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α-lipoic acid protects rat cortical neurons against cell death induced by amyloid and hydrogen peroxide through the Akt signalling pathway

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Abstract

Substantial evidence suggests that the accumulation of β-amyloid (Aβ)-derived peptides contributes to the aetiology of Alzheimer's disease (AD) by stimulating formation of free radicals. Thus, the antioxidant α-lipoate, which is able to cross the blood–brain barrier, would seem an ideal substance in the treatment of AD. We have investigated the potential effectiveness of α-lipoic acid (LA) against cytotoxicity induced by Aβ peptide (31–35) (30 μM) and hydrogen peroxide (H_2O_2) (100 μM) with the cellular 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) reduction and fluorescence dye propidium iodide assays in primary neurons of rat cerebral cortex. We found that treatment with LA protected cortical neurons against cytotoxicity induced by Aβ or H_2O_2. In addition, LA-induced increase in the level of Akt in the neurons was observed by Western blot. The LA-induced neuroprotection and Akt increase were attenuated by pre-treatment with the phosphatidylinositol 3-kinase inhibitor, LY294002 (50 μM). Our data suggest that the neuroprotective effects of the antioxidant LA are partly mediated through activation of the PKB/Akt signaling pathway.

Keywords: Lipoic acid; Cell death; Alzheimer's disease; Antioxidants; Apoptosis; Neuron

Amyloid peptide beta (Aβ), a major component of neuritic plaques in Alzheimer's disease (AD) brain, accumulates abundantly in the areas subserving information acquisition and processing and memory formation. Aβ directly induces neuronal cell death [9,17]. Substantial evidence suggests that the neurotoxicity of Aβ is associated with oxidative stress [1,2,6] and with the generation of reactive oxygen species that damage neuronal membrane lipids, proteins and nucleic acids [5]. Aβ stimulates formation of free radicals and is capable of generating hydrogen peroxide (H_2O_2) by metal ion reduction [8]. In senile plaques of AD brains, microglia produce free radicals [12], and lipid peroxidation has been observed in the frontal lobe of patients with AD [12]. Thus, both reductions of amyloid synthesis and antioxidants have been proposed in the treatment of the presumed Aβ-mediated neurotoxicity [12] in Alzheimer's disease.

The possible therapeutic efficacy of the antioxidant α-lipoic acid (LA) has been suggested by in vitro and in vivo animal studies [12]. LA, a low molecular weight substance which is absorbed from the diet, is able to cross the blood–brain barrier [12]. LA is reduced to dihydrolipoate in target cells and tissue, including brain, where it regenerates through redox cycling other antioxidants like vitamin C and vitamin E and raises levels of intracellular glutathione, an important thiol antioxidant [12]. In vitro, animal, and preliminary human studies indicate that LA has neuroprotective effects on Aβ-mediated cytotoxicity and may be effective in numerous neurodegenerative disorders [12].

Physiologic levels of Aβ activate phosphatidylinositol 3-kinase (PI3-K) [10]. Similarly, compounds with antioxidant properties, such as estrogen, protect neurons against Aβ-

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and glutamate-induced cytotoxicity and increase activation of Akt, an effector immediately downstream of PI3-K [7,17]. These results suggest that the PI3-K cascade is involved in neuroprotection [7,17]. To explore the basis for the neuroprotective effects of antioxidants, we have investigated an alternative mechanism in which LA protects neurons against toxicity induced by $A\beta_{31-35}$ and H_2O_2 through Akt signaling in rat primary cultured cortical neurons. Neuronal cultures were prepared from cerebral cortex of E19 fetuses obtained from Sprague–Dawley rats as described previously [16,17].

To examine Aβ- and H_2O_2-induced toxicity and LA-induced neuroprotection, cells were incubated with $A\beta_{31-35}$ (0–30 μM), H_2O_2 (0–100 μM), LA (1–10 μM), LA (1–10 μM) + $A\beta_{31-35}$ (30 μM), or LA (1–10 μM) + H_2O_2 (100 μM) at 37°C. The pre-treatment time for LA was 48 h. The treatment times for $A\beta_{31-35}$ and H_2O_2 were 48 h. Cell viability was quantified using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) colorimetric assay, described elsewhere [17]. The extent of neuronal injury was estimated by assessing the extent of neuronal uptake of the fluorescent dye propidium iodide (PI) (procedure described elsewhere) [9].

Survival of vehicle-treated control groups not exposed to either $A\beta_{31-35}$, H_2O_2, or LA was defined as 100%. The numbers of surviving, dead, or apoptotic cells in the treated groups were expressed as a percent of control groups. One-way ANOVA was used to compare control and treated groups, with $P < 0.05$ considered statistically significant.

As estimated by the MTT assay, 48-h treatment with $A\beta_{31-35}$ significantly decreased cell survival at 20–30 μM concentrations (Fig. 1A). This toxic effect of $A\beta_{31-35}$ agreed with previous data in primary cerebral cortical cells [7,17]. Pre-treatment of cells with LA (48 h) protected cells against the toxicity induced by $A\beta_{31-35}$ (Fig. 1B). These effects were significant at a concentration of 5 μM with a maximal effect obtained at 10 μM (Fig. 1B). Similarly, the $A\beta_{31-35}$ (30 μM)-induced increase in PI uptake was attenuated by pre-treatment with LA (5–10 μM) (Fig. 1C). The MTT and PI values in the group treated with LA (10 μM) alone were not significantly different from vehicle control (104 ± 3 versus 100 ± 2 for MTT; $P > 0.05$; 106 ± 2 versus 96 ± 3 for PI; $P > 0.05$). These data suggest that LA has little or no effect by itself on cell survival.

Because H_2O_2 is involved in Aβ toxicity [1], we studied the effects of LA on H_2O_2-induced cell death. As previously reported in cortical neurons [16,17], a 48-h exposure to H_2O_2 (100 μM) decreased cell survival as indicated by the MTT assay (Fig. 1D). The toxicity induced by H_2O_2 was reduced by pre-treatment with LA, significant at 5 μM and maximal at 10 μM (Fig. 1E,F). Fig. 2 summarizes the morphological features of exposure to vehicle, LA (10 μM), H_2O_2 (100 μM), and the combination of LA (10 μM) and H_2O_2 (100 μM).

We investigated the changes in the levels of phosphorylated Akt in neuronal cell cultures that were exposed to 10 μM LA for 5, 10, 30 min, 1, 48 and 72 h, respectively, examined by Western blot (Fig. 3A). 10 μM LA-induced elevation of Akt occurred as early as 10 min and reached a maximal level at 1 h (Fig. 3A). By contrast, 48-h treatment with $A\beta_{31-35}$ (30 μM) and H_2O_2 (100 μM) resulted in decreases in the levels of phosphorylated Akt in cultures (Fig. 3B). A 48-h pre-treatment with LA (10 μM) significantly reversed the $A\beta_{31-35}$- and H_2O_2-induced reduction of Akt in neuronal cell cultures (Fig. 3C). Pre-treatment (30 min) with the PI3-K inhibitor LY294002 (50 μM) significantly blocked LA-induced increase in the level of Akt (Fig. 3C) and attenuated LA-induced neuroprotection against $A\beta_{31-35}$ (Fig. 3E) and H_2O_2 (data not shown).

The present study indicates that LA is able to protect cerebral cortical neurons against Aβ peptide- and H_2O_2-induced toxicity [14]. Such protection by LA may be mediated by Akt

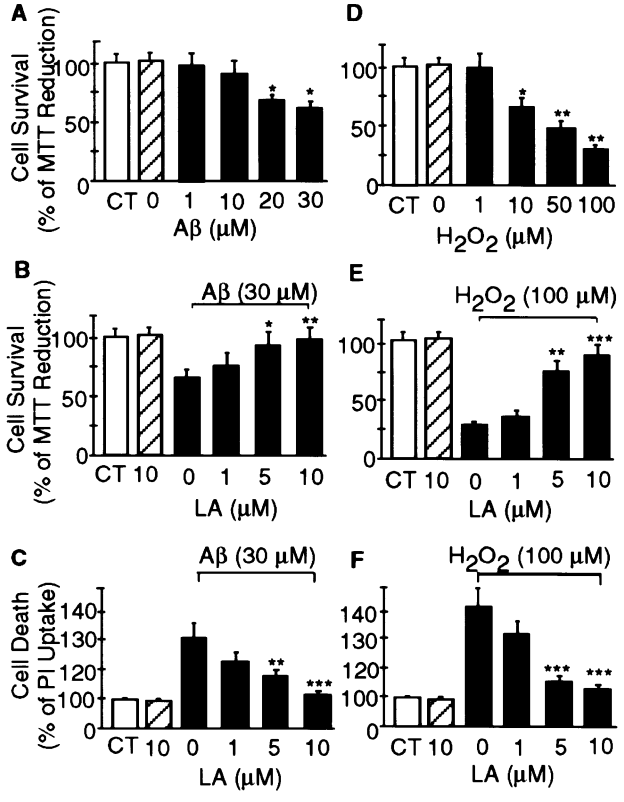

Fig. 1. The effects of $A\beta_{31-35}$, H_2O_2 and LA on neuronal survival or death in cortical neurons. (A) Effect of $A\beta_{31-35}$ on cell survival examined by MTT. (B) Effect of LA on Aβ-diminished cell survival examined by MTT. (C) Effect of LA on $A\beta_{31-35}$ -induced cell death examined by PI assay. (D) Effect of H_2O_2 on survival examined by MTT. (E) Effect of LA on H_2O_2-diminshed cell survival examined by MTT. (F) Effect of LA on H_2O_2-induced cell death examined by PI assay. Pre-treatment with LA revealed a dose-dependent increase in neuronal survival (B, E) and decrease in cell death (C, F), compared to that when cells were exposed to $A\beta_{31-35}$ or H_2O_2 alone. Percentage cell survival or death was relative to non-treated controls (0% toxicity). Values represent mean ± SD of three separate experiments, each performed in duplicate. CT, normal controls; O, vehicle; hatched bars in B,C,E and F, LA alone (10μm) $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

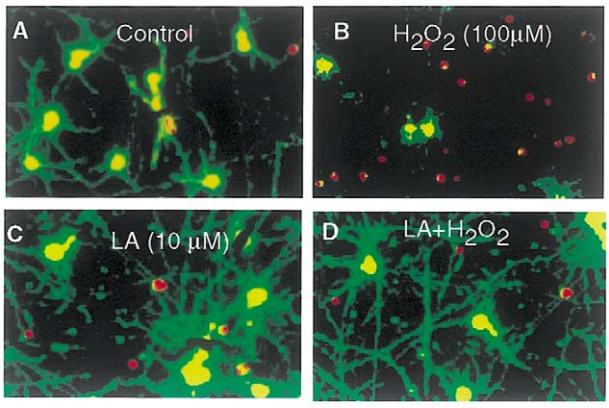

Fig. 2. The effects of LA and H_2O_2 on neuronal survival were revealed by microscopic examinations. (A) Control; (B) neurons treated with H_2O_2 (100 μM); (C) neurons treated with LA (10 μM); (D) neurons treated with LA (10 μM) and H_2O_2 (100 μM). The viability of cells was evaluated using the live/dead viability/cytotoxicity kit (Roche Molecular Biochemicals, Germany). The kit uses the terminal deoxyonucleotidyl transferase-mediated dUTP nick end labeling technique to label apoptosis-induced DNA strand breaks. Immobilized cells were stained following the instructions supplied with the kit. Live cells stain green, while dead cells stain red.

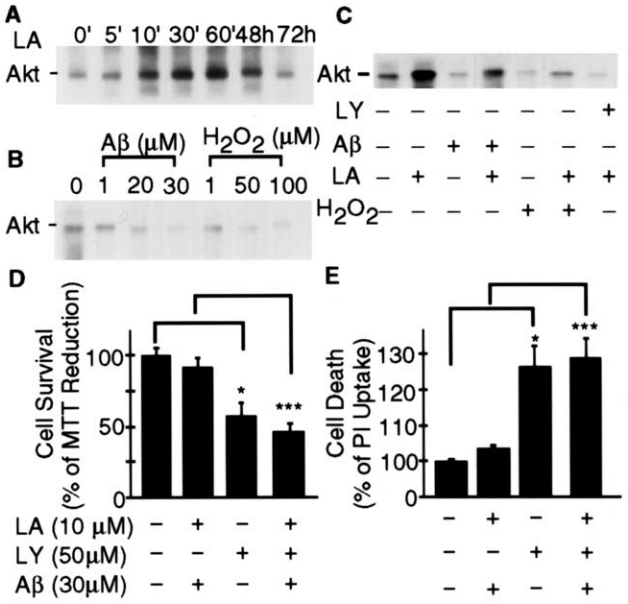

Fig. 3. The effects of LA, Aβ, H_2O_2 and LY294002 on the level of activated Akt and cell death in neuronal cultures. (A) Time course of LA (10 μM)-induced elevation of Akt; (B) the effect of $A\beta_{31-35}$ and H_2O_2 on the level of Akt; (C) the effect of LA (10 μM) on the $A\beta_{31-35}$ (30 μM)- and H_2O_2 (100 μM)-induced levels of Akt, and the effects of a 30 min-pre-treatment of LY294002 in neuronal cell cultures. LA blocked Aβ- and H_2O_2 -induced reduction of Akt. LY294002 (50 μM) prevented the LA-induced increase in the level of Akt. (D,E) the effect of LA (10 μM), $A\beta_{31-35}$ (30 μM) and LY294002 (100 μM) on cell survival. Values represent mean ± SD of three separate experiments, each performed in duplicate. $^*P < 0.05$, $^{***}P < 0.001$.

signaling, since its effects can be blocked by a PI3-K inhibitor, LY294002. To our knowledge, this is the first demonstration of the neuroprotective activity of LA associated with Akt signaling in cultured neuronal cells. These data are of particular interest in the clinical context, given that the AB-peptide probably plays a role in the neurodegenerative process occurring in AD [6,12], associated with the loss of cerebral cortical neurons [9,17], thus suggesting the potential effectiveness of LA in the treatment of AD [12].

To date, there is limited agreement in the literature concerning the cellular pathways involved in Aβ-induced cell death [15]. One hypothesis, however, suggests that Aβ-neurotoxicity results from induction of free radicals, presumably initiated either directly by Aβ peptides themselves [6,8] or indirectly through increases in intracellular production of reactive oxygen species [1,12]. Although there is a report indicating that Aβ neurotoxicity is not protected against by antioxidants [13], many studies demonstrate that higher levels of H_2O_2 occur in the CNS in AD and, further, can be attenuated by antioxidants [1,11]. Aβ produces hydrogen peroxide (H_2O_2), a hydroxyl radical donor [5], through metal ion reduction, with concomitant release of thiobarbituric acid-reactive substances, a process probably mediated by formation of hydroxyl radicals [2,8]. Free radicals peroxidize membrane lipids and oxidize proteins producing drastic cellular damage [4,16]. Thus, exposure to H_2O_2 may mediate Aβ peptide-induced toxicity [1,6] and cell death. Here, we show that LA can inhibit such H_2O_2- and Aβ-induced cell death.

A central role of the PI3-K pathway in neuronal survival was suggested by the observation that PI3-K inhibitors block the survival effect of NGF [3]. Akt activation results in the phosphorylation of several apoptosis-regulating proteins. For example, activation of Akt induces the phosphorylation of Bad, a pro-apoptotic member of the Bcl-2 family, and inhibits Bad's pro-apoptotic activity [3].

We observed that the level of phosphorylated Akt was rapidly increased by LA within 15 min and was maintained at relatively high levels at 48 h, when the neuroprotection was observed. Long-term LA exposure (with concomitant Akt activation) may, therefore, be necessary for the neuroprotection. This hypothesis is confirmed by our observation that a 1-h pre-treatment with LA (10 μM) did not protect neurons against cell death induced by $A\beta_{31-35}$ (30 μM) (data not shown). Although further investigation is needed to reveal the precise mechanism for lipoate neuroprotection, especially in Aβ-induced neurotoxicity, the present results suggest the possibility that LA may protect neurons from $A\beta_{31-35}$-induced neurotoxicity through the PKB/Akt signaling pathway, thereby maintaining the integrity of the nervous system and reducing the risk of AD-related toxicity.

[1] Behl, C., Davis, J.B., Lesley, R. and Schubert, D., Hydrogen peroxide mediates amyloid beta protein toxicity, Cell, 77 (1994) 817–827.
[2] Bus, A.I., Huang, X. and Fairlie, D.P., The possible origin of free radicals from amyloid beta peptides in Alzheimer's disease, Neurobiol. Aging, 20 (1999) 335–337.

[3] Cross, T.G., Scheel-Toellner, D., Henriquez, N.V., Deacon, E. and Salmon, M., Serine/threonine protein kinases and apoptosis, Exp. Cell Res., 256 (2000) 34–41.
[4] Gabbita, S.P., Subramaniam, R., Allouch, F., Carney, J.M. and Butterfield, D.A., Effects of mitochondrial respiratory stimulation on membrane lipids and proteins: an electron paramagnetic resonance investigation, Biochim. Biophys. Acta., 1372 (1998) 163–173.
[5] Halliwell, B. and Gutteridge, J.M., Biologically relevant metal ion-dependent hydroxyl radical generation. An update, FEBS. Lett., 307 (1992) 108–112.
[6] Harris, M.E., Hensley, K., Butterfield, D.A., Leedle, R.A. and Carney, J.M., Direct evidence of oxidative injury produced by the Alzheimer's beta-amyloid peptide (1–40) in cultured hippocampal neurons, Exp. Neurol., 131 (1995) 193–202.
[7] Honda, K., Sawada, H., Kihara, T., Urushitani, M., Nakamizo, T., Akaike, A. and Shimohama, S., Phosphatidylinositol 3-kinase mediates neuroprotection by estrogen in cultured cortical neurons, J. Neurosci. Res., 60 (2000) 321–327.
[8] Huang, X., Atwood, C.S., Hartshorn, M.A., Multhaup, G., Goldstein, L.E., Scarpa, R.C., Cuajungco, M.P., Gray, D.N., Lim, J., Moir, R.D., Tanzi, R.E. and Bush, A.I., The A beta peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction, Biochemistry, 38 (1999) 7609–7616.
[9] Huang, H., Ou, H. and Hsieh, S., Antioxidants prevent amyloid peptide-induced apoptosis and alteration of calcium homeostasis in cultured cortical neurons, Life Sci., 66 (2000) 1879–1892.
[10] Luo, Y., Sunderland, T. and Wolozin, B., Physiologic levels of beta-amyloid activate phosphatidylinositol 3-kinase with the involvement of tyrosine phosphorylation, J. Neurochem., 67 (1996) 978–987.
[11] Mattson, M.P., Free radicals and disruption of neuronal ion homeostasis in AD: a role for amyloid beta-peptide, Neurobiol. Aging, 16 (1995) 679–682.
[12] Packer, L., Tritschler, H.J. and Wessel, K., Neuroprotection by the metabolic antioxidant alpha-lipoic acid, Free Radic. Biol. Med., 22 (1997) 359–378.
[13] Pike, C.J., Ramezan-Arab, N. and Cotman, C.W., Beta-amyloid neurotoxicity in vitro: evidence of oxidative stress but not protection by antioxidants, J. Neurochem., 69 (1997) 1601–1611.
[14] Ratan, R.R. and Baraban, J.M., Apoptotic death in an in vitro model of neuronal oxidative stress, Clin. Exp. Pharmacol. Physiol., 22 (1995) 309–310.
[15] Yankner, B.A., Duffy, L.K. and Kirschner, D.A., Neurotrophic and neurotoxic effects of amyloid protein: reversal by tachykinin neuropeptides, Science, 25 (1990) 279–282.
[16] Whittemore, E.R., Loo, D.T. and Cotman, C.W., Exposure to hydrogen peroxide induces cell death via apoptosis in cultured rat cortical neurons, NeuroReport, 5 (1994) 1485–1488.
[17] Zhang, L., Rubinow, D.R., Xaing, G., Li, B., Chang, Y.H., Maric, D., Barker, J.L. and Ma, W., Estrogen protects against β-amyloid-induced neurotoxicity in rat hippocampal neurons by activation of Akt, NeuroReport, 12 (2001) 1919–1923.