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Mood stabilizers commonly restore staurosporine-induced increase of p53 expression and following decrease of Bcl-2 expression in SH-SY5Y cells

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Abstract

Adult neurogenesis in dentate gyrus (DG) is involved in the action mechanism of mood stabilizers. However, it is poorly understood how mood stabilizers affect adult neurogenesis in DG. Neurogenesis consists of proliferation, survival (anti-apoptosis) and differentiation of neural precursor cells in adult DG. Using *in vitro* culture of adult rat DG-derived neural precursor cells (ADP), we have already shown that four mood stabilizers, such as lithium (Li), valproate (VPA), carbamazepine (CBZ) and lamotrigine (LTG), commonly decrease staurosporine (STS)-induced apoptosis of ADP. These suggest that the common anti-apoptotic effect of mood stabilizers could be involved in mood-stabilizing effects.

Past studies have shown that Li and VPA increase the expression of Bcl-2, an anti-apoptotic gene. In addition, it has been shown that Li decreases the expression of p53, which plays a prominent role in apoptosis and regulates the expression of Bcl-2. Therefore, p53 and Bcl-2 can be considered to mediate the common anti-apoptotic effects of Li, VPA, CBZ and LTG.

To elucidate the molecular mechanism underlying the common anti-apoptotic effects of mood stabilizers, we investigated the effects of Li, VPA, CBZ and LTG on STS-induced expression changes of p53, Bcl-2 and other p53-related molecules using SH-SY5Y cells as a model of neural precursor-like cells. STS increased the expression of p53 and decreased that of Bcl-2. These effects of STS on p53 and Bcl-2 are restored by all of Li, VPA, CBZ and LTG. In addition, p53 overexpression decreased the expression of Bcl-2. Taken

together, these results suggest that p53 and Bcl-2 may be involved in a part of mood-stabilizing effects.

Key words: apoptosis, hippocampus, dentate gyrus, adult neurogenesis, Bcl-2

Abbreviations: BD, bipolar disorder; Li, lithium; VPA, valproate; CBZ, carbamazepine; LTG, lamotrigine; DG, dentate gyrus; STS, staurosporine; ADP, adult rat dentate gyrus-derived neural precursor cell

1. Introduction

Bipolar disorder (BD), characterized by recurrent periods of mania and depression, affects approximately 1% of the world population (Weissman et al, 1996). Although mood stabilizers such as lithium (Li), valproate (VPA), carbamazepine (CBZ), and lamotrigine (LTG) are commonly used for treatment of BD patients (Goodwin, 2003), their effects are often inadequate. Moreover, many BD patients have suffered from pervasive and chronic symptoms, multiple episode recurrences and marked functional disability (Giltin, 2006). Thus, BD remains a major health concern; the development of new treatments that are more effective for BD is ardently desired.

Although there is still no consensus on the pathophysiology of BD (Kato et al, 2007), the investigation of the action mechanisms of mood stabilizers is expected to lead to elucidating it. Recent studies suggest that adult neurogenesis in dentate gyrus (DG) of hippocampus may be involved in the action mechanism of mood stabilizers (Schlosser et al, 2007). Neurogenesis consists of three phenomena such as proliferation, survival (anti-apoptosis) and differentiation of neural precursor cell. However, it remains unclear which phenomenon is involved in the action mechanism of mood stabilizers. To investigate it, we established *in vitro* culture system of adult rat DG-derived neural precursor cell (ADP) (Boku et al, 2009). Using ADP, we showed that all of Li, VPA, CBZ and LTG decrease staurosporine (STS)-induced apoptosis. On the other hand, they have varied effects on dexamethasone-induced decreases of proliferation and retinoic acid-induced differentiation (Boku et al, 2011). STS is a common inhibitor of protein kinase C and often used as an inducer of apoptosis (Sanchez et al, 1992). These results suggest that the

common anti-apoptotic effects of mood stabilizers may be involved in the action mechanism of mood stabilizers.

The role of apoptosis of neural precursor cells in the pathophysiology of BD is still poorly understood. Past studies have suggested that stress, a crucial component in the emergence of affective disorders such as BD and depression (Kessler, 1997), reduces survival of neural precursor cells in adult DG without altering their proliferation (Thomas et al, 2007). In addition, reducing survival of neural precursor cells increases the level of glucocorticoids, stress-related hormones, and depressive behaviors (Snyder et al, 2011). These past studies suggest that survival of neural precursor cells in adult DG may play a role in the pathophysiology of BD via the regulation of stress. Therefore, elucidating the detailed mechanism underlying the anti-apoptotic effects of mood stabilizers may lead to further understanding the action mechanism of them and the pathophysiology of BD.

STS induces apoptosis through activating internal pathway via mitochondria (Ferrari et al, 1998). In this pathway, p53 regulates the transcription of Bcl-2 family genes. p53 is well known to play a prominent role in apoptosis through regulating the transcription of many apoptosis-related genes (Riley et al, 2008). Bcl-2 family genes contain pro-apoptotic genes and anti-apoptotic genes. The transcription of pro-apoptotic Bcl-2 family genes is activated by p53 and that of anti-apoptotic Bcl-2 family genes is inhibited by p53 (Miyashita et al, 1994). In Bcl-2 family genes, the anti-apoptotic gene Bcl-2 is increased in adult rat frontal cortex by chronic administration of Li, VPA, CBZ and LTG (Chen et al, 1999; Chang et al, 2009). This *in vivo* study suggests that Bcl-2 may be involved in the action mechanism of four mood stabilizers. Some *in vitro* studies have also shown that Li and VPA increase the

expression of Bcl-2 (Chen et al, 1999; Chen and Chuang, 1999) and that Li decreases the expression of p53 (Chen and Chuang, 1999; Lu et al, 1999). However, the effects of CBZ and LTG on Bcl-2 expression and those of VPA, CBZ and LTG on the expression of p53 in neural culture cells are not known yet.

Here we investigated the mechanism of the common effects of mood stabilizers on STS-induced apoptosis, using human neuroblastoma-derived SH-SY5Y cells as a model of neural precursor-like cells.

2. Materials and Methods

2.1 Drugs

Staurosporine (STS) was kindly donated by Asahi-Kasei (Shizuoka, Japan); Lithium chloride (Li) was purchased from Wako Pure (Osaka, Japan); Sodium valporate (VPA) was kindly donated by Kyowa-Hakko (Tokyo, Japan); Carbamazepine (CBZ) was kindly donated by Nihon Ciba-Geigy (Tokyo, Japan); Lamotrigine (LTG) was kindly donated by Glaxo Smith Kline (London, UK).

2.2 Cell culture

SH-SY5Y cells (ATCC) were maintained in DMEM/F-12 Ham (Sigma, St. Louis, MO) with 10 % fetal bovine serum (Invitrogen, Carlsbad, CA), 100 µg/ml penicillin-streptomycin (Invitrogen) and 100 mM sodium pyruvate (Invitrogen) in a humidified atmosphere of 5 % CO₂.

2.2 TUNEL assay

2×10^4 cells/well were put in laminin–ornithine coated 8-chamber slides (Lab-Tek II;

Nalge Nunc International, Naperville, IL). After overnight incubation, cells were treated with STS, PHT, Li, VPA, CBZ, and/or LTG. After 3 days, cells were fixed in 4% paraformaldehyde for 15 min. Permeabilization was performed with PBS containing 0.2% Triton X-100 for 30 min. Subsequently, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was performed with a DeadEnd Fluorometric TUNEL System (Promega, Madison, WI), as described in the manufacturer's manual. Fluorescent signals were detected using a fluorescence microscope system (IX-71; Olympus, Tokyo, Japan). The quantities of both TUNEL and 4',6-diamino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA) signals were counted in four randomly selected fields/ well. Then the ratio of TUNEL signals/ DAPI signals was calculated. Statistical analysis was performed using unpaired *t*-test. Significance was defined as $p < 0.05$. Data are expressed as means $\pm$ SEM.

2.3 Total RNA Isolation and Quantitative RT- PCR

The concentration used in the experiments for each drug is as follows: STS: 300nM; Li: 1mM; VPA: 0.3mM; CBZ: 3uM; LTG: 30nM. These concentrations were determined by our previous study with ADP (Boku et al, 2011). 2×10^5 cells/well were put on 6-well plates in maintenance medium. After overnight incubation, cells were treated with each drug for 3 or 6 hours. Total RNA was extracted from cells with RNeasy extraction kit (Qiagen, Hilden, Germany) and converted to cDNA with Quantitect Reverse Transcription kit (Qiagen). PCR was performed with SYBR GreenER qPCR SuperMix (Invitrogen) in ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster, CA). The conditions of PCR were: 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of

95 °C for 15 sec and 60 °C for 1 min. The sequences of forward and reverse primers used were: *GGT GCA TGG CCG TTC TTA* and *TCG TTC GTT ATC GGA ATT AAC C* for 18s rRNA, *AGG CCT TGG AAC TCA AGG AT* and *GGT AGA CTG ACC CTT TTT GGA C* for p53, *AGT ACC TGA ACC GGC ACC T* and *GGC CGT ACA GTT CCA CAA A* for Bcl2, *AGC CTT GGA TCC AGG AGA A* and *GCT GCA TTG TTC CCA TAG AGT* for Bcl-XL, *CCT GCC CTC TGC TTC TGA* and *CTG CTG ATG GCG GTA AAA A* for BAK, *CAA GAC CAG GGT GGT TGG* and *CAC TCC CGC CAC AAA GAT* for BAX, respectively. 18s rRNA was used as a control. The results were analyzed by using SDS 2.0 software (Applied Biosystems). Statistical analysis was performed by unpaired *t*-test. Significance was defined as $p < 0.05$. Data are expressed as the means $\pm$ SEM.

2.4 Overexpression of p53

pSG5 vector (Clontech, La Jolla, CA) containing mouse p53 was kindly donated by Masayuki Mizui (Osaka University, Osaka, Japan). 2×10^5 cells/well were put on 6-well plates in medium without any antibiotics. After overnight incubation, transfection of pSG5-p53 or pSG5 (used as a control) was performed using Lipofectamine PLUS (Invitrogen). Cells were lysed 2 days after transfection with Mammalian Cell Lysis Kit (Sigma). First, the protein expression of p53 was checked with Western Blotting. Western Blotting of p53 was performed using anti-mouse p53 antibody (1:1000, Biovision, Mountain View, CA) as described in our past study (Boku et al, 2009). Then, the effect of p53 overexpression on the expression of Bcl-2 was examined with quantitative RT-PCR and Western Blotting. Quantitative RT-PCR of Bcl-2 was performed as described above. Statistical analyses were performed by unpaired *t*-test. Significance was defined as $p < 0.05$.

Data are expressed as the means $\pm$ SEM. Western Blotting of Bcl-2 was performed with anti-rabbit Bcl-2 antibody (1:200, Cell Signaling, Danvers, MA) as described in our past study (Boku et al, 2009). The pictures were converted to digital files and the intensity of each band was analyzed with Image J (National Institutes of Health, Bethesda, MD). Statistical analyses were performed by unpaired *t*-test. Significance was defined as $p < 0.05$. Data are expressed as the means $\pm$ SEM.

3. Results

3.1 Mood stabilizers decreased STS-induced apoptosis in SH-SY5Y cells

To check the applicability of SH-SY5Y cells as a model of neural precursor cells for investigating the mechanism underlying the effects of mood stabilizers on STS-induced apoptosis, the effect of mood stabilizers on STS-induced apoptosis in SH-SY5Y cells were examined using TUNEL assay. As in the case of our previous study (Boku et al, 2011), around 70% of cells were TUNEL-positive with 300 nM STS (Figure 1). Moreover, all of 1 mM Li, 300 μ M VPA, 3 μ M CBZ, and 30 nM LTG significantly decreased the ratio of TUNEL-positive cells to around 40% (unpaired *t*-test, Li: $p < 0.01$, $t = 4.212$, $F_{7,7} = 1.863$; VPA: $p < 0.05$, $t = 2.854$, $F_{7,7} = 1.109$; CBZ: $p < 0.01$, $t = 4.035$, $F_{7,7} = 3.705$; LTG: $p < 0.01$, $t = 3.964$, $F_{7,7} = 1.646$, Figure 1). These results mimic our previous study (Boku et al, 2011) for the effects of mood stabilizers on STS-induced apoptosis in ADP. Therefore, SH-SY5Y may have the applicability as a model of neural precursor cells for investigating the mechanism underlying the effects of mood stabilizers on STS-induced apoptosis.

3.2 Mood stabilizers restore STS-induced increase of p53

It has been shown that STS increases the expression of p53 in some cell lines, including SH-SY5Y cells (Tieu et al, 1999). Thus, we first examined the effects of STS on the expression of p53 with quantitative RT-PCR under our culture condition. 300nM STS significantly increased mRNA expression of p53 at 3 hours after treatment (unpaired *t*-test, $p<0.01$, $t=7.326$, $F_{8,8}=1.964$, Figure 2A), but had no effect on it at 6 hours ($p>0.05$, $t=1.089$, $F_{8,8}=1.886$, Figure 2A), 12 and 24 hours (data not shown) after treatment.

Our previous study has shown that four common mood stabilizers, such as Li, VPA, CBZ and LTG, inhibit STS-induced apoptosis (Boku et al, 2011). It suggests the possibility that mood stabilizers may inhibit some STS-increased gene expressions. Therefore, we examined the effects of these mood stabilizers on STS-induced expression of p53 with quantitative RT-PCR. All of 1 mM Li, 300 μ M VPA, 3 μ M CBZ and 30 nM LTG significantly restored STS-induced increase of p53 at 3 hours after treatment (one-ANOVA and Bonferroni's post hoc test, STS vs STS+Li: $p<0.01$, $t=6.922$, $F_{8,8}=2.269$; STS vs STS+VPA: $p<0.01$, $t=7.815$, $F_{8,8}=3.865$; STS vs STS+CBZ: $p<0.01$, $t=6.321$, $F_{8,8}=1.821$; STS vs STS+LTG: $p<0.01$, $t=7.419$, $F_{8,8}=2.018$, Figure 2B). In addition, all of them had no effect on the expression of p53 in the absence of STS (Control vs Li: $p>0.05$, $t=0.7238$, $F_{8,8}=2.194$; Control vs VPA: $p>0.05$, $t=2.977$, $F_{8,8}=1.960$; Control vs CBZ: $p>0.05$, $t=0.3540$, $F_{8,8}=1.315$; Control vs LTG: $p>0.05$, $t=0.7341$, $F_{8,8}=2.784$, Figure 2B).

3.3 Effects of STS on the expressions of apoptosis-related p53 target genes

Following p53, we examined the effects of STS on the expressions of common four Bcl-2 family genes, such as Bcl-2, Bcl-XL, BAK, and BAX with quantitative RT-PCR at 6 hours after treatment. The expression of Bcl-2, an anti-apoptotic Bcl-2 family gene, was

significantly decreased (unpaired *t*-test, $p<0.01$, $t=14.95$, $F_{8,8}=3.890$, Figure 3), but those of Bcl-XL, BAK and BAX were not altered (Bcl-XL: $p>0.05$, $t=1.561$, $F_{8,8}=4.892$; BAK: $p>0.05$, $t=1.319$, $F_{8,8}=16.40$; BAX: $p>0.05$, $t=0.5251$, $F_{8,8}=1.681$, Figure 3).

3.4 Mood stabilizers restore STS-induced decrease of Bcl-2

Next, we examined whether STS-induced decrease of Bcl-2 is restored by Li, VPA, CBZ and LTG with quantitative RT-PCR. All of 1mM Li, 300 μ M VPA, 3 μ M CBZ and 30 nM LTG significantly restored STS-induced decrease of Bcl-2 expression at 6 hours after treatment (one-ANOVA and Bonferroni's post hoc test, STS vs STS+Li: $p<0.01$, $t=3.807$, $F_{8,8}=15.58$; STS vs STS+VPA: $p<0.01$, $t=7.430$, $F_{8,8}=4.253$; STS vs STS+CBZ: $p<0.01$, $t=5.218$, $F_{8,8}=6.504$; STS vs STS+LTG: $p<0.01$, $t=3.550$, $F_{8,8}=12.24$, Figure 4). In addition, Li, CBZ and LTG had no effect on the expression of Bcl-2 (Control vs Li: $p>0.05$, $t=0.4641$, $F_{8,8}=3.786$; Control vs CBZ: $p>0.05$, $t=2.392$, $F_{8,8}=3.394$; Control vs LTG: $p>0.05$, $t=2.035$, $F_{8,8}=14.85$, Figure 4) and VPA slightly decreased it in the absence of STS ($p<0.05$, $t=4.448$, $F_{8,8}=1.835$, Figure 4).

3.5 p53 overexpression decreases the expression of Bcl-2 in SH-SY5Y cells

It has been shown that p53 decreases the expression of Bcl-2 in SH-SY5Y cells (Chen and Chuang, 1999). To examine whether p53 decreases the expression of Bcl-2 in our culture condition, we examined the effects of p53 overexpression on the expression of Bcl-2 with the transfection of pSG5-p53 vector. p53 overexpression was confirmed with Western Blotting at 2 days after transfection of pSG5-p53 vector (Figure 5A). Next, we examined the effects of p53 overexpression on Bcl-2 expression with quantitative RT-PCR and Western Blotting. p53 overexpression significantly decreased mRNA expression of

Bcl-2 at 2 days after transfection (unpaired t -test, $p<0.05$, $t=3.188$, $F_{8,8}=1.485$, Figure 5B). p53 overexpression also significantly decreased protein expression of Bcl-2 at 2 days after transfection (unpaired t -test, $p<0.01$, $t=3.361$, $F_{8,8}=2.124$, Figure 5C).

4. Discussion

Using SH-SY5Y cells, here we have shown that STS increases the expression of p53 and decreases the expression of Bcl-2, that all of four common mood stabilizers such as Li, VPA, CBZ and LTG restore STS-induced increase of p53 at 3 hour after treatment and STS-induced decrease of Bcl-2 at 6 hour after treatment, and that p53 overexpression decreases the expression of Bcl-2. Taken together, our results suggest that four mood stabilizers commonly restore STS-induced increase of p53 and following decrease of Bcl-2 in SH-SY5Y cells. In the case of Li, it has already been shown that Li decreases the expression of p53 in neural culture cells (Chen and Chuang, 1999; Lu et al, 1999) and that Li increases that of Bcl-2 in neural culture cells (Chen et al, 1999; Chen and Chuang, 1999). In the case of VPA, it has been shown that VPA increases the expression of Bcl-2 as in the case of Li in neural culture cells (Chen et al, 1999), but it remains unclear whether VPA decreases the expression of p53. In the cases of CBZ and LTG, there are no past *in vitro* studies about their effects on the expressions of p53 and Bcl-2. Therefore, this is the first *in vitro* study indicating that four common mood stabilizers have the common effects on the expressions of p53 and Bcl-2.

Originally, we found the common effect of mood stabilizers on STS-induced apoptosis in ADP (Boku et al, 2011). However, it is quite difficult to get enough amounts of ADP

cells for investigating the involvement of many apoptotic-related genes in the anti-apoptotic effects of mood stabilizers, because the proliferation potency of ADP is limited and the culture of ADP is cumbersome and time-consuming (Boku et al, 2009). In addition, it is also quite difficult to transfect plasmid or siRNA into ADP because most of reagents for transfection of plasmids or siRNA are toxic to ADP. Therefore, an applicable model for ADP was required for investigating the mechanisms underlying the effects of mood stabilizers on ADP. The definitions of neural precursor cells are self-renewal and differentiation potency, so a model of ADP is at least needed to fulfill these definitions of neural precursor cells. SH-SY5Y cells have self-renewal activity and can differentiate into neurons (Påhlman et al, 1990). Moreover, bFGF increases proliferation of SH-SY5Y cells in a serum-free condition, as in the cases of *in vitro* culture of neural precursor cells (Murphy et al, 1990; Ray and Gage, 2006; Babu et al, 2007; Boku et al, 2009). Therefore, SH-SY5Y cells may fulfill the definitions of neural precursor cells and their proliferation may be similarly affected by bFGF as in the case of ADP proliferation. Moreover, all of Li, VPA, CBZ and LTG also decreased STS-induced apoptosis in SH-SY5Y cells in the case of ADP. Taken together, to make a short list of genes involved with the anti-apoptotic effects of mood stabilizers, we used SH-SY5Y cells as a model of ADP in this study.

In our past study (Boku et al, 2011) and this one, STS was used as an inducer of apoptosis. It is well known that apoptosis has two pathways: the internal pathway via mitochondria and the external pathways via death receptors (Adams, 2003). STS is an inducer of internal pathway (Ferrari et al, 1998). Past studies have suggested that the internal pathway-related factors (e.g., GSK-3 β , Bcl-2) might be candidates of anti-apoptotic

effects of mood stabilizers (Chen et al, 1999; Jope and Bijur, 2002; Li et al, 2002; Chang et al, 2008). In addition, the internal pathway, but not the external pathway, is involved in apoptosis of neural progenitor cells (Exdahl et al, 2003; Ceccatelli et al, 2004). Our unpublished data also showed that tumor necrosis factor- α , a ligand of death receptors and an inducer of the external pathway, did not induce apoptosis on ADP (data not shown). Therefore, we chose STS as an inducer of apoptosis and focused on the internal pathway in this study. However, STS is not a physiological inducer of the internal pathway-mediated apoptosis. To confirm our results, further investigation using more physiological inducers of apoptosis, such as mitogen or insulin withdrawal and glucose deprivation, may be required.

The expression level of p53 is tightly regulated by both transcriptional mechanisms and post-translational mechanisms. Most of past studies have shown that post-translational mechanism is critical for the regulation of the expression of p53 and well understood (Kruse and Gu, 2009). On the other hand, it is poorly understood how the expression of p53 is transcriptionally regulated. However, some studies suggest that CCAAT/enhancer-binding protein β (C/EBP β), a transcription factor, directly binds to p53 promoter and regulates its expression (Boggs and Reisman, 2006; Boggs and Reisman, 2007). In addition, STS increases the expression of C/EBP β (Hecker et al, 1997). These studies and our results suggest that C/EBP β is involved in STS-induced increase of p53 and that C/EBP β may be a potential common target of mood stabilizers. In fact, Li decreases the expression of p53 (Davenport et al, 2010) and inhibits the transcriptional activity of C/EBP β through inhibiting the activity of GSK-3 β and following decrease of the

phosphorylation of C/EBP β (Piwien-Pilipuk, 2001). On the other hand, it remains unclear the effects of VPA, CBZ and LTG on the activity and expression of C/EBP β . Therefore, we will investigate the effects of four mood stabilizers on the activity and expression of C/EBP β in the next step.

The involvement of p53 and Bcl-2 on the pathophysiology of BD and the action mechanism of mood stabilizers remain unclear. A postmortem study has shown that the expression of Bcl-2 is decreased in the frontal cortex of BD patients compared with healthy controls (Kim et al, 2010). In addition, some past studies have shown that TNF- α and its receptors, which can induce apoptosis via the external pathway, are increased in peripheral blood in BD patients compared with healthy controls (Kauer-Sant'Anna et al, 2009; Barbosa et al, 2010). These suggest that both the internal pathway and the external pathway might be involved in the pathophysiology of BD. As past studies and our present study have shown, the internal pathway, which is activated by STS and mediated by p53 and Bcl-2, may be involved in the action mechanism of mood stabilizers. On the other hand, there seems to be no study for investigating the effects of mood stabilizers on the external pathway to our knowledge, and TNF- α does not induce apoptosis in ADP (our unpublished data). Taken together, both the internal pathway and the external pathway might be involved in the pathophysiology of BD, but only the internal pathway might be involved in the action mechanism of mood stabilizers. However, this discussion is speculative. To clarify the roles of the internal pathway and external pathway in the pathophysiology of BD and the action mechanism of mood stabilizers, the investigation using conditional knockout and/or transgenic mice of each pathway-related genes is surely required.

5. Conclusion

Here we have shown that p53 and Bcl-2 may be involved in the common effects of mood stabilizers on STS-induced apoptosis using SH-SY5Y cells as a model for neural precursor-like cells. However, the detailed mechanisms underlying them and *in vivo* functional significance of these results remain unclear. To investigate them may lead to further understanding of the action mechanisms of mood stabilizers and the pathophysiology of BD.

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Figure legends

Figure 1: Mood stabilizers decrease STS-induced apoptosis in SH-SY5Y cells

All four mood stabilizers decreased STS-induced ADP apoptosis. TUNEL assay was performed 3 days after drug treatments for four independent cultures. The quantities of both TUNEL and DAPI signals were counted in four randomly selected fields/ well. Then the percentage of TUNEL signals/ DAPI signals was calculated. Data are expressed as means $\pm$ SEM. Statistical analysis was performed using unpaired *t*-test. Significance was defined as $p < 0.05$, compared with STS.

Figure 2: Mood stabilizers restores STS-induced increase of p53

A. mRNA expression of p53 was significantly increased by 300 nM STS at 3 hours, but not 6 hours after treatment. RNA isolation was performed 3 hours and 6 hours after treatment. Values are shown as the ratio of p53 versus 18s rRNA. Data are shown as means $\pm$ SEM of nine independent samples. Statistical analysis was performed using unpaired *t*-test. Significance was defined as $p < 0.05$, compared with control.

B. STS-increased mRNA expression of p53 was significantly restored by 1 mM Li, 300 μ M VPA, 3 μ M CBZ and 30 nM LTG. RNA isolation was performed 3 hours after treatment. Values are shown as the ratio of p53 versus 18s rRNA. Data are shown as means $\pm$ SEM of nine independent samples. Statistical analysis was performed using one-way ANOVA and Bonferonni's post hoc test. Significance was defined as $p < 0.05$, compared with control or STS.

Figure 3: Effects of STS on the expressions of apoptosis-related p53 target genes

mRNA expression of Bcl-2 was significantly decreased by 300 nM STS, but those of Bcl-XL, BAK and BAX were not altered. RNA isolation was performed 6 hours after treatment. Values are shown as the ratio of Bcl-2, Bcl-XL, BAK or BAX versus 18s rRNA. Data are shown as means $\pm$ SEM of nine independent samples. Statistical analysis was performed using unpaired *t*-test. Significance was defined as $p < 0.05$, compared with control.

Figure 4: Mood stabilizers restores STS-induced decrease of Bcl-2

All of 1 mM Li, 300 μ M VPA, 3 μ M CBZ and 30 nM LTG significantly restored STS-induced decrease of Bcl-2. RNA isolation was performed 6 hours after treatment. Values are shown as the ratio of Bcl-2 versus 18s rRNA. Data are shown as means $\pm$ SEM

of nine independent samples. Statistical analysis was performed using one-way ANOVA and Bonferonni's post hoc test. Significance was defined as $p < 0.05$, compared with control or STS.

Figure 5: p53 overexpression decreases the expression of Bcl-2 in SH-SY5Y cells

A. p53 overexpression with pSG5-p53 vector. pSG5 vector without any insert sequence was used as a mock. Preparation of cell lysates was performed 2 days after transfection.

B. p53 overexpression actually decreases mRNA expression of Bcl-2. RNA isolation was performed 2 days after transfection. Values are shown as the ratio of Bcl-2 versus 18s rRNA. Data are shown as means $\pm$ SEM of six independent samples. Statistical analysis was performed using unpaired *t*-test. Significance was defined as $p < 0.05$, compared with mock.

C. p53 overexpression actually decreases protein expression of Bcl-2. Preparation of cell lysates was performed 2 days after transfection. Values are shown as the ratio of Bcl-2 versus GAPDH. Data are shown as means $\pm$ SEM of six independent samples. Pictures are shown from typical experiment. Statistical analysis was performed using unpaired *t*-test. Significance was defined as $p < 0.05$, compared with mock.

Figure 1

Figure 2

A

B

Figure 3

Figure 4

Figure 5