Prospective Clinical Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
49(5) 1–13

DOI: 10.1177/03000605211012213
journals.sagepub.com/home/imr
SAGE

Effects of alpha-lipoic acid treatment on serum progranulin levels and inflammatory markers in diabetic neuropathy

Bíborka Nádró[1], Hajnalka Lőrincz[1], Ágnes Molnár[1], Anita Szentpéteri[1], Eszter Zöld[2], Ildikó Seres[1], Dénes Páll[1], György Paragh[1], Péter Kempler[3], Mariann Harangi[1] and Ferenc Sztanek[1]

Abstract

Objectives: Progranulin (PGRN) is a secreted growth factor that helps to regulate neuronal survival by blocking tumor necrosis factor-alpha (TNFα) receptors. The antioxidant alpha-lipoic acid (ALA) is used in diabetic neuropathy to improve nerve conduction and relieve neuropathic pain, but its effects on PGRN levels have not yet been elucidated.

Methods: In this prospective study, 54 patients with type 2 diabetes and peripheral neuropathy received 600 mg of ALA daily for 6 months. Twenty-four patients with diabetes without neuropathy were also included in the study. Serum PGRN and TNFα levels were determined using enzyme-linked immunosorbent assays. In addition, current perception threshold (CPT) testing was used to assess sensory neuropathy.

Results: After ALA treatment, serum PGRN levels were significantly increased and CPT values were significantly improved. Furthermore, there were significant positive correlations among TNFα, ICAM-1, and PGRN levels both before and after ALA treatment. A significant negative correlation was observed between the improvements in CPT and the PGRN levels. Furthermore, ICAM-1 levels were an independent predictor of PGRN levels.

[1]Department of Internal Medicine, University of Debrecen Faculty of Medicine, Debrecen, Hungary
[2]Department of Ophthalmology, University of Debrecen Faculty of Medicine, Debrecen, Hungary
[3]First Department of Internal Medicine, Semmelweis University Faculty of Medicine, Budapest, Hungary

Corresponding author:
Mariann Harangi, Department of Internal Medicine, University of Debrecen Faculty of Medicine, Nagyerdei krt. 98., Debrecen H-4032, Hungary.
Email: harangi@belklinika.com

Conclusions: Changes in serum PGRN levels indicate that ALA treatment may have beneficial effects on endothelial function and neuronal inflammation.

Keywords
Diabetic neuropathy, cardiac autonomic neuropathy, progranulin, intercellular adhesion molecule-1, alpha-lipoic acid, tumor necrosis factor-alpha, type 2 diabetes

Date received: 8 December 2020; accepted: 25 March 2021

Introduction

Diabetic neuropathy is a common and progressive microvascular complication in type 2 diabetes that affects the motor, sensory, or autonomic nerves. The pathological mechanisms of diabetic neuropathy are generally accepted to be multifactorial, although the exact cellular mechanisms are not yet fully understood.[1] The main components are likely to be the hyperglycemia-induced activation of alternative metabolic pathways (including increased polyol and hexosamine pathways), protein kinase C activation, mitochondrial dysfunction, chronic inflammation, formation of reactive oxygen species, and inefficient generation of endothelial nitric oxide leading to endothelial dysfunction.[2] In the early stages of diabetic sensorimotor peripheral neuropathy, the degeneration of small nerve fibers is closely associated with endoneurial microangiopathy.[3] Excessive oxidative stress, activation of alternative metabolic pathways, and inadequate antioxidant protection appear to contribute to endothelial dysfunction and changes in endoneurial blood flow with subsequent hypoxia, thus causing further nerve damage during the progression of diabetic neuropathy.[4] Although diabetic neuropathy has been traditionally considered a manifestation of diabetes-associated microangiopathy, insights into the underlying cellular abnormalities that occur in both endothelial cells and neurons show similarities, suggesting that concomitant mechanisms may be involved.[5,6]

Progranulin (PGRN) is a cysteine-rich glycosylated protein that was initially identified as a growth factor. It is involved in numerous physiological and pathological processes, such as inflammation, neuronal cell growth, tumorigenesis, and wound healing.[7,8] Full-length PGRN and granulin peptides seem to have opposite effects on inflammation:[9] granulin peptides stimulate epithelial cells to secrete proinflammatory cytokines, whereas PGRN inhibits the inflammatory response.[10,11] PGRN might inhibit neutrophil activation by binding directly to tumor necrosis factor receptor (TNFR) and disturbing the interaction between tumor necrosis factor-alpha (TNFα) and TNFR.[10,12] Thus, PGRN may inhibit TNFα-induced activation of the nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathway.[13]

Alpha-lipoic acid (ALA) is an essential cofactor for mitochondrial oxidative metabolism, and has beneficial effects on diabetic neuropathy. Because of its properties as a potent antioxidant, ALA may improve nerve conduction velocity and protect the peripheral nerves from hyperglycemia-induced oxidative stress by enhancing cellular glutathione synthesis.[14]

ALA functions as an inhibitor of advanced glycation end products that might decrease the activity of NF-κB as well as the expression of proinflammatory cytokines, including interleukin-1, interleukin-6, and TNFα.[3,15] However, the effects of ALA on serum PGRN and TNFα levels, and their correlations with the severity of diabetic neuropathy, are not yet completely understood.

The aim of our study was to assess the relationship between inflammation and endothelial dysfunction markers and PGRN levels in patients with type 2 diabetes with peripheral neuropathy after 6 months of ALA treatment. Moreover, we explored the association between changes in PGRN levels and the severity of peripheral sensory neuropathy after ALA treatment.

Materials and methods

Study population

This prospective study was performed in patients with type 2 diabetes with neuropathy as well as in age- and sex-matched control subjects who had diabetes without neuropathy. All subjects underwent a detailed neurological assessment to identify patients with diabetic neuropathy. Distal sensorimotor polyneuropathy was diagnosed by the presence of neuropathic symptoms and by vibration perception thresholds on the hallux of both feet using a 128-Hz tuning fork. Detection of neuropathic pain was performed according to the DN4 (Douleur Neuropathique en 4 Questions) questionnaire assessing numbness, pain, hypoesthesia, and tingling sensations. The Semmes–Weinstein monofilament test was used to identify sensory changes in protective sensations of the feet.[16] All patients were treated for 6 months with 600 mg ALA (WÖRWAG Pharma GmBH, Böblingen, Germany) administered daily by the oral route. In addition, all patients were adequately controlled with oral antidiabetic drugs (metformin, sulfonylurea, and/or dipeptidyl peptidase-4 inhibitors); subjects on insulin therapy were excluded. Patients with a previous history of diabetic proliferative retinopathy, diabetic nephropathy (estimated glomerular filtration rate < 60 mL/minute/1.73 m^2 and/or persistent albuminuria), or type 1 diabetes were not included in the study.

We excluded subjects with alcoholism, known liver diseases, autoimmune and endocrine diseases, or neurological and hematological disorders that may be associated with peripheral polyneuropathy. Subjects with prior cardiovascular disease, established coronary artery disease or myocardial infarction, or severe congestive heart failure (New York Heart Association class III–IV) were not included in the study, and smokers, pregnant women, and subjects with established malignancies were also excluded. All patients were recruited from the Diabetic Neuropathy Center of Debrecen, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary. All participants provided written informed consent. The study protocol was approved by the Regional and Institutional Ethics Committee, University of Debrecen, Clinical Center (UDCC REC/IEC; 4775-2017) and by the Medical Research Council of Hungary, National Scientific and Ethical Committee (5287-2/2019/EÜIG). We followed the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) Statement guidelines for reporting observational studies. The study was performed in accordance with the Declaration of Helsinki.

Blood sampling

Venous blood samples were drawn after overnight fasting and sera were prepared immediately. Routine laboratory

investigations (triglycerides, total cholesterol, low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C], creatinine, uric acid, glucose, and hemoglobin A1c [HbA1c]) were performed with fresh sera using the Cobas c501 autoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany) in the Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary. Non-HDL-C was calculated as the total cholesterol minus HDL-C. The sera for enzyme activity measurements and for enzyme-linked immunosorbent assay (ELISA) determinations were stored at −70°C until analysis. Reagents were purchased from Roche Diagnostics GmbH and tests were performed according to the manufacturer's recommendations.

PGRN measurement

Serum PGRN levels were measured using a commercially available competitive ELISA kit (BioVendor, Brno, Czech Republic) with intra-assay coefficients of variability (CVs) ranging from 3.4% to 4.4% and inter-assay CVs ranging from 6.4% to 7.9%. Measurements of PGRN levels in sera were performed according to the manufacturer's instructions. Values were expressed as ng/mL.

TNFα measurement

Serum levels of TNFα were assessed using a TNFα ELISA kit (R&D Systems Europe Ltd., Abington, UK). Measurements of TNFα levels in the sera were performed according to the manufacturer's recommendations. The intra-assay CVs ranged from 1.9% to 2.2% and the inter-assay CVs ranged from 6.2% to 6.7%. Values were expressed as pg/mL.

Oxidized low-density lipoprotein (oxLDL) measurement

Serum concentrations of oxLDL were detected using a commercial sandwich ELISA kit (Mercodia AB, Uppsala, Sweden). The kit was based on the direct sandwich technique, in which two monoclonal antibodies are directed against separate antigenic determinants on the oxidized apolipoprotein B molecule. The intra- and inter-assay CVs ranged from 5.5% to 7.3% and 4% to 6.2%, respectively. The sensitivity was < 1 mU/L.

Intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) measurement

The ICAM-1 and VCAM-1 levels were measured using human soluble ICAM-1 and VCAM-1 sandwich ELISA kits (R&D Systems Europe Ltd.). ELISA procedures were performed according to the manufacturer's instructions. The intra- and inter-assay CVs ranged from 3.7% to 5.2 % and 4.4% to 6.7%, respectively, for ICAM-1, and from 2.3% to 3.6% and 5.5% to 7.8%, respectively, for VCAM-1. Values were expressed as ng/mL.

Assessment of autonomic and peripheral nerve function

All participants underwent detailed assessments of peripheral neuropathy (DN4 questionnaire to screen for neuropathic pain syndrome, vibration perception threshold, and quantitative sensory testing) and *in vivo* corneal confocal microscopy by an ophthalmologist for the diagnosis of diabetic sensorimotor polyneuropathy. Peripheral sensory nerve function was assessed by current perception threshold testing (CPT) using a Neurometer® (Neurotron Inc., Baltimore, MD, USA). It has been previously reported that this neurodiagnostic

device is capable of detecting peripheral sensory neuropathy in various diseases, including diabetes mellitus.[17] Neurometer® CPT testing involved the delivery of sinusoidal alternating current stimuli at three different frequencies: 5 Hz, 250 Hz, and 2000 Hz, to assess the function of small unmyelinated C-fibers, small myelinated Aß fibers, and large myelinated Aß fibers, respectively. An intensity alignment was conducted to approach the sensory threshold with a $\pm 50\,\mu A$ range, out of a total range of 0 to 9.99 mA.[18,19] Current stimuli were applied to the dorsal surfaces of the distal phalanges of the index finger and hallux unilaterally via two small electrodes. The intensity was increased until the participants experienced a painless sensation. Neurometer® CPT testing automatically adjusts the level of stimulation based on the patient's response. Participants were presented with five to seven randomly generated sets of stimuli above and below their level of perception, and were asked to choose which of the two stimuli felt stronger using an automated forced choice protocol. A CPT value (mA) based on the minimal current perceived was calculated once a sufficient number of correct consecutive responses had been obtained.

Autonomic function was assessed using Ewing's five standard cardiovascular reflex tests: changes in heart rate during deep inspiration and expiration, heart rate responses to standing up (30/15 ratio), the Valsalva maneuver, systolic blood pressure fluctuation to standing up, and changes in diastolic pressure during a sustained handgrip.[20] A score was created to express the severity of autonomic neuropathy based on the results of the five tests (for each test, normal: 0, borderline: 1, abnormal: 2). The composite autonomic score (CAS) ranged from 0 to 10. A CAS of 0 to 1 was taken as normal, 2 to 3 as mild autonomic dysfunction, 4 to 6 as moderate autonomic dysfunction, and 7 to 10 as severe autonomic dysfunction.

Statistical analysis

Statistical analyses were performed using Statistica® 13.5.0.17 software (TIBCO Software Inc., Palo Alto, CA, USA). Normality of distribution was tested using the Kolmogorov–Smirnov test. For data with normal distributions, the differences between anthropometry and laboratory parameters in controls and patients before ALA were analyzed using the unpaired *t*-test. Data are expressed as the mean ± standard deviation. For data with non-normal distributions, differences were analyzed using the Mann–Whitney *U*-test. These data are presented as the median (interquartile range). Differences before and after ALA treatment were determined using the paired *t*-test (for data with normal distributions) or the Wilcoxon matched pairs test (for data with non-normal distributions). The Pearson correlation coefficient was used to investigate the relationship between variables. A multiple regression analysis (backward stepwise method) was performed to determine the best independent predictor of PGRN. Values of $p < 0.05$ were considered to be statistically significant.

Results

Clinical and laboratory data from patients with diabetes with and without neuropathy

Fifty-four patients with type 2 diabetes with neuropathy (22 men and 32 women, mean age: 64.1 ± 8.7 years; mean known type 2 diabetes duration before the initiation of our study: 12.4 years [interquartile range: 4.1–14.7 years], and duration of diabetic neuropathy: 3.2 ± 1.4 years) were enrolled in the study. In addition, 24 age- and sex-matched

control subjects, who had diabetes without neuropathy, were also enrolled in the study (mean known type 2 diabetes duration before the initiation of our study: 12.1 years [interquartile range: 4.0–14.6 years]. The clinical and laboratory characteristics of the patients with diabetes with and without neuropathy are summarized in Table 1. There were no significant differences in age, body mass index, waist circumference, or levels of glucose, creatinine, uric acid, HbA1c, ICAM-1, PGRN, or oxLDL and other lipid parameters between patients with and without neuropathy. TNFα levels were significantly higher in patients with neuropathy compared with controls.

Table 1. Clinical and laboratory characteristics of patients

	Patients with diabetes with neuropathy before ALA treatment	Patients with diabetes with neuropathy after ALA treatment	Control patients with diabetes without neuropathy	*p*-value
Number of patients (male/female)	54 (22/32)		24 (11/13)	
Age of patients (years)	64.15±8.66		63.58 ± 5.12	n.s.
BMI (kg/m^2)	30.02±3.29	29.95 ±3.73	29.50 ± 2.86	n.s.
Waist circumference (cm)	102.3±12.7	102.4 ±13.2	101.1 ± 10.4	n.s.
hsCRP (mg/L)	2.1 (0.8–3.6)	2.8 (0.75–5.15)	1.25 (0.9–2.25)	n.s.
Glucose (mmol/L)	7.34 ±2.18	7.51 ±2.60	7.44 ± 1.36	n.s.
Creatinine (μmol/L)	72.61 ±16.97	74.75 ±14.65	75.17 ± 20.97	n.s.
Uric acid (μmol/L)	296.51 ±76.44	304.33 ±77.69	316.13 ± 57.37	n.s.
Total cholesterol (mmol/L)	4.84 ±1.16	4.76 ±1.24	4.90 ± 1.17	n.s.
HDL-C (mmol/L)	1.38 ±0.37	1.38 ±0.44	1.26 ± 0.33	n.s.
LDL-C (mmol/L)	2.98 ±0.97	2.87 ±1.16	2.84 ± 1.07	n.s.
Non-HDL-C (mmol/L)	3.47 ±1.08	3.38 ±1.19	3.63 ± 1.19	n.s.
HbA1C (%)	6.94 ±0.93	6.84 ±1.04	6.78 ± 0.75	n.s.
Progranulin (ng/mL)	**34.89±7.13**	**36.23±7.93**	**33.13 ± 7.35**	$p < 0.05$[a]
VCAM-1 (ng/mL)	820 (660–992)	836.3 (674.3–929.6)	729.2 (653.8–847)	n.s.
ICAM-1 (ng/mL)	210.8 (184.4–247.3)	216.8 (194.4–253.1)	213.3 (189.4–239.4)	n.s.
oxLDL (U/L)	63.6 (50.7–91.1)	63.36 (45.59–89.77)	70.76 (59.18–99.46)	n.s.
TNFα (pg/mL)	1.18 ±0.36	1.05 ±0.50	0.75 ± 0.29	$p = 0.003$[a], $p < 0.001$[b]
Current perception threshold (by Neurometer, mA)	0.473 ±0.171	0.409 ±0.154	0.375 ± 0.124	$p < 0.05$[a], $p < 0.05$[b]
CAS	2.67 ±1.05	1.56 ±1.24	1.13 ± 0.77	$p < 0.01$[a], $p < 0.01$[b]

[a]$p < 0.05$ between patients with diabetes with neuropathy before and after ALA treatment (Student's paired *t*-test or Wilcoxon matched pairs test).

[b]$p < 0.05$ between patients with diabetes with neuropathy before treatment and controls (Student's unpaired *t*-test or Mann–Whitney *U*-test).

Unless otherwise stated, all values are presented as the mean ± standard deviation or as the median (interquartile range).
ALA, alpha-lipoic acid; BMI, body mass index; CAS, composite autonomic score; HbA1C, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; ICAM-1, intercellular cell adhesion molecule 1; LDL-C, low-density lipoprotein cholesterol; non-HDL-C, total cholesterol minus HDL-C, n.s., non-significant; oxLDL, oxidized low-density lipoprotein; TNFα, tumor necrosis factor-alpha; VCAM-1, vascular cell adhesion molecule 1.

Effects of ALA treatment in patients with diabetes with neuropathy

Patients with diabetes with neuropathy were treated with oral ALA daily for 6 months. TNFα levels were significantly lower after ALA treatment ($p = 0.003$). In addition, significant improvements in CPT and CAS were observed after ALA treatment ($p < 0.05$ and $p < 0.01$, respectively). The levels of PGRN were significantly higher after ALA treatment (before ALA: 34.89 ± 7.13 ng/mL vs. after ALA: 36.23 ± 7.93 ng/mL; $p < 0.05$) (Table 1 and Figure 1).

There was a significant negative correlation between PGRN and HDL-C levels ($r = -0.38$, $p = 0.005$) and a significant positive correlation between PGRN and non-HDL levels ($r = 0.29$, $p < 0.05$) before ALA treatment (data not shown).

Significant positive correlations were demonstrated between PGRN levels and ICAM-1 ($r = 0.45$, $p = 0.001$), oxLDL ($r = 0.36$; $p = 0.009$), and TNFα ($r = 0.37$, $p = 0.007$) levels in patients with diabetic neuropathy before ALA treatment (Figure 2). There were also significant positive correlations between PGRN levels and ICAM-1 ($r = 0.49$; $p < 0.001$), VCAM-1 ($r = 0.27$; $p = 0.05$), and TNFα ($r = 0.29$; $p = 0.038$) levels after ALA treatment (Figure 3). Moreover, a significant negative correlation was revealed between the changes in CPT and PGRN levels ($r = -0.31$; $p = 0.037$) (Figure 4).

Multiple regression analysis with PGRN levels as the dependent variable

To test whether the associations detected in the univariate analyses were independent of other inflammatory parameters, we performed a multiple regression analysis using PGRN levels as the dependent variable. The model included ICAM-1, VCAM-1, oxLDL, and TNFα levels. Using the backward stepwise analysis, PGRN levels were predicted by serum ICAM-1 levels both before (beta = 0.439; $p < 0.001$) and after (beta = 0.488; $p < 0.001$) ALA treatment.

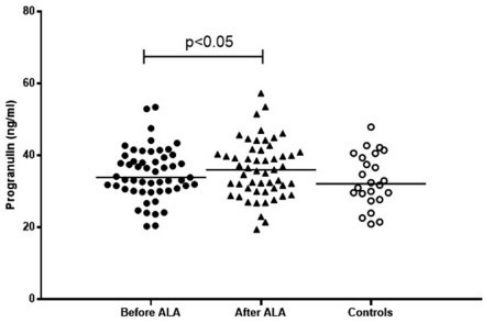

Figure 1. Serum concentrations of progranulin in patients with type 2 diabetes with neuropathy before and after 6 months of 600 mg/day alpha-lipoic acid (ALA) treatment (before ALA: 34.89 ± 7.13 ng/mL; after ALA: 36.23 ± 7.93 ng/mL; $p < 0.05$) and in diabetic controls without neuropathy (33.13 ± 7.35 ng/mL)

Discussion

Progranulin is expressed not only in myeloid- and lymphoid-derived cell lines, but also in neurons and microglia.[21] Moreover, dedifferentiated Schwann cells, which are the primary glial cells in the peripheral nervous system, also express and secrete PGRN. This PGRN functions as a paracrine factor to promote the survival and axonal growth of neighboring neurons after injury.[22] PGRN has been hypothesized to directly bind to TNFR and suppress TNFα-mediated inflammation. PGRN is reportedly a TNFα antagonist and, based on cell-free binding studies, PGRN has a relatively high affinity for the receptors TNFR1 and TNFR2, which even exceeds the physiological effects of TNFα.[12] However, it must be noted that other research groups have failed to support a

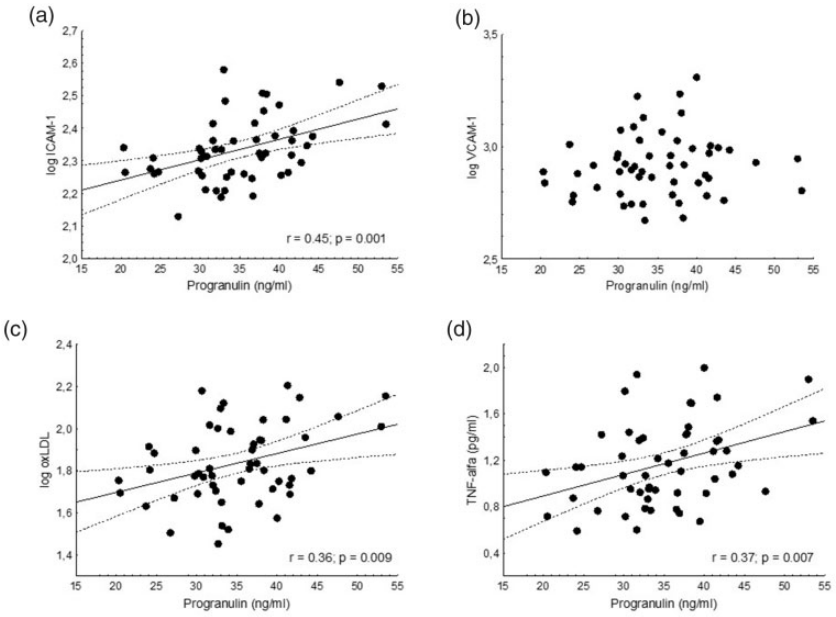

Figure 2. Correlations between serum levels of progranulin and (a) intercellular adhesion molecule-1 (ICAM-1), (b) vascular adhesion molecule-1 (VCAM-I), (c) oxidized LDL (oxLDL), and (d) tumor necrosis factor-alpha (TNFα) in patients with type 2 diabetes with neuropathy before 6 months of 600 mg/day alpha-lipoic acid treatment

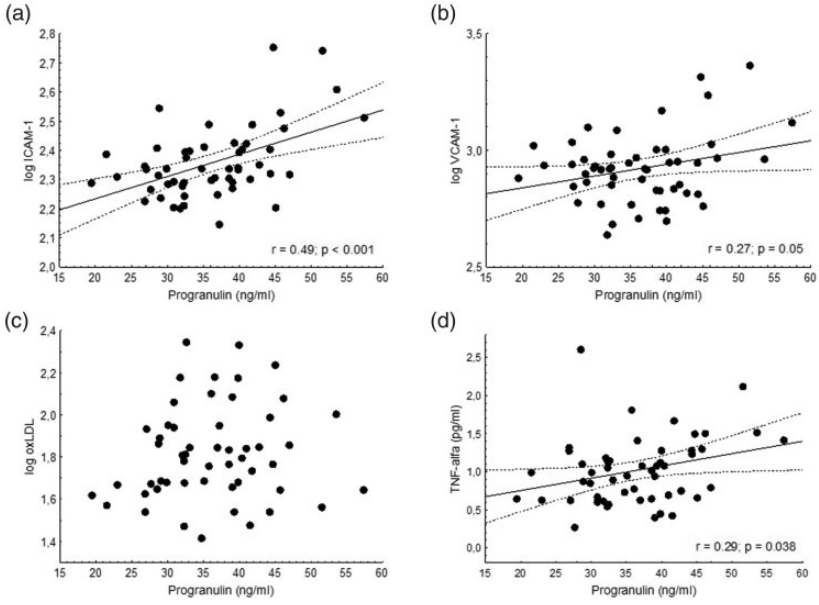

Figure 3. Correlations between serum levels of progranulin and (a) intercellular adhesion molecule-1 (ICAM-1), (b) vascular adhesion molecule-1 (VCAM-I), (c) oxidized LDL (oxLDL), and (d) tumor necrosis factor-alpha (TNFα) in patients with type 2 diabetes with neuropathy after 6 months of 600 mg/day alpha-lipoic acid treatment

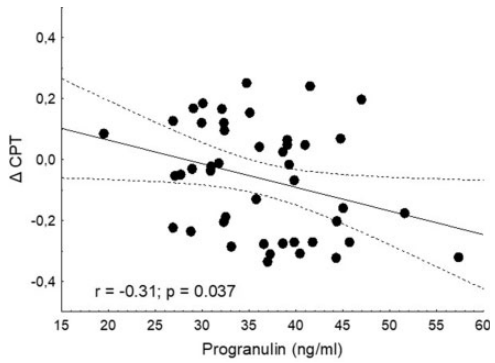

Figure 4. Correlation between improvements in the current perception threshold (CPT), measured using the Neurometer®, and serum progranulin levels in patients with type 2 diabetes with neuropathy after 6 months of 600 mg/day alpha-lipoic acid treatment

direct inhibitory effect of PGRN on TNFα and TNFR interaction.[23,24]

PGRN has an anti-inflammatory effect as an antagonist of the TNFα signaling pathway; this result is consistent with previously reported data.[25] TNFα plays a major role in the inflammatory response of vascular endothelial cells via the induction of cell adhesion molecules, including VCAM-1 and ICAM-1, which induce neutrophil adhesion to endothelial cells. Therefore, PGRN has a dual mechanism of action: by suppressing neutrophil recruitment, it both inhibits neutrophil chemotaxis by reducing TNFα-induced ICAM-1 expression and ameliorates endothelial inflammation.[26] A previous has study reported that higher PGRN levels are associated with more microvascular complications in patients with type 2 diabetes, including patients with diabetic nephropathy, neuropathy, and retinopathy.[27] To date, however, there are no data regarding the effects of ALA on PGRN levels among patients with type 2 diabetes. Thus, this is the first report of the effects of ALA treatment on PGRN levels in diabetic neuropathy. Our findings indicate that ALA treatment has beneficial effects on sensory symptoms and neuropathic deficits in patients with diabetes.

The endothelium regulates inflammatory processes in the walls of blood vessels by producing biologically active agents.[6] In diabetic neuropathy, the activation of alternative metabolic pathways is strongly associated with intracellular hyperglycemia-induced oxidative stress,[28] and endothelial cells are unable to compensate for increased oxidative stress with nitric oxide production, which may lead to increased oxLDL.[29,30] Other investigators have reported that oxLDL-induced activation of NF-$\kappa\beta$ attenuates the expression of cell-adhesion molecules (e.g., ICAM-1 and VCAM-1) and provokes inflammation in endothelial cells.[31] These processes in diabetic neuropathy lead to functional changes of the vasa vasorum, thus causing neuronal ischemia and direct axonal damage.[32] It has been reported that PGRN may alleviate neuronal injury induced by ischemia–reperfusion in mice via the inhibition of neutrophil recruitment, resulting in the decreased activation of NF-κB and matrix metalloproteinase-9.[33]

We identified a significant positive correlation between PGRN and TNFα levels in patients with diabetic neuropathy. As has previously been reported, PGRN directly binds to TNFR *in vitro*,[12] and we hypothesize that a compensatory increase in PGRN levels occurs as a result of TNFα-induced activation of NF-κB, which is associated with neuroinflammation and oxidative stress in diabetic neuropathy.[34] In the present study, we also revealed a positive correlation between PGRN and ICAM-1 levels, as well as between PGRN and oxLDL levels. Correlations among these markers indicate that the action of PGRN may play an important role in inflammatory processes by inhibiting TNFα-induced activation of the NF-κB and MAPK

signaling pathway, as a competitive antagonist of TNFR. Furthermore, because of its anti-inflammatory effects, PGRN may be a useful marker for assessing the levels of oxidative stress in diabetic neuropathy.[35] We hypothesize that the elevation in PGRN levels may represent a compensatory response in diabetic neuropathy to reduce the NF-κB-mediated expression of chemokines and intercellular adhesion molecules (e.g., ICAM-1 and VCAM-1) and to protect endothelial cells from atherosclerotic inflammation.[13,36]

Although the correlation was not significant, the positive tendency between PGRN and LDL-C levels and the significant negative correlation between PGRN and HDL-C concentrations support the aforementioned findings. The levels of non-HDL-C, which includes all cholesterol present in lipoprotein particles that are considered to be atherogenic, including LDL, very-LDL, very-LDL remnants, and lipoprotein(a), correlated positively with PGRN levels. We propose that alterations in PGRN levels may be closely related to endothelial dysfunction and dyslipidemia in diabetic neuropathy.[37] Previous research has demonstrated that PGRN is protected from degradation by its binding proteins, including secretory leukocyte protease inhibitor and apolipoprotein A-I, which is the predominant protein in plasma HDL.[10] Other investigators have suggested that the anti-inflammatory effects of HDL on macrophages may be caused by the suppression of PGRN cleavage and granulin production.[38] The significant positive correlation between PGRN and HDL-C levels in the present study thus supports the protective role of HDL in PGRN cleavage. However, further studies are necessary to validate our conclusions.

Some limitations of our study should be noted. The relatively small size of the study population and the low number of men in our sample may reduce the power of the analysis; however, the significant correlations between inflammatory markers, CPT, and PGRN levels highlight the association between PGRN and neuronal repair. Therefore, larger studies with long-term follow-up in this patient population are necessary to reveal the effects of PGRN on neuronal repair mechanisms in diabetes-induced oxidative stress.

Conclusions

Our results highlight the important role of ALA administration in the treatment of diabetic neuropathy. PGRN may be responsible for tissue repair, including neuronal and vascular repair, of damage caused by oxidative and inflammatory processes that are associated with type 2 diabetes. Therefore, PGRN might be elevated in patients with microvascular complications because of a subsequent increase in PGRN expression in response to injury. The antioxidant and anti-inflammatory effects of ALA may modify the PGRN response by relieving oxidative stress and inflammation in diabetic neuropathy. Therefore, changes in serum PGRN levels may indicate a beneficial effect of ALA treatment on endothelial function and neuronal inflammation. Monitoring serum PGRN levels during any new anti-inflammatory therapy that improves these processes may provide additional information about the efficacy of these agents. There may be potential therapeutic applications for PGRN levels in patients with diabetes, or novel treatment options that increase PGRN in these patients. However, further studies are needed to clarify the importance of PGRN in neuronal repair among patients with diabetic neuropathy.

Acknowledgements

We are indebted to the subjects of this study for their cooperation.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Research, Development and Innovation Office (NKFIH), grant number K115723; the Hungarian Diabetes Association; and the University of Debrecen, Doctoral School of Health Sciences. This article was also supported by the European Union and was co-financed by the European Social Fund, grant no. EFOP-3.6.2-16-2017-00009 (Establishing Thematic Scientific and Cooperation Network for Clinical Research).

Author contributions

FS, MH, and GP conceived and designed the study. BN, HL, AS, and AM performed the experiments. IS and EZ interpreted and analyzed the data. PK and DP provided guidance and feedback. FS and MH wrote the manuscript. All authors read and agreed to the final version of the manuscript.

ORCID iD

Mariann Harangi https://orcid.org/0000-0001-9761-9595

References

1. Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. *Diabetes Care* 2010; 33: 2285–2293. DOI: 10.2337/dc10-1303.
2. Edwards JL, Vincent AM, Cheng HT, et al. Diabetic neuropathy: mechanisms to management. *Pharmacol Ther* 2008; 120: 1–34. DOI: 10.1016/j.pharmthera.2008.05.005.
3. Coppey LJ, Gellett JS, Davidson EP, et al. Effect of antioxidant treatment of streptozotocin-induced diabetic rats on endoneurial blood flow, motor nerve conduction velocity, and vascular reactivity of epineurial arterioles of the sciatic nerve. *Diabetes* 2001; 50: 1927–1937. DOI: 10.2337/diabetes.50.8.1927.
4. Hosseini A and Abdollahi M. Diabetic neuropathy and oxidative stress: therapeutic perspectives. *Oxid Med Cell Longev* 2013; 2013: 168039. DOI: 10.1155/2013/168039.
5. Vincent AM, Callaghan BC, Smith AL, et al. Diabetic neuropathy: cellular mechanisms as therapeutic targets. *Nat Rev Neurol* 2011; 7: 573–583. DOI: 10.1038/nrneurol.2011.137.
6. Rask-Madsen C and King GL. Vascular complications of diabetes: mechanisms of injury and protective factors. *Cell Metab* 2013; 17: 20–33. DOI: 10.1016/j.cmet.2012.11.012.
7. Bateman A and Bennett HP. Granulins: the structure and function of an emerging family of growth factors. *J Endocrinol* 1998; 158: 145–151. DOI: 10.1677/joe.0.1580145.
8. Van Damme P, Van Hoecke A, Lambrechts D, et al. Progranulin functions as a neurotrophic factor to regulate neurite outgrowth and enhance neuronal survival. *J Cell Biol* 2008; 181: 37–41. DOI: 10.1083/jcb.200712039.
9. Kessenbrock K, Fröhlich L, Sixt M, et al. Proteinase 3 and neutrophil elastase enhance inflammation in mice by inactivating antiinflammatory progranulin. *J Clin Invest* 2008; 118: 2438–2447. DOI: 10.1172/JCI34694.
10. Zhu J, Nathan C, Jin W, et al. Conversion of proepithelin to epithelins: roles of SLPI and elastase in host defense and wound repair. *Cell* 2002; 111: 867–878. DOI: 10.1016/s0092-8674(02)01141-8.
11. Jin FY, Nathan C, Radzioch D, et al. Secretory leukocyte protease inhibitor: a macrophage product induced by and antagonistic to bacterial lipopolysaccharide. *Cell* 1997; 88: 417–426. DOI: 10.1016/s0092-8674(00)81880-2.
12. Tang W, Lu Y, Tian QY, et al. The growth factor progranulin binds to TNF receptors and is therapeutic against inflammatory arthritis in mice. *Science* 2011; 332: 478–484. DOI: 10.1126/science.1199214.
13. Jian J, Konopka J and Liu C. Insights into the role of progranulin in immunity, infection, and inflammation. *J Leukoc Biol* 2013; 93: 199–208. DOI: 10.1189/jlb.0812429.

14. Tibullo D, Li Volti G, Giallongo C, et al. Biochemical and clinical relevance of alpha lipoic acid: antioxidant and anti-inflammatory activity, molecular pathways and therapeutic potential. *Inflamm Res* 2017; 66: 947–959. DOI: 10.1007/s00011-017-1079-6.
15. Papanas N and Ziegler D. Efficacy of α-lipoic acid in diabetic neuropathy. *Expert Opin Pharmacother* 2014; 15: 2721–2731. DOI: 10.1517/14656566.2014.972935.
16. Meijer JW, Smit AJ, Sonderen EV, et al. Symptom scoring systems to diagnose distal polyneuropathy in diabetes: the Diabetic Neuropathy Symptom score. *Diabet Med* 2002; 19: 962–965. DOI: 10.1046/j.1464-5491.2002.00819.x.
17. American Association of Electrodiagnostic Medicine (AAEM) Equipment and Computer Committee (1993–1996). Technology review: the Neurometer current perception threshold (CPT). *Muscle Nerve* 1999; 22: 523–531. DOI: 10.1002/(SICI)1097-4598(199904)22:4<523::AID-MUS16>3.0.CO;2-1.
18. Matsutomo R, Takebayashi K and Aso Y. Assessment of peripheral neuropathy using measurement of the current perception threshold with the Neurometer in patients with type 2 diabetes mellitus. *J Int Med Res* 2005; 33: 442–453. DOI: 10.1177/147323000503300410.
19. Masson EA and Boulton AJ. The Neurometer: validation and comparison with conventional tests for diabetic neuropathy. *Diabet Med* 1991; 8: S63–S66. DOI: 10.1111/j.1464-5491.1991.tb02159.x.
20. Ewing DJ and Clarke BF. Diagnosis and management of diabetic autonomic neuropathy. *Br Med J (Clin Res Ed)* 1982; 285: 916–918. DOI: 10.1136/bmj.285.6351.1353-a.
21. Daniel R, He Z, Carmichael KP, et al. Cellular localization of gene expression for progranulin. *J Histochem Cytochem* 2000; 48: 999–1009. DOI: 10.1177/002215540004800713.
22. Hyung S, Im SK, Lee BY, et al. Dedifferentiated Schwann cells secrete progranulin that enhances the survival and axon growth of motor neurons. *Glia* 2019; 67: 360–375. DOI: 10.1002/glia.23547.
23. Etemadi N, Webb A, Bankovacki A, et al. Progranulin does not inhibit TNF and lymphotoxin-α signalling through TNF receptor 1. *Immunol Cell Biol* 2013; 91: 661–664. DOI: 10.1038/icb.2013.53.
24. Chen X, Chang J, Deng Q, et al. Progranulin does not bind tumor necrosis factor (TNF) receptors and is not a direct regulator of TNF-dependent signaling or bioactivity in immune or neuronal cells. *J Neurosci* 2013; 33: 9202–9213. DOI: 10.1523/JNEUROSCI.5336-12.2013.
25. Jian J, Li G, Hettinghouse A, et al. Progranulin: a key player in autoimmune diseases. *Cytokine* 2018; 101: 48–55. DOI: 10.1016/j.cyto.2016.08.007.
26. McCoy MK and Tansey MG. TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease. *J Neuroinflammation* 2008; 5: 45. DOI: 10.1186/1742-2094-5-45.
27. Albeltagy ES, Hammour AE and Albeltagy SA. Potential value of serum progranulin as a biomarker for the presence and severity of micro vascular complications among Egyptian patients with type 2 diabetes mellitus. *J Diabetes Metab Disord* 2019; 18: 217–228. DOI: 10.1007/s40200-019-00406-1.
28. Pop-Busui R, Ang L, Holmes C, et al. Inflammation as a therapeutic target for diabetic neuropathies. *Curr Diab Rep* 2016; 16: 29. DOI: 10.1007/s11892-016-0727-5.
29. Gomes-Neto AW, Osté MCJ, Sotomayor CG, et al. Fruit and vegetable intake and risk of posttransplantation diabetes in renal transplant recipients. *Diabetes Care* 2019; 42: 1645–1652. DOI: 10.2337/dc19-0224.
30. Sedaghat A, Shahbazian H, Rezazadeh A, et al. The effect of soy nut on serum total antioxidant, endothelial function and cardiovascular risk factors in patients with type 2 diabetes. *Diabetes Metab Syndr* 2019; 13: 1387–1391. DOI: 10.1016/j.dsx.2019.01.057.
31. Wei Q, Tu Y, Zuo L, et al. MiR-345-3p attenuates apoptosis and inflammation caused by oxidized low-density lipoprotein by targeting TRAF6 via TAK1/p38/NF-kB

signaling in endothelial cells. *Life Sci* 2020; 241: 117142. DOI: 10.1016/j.lfs.2019.117142.
32. Roustit M, Loader J, Deusenbery C, et al. Endothelial dysfunction as a link between cardiovascular risk factors and peripheral neuropathy in diabetes. *J Clin Endocrinol Metab* 2016; 101: 3401–3408. DOI: 10.1210/jc.2016-2030.
33. Thurner L, Stöger E, Fadle N, et al. Proinflammatory progranulin antibodies in inflammatory bowel diseases. *Dig Dis Sci* 2014; 59: 1733–1742. DOI: 10.1007/s10620-014-3089-3.
34. Kuan-Hong W and Bai-Zhou L. Plumbagin protects against hydrogen peroxide-induced neurotoxicity by modulating NF-κB and Nrf-2. *Arch Med Sci* 2018; 14: 1112–1118. DOI: 10.5114/aoms.2016.64768.
35. Sandireddy R, Yerra VG, Areti A, et al. Neuroinflammation and oxidative stress in diabetic neuropathy: futuristic strategies based on these targets. *Int J Endocrinol* 2014; 2014: 674987. DOI: 10.1155/2014/674987.
36. Nicoletto BB and Canani LH. The role of progranulin in diabetes and kidney disease. *Diabetol Metab Syndr* 2015; 7: 117. DOI: 10.1186/s13098-015-0112-6.
37. Yoo HJ, Hwang SY, Hong HC, et al. Implication of progranulin and C1q/TNF-related protein-3 (CTRP3) on inflammation and atherosclerosis in subjects with or without metabolic syndrome. *PLoS One* 2013; 8: e55744. DOI: 10.1371/journal.pone.0055744.
38. Okura H, Yamashita S, Ohama T, et al. HDL/apolipoprotein A-I binds to macrophage-derived progranulin and suppresses its conversion into proinflammatory granulins. *J Atheroscler Thromb* 2010; 17: 568–577. DOI: 10.5551/jat.3921.