Meclizine-induced enhanced glycolysis is neuroprotective in Parkinson disease cell models

Chien Tai Hong¹,², Kai-Yin Chau¹ & Anthony H. V. Schapira¹

Meclizine is a well-tolerated drug routinely used as an anti-histamine agent in the management of disequilibrium. Recently, meclizine has been assessed for its neuroprotective properties in ischemic stroke and Huntington disease models. We found that meclizine protected against 6-hydroxydopamine-induced apoptosis and cell death in both SH-SY5Y cells and rat primary cortical cultures. Meclizine increases the level of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), which activates phosphofructokinase, a rate-determining enzyme of glycolysis. This protection is therefore mediated by meclizine’s ability to enhance glycolysis and increase mitochondrial hyperpolarization. Meclizine represents an interesting candidate for further investigation to re-purpose for its potential to be neuroprotective in Parkinson disease.

Parkinson disease (PD) is the second most common neurodegenerative disease¹. The pathological hallmark of PD is the degeneration of dopaminergic neurons in the midbrain substantia nigra (SN) with Lewy body deposition². Clinically, PD leads to motor and non-motor symptoms, due to both dopaminergic and non-dopaminergic deficits that result in disability and a significant reduction in quality of life³. Although several of symptomatic treatments are available, there is no therapy able to slow disease progression.

Mitochondrial dysfunction is recognized as a significant feature of PD pathogenesis⁴–⁶. In addition to their role in bioenergetics, mitochondria are involved in mediating apoptosis. Several apoptotic markers, including Bax, caspase 9 and caspase-3 have been identified in SNpc of PD⁷–¹⁰. Increased reactive oxygen species (ROS) and depolarization of the mitochondrial membrane potential (ψm) are believed to trigger the intrinsic apoptotic pathway by increasing the mitochondrial outer membrane permeability (MOMP). Release of mitochondrial proteins including cytochrome c, takes place after MOMP and initiate the apoptotic cell death cascade¹¹.

In the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and β-amyloid toxicity models, increased glycolysis has been suggested to be able to restore cellular ATP synthesis, control ROS production, and maintain ψm in order to protect cell death¹²–¹⁷. Meclizine, is a widely-used antiemetic, and has been shown to enhance glycolysis and protect against neuronal death in stroke and Huntington disease models¹⁸,¹⁹.

In the present study, we demonstrate the neuroprotective effect of meclizine in cell models of PD. Our data show that the protective mechanism of meclizine involves increased glycolysis without altering oxidative phosphorylation and total ATP levels, the maintenance of mitochondrial hyperpolarization and the inhibition of apoptosis. We found that meclizine enhances glycolysis by increasing the activity 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) activity, which mediates the synthesis of fructose 2,6-bisphosphate to activate phosphofructokinase.

Results

Meclizine protected 6-OHDA induced apoptosis and cell death in primary cortical neurons. The protection of meclizine was tested in primary rat cortical cultures highly enriched with neurons (supplementary S1). 6-OHDA induced a dose-dependent increase of Fluoro-jade C (FJ-C) stain, which reflected the neuronal death (supplementary S2A,B). The concentration of 10μM 6-OHDA was chosen because of remarkable but not overwhelming effect of cell death (21.10 ± 5.37% FJ-C stained cells compared with no-toxin control: 6.65 ± 0.67% FJ-C stained cells). Compared with control, 3.125μM of meclizine treatment, which was determined by the dose-dependent experiments of the protection of meclizine against 6-OHDA (supplementary S2C), for 24 hours did not increase the neuronal death detected by FJ-C stain. Upon 10μM of 6-OHDA

¹Department of Clinical Neurosciences, UCL Institute of Neurology, University College London, UK. ²Department of Neurology, Shuang Ho Hospital, Taipei Medical University Taiwan. Correspondence and requests for materials should be addressed to A.H.V.S. (email: a.schapira@ucl.ac.uk)
treatment for 24 hours, 3.125 μM meclizine significantly reduced the neuronal death release from 20.38 ± 1.57% to 12.68 ± 0.74% (p < 0.001) (Fig. 1A). Meclizine was applied at the same time with 6-OHDA. (N.S., non-significant, ***p < 0.001). (B) In primary rat cortical cultures enriched with neurons treated with 10 μM 6-OHDA for 24 hours, 3.125 μM meclizine co-treatment significantly protected against cytotoxicity (control: 10.8 ± 1.4%, meclizine: 6.8 ± 0.9%, *p < 0.05, n = 8). Meclizine alone did not produce extra cell death. (C) The protection of meclizine against 10 μM 6-OHDA treatment on rat primary cortical culture cells for 24 hours was confirmed by PI binding assay (control: 11.5 ± 0.7%, meclizine: 6.6 ± 0.7%, p < 0.001, n = 10). Data were presented as mean ± S.E.M. and statistic analysis was performed by two-tailed Student's t-test. (N.S., non-significant, ***p < 0.001). (D) In primary rat cortical cultures treated with 20 μM 6-OHDA for 6 hours, pre-treatment with 3.125 μM meclizine for 24 hours significantly decreased the percentage of apoptotic cells induced by 6-OHDA (control: 12.4 ± 0.6%, meclizine: 8.8 ± 0.4%, ***p < 0.001, n = 10).

Meclizine protected apoptosis and death in PD cellular model. SH-SY5Y cells are widely used as a dopaminergic cell model and express the dopamine transporter and dopamine synthesis capacity. Meclizine treatment alone up to 12.5 μM for 48 hours did not induce cell death in SH-SY5Y cells. Upon 30 μM of 6-OHDA treatment for 48 hours, co-treatment with an increasing level of meclizine up to 12.5 μM produced a dose-dependent reduction of LDH release in SH-SY5Y cells; this became significant at 3.125 μM and 12.5 μM, from 20.6 ± 1.2% to 13.3 ± 0.6 and 12.2 ± 0.4% respectively (Fig. 2A). The anti-apoptotic effect of meclizine was also evaluated in SH-SY5Y cells. Meclizine treatment at 12.5 μM for 24 hours alone did not alter the caspase-3 activity. SH-SY5Y cells pre-treated with 12.5 μM of meclizine for 24 hours significantly down-regulated the activation of caspase-3 induced by 6-OHDA by 19% (Fig. 2B).

Meclizine hyperpolarized mitochondria and prevented the depolarization induced by 6-OHDA. 6-OHDA causes increased ROS production and this can be measured as a significant fall in

---

**Figure 1.** Meclizine protected 6-hydroxydopamine (6-OHDA) induced apoptosis and death in primary rat cortical cultures. (A) primary rat cortical cultures enriched with neurons, 3.125 μM meclizine treatment for 24 hours did not increase the spontaneous neuronal death but significantly reduced the percentage of FJC positive cells against 10 μM 6-OHDA treatment for 24 hours (control: 20.4 ± 1.6%, meclizine: 12.7 ± 0.7%, p < 0.001, n = 10). Meclizine was applied at the same time with 6-OHDA. (N.S., non-significant, ***p < 0.001). (B) In primary rat cortical cultures enriched with neurons treated with 10 μM 6-OHDA for 24 hours, 3.125 μM meclizine co-treatment significantly protected against cytotoxicity (control: 10.8 ± 1.4%, meclizine: 6.8 ± 0.9%, *p < 0.05, n = 8). Meclizine alone did not produce extra cell death. (C) The protection of meclizine against 10 μM 6-OHDA treatment on rat primary cortical culture cells for 24 hours was confirmed by PI binding assay (control: 11.5 ± 0.7%, meclizine: 6.6 ± 0.7%, p < 0.001, n = 10). Data were presented as mean ± S.E.M. and statistic analysis was performed by two-tailed Student's t-test. (N.S., non-significant, ***p < 0.001). (D) In primary rat cortical cultures treated with 20 μM 6-OHDA for 6 hours, pre-treatment with 3.125 μM meclizine for 24 hours significantly decreased the percentage of apoptotic cells induced by 6-OHDA (control: 12.4 ± 0.6%, meclizine: 8.8 ± 0.4%, ***p < 0.001, n = 10).
aconitase activity (see supplementary S3)\(^2\). Meclizine's mechanism of action did not include any protection of aconitase, implying that it is not an anti-oxidant (supplementary S4). 6-OHDA also causes a fall in $\psi_m$ and prevention of this is considered anti-apoptotic\(^{12,22}\). $\psi_m$ was measured by TMRM fluorescence with adjustment for mitochondrial content. In rat primary cortical neurons, we found that 1 $\mu$M of meclizine treatment for 48 hours increased net fluorescence of TMRM compared with control, which reflects the mitochondrial hyperpolarization. Meanwhile, meclizine treatment on rat primary cortical neurons prevented 6-OHDA induced depolarization on rat primary cortical neurons, which would favour an anti-apoptotic action (Fig. 3A,B). In similar, we noticed that meclizine at 12.5 $\mu$M not only induced a 32% increase (p < 0.001) in $\psi_m$ in SH-SY5Y cells, but also prevented 100 $\mu$M 6-OHDA treatment for one hour induced mitochondrial depolarization and maintained $\psi_m$ at basal levels (Fig. 3C,D).

Mitochondrial hyperpolarization and anti-apoptotic effect of meclizine are due to increased glycolysis. SH-SY5Y cells treated with 12.5 $\mu$M meclizine for 48 hours significantly increased the glycolytic activity as measured by extracellular acidification rate, by 157% (p < 0.01) (Fig. 4A). This was confirmed by the use of glycolytic inhibitors 2-deoxy-D-glucose (2DG) and 3-bromopyruvate (3BP) that blocked the meclizine-induced increase in glycolysis (Fig. 4A). The increase in glycolysis did not affect oxidative phosphorylation or total ATP levels (supplementary S4).

10 $\mu$M of 2DG or 5 $\mu$M of 3BP prevented the mitochondrial hyperpolarization seen with meclizine treatment (Fig. 4B). Lastly, we investigated whether the meclizine-mediated neuroprotection against 6-OHDA is glycolysis-dependent in SH-SY5Y cells. LDH release measurements showed that 10 $\mu$M of 2DG or 5 $\mu$M of 3BP did not alter the cytotoxicity produced by 6-OHDA at 30 $\mu$M treated for 48 hours. In line with the data shown in Fig. 1A, co-treatment with 12.5 $\mu$M meclizine significantly decreased the LDH release induced by 6-OHDA. However, co-administration with the glycolytic inhibitors resulted in significant attenuation of protection against 6-OHDA (Fig. 4C).

Meclizine enhanced glycolysis through increased levels of PFKFB3. An increase in glycolysis may be HIF-1$\alpha$-dependent, but levels of this protein were not increased with meclizine treatment (Fig. 5A). Alternatively, increased expression of glycolytic enzymes can also enhance glycolysis, but we found that 12.5 $\mu$M meclizine treatment for 48 hours did not alter the protein levels of: HK1, HK2, PFK and PKM1/2 (Fig. 5B).
PFKFB is a bifunctional enzyme that can catalyze the conversion of fructose-6-phosphate to fructose 2,6-bisphosphate (F2,6 P2) and vice versa. PFKFB Isoform 3 predominately effects the synthesis of F2,6 P2, which is a powerful allosteric activator of PFKP and glycolysis23. 12.5 μM meclizine treatment for 48 hours significantly increased the protein level of 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) in SH-SY5Y cells (139.5 ± 12.3%, p < 0.01). 100 μM 6-OHDA treatment for 6 hours on control SH-SY5Y cells resulted in a reduction of PFKFB3 protein level (96.3 ± 6.0%) while pre-treatment of 12.5 μM meclizine for 48 hours prevented the reduction of PFKFB3 protein level caused by 6-OHDA (106.2 ± 5.5%) (Fig. 5C,D). These effect was seen on rat primary cortical neurons as well (Supplementary S5).

Discussion
The present study demonstrates that meclizine, a glycolysis-enhancing agent, protects neuronal death in the 6-OHDA cell toxicity model. Meclizine hyperpolarizes mitochondria to prevent 6-OHDA induced apoptosis and mitochondrial depolarization and these effects are reduced by inhibition of meclizine-induced glycolysis. Meclizine increases glycolysis through increased levels of PFKFB3, which activates the key enzyme, PFK to enhance glycolysis.

Glycolysis is less efficient for ATP synthesis than oxidative phosphorylation. Neurons predominantly rely on oxidative phosphorylation for ATP generation and are not able to up-regulate glycolysis under respiratory inhibition24. However, it has been reported that glycolysis is responsible for neuronal vesicle transport in axonal
regions\textsuperscript{25}. In the present study, meclizine increases glycolysis but does not induce apoptosis and cell death in neuronal cells, which also support the notion that increasing glucose metabolism by glycolysis does not compromise the neuronal cells.

In the present study, meclizine increased cellular glycolysis, hyperpolarized mitochondria and protected against 6-OHDA toxicity. These effects were prevented by glycolysis inhibitors. Although hyperpolarization is also able to result from complex V inhibition, it is unlikely that meclizine hyperpolarizes mitochondria through this mechanism because complex V inhibition blocks oxidative phosphorylation and is toxic to the cells, whereas we saw no cytotoxicity upon meclizine treatment.

6-OHDA induces oxidative damage and apoptotic cell death and is widely-used in experimental PD models. Mitochondrial depolarization is a universal and early phenomenon of 6-OHDA induced apoptosis\textsuperscript{26-28}. Meclizine enhanced glycolysis by increasing levels of PFKFB3 and protects against the fall in $\psi_m$ induced by 6-OHDA treatment and initiation of the caspase cascade.

We have demonstrated that the anti-emetic meclizine increases glycolysis by increasing the levels of PFKFB3. This shift in energy metabolism is able to protect against the fall in $\psi_m$ induced by 6-OHDA and reduce apoptotic cell death. We suggest that these results support further evaluation and potential re-purposing of meclizine as a modulator of energy metabolism for neuroprotection in PD.

**Material and Methods**

**Cell models and treatments.** SH-SY5Y cells were maintained as previously described\textsuperscript{29} with reagents supplied from Life Technologies (Paisley, UK). The method for setting up E18 primary rat cortical cultures enriched with neurons was adapted from previous well-established protocols\textsuperscript{30}, and they were used 7 days post-culture. Meclizine was obtained from 2 independent sources namely Tocris Bioscience (Bristol, UK) and Santa Cruz Biotechnology (Heidelberg, Germany), and was dissolved in DMSO to produce a 12.5 mM stock. 6-hydroxydopamine (6-OHDA) was purchased from Sigma-Aldrich (Dorset, UK).
Protein analysis. Whole cell lysates for Western blot analysis were prepared in 10 mM Tris–HCl pH 7.5, 0.1% of SDS and in the presence of protease inhibitors (Fisher Scientific, Loughborough, UK), followed by DNAse I digestion (Promega, Southampton, UK). Protein samples were separated under reducing conditions by SDS-PAGE using the Novex system (Life Technologies), transferred to PVDF (Millipore, Watford, UK) and were analyzed by a standard Western blot protocol using Amersham ECL Western Blotting Detection Reagent and Amersham Hyperfilm (GE Healthcare, Little Chalfont, UK). The following antibodies were used: anti-PFKFB3 antibody, anti-hypoxia inducible factor (HIF)-1α antibody, anti-phosphofructokinase (PFK) antibody, anti-pyruvate kinase (PK) antibody, and hexokinase 1 and 2 antibody (HK1 & 2) (New England Biolabs, Hitchin, UK). Expression level of the above targets was corrected by the level of β-actin and normalized to non-treatment (NT) that is set as 100%.

Protein concentration was determined by bicinchoninic acid assay (BCA) according to manufacturer's instructions (Fisher Scientific).

Apoptosis and cell death analysis. Caspase-3 activity was measured from cell lysates according to the manufacturer's instruction of the EnzChek® Caspase-3 Assay Kit #2 (Life Technologies) which has been described previously31. The caspase-3 activity was corrected by the total protein level of the cell lysate and normalized to non-treatment (NT) that is set as 100%.

Lactate dehydrogenase (LDH) release assay (Roche, Burgess Hill, UK) was used to quantify the cell death and the protocols had been described32. The percentage LDH release was obtained from the experimental to the Triton X-100 treated total release. For the detection of neuronal death in primary culture, cells were stained with 0.001% Fluoro-Jade C (FJ-C)(Millipore) after fixed with 4% paraformaldehyde. Positive stained cells were counted under an epifluorescent microscope by examiner blinded to the condition.

Ψm analysis. Maximal steady state mitochondrial fluorescence upon Tetramethylrhodamine, methyl ester (TMRM, Life Technologies) staining at 25 nM was measured from <1.5μm confocal images obtained from the Zeiss 510 laser scanning microscope after z-projection, as previously described33,34. Images obtained from SH-SY5Y cells were analyzed by ImageJ software (National Institutes of Health, Maryland, USA) following the procedure described34.

Figure 5. Increased glycolysis upon meclizine treatment was associated with increased PFKFB3 in SH-SY5Y cells. (A) Representative Western blot images demonstrate that meclizine treatment at 12.5 μM for 48 hours did not increase the protein level of hypoxia-inducible factor 1α (HIF-1α). (B) Representative Western blot images support that meclizine treatment at 12.5 μM for 48 hours did not alter the protein level of certain key glycolytic enzymes, including hexokinase 1 (HK1), hexokinase 2 (HK2), phosphofructokinase (PFK), and pyruvate kinase isozymes M1/M2 (PKM1/2). (C,D) Representative Western blot images and densitometry analysis revealed that 12.5 μM meclizine treatment for 48 hours significantly increased the protein level of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) in SH-SY5Y cells (139.5 ± 12.3%, p < 0.01). 100 μM 6-OHDA treatment for 6 hours on control SH-SY5Y cells resulted in a slight reduction of PFKFB3 protein level (96.3 ± 6.0%). Pre-treatment of meclizine prevents the reduction of PFKFB3 protein level caused by 6-OHDA (106.2 ± 5.5%). The protein expression amount was normalized by control SH-SY5Y cells. Data were presented as mean ± S.E.M. and the n = 6, **p < 0.01.
previously described analysis protocols\(^5\). Briefly, raw images were background corrected, linearly contrast optimized, applied with a \(7 \times 7\) ‘top hat’ filter, subjected to twice \(3 \times 3\) median filter, and then threshold, to generate binary images. Mitochondrial content per cell was estimated based on mitochondrial occupancy of these binary images. Mitochondrial fluorescence of each cell from the background corrected images was corrected by the mitochondrial content to produce the \(\Psi_{m}\).

For the rat primary cortical neurons, net TMRM fluorescence is obtained by the total TMRM fluorescence minus the background TMRM fluorescence.

Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR).

OCR and ECAR were measured by the XF extracellular flux analyzer following manufacturer’s instructions (Seahorse Bioscience, MA, US). For measuring OCR, 25 mM glucose, 1\(\mu\)g/ml oligomycin and 2\(\mu\)M rotenone were loading sequentially whereas for ECAR, 25 mM glucose, 1\(\mu\)g/ml oligomycin and 100\(\mu\)M 2DG were applied.

**Aconitase assay.** Aconitase enzymatic activities were measured as described\(^6\), subsequently activity was corrected by protein amount.

**Total ATP measurement.** Total ATP was measured using the ATP Bioluminesence Assay kit CLSII (Roche, Mannheim, Germany), from the cell lysates and that was corrected by the protein level.

**Statistics.** All data were presented as mean ± standard error of mean (S.E.M.). Statistics was performed by either two-tailed Student’s t-test or one-way ANOVA with post-hoc analysis. \(p\) value less than 0.05 is considered as statistically significant.

**References**

1. de Lau, L. M. & Breteler, M. M. Epidemiology of Parkinson’s disease. *Lancet Neurol.* 5, 525–535, doi: 10.1016/S1474-4422(06)70471-9 (2006).

2. Lewis, P. D. Parkinsonism—neuropathology. *Br Med J* 3, 690–692 (1971).

3. Rahman, S. et al. Quality of life in Parkinson’s disease: the relative importance of the symptoms. *Mov Disord.* 23, 1438–1434, doi: 10.1002/mds.21667 (2008).

4. Parker, W. D. Jr., Parks, J. K. & Swerdlow, R. H. Complex I deficiency in Parkinson’s disease. *Brain Res.* 1189, 215–218, doi: 10.1016/j.brainres.2007.10.061 (2008).

5. Schapira, A. H. et al. Mitochondrial complex I deficiency in Parkinson’s disease. *Lancet* 333, 1269, doi: 10.1016/S0140-6736(89)92366-0 (1989).

6. Schapira, A. H. Mitochondrial membrane permeability transition and cell death. *Biochim. Biophys. Acta* 1757, 1297–1300, doi: 10.1016/j.bbadis.2006.03.017 (2006).

7. Bonnet, S. et al. A mitochondria-K⁺ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. *Cancer Cell* 11, 37–51, doi: 10.1016/j.ccc.2006.10.020 (2007).

8. Heidt, B. G., Houston, M. A. & Augenlicht, L. H. The intrinsic mitochondrial membrane potential of colon cancer cells is linked to the probability of tumor progression. *Cancer Res.* 65(21), 9861–7, doi: 10.1158/0008-5472.CAN-05-2444 (2005).

9. Mogi, M. et al. Caspase activities and tumor necrosis factor receptor R1 (p55) level are elevated in the substantia nigra from parkinsonian brain. *J. Neural Transm.* 107(3), 335–341 (2000).

10. Tatton, N. A. Increased caspase 3 and Bax immunoactivity accompany nuclear GAPDH translocation and neuronal apoptosis in Parkinson’s disease. *Exp. Neurol.* 166, 29–43, doi: 10.1006/exnr.2000.7489 (2000).

11. Tsujimoto, Y., Nakagawa, T. & Shimizu, S. Mitochondrial membrane permeability transition and cell death. *Biochim. Biophys. Acta* 1757, 1297–1300, doi: 10.1016/j.bbadis.2006.03.017 (2006).

12. Williams, Z. R., Goodman, C. R. & Soliman, K. F. Anaerobic glycolysis protection against 1-methyl-4-phenylpyridinium toxicity through anaerobic glycolysis in neuroblastoma cells. *Brain Res.* 962, 48–60, doi: 10.1016/S0006-8993(02)03695-8 (2003).

13. Newington, J. T. et al. Amyloid beta resistance in nerve cell lines is mediated by the Warburg effect. *PLoS One.* 6, e19191, doi: 10.1371/journal.pone.0019191 (2011).

14. Newington, J. T. et al. Overexpression of pyruvate dehydrogenase kinase 1 and lactate dehydrogenase A in nerve cells confers resistance to amyloid beta and other toxins by decreasing mitochondrial respiration and reactive oxygen species production. *J. Biol. Chem.* 287, 37245–37258, doi: 10.1074/jbc.M111.266195 (2012).

15. Gohil, V. M. et al. Nutrient-sensitized screening for drugs that shift energy metabolism from mitochondrial respiration to glycolysis. *Nat. Biotechnol.* 28, 249–255, doi: 10.1038/nbt.1606 (2010).

16. Gohil, V. M. et al. Medlines is neuroprotective in models of Huntington’s disease. *Hum. Mol. Genet.* 20, 294–300, doi: 10.1093/hmg/ddq464 (2011).

17. Gomez-Lazaro, M. et al. 6-Hydroxydopamine activates the mitochondrial apoptosis pathway through p38 MAPK-mediated, p53-independent activation of Bax and PUMA. *J. Neurochem.* 104, doi: 10.1111/j.1471-4159.2007.05115.x, 1599–161 (2008).

18. Kuch, M. S., Horbinski, C., Patel, M. & Chu, C. T. 6-Hydroxydopamine induces mitochondrial ERK activation. *Free Radic. Biol. Med.* 43, 372–383, doi: 10.1016/j.freeradbiomed.2007.04.028 (2007).

19. Kroemer, G., Galluzzi, L. & Brenner, C. Mitochondrial membrane permeabilization in cell death. *Physiol Rev.* 87, 99–163, doi: 10.1152/physrev.00123.2007 (2007).

20. Bolanos, J. P., Almeida, A. & Moncada, S. Glycolysis: a bioenergetic or a survival pathway? *Trends Biochem. Sci.* 35, 145–149, doi: 10.1016/j.tibs.2009.10.006 (2010).

21. Almeida, A., Almeida, J., Bolanos, J. P. & Moncada, S. Different responses of astrocytes and neurons to nitric oxide: the role of glycolytically generated ATP in astrocyte protection. *PNAS* 98, 15284–15289, doi: 10.1073/pnas.201560998 (2001).

22. Zala, D. et al. Vesicular glycolysis provides on-board energy for fast axonal transport. *Cell 152*, 479–91, doi: 10.1016/j.cell.2012.12.029 (2013).

23. Lopes, F. M. et al. Comparison between proliferative and neuron-like SH-SYSY cells as an in vitro model for Parkinson disease studies. *Brain Res.* 1337, 85–94, doi: 10.1016/j.brainres.2010.03.102 (2010).
27. Tirmenstein, M. A. et al. Effects of 6-hydroxydopamine on mitochondrial function and glutathione status in SH-SY5Y human neuroblastoma cells. *Toxicol. In Vitro* **19**, 471–479, doi: 10.1016/j.tiv.2005.01.006 (2005).
28. Tovilovic, G. et al. Arylpiperazine-mediated activation of Akt protects SH-SY5Y neuroblastoma cells from 6-hydroxydopamine-induced apoptotic and autophagic death. *Neuropharmacology* **72**, 224–235, doi: 10.1016/j.neuropharm.2013.04.037 (2013).
29. Chau, K. Y., Korlipara, L. V., Cooper, J. M. & Schapira, A. H. Protection against paraquat and A53T alpha-synuclein toxicity by cabergoline is partially mediated by dopamine receptors. *J. Neurol. Sci.* **278**, 44–53, doi: 10.1016/j.jns.2008.11.012 (2009).
30. Xu, S. Y. et al. A modified technique for culturing primary fetal rat cortical neurons. *J. Biomed. Biotechnol.* **2012**, 803930, doi: 10.1155/2012/803930 (2012).
31. Chau, K. Y., Cooper, J. M. & Schapira, A. H. Rasagiline protects against alpha-synuclein induced sensitivity to oxidative stress in dopaminergic cells. *Neurochem. Int.* **57**, 525–9, doi: 10.1016/j.neuint.2010.06.017 (2010).
32. Cleeter, M. W. et al. Glucocerebrosidase inhibition causes mitochondrial dysfunction and free radical damage. *Neurochem. Int.* **62**, 1–7, doi: 10.1016/j.neuint.2012.10.010 (2013).
33. Duchen, M. R., Surin, A. & Jacobson, J. Imaging mitochondrial function in intact cells. *Methods Enzymol.* **361**, 35–389 (2003).
34. Gandhi, S. et al. PINK1-associated Parkinson's disease is caused by neuronal vulnerability to calcium-induced cell death. *Mol. Cell* **33**, 627–638, doi: 10.1016/j.molcel.2009.02.013 (2009).
35. Koopman, W. J., Visch, H. J., Smeitink, J. A. & Willems, P. H. Simultaneous quantitative measurement and automated analysis of mitochondrial morphology, mass, potential, and motility in living human skin fibroblasts. *Cytometry A* **69**, 1–12, doi: 10.1002/cyto.a.20198 (2006).
36. Gu, M. et al. Pramipexole protects against apoptotic cell death by non-dopaminergic mechanisms. *J. Neurochem.* **91**, 1075–1081, doi: 10.1111/j.1471-4159.2004.02804.x (2004).

**Acknowledgements**

This work was supported by the Wellcome Trust/Medical Research Council (MRC) Joint Call in Neurodegeneration award (WT089698). We also acknowledge the support of the Kattan Trust and Taiwan Minister of Education. AHVS is a NIHR Senior Investigator and is supported by the NIHR BRC grant to UCLH.

**Author Contributions**

Author C.T.H. study design, experiment conduct (all Figs except 3A,B and 5C,D), manuscript drafting K.-Y.C. study design, experiment conduct (Figs 3A,B and 5C,D), manuscript drafting A.H.V.S. study design, manuscript revision.

**Additional Information**

Supplementary information accompanies this paper at http://www.nature.com/srep

**Competing financial interests:** The authors declare no competing financial interests.

**How to cite this article:** Hong, C. T. et al. Meclizine–induced enhanced glycolysis is neuroprotective in Parkinson disease cell models. *Sci. Rep.* **6**, **25344**: doi: 10.1038/srep25344 (2016).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/