It has been demonstrated that blocking and attenuation of neurodegeneration in α-synuclein models occurs with curcumin (AS3T, A30P, E46K) and α-synuclein multiplications (Gorman, 2008; Yu and Lyubchenko, 2009), as well as neurotoxin-related mitochondrial dysfunction (Sherer et al., 2003) are important in the biology of Parkinson’s disease (PD). Recently, defective degradation of the abnormal proteins has emerged as the leading mechanism of cell death in PD and other neurodegenerative disorders (Pan et al., 2009; Ramí, 2009). Autophagy is involved not only in clearing misfolded proteins but also in injured mitochondria (Rubinsztein, 2006). Inhibition of autophagy may cause the increase of mitochondrial load, such as mitochondrial complex IV and cytochrome c, which may be protective for PD (Pan et al., 2009; Ramí, 2007), or cause the delay in the clearance of misfolded proteins (Hara et al., 2006). We have recently reported that compounds clearing misfolded proteins or injured mitochondria may be protective for PD (Pan et al., 2009; Wu et al., 2010).

Curcumin (1,7-bis[4-hydroxy-3-methoxy phenyl]-1,6-hepadiene-3,5-dione), is a widely studied natural phenolic nonsteroidal compound with a variety of biological activities (Cancer Res., 2009). A recent report of the anti-apoptotic effects of curcumin have been reported (Chen et al., 2008) and the neuroprotective properties of curcumin have also been demonstrated in the toxin model of PD (Zbarsky et al., 2005). Additionally, it has been reported that curcumin has inhibitory effects on the aggregation of α-synuclein (Pandey et al., 2008). Recent reports that curcumin induces autophagy in malignant glioma cells (Aoki et al., 2007) led us to hypothesize that curcumin may have beneficial effects on PD via enhanced clearance of injured mitochondrial and α-synuclein through autophagy induction.

In this study, we explored the role of curcumin in the induction of autophagy and determined neuroprotective effects of curcumin on mitochondrial complex I inhibitor 1-methyl-4-phenylpyridinium ions (MPP+)-induced apoptosis and on the degradation of α-synuclein.

Stable inducible PC12 cell lines expressing HA-tagged A30P, A33T mutant or wild-type α-synuclein respectively (kind gift of professor David Rubinsztein from Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge), were maintained at Dulbecco’s modified Eagle’s medium (DMEM) containing 10% horse serum, 5% fetal bovine serum, 75 µg/ml hygromycin B, and 100 µg/ml G418 at 37°C, 10% CO2. The expression of transgenes including A30P, A33T mutant or wild-type α-synuclein from Department of Medical Genetics, Cambridge Institute for Medical Research, University of Cambridge (kind gift of professor David Rubinsztein) were differentiated with NGF (100 ng/ml) for 5 days followed by addition of MPP+ (500 µM) for another 24 h. Total proteins were isolated and subjected to immunoblotting assay. The expression of transgene (Fig. 2A) and the increase of histone-associated DNA fragments as determined by ELISA assay (Fig. 4B), which was partially blocked by curcumin pretreatment (Fig. 4A, 4B). Moreover, the neuroprotective effect of curcumin on MPP+-induced apoptosis was inhibited by autophagy inhibitor 3MA (Fig. 4A, 4B). Additionally, we found that curcumin treatment decreased mitochondrial complex IV and cytochrome c as determined by immunoblotting assay (Fig. 4C), suggesting that curcumin may enhance the degradation of mitochondrial α-synuclein.

Furthermore, immunoblotting assay demonstrated that curcumin caused inhibition of mTOR activity by showing the reduction of its downstream proteins, such as p-70S6K, S6 and p-4E-BP, suggesting that mTOR signaling pathway is involved in the induction of autophagy by curcumin.

Our results showed that the induction of autophagy by curcumin was concentration-dependent and that the role of curcumin in protecting against MPP+-induced apoptosis and in accelerating the clearance of mutant α-synucleins is possibly through the induction of autophagy. Our findings suggest a potential therapeutic role of curcumin in PD.

References