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\textsubscript{2}O\textsubscript{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\textsubscript{2}O\textsubscript{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 neurotic 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\textsubscript{2}O\textsubscript{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 anti-oxidants 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-
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 αβ31–35 and H2O2 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 αβ31–35 and H2O2-induced toxicity and LA-induced neuroprotection, cells were incubated with αβ31–35 (0–30 μM), H2O2 (0–100 μM), LA (1–10 μM), LA (1–10 μM) + αβ31–35 (30 μM), or LA (1–10 μM) + H2O2 (100 μM) at 37°C. The pre-treatment time for LA was 48 h. The treatment times for αβ31–35 and H2O2 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 αβ31–35, H2O2, 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 αβ31–35 significantly decreased cell survival at 20–30 μM concentrations (Fig. 1A). This toxic effect of αβ31–35 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 αβ31–35 (Fig. 1B). These effects were significant at a concentration of 5 μM with a maximal effect obtained at 10 μM (Fig. 1B). Similarly, the αβ31–35 (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 H2O2 is involved in αβ toxicity [1], we studied the effects of LA on H2O2-induced cell death. As previously reported in cortical neurons [16,17], a 48-h exposure to H2O2 (100 μM) decreased cell survival as indicated by the MTT assay (Fig. 1D). The toxicity induced by H2O2 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), H2O2 (100 μM), and the combination of LA (10 μM) and H2O2 (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 αβ31–35 (30 μM) and H2O2 (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 αβ31–35- and H2O2-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 αβ31–35 (Fig. 3E) and H2O2 (data not shown).

The present study indicates that LA is able to protect cerebral cortical neurons against αβ peptide- and H2O2-induced toxicity [14]. Such protection by LA may be mediated by Akt
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 Aβ-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 H2O2 occur in the CNS in AD and, further, can be attenuated by antioxidants [1,11]. Aβ produces hydrogen peroxide (H2O2), 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 H2O2 may mediate Aβ peptide-induced toxicity [1,6] and cell death. Here, we show that LA can inhibit such H2O2- 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 Aβ31–35 (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 Aβ31–35-induced neurotoxicity through the PKB/Akt signaling pathway, thereby maintaining the integrity of the nervous system and reducing the risk of AD-related toxicity.
