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Peripheral Biomarkers of Parkinson's Disease Progression and Pioglitazone Effects

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Abstract

Pioglitazone, an oral hypoglycemic agent, recently failed to show promise as a disease-modifying agent in a 44-week phase 2 placebo-controlled study in 210 Parkinson's disease (PD) subjects. We analyzed peripheral biomarkers, including leukocyte PGC-1α and target gene expression, plasma interleukin 6 (IL-6) as a marker of inflammation, and urine 8-hydroxydeoxyguanosine (8OHdG) as a marker of oxidative DNA damage. Baseline or changes from baseline in biomarker levels were not associated with the rate of progression of PD. Pioglitazone did not significantly alter biomarker levels. Other agents that more effectively target these mechanisms remain of potential interest as disease modifying therapies in PD.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

SUPPLEMENTARY MATERIAL

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Keywords

PGC-1alpha; oxidative stress; inflammation; Parkinson's disease; biomarker; pioglitazone; 8-hydroxydeoxyguanosine; 8OHdG; IL-6; interleukin-6; cytokine

INTRODUCTION

Mechanisms hypothesized to contribute to neurodegeneration in Parkinson's disease (PD) include mitochondrial dysfunction, oxidative stress, and inflammation [1]. Mitochondrial dysfunction may result in part from deficiency of PGC-1α [2–4], a transcriptional coactivator that regulates expression of genes required for mitochondrial biogenesis while also upregulating multiple antioxidant pathways [5]. Levels of 8OHdG, a marker of oxidative DNA damage, and other markers of oxidative damage are increased in the substantia nigra (SN) [6], peripheral blood [7–9] and urine [10] in PD, including early untreated PD [7], and correlate with disease stage [9]. Interleukin 6 (IL-6) is a pro-inflammatory cytokine that can induce neuronal death [11] and is elevated in the brain [12] and serum [13, 14] in PD. IL-6 levels correlate with disease severity but not with levodopa treatment [13].

Pioglitazone is an oral hypoglycemic agent that enhances mitochondrial energy metabolism and antioxidant defenses, in part by upregulating PGC-1α activity, which has been demonstrated in skeletal muscle [15] and in human SH-SY5Y cells [16]. Pioglitazone can decrease urine 8OHdG in patients with type 2 diabetes [17] and serum IL-6 levels in patients with myotonic dystrophy and diabetes [18]. IL-6 levels also are decreased by pioglitazone in a rat model of spinal cord injury [19], a focal cerebral ischemia rat model [20], and in a rat model of iron-induced oxidative damage in the brain [21]. Pioglitazone is neuroprotective against cerebral ischemia in rodents and against MPTP in rodents [22–24] and non-human primates [25]. Pioglitazone was studied for potential disease-modifying effects in PD (the "FS-ZONE" study) [26]. In conjunction with this study, we analyzed peripheral biomarkers of relevance to potential mechanisms of neuroprotection by pioglitazone.

METHODS

Study design

The FS-ZONE study is a phase 2 placebo-controlled futility-design study of 210 early PD patients to assess the impact of pioglitazone on the clinical progression of PD. Subjects were required to be within 5 years of diagnosis of PD and were required to be on either 1 mg/day of rasagiline or 10 mg/day of selegiline. All study procedures were approved by each participating institution's local Institutional Review Board. Biomarker samples were collected at baseline, 16 weeks and 44 weeks. Plasma and urine were stored frozen until assayed. A blood sample was processed immediately through the LeukoLOCK filter system (Ambion) to stabilize RNA, which was later extracted and stored at −80 Celsius until assayed. Samples from all 3 time points were run at the same time for each subject (in 3 or 4 batches for each biomarker) to limit the impact of batch to batch variability. Results are shown in Supplemental Table 1. Gene expression (qRT-PCR) analyses were performed by

Asuragen, Inc. (Supplemental Table 2). Plasma IL-6 levels were analyzed through the Massachusetts General Hospital Clinical Research Laboratory Core Facility using the Human IL-6 Cytokine Assay (MesoScale Discovery), which is a multi-array 96-well small spot plate assay with a dynamic range of 0.163 – 25.00 pg/ml [27]. The intraplate variability (% CV) for this assay is 4.8 and the inter-plate %CV is 15.7, which are better than alternative methods [27]. 8-OHdG in urine was measured at the Behavioral Medicine Core Facility at the University of Pittsburgh using a competitive ELISA assay (Stress-Marq Biosciences, Inc, Victoria, Canada) as previously described [28]. Samples were measured in duplicate and normalized to urinary creatinine levels. The sensitivity of detection is 30pg/ml.

Statistical methods

Levels of biomarkers were compared between treatment arms using an F-test. To assess whether baseline biomarker levels predict disease progression, multiple linear regression models were fit on the change in total UPDRS (parts I, II, III) (dependent variable) adjusted for age, disease duration, gender and treatment group, and batch (for the 8OHdG model due to differences between batches for this biomarker). Change in total UPDRS was defined as the difference from baseline to 44 weeks or the last visit before the additional symptomatic therapy was initiated. To assess if changes in biomarker levels correlate with the effect of treatment on disease progression, a multiple linear regression of the change in total UPDRS was fit with change in biomarker levels, treatment group, and the interaction of biomarker change and treatment group as independent variables. Pearson's correlation coefficient was used to assess reliability of measurements between baseline and follow-up time points. The test-retest reliability of the biomarker measurements was estimated using the Intraclass Correlation Coefficient (ICC).

RESULTS

Association of baseline biomarker levels with rate of progression of PD

There were no significant associations between baseline levels of expression of PGC-1α and 3 of its target genes (CYC, ERRα, or PRDX3) in peripheral leukocytes, or baseline levels of plasma IL-6 or urinary 8OHdG, and the rate of progression of PD as measured by the change in the total UPDRS (Table 1). Results were similar when the Total UPDRS change was modeled longitudinally, including measures at 16, 28, 44 weeks (not shown).

A subset of samples had undetectable levels of gene expression, defined as within the range of no RNA control samples (Ct >36.68), despite RNA yields and quality that met our standards. This might occur due to technical problems for those few samples, or due to unusually low levels of expression of the gene of interest. Undetectable expression occurred most commonly for PGC-1α (n = 15 at baseline), which normally is expressed at low levels. Thus, a theoretical concern is that exclusion of these samples with undetectable levels of PGC-1α might reduce our ability to detect an association of low levels of PGC-1α expression on the rate of progression of PD. However, this was not the case as the mean (95% CI) progression for these 15 subjects was smaller than for 131 participants with detectable baseline PGC-1α levels (3.5; 0.44–6.63 compared to 4.7; 3.31–6.06), although not statistically significantly different.

Impact of pioglitazone on biomarkers

There were no significant differences between treatment groups in the change from baseline at 16 or 44 weeks for levels of expression of PGC-1α and its target genes, 8OHdG, or IL-6 (Table 2). However, mean IL-6 levels were significantly lower at 16 and 44 weeks compared to baseline in all 3 treatment groups, including placebo. In order to assay samples from all time points from each subject in the same batch, baseline sample were stored for 16 and 44 weeks longer than the 16 and 44 week samples, respectively. If increasing storage time tends to increase the apparent IL-6 levels detected with the Human IL-6 Cytokine Assay (e.g. through epitope unmasking), then this could lead to the observed increase over time in IL-6 levels. Indeed, there was a weak but statistically significant ($r = 0.095$, $p = 0.019$) association for higher IL-6 levels with longer storage times (Supplemental Figure), potentially accounting for the change over time in all treatment groups.

Association of change in biomarker levels and progression of PD

The association of the change in biomarker levels and PD progression as measured by the change in total UPDRS score was assessed via a multiple linear regression. A model was fit simultaneously with indicators for PGC-1α, CYCS, ERRα and PRDX3, treatment group and interaction terms with treatment group ($N = 79$). There were no significant effects for PGC-1α, ERRα or PRDX3 (F-test > 0.05 in each case), and no consistent associations of the change from baseline in gene expression levels and the change in total UPDRS. A similar model was fit for IL-6, and neither the main effect of the change in IL-6 ($F_{1,180} = 0.17$, $p = 0.68$) nor the interaction term was statistically significant ($F_{2,180} = 0.01$, $p = 0.99$). Likewise, when 8OHdG was included (adjusting for batch), neither the main effect of the change in 8OHdG ($F_{1,166} = 0.22$, p-value = 0.64) nor the interaction term was statistically significant ($F_{2,166} = 0.70$, $p = 0.50$).

Validation of measurements

Analyses of correlations between subjects' baseline results with their 16 and 44 week results supported the reliability of these data (Supplementary Table 3). For each biomarker, a subset of subjects' samples was randomly selected and replicate aliquots from the same time point were measured. In every case, the biomarkers in this study showed excellent (ICC > 0.75) or fair to good (ICC > 0.4 but <0.75) reproducibility [29] (Supplemental Table 4).

DISCUSSION

Pioglitazone has neuroprotective effects in various animal models of neurodegeneration that may result from upregulation of PGC-1α [15, 16] as well as anti-inflammatory [18–20] and antioxidant [17] effects. However, pioglitazone was futile in the FS-ZONE study with respect to its potential as a disease-modifying therapy for PD [26]. Consistent with this, pioglitazone did not significantly upregulate expression of PGC-1α or its target genes in blood leukocytes, and lacked clear anti-inflammatory or antioxidant effects. Thus, both the clinical and the biomarker data support lack of promise for pioglitazone as a potential neuroprotective agent in PD. Interestingly, although a recent epidemiological study found a lower risk of PD among individuals exposed to glitazone drugs, this association was not significant in a subanalysis specifically of pioglitazone [30].

This study also tested the sensitivity of these biomarkers as predictors and measures of PD progression, independently of pioglitazone effects. Low PGC-1α activity may play a role in the pathogenesis of PD [2, 3, 5, 31], and thus we predicted faster progression in subjects with lower baseline PGC-1α activity. Similarly, inflammation [1, 32] and oxidative stress [1, 6] may contribute to PD pathogenesis, and so we predicted faster progression in patients with higher levels of a pro-inflammatory cytokine (IL-6) in plasma, or of a marker of oxidative DNA damage (8OHdG) in urine. Contrary to these predictions, there were no significant associations of baseline levels of these biomarkers, or the magnitude of the changes from baseline, with the rate of progression of PD. Thus, these peripheral biomarkers are not useful for predicting or monitoring the rate of progression of PD.

A limitation of this study is that peripheral biomarkers may not reflect levels in the brain. Although pioglitazone has been shown to induce PGC-1α activity [15, 16] and to have anti-inflammatory [18–21] and antioxidant effects, prior studies have not tested whether or not these effects are detectable in peripheral blood cells in PD patients or in PD animal models. Also, we could not detect changes that might occur over longer than 44 weeks or later in the disease course. Furthermore, this study did not include control subjects and so we could not determine if these biomarkers have value as diagnostic biomarkers of PD. An additional limitation is that we did not measure other possible biomarkers of pioglitazone action, such as circulating adiponectin [33, 34].

In this study, the selected peripheral biomarkers proved to be unhelpful for predicting or monitoring the rate of progression of PD. We also found no evidence for target engagement by pioglitazone in these study subjects. Therefore, other agents that more effectively target these mechanisms remain of potential interest for future studies of neuroprotection in PD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Multiple linear regression models of total UPDRS change from baseline (Dependent Variable)

Multiple linear model of 44 week total UPDRS change*, *N* = 128			
Independent variables	**Parameter estimate**	**SE**	***t*-test, *p*-value (two-sided)**
Baseline PGC-1α	0.352	0.3	0.24
Baseline CYCS	0.298	2.39	0.9
Baseline ERRα	0.463	0.66	0.49
Baseline PRDX3	−0.686	1.05	0.52
Multiple linear model of 44 week total UPDRS change*, *N* = 204			
Baseline IL-6	0.33	0.49	0.65
Multiple linear model of 44 week total UPDRS change*, *N* = 202			
Baseline 8OHdG	1.64	1.10	0.14

All models are adjusted for age, disease duration, gender, and treatment group. SE = Standard Error.

*Total UPDRS Change from baseline to 44 weeks, or Last Value Carried Forward.

Table 2

Mean change from baseline at 16 and 44 weeks by treatment group. Genes regulated by PGC-1α, IL-6, 8OHdG (ng/ml)/creatine (ug/ml)

Biomarker	Week	Placebo (0 mg) (*N* = 71 enrolled)			15 mg Pioglitazone (*N* = 72 enrolled)			45 mg Pioglitazone (*N* = 67 enrolled)				F-test, *p*-value
		N	Mean Fractional Change*	SD	N	Mean Fractional Change*	SD	N	Mean Fractional Change*	SD	
PGC-1α	16	32	−0.17	1.11	28	−0.01	1.2	32	0.07	1.31	0.74
	44	26	0.76	1.67	28	0.13	1.55	28	0.58	1.05	0.26
CYCS	16	45	0.32	1.39	32	−0.01	0.53	37	0.3	0.68	0.31
	44	31	0.61	0.92	31	0.28	0.65	36	0.47	0.73	0.24
ERRα	16	42	0.05	4.62	29	−0.69	2.82	35	−0.53	1.53	0.62
	44	27	−1.33	5.67	30	−0.81	2.2	29	−1.23	3.11	0.87
PRDX3	16	45	0.07	0.8	32	−0.03	0.48	38	0.55	1.96	0.11
	44	32	0.43	0.99	31	0.19	0.59	37	0.47	1.15	0.46
Biomarker	Week	N	Mean Change≠	SD	N	Mean Change≠	SD	N	Mean Change≠	SD	F-test, *p*-value
IL-6	16	68	−0.17	0.82	64	−0.2	0.8	63	0	0.54	0.26
	44	62	−0.22	0.93	62	−0.27	0.84	62	−0.12	0.35	0.53
Biomarker	Week	N	Mean Change≠	SD	N	Mean Change≠	SD	N	Mean Change≠	SD	F-test, *p*-value
8OHdG	16	64	0.08	0.75	62	0.02	0.3	60	0.07	0.36	0.77**
	44	59	0.05	0.43	56	0.05	0.26	59	0.09	0.28	0.54**

SD = Standard Deviation.

*The Fractional Change is the fold change minus 1 for positive fold changes, and plus 1 for negative fold changes (see methods for details).

≠Change is the difference in the post – baseline measurement. F-test for the null hypothesis that all three treatment group mean (fractional) changes are equal.

**For 8OHdG, the F-test is a partial F-test adjusted for batch.