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Short communication

Niacin modulates macrophage polarization in Parkinson's disease

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ABSTRACT

Neuroinflammation remains a central piece in Parkinson's disease (PD) pathophysiology. However, mechanisms by which PD links to the neuroinflammation remain elusive.

Here, for the first time, we report that lower dose of niacin in PD patients may affect macrophage polarization from M1 (pro-inflammatory) to M2 (counter-inflammatory) profile through the niacin receptor GPR109A. Skew in the peripheral macrophages were accompanied by improved quality of life assessments in patients. Low dose niacin supplementation may be beneficial in PD, boosting anti-inflammatory processes and suppressing inflammation. Varied niacin dosages for longer durations may further reveal the potential role of anti-inflammatory interventions in PD progression.

1. Introduction

Parkinson's disease is a progressive neurodegenerative disorder. Aging, genetic susceptibility and environmental factors play pivotal roles in its initiation, and progression. The main cause of PD still remains unknown and there is no cure (Moehle and West, 2015).

Among all mechanisms and factors proposed to be involved in PD pathology, inflammation is universally thought to play a central role in the initiation and progression of PD (Moehle and West, 2015; Bartels et al., 2010). Acute inflammatory responses may initially be beneficial, however, chronic inflammation exacerbates brain damage. As a part of innate immunity, macrophages and neutrophils are known to cross the leaky blood brain barrier, secrete cytokines (e.g., interleukins, tumor necrosis factor, interferon gamma) that in turn can initiate and regulate inflammatory responses leading to neurodegenerative damage. Due to lineage proximity to microglia, macrophages have attracted increasing attention in relation to the onset and progression of PD (Moehle and West, 2015; Lee et al., 2017; Zhao et al., 2014). Macrophages can be divided into two classes of M1 (pro-inflammatory) and M2 (counter-inflammatory) subtypes. The notion of macrophage polarization and skewing from M1 to M2 type may be a plausible modality in containing the chronic inflammation and slowing the progression of PD (Moehle and West, 2015).

Our previous studies have demonstrated that niacin supplementation may influence the course of PD (Wakade et al., 2014; Wakade and Chong, 2014). Niacin can play a significant role in triggering and boosting anti-inflammatory immune responses in humans and animal models (Feingold et al., 2014). Niacin is a ligand for hydroxycarboxylic acid receptor 2 (HCAR2, also known as GPR109A). The counter inflammatory role of GPR109A has been already proposed (Salem and Wadie, 2017). Several studies have suggested that niacin's effects maybe mediated via GPR109A/HCA2, which is highly expressed in adipose tissue and macrophages (Blad et al., 2012; Ganapathy et al., 2013; Singh et al., 2014a).

Here in this study we demonstrated for the first time in PD patients, that niacin supplementation through its receptor, GPR109A, may alter the macrophages polarization from M1 to M2 profile. Most importantly, our findings indicated an improvement in quality of life for PD patients.

2. Material and methods

2.1. Participants

46 patients diagnosed with PD participated in the study. Selection

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criteria and sampling were carried out in accordance with the Declaration of Helsinki and approved by the institutional review board. All participants gave informed consent before participating in the study. The mean (+/− standard deviation) age for the placebo, 100 mg niacin and 250 mg niacin groups were 62 (Zhao et al., 2014), 64 (Wakade et al., 2014), and 61 (Wakade and Chong, 2014) years. The duration of disease in years for these groups was 7 (Wakade and Chong, 2014), 5 (Zhao et al., 2014), and 5 (Lee et al., 2017) years, respectively. The H&Y scores were 1.9 (0.8), 2 (0.9), and 2.1 (0.7) respectively.

2.2. Blood collection and sample preparation

Eight ml blood samples were collected by venipuncture following standard procedures and processed within 4 h as described earlier (Wakade et al., 2014). Briefly, purple-top ethylenediaminetetraacetic acid (EDTA) tubes were used to collect the blood sample and immediately kept in ice. Whole blood was spun and leukocytes (WBCs) were then collected and placed in fresh tubes, re-suspended in 4 ml of ACK Lysing Buffer (Lonza cat # 10-548E) and incubated for 10 min at room temperature before being spun again at 300 × g for 5 min. The process was repeated one more time. Supernatant was discarded from the clean WBCs pellet. WBCs pellets were washed twice with 1 ml of PBS and WBCs pellets were then stored at −80 °C until further analyses (Wakade et al., 2014).

2.3. Analytical flow cytometry

As described previously (Stranahan et al., 2016), whole blood cells were incubated with antibodies for surface markers including CD11b, CD68, F4/80 (M1), CD206 (M2), and GPR109a. Next, cells were fixed and permeabilized using fix/perm concentrate (eBioScience) before incubation with antibodies for intracellular staining of IL-10 (functional M2 s). Cells were then washed and run through a four-color flow cytometer (FACS Calibur). Data were collected using Cell Quest software. Samples were double-stained with control IgG (isotypes) and cell markers to assess any spillover signal of fluorochromes. Gating excluded dead cells and debris using forward and side scatter plots.

2.4. Western blot analysis

Venous blood samples (10 ml/subject) were collected from each patient during each visit at the beginning of the study and three month completion of the niacin treatment according to Augusta University, IRB approval for biochemical analysis of GPR109A receptor protein level by using western blots. WBCs were separated as described previously (Perry, 2012). WBCs were subjected to lysis by RIPA buffer with a protease inhibitor cocktail and protein concentration was measured by Bradford reagent (Bio-Rad). Again the samples were subjected to western blot using Bio-Rad 4–15% SDS-PAGE then transferred to PVDF membrane followed by incubation with respective antibody overnight and developed with an ECL kit (Giri et al., 2007).

2.5. Behavioral analysis

Three questionnaire forms were used to evaluate the subjects' quality of life: the Rapid Assessment of Postural Instability in Parkinson's disease (RAPID) (Chong et al., 2011; Jenkinson and Fitzpatrick, 2007), the Parkinson's Disease Quality of Life (PDQ-8) (Jenkinson and Fitzpatrick, 2007) and the Parkinson's Disease Sleep Scale (Chaudhuri et al., 2002). The investigator blinded to the protocol administered the questionnaires. The interview was carried out in a quiet environment in presence of a caregiver. The subjects were seen in the morning after breakfast and taking PD medications. The total score from each questionnaire was combined to produce a composite quality of life aggregate for each subject.

2.6. Statistical analysis

Statistical analysis was carried out using GraphPad Prism 5. One-way ANOVA with Tukey's post hoc test, two-way ANOVA, the Kruskal–Wallis test, two-sample Student's unpaired *t*-test, or Pearson's correlation was used as appropriate, and all correlations were corrected for corresponding variables. Normality of distribution was evaluated using the D'Agostino and Pearson omnibus normality test. Statistical significance was set at $p < 0.05$. Cutoff scores were based on the highest sensitivity and specificity combinations for $\chi 2$ analysis. The composite quality of life score was analyzed with a 2 (Period) × 3 (Group) Mixed ANOVA.

3. Results

3.1. Niacin skews the macrophage polarization from M1 type towards M2

Flow cytometry analysis of whole blood of PD patients showed that niacin treatment significantly increased the number of M2 type macrophages (CD11b+, CD68+, F4/80+, CD206+, IL-10+) resulting in a higher ratio of M2:M1 (CD11b + F4/80+, CD206-, TNFα macrophages (Fig. 1A–C panels). As shown, the most significant rate of M2 polarization was obtained when niacin was administered at the 250 mg dose, followed by 100 mg and the rate was unchanged from baseline in the placebo group.

3.2. Niacin reduces the expression level of GPR109a on macrophages in PD

As shown in Figs. 1 (D, E) and 2, niacin treatment decreased the expression of GPR109A receptor on macrophages significantly in PD subjects, while the highest decrease was seen in patients taking 250 mg of niacin followed by 100 mg of niacin. Importantly, the most significant reduction of GPR109A expression was detected on type 2 macrophages (Fig. 1D).

3.3. Niacin improves quality of life

Our findings demonstrated improvement in the quality-of-life composite score in the group treated with 100 mg niacin compared to the placebo group ($p < .0065$). Many PD subjects also reported increased energy levels and improved mood (Fig. 3).

4. Discussion

This is the first demonstration that macrophage polarization is associated with PD. Our findings support the notion that treatment with niacin can be a therapeutic modality in the treatment of PD patients. Through its receptor, GPR109A, niacin alters the macrophage polarization from M1 (pro-inflammatory) to M2 (counter-inflammatory) profile. In fact, some studies suggested that Gpr109a signaling may promote anti-inflammatory properties in certain macrophages and dendritic cells and enabled them to induce differentiation of Treg cells and IL-10-producing T cells (Singh et al., 2014b). Engagement of GPR109A may cause the down-regulation of the NFκB signaling pathway, antioxidant mechanisms, boosting mitochondrial NAD, which may explain how niacin produces macrophage skew towards M2 profile. In fact, it is very plausible that suppression of NF-κB signaling pathway and counter inflammatory functions of M2 macrophages through induction of IL-10 and TGFβ signaling eliciting the niacin-induced neuroprotective effects in PD patients (Moehle and West, 2015).

Furthermore, this study provides evidence to suggest that niacin may improve the life quality of PD patients. Although, both low (100 mg) and high (250 mg) doses of niacin had beneficial effects, however, the lower dose showed more protective effects compared to the higher dose. The patterns of cellular and molecular changes compared to the clinical outcome were not coherent after niacin treatment.

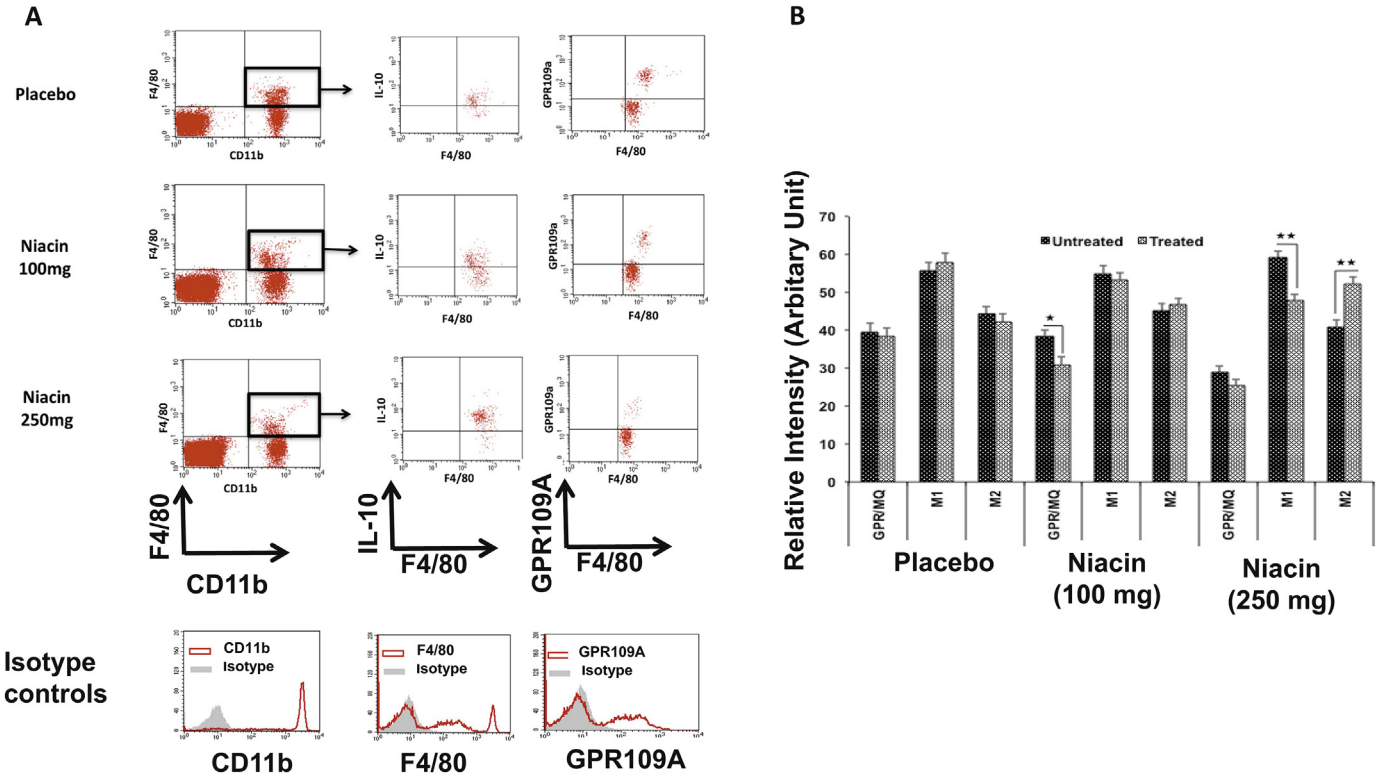

Fig. 1. Niacin treatment in PD patients skews the macrophage polarization from M1 type towards M2 macrophages. A) Flow cytometry analysis of macrophages in the peripheral blood of PD patients receiving low (100 mg) and high (250 mg) of niacin. While both low and high doses of niacin reduced the level of GPR109A expression on macrophages compared to placebo, however, the effect of high dose was more significant. Histograms at the bottom show the isotype controls (grey filled graphs) versus actual specific antibodies (red not-filled graphs
B) Bar graph demonstrating M1/M2 ratio based on the absolute values in all groups. ** representing significant difference between groups ($p < 0.05$)). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

This disconnect between the dose effect on the clinical improvement may be due to the short treatment duration. A longer study may produce a stronger association between them. Nevertheless, our findings suggested that niacin, at the very least may slow the disease progression in long term which would be a very desirable outcome in PD.

5. Conclusion

Despite all controversies about the role and impact of niacin on different diseases, our findings for the first time provides the rationale for further investigations to help better understand and define the potential therapeutic role of niacin in the treatment of PD patients. The interaction between niacin and macrophages as an important cellular component of innate immunity provides a solid base from which the potential of niacin as safe and non-expensive immunotherapeutic target may be further explored. These results may have implications for an immunotherapeutic approach to treat PD patients by niacin supplementation targeting macrophages as crucial component of immune system.

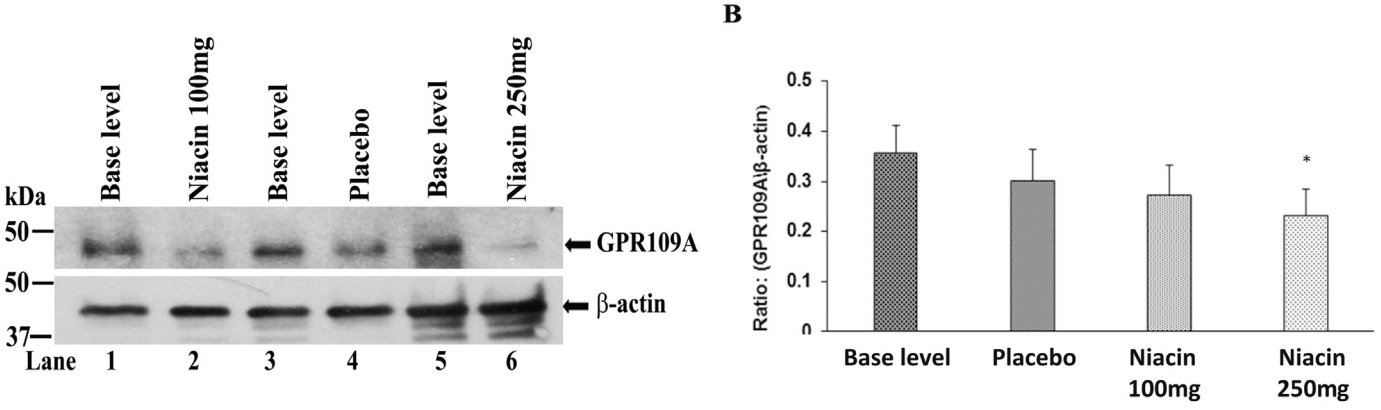

Fig. 2. GPR109A protein expression in Parkinson Disease attenuated by niacin (Vitamin B3). (A) Demonstrates representative expressions of GPR109A at base level vs treated: placebo, niacin 100 mg and niacin 250 mg groups. The lower panel indicates β-actin as the loading control. (B) Bar diagram demonstrates densitometry analyses of GPR109A Western blots in placebo, niacin 100 mg and niacin 250 mg groups using ImageJ software. Results are expressed as means ± SEM from fifteen independent subjects of each group. *Significant ($p < 0.05$) using Students *t*-test.

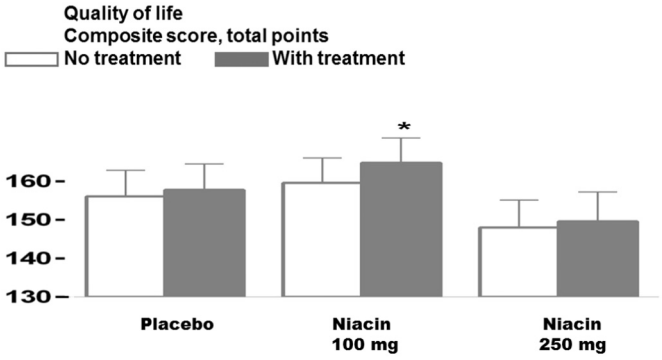

Fig. 3. Quality of life composite score. Improvement in the quality-of-life composite score was shown in the group treated with niacin compared to the placebo group ($p < 0.0065$).

Author contributions

BB: Study conception and design, acquisition of data, analysis and interpretation of data and drafting the article. BG: acquisition of data, analysis and interpretation of data and drafting the article. AM and NS: acquisition of data. JM: Clinical interpretation, study conception, design and drafting the article RKC and CW: Study conception and design, acquisition of data, analysis and interpretation of data and drafting the article.

Disclosure

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Conflict of interest

Authors declare that they have no conflict of interest.

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