Neurotoxicology and Teratology 34 (2012) 344–349

Contents lists available at SciVerse ScienceDirect

Neurotoxicology and Teratology

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

Glyphosate induced cell death through apoptotic and autophagic mechanisms

Ya-xing Gui [a,1], Xiao-ning Fan [a,1], Hong-mei Wang [a], Gang Wang [a,*], Sheng-di Chen [a,b,*]

[a] Department of Neurology & Institute of Neurology, Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
[b] Lab of Neurodegenerative Diseases & key Laboratory of Stem Cell Biology, Institute of Health Science, Shanghai Institutes of Biological Sciences, Chinese Academy of Science & Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

ARTICLE INFO

Article history:
Received 17 October 2011
Received in revised form 7 March 2012
Accepted 27 March 2012
Available online 4 April 2012

Keywords:
Glyphosate
Apoptosis
Autophagy
Beclin-1
Parkinsonism

ABSTRACT

Herbicides have been recognized as the main environmental factor associated with human neurodegenerative disorders such as Parkinson's disease(PD). Previous studies indicated that the exposure to glyphosate, a widely used herbicide, is possibly linked to Parkinsonism, however the underlying mechanism remains unclear. We investigated the neurotoxic effects of glyphosate in differentiated PC12 cells and discovered that it inhibited viability of differentiated PC12 cells in dose-and time-dependent manners. Furthermore, the results showed that glyphosate induced cell death via autophagy pathways in addition to activating apoptotic pathways. Interestingly, deactivation of Beclin-1 gene attenuated both apoptosis and autophagy in glyphosate treated differentiated PC12 cells, suggesting that Beclin-1 gene is involved in the crosstalk between the two mechanisms.

1. Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative disorders, and characterized by progressive cell loss confined mostly to dopaminergic neurons of the substantia nigra. Several factors, including mitochondrial dysfunction, oxidative stress, altered protein handling and inflammatory change, are involved in the degenerative process through apoptosis and/or autophagic pathways (Anglade et al., 1997; Bredesen et al., 2006). Numerous apoptotic pathways are activated in response to PD-induced dysfunction in several systems, including the c-Jun N-terminal kinase (JNK) and p53 activation, cell cycle re-entry, and bcl-2 family signaling. Meanwhile, autophagy may act as an initial protective response, but excessive autophagy eventually pushed towards cell death during the disease progression(Levy et al., 2009; ubinsztein, 2006). Therefore, PD is currently viewed as a multifactorial disease. Epidemiologically, environmental exposure plays an causal role in the etiology of PD(Dauer and Przedborski, 2003; Patel and Schier, 2006; Peng et al., 2010). N-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine(MPTP), rotenone, paraquat(Bove et al., 2005; Cannon and Greenamyre, 2010) have been found trigger apoptosis and/or excessive autophagy of dopaminergic neurons to induce PD development.

Recently, another environmental neurotoxin, glyphosate, have also been reported involved in the pathogenesis of PD. Negga and his co-workers found that the exposure to glyphosate pesticides could result in neurodegeneration in Caenorhabditis elegans(Negga et al., 2011). Furthermore, our case report (Wang et al., 2011) and a case described by Barbosa et al. (2001) indicated that the exposure to glyphosate is possibly linked to Parkinsonism. Glyphosate is a broad-spectrum systemic herbicide used to kill weeds, especially annual broadleaf weeds and grasses known to compete with crops grown widely. Glyphosate's mode of action is to interfer with the synthesis of the amino acids phenylalanine, tyrosine and tryptophan. Animal exposures to glyphosate were observed that it may cause loss of mitochondrial transmembrane potential and result in oxidative stress to liver and brain(Astiz et al., 2009; Peixoto, 2005).

Therefore, the mechanism of glyphoste-induced neurotoxic effects needs to be determined. In this study, we investigated the neurotoxicity of glyphosate in differentiated rat pheochromocytoma PC12 cells and further explored whether the two important cell death pathway, apoptosis and autophagy involved in the glyphosate-induced toxicity mechanism.

2. Materials and methods

2.1. Cell culture and transfection

Differentiated PC12 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM, GIBCO) supplemented with 10% fetal bovine serum (FBS, PAA), 100 U/ml penicillin, and 100 μg/ml Streptomycin (GIBCO). All cells were maintained in a humidified 5% CO2 containing atmosphere at 37 °C. For transfection experiments, PC12 cells were

* Corresponding authors at: Department of Neurology & Institute of Neurology, Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine, 197 Ruijin 2nd Road, Shanghai 200025, China. Tel./fax: +86 21 64454473.
E-mail addresses: wgneuron@hotmail.com (G. Wang), Chen_sd@medmail.com.cn (S. Chen).
[1] Contributed equally to this work.

0892-0362/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.ntt.2012.03.005

plated the day before transfection to achieve ~50% confluency. Cells were transiently transfected with enhanced green fluorescent protein vector (pEGFP) or GFP–LC3 expression construct using Lipofectamine 2000 (Invitrogen USA) according to the manufacturer's instructions. The plasmid DNA/Lipofectamine was mixed as the ratio of 1:3. pEGFP empty vector was purchased from Clontech (Palo Alto, CA, USA). The LC3 cDNA was kindly provided by Prof. T. Yoshimori and N. Mizushima(National Institute for Basic Biology, Japan). For the co-treatment experiments, PC12 cells were treated with plasmid and glyphosate, as well as siRNA, or inhibitors. .

2.2. Reagents and antibodies

2,5-diphenyltetrazoliumbromide (MTT) and lipofectamine 2000 were sourced from Invitrogen (Carlsbad, CA, USA). Glyphosate, dimethyl sulfoxide (DMSO), 4′, 6′-diamidino-2-phenylindole (DAPI) and 3-methyladenine (3-MA) were obtained from Sigma Aldrich (St. Louis, MO, USA). Caspase inhibitor z-VAD was obtained from Calbiochem (La Jolla, CA, USA). Glyphosate was dissolved in DMSO as a stock solution at −20 °C and diluted with culture medium to various working concentrations. 3-MA was dissolved in double distilled water and titrated to 1 mM while zVAD was dissolved in DMSO and used at 20 μM as indicated. FITC Annexin V Apoptosis Detection Kit was obtained from BD Pharmingen (San Diego, CA). Antibodies against microtubule associated protein light chain 3 (LC3), Beclin-1 and β-actin were purchased from Sigma Aldrich. BAX and Bcl-2 antibodies were obtained from Abcam (Cambridge, UK). Species-specific anti-IgG antibodies conjugated to HRP were obtained from Jakson Immuno Research Laboratories (USA).

2.3. siRNA mediated silencing of Beclin-1

Cells were transfected with Beclin-1 siRNA or scrambled siRNA (Santa Cruz, CA, USA) using Lipofectamine 2000 reagent according to the manufacturer's instructions. After 48 h, cells were subjected to various treatments. In order to evaluate the effects of Beclin-1 siRNA or scrambled siRNA on GFP–LC3 puncta formation, cells were cotransfected with GFP–LC3 and either Beclin-1 siRNA or scrambled siRNA following various treatments and observed by confocal laser scanning microscopy. The Beclin-1 siRNA sequence (5′-CAGTTTGGA-CAATCAATA-3′) efficiently targeted.

2.4. Cell viability assay

Cell viability was assessed by the MTT assay. PC12 cells after various treatment were plated in 96-well culture plates (1.0×10^4 cells per well). 5 g/l MTT solution was added to the culture medium 4 h before the end of treatments. After that DMSO was added to each well to dissolve the dark blue crystals and the absorbance at 570 nm was interpreted on a microplate reader (Safire,TECAN).

2.5. Apoptosis detection

Nuclei were stained with DAPI to detect chromatin condensation or nuclear fragmentation, which were characteristics of apoptosis. Following various treatments, cells were fixed with 4% paraformaldehyde for 20 min and then stained with DAPI for 10 min away from light at room temperature. The cells were washed twice with PBS before being viewed under confocal laser scanning microscopy (Nikon,A1R) with excitation/emission spectra set at 350/460 nm respectively. Two hundred cells were counted and the percentage of apoptotic cells was taken as the mean of three independent experiments. The cell apoptosis was further determined using FITC Annexin V Apoptosis Detection Kit according to the manufacturer's recommendations. Cells were then analyzed by flow cytometry (FCM) on a linear scale, to detect apoptosis using a Becton Dickinson FACS Array.

2.6. Autophagy detection

For LC3 punctate analyses experiments, cells were transiently transfected with EGFP empty vector or GFP–LC3 expression vector. 24 h after transfection, the cells were subjected to various treatments before being observed under confocal laser scanning microscopy. In each experiment, no fewer than 200 transfected cells were counted. Only cells with at least five dots were scored as GFP–LC3-positive. The percentage of the positive cells was thus determined and expressed as the mean of four independent experiments. For western blot analyses, LC3-II/LC3-I ratio was evaluated by band density analysis as the marker of cell autophagy.

2.7. Western blot analysis

Cells after various treatment were lysed in ice-cold RIPA lysis buffer (1% Triton X100, 0.5% sodium deoxycholate, 0.1% SDS, 1% NP40, 50 mM TrisHCl, pH 7.4) supplemented with protease inhibitor cocktail and phenylmethanesulfonyl fluoride for 30 min, followed by centrifugation at 12,000 g for 30 min at 4 °C before collecting the supernatants. Protein extracts were quantified and equal amounts of lysates were resolved by SDS-PAGE, and then transferred into PVDF membrane (Millipore). After blocking with 3% BSA, appropriate primary antibodies and secondary antibodies were applied. The signals were developed with Immobilon Western Chemiluminescent HRP Substrate (Millipore). Band density values of interested bands were normalized to loading control and quantitative analyses were performed by imageJ software (Wayne Rasband, NIH).

2.8. Statistical analysis

Statistical analyses were done by SPSS software using a one-way ANOVA, followed by a two-tailed Student's *t*-test or multiple comparison test where appropriate. A $P<0.05$ was considered significant for all analyses.

3. Results

3.1. Glyphosate induced cell death in dose-and time-dependent manners

Chronic exposure of PC12 cells to glyphosate resulted in a concentration- and time-dependent decrease of cell viability assayed by MTT, which indicated a reduction in the mitochondria of living cells (Fig. 1). After treatment with glyphosate for 24 h, the inhibitory rate of glyphosate on the viability of PC12 cells increased to 10%–25% when the concentration was increased from 10 mM to 40 mM. When the incubation time was prolonged to 72 h, the inhibitory rate reached to about 50%.

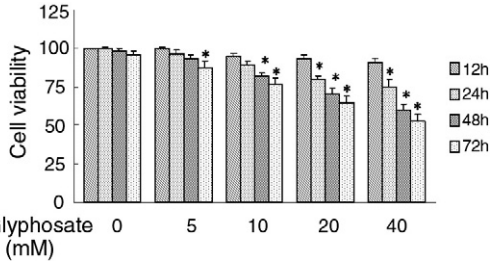

Fig. 1. Effects of glyphosate on viability of PC12 cells. PC12 cells following exposure to various concentrations of glyphosate at different lengths of time and cell viability were analyzed with the MTT assay. Results (mean ± SD) from three independent experiments were quantitatively analyzed. *$P<0.05$ compared to the control.

3.2. Glyphosate induced apoptosis in neuronal differentiated PC12 cells

Observed typical morphologic changes of apoptosis associated with glyphosate exposure in a time-dependent manner, included cell shrinkage, disappearance of neurites, nuclear condensation and DNA fragmentation using DAPI staining (Fig. 2A). Additionally, FACS via Annexin-V-PI double staining and Westeren blot were used to detect apoptotic cells and apoptotic proteins, respectively. We found that glyphosate increased apoptosis percentage in a time dependent manner compared with the untreated control (Fig. 2B, D), consistent with previous DAPI staining results. Meanwhile, Western blot experiment revealed that the expression of anti-apoptotic protein Bcl-2 was decreased in comparison with increasing of proapoptotic protein Bax after glyphosate treatment for 48 h (Fig. 4 C).

However, when PC12 cells were pretreated with the pan-caspase inhibitor Z-VAD for 1 h before glyphosate treatment, the cell viability assessment suggested that Z-VAD only partially inhibited the glyphosate-induced cytotoxicity (Fig. 4B). This phenomenon implies the existence of other cell death modes.

3.3. Glyphosate activated autophagic pathway in neuronal differentiated PC12 cells

To determine whether autophagy involved in glyphosate-induced neurotoxicity, we investigated the possible effects of autophagy Inhibitor, 3-MA, on glyphosate-induced cell death and MTT assay showed that pretreatment of PC12 cells with 3-MA markedly suppressed glyphosate-induced cell death (Fig. 4B). Furthermore, because autophagy marker GFP–LC3 would redistribute from a diffuse cytoplasmic pattern to punctate structures once autophagy is initiated (Maiuri et al., 2007), we examined LC3 immunoreactivity and found that punctate patterns of GFP–LC3 were observed in almost half of glyphosate treated cells compared with largely diffuse distribution in the control groups (Fig. 3A,).

In addition, we measured the expressions of two forms of LC3 proteins (LC3-I and LC3-II) and Beclin-1 levels by Western blot. Recent study indicated that LC3-II migrates faster than LC3-I on SDS-PAGE, and is detected more readily by Western blot (Kabeya et al., 2000). The results showed that an apparent increase in the level of LC3-II protein and Beclin-1 were observed in PC12 cells in a time dependent manner after treatment with glyphosate in response to increased LC3-II/LC3-I and Beclin-1/Actin protein expression ratio (Fig. 3B–D).

3.4. Down-regulation of Beclin-1 attenuated glyphosate induced autophagy and apoptosis

In order to determine whether Beclin-1 was required to activate both autophagy and apoptosis mechanisms in glyphosate-induced toxicity, the cell viability, LC3 immunoreactivity and proteins expressions were measured after knockdown of Beclin-1 expression by siRNA, respectively. We found that the inhibition of Beclin-1 caused increasing cell viability was more evident than 3-MA or z-VAD pretreatment (Fig. 4B) and reduced 34% of GFP–LC3 punctuate formulation compared with both scrambled siRNA transfected controls and z-VAD controls after glyphosate treatment, similar with the effect

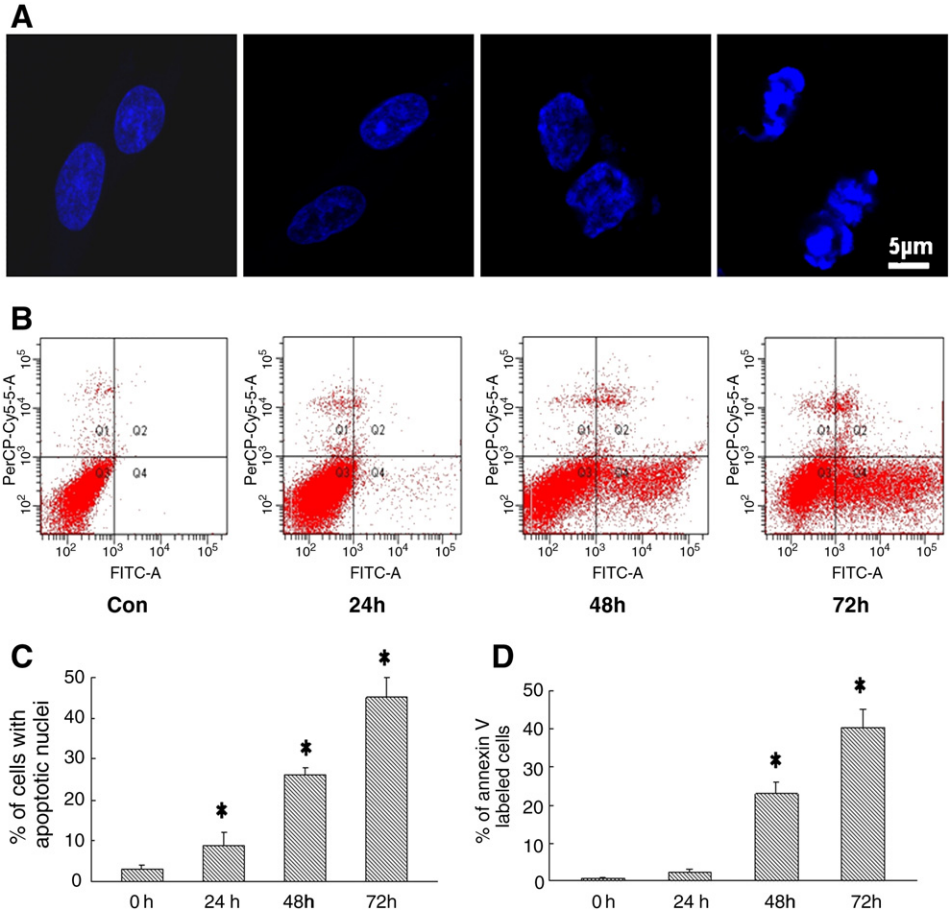

Fig. 2. Time-dependent apoptosis by glyphosate in PC12 cells. Glyphosate(40 mM) were incubated for 24, 48, or 74 h. (A) PC12 cells were treated or untreated with glyphosate and nuclei were stained with DAPI to detect apoptosis morphologically by confocal microscopy. Scar bar, 5 μm. (B) Representative flow cytometry graphs for cell apoptosis determination by annexin V/PI staining. (C, D) Results (mean ± SD) from three independent experiments were quantitatively analyzed. *$P<0.05$ compared to the control.

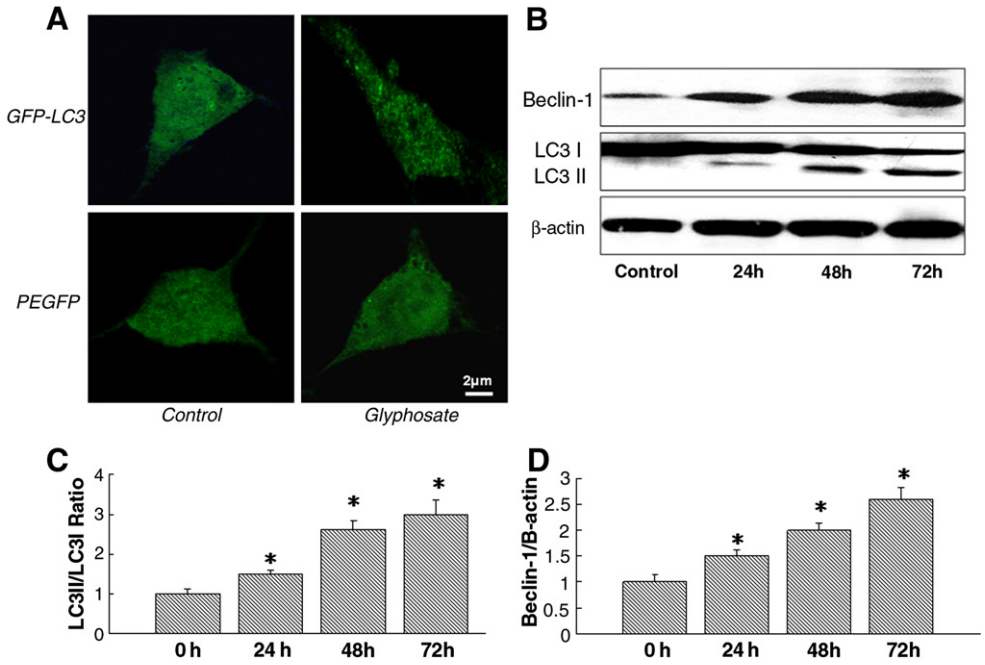

Fig. 3. Effects of glyphosate on inducing autophagy in PC12 cells. Cells were transiently transfectd with GFP–LC3 expression vector or GFP empty vector and then were untreated or treated with glyphosate (40 mM) for 48 h. (A) Representative confocal photos demonstrated that glyphosate increase punctuate GFP–LC3 in PC12 cells while dots failed to be found in GFP alone cells treated with glyphosate. Scale bars: 2 μm. Representative Western blot images(B) and quantification analysis(C,D) showed that glyphosate promoted the conversion of LC3-Ito LC3-II and upregulated Beclin-1 expression in the time-dependent manner. β-actin was used as a loading control. The data of three independent experiments are expressed as mean ± SD. $^{*}P<0.05$ compared to the control.

of 3-MA treatment (Fig. 4A). However, pretreatment with z-VAD demonstrated no significant influence on glyphosate-induced autophagy (Fig. 4A). As expected, Western blot analysis demonstrated that Beclin-1 knockdown decreased LC3-II levels and more importantly, reduced Bax/Bcl-2 ratio, which is a key determining factor for apoptosis in glyphosate treated PC12 cells (Fig. 4 C–F).

4. Discussion

This study demonstrated that glyphosate induced both apoptotic and autophagic cell death in neuronal differentiated PC12 cells, and elucidated the involvement of Beclin-1 in the crosstalk between these two mechanisms.

As a commonly used herbicide, glyphosate has been reported with the potential to cause a variety of acute and chronic adverse health effects (Astiz et al., 2009; Zhuang et al., 2009). A series of experiments have proven that it can induce DNA damage in human fibroblasts (Oberstein et al., 2007), and resulted in apoptosis in several cell-lines via mitochondrial disruption(Benachour and Seralini, 2009). Furthermore, prior case reports indicated that exposure to glyphosate was related with Parkinsonism(Barbosa et al., 2001; Wang et al., 2011), but the underlying mechanism of the association is still unknown.

Apoptosis is an energy-dependent, natural, genetically-controlled process tightly regulated by the interplay of the Bcl-2 family proteins characterized by the presence of up to 4 conserved 'Bcl-2 homology' (BH) domains. Among the members of the Bcl-2 family, Bcl-2 protein acts to suppress cell death and Bax protein promotes cell death. Bax/Bcl-2 index was confirmed as a significant independent prognostic factor for cell apoptosis (Gazzaniga et al., 1996). In this study, glyphosate treatment produced high Bax and low Bcl-2 protein expression, and thus increased the Bax/Bcl-2 ratio, which is consistent with our hypothesis that glyphosate induced apoptosis in neuronal differentiated PC12 cells. This notion was further supported by nuclei morphological stains and Annexin-V-FITC/PI double staining assay analyses. However, cell viability assay showed that blockage of apoptosis by the pan caspase inhibitor z-VAD only decreased glyphosate-induced cell death partially, and prompting the involvement of other mechanisms apart from apoptosis in glyphosate-induced cell death.

As one of the two major programmed cell death pathways in eukaryotes, autophagy has been known for a long time. Unlike apoptosis, its involvement in the pathogenesis of PD has been investigated only recently (Schapira and Jenner, 2011). Autophagy is a process by which cytoplasmic constituents and entire organelles are targeted to lysosomes for degradation(Eskelinen and Saftig, 2009). It is a complex cellular process with a dual-effect. In most circumstances, autophagy promoted cell survival and defensive stress response. However, it may also accelerate cell death through excessive self-digestion and degradation of essential cellular constituents. LC3, a mammalian homologue of Atg8, is a specific marker for autophagosomes. Once autophagy is initiated, cytosolic LC3I form (18 kDa) covalently conjugates to a lipidated, autophagosome associated LC3II form (16 kDa) (Trincheri et al., 2008). Therefore, GFP–LC3 would redistribute from a diffuse cytosolic staining to a cytoplasmic punctuate pattern upon autophagy stimulation. Our results showed that after incubation with glyphosate, GFP–LC3 in PC12 cells displayed significant punctate expression pattern. Consistent with the fluorescent experiments, a time-dependent conversion of LC3-I to LC3-II was observed in glyphosate-treated PC12 cells by Western blot. Moreover, glyphosate induced cell death in PC12 cells was partially blocked by 3-MA, an autophagy inhibitor, suggesting the existence of autophagic mechanism in glyphosate-treated PC12 cells.

Although apoptosis and autophagy are classified separately and present with distinct morphological features, these two types of cell death are thought to have extremely complex interrelationships and can overlap at the level of various signaling steps(Kurz et al., 2010). It has been reported that numerous stimuli can induce either apoptosis or autophagy, or both; and morphologic features of both cell death mechanisms can be observed concurrently in the same cell (Maiuri et al., 2007; Zhuang et al., 2009). Autophagy may act as a cytoprotective mechanism favouring stress adaptation and suppress apoptosis. In specific conditions, it may also promote or be in collaboration with

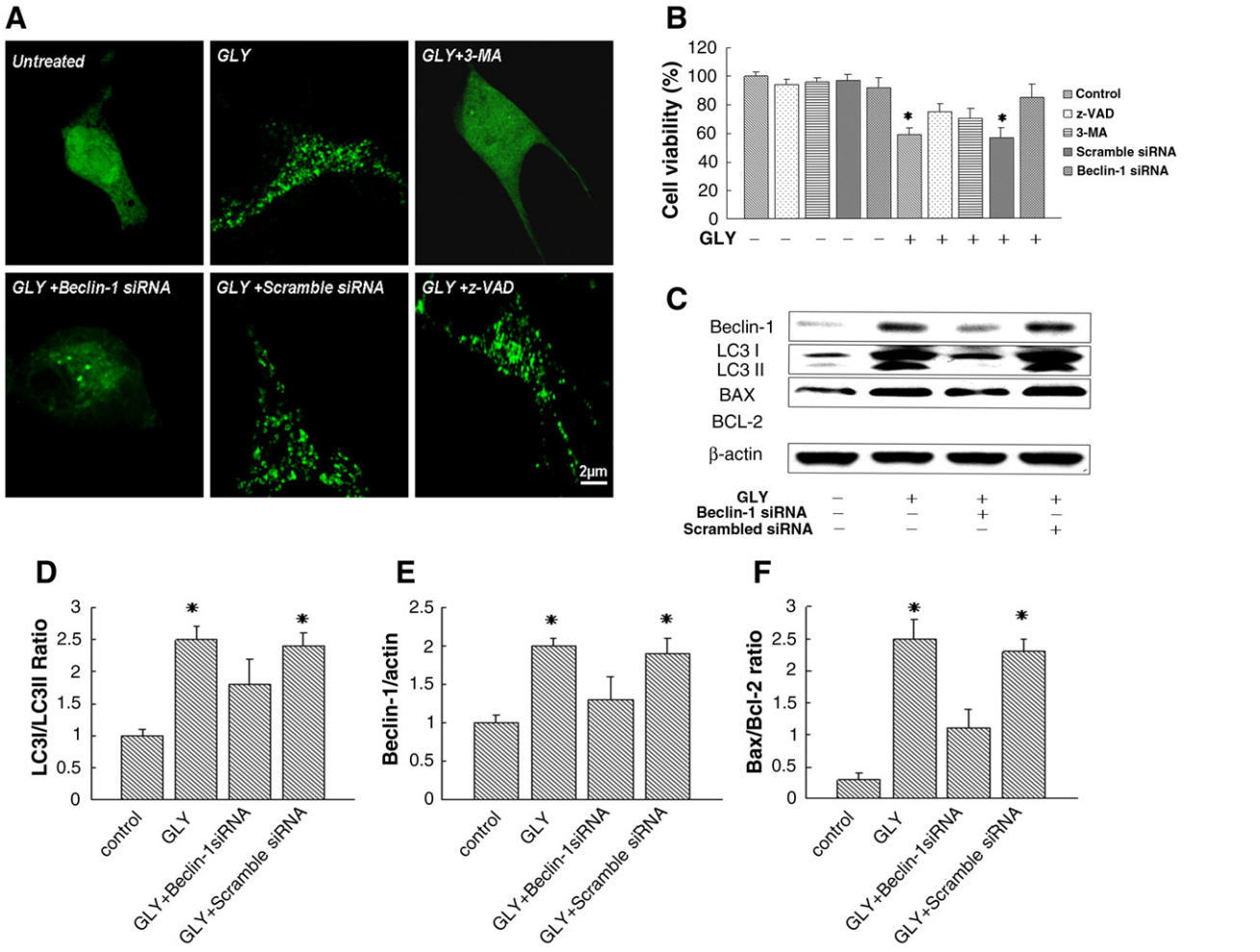

Fig. 4. Beclin-1 was involved in glyphosate-induced cell death in PC cells. Cells were co-transfected with GFP–LC3 and Beclin-1 siRNA or scrambled siRNA, or transfected with GFP–LC3 after pretreated with z-VAD or 3-MA, and further treated with glyphosate (40 mM) for 48 h. Representative confocal photos (A) demonstrated that Beclin-1 siRNA and 3-MA reduced glyphosate-induced GFP- LC3 puncta formation,whilst scrambled siRNA and z-VAD caused no significant difference in glyphosate-treated cell. Scale bars: 2 μm. GLY: glyphosate. (B) Cell viability after various treatments was evaluated by the MTT assay. Representative Western blot images (C) and quantification analysis (D,E,F) showed that Beclin-1 knockdown attenuated glyphosate-induced changes on LC3-II/LC3-I ratio, Beclin-1 expression and Bax/bcl-2 index in PC12 cells. The data of three independent experiments are expressed as mean ± SD. $^{*}P<0.05$ compared to the control.

apoptosis(Gazzaniga et al., 1996). Similarly, regulators of apoptosis activation also function as autophagy regulators. Some upstream signaling molecules are common to both processes and the interactions between them may arise as a result of signaling crosstalk(Levine and Yuan, 2005). Therefore, the existence of crosstalk between glyphosate-induced apoptotic and autophagic cell death needed to be further elicited.

Recent studies have suggested that a functional and physical Beclin-1/Bcl-2 interaction being possibly involved in the molecular mechanisms for the crosstalk between apoptosis and autophagy (Chang et al., 2010; Maiuri et al., 2007). Prior studies have shown that Beclin-1/Atg6 is a subunit of the class III PI3-kinase complex and plays important roles in the processes of autophagy (Furuya et al., 2005; Oberstein et al., 2007). Beclin-1 has been identified originally as a Bcl-2-interacting protein, which forms a complex along with hVps34 and Vps15 in charge of autophagic initiation in mammals. It possesses a Bcl-2 homology-3 domain (BH3), which is necessary for its binding to the BH3 receptor domain of Bcl-2 or Bcl-xL. Bcl-2 and Bcl-xL operate as critical nodes in complex networks to integrate information and make ultimate decisions on whether to initiate apoptosis. Although Bcl-2 and Bcl-xL are well-described in the apoptotic pathway, they appear to be important factors in suppressing autophagy by binding to the protein Beclin-1 and can therefore aid cells to evade cell death by autophagy (Cho et al., 2009; Levine et al., 2008; Liang et al., 1999).

Having established in our study that Beclin-1 was involved in glyphosate induced autophagy in PC12 cells, we hypothesized that it also plays a role in glyphosate induced cytotoxicity and investigated the possibility of its involvement in cell apoptosis. Our results demonstrated that glyphosate caused an increased expression of the Beclin-1 gene, indicating that Beclin-1 associated autophagy was also involved in glyphosate induced death in PC12 cells. Furthermore, Beclin-1 knockdown by siRNA not only reduced GFP–LC3 puncta and LC3 II levels, but also apparently reversed glyphosate induced higher Bax/Bcl-2 ratio. MTT assay results further revealed that Beclin-1 knockdown improved the viability of PC12 cells treated with glyphosate more effectively than 3-MA or z-VAD pretreatment. These data presented here highlighted the effect of Beclin-1 on regulating both autophagic and apoptotic pathways. However, the interplay between autophagy and apoptosis, including caspase activation, Bcl-2 family proteins post-translational modifications and other possible upstream signals alterations induced by glyphosate needs to be clarified by in-depth studies in future.

To summarize, our results demonstrate that glyphosate induces not only apoptosis, but autophagy in PC12 cells. Beclin-1 has been identified as the protein potentially regulating the two pathways. This also provides a novel link between use of herbicide, glyphosate and PD.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

We would like to thank Dr. Yue Huang at Neuroscience Research Australia, University of New South Wales, and Prof. Robert Chen, University of Toronto for critical reading of the manuscript. This work was supported by grants from the National Program of Basic Research (2010CB945200, 2011CB504104) of China.

References

Anglade P, Vyas S, Javoy-Agid F, Herrero MT, Michel PP, Marquez J, et al. Apoptosis and autophagy in nigral neurons of patients with Parkinson's disease. Histol Histopathol 1997;12:25. [PubMed: 9046040].

Astiz M, de Alaniz MJ, Marra CA. Effect of pesticides on cell survival in liver and brain rat tissues. Ecotoxicol Environ Saf 2009;72:2025–32. [PubMed: 19493570].

Barbosa ER, Leiros da Costa MD, Bacheschi LA, Scaff M, Leite CC. Parkinsonism after glycine-derivate exposure. Mov Disord 2001;16:565–8. [PubMed: 11391760].

Benachour N, Seralini GE. Glyphosate formulations induce apoptosis and necrosis in human umbilical, embryonic, and placental cells. Chem Res Toxicol 2009;22: 97-105. [PubMed: 19105591].

Bove J, Prou D, Perier C, Przedborski S. Toxin-induced models of Parkinson's disease. NeuroRx 2005;2:484–94. [PubMed: 16389312].

Bredesen DE, Rao RV, Mehlen P. Cell death in the nervous system. Nature 2006;443: 796. [PubMed: 17051206].

Cannon JR, Greenamyre JT. Neurotoxic in vivo models of Parkinson's disease recent advances. Prog Brain Res 2010;184:17–33. [PubMed: 20887868].

Chang NC, Nguyen M, Germain M, Shore GC. Antagonism of Beclin 1-dependent autophagy by BCL-2 at the endoplasmic reticulum requires NAF-1. EMBO J 2010;29:606–18. [PubMed: 20010695].

Cho DH, Jo YK, Hwang JJ, Lee YM, Roh SA, Kim JC. Caspase-mediated cleavage of ATG6/Beclin-1 links apoptosis to autophagy in HeLa cells. Cancer Lett 2009;274: 95-100. [PubMed: 18842334].

Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. Neuron 2003;39:889–909. [PubMed: 12971891].

Eskelinen EL, Saftig P. Autophagy: a lysosomal degradation pathway with a central role in health and disease. Biochim Biophys Acta 2009;1793:664–73. [PubMed: 18706940].

Furuya N, Yu J, Byfield M, Pattingre S, Levine B. The evolutionarily conserved domain of Beclin 1 is required for Vps34 binding, autophagy and tumor suppressor function. Autophagy 2005;1:46–52. [PubMed: 16874027].

Gazzaniga P, Gradilone A, Vercillo R, Gandini O, Silvestri I, Napolitano M, et al. Bcl-2/bax mRNA expression ratio as prognostic factor in low-grade urinary bladder cancer. Int J Cancer 1996;69:100–4. [PubMed: 8608975].

Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, et al. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. EMBO J 2000;19:5720–8. [PubMed: 11060023].

Kurz T, Eaton JW, Brunk UT. Redox activity within the lysosomal compartment: implications for aging and apoptosis. Antioxid Redox Signal 2010;13:511–23. [PubMed: 20039839].

Levine B, Yuan J. Autophagy in cell death: an innocent convict? J Clin Invest 2005;115: 2679–88. [PubMed: 16200202].

Levine B, Sinha S, Kroemer G. Bcl-2 family members: dual regulators of apoptosis and autophagy. Autophagy 2008;4:600–6. [PubMed: 18497563].

Levy OA, Malagelada C, Greene LA. Cell death pathways in Parkinson's disease: proximal triggers, distal effectors, and final steps. Apoptosis 2009;14:478. [PubMed: 19165601].

Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, et al. Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 1999;402:672–6. [PubMed: 10604474].

Maiuri MC, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: crosstalk between autophagy and apoptosis. Nat Rev Mol Cell Biol 2007;8:741–52. [PubMed: 17717517].

Negga R, Rudd DA, Davis NS, Justice AN, Hatfield HE, Valente AL, et al. Exposure to Mn/Zn ethylene-bis-dithiocarbamate and glyphosate pesticides leads to neurodegeneration in Caenorhabditis elegans. Neurotoxicology 2011;32:331–41. [PubMed: 21376751].

Oberstein A, Jeffrey PD, Shi Y. Crystal structure of the Bcl-XL-Beclin 1 peptide complex: Beclin 1 is a novel BH3-only protein. J Biol Chem 2007;282:13123–32. [PubMed: 17337444].

Patel MM, Schier JG. Medical toxicology and public health: update on research and activities at the Centers for Disease Control and Prevention and the Agency for Toxic Substances and Disease Registry. J Med Toxicol 2006;2:169. [PubMed: 18072139].

Peixoto F. Comparative effects of the Roundup and glyphosate on mitochondrial oxidative phosphorylation. Chemosphere 2005;61:1115–22. [PubMed: 16263381].

Peng J, Oo ML, Andersen JK. Synergistic effects of environmental risk factors and gene mutations in Parkinson's disease accelerate age-related neurodegeneration. J Neurochem 2010;115:1363–73. [PubMed: 21039522].

Schapira AH, Jenner P. Etiology and pathogenesis of Parkinson's disease. Mov Disord 2011;26:1049–55. [PubMed: 21626550].

Trincheri NF, Follo C, Nicotra G, Peracchio C, Castino R, Isidoro C. Resveratrol-induced apoptosis depends on the lipid kinase activity of Vps34 and on the formation of autophagolysosomes. Carcinogenesis 2008;29:381–9. [PubMed: 18048384].

ubinsztein DC. The roles of intracellular protein-degradation pathways in neurodegeneration. Nature 2006;443:780. [PubMed: 17051204].

Wang G, Fan XN, Tan YY, Cheng Q, Chen SD. Parkinsonism after chronic occupational exposure to glyphosate. Parkinsonism Relat Disord 2011;17:486–7. [PubMed: 21367645].

Zhuang W, Qin Z, Liang Z. The role of autophagy in sensitizing malignant glioma cells to radiation therapy. Acta Biochim Biophys Sin (Shanghai) 2009;41:341–51. [PubMed: 19430698].