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LETTER TO THE EDITOR

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Inhibitory Effect of Bcl-xL Gene on Toxicity Induced by Sodium Nitroprusside in SH-SY5Y cells

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Nitric oxide (NO) at physiological concentration is an intracellular messenger molecule in central nervous system (CNS). It plays a pivotal role in maintaining normal physiological function. While excessive NO produced by inducible NO synthase may lead to a variety of neurological diseases, such as Alzheimer's disease, Parkinson's disease, multiple sclerosis and cerebral vascular disease, and so on [1,2]. It has been demonstrated that neural damage triggered by NO donor sodium nitroprusside

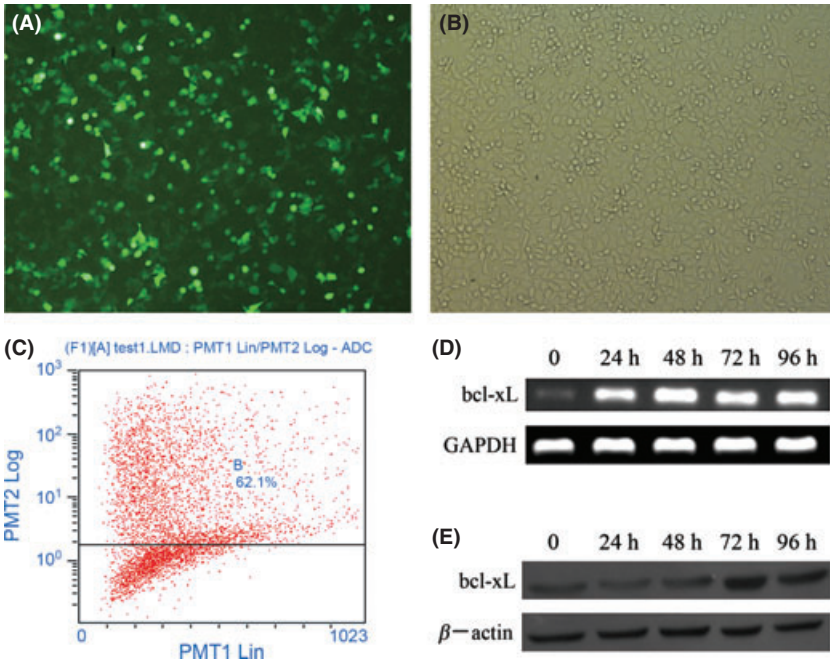

Figure 1 Overexpression of Bcl-xL in SH-SY5Y cells. (**A**) The transfected cells were analyzed under a fluorescent microscope. (**B**) The morphology of SH-SY5Y cells was observed under bright field microscope. (**C**) Transfection efficiency was detected 48h after transfection by flow cytometry. (**D**) RT-PCR and (**E**) Western blotting were employed to detect the mRNA and protein expression of Bcl-xL 24, 48, 72, and 96h after transfection, respectively.

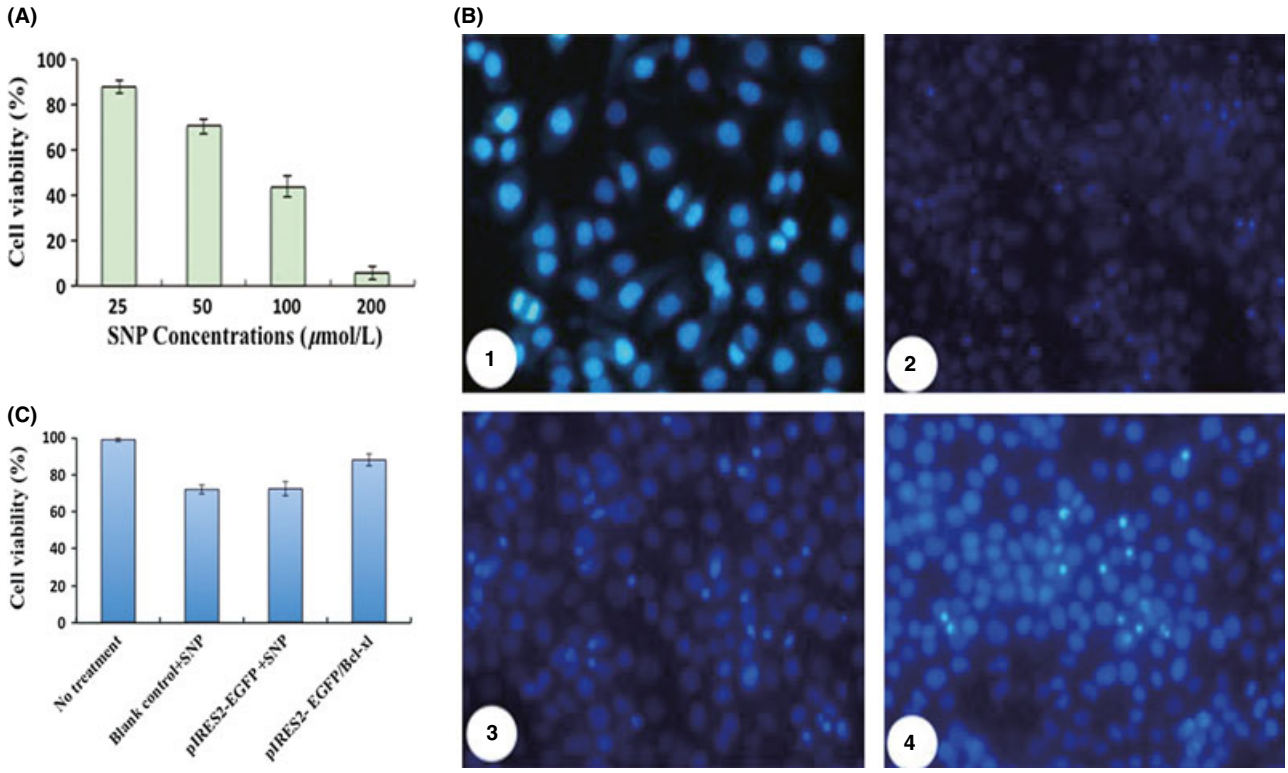

Figure 2 Effect of Bcl-xL on cell viability in SH-SY5Y cells treated with SNP was assessed by MTT assay. (**A**) The effect of sodium nitroprusside (SNP) on cell viability in SH-SY5Y cells was evaluated by MTT assay. Data were presented as mean ± SD. (**B**) Cell apoptosis detected by Hoechst 33258 staining. (1) SH-SY5Y cells without treatment (2) SH-SY5Y cells treated with SNP (3) SH-SY5Y cells transfected with pIRES2-EGFP and then treated with SNP (4) SH-SY5Y cells transfected with pIRES2-EGFP/Bcl-xL and treated with SNP. (**C**) MTT assay was employed to calculate the effect of Bcl-xL on cell viability in SH-SY5Y cells treated with SNP.

(SNP) is related to oxidative stress, apoptosis, and inflammatory responses. However, the mechanisms involved in NO-induced apoptosis of nerve cells are poorly elucidated.

Bcl-2 was used to be an attractive gene for reducing apoptosis [3]. However, some researchers found that overexpression of Bcl-2 did not fight against but promoted apoptosis of cells. The most rational explanation is that Bcl-2 has distinct effects in different cells [4]. Thus, Bcl-xL attracts more and more attention in the field of resisting apoptosis as it has similar structure to Bcl-2 [5]. A growing body of studies has demonstrated that Bcl-xL exerted satisfactory anti-apoptotic effects on various cells, including neurons [6,7]. Furthermore, González-García M's study showed that Bcl-2 expressed much less than Bcl-xL in the mature CNS. Therefore, Bcl-xL may play a central role in protecting cells from apoptosis in CNS, while Bcl-2 seems to be inessential for neurons [8]. Thus, we aimed to investigate the potential of Bcl-xL to block cytotoxicity induced by NO in neurons.

In this study, the eukaryotic expression vector pIRES2-EGFP/Bcl-xL was constructed and then delivered into SH-SY5Y human neuron-like cells. Transfection efficiency was detected by fluorescence microscopy and flow cytometry. RT-PCR and Western blotting were employed to evaluate the expression of Bcl-xL at multiple time points after transfection. Furthermore, SNP, an apoptosis-inducing agent, was used as NO donor to treated SH-SY5Y cells transfected with pIRES2-EGFP/Bcl-xL or pIRES2-EGFP plasmids. Cytotoxicity of SNP was analyzed by MTT assay. And the anti-apoptotic effect of Bcl-xL on cells exposed to SNP was observed by Hoechst 33258 immunohistochemistry staining. Data were expressed as mean ± standard deviation (SD) and analyzed with SPSS 13.0 (SPSS Inc., Chicago, IL, USA). One-way analysis of variance was used for establishing differences among groups. Intergroup comparisons were made by Duncan's multiple range test. Differences were regarded as significant if $P < 0.05$.

Twenty-four hour after transfection, green fluorescence from EGFP fusion protein was observed in the images of SH-SY5Y cells under fluorescence microscope (Figure 1A,B), which demonstrated that pIRES2-EGFP/Bcl-xL recombinant vectors were successfully delivered into SH-SY5Y cells. Moreover, the results of flow cytometry showed that the transfection efficiency was approximately 63.5 ± 3.8% (Figure 1C).

To detect the expression of Bcl-xL after transfection, the mRNA and the protein of Bcl-xL at different time points were analyzed. The result of RT-PCR (Figure 1D) showed that specific PCR fragments of Bcl-xL were about 700 bp. The mRNA expression of Bcl-xL gradually strengthened and reached the peak at 48h after transfection. Western blotting analysis (Figure 1E) suggested that the expression of Bcl-xL protein gradually increased and reached the peak at 72 h, but slightly decreased at 96 h after transfection.

Then SNP was employed as a NO donor to treat cells. The results of MTT test suggested that as the concentration of SNP

increased, cell viability decreased in a dose-dependent manner (Figure 2A). After transfected with pIRES2-EGFP plasmid and pIRES2-EGFP/Bcl-xL plasmid for 48 h, SH-SY5Y cells were incubated with 50 μmol/L SNP for 24 h. Cells without transfection exposed to SNP were used as control. Furthermore, cells in each group were observed under fluorescene microscopy after Hoechst 33258 staining. The results showed that SH-SY5Y cells without treatment displayed well-distributed blue color in neucleus (Figure 2B-1), while 50 μmol/L SNP could induce apoptosis in about 27.9 $\pm$ 2.4% SH-SY5Y cells with apoptotic bodies and pyknotic neuclei. Furthermore, cells transfected with pIRES2-EGFP plasmids (Figure 2B-3) could not reduce the per cent of apoptotic cells (27.5 $\pm$ 3.8%), but overexpression of Bcl-xL could rescue the cell death via alleviating percentage of apoptosis to about 11.9 $\pm$ 3.3%(Figure 2B-4). The results of MTT assay (Figure 2C) revealed that after the exposure to SNP, the cell viability of cells transfected with pIRES2-EGFP/Bcl-xL plasmids was significantly higher than cells treated with or without pIRES2-EGFP empty vector, and the differences had statistical significance ($P < 0.05$), while the transfection of pIRES2-EGFP empty vector could not rescue the cell viability of cells exposed to SNP ($P > 0.05$).

NO serves as a double-edged sword for deciding the fate of mammalian cells. Excessive production of NO is an important inflammatory mediator and leads to damage and even death of neurons in CNS. A mechanism of NO-induced neural cytotoxicity might involve the activation of oxidative stress. High dose of NO interacts with O_2^- to form ONOO-, which destructs the integrity and metabolism of mitochondria. Bax and Bak, the pro-apoptotic members of the Bcl-2 family, can change the permeability of the outer mitochondrial membrane and lead to the release of apoptotic factors into the cytoplasm. Bcl-xL can directly form heterodimers with Bax to block its translocation to the mitochondria, thus inhibiting apoptotic cascade via regulation mitochondrial membrane conductance and permeability [9]. Moreover, Bcl-xL promoter contains two GATA elements. A recent study revealed that SNP reduced Bcl-xL expression by decreasing the GATA-4 DNA-binding activity in lung vascular smooth muscle [10]. Therefore, we wondered whether Bcl-xL had protective effect on NO-induced cellular damage.

The results in this study demonstrated that overexpression of Bcl-xL could partially reduce the cell loss induced by SNP in SH-SY5Y cells. Although the significance of our findings should be validated in further studies using *in vivo* animal models, this study still provides new strategies for gene therapy of neurological diseases.

Acknowledgments

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References

1. Moncada S, Bolanos JP. Nitric oxide, cell bioenergetics and neurodegeneration. *J Neurochem* 2006;**97**:1676–1689.
2. Chen XM, Wang NN, Zhang TY, Wang F, Wu CF, Yang JY. Neuroprotection by sildenafil: neuronal networks potentiation in acute experimental stroke. *CNS Neurosci Ther* 2014;**20**:40–49.
3. Zhao ZY, Luan P, Huang SX, et al. Edaravone protects HT22 neurons from H_2O_2-induced apoptosis by inhibiting the MAPK signaling pathway. *CNS Neurosci Ther* 2013;**19**:163–169.
4. Del Bufalo D, Biroccio A, Trisciuoglio D, et al. Bcl-2 has differing effects on the sensitivity of breast cancer cells depending on the antineoplastic drug used. *Eur J Cancer* 2002;**38**:2455–2462.
5. Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. *Science* 1998;**281**:1322–1326.
6. Chien CT, Shyue SK, Lai MK. Bcl-xL augmentation potentially reduces ischemia/reperfusion induced proximal and distal tubular apoptosis and autophagy. *Transplantation* 2007;**84**:1183–1190.
7. Doeppner TR, El Aanbouri M, Dietz GP, Weise J, Schwarting S, Bahr M. Transplantation of TAT-Bcl-xL-transduced neural precursor cells: long-term neuroprotection after stroke. *Neurobiol Dis* 2010;**40**:265–276.
8. Gonzalez-Garcia M, Garcia I, Ding L, et al. bcl-x is expressed in embryonic and postnatal neural tissues and functions to prevent neuronal cell death. *Proc Natl Acad Sci U S A* 1995;**92**:4304–4308.
9. Antignani A, Youle RJ. How do Bax and Bak lead to permeabilization of the outer mitochondrial membrane? *Curr Opin Cell Biol* 2006;**18**:685–689.
10. Suzuki YJ, Nagase H, Wong CM, et al. Regulation of Bcl-xL expression in lung vascular smooth muscle. *Am J Respir Cell Mol Biol* 2007;**36**:678–687.