Biochemical and Biophysical Research Communications 451 (2014) 142–147

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

1,25-Dyhydroxyvitamin D_3 attenuates rotenone-induced neurotoxicity in SH-SY5Y cells through induction of autophagy

Wooyoung Jang [a,*,1], Hee Ju Kim [b,1], Huan Li [b], Kwang Deog Jo [a], Moon Kyu Lee [a], Sun Hong Song [c], Hyun Ok Yang [b,*]

[a] Department of Neurology, Gangneung Asan Hospital, University of Ulsan College of Medicine, Bangdong-ri, Sacheon-myeon, Gangneung-si, Gangwon-do 210-711, Republic of Korea
[b] Natural Medicine Center, Korea Institute of Science and Technology, Gangneung 210-340, Republic of Korea
[c] Department of Rehabilitation Medicine, Gangneung Asan Hospital, University of Ulsan College of Medicine, Bangdong-ri, Sacheon-myeon, Gangneung-si, Gangwon-do 210-711, Republic of Korea

ARTICLE INFO

Article history:
Received 12 July 2014
Available online 29 July 2014

Keywords:
Calcitriol
Parkinson's disease
Autophagy
Neuroprotection

ABSTRACT

Background and objectives: Dysregulation of the autophagy pathway has been suggested as an important mechanism in the pathogenesis of Parkinson's disease (PD). Therefore, modulation of autophagy may be a novel strategy for the treatment of PD. Recently, an active form of vitamin D_3 has been reported to have neuroprotective properties. Therefore, we investigated the protective, autophagy-modulating effects of 1,25-dyhydroxyvitamin D_3 (calcitriol) in an in vitro model of Parkinson's disease.
Methods: An in vitro model of Parkinson's disease, the rotenone-induced neurotoxicity model in SH-SY5Y cells was adapted. We measured cell viability using an MTT assay, Annexin V/propidium iodide assay, and intracellular reactive oxygen species levels and analyzed autophagy-associated intracellular signaling proteins by Western blotting.
Results: Rotenone treatment of SH-SY5Y cells reduced their viability. This treatment also increased reactive oxygen species levels and decreased levels of intracellular signaling proteins associated with cell survival; simultaneous exposure to calcitriol significantly reversed these effects. Additionally, calcitriol increased levels of autophagy markers, including LC3, beclin-1, and AMPK. Rotenone inhibited autophagy, as indicated by decreased beclin-1 levels and increased mTOR levels, and this effect was reversed by calcitriol treatment.
Discussion: Calcitriol protects against rotenone-induced neurotoxicity in SH-SY5Y cells by enhancing autophagy signaling pathways such as those involving LC3 and beclin-1. These neuroprotective effects of calcitriol against rotenone-induced dopaminergic neurotoxicity provide an experimental basis for its clinical use in the treatment of PD.

1. Introduction

Parkinson's disease (PD) is a relatively common degenerative disorder characterized by resting tremors, rigidity, bradykinesia, and a loss of postural reflex and is associated with functional disability and a reduced quality of life in the elderly [1]. Although dopaminergic medications, including L-3,4-dihydrophenylalanine (L-DOPA) and dopamine agonists, remain the standard treatment for PD, they only provide symptomatic treatment without slowing or stopping the progression of the disease [1–3]. Furthermore, experts continue to debate whether L-DOPA is detrimental to dopaminergic neurons or could even accelerate the degeneration process [4,5]. Therefore, the development of neuroprotective therapies that retard, halt, or reverse disease progression is essential for the treatment of PD patients.

The pathogenesis of PD is likely multifactorial, including genetic and environmental factors. Cell dysfunction and death in PD also involve several biochemical factors, such as free radicals, mitochondrial dysfunction, excitotoxicity, and inflammation [6,7]. Of particular relevance, abnormal protein degradation is associated with Lewy bodies, which are a hallmark pathologic feature of PD [8].

Autophagy is a lysosome-mediated catabolic pathway that is responsible for the bulk degradation of cytosolic proteins and

* Corresponding authors. Fax: +82 31 660 3197 (W. Jang). Fax: +82 33 650 3529 (H.O. Yang).
E-mail addresses: neveu@gnah.co.kr (W. Jang), hoyang@kist.re.kr (H.O. Yang).
[1] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.07.081
0006-291X/

organelles in a non-specific manner [9,10]. Recently, dysregulation of the autophagy pathway has been reported in the brains of PD patients and in animal models of PD [10–12]. Therefore, modulation of autophagy may be a novel strategy for the treatment of PD.

1,25-Dyhydroxyvitamin D_3 (hereafter, calcitriol), which is a biologically active form of vitamin D_3, is known to be a primary regulator of calcium homeostasis that affects bone metabolism [13]. However, the broad distribution of the vitamin D receptor (VDR) suggests that calcitriol may have additional functions beyond calcium homeostasis, and recent evidence has demonstrated that calcitriol has effects in several neurological disorders [14–16]. The activation of VDR signaling affects various processes, including calcium metabolism, apoptosis, inflammation, immunomodulation, and detoxification [17–20]. In addition, vitamin D_3 signaling is associated with the regulation of autophagy through various mechanisms [21,22].

Because calcitriol plays a critical role in regulating autophagy to prevent the accumulation of misfolded proteins and the degeneration of dopaminergic neurons, we hypothesized that calcitriol may reduce rotenone-induced neurotoxicity in SH-SY2Y cells as an in vitro model of PD. In the current study, we investigated the concentration-dependent effects of calcitriol on autophagy markers in SH-SY2Y cells and whether calcitriol protects SH-SY2Y cells from rotenone-induced neurotoxicity together with the underlying mechanisms of protection, including autophagy.

2. Materials and methods

2.1. Cell culture and chemicals

The human neuroblastoma cell line SH-SY5Y was obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were grown in Dulbecco's modified Eagle medium:Ham's F12 (1:1 mixture) (Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Grand Island, NY, USA), 100 units/ml penicillin, and 100 mg/ml streptomycin (Grand Island, NY, USA) in a 5% CO_2 incubator at 37 °C. The calcitriol was purchased from Cayman (Ann Arbor, Michigan, USA) and dissolved in DMSO (Sigma, USA) to prepare a 5 mM stock solution. To treat the cells, calcitriol was diluted in the culture medium to the appropriate concentration. Rotenone was obtained from Sigma (Sigma, USA).

2.2. MTT assay

Cell viability was measured by a quantitative colorimetric assay using MTT. The MTT assay provided a sensitive measurement of the metabolic status of the cells, particularly the status of the mitochondria, which may reflect early redox changes. Briefly, exponentially growing cells were seeded in a 96-well plate at a density of 5×10^4 cells/well. The cells were then pre-treated with calcitriol for 2 h. After the pre-treatment period, rotenone was added to the culture medium to reach a final concentration of 200 nM, and the cells were incubated for 24 h. The control cells were not treated with calcitriol or rotenone. After incubation for 24 h, 10 μl of the MTT assay kit reagent was added to each well, and the cells were incubated for an additional hour. The absorbance for each reaction product was measured with a microplate reader at a wavelength of 450 nm. The results are expressed as a percentage of the MTT absorbance of the control cells, i.e., the MTT absorbance on the control cells was set to 100%.

2.3. Immunoblot analysis

Whole-cell lysates were prepared by incubating the cells in RIPA buffer (Beverly, MA, USA) supplemented with a protease inhibitor cocktail (Roche, Mannheim, Germany) according to the manufacturer's instructions. Briefly, the cells were harvested by centrifugation at 13,200 rpm for 5 min and washed in PBS (pH 7.2). The pellets were solubilized in the same volume of mitochondrial lysis buffer, kept on ice, vortexed for 5 min and centrifuged at 13,200×*g* for 20 min at 4 °C. Equal amounts of the total lysate protein were loaded and separated on a 15% SDS–PAGE gel. The proteins were electrophoretically transferred to a PVDF membrane, and the membrane was blocked in 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 1 h. Then, the membranes were incubated in the presence of primary antibodies against one of the following proteins: LC3, beclin-1, mTOR, caspase 3, cleaved caspase 3, Bak, Bax, or GAPDH from Cell Signaling (Beverly, MA, USA) at 4 °C overnight. The membranes were then washed three times with TBST and probed with the corresponding HRP-conjugated secondary antibodies at room temperature for 1 h. Probe detection was conducted using enhanced ECL Advance Western Blotting Detection Reagents (GE Healthcare, Buckinghamshire, UK) and LAS-4000 film (Fujifilm, Tokyo, Japan).

2.4. Measurement of intracellular reactive oxygen species (ROS)

Levels of intracellular ROS were estimated following treatment with the various compounds using 2′,7′-dichlorofluorescein diacetate (H2DCFDA) (Sigma, USA) as a fluorescent probe. SH-SY5Y cells were exposed to calcitriol and rotenone for 24 h, and the culture medium was replaced by fresh serum-free medium containing 20 μM H2DCFDA. The DCF fluorescence intensity was determined by flow cytometry using a (BD Biosciences, USA) and CellQuestPro software.

2.5. Annexin V/propidium iodide assay

SH-SY5Y cells were incubated with drugs for 24 h. The supernatant and cells that adhered to the plate were collected and washed with PBS. The cells were diluted to 10^6 cells/ml in Annexin V binding buffer and stained with fluorescein isothiocyanate Annexin V and propidium iodide (PI; BD Pharmingen) according to the manufacturer's protocol. The stained cells were detected by flow cytometry (BD Biosciences, USA) and the data were analyzed by CellQuestPro (BD Biosciences, USA).

2.6. Statistical analysis

Statistical data are expressed as the mean ± SD, and Student's *t*-test was used to determine statistical significance. $p < 0.05$ and $p < 0.01$ were considered statistically significant.

3. Results

3.1. Effects of calcitriol and rotenone in SH-SY5Y cells

To evaluate the effects of calcitriol on rotenone-induced neurotoxicity in SH-SY5Y cells, we treated the cells for 24 h with several concentrations of calcitriol (0.0 μM, 0.3 μM, 0.63 μM, 1.25 μM, 2.5 μM, 5 μM, 10 μM, 15 μM and 20 μM). Cell viability started to decrease at calcitriol concentrations greater than 15 μM (Fig. 1A). SH-SY5Y cells were then incubated with 200 nM rotenone and treated simultaneously with several concentrations of calcitriol (0.0 μM, 0.63 μM, 1.25 μM, 2.5 μM, 5 μM and 10 μM). Compared to the controls, the viability of SH-SY5Y cells treated with 200 nM rotenone was significantly decreased; however, combined treatment with calcitriol at levels up to 10 μM increased cell viability in a concentration-dependent manner (Fig. 1B).

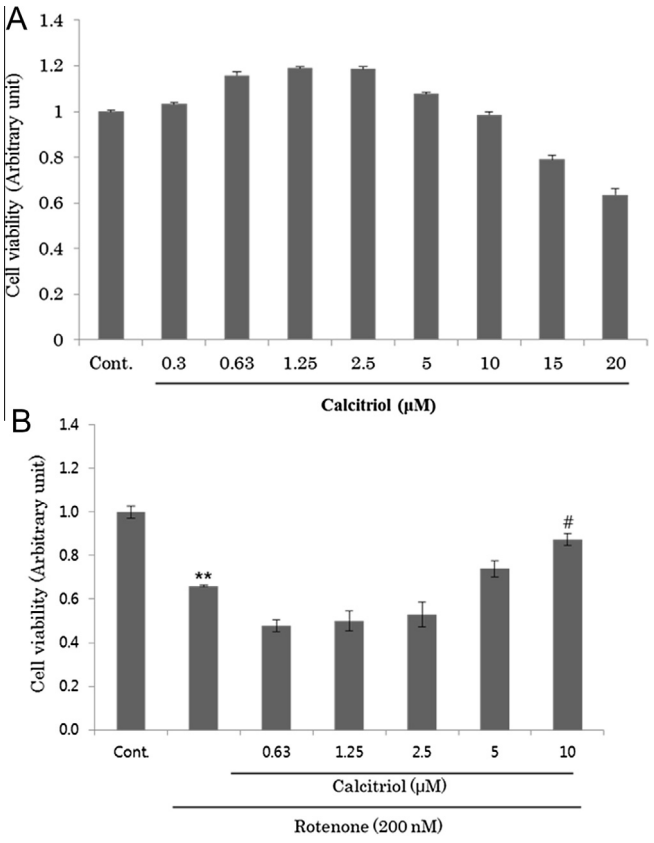

Fig. 1. Protective effect of calcitriol in rotenone-induced SH-SY5Y cells. (A) SH-SY5Y cells were treated with calcitriol at concentrations of 0.3 μM, 0.63 μM, 1.25 μM, 2.5 μM, 5 μM, 10 μM, 15 μM and 20 μM for 24 h. The cell viability was measured by the MTT assay and the results were expressed as a percentage of the absorbance of the control. Data represent the mean ± SD ($n = 3$). (B) Neuroprotective effects of calcitriol on rotenone-induced cytotoxicity were determined by MTT assay. Cells were treated with 200 nM rotenone alone, or rotenone and calcitriol for 24 h. SH-SY5Y cells were pre-treated with calcitriol at concentrations of 0.63 μM, 1.25 μM, 2.5 μM, 5 μM and 10 μM for 2 h followed by the addition of rotenone 200 nM for 24 h. The cell viability was measured by the MTT assay. Data represent the mean ± SD ($n = 3$). **Significantly different ($p < 0.01$) from control. #Significantly different ($p < 0.05$) from the rotenone-treated group.

3.2. Anti-apoptotic effects of calcitriol against rotenone-induced neurotoxicity

To evaluate the effect of calcitriol on rotenone-induced neurotoxicity, Annexin V/PI staining and flow cytometry were applied. Annexin V/PI staining with flow cytometry showed similar results to the MTT assay. Fig. 2 shows that SH-SY5Y cells with 200 nM rotenone showed a significantly increasing proportion of early apoptotic cells compared with the controls. However, this shift was reversed with combined treatment with 10 μM calcitriol. 10 μM calcitriol alone had no significant effect on the proportion of early and late apoptotic cells compared with the controls.

3.3. Effects of rotenone and calcitriol on ROS in SH-SY5Y cells

To determine whether calcitriol has an anti-oxidant effect against rotenone-induced ROS production, ROS levels in SH-SY5Y cells were assessed after the following treatments: (1) no treatment (control), (2) 10 μM calcitriol, (3) 200 nM rotenone, and (4) 200 nM rotenone with calcitriol (5 μM and 10 μM).

As shown in Fig. 3, ROS levels were markedly increased in SH-SY5Y cells treated with 200 nM rotenone compared to the controls ($p < 0.01$). We also found that co-treatment with 5 μM and 10 μM calcitriol greatly reduced ROS formation (Supplementary Fig. 1). Treatment with 10 μM calcitriol alone did not significantly increase ROS formation compared to the controls.

3.4. Effects of rotenone and calcitriol on levels of intracellular signaling proteins associated with apoptosis, including Bak, Bax and activated caspase-3

SH-SY5Y cells were immunoblotted to determine the effects of rotenone and calcitriol on several intracellular signaling proteins, such as Bak, Bax, and cleaved caspase-3. Treatment with 200 nM rotenone increased the immunoreactivity (IR) of Bak, Bax, and activated caspase-3 compared with the untreated control, whereas combined treatment with calcitriol significantly inhibited the rotenone-mediated IR elevation (Fig. 3).

3.5. Effects of rotenone and calcitriol on levels of intracellular signaling proteins associated with autophagy

Compared with the untreated control, the IR of LC3 and beclin-1, which are basic markers of autophagy, were significantly increased in SH-SY5Y cells treated with calcitriol at different concentrations (0.0 μM, 0.63 μM, 1.25 μM, 2.5 μM, 5 μM and 10 μM) in a concentration-dependent manner at levels up to 10 μM (Supplementary Fig. 2A). We also observed that calcitriol monotreatment significantly increased the expression of AMPK, an upstream regulator of autophagy in SH-SY5Y cells, in a concentration-dependent manner (Supplementary Fig. 2B).

Single treatment with 200 nM rotenone increased mTOR expression and decreased beclin-1 expression, indicating suppression of autophagy; however, combined treatment with calcitriol (2.5 μM, 5 μM, and 10 μM) restored the expression of these autophagy-enhancing markers (Fig. 4). Treatment with 200 nM rotenone increased LC3 expression compared with the untreated control, and combined treatment with calcitriol also resulted in a significant increase of LC3 expression in a concentration-dependent manner (Fig. 4).

4. Discussion

Investigations into the pathogenesis of PD appear to be converging on oxidative stress, mitochondrial dysfunction and protein aggregation [6,8]. In particular, it is well-established that alpha synuclein aggregation and mitochondrial dysfunction are closely related to the pathogenesis of PD [23,24]. Emerging evidence suggests that dysregulated autophagy results in the accumulation of abnormal proteins or damaged organelles in PD models [10–12]. First, not only the ubiquitin proteasome pathway but also the alpha synuclein pathway is degraded by the autophagy lysosomal pathway [25,26]. Furthermore, a mutant form of alpha synuclein that is excluded from chaperone-mediated autophagy (CMA) because of higher affinity than wild-type alpha synuclein to the lysosomal-associated membrane protein 2A (LAMP2A) causes compensatory activation of macroautophagy [27]. However, this compensatory pathway cannot be sufficient to sustain an efficient rate of protein aggregation. Second, autophagy also participates in mitochondrial turnover [28]. The products of the PINK-1 and Parkin genes are associated with the elimination of damaged mitochondria through the mitophagy pathway [29]. Therefore, mutation of these genes could result in a failure to degrade damaged mitochondria and result in an accumulation of mitochondria and excess reactive oxygen species that could lead to neurodegeneration. These findings suggest that both autophagy activation and lysosomal clearance can contribute to the pathogenesis of PD and that activation of autophagy may be a new therapeutic target

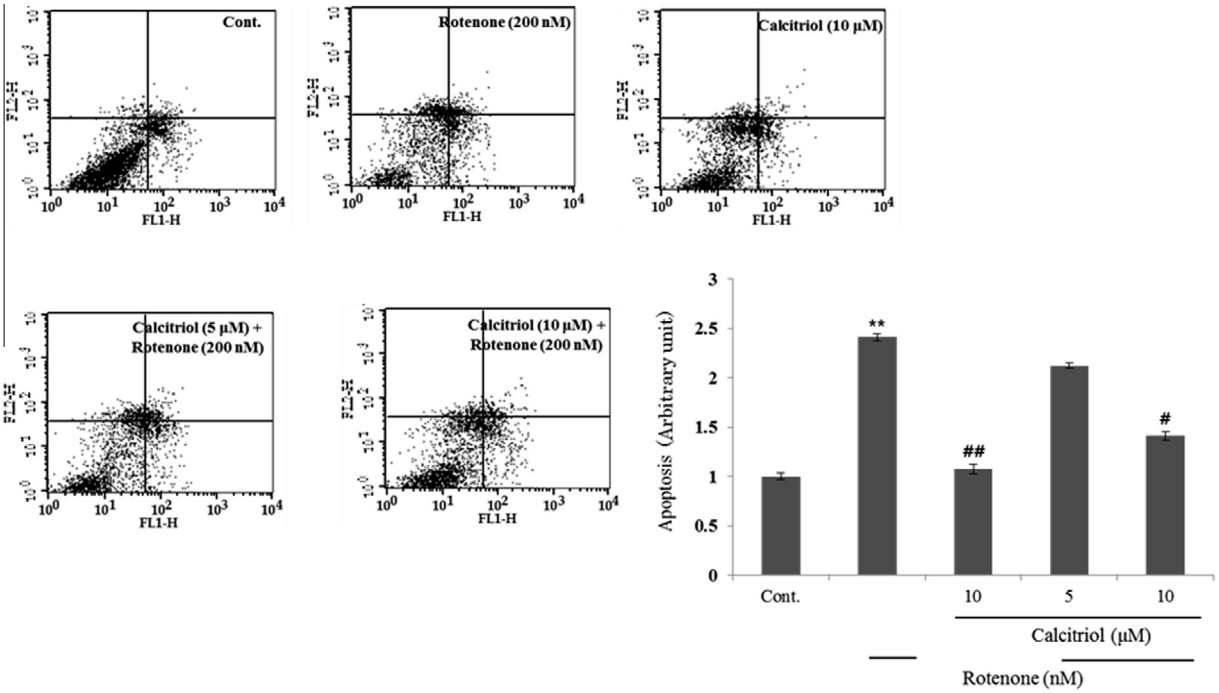

Fig. 2. Effects of autophagy-related drugs on rotenone-induced apoptosis in SH-SY5Y. FACS analysis of annexin V (AV) and propidium iodide (PI) binding. SH-SY5Y cells were treated for 24 h with vehicle, rotenone, calcitriol, or calcitriol (5 μM and 10 μM) + rotenone. PI and AV-FITC fluorescence was measured by flow cytometry and analyzed (dot-plots). Viable (AV−/PI−), early apoptotic (AV+/PI−), apoptotic (AV+/PI+) and residual damaged (AV−/PI+) cells are shown in the respective quadrants.

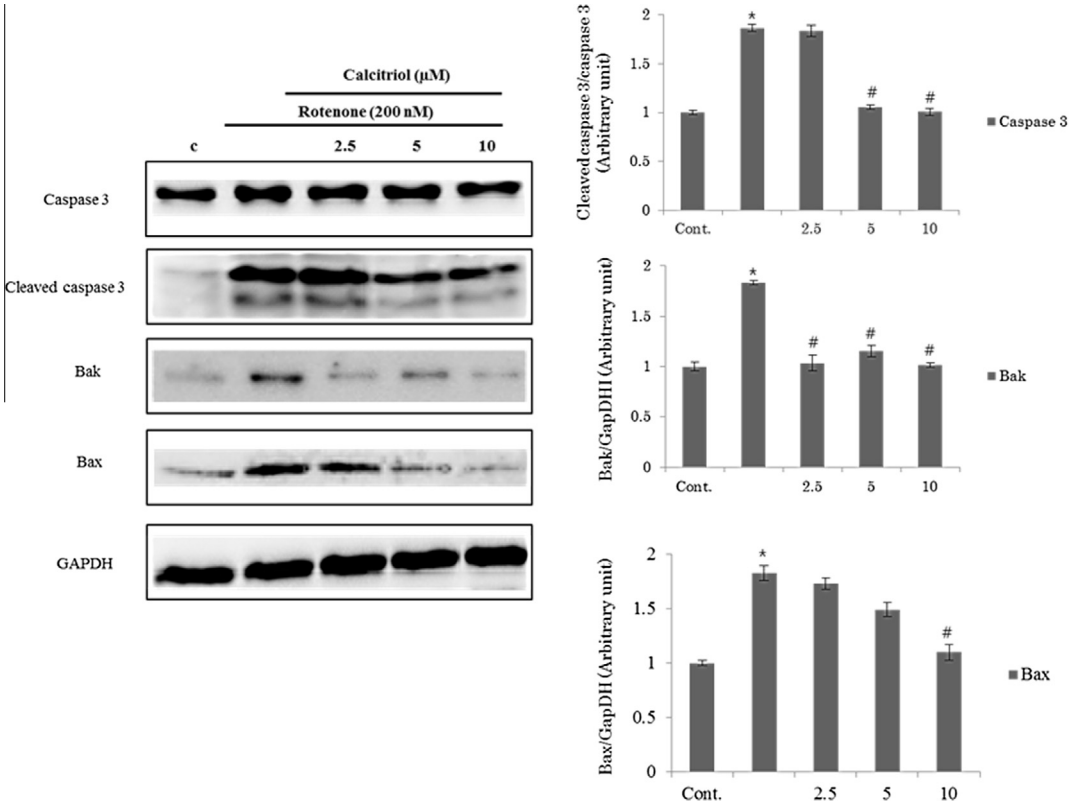

Fig. 3. Calcitriol protects against rotenone-induced apoptosis in SH-SY5Y cells. SH-SY5Y cells were pre-treated with calcitriol at concentrations of 2.5 μM, 5 μM, and 10 μM for 2 h followed by addition of 200 nM rotenone for 24 h. Induction of apoptosis by calcitriol in SH-SY5Y cells was determined by measuring the Caspase 3, Bak and Bax protein levels using an immunoblotting assay. GAPDH was used as an equal loading control ($n = 3$). Significantly different ($^{*}p < 0.05$) from control and significantly different ($^{\#}p < 0.05$) from rotenone-treated group.

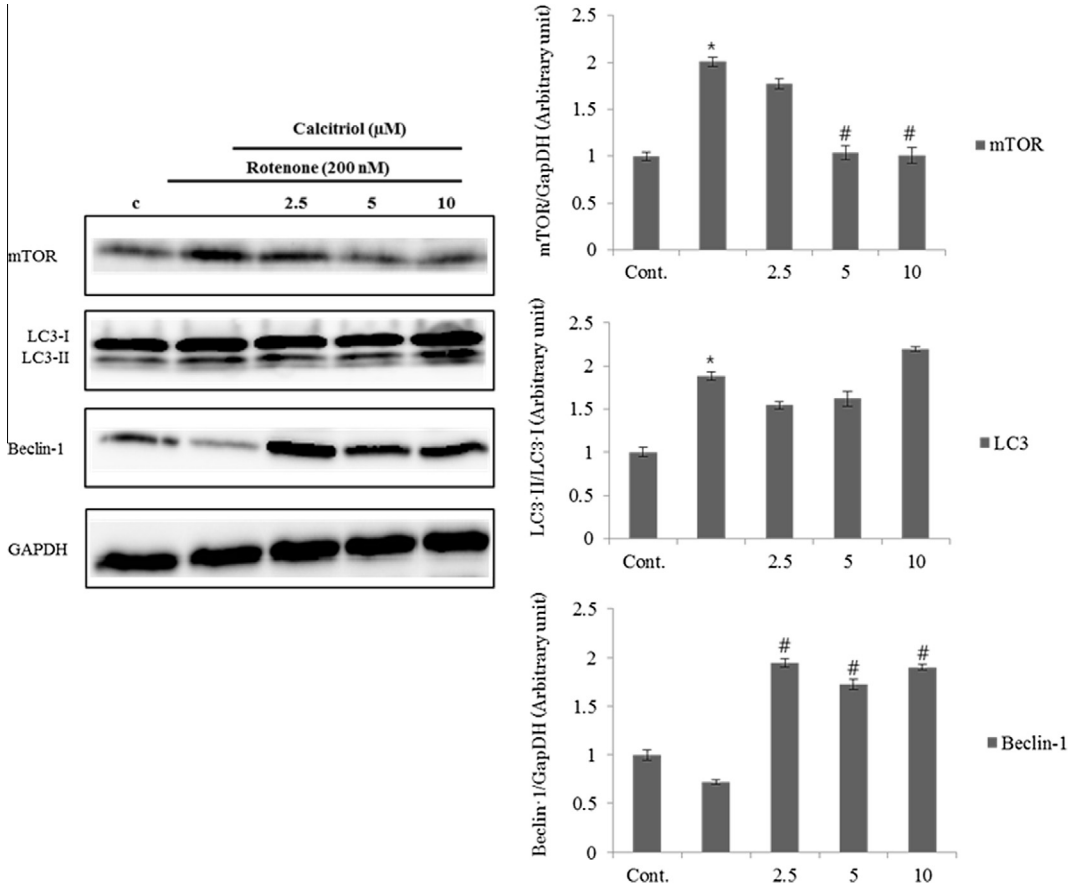

Fig. 4. Induction of autophagy-lysosomal upstream pathway by calcitriol in SH-SY5Y cells. SH-SY5Y cells were pre-treated with calcitriol at concentrations of 2.5 μM, 5 μM, and 10 μM for 2 h, followed by addition of 200 nM rotenone for 24 h. The induction of autophagy by calcitriol in SH-SY5Y cells was determined by measuring the mTOR, LC3II and beclin-1 protein levels using an immunoblotting assay. GAPDH was used as an equal loading control ($n = 3$). *Significantly different ($p < 0.05$) from control #significantly different ($p < 0.05$) from rotenone-treated group.

in PD. In this study, we demonstrated that calcitriol significantly attenuates rotenone-induced neurotoxicity in SH-SY5Y cells through reduced ROS formation and augmented autophagy induction. Our results suggest that calcitriol acts as an autophagy enhancer in an in vitro PD model and modulates the microenvironment that leads to neuronal death. Therefore, calcitriol may be a candidate disease-modifying agent in PD.

Recent epidemiologic studies indicate that low vitamin D_3 levels could play an important role in the pathogenesis and progression of PD [30–32]. Evatt et al. suggested that chronic vitamin D_3 insufficiency is more frequently found in early PD patients than in normal healthy controls [30,31]. The importance of calcitriol in PD is also supported by animal studies showing that calcitriol attenuates neuronal toxicity induced by 6-hydroxydopamine in rats and that vitamin D receptor (VDR)-knockout mice have motor impairments that affect locomotor behavior [20,33]. Furthermore, Newmark and Newmark hypothesized that chronic insufficiency of vitamin D_3 may lead to abnormal function of SN cells and may even lead to degeneration [34]. Vitamin D_3 induces autophagy in a multi-step process affecting various intracellular signaling pathways, such as those including Bcl-2, mTOR, beclin-1, the class III phosphatidylinositol 3-kinase complex (PI3 KC3), and cathelicidin [9,35–38]. Vitamin D_3 increases free cytosolic calcium levels, leading to decreased mTOR induction [39]. Inhibition of mTOR induces autophagy. Vitamin D_3 also regulates the nucleation process of autophagy through the beclin-1 and PI3 KC4 pathways, increasing lysosome function to promote maturation and degradation in the autophagy process [37]. In our study, treatment with calcitriol alone in SH-SY5Y cells enhanced the expression of AMPK in addition to LC3 and beclin-1, which indicates that calcitriol enhances autophagy not only through the mTOR-dependent pathway, as shown in previous studies, but also through mTOR-independent pathways, including AMPK, leading to the direct phosphorylation of ULK-1 and beclin-1 [40]. Consequently, vitamin D_3 signaling affects autophagy at several steps. Calcitriol thus displays potential as a neuroprotective agent through enhancement of autophagy in PD, and this application will require further investigation.

Our study revealed that calcitriol significantly ameliorated rotenone-induced apoptosis in SH-SY5Y cells and reduced the levels of markers of apoptosis induction and autophagy inhibition. To the best of our knowledge, this is the first study to show that calcitriol has neuroprotective effects through activation of autophagy in an in vitro model of PD. Because multiple recent studies have reported that autophagy is a key mechanism associated with dopaminergic cell death and may play an important role in the pathogenesis of PD, autophagy enhancement by calcitriol treatment may be a potential therapeutic treatment for PD [11,12,29,41]. However, there have also been reports that autophagy enhancement may be related to neuronal cell loss, and these two aspects of autophagy pose a challenge to the application of autophagy-inducing drugs for neurodegenerative diseases.

Notably, we found that calcitriol inhibited ROS generation induced by rotenone. Calcitriol is widely known to offer neuroprotection through multiple mechanisms, including the upregulation of glial cell line-derived neurotrophic factor (GDNF) and nerve growth factor (NGF), the downregulation of L-type calcium

channels and antioxidant effects through enhancement of the glutathione level [18–20,42]. ROS generation is essential to trigger autophagy induction, and rotenone is a mitochondrial complex I inhibitor that causes mitochondrial damage [9,12,28]. Considering that mitochondria are essential for ROS production and that damaged mitochondria are removed by the autophagy-lysosomal system, we assume that calcitriol could decrease ROS production by autophagy enhancement to degrade disabled mitochondria.

There were some limitations in this study: (1) This study was performed under in vitro conditions; therefore, these results may be different from those observed under in vivo conditions or in clinical trials, in which various additional factors may have roles; (2) only limited types of autophagy markers were used for evaluating the mechanism of calcitriol-mediated autophagy regulation, and (3) the toxicity model of PD is not fully compatible with the microenvironment of dopaminergic cells in PD patients.

In conclusion, we have shown that calcitriol has neuroprotective effects in SH-SY5Y cells against rotenone-induced neurotoxicity and that the mechanisms of neuroprotection appear to be associated with enhanced autophagy. These neuroprotective effects of calcitriol on rotenone-induced dopaminergic neurotoxicity provide an experimental basis for its clinical use for the treatment of PD.

Disclosure statement

None of the authors have anything to disclose regarding this manuscript and all authors have no potential conflicts of interest to report concerning this article.

Acknowledgments

This work was funded by the Gangneung Asan Hospital Biomedical Research Center Promotion Fund.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.07.081.

References

[1] J.G. Nutt, G.F. Wooten, Clinical practice. Diagnosis and initial management of Parkinson's disease, N. Engl. J. Med. 353 (2005) 1021–1027.
[2] S. Fahn, Parkinson study group, Does levodopa slow or hasten the rate of progression of Parkinson's disease?, J Neurol. 252 (2005) IV37–IV42.
[3] L. Parkkinen, S.S. O'Sullivan, M. Kuoppamäki, et al., Does levodopa accelerate the pathologic process in Parkinson disease brain?, Neurology 77 (2011) 1420–1426
[4] J. Lipski, R. Nistico, N. Berretta, et al., A scapegoat for accelerated neurodegeneration in Parkinson's disease?, Prog Neurobiol. 94 (2011) 389–407.
[5] T. Müller, H. Hefter, R. Hueber, et al., Is levodopa toxic?, J Neurol. 251 (2004) VI44–VI46.
[6] J.L. Eriksen, Z. Wszolek, L. Petrucelli, Molecular pathogenesis of Parkinson disease, Arch. Neurol. 62 (2005) 353–357.
[7] A.V. Schapira, C. Olanow, Neuroprotection in Parkinson disease: mysteries, myths, and misconceptions, JAMA 291 (2004) 358–364.
[8] D.T. Dexter, P. Jenner, Parkinson disease: from pathology to molecular disease mechanisms, Free Radic. Biol. Med. 62 (2013) 132–144.
[9] D. Glick, S. Barth, K.F. Macleod, Autophagy: cellular and molecular mechanisms, J. Pathol. 221 (2010) 3–12.
[10] C.C. Tan, J.T. Yu, M.S. Tan, et al., Autophagy in aging and neurodegenerative diseases: implications for pathogenesis and therapy, Neurobiol. Aging 35 (2014) 941–957.
[11] M.A. Lynch-Day, K. Mao, K. Wang, et al., The role of autophagy in Parkinson's disease, Cold Spring Harb. Perspect. Med. 2 (2012) a009357.
[12] N. Xiong, J. Xiong, M. Jia, et al., The role of autophagy in Parkinson's disease: rotenone-based modeling, Behav. Brain Funct. 9 (2013) 1–12.
[13] C. Annweiler, A.M. Schott, G. Berrut, et al., Vitamin D and ageing: neurological issues, Neuropsychobiology 62 (2010) 139–150.
[14] D.A. Fernandes de Abreu, D. Eyles, F. Féron, Vitamin D, a neuro-immunomodulator: implications for neurodegenerative and autoimmune diseases, Psychoneuroendocrinology 34 (Suppl. 1) (2009) S265–S277.
[15] E. Garcion, N. Wion-Barbot, C.N. Montero-Menei, et al., New clues about vitamin D functions in the nervous system, Trends Endocrinol. Metab. 13 (2002) 100–105.
[16] P. Welsh, N. Sattar, Vitamin D and chronic disease prevention, BMJ 348 (2014) g2280.
[17] B. Klotz, B. Mentrup, M. Regensburger, et al., 1,25-Dihydroxyvitamin D3 treatment delays cellular aging in human mesenchymal stem cells while maintaining their multipotent capacity, PLoS One 7 (2012) e29959.
[18] K. Shinpo, S. Kikuchi, H. Sasaki, et al., Effect of 1,25-dihydroxyvitamin D3 on cultured mesencephalic dopaminergic neurons to the combined toxicity caused by L-buthionine sulfoximine and 1-methyl-4-phenylpyridine, J. Neurosci. Res. 62 (2000) 374–382.
[19] H. Taniura, M. Ito, N. Sanada, et al., Chronic vitamin D3 treatment protects against neurotoxicity by glutamate in association with upregulation of vitamin D receptor mRNA expression in cultured rat cortical neurons, J. Neurosci. Res. 83 (2006) 1179–1189.
[20] J.Y. Wang, J.N. Wu, T.L. Cherng, et al., Vitamin D(3) attenuates 6-hydroxydopamine-induced neurotoxicity in rats, Brain Res. 904 (2001) 67–75.
[21] S. Wu, J. Sun, Vitamin D, vitamin D receptor, and macroautophagy in inflammation and infection, Discov. Med. 11 (2011) 325–335.
[22] J.M. Yuk, D.M. Shin, H.M. Lee, et al., Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin, Cell Host Microbe 6 (2009) 231–243.
[23] R. Banerjee, A.A. Starkov, M.F. Beal, et al., Mitochondrial dysfunction in the limelight of Parkinson's disease pathogenesis, Biochim. Biophys. Acta 1792 (2009) 651–663.
[24] S.J. Lee, Alpha-synuclein aggregation: a link between mitochondrial defects and Parkinson's disease?, Antioxid Redox Signal. 5 (2003) 337–348.
[25] J.L. Webb, B. Ravikumar, J. Atkins, et al., Alpha-synuclein is degraded by both autophagy and the proteasome, J. Biol. Chem. 278 (2003) 25009–25013.
[26] M. Xilouri, T. Vogiatzi, L. Stefanis, Alpha-synuclein degradation by autophagic pathways: a potential key to Parkinson's disease pathogenesis, Autophagy 4 (2008) 917–919.
[27] A.M. Cuervo, L. Stefanis, R. Fredenburg, et al., Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy, Science 305 (2004) 1292–1295.
[28] J. Lee, S. Giordano, J. Zhang, Autophagy, mitochondria and oxidative stress: cross-talk and redox signalling, Biochem. J. 441 (2012) 523–540.
[29] E. Deas, N.W. Wood, H. Plun-Favreau, Mitophagy and Parkinson's disease: the PINK1–parkin link, Biochim. Biophys. Acta 2011 (1813) 623–633.
[30] M.L. Evatt, M.R. Delong, N. Khazai, et al., Prevalence of vitamin D insufficiency in patients with Parkinson disease and Alzheimer disease, Arch. Neurol. 65 (2008) 1348–1352.
[31] M.L. Evatt, M.R. DeLong, M. Kumari, et al., High prevalence of hypovitaminosis D status in patients with early Parkinson disease, Arch. Neurol. 68 (2011) 314–319.
[32] P. Knekt, A. Kilkkinen, H. Rissanen, et al., Serum vitamin D and the risk of Parkinson disease, Arch. Neurol. 67 (2010) 808–811.
[33] T.H.J. Burne, J.J. McGrath, D.W. Eyles, et al., Behavioural characterization of vitamin D receptor knockout mice, Behav. Brain Res. 157 (2005) 299–308.
[34] H.L. Newmark, J. Newmark, Vitamin D and Parkinson's disease–a hypothesis, Mov. Disord. 22 (2007) 461–468.
[35] J.E. Chipuk, T. Moldoveanu, F. Llambi, et al., The BCL-2 family reunion, Mol. Cell 37 (2010) 299–310.
[36] M. Hoyer-Hansen, L. Bastholm, I.S. Mathiasen, et al., Vitamin D analog EB1089 triggers dramatic lysosomal changes and Beclin 1-mediated autophagic cell death, Cell Death Differ. 12 (2005) 1297–1309.
[37] M. Saji, M.D. Ringel, The PI3K-Akt-mTOR pathway in initiation and progression of thyroid tumors, Mol. Cell. Endocrinol. 321 (2010) 20–28.
[38] S. Sinha, B. Levine, The autophagy effector Beclin 1: a novel BH3-only protein, Oncogene 27 (2008) S137–S148.
[39] J. Wang, H. Lian, Y. Zhao, et al., Vitamin D3 induces autophagy of human myeloid leukemia cells, J. Biol. Chem. 283 (2008) 25596–25605.
[40] J. Kim, M. Kundu, B. Viollet, et al., AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1, Nat. Cell Biol. 13 (2011) 132–141.
[41] H. Zhang, C. Duan, H. Yang, Defective autophagy in Parkinson' disease: lessons from genetics, Mol. Neurobiol. (2014), http://dx.doi.org/10.1007/s12035-014-8787-5 (Epub ahead of print).
[42] L.D. Brewer, V. Thibault, K.-C. Chen, et al., Vitamin D hormone confers neuroprotection in parallel with downregulation of L-type calcium channel expression in hippocampal neurons, J. Neurosci. 21 (2001) 98–108.