Role of Peroxynitrite in the Development of Diabetic Peripheral Neuropathy

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Approximately 50% of patients with diabetes (DM) will eventually develop neuropathy. Large-scale studies have demonstrated that the regulation of blood glucose levels aids in the prevention and progression of diabetic peripheral neuropathy (DPN), but glycemic control does not impact established neuropathy or its severity (1). Causative factors also include microvascular insufficiency, defective neurotrophism, abnormal lipid metabolism, and oxidative and nitrosative stress (2). A key component of nitrosative stress, nitrotyrosine (NT), has multiple cytotoxic effects. Accumulation of NT has been identified in several neural structures in both rodents and humans (3), reflected by raised NT concentrations in the plasma of subjects with DM (4).

The aim of this study was to evaluate the relationship between NT concentrations with the presence and severity of possible/probable (PP-DM-DPN) and established (DM-DPN) neuropathy in subjects with DM compared with healthy control subjects (HCs). The study population consisted of 49 patients. Patient demographics are presented in Table 1. Subjects with DM were further stratified based on the presence of neuropathy using the current Toronto Expert Panel guidelines (5). Individuals were assessed using clinical neuropathy scores and nerve conduction studies (NCS) to measure nerve conduction velocities (CV), latencies, and amplitudes of specific nerves.

NT was found in the serum of all patients with significant mean differences in concentrations between all DM groups when compared with HCs; specifically, DM-DPN ($P = 0.006$), PP-DM-DPN ($P = 0.028$), and diabetic patients without DPN (DM–Non-DPN) ($P = 0.046$). Total neuropathy scores (TNS) were significantly higher in the neuropathy groups, as shown in Table 1. Significant differences were found for all three subcategories of the TNS (motor, sensory, and symptom scores) (Table 1). A positive correlation was found between NT concentrations and TNS ($r = 0.676$, $P = 0.022$) and symptom score ($r = 0.673$, $P = 0.026$) in the PP-DM-DPN.

Significant correlations also were found between NT concentrations and peroneal nerve ankle amplitude ($r = -0.408$, $P = 0.018$), above fibula amplitude ($r = -0.375$, $P = 0.035$), and above fibula CV ($r = -0.486$, $P = 0.005$) for the DM groups. Moreover, NT levels correlated significantly with NCS in the DM-DPN for peroneal nerve CV ($r = -0.656$, $P = 0.0005$) and peroneal nerve amplitude ($r = -0.491$, $P = 0.01$).

The findings in this study show that the circulating levels of NT are significantly raised in patients with DM when compared with HCs, with the strongest correlations seen with clinical and objective measures of neuropathy severity in patients with PP-DM-DPN and DM-DPN. This suggests that NT and nitrosative stress could play a role in the development of DPN and could provide a biomarker for DPN progression. These findings concur with previous animal and clinical studies demonstrating that nitrosative stress seems to have the largest impact in patients during the earlier stages of metabolic disease (2,4,6) and seems to be a potential target for intervention. This is a small, cross-sectional observational study, and larger scale trials are needed to determine longitudinal predictive value and the impact of the reduction of NT in progression and severity of neuropathy.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.
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Table 1—Demographic characteristics for study population

	HC ($N = 15$)	DM–Non-DPN ($N = 12$)	PP-DM-DPN ($N = 11$)	DM-DPN ($N = 11$)
Baseline characteristics				
Sex (F/M)	6/9	3/9	2/9	6/5
Age (years)	50.4 ± 2.11	45.5 ± 4.25	55.73 ± 2.07	57.91 ± 1.53#
Duration of DM (years)		7.75 ± 3.34	9.91 ± 3.56	10.91 ± 2.63
BMI (kg/m^2)	27.46 ± 1.12	28.48 ± 1.38	30.42 ± 1.58	32.15 ± 1.83*
Systolic blood pressure (mmHg)	121.6 ± 2.15	116.75 ± 5.68	122.09 ± 6.10	130.82 ± 5.13#
Diastolic blood pressure (mmHg)	75.73 ± 2.56	74.17 ± 3.76	80.0 ± 3.96	78.55 ± 2.60
HDL (mg/dL)	55.93 ± 4.02	60.0 ± 5.61	52.09 ± 5.51	49.0 ± 5.02
LDL (mg/dL)	121.0 ± 7.07	85.81 ± 10.61*	90.64 ± 8.06*	74.36 ± 7.38*
Triglycerides (mg/dL)	87.82 ± 9.16	123.82 ± 29.28	90.40 ± 13.22	110.63 ± 24.00
HbA_{1c} (%)	5.72 ± 0.09	7.23 ± 0.48	9.4 ± 0.77	8.27 ± 0.51
Glucose (mg/dL)	83.17 ± 1.88	124.36 ± 12.17	207.20 ± 25.06*	158.13 ± 22.65*
Insulin (μIU/mL)	5.90 ± 1.20	10.80 ± 2.52	13.71 ± 5.23*	26.76 ± 9.58*
NT concentrations (pmol/mg protein)	3.14 ± 0.32	4.32 ± 0.44*	4.47 ± 0.46*	4.81 ± 0.47*
Clinical neuropathy assessment				
TNS	0.60 ± 1.15	0.56 ± 1.28	5.73 ± 1.34*,#	9.50 ± 1.34*,+,#
Total sensory score	0.07 ± 0.48	0.08 ± 0.53	1.55 ± 0.56*	3.14 ± 0.56*,+,#
Total motor score	0.17 ± 0.32	0.19 ± 0.36	1.45 ± 0.38*,#	2.27 ± 0.38*,#
Total symptom score	0.37 ± 0.46	0.29 ± 0.51	2.73 ± 0.53*,#	4.09 ± 0.53*,#
NCS				
Ulnar motor amplitude	6.11 ± 0.43	5.72 ± 0.75	6.41 ± 0.62	3.61 ± 0.46*,+
Ulnar motor CV	44.52 ± 2.47	51.21 ± 1.92*	50.24 ± 2.07	43.11 ± 2.05*,+
Ulnar sensory amplitude	32.45 ± 5.45	29.08 ± 6.88	29.26 ± 6.33	14.35 ± 2.24*
Ulnar sensory CV	46.56 ± 1.12	49.11 ± 1.32	45.36 ± 1.87	42.8 ± 3.39+
Peroneal ankle amplitude	4.45 ± 0.50	4.91 ± 0.56	3.47 ± 0.58	3.51 ± 0.61
Peroneal below fibula CV	45.71 ± 1.48	45.60 ± 1.66	40.35 ± 1.73*,#	38.17 ± 1.81*,#
Peroneal below fibula amplitude	4.65 ± 0.53	4.48 ± 0.55	3.17 ± 0.57	3.21 ± 0.49
Peroneal above fibula CV	46.15 ± 1.13	48.06 ± 1.99	42.50 ± 3.14	45.41 ± 1.11
Peroneal above fibula amplitude	4.03 ± 0.26	4.54 ± 0.57	3.47 ± 0.52	3.82 ± 0.24

Data are presented as mean ± SEM or *n* (%). Comparisons between groups were analyzed using Kruskal-Wallis one-way ANOVA. Wilcoxon rank sum test was used for less than two groups. *$P < 0.05$ vs. HCs; +$P < 0.05$ vs. PP-DM-DPN; #$P < 0.05$ vs. DM–Non-DPN.

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