GM604 for Parkinson's Disease treatment: Pre-clinical findings and results from a pilot placebo-controlled trial

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INTRODUCTION

Parkinson's disease (PD) is a progressive motor disorder caused by death of dopaminergic neurons in the substantia nigra pars compacta. Clinical manifestations of PD overlap with other movement disorders, but the disease is classically characterized by resting tremor, rigidity, akinesia, postural instability, and shuffling gait. Multiple PD drugs are available, but most only augment dopamine signaling to control symptoms. Slowing the underlying neurodegeneration remains a longstanding challenge for drug development.

GM604 (GM6) is an oligopeptide containing 6 amino acids, corresponding to the active site of an embryonic-stage protein functioning within the developing nervous system. GM6 has been shown to cross the blood-brain barrrier (Yu et al. 2008, Brain Res. 1238:143-153) and its safety and tolerability has been demonstrated in multiple phase 1 and 2 clinical trials. It has been proposed that GM6 triggers activation of pro-survival pathways mediating neural development, which may in turn have beneficial effects for treatment of multiple neurodegenerative diseases.

The purpose of this study was to evaluate GM6 as a PD drug candidate, utilizing microarray analysis with *in vitro* and *in vivo* approaches to model PD pathogenesis. We additionally report blood biomarker findings from a pilot double-blinded, randomized, placebo-controlled trial enrolling a small number of PD patients (*n* = 6).

RESULTS

Salsolinol (SAL) is a neurotoxin byproduct of dopamine metabolism elevated in CSF from PD patients. We evaluated viability of dopaminergic human neuroblastoma SH-SY5Y cells 24 hours after treatment with GM6, SAL (100 µM), or the combination GM6+SAL (Figure 1A). SAL significantly decreased cell survival by ~60% (P < 0.05). This effect was abrogated, however, in cells treated concurrently with GM6 (Figure 1A). Concurrent GM6 treatment led to dose-dependent rescue of cell survival, with complete recovery of cell viability at the highest GM6 dose (10 mg/ml) (Figure 1A). Consistent with these results, GM6 also partially rescued survival of rat cortical neuronal cells treated with post-mortem CSF from PD patients (Figure 1B).

We next used DNA microarrays to identify genes altered in SH-SY5Y cells treated for 48 hrs. with GM6 (*n* = 2 per group, GM6 or vehicle). We identified > 1000 genes altered by GM6 (FDR < 0.10; 2-fold expression change), but focused on 104 genes associated with the PD pathway as annotated by the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Figure 2). **Strikingly, nearly 80% of the KEGG PD pathway genes were decreased by GM6 (FC < 1).** This down-regulation of PD-associated genes was statistically significant based upon simulation-based comparisons to randomly sampled sets of SH-SY5Y-expressed genes (Figure 3A and 3B; P < 0.001).

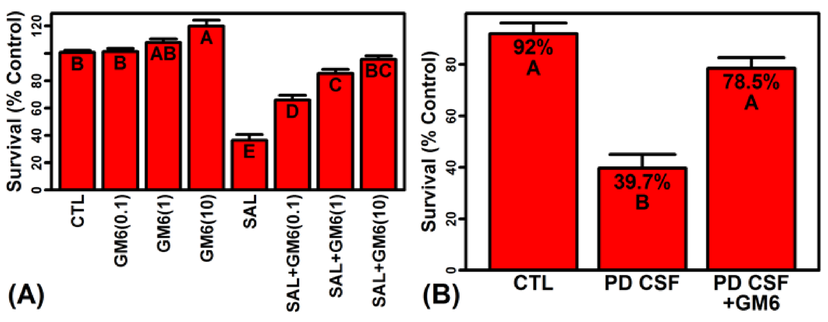

Figure 1. GM6 prevents cell death following treatment with salsolinol or post-mortem CSF from PD patients. (A) Salsolinol. SH-SY5Y cells were untreated (CTL) or treated for 24 hours with GM6 (0.1, 1, or 10 mg/ml) or GM6 + salsolinol (100 µM) (*n* = 10/group). (B) PD post-mortem CSF. Sprague Dawley rat cortical neuronal cells were treated with post-mortem CSF from control subjects or PD patients (*n* = 5/group). In (A) and (B), thiazolyl blue (MTT) assays were used to assess cell viability. Groups without the same letter differ significantly (P < 0.05, Tukey HSD test).

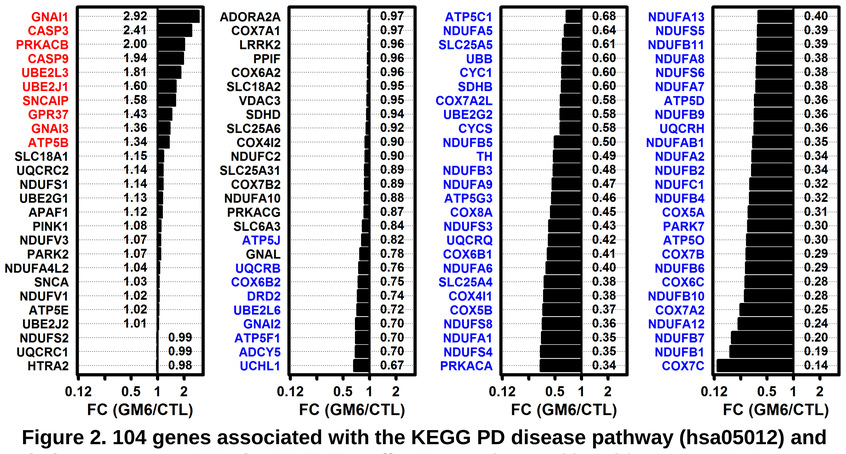

Figure 2. 104 genes associated with the KEGG PD disease pathway (hsa05012) and their response to GM6 in SH-SY5Y cells. Genes shown with red font were GM6-increased (FDR < 0.10) and genes shown with blue font were GM6-decreased (FDR < 0.10). Fold-change estimates (GM6/CTL) are indicated for each gene.

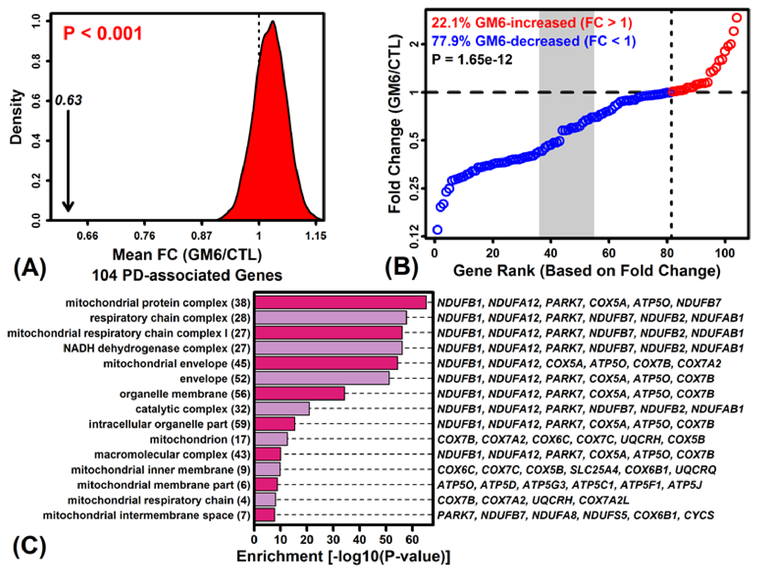

Figure 3. PD-associated genes are predominantly repressed by GM6 in SH-SY5Y cells and associated with mitochondria. (A) Simulation analyses. Sets of 104 SH-SY5Y-expressed genes were sampled at random. The histogram shows average FC among sets of 104 randomly sampled genes (arrow: observed average FC among 104 PD-associated genes). (B) FC estimates. The proportion of GM6-decreased genes (FC < 1) was significantly larger than expected (P = 1.65 x 10^{-12}; Fisher's exact test). (C) GO CC terms enriched among PD-associated GM6-decreased genes (conditional hypergeometric test; left margin parentheses: no. of GM6-decreased genes associated with each term; right margin: example GM6-decreased genes associated with each term).

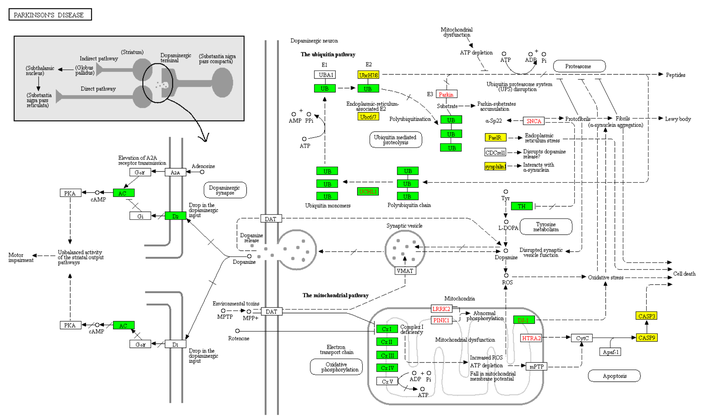

Figure 4. KEGG PD pathway (hsa05012). Yellow boxes: GM6-increased genes (FDR < 0.10). Green boxes: GM6-decreased genes (FDR < 0.10).

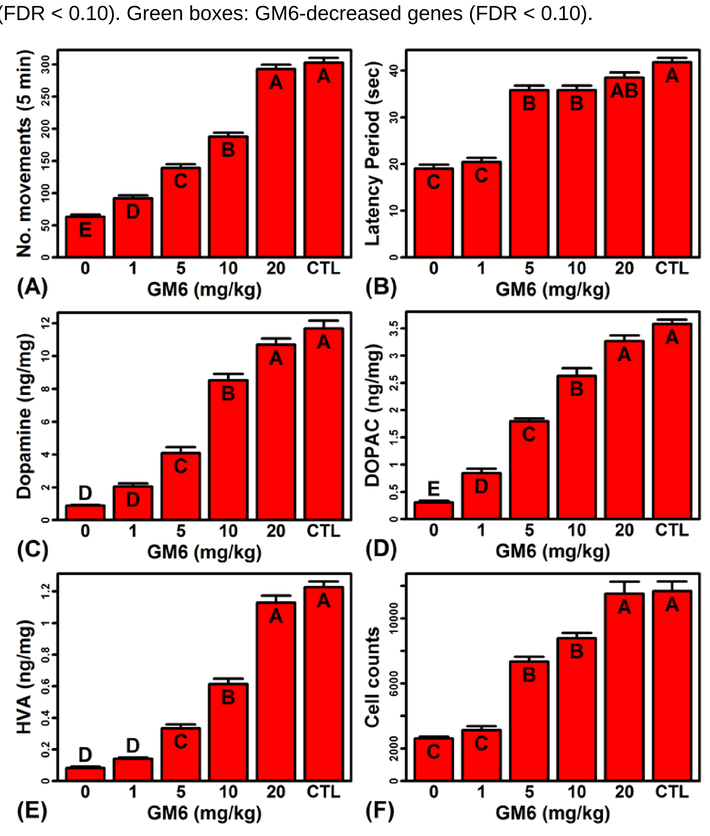

Figure 5. GM6 improves behavioral, biochemical, and histological features in the 6-OHDA mouse model. Male C57BL/6 mice received intracerebral injection of 6-OHDA or solvent (CTL). Treated mice received GM6 twice daily (i.v.) over 5 days after 6-OHDA treatment (0, 1, 5, 10 or 20 mg/kg in saline). At 1 week following 6-OHDA treatment, we evaluated (A) spontaneous activity, (B) rotarod performance, (C) brain dopamine, (D) brain DOPAC, (E) brain HVA, and (F) number of tyrosine hydroxylase positive neurons in the substantia nigra pars compacta (*n* = 10/group). Groups without the same letter differ significantly (P < 0.05, Tukey HSD test).

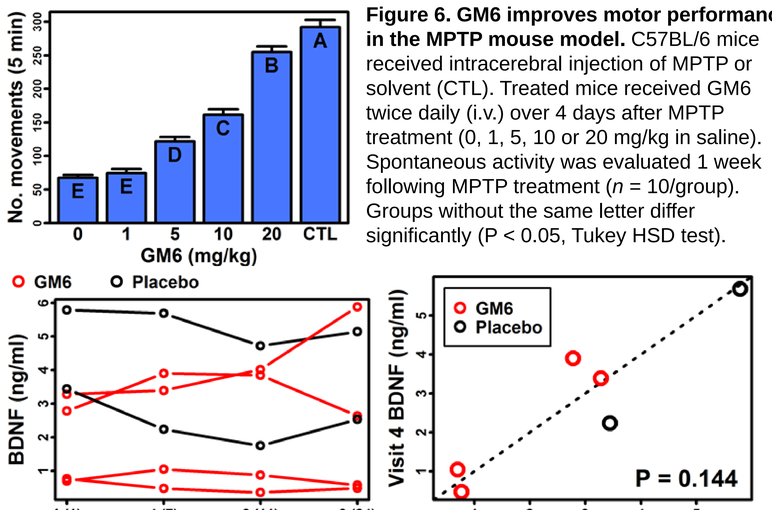

Figure 6. GM6 improves motor performance in the MPTP mouse model. C57BL/6 mice received intracerebral injection of MPTP or solvent (CTL). Treated mice received GM6 twice daily (i.v.) over 4 days after MPTP treatment (0, 1, 5, 10 or 20 mg/kg in saline). Spontaneous activity was evaluated 1 week following MPTP treatment (*n* = 10/group). Groups without the same letter differ significantly (P < 0.05, Tukey HSD test).

Figure 7. Short-term GM6 treatment improves BDNF blood levels in PD patients (Phase 2A trial). PD patients received 6 doses of GM6 (*n* = 4) or placebo (*n* = 2) over 2 weeks. BDNF was evaluated at baseline (visit 1), after 4 doses (visit 4), after 6 doses (visit 6) and 10 weeks post-treatment (visit 8). We analyzed the change in BDNF blood level for each patient relative to baseline at visits 4, 6, and 8 (one-tailed paired t-test).

Overall, 61 of 104 PD-associated genes were significantly repressed by GM6 (FDR < 0.10; FC < 1.0; Figure 2). These genes were largely associated with the mitochondrial protein complex and respiratory chain (Figure 3C) and included genes associated with each mitochondrial complex (Figure 4).

To assess whether GM6 could prevent dopaminergic neuron loss *in vivo*, we utilized 2 PD mouse models (Figures 5 and 6). First, intracerebral 6-hydroxydopamine (6-OHDA) injection was used to damage nigrostriatal dopaminergic neurons in C57BL/6 mice (Simola et al. 2007, Neurotox Res 11:151-67). 6-OHDA-treated mice were then provided varying doses of GM6 for 5 consecutive days post-treatment (0, 1, 5, 10 or 20 mg/kg). **GM6 at the highest dose almost completely abrogated 6-OHDA effects**, leading to improved motor performance (Figures 5A and 5B), increased numbers of TH-positive neurons in the substantia nigra (Figure 5F), and increased brain levels of dopamine, 3,4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (Figures 5C – 5E). The experiment was repeated using an alternative PD model, i.e., mice injected with injected with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; Meredith and Rademacher 2011, J Parkinsons Dis 1: 19–33.) (Figure 6). Results mirrored those from the 6-OHDA experiment, with dose-dependent improvement in motor performance (Figure 6) and corresponding increases in TH-positive neurons, brain dopamine, DOPAC and HVA (data not shown).

SUMMARY

Parkinson's is a debilitating disease for which existing drugs primarily control symptoms without preventing underlying neurodegeneration. This study used multiple experimental models to evaluate GM6 as a PD drug candidate.

We here demonstrate protective effects of GM6 against salsolinol, 6-OHDA, MPTP, and post-mortem CSF from PD patients (Figures 1, 5 and 6). It is notable that these treatments elicit neuron damage by generating reactive oxygen species (salsolinol, 6-OHDA) or by inhibition of mitochondria complex I (MPTP). Consistent with this, our microarray data show that GM6 significantly down-regulates expression of > 40 mitochondrial genes associated with PD (Figures 2 – 4). This may reflect decreased mitochondrial abundance or activity in GM6-treated cells. Potentially, therefore, protective effects of GM6 against post-mortem PD CSF, salsolinol, 6-OHDA and MPTP may involve attenuation of mitochondrial dysfunction, e.g., decreased ROS production and/or dampening of intrinsic apoptosis cascades.

We have completed a pilot phase 2A placebo-controlled study to evaluate GM6 safety and potential evidence of benefit (*n* = 6 patients). GM6 was well-tolerated and we observed favorable changes in blood biomarkers (e.g., blood-derived neurotrophic factor, BDNF; Figure 7). Long-term data based upon larger patient cohorts appear justified and are needed to fully address clinical efficacy of GM6 in this context.