LETTER TO THE EDITOR

Effect of Edaravone on $A\beta_{1-40}$ Induced Enhancement of Voltage-Gated Calcium Channel Current

Feng He,¹ Ping Luan,² Rui He,³ Zhong-Yan Zhao,⁴ Zhi-Qing Sun,¹ Feng-Yuan Che,¹ Yi-Gang Xing⁴ & Jun Liu⁴

¹ Department of Neurology, Linyi People's Hospital, Linyi City, Shandong, China

² Medical School, Shenzhen University, Shenzhen, Guangdong, China

³ Department of Medical Insurance, Xinwen Mining Group Centre Hospital, Tai'an, Shandong, China

⁴ Department of Neurodegenerative Diseases and Aging, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangdong, China

Correspondence

Dr. Jun Liu, Department of Neurodegenerative Diseases and Aging, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangdong 510120, China.

Tel.: 86-20-81332621;

Fax: 86-20-81332620;

E-mail: docliujun@hotmail.com

Dr. Ping Luan, Medical School, Shenzhen University, Shenzhen, Guangdong 518060, China.

Tel.: 86-755-26958869;

Fax: 86-755-86671906;

E-mail: Luanping@mail.com

Received 13 November 2011; accepted 14 November 2011

doi: 10.1111/j.1755-5949.2011.00283.x

β -Amyloid ($A\beta$) damages nerve cells through a variety of biological effects, including oxidative stress and calcium steady-state imbalance [1]. $A\beta$ promotes voltage-gated calcium channels, causing Ca^{2+} influx, resulting in intracellular calcium overload [2]; and free radicals increase voltage-gated calcium channel (VGCC)-mediated Ca^{2+} current [3]. These findings suggest that $A\beta$ may achieve this effect via free radicals. Edaravone (EDA) is a hydroxyl radical scavenger, and has certain antioxidant capacity. There has been no report on whether EDA can affect the enhancement effect of VGCC-currents induced by $A\beta$. In this study, PC12 cells were cocubated with EDA and $A\beta_{1-40}$ to examine potential effects of EDA on the enhancement of VGCC-currents induced by $A\beta$.

PC12 cells were exposed to $A\beta_{1-40}$ (final concentration of $1 \mu\text{mol/L}$), in the absence or presence of EDA (1, 10, 100, or $300 \mu\text{mol/L}$). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to examine cell viability. Whole-cell patch-clamp recording technique was used to measure calcium current, membrane capacitance. Current density was defined as (current/membrane capacitance). Data were statistically analyzed by one-way ANOVA.

$A\beta_{1-40}$ exposure significantly decreased the OD value at 570 nm (Figure 1). EDA alone did not affect the OD value. EDA attenuated the effects of $A\beta_{1-40}$ at 100 and $300 \mu\text{mol/L}$, but not

Figure 1 Optical density at 570 nm under different treatment conditions. Cells were exposed to $1 \mu\text{mol/L}$ $A\beta_{1-40}$, in the absence or presence of different concentrations of EDA for 12 h. OD was analyzed by MTT assay. The data showed that EDA significantly increased the OD value at 100 and $300 \mu\text{mol/L}$, but not at 1 and $10 \mu\text{mol/L}$. * $P < 0.01$ versus $A\beta_{1-40}$ alone.

Figure 2 Electrophysiological results. Panel **A**, Characteristics of VGCC. The inward current of cell exposed to 1 $\mu\text{mol/L}$ A β_{1-40} was highest, followed by incubation with 1 $\mu\text{mol/L}$ A β_{1-40} and 100 $\mu\text{mol/L}$ EDA. The inward current was observed decreased, close to the amplitude of control group and the group treated with EDA alone. Using CdCl₂ (150 $\mu\text{mol/L}$ CdCl₂) could block all inward currents. Panel **B**, VGCC currents and current-voltage curve.

The peak inward current appeared when the membrane potential was +20 mV. The average peak current value of the A β_{1-40} group was the largest. In comparison with other groups, 100 $\mu\text{mol/L}$ EDA attenuated the effect of A β_{1-40} ($P < 0.01$). EDA alone had no effects on the average peak current value.

at 1 and 10 $\mu\text{mol/L}$. A β_{1-40} treatment increased the peak current and current density (from 318.59 pA and 29.52 pA/pF to 580.54 pA and 56.85 pA/pF; $P < 0.01$ for both). CdCl₂ (150 $\mu\text{mol/L}$ in the extracellular solution) blocked the inward current. Effects of A β_{1-40} were blocked by EDA (100 $\mu\text{mol/L}$). EDA treatment alone did not affect current peak or density. Cell inward currents were observed generally larger in the A β_{1-40} group, and the value was significantly reduced by EDA (Figure 2, panel A). The i-v curves of VGCC were V-shaped in all groups, and the channel was activated when the membrane potential was approximately -40 mV and the peak inward current appeared at +20 mV (Figure 2, panel B).

A β directly generates free radicals, but can also combine with metal ions (Fe^{3+} , Cu^{2+}) to generate hydroxyl radical and other reactive oxygen species (ROS) from hydrogen peroxide (H_2O_2) via Fenton reaction [4]. H_2O_2 can promote the enhancement of L-VGCC Ca^{2+} current amplitudes [5]. EDA is a radical scavenger, and can reduce the damaging effects of lipid peroxidation. The results from this study showed that EDA could attenuate A β_{1-40} -induced cytotoxicity in a concentration-dependent manner. In the whole-cell recording, A β_{1-40} markedly enhanced the VGCC currents. Consistent with previous reports [6], the current was blocked with CdCl₂. Using 100 $\mu\text{mol/L}$ EDA incubation alone no

obvious change was observed on amplitude of VGCC current that prompted EDA have no effect on normal cell VGCC current. The effect of A β_{1-40} on VGCC-current in PC12 cells was abolished by the presence of 100 $\mu\text{mol/L}$ EDA, indicating an important role of Ca^{2+} overload. These results suggest that EDA could attenuate Ca^{2+} overload, and mitigates the changes associated with A β generation [7].

Acknowledgments

This study was supported by grant to Jun Liu from the National Natural Science Foundation of China (No. 30970966), the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry and the grants to Ping Luan from the Technology Planning Project of Guangdong Province (No. 2010B031600306) and Scientific Research Fund of Shenzhen University (No. 201051).

Conflict of Interests

The authors have no conflict of interest.

References

- Holtrian C. Possible causes of Alzheimer's disease: Amyloid fragments, free radicals, and calcium homeostasis. *Neurobiol Dis* 1998;**5**:129–141.
- MacManus A, Ramsden M, Murray M, et al. Enhancement of $^{45}\text{Ca}^{2+}$ influx and voltage-dependent Ca^{2+} channel activity by β -amyloid-(1–40) in rat cortical synaptosomes and cultured cortical neurons. Modulation by the proinflammatory cytokine interleukin-1 β . *J Biol Chem* 2000;**275**:4713–4718.
- Akaishi T, Nakazawa K, Sato K, et al. Hydrogen peroxide modulates whole cell Ca^{2+} currents through L-type channels in cultured rat dentate granule cells. *Neurosci Lett* 2004;**356**:25–28.
- Lynch T, Cherny RA, Bush AI. Oxidative processes in Alzheimer's disease: The role of abeta-metal interactions. *Exp Gerontol* 2000;**35**:445–451.
- Hudasek K, Brown ST, Fearon IM. H_2O_2 regulates recombinant Ca^{2+} channel $\alpha 1\text{C}$ subunit but does not mediate their sensitivity to acute hypoxia. *Biochem Biophys Res Commun* 2004;**318**:135–141.
- He LM, Chen LY, Lou XL, et al. Evaluation of β -amyloid peptide 25–35 on calcium homeostasis in cultured rat dorsal root ganglion neurons. *Brain Res* 2002;**939**:65–75.
- Green KN, Smith IF, Laferla FM. Role of calcium in the pathogenesis of Alzheimer's disease and transgenic models. *Subcell Biochem* 2007;**45**:507–521.