type 1 diabetes mellitus enrolled 2 to 22 months after diagnosis in a longitudinal study of peripheral nerve function and oxidative stress (Table 1) [10]. Patients with other systemic illnesses or a history of alcohol abuse were excluded. Patients were instructed on home glucose monitoring and taught to adjust their insulin doses so as to maintain optimal glycemic control. Twenty of the 37 patients maintained their hemoglobin A_1 (HbA_1) within the guidelines recommended by the American Diabetes Association (less than 1% higher than the upper limit of normal of the nondiabetic population). Mean HbA_1 concentrations were 8.2% ± 0.32% at year 1, 9.2% ± 0.29% at year 2, and 8.9% ± 0.29% at year 3.

Forty-one nondiabetic age- and sex-matched control subjects were also studied.

2.2. Patients in protocol 2

Fifteen diabetic patients (male, 7; female, 8) with chronic diabetes participated by collecting urine for the measurement of 8-OHdG. None had increased cholesterol or hypertension, or were being treated with cholesterol-lowering or hypertensive drugs. None smoked cigarettes or had renal disease. Patients with microalbuminuria were excluded. Three patients had retinopathy, and 2 had symptoms of neuropathy. The mean duration of diabetes was 9.2 years (range, 5-44 years). Mean concurrent glycosylated hemoglobin (n = 13) was 8.8% (range, 6.4%-12.6%). Ten age- and sex-matched controls also collected urine for the measurement of 8-OHdG.

2.3. Measurement of MDA

The thiobarbituric acid derivative of MDA was formed by heating an acidified aliquot of urine as previously described [10].

Table 1
Clinical characteristics of patients

	Diabetic patients	Healthy control subjects
n (male/female)	37 (10/27)	41 (14/27)
Age (y)	20.3 (10-40)[a]	21.0 (10-42)[b]
Disease duration at initial evaluation (mo)	10.4 (2-22)	

[a] At diagnosis.
[b] At time of study.

Table 2
Excretion of MDA and 8-OHdG in early diabetes

	Control subjects	Diabetic patients' evaluation		
		1st	2nd	3rd
MDA excretion (μmol/24 h)	1.64 ± 0.13	2.56 ± 0.29	2.54 ± 0.21	2.55 ± 0.19[a]
8-OHdG excretion (nmol/24 h)	16.2 ± 1.1	15.6 ± 1.1	15.7 ± 0.86	16.6 ± 1.3
MDA excretion (μmol/g creatinine)	1.51 ± 0.11	2.43 ± 0.31	2.39 ± 0.24	1.93 ± 0.15[a]
8-OHdG excretion (nmol/g creatinine)	13.8 ± 0.61	13.7 ± 0.72	14.0 ± 0.79	12.0 ± 0.72

[a] The MDA excretion was greater in the diabetic patients than in the control subjects; P less than .005.

2.4. Measurement of 8-OHdG

Aliquots of urine were sent to laboratories at ESA, Chelmsford, MA, where 8-OHdG was measured by column-switching high-performance liquid chromatography (HPLC) [11] followed by coulometric analysis [12]. The 8-OHdG was first retained by a C8 column (YMC America, Allentown, PA; 3 μm, 4.6 × 150 mm), then back flushed onto the first of 2 carbon columns with mobile phase A containing 0.1 mol/L lithium acetate, pH 6.4, MeOH 4%. The 8-OHdG was retained by the first carbon column, whereas interferences were removed by the mobile phase. Switching a second valve to change to mobile phase B (0.1 mol/L lithium acetate, pH 3.3, acetonitrile 4.5%) moved the 8-OH along to the second carbon column. Switching the valve for mobile phase C, which is identical to B except for the addition of adenosine, moved the band containing the 8-OHdG to a C18 column (Tosoh Biosciences, Montgomery, PA; C18 ODS 80 TM), where the final separation occurred. The peak was detected using coulometric cells in series where the first cell (40 mV) removed low oxidation potential interferences before the sensor cells (60 and 165 mV) quantitatively measured the 8-OHdG.

3. Results

3.1. Protocol 1

The excretion of MDA was increased in the diabetic patients throughout the study (Table 2). Malondialdehyde excretion correlated with the HbA_1 at the first ($P < .01$, $r = .39$) and second evaluation ($P < .025$, $r = .37$) and with the pooled data for all evaluations ($P < .025$, $r = .34$). Diabetic female subjects had higher MDA excretion than diabetic male subjects at the first and second evaluation ($P < .01$) [10]. 8-Hydroxydeoxyguanosine excretion was not elevated in the diabetic patients. The slight decrease in 8-OHdG and MDA at the third evaluation reflects the increased excretion of creatinine at that evaluation [10]. There was no correlation between MDA excretion and 8-OHdG excretion. Neither parameter correlated with

Table 3
Excretion of 8-OHdG in patients with long-standing diabetes

	Diabetic patients	Control subjects
n (male/female)	15 (7/8)	10 (5/5)
Age (range)	40.4 (21-63)	43.6 (21-66)
8-OHdG excretion (nmol/g creatinine)	12.6 ± 1.3	14.6 ± 1.6

nitrosative stress (the sum of nitrate and nitrite or NOX) or with an alternative index of lipid peroxidation (isoprostaglandin $F_{2\alpha}$).

3.2. Protocol 2

8-Hydroxydeoxyguanosine excretion in the diabetic patients was 12.6 ± 1.3 nmol/g creatinine, which was not significantly different than that in the control subjects who excreted 14.6 ± 1.6 nmol/g creatinine (Table 3). 8-Hydroxydeoxyguanosine excretion in the diabetic patients did not correlate with glycosylated hemoglobin or duration of diabetes.

4. Discussion

Many adverse consequences have been attributed to the oxidative stress associated with chronic hyperglycemia including lipid peroxidation, and the oxidative damage to DNA, as reflected by the presence of increased 8-OHdG [2,3]. Although there was evidence of enhanced lipid peroxidation in the present study, as reflected by the increased MDA excretion, we failed to confirm an increase in 8-OHdG excretion. It is possible that the discrepancy between our results and those previously published stems from the technical difficulties associated with measuring 8-OHdG. Leionen et al [3] measured 8-OHdG with an enzyme-linked immunosorbent assay method and estimated its 24-hour excretion to be 49.6 μg (175 nmol), approximately 10 times greater than what we observed. Other investigators have used HPLC and shown that diabetic patients have increased excretion of 8-OHdG [13,14]. Recent technical reports, however, have challenged the validity of some of the published HPLC separations and electrochemical analyses of 8-OHdG [11,12]. Recent refinements in HPLC-based electrochemical analyses have made it possible to expose the HPLC eluate to a sequential gradient of electrical potentials, which makes it possible to differentiate compounds with identical retention times but different susceptibilities to oxidation. Applying this approach to urine samples has revealed that, when only 1 HPLC column is used, there are multiple compounds with similar retention time to 8-OHdG but different electrical properties. The problem has been resolved by using 3 sequential HPLC columns with different properties as described above ("Research designs and methods"). The purified compound in the final eluate has identical electrical properties to native 8-OHdG. It is therefore possible that the discrepancy between the present results and those previously published reflects the more extensive purification of the 8-OHdG and the elimination of interfering compounds in this study.

An alternative explanation for our results is that the diabetic patients in this study were younger, were healthier, and had diabetes for only a few years. Previous studies have been performed on older patients with chronic disease, multiple cardiovascular risk factors, and complications [2,3,13,14]. The patients in protocol 1, by contrast, had hyperglycemia, but most had normal blood pressure, normal lipids, and no evidence of vascular disease. Hypertension, dyslipidemia, and vascular disease have all been linked to reactive oxygen; so it is frequently difficult to interpret biochemical evidence of oxidative stress in chronic diabetes or assess its relation to hyperglycemia. These problems were not prevalent in our protocol 1 patient population, which thus provides a unique opportunity to study the relationship between oxidative stress and glycemic control.

Protocol 2 was performed to determine whether the 8-OHdG as measured in the column-switching procedure was increased in chronic diabetes in patients similar to those included in previous clinical studies [2,3,13]. We observed that 8-OHdG excretion was not increased in this cohort of diabetic patients (Table 3). We believe, therefore, that the previous reports of increased urinary 8-OHdG in diabetic patients resulted from the use of nonspecific methods for measuring this compound. It is possible, however, that oxidative damage to DNA occurs in highly specialized tissues such as mononuclear cells [2] or the pancreatic islets [15]; but insufficient 8-OHdG is formed to change the urinary excretion of this compound that reflects DNA metabolism in the whole patient.

In summary, young adults with recent-onset type 1 diabetes mellitus have increased lipid peroxidation, as evidenced by increased MDA excretion, but no evidence of oxidative damage to DNA, as assessed from the excretion of 8-OHdG.

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