Biochemical and Biophysical Research Communications 440 (2013) 359–363

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

A compound CP-31398 suppresses excitotoxicity-induced neurodegeneration

Takeshi Fujiwara [a,*], Koji Morimoto [b]

[a] Biomolecular Dynamics Laboratory, Department of Biomolecular Networks, Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan
[b] Department of Breast and Endocrine Surgery, Graduate School of Medicine, Osaka University, Osaka, Japan

ARTICLE INFO

Article history:
Received 13 August 2013
Available online 26 August 2013

Keywords:
CP-31398
Excitotoxicity
Mitochondrial dysfunction
Neurodegeneration
p53

ABSTRACT

Neurodegeneration causes dysfunction and degeneration of neurons and is triggered by various factors including genetic defects, free radicals, injury, and glutamate excitotoxicity. Among those, glutamate excitotoxicity is implicated in chronic disorders including AD and ALS, and in acute insults in the CNS including traumatic brain injury. Neurological disorders show hallmark morphological abnormalities such as axon degeneration and cell body death. The molecular mechanisms underlying excitotoxicity-induced neurodegeneration are complex and deciphering a molecular mechanism from one angle is beneficial to understand the process, however, still difficult to develop strategies to suppress excitotoxicity-induced degeneration due to existence of other mechanisms. Thus, directly identifying compounds that can modulate excitotoxicity-induced neurodegeneration and subsequently clarifiying the molecular mechanism is a valid approach to develop effective strategies to suppress neurodegeneration. We searched for compounds that can suppress excitotoxicity-induced neurodegeneration and found that CP-31398, a known compound that can rescue the structure and function of the tumor suppressor protein p53 mutant form and stabilize the active conformation of the p53 wild-type form, suppresses excitotoxicity-induced axon degeneration and cell body death. Moreover, CP-31398 suppresses mitochondrial dysfunction which has a strong correlation with excitotoxicity. Thus, our findings identify a compound that can serve as a novel modulator of neurodegeneration induced by glutamate excitotoxicity.

1. Introduction

Neurodegenerative disorders involve degeneration of axons, synapse loss, and cell body death. The spatiotemporal sequence and the causal relationships of these events are difficult to determine and remain obscure. Within that regard, axon degeneration and loss seem to precede the appearance of symptoms and the loss of neuronal cell bodies in various neurodegenerative disease [1]. Axon degeneration is an active, controlled, and versatile process of the axonal compartment competent for self-destruction. It can be observed in conditions such as neurodegenerative disease, brain injury, and pruning during neuronal development [1]. Particularly in neurodegenerative disease, the degenerative process of axon is likely to be distinct from that of cell body death [2]. This notion is exemplified by studies in a mice model of glaucoma showing that neuronal cell body protection by *Bax* (Bcl-2 associated X) deletion cannot prevent axon degeneration and death [3], and in progressive motor neuronopathy (*pmn*) mice showing synapse loss and axon degeneration despite the rescue of the cell bodies by overexpression of Bcl-2 [4]. In addition, in mice models of PD, axonal protection by Wld^S (slow Wallerian degeneration protein) cannot prevent cell body death [5,6]. However, in ALS, the events that trigger synapse loss could occur in axons or the cell bodies suggesting that synapse loss observed in neurodegenerative disease could be triggered from distant subcellular sites [2,7,8]. Taken together, mechanisms involved in degeneration of axons, synapse loss, and cell body death are likely to be distinct from one another with a possibility of some overlapping mechanisms.

Various factors including excessive glutamate exposure could trigger neurodegeneration [9]. Glutamate is an excitatory neurotransmitter in the CNS that plays a pivotal role in long-term potentiation and cognitive functions such as learning and memory. However, exposure to excessive glutamate overactivates glutamate receptors and triggers neurodegenerative processes known as excitotoxicity that results in damage and loss of axons, synapse, and the cell body [10]. Excitotoxicity is linked to chronic neurological disorders, such as AD, PD, and ALS, and acute hypoxic–ische-

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; CP-31398, N′-[2-[2-(4-methoxyphenyl)ethenyl]-4-quinazolinyl]-N,N-dimethyl-1, 3-propanediamine dihydrochloride; CNS, central nervous system; DAPI, 4′,6′-diamidino-2-phenylindole; DIV, days *in vitro*; GFP, green fluorescent protein; PD, Parkinson's disease; PI, propidium iodide.

* Corresponding author. Address: Biomolecular Dynamics Laboratory, Department of Biomolecular Networks, Graduate School of Frontier Biosciences, Osaka University, Yamada-oka 1-3, Suita 565-0871, Osaka, Japan. Fax: +81 6 6879 4609.
E-mail address: fujiwara@anat3.med.osaka-u.ac.jp (T. Fujiwara).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.08.052

mic brain injury [11]. The mechanisms underlying excitotoxicity is a complex issue, however, the most upstream cue to neuronal death is regarded as overactivation of glutamate receptors, especially the NMDA subtype. Overactivated NMDA receptors trigger calcium influx, and lead to numerous events that are detrimental to normal neuronal function including acute mitochondrial dysfunction and free radical production [9,11]. Hence, NMDA receptor antagonists could potentially be of therapeutic benefit in chronic and acute neurological disorders that manifest excessive NMDA receptor activities [12].

At present, memantine, is the most successful compound that is linked to excitotoxicity as it blocks excessive NMDA receptor activity without affecting the normal activity and is used for treatment of patients with moderate-to-severe AD [13,14]. However, the treatment is symptomatic but not causative. Many other pharmacological approaches attempting to block excitotoxicity including antagonists of AMPA and kainate receptors, and intracellular free radical scavengers and antioxidants have shown no clinical benefit with treatments of patients with neurological disorders [9]. Thus, identification of alternative compounds is still in need to attenuate neuronal damage observed in chronic and acute neurological disorders linked to excitotoxicity.

Here, we identify a compound, CP-31398, as a novel pharmacological modulator of excitotoxicity-induced neurodegeneration. CP-31398 is originally developed as an anti-cancer compound and is known to regain the structure and function of mutant p53 and stabilize active conformation of wild-type p53 [15,16]. Using primary hippocampal cultures, we find that treatment with CP-31398 suppresses excitotoxicity-induced axon degeneration, cell body death, and mitochondrial dysfunction. The protective effect by CP-31398 was greater on axons compared with that on the cell bodies from degeneration.

2. Materials and methods

2.1. Antibodies and chemicals

Primary antibodies used are rat monoclonal anti-GFP (Nacalai Tesque) and mouse monoclonal anti-βIII-tubulin (Covance). Secondary antibodies are Alexa Fluor® 488 or 568 fluorescents (Invitrogen). Chemicals used are CP-31398 (Tocris bioscience), PI (Invitrogen), and DAPI dihydrochloride hydrate (Sigma).

2.2. Neuron culture, immunofluorescence, and transfection

Preparation of primary Wistar rat embryonic hippocampal neurons and culture conditions for indirect immunofluorescence labeling of transfected neurons were performed as previously described [17,18]. Procedures were approved by the Osaka University Institutional Guidelines for the Care and Use of Laboratory Animals. Transfection was performed using AMAXA Nucleofector transfection system (Lonza) with 4 μg of plasmid pEGFP-C1 (Clontech) and approximately 3×10^6 of cells. Images were captured by confocal laser microscopy FV1000 system (Olympus) with 40× and 60× oil-immersion objective lenses, and with 2× or 3× zoom.

2.3. Neurite beading and cell death analyses

Hippocampal cultures subjected for neurite beading analysis were incubated for 8 DIV at 37 °C, 5% CO_2, and treated with 50 μM glutamate (Sigma), 10 μM CP-31398, or vehicle in combinations as described in the Results section. Analysis was carried on by observing visually-isolated transfected neurons, and a bead was defined as previously described [17]. For cell body death analysis, cells of approximately 3×10^4 were seeded on 13 mm diameter glass cover slips (Matsunami) placed in 24-well dishes (Nunc) pre-coated with 0.05 mg/ml poly-L-lysine (Sigma), and grown for 8 DIV at 37 °C, 5% CO_2. Hippocampal cultures were incubated with 5 μg/ml of PI for 30 min prior to fixation. Quantification for cell body death was achieved by measuring the ratio of PI-incorporated βIII-tubulin-positive neurons within DAPI-labeled βIII-tubulin-positive neurons.

2.4. Formazan dye assay

Mitochondrial dehydrogenase activity of hippocampal cultures treated with 50 μM glutamate, 10 μM CP-31398, or vehicle in combinations as described in the Results section for 1, 3, and 6 h, was measured using tetrazolium salt, WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] (Cell Count Reagent SF, Nacalai Tesque). WST-8 was added for 3 h prior to terminating the reaction by 1% (w/v) sodium dodecyl sulfate. For formazan dye assay, cells were seeded at approximately 7×10^4 on each well of 24-well dishes pre-coated with 0.05 mg/ml poly-L-lysine and incubated for 8 DIV at 37 °C, 5% CO_2. Assay was performed mainly under manufacture's instruction and the values of optical density (O.D.) at 450 nm wavelength were measured by microplate reader (Dainippon Pharma Co.) for formazan dye production.

2.5. Statistics

Statistical analyses were done by one-way ANOVA with Bonferroni/Dunn post test. In all instances, a value of $p < 0.05$ was considered significant.

3. Results

3.1. CP-31398 suppresses axon degeneration

We have previously reported that p53 function could modulate the process of axon degeneration by administration of p53 function inhibitors, Pifithrin-α and -μ [19]. Pifithrin-α inhibits p53-mediated transactivation, by which pro-apoptotic and cell cycle regulators are produced [20]. Pifithrin-μ inhibits the association of p53 to mitochondria by reducing the affinity of cytoplasmic p53 with anti-apoptotic Bcl-X_L and Bcl-2 proteins without affecting p53-mediated transactivation [21]. Inhibition of p53-mediated transactivation by Pifithrin-α suppresses excitotoxicity-induced axon degeneration, which is further suppressed by additional treatment with taxol [19]. On the contrary, inhibition of p53 association with mitochondria by Pifithrin-μ induces axon degeneration in a normal condition, suggesting that cytoplasmic p53 supports axonal integrity rather than inducing apoptosis in neurons [19]. Thus, we hypothesized that stabilization and retention of cytoplasmic p53 function in neurons could protect axons from excitotoxic insults. We took a pharmacological approach and used the styrylquinazoline compound, CP-31398, that has previously been shown to regain the structure and function of mutant p53 as well as stabilize the active conformation of wild-type p53 [15,16,22]. First, we assessed whether CP-31398 administration has an impact on excitotoxicity-induced axon degeneration by analyzing the neurite beading phenotype. A focal bead-like swelling phenotype in neurites is an early neurodegenerative feature in acute and chronic neurological disorders [23]. Previous studies have used CP-31398 at a range of 0.5–35 μM in *in vitro* studies using cell lines [24,25]. Since CP-31398 has not been used in studies with primary cultured neurons, we first determined the optimal concentration of CP-31398 with rat embryonic hippocampal cultures at 8 DIV. Treatment with 10 μM concentration for 3 h had no notable effect

showing less than 2% the number of neurons with beading neurites (unpublished observation, T.F.). Treatment with 25, 50, and 100 μM concentrations for 3 h showed approximately 8%, 12%, and 47% the number of neurons with beading neurites, respectively, inducing degeneration (unpublished observation, T.F.). For assessing the impact on excitotoxicity-induced axon degeneration, we selected the concentration of 10 μM for our study. We analyzed GFP-overexpressing visually-isolated neurons that contained beading neurites. In 8 DIV hippocampal cultures, simultaneous treatment with glutamate and CP-31398, and with glutamate alone for 3 h showed approximately 2.4% and 43.6% the number of neurons with beading neurites, respectively (Fig. 1A and B). Treatments with CP-31398 alone, and with vehicle alone for 3 h showed approximately 3.3% and 1.0% the number of neurons with beading neurites, respectively (Fig. 1A and B). Thus, CP-31398 suppressed more than 90% the phenotype of excitotoxicity-induced axon degeneration compared with glutamate treatment alone, and CP-31398 treatment alone had no notable effect on axonal integrity (Fig. 1B). This result indicates that CP-31398 effectively protects axon from excitotoxicity-induced degeneration.

3.2. CP-31398 suppresses cell body death

In a number of neurodegenerative conditions, the mechanism of axon degeneration and cell body death is likely to be distinct [2]. Based on this notion, we assessed whether CP-31398 administration also has an impact on excitotoxicity-induced cell body death by PI incorporation analysis. In 8 DIV hippocampal cultures, simultaneous treatment with glutamate and CP-31398, and with glutamate alone for 3 h showed approximately 24.0% and 50.5% the number of PI-incorporated neurons, respectively (Fig. 2A and B). Treatments with CP-31398 alone, and with vehicle alone for 3 h showed approximately 5.9% and 1.5% the number of PI-incorporated neurons, respectively (Fig. 2A and B). Thus, CP-31398 suppressed more than 50% the phenotype of excitotoxicity-induced cell body death compared with glutamate treatment alone, and CP-31398 treatment alone had no notable effect on the cell body (Fig. 2B). This result indicates that CP-31398 effectively protects the cell body from excitotoxicity-induced death, however, in a less degree compared with that observed in axons.

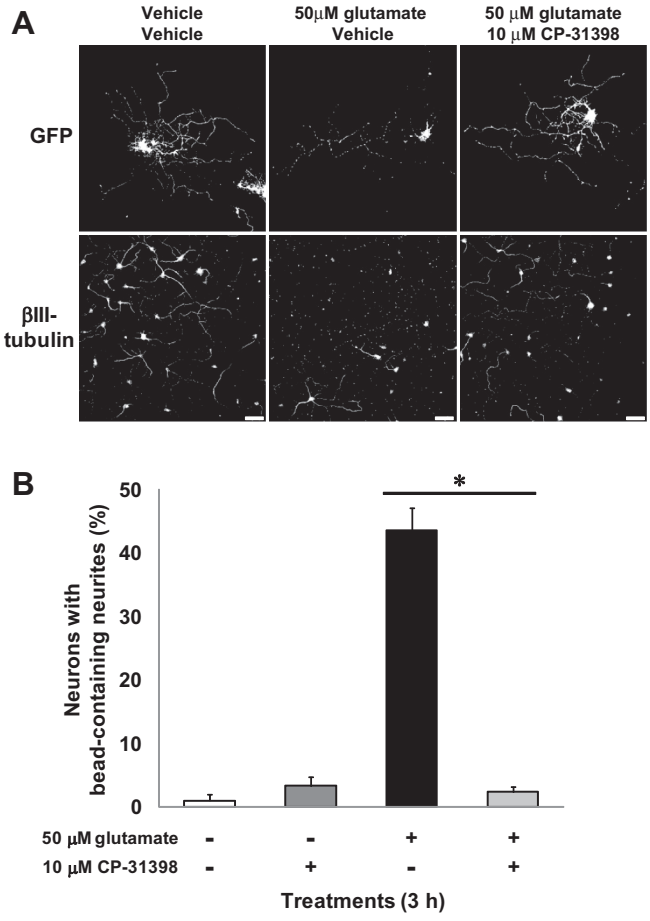

Fig. 1. CP-31398 suppresses axon degeneration. (A) Representative images of GFP vector-transfected 8 DIV hippocampal neurons treated with vehicle, 50 μM glutamate, and 50 μM glutamate + 10 μM CP-31398 for 3 h and labeled for GFP, βIII-tubulin, and DAPI. GFP- and βIII-tubulin-labeled images are presented. Bars: 60 μM. (B) Quantification of neurons containing beading neurites (>196 cells counted for each condition). GFP vector-transfected 8 DIV hippocampal neurons treated with vehicle, 10 μM CP-31398, 50 μM glutamate, and 50 μM glutamate + 10 μM CP-31398 for 3 h showed 1.0%, 3.3%, 43.6%, and 2.4%, respectively (*$p < 0.01$). All statistics represent mean ± SEM of 3 independent experiments.

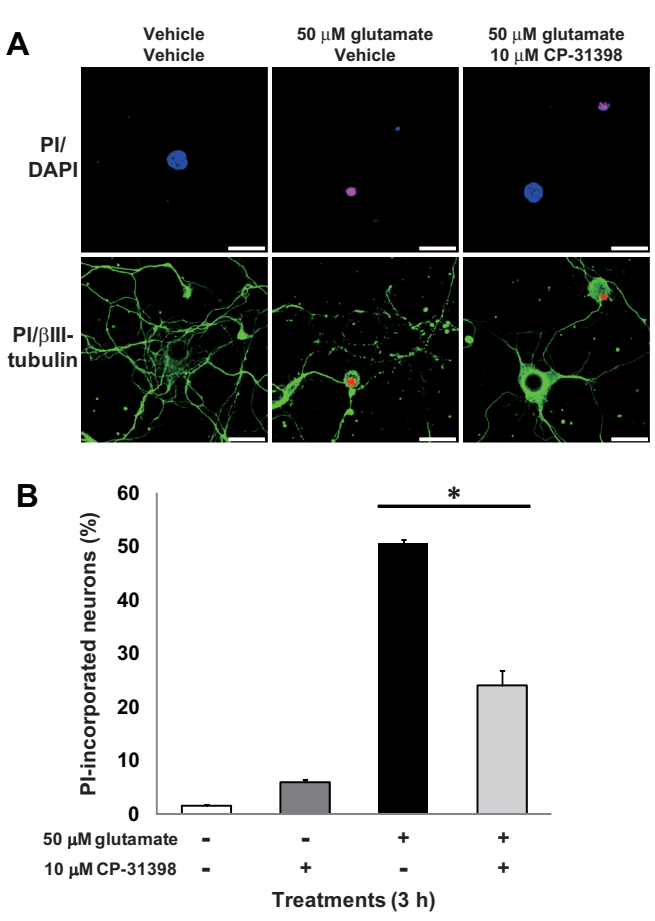

Fig. 2. CP-31398 suppresses cell body death. (A) Representative images of 8 DIV hippocampal neurons treated with vehicle, 50 μM glutamate, and 50 μM glutamate + 10 μM CP-31398 for 3 h and PI (red) for 30 min labeled for βIII-tubulin (green) and DAPI (blue). Merged images of PI/DAPI and PI/βIII-tubulin are presented. Bars: 20 μm. (B) Quantification of PI-incorporated neurons (>733 cells counted for each condition). PI-incorporated hippocampal neurons treated with vehicle, 10 μM CP-31398, 50 μM glutamate, and 50 μM glutamate + 10 μM CP-31398 for 3 h showed 1.5%, 5.9%, 50.5%, and 24.0%, respectively (*$p < 0.01$). All statistics represent mean ± SEM of 3 independent experiments.

3.3. CP-31398 suppresses mitochondrial dysfunction

The function of mitochondria has a strong correlation with excitotoxicity as the majority of increased intracellular calcium is sequestered into mitochondria leading to mitochondrial toxicity, metabolic acidosis, and free radical generation [26,27]. Inhibition of p53-mediated transactivation by Pifithrin-α suppresses excitotoxicity-induced mitochondrial dysfunction [19]. On the contrary, inhibition of p53 association with mitochondria by Pifithrin-μ has no notable effect on excitotoxicity-induced mitochondrial dysfunction [19]. From these previous findings, the role of cytoplasmic p53 function on neuronal mitochondrial function in excitotoxicity remains obscure. Thus, we assessed the impact of p53 stabilization by CP-31398 on excitotoxicity-induced mitochondrial dysfunction in 8 DIV hippocampal cultures. We measured the activity of mitochondrial dehydrogenase, and the ratio of measured values compared with vehicle treated control was quantified. Treatments with vehicle alone, CP-31398 alone, glutamate and CP-31398, and glutamate alone for 1 h, showed similar activities with ratios of 1.00, 0.95, 1.00, and 0.85, respectively (Fig. 3). On the contrary, treatments with glutamate and CP-31398, and glutamate alone for 3 h showed mitochondrial dehydrogenase activities with ratios of 0.59 and 0.42, respectively, an approximately 40% increase of mitochondrial function by CP-31398 treatment compared with glutamate treatment alone (Fig. 3). No notable alterations were observed in mitochondrial dehydrogenase activities treated with vehicle alone, and CP-31398 alone showing ratios of 1.00 and 0.84, respectively (Fig. 3). In 6 h treatment, vehicle alone, CP-31398 alone, glutamate and CP-31398, and glutamate alone showed mitochondrial dehydrogenase activities with ratios of 1.00, 0.76, 0.30, and 0.16, respectively (Fig. 3). A more than 80% increase of mitochondrial function was observed by CP-31398 treatment compared with glutamate treatment alone. A moderate reduction of mitochondrial dehydrogenase activity was detected with CP-31398 treatment in a normal condition. This result indicates that CP-31398 effectively protects mitochondria from excitotoxicity-induced dysfunction.

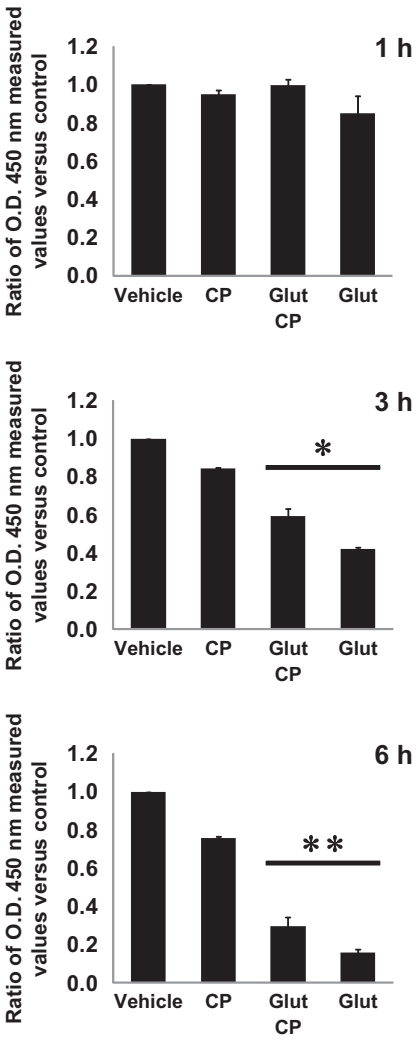

Fig. 3. CP-31398 suppresses mitochondrial dysfunction. Mitochondrial dehydrogenase activity was analyzed using tetrazolium salt, WST-8, and formazan dye production was quantified by measuring O.D. 450 nm wavelength. The ratio of measured values of each condition compared with control (vehicle) is presented. With 1 h treatment of vehicle, 10 μM CP-31398 (CP), 50 μM glutamate (Glut) + 10 μM CP-31398 (CP), and 50 μM glutamate (Glut) showed 1.00, 0.95, 1.00, and 0.85, respectively. With 3 h treatment of vehicle, CP, Glut + CP, and Glut showed 1.00, 0.84, 0.59, and 0.42, respectively ($^{*}p < 0.01$). With 6 h treatment of vehicle, CP, Glut + CP, and Glut showed 1.00, 0.76, 030, and 0.16, respectively ($^{**}p < 0.05$). Statistics represent mean ± SEM of 3 independent experiments and 16 independent sets of samples.

4. Discussion

Our results demonstrate for the first time that CP-31398, a compound that is known to regain the structure and function of mutant p53 as well as stabilize the active conformation of wild-type p53, effectively suppresses axon degeneration, cell body death, and mitochondrial dysfunction of neurons. CP-31398 is originally developed as an anti-cancer styrylquinazoline compound which exerts its' activity to rescue the structure and function of mutant p53, inhibit human tumor xenografts *in vivo*, enhance wild-type p53 expression and activity, restore DNA-binding activity of p53, block ubiquitination and degradation of p53 without affecting Mdm2 binding ability, and induce p21 and Bax expressions as well as apoptosis in p53-mutant carrying cells [15,22,24,28,29]. Despite the culminating notion that CP-31398 is a p53 modulator to eliminate tumors by inducing p53-dependent apoptosis from rigorous studies with cancer cell lines, whether CP-31398 affects neurons in the same manner remained an open question.

In our present study in primary hippocampal cultures, CP-31398 treatment alone has no notable effect on the morphology of axons and the cell body, and only a moderate effect on the activity of mitochondrial dehydrogenase with prolonged treatment in a normal condition. In our previous findings, inhibition of p53-mediated transactivation by Pifithrin-α protected axons from degeneration and attenuated the loss of mitochondrial dehydrogenase activity in an excitotoxic condition [19]. If the active conformation of wild-type p53 in hippocampal neurons is stabilized by CP-31398, p53-mediated transactivation would enhance and result in exacerbation of excitotoxicity-induced degeneration of axons and the cell bodies, which is not the case. On the other hand, we have previously shown that inhibition of cytoplasmic p53 association with mitochondria by Pifithrin-μ induces axon degeneration in a normal condition [19]. In this case, stabilization of the active conformation of cytoplasmic p53 could protect axons and the cell bodies from excitotoxicity-induced degeneration. Taken together, the effect of CP-31398 might weigh more on the function of the cytoplasmic p53 than that of the nucleic p53.

Up to this point, there is no evidence that CP-31398 directly associates with p53. This fact renders room for a possibility that the effect of CP-31398 on p53 function is secondary and that proper target factors of CP-31398 still remain unidentified. It has previously been reported that the absence of p53 protects neuronal cells in the CNS from being damaged and eliminated by excitotoxicity in *in vivo* and *in vitro* studies [30,31]. p53 wild-type (p53 +/+),

heterozygous (p53 +/−), and deficient (p53 −/−) mice in a kainate-induced excitotoxicity model which is associated with well defined patterns of neuronal cell loss, was used for evaluation of p53-dependent cell death *in vivo*. A majority of p53 wild-type and heterozygous mice exhibited extensive cell loss in the hippocampus including subregions CA1, CA3, the hilus, and the subiculum, as well as in the amygdala, cerebral cortex, caudate-putamen, and thalamus [30]. In marked contrast, a majority of p53 deficient mice displayed no signs of neuronal cell damage, and a minor population of mice showed mild-to-moderate cell damage confined to the CA1 and CA3b regions of the dorsal hippocampus [30]. Thus, a single copy of the p53 gene is sufficient to confer neuronal cell vulnerability and the absence of p53 causes almost complete neuroprotection from kainate-induced excitotoxicity. A more precise study to determine the protective effect of the absence of p53 in neurons was performed with isolated primary cultured neurons from the hippocampus and cerebral cortex of p53 wild-type, heterozygous, and deficient mice, combined with excessive exposure to glutamate or kainate [31]. Neurons that contained at least one copy of the p53 gene were severely damaged by glutamate- or kainate-induced excitotoxicity. On the contrary, neurons deficient in p53 exhibited little-to-no damage in response to excitotoxicity [31]. Overexpression of p53 using adenovirus-mediated transduction in p53 deficient mice was sufficient to promote death of hippocampal neurons in the absence of excitotoxins [31]. Thus, consistent with the *in vivo* study, the absence of p53 causes almost complete neuroprotection from excitotoxicity. If p53 is the direct target of CP-31398, our results showing that CP-31398 protects neurons from excitotoxicity-induced axon degeneration, cell body death, and mitochondrial dysfunction, contradicts these previous findings based on genetically defined mice and primary cultured neurons. It is most likely that p53 is not the direct target of CP-31398 and that other bona fide target factors are unidentified and still elusive. As CP-31398 protects axons more effectively than the cell bodies from excitotoxicity, direct target factors might reside in the axonal compartment and activate molecular pathways to suppress excitotoxicity-induced degeneration as the primary effect. Identifying proper CP-31398 target factors (e.g. proteins and lipids) could contribute to decipher and/or identify more detailed molecular mechanisms that could effectively modulate the process of excitotoxicity-induced axon degeneration, cell body death, and mitochondrial dysfunction. Importantly, future studies involving CP-31398 in the modulation of neurodegenerative processes require *in vivo* analyses using rodents as a model to carefully investigate the toxicity of CP-31398 in normal conditions, and to investigate the effectiveness of CP-31398 in rodent models of ischemia or kainite-induced seizures that induce neuronal damage.

Acknowledgments

We thank Drs. Y. Yoneda and Y. Shima for their support. This work was supported in part by The Osaka University Global COE Program (Frontier Biomedical Science Underlying Organelle Network Biology), The Kurata Memorial Hitachi Science and Technology Foundation, Takeda Science Foundation, and The Japan Health Foundation.

References

[1] M.A. Hilliard, Axonal degeneration and regeneration: a mechanistic tug-of-war, J. Neurochem. 108 (2009) 23–32.
[2] L. Conforti, R. Adalbert, M.P. Coleman, Neuronal death: where does the end begin?, Trends Neurosci 30 (2007) 159–166.
[3] R.T. Libby, Y. Li, O.V. Savinova, J. Barter, R.S. Smith, R.W. Nickells, S.W.M. John, Susceptibility to neurodegeneration in a glaucoma is modified by Bax gene dosage, PLoS Genet. 1 (2005) 17–26.
[4] Y. Sagot, M. Duboisdauphin, S.A. Tan, F. Debilbao, P. Aebischer, J.C. Martinou, A.C. Kato, Bcl-2 overexpression prevents motoneuron cell body loss but not axonal degeneration in a mouse model of a neurodegenerative disease, J. Neurosci. 15 (1995) 7727–7733.
[5] A. Sajadi, B.L. Schneider, P. Aebischer, Wld(s)-mediated protection of dopaminergic fibers in an animal model of Parkinson disease, Curr. Biol. 14 (2004) 326–330.
[6] D.M. Hasbani, K.L. O'Malley, Wld(S) mice are protected against the Parkinsonian mimetic MPTP, Exp. Neurol. 202 (2006) 93–99.
[7] S. Pun, A.F. Santos, S. Saxena, L. Xu, P. Caroni, Selective vulnerability and pruning of phasic motoneuron axons in motoneuron disease alleviated by CNTF, Nat. Neurosci. 9 (2006) 408–419.
[8] C. Raoul, E. Buhler, C. Sadeghi, A. Jacquier, P. Aebischer, B. Pettmann, G. Haase, Chronic activation in presymptornatic amyotrophic lateral sclerosis (ALS) mice of a feedback loop involving Fas, Daxx, and FasL, Proc. Natl. Acad. Sci. USA 103 (2006) 6007–6012.
[9] A. Lau, M. Tymianski, Glutamate receptors, neurotoxicity and neurodegeneration, Pflugers. Arch-Eur. J. Physiol. 460 (2010) 525–542.
[10] M. Tymianski, M.P. Charlton, P.L. Carlen, C.H. Tator, Source specificity of early calcium neurotoxicity in cultured embryonic spinal neurons, J. Neurosci. 13 (1993) 2085–2104.
[11] S.A. Lipton, Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond, Nat. Rev. Drug Discov. 5 (2006) 160–170.
[12] J.A. Kemp, R.M. McKernan, NMDA receptor pathways as drug targets, Nat. Neurosci. 5 (2002) 1039–1042.
[13] J.W. Johnson, S.E. Kotermanski, Mechanism of action of memantine, Curr. Opin. Pharmacol. 6 (2006) 61–67.
[14] R. Howard, R. McShane, J. Lindesay, C. Ritchie, A. Baldwin, R. Barber, A. Burns, T. Dening, D. Findlay, C. Holmes, A. Hughes, R. Jacoby, R. Jones, R. Jones, I. McKeith, A. Macharouthu, J. O'Brien, P. Passmore, B. Sheehan, E. Juszczak, C. Katona, R. Hills, M. Knapp, C. Ballard, R. Brown, S. Banerjee, C. Onions, M. Griffin, J. Adams, R. Gray, T. Johnson, P. Bentham, P. Phillips, Donepezil and memantine for moderate-to-severe Alzheimer's disease, N. Engl. J. Med. 366 (2012) 893–903.
[15] B.A. Foster, H.A. Coffey, M.J. Morin, F. Rastinejad, Pharmacological rescue of mutant p53 conformation and function, Science 286 (1999) 2507–2510.
[16] R. Takimoto, W.G. Wang, D.T. Dicker, F. Rastinejad, J. Lyssikatos, W.S. El-Deiry, The mutant p53-conformation modifying drug, CP-31398, can induce apoptosis of human cancer cells and can stabilize wild-type p53 protein, Cancer Biol. Ther. 1 (2002) 47–55.
[17] T. Fujiwara, K. Morimoto, A. Kakita, H. Takahashi, Dynein and dynactin components modulate neurodegeneration induced by excitotoxicity, J. Neurochem. 122 (2012).
[18] T. Fujiwara, K. Morimoto, Cooperative effect of p150Glued and microtubule stabilization to suppress excitotoxicity-induced axon degeneration, Biochem. Biophys. Res. Commun. 424 (2012) 82–88.
[19] T. Fujiwara, K. Morimoto, Inhibition of p53 transactivation functionally interacts with microtubule stabilization to suppress excitotoxicity-induced axon degeneration, Biochem. Biophys. Res. Commun. 427 (2012) 100–106.
[20] P.G. Komarov, E.A. Komarova, R.V. Kondratov, K. Christov-Tselkov, J.S. Coon, M.V. Chernov, A.V. Gudkov, A chemical inhibitor of p53 that protects mice from the side effects of cancer therapy, Science 285 (1999) 1733–1737.
[21] E. Strom, S. Sathe, P.G. Komarov, O.B. Chernova, I. Pavlovska, I. Shyshynova, D.A. Bosykh, L.G. Burdelya, R.M. Macklis, R. Skaliter, E.A. Komarova, A.V. Gudkov, Small-molecule inhibitor of p53 binding to mitochondria protects mice from gamma radiation, Nat. Chem. Biol. 2 (2006) 474–479.
[22] Y. Luu, J. Bush, K.J. Cheung, G. Li, The p53 stabilizing compound CP-31398 induces apoptosis by activating the intrinsic bax/mitochondrial/caspase-9 pathway, Exp. Cell Res. 276 (2002) 214–222.
[23] H. Takeuchi, T. Mizuno, G.Q. Zhang, J.Y. Wang, J. Kawanokuchi, R. Kuno, A. Suzumura, Neuritic beading induced by activated microglia is an early feature of neuronal dysfunction toward neuronal death by inhibition of mitochondrial respiration and axonal transport, J. Biol. Chem. 280 (2005) 10444–10454.
[24] W.G. Wang, R. Takimoto, F. Rastinejad, W.S. El-Deiry, Stabilization of p53 by CP-31398 inhibits ubiquitination without altering phosphorylation at serine 15 or 20 or MDM2 binding, Mol. Cell. Biol. 23 (2003) 2171–2181.
[25] M.J. Demma, S. Wong, E. Maxwell, B. Dasmahapatra, CP-31398 restores DNA-binding activity to mutant p53 in vitro but does not affect p53 homologs p63 and p73, J. Biol. Chem. 279 (2004) 45887–45896.
[26] S.A. Thayer, G.J. Wang, Glutamate-induced calcium loads: effects on energy metabolism and neuronal viability, Clin. Exp. Pharmacol. Physiol. 22 (1995) 303–304.
[27] G.J. Wang, S.A. Thayer, Sequestration of glutamate-induced Ca2+ loads by mitochondria in cultured rat hippocampal neurons, J. Neurophysiol. 76 (1996) 1611–1621.
[28] W. Wang, S.-H. Kim, W.S. El-Deiry, Small-molecule modulators of p53 family signaling and antitumor effects in p53-deficient human colon tumor xenografts, Proc. Natl. Acad. Sci. USA 103 (2006) 11003–11008.
[29] X. Tang, Y. Zhu, L. Han, A.L. Kim, L. Kopelovich, D.R. Bickers, M. Athar, CP-31398 restores mutant p53 tumor suppressor function and inhibits UVB-induced skin carcinogenesis in mice, J. Clin. Invest. 117 (2007) 3753–3764.
[30] R.S. Morrison, H.J. Wenzel, Y. Kinoshita, C.A. Robbins, L.A. Donehower, P.A. Schwartzkroin, Loss of the p53 tumor suppressor gene protects neurons from kainate-induced cell death, J. Neurosci. 16 (1996) 1337–1345.
[31] H. Xiang, D.W. Hochman, H. Saya, T. Fujiwara, P.A. Schwartzkroin, R.S. Morrison, Evidence for p53-mediated modulation of neuronal viability, J. Neurosci. 16 (1996) 6753–6765.