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Two Glycoconjugates of Dopamine, IPX-750 and IPX-760, Attenuate α-Synuclein Oligomerization

Alex Harrelson, Neena Xavier, Khushbu Viralkumar Patel, Yi En Huang, Joe Garner, Kathy Nugent, M. Tino Unlap*, Zainab Suleiman, Tawnya Yates, Dina Avery

Departments of Clinical and Diagnostic Sciences, University of Alabama at Birmingham, Birmingham, USA

***Corresponding author:** M. Tino Unlap, Department of Clinical and Diagnostic Sciences, SHPB 443, University of Alabama at Birmingham, Birmingham, AL 35294, USA. Tel: +12059347382; Email: unlap@uab.edu

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Abstract

Alpha-synuclein (α-synuclein) is a small protein that is expressed in neurons in the substantia nigra. Its function in the healthy brain is currently unknown, but has been found to be the major constituent of Lewy bodies, protein aggregations that are a hallmark of Parkinson's disease. The effect of IPX-750 or IPX-760, two glycoconjugates of dopamine, on α-synuclein aggregation was tested in a cell base assay Tet-off cell line consisting of neurons co-expressing α-synuclein proteins conjugated to the amino terminal (α-synuclein-nGL) or the carboxyl region (α-synuclein-cGL) of the Gaussia luciferase protein. Aggregation of two α-synuclein proteins brings the two regions of Gaussia luciferase together which results in increased fluorescence that is captured by a microplate reader. The effect of each drug on α-synuclein aggregation was tested by growing cells for 24hrs in the absence of tetracycline to allow the expression of α-synuclein-nGL and α-synuclein-cGL. Cells were treated with IPX-750 or IPX-760 at 0.01, 0.05, and 0.1μM followed by fluorescence assay at 24hrs. The effect of each treatment was compared between treated and non-treated samples and expressed as % aggregation reduction. Our results showed that IPX-750 reduced α-synuclein aggregation by 5, 15 and 16%, respectively, while IPX-760 reduced α-synuclein aggregation by 18, 17, and 18%, respectively. These results demonstrate that IPX-750 and 760 are potential candidates for Parkinson disease treatment.

Keywords: Alpha Synuclein; Dopamine; IPX-750; IPX-760; Lewy Bodies; Parkinson's Disease

Introduction

The pathological feature of Parkinson's disease is the degeneration of dopaminergic neurons in the Substantia Nigra Pars Compacta (SNpc). This loss of the nigrostriatal neurons leads to a deficiency in dopamine in the striatum and nigra, which aids in motor movement [1,2]. Other features include the depigmentation of neuromelanin in the dopaminergic neurons, increased glial cells in the nigra, and the presence of the cytoplasmic inclusions called Lewy bodies [1-3]. Parkinson's symptoms are not encountered until there is approximately an 80% reduction in dopamine and approximately a 60% decrease in dopaminergic neurons. While Parkinson's disease can develop at any age, the average age of onset is 55-60 years old. At age 60, the incidence of the disease is 20 per 100,000/year and increases to 120 per 100,000/year by age 70 [1]. Early symptoms of Parkinson's include tremors at rest, bradykinesia, and rigidity. Generally, symptoms are seen to gradually worsen with time. Parkinson's patients may also develop stooped posture, lose postural reflexes, and experience freezing (the inability to begin voluntary movement) [1-3].

Since the 1960's, the most common treatment for Parkinson's disease is levodopa/carbidopa replacement therapy [1-6]. However, levodopa therapy has multiple side-effects and crosses the Blood-Brain Barrier (BBB) poorly [1-3,7,8]. Prolonged levodopa therapy has been shown to shorten the periods of beneficial response and requires more frequent dosages. Many patients develop levodopa-related motor fluctuations ("Wearing Off" phenomena) and dyskinesias with chronic levodopa therapy [1-3,9]. Levodopa is also incapable of treating Parkinson's symptoms that develop as the disease progresses (e.g. freezing, falling, loss of postural reflexes) [1,2]. These limitations illustrate the need for drug development with greater efficacies and with decreased side-effects. Peripheral dopamine does not cross the blood-brain barrier; therefore, the brain and central nervous system must rely on endogenous dopamine synthesis [10-14]. Glycon LLC has developed a dopamine gluconamine (IPX-750) that is designed to cross the blood-brain barrier [10]. Gluconamine conjugation should increase dopamine uptake via glycosyl transporters,

dopamine transporters, and dopamine receptors in luminal cells in the GI tract, endothelial cells in the BBB, and dopaminergic neural cells in the substantia nigra [15]. Preliminary studies in three animal models have shown the gluconamine conjugation increases dopamine transport and IPX-750 exhibits biological activity at D1 and D5 receptors [15,16]. These preliminary findings indicate that IPX-750 has potent anti-parkinsonian effects [16].

While the pathogenesis of Parkinson's disease still remains to be fully defined, there is increasing evidence for the role of α-synuclein involvement in the development of Parkinson's disease. Alpha-synuclein, a small protein, is expressed at presynaptic terminals in the central nervous system [17,18]. The role of α-synuclein in healthy cells is presently not well understood; however, α-synuclein dysfunction is observed in all forms of Parkinson's disease [17-19]. Genetic and environmental factors may trigger α-synuclein dysfunction, resulting in cellular toxicity and death in dopaminergic cells [17]. Evidence suggests that cell toxicity may be induced by α-synuclein aggregation [17,18]. Therefore, due to IPX-750 having shown anti-parkinsonian effects, this study was conducted to test two hypotheses that the glycoconjugates, IPX-750 and IPX-760, will reduce α-synuclein aggregation.

Materials and Methods

Cell Culture

A cell-based Protein Complementation Assay which consists of fragments of the Gaussia Luciferase protein fused with the α-synuclein protein was used to assess the effect of IPX-750 and IPX-760 on α-synuclein aggregation. Four sets of experiments were performed. In the first set, cells were treated with IPX-750 or IPX-760 at 0, 0.1, 1, and 10μM for 24hrs followed by addition of 20μM of coelenterazine and luminescence measurement. The effect of each treatment was determined by comparing luminescence in treated with non-treated samples using Analysis of Variance (ANOVA). Means ± SEM (n=8), *$p \leq 0.01$. The second set of experiments was a repeat of the first set, except that cells were treated before α-synuclein protein expression. Cells were cultured in the presence of tetracycline, which shut down production of α-synuclein. Then media was changed to remove tetracycline. Cells were then counted and plated. IPX-750 and 760 were added to the cells using the same concentrations that were used previously (0, 0.1, 1, 10μM). Cells were incubated for 24hrs. On the next day, the cells were changed into fresh media and treated with the same treatments. The cells were then incubated for another 24hrs and luminescence assay was performed.

The third set of experiments was a repeat of the first set (i.e. cell culture in the absence of tetracycline, to ensure α-synuclein expression) but with lower concentrations of the drugs. The cells were stripped, counted, and plated. Cells were incubated for 24hrs and then treated with 0, 0.01, 0.05 and 0.1μM of IPX-750 or IPX-760. Following treatment, cells were incubated for 24hrs. The next day, luminescence assay was performed. The fourth set of experiments was a repeat of the third set, except that cells were treated before α-synuclein expression. Cells were cultured in the presence of tetracycline, which shut down production of α-synuclein. Cells were then stripped, counted plated in the absence of tetracycline but in the presence of IPX-750 and 760 using the same concentrations that were used previously (0, 0.01, 0.05, 0.1μM). Cells were incubated for 24hrs and the next day, changed into fresh media and treated with the same treatments. Cells were incubated for another 24hrs and luminescence assay was performed.

Results

The results show that IPX-750 and IPX-760 had significant effects on α-synuclein. A p-value less than or equal to 0.01 was considered significant for all experiments. The first set of experiments tested the ability of IPX-750 and IPX-760 to reduce, while the second set of experiments tested the ability of the drugs to prevent α-synuclein aggregation. The drug concentrations used were 0,0.1, 1.0and 10μM. Figure 1 shows the results of IPX-750 treatment on α-synuclein, without adding tetracycline (left) and after adding tetracycline (right). Similarly, Figure 2 shows the results of IPX-760 treatment on α-synuclein, without adding tetracycline (left) and after adding tetracycline (right). For both sets of experiments, there was a significant decrease in fluorescent units with the increase in drug concentration. This indicates a decrease in α-synuclein aggregation, as the luciferase protein illuminates only after α-synuclein aggregation (i.e. more aggregation results in more fluorescence units).

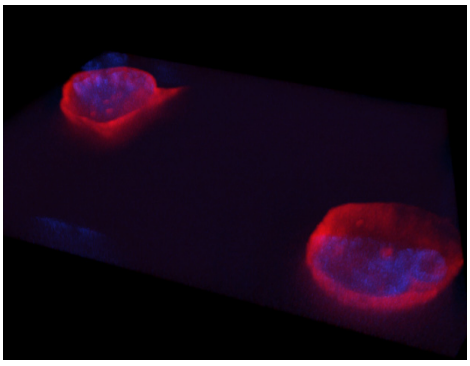

Figure 1: α-synuclein is overexpressed in neurons. A cell based neuron Tet-off bimolecular fluorescence complementation (BiFC) assay was generated by fusing the N- and C- terminal fragments of the Gaussia luciferase protein with the α-synuclein protein. Cells were cultured in media containing tetracycline. Prior to initiating experiments, tetracycline was removed to allow expression of the protein. Cells were plated on cover slips and α-synuclein was detected using immunofluorescence as described in the Methods section.

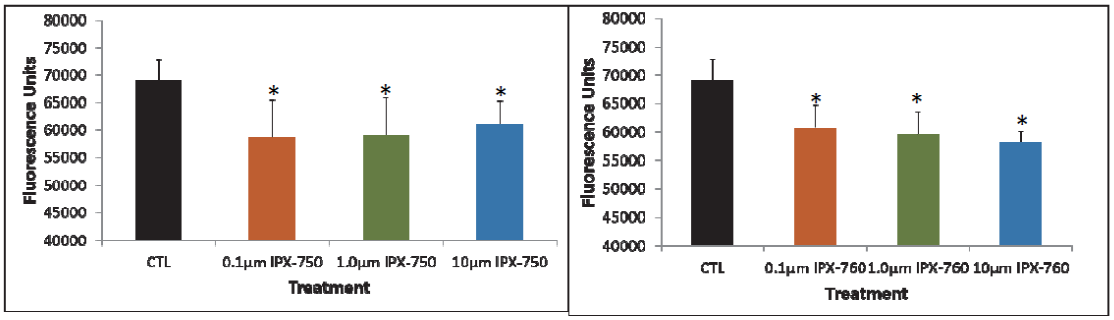

Figure 2: High concentrations of IPX-750 and 760 attenuate α-synuclein oligomerization. A cell based bimolecular fluorescence complementation (BiFC) assay which consists of fragments of the Gaussia Luciferase protein fused with the α-synuclein protein was used to assess the effect of IPX-750 and IPX-760 on α-synuclein oligomerization. Cells were treated with IPX-750 or IPX-760 at 0, 0.1, 1, or 10µM for 24hrs followed by addition of 20µM of coelenterazine and luminescence measurement. The effect of each treatment was determined by comparing luminescence in treated with non-treated samples using Analysis of Variance (ANOVA). Means ± SEM (n=8), *p ≤ 0.01.

The third and fourth set of experiments involved the treatment of the cells with 0, 0.01, 0.05 and 0.1µM IPX-750 and IPX-760, in order to test the drugs' ability to reduce α-synuclein aggregation, while the fourth set of experiments tested their ability to prevent α-synuclein aggregation. Figure 3 shows the results of IPX-750 treatment on α-synuclein, without adding tetracycline (left) and after adding tetracycline (right), and Figure 4 shows the results of IPX-760 treatment on α-synuclein, without adding tetracycline (left) and after adding tetracycline (right). As shown in the figures, fluorescent units decreased with increasing drug concentration. This shows that the drugs had a significant effect in reducing and preventing α-synuclein aggregation. Figure 3

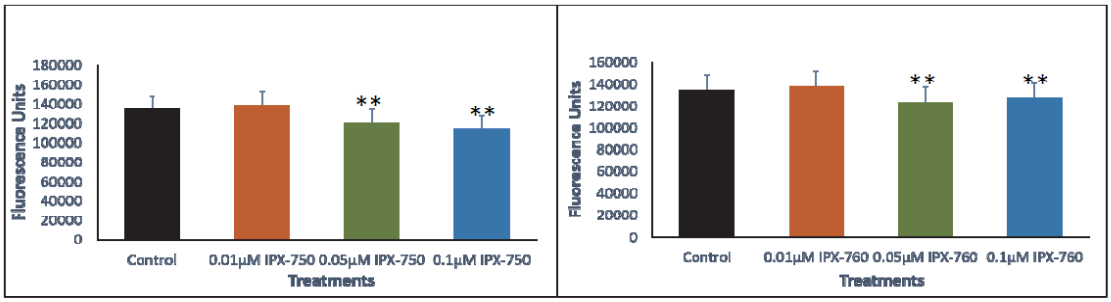

Figure 3: Low concentrations of IPX-750 and 760 attenuate α-synuclein oligomerization. A cell based bimolecular fluorescence complementation (BiFC) assay which consists of fragments of the Gaussia Luciferase protein fused with the α-synuclein protein was used to assess the effect of IPX-750 and IPX-760 on α-synuclein oligomerization. Cells were treated with IPX-750 or IPX-760 at 0, 0.01, 0.05, or 0.1µM for 24hrs followed by addition of 20µM of coelenterazine and luminescence measurement. The effect of each treatment was determined by comparing luminescence in treated with non-treated samples using Analysis of Variance (ANOVA). Means ± SEM (n=8), **p ≤ 0.001

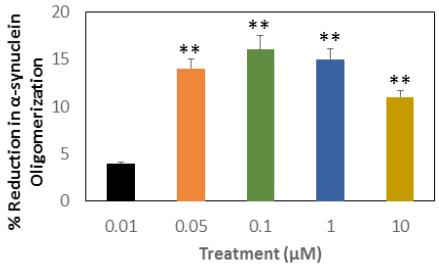

Figure 4: IPX-750 attenuates α-synuclein oligomerization. Percent reduction in α-synuclein oligomerization, a condition that leads to Parkinson disease, was measured in response to IPX-750 treatment. Cells expressing the α-synuclein protein were grown in 96 well plates and treated with IPX-750 at 0, 0.01, 0.05, 0.1, 1, and 10µM for 24hrs followed by measurement of fluorescence which was indicative of aggregation. The effect of treatment on aggregation was evaluated by comparing fluorescence in non-treated cells (0) with treated (0.01, 0.05, 0.1, 1, 10µM) cells. Data were analyzed for statistical significance using ANOVA. Values are means ±SE (n=16); ** P < 0.001 compared with non-treated (0).

Discussion

Neurodegenerative diseases such as Parkinson's Disease (PD) are characterized by the formation of protein aggregates that are initiated by the formation of soluble oligomers followed by the formation of protofibrils and insoluble fibrils [1]. The toxic effects of these structures is alleviated by the cell's Protein Quality Control System (PQCS) which is made up of folding, holding, and unfolding chaperones [2-4]. Folding chaperones mediate the folding of proteins into their 3-dimensional structures, unfolding chaperones removes proteins from soluble protein aggregates and holding chaperones hold proteins and prevent them from aggregating until folding chaperones can mediate their proper folding. Without a proper functioning PQCS, the formation of fibrillary aggregates eventually leads to cell death. α-synuclein misfolding leads to oligomerization and fibrillization that are thought to be critical events for the onset and progression of Parkinson's disease. α-synuclein fibrils are a major component of Lewy Bodies (LBs) that are the cause of neuronal death in the Substantia nigra that leads to the symptoms that are hallmarks of PD [5,6]. However, recent data show that oligomeric and prefibrillar intermediates are the more toxic species in Parkinson's disease [7,8,9].

Therefore, this study was initiated in order to study the effect of two glycoconjugates of dopamine, IPX-750 and IPX-760, on α-synuclein oligomerization. We used a bimolecular fluorescence complementation (BiFC) assay using a neuronal model for α-synuclein oligomerization [10]. This system consists of neuronal cells expressing α-synuclein proteins conjugated to either the N-terminus (1-94) or C-terminus (95-185) of the Gaussia luciferase enzyme [7,11]. Studies using this cell model of α-synuclein oligomerization demonstrate that stabilization of α-synuclein oligomers via BiFC results in increased cytotoxicity [7,8,12,13]. The oligomerization of α-synuclein in this system can be easily assessed using luminescence by incubating cells in the presence of the luciferase substrate, coelentrazene, and measuring chemiluminescence using a microplate reader.

Cells were seeded in the absence of tetracycline for 24 hours to allow expression and aggregation of α-synuclein followed by treatment with IPX-750 and IPX-760 at 0, 0.1, 1.0, and 10 μM (high) or 0, 0.01, 0.05, and 0.1 μM (low) for 24 hours. Coelentrazene was added and chemiluminescence was measured in a microplate reader. Our results showed that both glycoconugates of dopamine attenuate α-synuclein oligomerization at 0, 0.05, 0.1, 1.0 and 10 μM. This finding are significant since previous studies showed that IPX-750 crosses the blood brain barrier and IPX-750 attenuates symptoms of PD in three animal models of Parkinson's disease, OHDA-lesioned rats, MPTP-lesioned mice, and Nurr1 +/- knockout mice [14] and may represent one mechanism by which IPX-750 and perhaps IPX-760 attenuate the symptoms of PD in these animal models.

The mechanism at the molecular level for these two dopamine glycoconjugatesis not currently known. However, studies using the current cellular model system demonstrate that α-synuclein oligomer formation can be rescued by Hsp70 in a process that reduces the formation of α-synuclein oligomers. It is, therefore, possible that these two glycoconjugates could act in the same way as Hsp70 and reduce α-synuclein oligomerization [8,15]. Interestingly, mutations of the Glucocerebrosidase (GBA) gene are considered the most important risk factor for Parkinson Disease (PD). Studies show that the presence of a GBA mutation in homozygous or heterozygous form is associated with an approximately 20-fold increase in the risk for PD. Most studies suggest that 5-10% of PD patients have GBA mutations and is clinically indistinguishable from idiopathic PD, except for slightly earlier age of onset and a greater frequency of cognitive impairment. GBA mutations result in reduced enzyme activity and mutant protein may become trapped in the Endoplasmic Reticulum (ER) leading to unfolded protein response and ER associated degradation and stress. Of particular relevance to PD is the interaction of Glucocerebrosidase enzyme (GCase) with alpha-synuclein (SNCA). There appears to be a reciprocal relationship between GCase levels and those of SNCA. Thus, reduced GCase in GBA mutation PD brain is associated with increased SNCA, and increased SNCA deposition/oligomerization is associated with reduced GCase even in GBA wild-type PD brains. Finally, GBA mutations are also associated with an increased risk for dementia with Lewy bodies, another synucleinopathy.

References

1. Dauer W, Przedborski S (2003) Parkinson's disease: mechanisms and models. Neuron 39: 889-909.
2. Fahn S (2006) Levodopa in the treatment of Parkinson's disease. Journal of neural transmission. Supplementum s71: 1-15.
3. Gibbs WRG (1988) The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Ps 51: 745-752.
4. Pahwa R, Koller WC (1996) Treatment of Parkinson's disease with controlled-release Carbidopa/L-DOPA. Advances in neurology 69: 487-491.
5. Tyce GM, Dousa MK, Muenter MD (1990) MAO and L-dopa treatment of Parkinson's disease. Journal of neural transmission. Supplementum 29: 233-239.
6. Admani AK, Verma S, Cordingley GJ, Harris RI (1985) Patient benefits of l-dopa and a decarboxylase inhibitor in the treatment of Parkinson's disease in elderly patients. Pharmatherapeutica 4: 132-140.
7. Lopez Lozano JJ, Moreno Cano R (1995) Preparation of a levodopa/carbidopa solution in ascorbic acid (citridopa) and chromatographic and electrochemical assessment of its stability over 24 hours. Neurologia 10: 155-158.
8. Markham CH (1974) The "on-off" side effect of L-DOPA. AdvNeurol 5: 387-396.

9. Jankovic J (2002) Levodopa strengths and weaknesses. Neurology 58: S19-S32.

10. Fatehi MI, Gerhart DZ, Myers TG, Drewes LR (1987) Characterization of the blood-brain barrier: glycoconjugate receptors of 14 lectins in canine brain, cultured endothelial cells, and blotted membrane proteins. Brain Res 415: 30-39.

11. Walters A, Jackson-Lewis V, Fahn S (1984) Little effect of dimethyl sulfoxide on blood-brain barrier to dopamine. Experientia 40: 859-861.

12. Harris AP, Robinson R, Koehler RC, Traystman RJ, Gleason CA (2001) Blood-brain barrier permeability during dopamine-induced hypertension in fetal sheep. J Appl Physiol 91: 123-129.

13. Martel CL, Mackic JB, Adams JD Jr, McComb JG, et al. (1996) Transport of dopamine at the blood-brain barrier of the guinea pig: inhibition by psychotropic drugs and nicotine. Pharm Res 13: 290-295.

14. Hardebo JE, Edvinsson L, Owman C, Rosengren E (1977) Quantitative evaluation of the blood-brain barrier capacity to form dopamine from circulating L-DOPA. Acta Physiol Scand 99: 377-384.

15. Laine R, Unlap MT (2012) IPX-750, a dopamine gluconamine that binds D1/D5 receptors and has anti-parkinsonian effects in three animal models, is transported across the blood brain barrier. J Biotechnol Biomater 2:142.

16. Jiang C, Wan X, Jankovic J, Christian ST, Pristupa ZB, et al. (2004) Dopaminergic properties and experimental anti-parkinsonian effects of IPX750 in rodent models of Parkinson disease. Clin Neuropharmacol 27: 63-73.

17. Recchia A, Debetto P, Negro A, Guidolin D, Skaper SD, et al. (2004) α-synuclein and Parkinson's disease. FASEB 18: 617-626.

18. Cookson MR (2009) α-synuclein and neuronal cell death. Mol Neurodegener 4: 9.

19. Bartels T, Choi J G, Selkoe DJ (2011) α-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation. Nature 477: 107-110.