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Original Contribution

NEUROPROTECTIVE EFFECTS OF α-LIPOIC ACID AND ITS POSITIVELY CHARGED AMIDE ANALOGUE

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Abstract—Elevated levels of extracellular glutamate have been linked to reactive oxygen species mediated neuronal damage and brain disorders. Lipoic acid is a potent antioxidant that has previously been shown to exhibit neuroprotection in clinical studies. A new positively charged water soluble lipoic acid amide analog, 2-(N,N-dimethylamine) ethylamido lipoate·HCl (LA-plus), with a better cellular reduction and retention of the reduced form was developed. This novel antioxidant was tested for protection against glutamate induced cytotoxicity in a HT4 neuronal cell line. Glutamate treatment for 12 h resulted in significant release of LDH from cells to the medium suggesting cytotoxicity. Measurement of intracellular peroxides showed marked (up to 200%) increase after 6 h of glutamate treatment. Compared to lipoic acid, LA-plus was more effective in (1) protecting cells against glutamate induced cytotoxicity, (2) preventing glutamate induced loss of intracellular GSH, and (3) disallowing increase of intracellular peroxide level following the glutamate challenge. The protective effect of LA-plus was found to be independent of its stereochemistry. The protective function of this antioxidant was synergistically enhanced by selenium. These results demonstrate that LA-plus is a potent protector of neuronal cells against glutamate-induced cytotoxicity and associated oxidative stress. © 1999 Elsevier Science Inc.

Keywords—Thioctic acid, Antioxidants, Redox, Neurotoxicity, Free radicals

INTRODUCTION

Reactive oxygen species (ROS) represent a major contributor to brain damage in disorders such as epilepsy[1,2], head trauma[3], and ischemia reperfusion injuries [4–6]. Oxidative damage is also implicated in neurodegenerative diseases such as Huntington's[7], Alzheimer's[8], and Parkinson's [9–12]. In the pathogenesis of these diseases, oxidative damage may accumulate over a period of years, leading to massive neuronal loss.

Glutamate toxicity is a major contributor to pathological cell death within the nervous system and appears to be mediated by ROS [13]. There are two forms of glutamate toxicity: receptor-initiated excitotoxicity [14], and nonreceptor-mediated glutamate induced toxicity[15]. One model used to study oxidative stress related neuronal death is to inhibit cystine uptake by exposing cells to high levels of glutamate [16]. High glutamate levels block cystine uptake via the amino acid transporter Xc and impair reduced glutathione (GSH) cell homeostasis. The induction of oxidative stress by glutamate in this model has been demonstrated to be a primary cytotoxic mechanism in C6 glial cells [17,18], PC-12 neuronal cells [19,20], immature cortical neurons cells [16], and oligodendroglia cells [21]. Recently, the mitochondrial electron transport chain has been shown to be a source of ROS production during glutamate induced apoptosis in HT22 neuronal cells, a sub-clone of HT4 cells used in the current study [15].

Antioxidants such as α-tocopherol, probucol, and α-lipoic acid (LA) have been shown to protect these cells against glutamate cytotoxicity [16–18,22,23]. We sought to elucidate the neuroprotective properties of the novel water-soluble positively charged analogue of lipoic acid 2-(N,N-dimethylamine) ethylamido lipoate·HCl (LA-plus). Recently, it has been reported that, compared with LA, LA-plus is taken up and reduced by T-cells more

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effectively. As a result, LA-plus demonstrated more potent immunomodulatory activity [24].

In this investigation, mouse hippocampal neuronal HT4 cells [25] were exposed to elevated glutamate levels and several analogues of LA were tested for their efficacy as neuroprotectors.

MATERIALS AND METHODS

Materials

The following materials were obtained from the sources indicated in the corresponding adjacent parentheses: L-glutamic acid monosodium salt, NADH, lipoamide dehydrogenase, sodium selenite, lipoamide, (Sigma, St Louis, MO); dichlorodihydrofluorescein diacetate (DCFH-DA) (Molecular Probes, Eugene, Oregon); GLA-LA, R(+),S(−) LA and R(+) LA (ASTA Medica, Frankfurt, Germany); cholesteryl-lipoate was kindly provided by professor Manfred Schneider (University of Wuppertal, Germany); R(+) LA-plus was synthesized as described [24]. In addition, S(−) LA-plus and R(+),S(−) LA-plus were also prepared. For cell culture, Dulbecco's Modified Eagle Medium (Gibco, Gaithersburg, MD); fetal calf serum and penicillin and streptomycin (University of California, San Francisco, CA); and culture dishes 100 × 15 mm (Becton Dickinson) were used.

Cell culture

Mouse hippocampal HT4 cells, kindly provided by D.E. Koshland, Jr., University of California at Berkeley, were grown in Dulbecco's Modified Eagle Medium supplemented with 10% fetal calf serum, penicillin (100 U/ml) and streptomycin (100 μg/ml) at 37°C in a humidified atmosphere containing 95% air and 5 % CO_2. Confluent cells were trypsinized and subcultivated in culture dishes at a concentration of $6.8x10^4$ cells per/ml (5 ml per plate). The cells were cultured at the standard conditions described above. After 24 h of seeding, the culture medium was replaced with fresh medium supplemented with serum and antibiotic. The cells were then exposed to glutamate (10 mM) (according to a model of glutamate induced cytotoxicity to PC12 cells and HT22 cells) [15,26]. No change in medium pH was observed in response to addition of glutamate.

Selenium pretreatment

HT4 cells were seeded at $6.8x10^4$ cells/ml in the presence of 1 μM of sodium selenite. Cells were cultured for 24 h, after which the culture medium was replaced, and cells were exposed to 10 mM glutamate.

Antioxidant treatment

Stock solutions (50 mM) of LA and LA-plus were prepared in phosphate buffer saline pH 7.4. (PBS). Briefly, aqueous solution of LA was prepared by first dissolving LA in alkaline pH (1N NaOH). Then, PBS was added to the solution and pH was neutralized (1N HCl). LA-Plus was first dissolved in acidic pH (1N HCl). Then PBS was added and pH was neutralized using 1N NaOH. Lipoamide, GLA-LA, or cholesteryl-lipoate were dissolved in DMSO at concentrations such that the final concentration of the solvent in cell culture medium did not exceed 0.1% v/v. Respective controls were treated with an equal volume of DMSO. The antioxidants were added to the culture dishes 5–10 min before glutamate challenge.

Determination of cell viability

Quantitative assessment of cell viability was done by measuring lactate dehydrogenase (LDH) leakage [17, 23], a widely accepted measure of cell membrane integrity [17,23]. After completion of experiments, culture medium was removed from plates and centrifuged (500 g × 5 min). The cells that detached from the monolayer following glutamate treatment were separated by centrifugation of the culture medium. The supernatant medium was mixed with an equal volume of 5% (w/v) bovine serum albumin (BSA) solution in PBS to help stabilize LDH activity in the solution for storage at 4°. The pelleted detached cells were washed once with PBS and treated with a lysis buffer (0.5% v/v Triton X-100 in PBS). The resulting lysate was mixed with an equal volume of the BSA solution for storage at 4°C. Cells in monolayer were washed with PBS, treated with lysis buffer and BSA solution as described above for detached cells. Lactate dehydrogenase activity was measured from the samples within two days of storage. Cell viability was determined using the following formula: viability = LDH activity of attached cell / total LDH activity (medium + LDH activity of detached cells + LDH activity of attached cell) [17,23].

Determination of intracellular peroxides

Intracellular peroxides were detected using dichlorodihydrofluorescein-diacetate (DCFH-DA) [17]. Following treatment with or without antioxidants and glutamate, cells were washed three times with PBS. Monolayer cells were detached using trypsin, and centrifuged (600 g, 5 min). The cells were again washed with PBS and centrifuged, after which the cells were resuspended in PBS and incubated with DCFH-DA (25 μM) for 30 minutes at 37°C. Cells were then excited

with a 488 nm UV line argon-ion laser in a flow cytometer (XL, Coulter, FL, USA) and the dichlorofluorescein (DCF) emission was recorded at 530 nm. Data were collected from at least 10,000 cells.

High performance liquid chromatography–electrochemical detection

HT4 cells were washed with ice cold PBS, treated with 2% (w/v) monochloroacetic acid, and scraped. All samples were immediately frozen in liquid nitrogen and stored at −80°C, until high performance liquid chromatography (HPLC) analysis. Immediately before the assay, samples were thawed, vortexed, and then centrifuged at 15,000 g for 2 min. The clear supernatant was removed and injected to the HPLC system. High performance liquid chromatography–electrochemical (EC) detection of GSH was performed using an ESA (Chelmsford, MA) coulometric detector [17].

α-Lipoic acid and dihydrolipoic acid (DHLA), LA-plus and DHLA-plus from cell extracts were analyzed as described recently [24] with minor modifications as specified below. Cells were scraped from the plates and collected in acidic conditions (4% (w/v)-phosphoric acid). A C-18 RP (25 cm × 4.6 mm) column (Alltech) and a coulometric detector (ESA, Coulochem II, Chelmsford, MA, USA) were used. The mobile phase, consisting of 50 % (v/v) of solution A [50 mM NaH_2PO_4, (pH 2.7)], and 50% (v/v) of solution B [70 % (v/v) acetonitrile and 30 % (v/v) methanol] was delivered using an isocratic solvent delivery module (ESA) set at a flow rate of 1.2 ml/min. Retention times for LA and DHLA were 10.9 min and 12.6 min, respectively. For the determination of LA-plus and its corresponding reduced dithiol form, DHLA-plus, cells were extracted and analyzed as described for the determination of LA and DHLA except for the mobile phase that consisted of 65% (v/v) solution A and 35% (v/v) solution B, and the flow rate was set at 1.0 ml/min. Retention times for LA-plus and DHLA-plus were 7.2 min and 8.3 min, respectively.

Enzymatic reduction of LA and LA-plus by lipoamide dehydrogenase

The reaction mixture included 0.1 mM of NADH, 0.1 U/ml of lipoamide dehydrogenase, and 0.3 mM of LA or LA-plus at room temperature. The conversion rate of LA to DHLA or LA-plus to DHLA-plus was measured by monitoring the oxidation rate of NADH at 340 nm.

Statistics

Data are reported as mean ± standard deviation (SD) of three experiments except where indicated. Comparison among multiple groups were made by analysis of variance ANOVA. $p < .05$ was considered statistically significant.

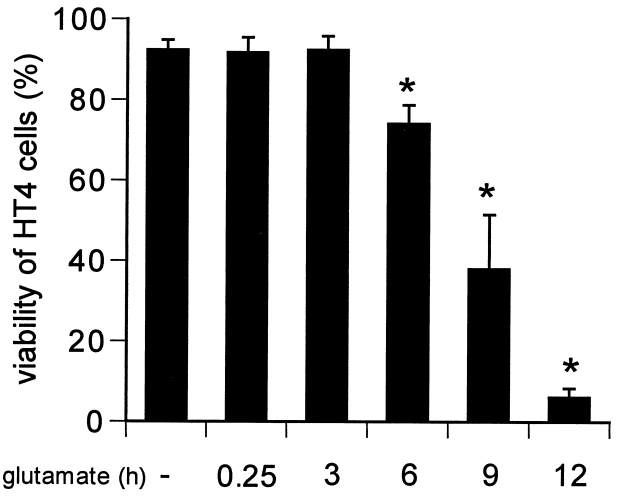

Fig. 1. Viability of HT4 neuronal cells following treatment with glutamate (10 mM). HT4 cells were treated with glutamate for 0.25, 3, 6, 9, or 12 h and cell viability was measured by LDH leakage. The control represents 12 h with no glutamate treatment. Control and glutamate 12 h bars are the means ± SD of 11 independent experiments while the rest of the bars are the means ± SD of at least three independent experiments. *$p < .05$, lowered compared to glutamate nontreated control.

RESULTS

Protection against glutamate induced cytotoxicity

Exposure of HT4 cells to 10 mM glutamate for 12 hours resulted in <90% loss of cell viability as was determined by release of LDH to the cell culture medium (Fig. 1) At this time-point, most cells were attached to the plate, although they appeared round and shrunken as observed visually using a light microscope. Our results show that LDH leakage occurred before any detachment of cells, since LDH activity in detached cells was found to be negligible (2.6±1.4 U/L) compared with the activity detected in the medium of glutamate treated cells (173±24 U/L).

Unless otherwise stated, cell viability in the presence or absence of antioxidants was investigated after 12 h of glutamate treatment.

Following glutamate challenge, cell viability was studied in the presence of thiol antioxidants R(+) LA or R(+) LA-plus (Fig. 2). R(+) LA-plus was more efficient in protecting the HT4 cells compared to R(+) LA against such challenge. R(+) LA-plus at 50 μM was sufficient to protect cells against glutamate cytotoxicity while R(+) LA at 100 μM did not provide total protection (Fig. 3A). We tested the amine part (N,N-dimethylethylenedia-

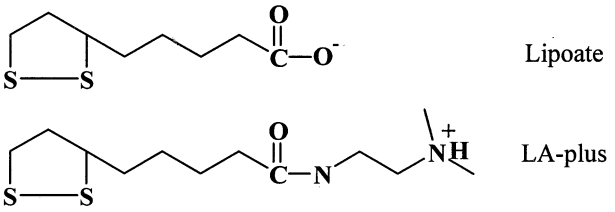

Fig. 2. Chemical structures. The ionized form of LA (lipoate), and LA-plus (the protonated form of 2-(N,N-dimethylamine) ethylamido lipoate) at physiological pH (7.4). The term plus indicates positive ionic charge in neutral aqueous solutions.

mine) of LA-plus (which was used for synthesis [24]) at a concentration of 50 μM. This resulted in no protection against the glutamate challenge (data not shown). Supplementation of the cell medium with 1 μM of sodium selenite for 24 h before the glutamate challenge resulted in a marginal protection against glutamate induced cytotoxicity (Fig. 3B). Both R(+) LA and R(+) LA-plus showed synergistic protective effects in interaction with selenium. R(+) LA-plus, at a concentration of 10 μM, in combination with 1 μM of selenium prevented 80% of the glutamate induced cytoxicity following 12 hours of glutamate exposure. R(+) LA-plus at a concentration of 20 μM, in combination with 1 μM of selenium, protected HT4 cells against glutamate treatment even up to 24 h (Fig. 3B). The combination of R(+) LA and selenium was less effective in protecting the HT4 cells against glutamate cytotoxicity compared to R(+) LA-plus and selenium (Fig. 3B).

Intracellular levels of peroxides and scavenging effects by R(+) LA or R(+) LA-plus

Using the probe DCFH-DA, accumulation of intracellular peroxides in glutamate treated cells was observed to reach a peak level 6 h after glutamate treatment (Fig. 4A). After 6 h of glutamate treatment, intracellular peroxide levels were significantly higher than those in corresponding controls. Following 8 h of glutamate exposure DCF fluorescence, representing the intracellular peroxides levels, declined from the 6 h peak most likely because of leakage of the fluorescent dye from cells. Peroxide levels at this time point were still significantly higher compared to the control levels (Fig. 4A).

R(+) LA-plus was significantly more effective compared to R(+) LA in scavenging intracellular peroxides that had accumulated in response to 6h of glutamate treatment (Fig. 4B).

Effect of R(+) LA or R(+) LA-plus on glutamate induced glutathione loss

A significant loss in cellular GSH was observed in HT4 cells treated with glutamate for 6 h (Fig. 5). R(+)

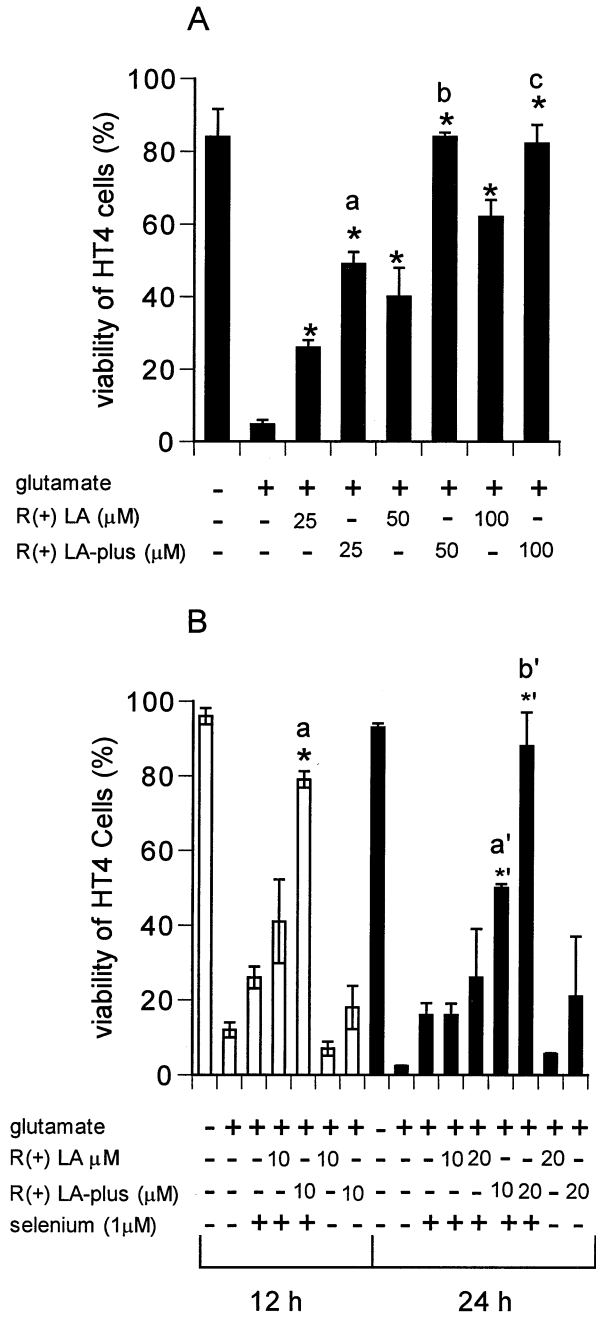

Fig. 3. Protection by R(+) LA or R(+) LA-plus against glutamate induced cytotoxicity. A) Viability of HT4 cells after treatment with glutamate (10 mM) for 12 h in the presence of different concentrations of the thiol antioxidants, R(+) LA and R(+) LA-plus. *$p < .05$, higher compared to glutamate treated control. [a]$p < .05$, higher compared with glutamate + LA 25 mM treated cells, [b]$p < .05$, higher compared with glutamate + LA 50 μM treated cells, [c]$p < .05$, higher compared with glutamate + LA 100 μM treated cells B) Viability of HT4 cells following treatment with glutamate (10 mM) for 12 h (open bars) or 24 h (solid bars). HT4 cells were treated or not with selenium for 24 h before glutamate treatment. *$p < .05$, higher compared with glutamate + selenium treated cells. [a]$p < .05$, higher compared with glutamate + selenium and LA treated cells. *′$p < .05$, higher compared with glutamate + selenium treated cells. [a]′$p < .05$, higher compared with glutamate + selenium and LA 10 μM treated cells. [b]′$p < .05$, higher compared with glutamate + selenium and LA 20 μM treated cells.

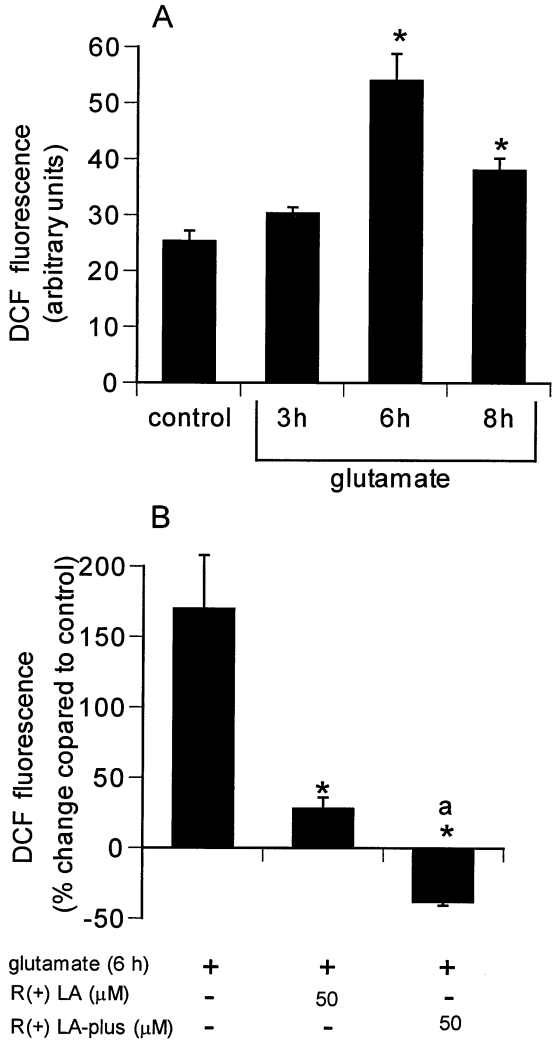

Fig. 4. Detection of Intracellular peroxide levels using the fluorescent probe DCFH-DA. (A) Mean DCF fluorescence in HT4 cells after treatment with glutamate 10 mM for 3, 6, or 8 h, and control (no glutamate treatment). *$p < .05$, higher compared with glutamate non-treated control. (B) Percent change in DCF fluorescence compared with control HT4 cells (no glutamate treatment) following exposure to 10 mM glutamate for 6 h in the presence or absence of R(+) LA or R(+) LA-plus. *$p < .05$, lower compared to glutamate treated control. [a]$p < .05$, lower compared with glutamate +LA-treated cells.

LA-plus at a concentration of 100 μM concentration, more effectively prevented such glutathione loss compared to the effect of 100 μM R(+) LA (Fig. 5). Neither

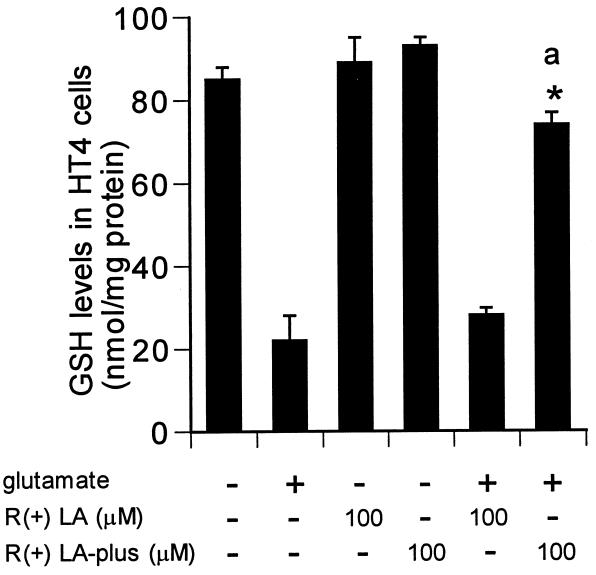

Fig. 5. Glutamate induced glutathione loss in HT4 cells. Cells were treated with glutamate 10 mM for 6 h with or without 100 μM of R(+) LA or R(+) LA-plus. *$p < .05$, higher compared with glutamate treated control. [a]$p < .05$, higher compared with glutamate + LA-treated cells.

LA nor LA-plus (100 μM) increased basal gluthatione levels in cells treated for 6 h (Fig. 5).

Cellular uptake and reduction of R(+) LA and R(+) LA-plus

Treatment of HT4 cells with 100 μM of either R(+) LA or R(+) LA-plus for 1 or 5 h after which HPLC analysis showed higher intracellular amounts of DHLA-plus compared with corresponding amounts of intracellular DHLA (Table 1).

Contribution of the amide bond to enhanced HT4 neuronal protective effects of LA-plus

The neuroprotective activities of R(+),S(−) LA, R(+),S(−) LA-plus and R(+),S(−) lipoamide were compared. Terminal amidation of lipoic acid was found to be a major contributor for the neuroprotective activity (Fig. 6). Amide bearing analogues provided a better

Table 1. Intracellular Levels of LA, DHLA, LA-Plus, and DHLA-Plus in HT4 Cells Treated With 100 μM of Either R(+)LA or R(+) LA-plus for 1 or 5 h

Time (h)	LA	DHLA	Ratio reduced/oxidized	LA-plus	DHLA-plus	Ratio reduced/oxidized
1	1059 ± 150	20 ± 5.9	0.018	859 ± 220	*188 ± 57	0.2
5	4674 ± 1300	44 ± 20	0.009	169 ± 15	*89 ± 19	0.5

Values are in pmol/mg protein.
*$p < .05$ compared with corresponding DHLA values.

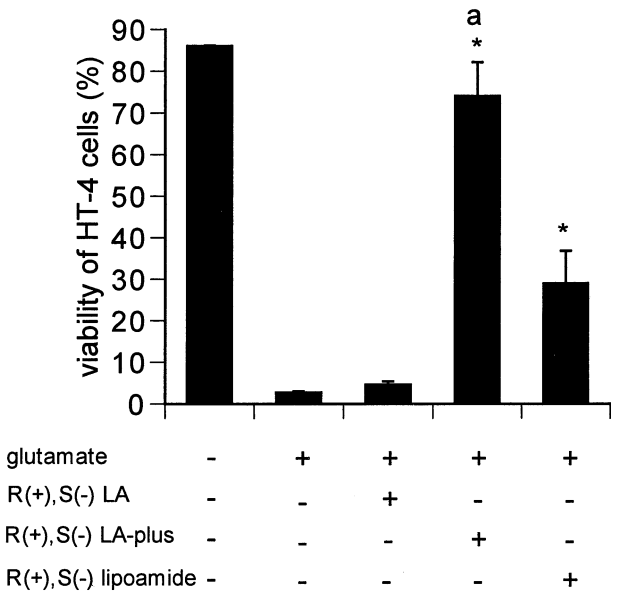

Fig. 6. Protective effects of amide analogues of LA against glutamate induced cytotoxicity. The amide analogues of LA or LA itself were added to the cell culture medium at a concentration of 50 μM. Cells were then treated with glutamate (10 mM) for 15 h, $^{*}p < .05$, higher compared with the glutamate + R(+),S(−) LA treated cells. $^{a}p < .05$, higher compared with the glutamate + R(+),S(−) lipoamide-treated cells.

protection than native lipoic acid. LA-plus was found to be a better neuroprotector than lipoamide (Fig. 6).

LA-plus non stereo-specific HT4 neuronal protective effect

The higher neuroprotective activity of LA-plus was found to be enantiomer independent. All of the stereoisomer analogues of LA-plus were effective in protecting against glutamate toxicity (Fig. 7A).

Lipoamide dehydrogenase specifically reduces lipoyllysine, lipoamide and, with less efficiency, R(+) lipoic acid. Therefore, we investigated whether the enhanced cell protection activity of LA analogues in HT4 cells is due to lipoamide dehydrogenase activity. Cell viability was correlated with the capacity (in vitro) of lipoamide dehydrogenase to utilize the different LA derivatives as substrate. No correlation was found between the protective effect of the various analogues of LA (including of LA itself) and their recognition by lipoamide dehydrogenase as substrates.

The rates of reduction of the various enantiomers of LA-plus and of R(+) LA by lipoamide dehydrogenase were as follows:R(+) LA-plus > R(+),S(−) LA-plus >> R(+) LA ≅ S(−) LA-plus (Fig. 7 B). In addition, the specific inhibitor of lipoamide dehydrogenase 5-methoxyindol-2-carboxilic acid [27] did not impair the cytoprotective effect of R(+) LA-plus (not shown).

DISCUSSION

LA and LA-plus are two dithiolane ring containing antioxidants bearing opposite charges at physiological pH. The difference between LA and LA-plus is that the cellular reduction of LA-plus is better, and its intracellular concentrations are higher compared to DHLA, which is known to be effluxed into the extracellular medium [28]. This property of higher intracellular levels of DHLA-plus compared to DHLA was consistently observed in other cell lines [28].

LA and LA-plus were observed to be efficient protectors against oxidative stress related damage to neuronal cells, inhibiting glutamate induced cytotoxicity. These compounds possess the same dithiolane ring structure, and have similar antioxidant activity. It is thus indicated that differences in cellular reduction of LA-plus and LA contributed to the enhanced protective effect of LA-plus reported here. Data in Table 1 and Fig. 3 show a correlation between the ability of the HT4 cells to reduce these compounds and protection against glutamate induced loss of cell viability. Higher cellular reduction of LA-plus may also explain the enhanced synergistic protection observed with selenium.

Antioxidant synergism

A synergistic interaction to maintain cell viability by selenium and the thiol antioxidants LA and LA-plus was demonstrated. The loss of intracellular GSH in glutamate treated cells is expected to impair hydroperoxide scavenging capacity of seleno enzymes such as glutathione peroxidase (GPx). However, it is known that GPx can also use other electron donors to decompose peroxides [29]. Selenite treatment alone had only a marginal influence to sustain cell viability. However, the protective effect of both LA-plus and LA to inhibit glutamate cytotoxicity was synergistically enhanced by selenite. A possible explanation to such synergism is that GPx and thioredoxin reductase in cells are known to be upregulated by selenium supplementation [30]. Moreover, dithiols such as glutaredoxin and thioredoxin efficiently donate electrons to GPx thus detoxifying hydroperoxides [29]. It is therefore likely that intracellular DHLA and DHLA-plus, which are dithiols, may serve as electron donors to glutathione peroxidase, the activity of which is expected to be upregulated in selenite treated cells [30]. The synergistic protective combination of selenium and LA or LA-plus indicated that production of intracellular peroxides is a key event in glutamate induced HT4 cytotoxicity.

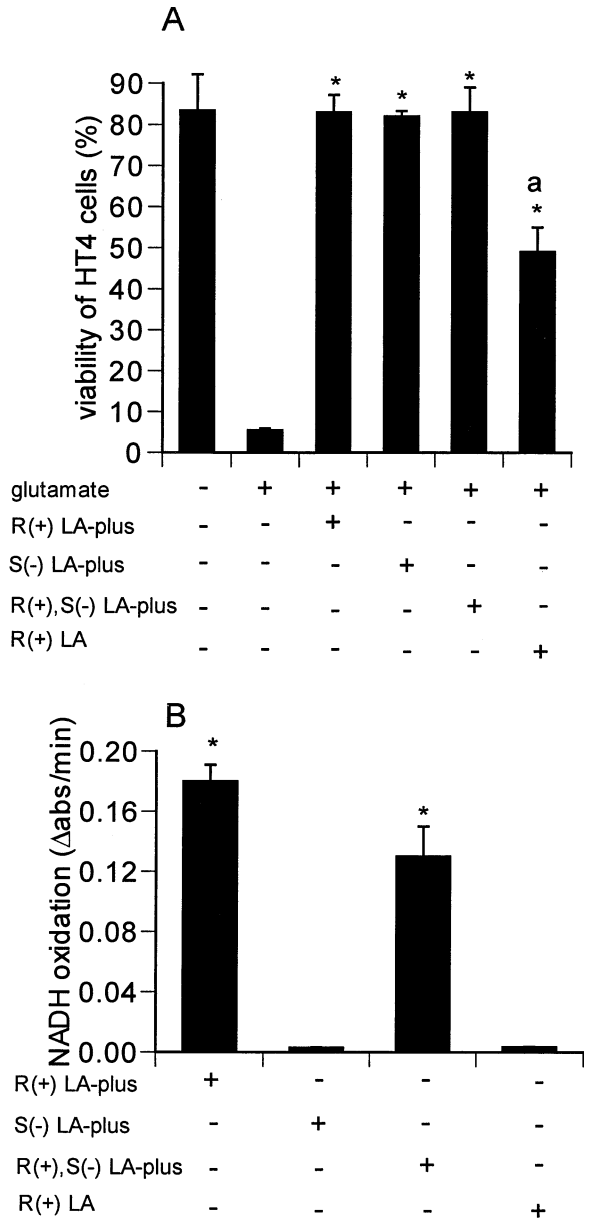

Fig. 7. Cytoprotective effect and lipoamide dehydrogenase enzymatic reduction of stereospecific analogues of LA-plus, (A) protection by different stereoisomers of LA-plus or R(+) LA against glutamate 10 mM–induced cytotoxicity (12-h incubation). α-Lipoic acid analogues were added at a final concentration of 50 μM. $*p < .05$, higher compared with glutamate treated control. $^{a}p < .05$, lowered compared with cells treated with glutamate + LA-plus different stereoisomers. (B) Reduction rates of LA analogues as assayed by monitoring NADH oxidation spectrophotometrically at 340 nm by 0.1 units/ml of lipoamide dehydrogenase. The substrates for the enzymatic reduction were 300 μM of the R(+) stereoisomer of LA or 300 μM of the different stereoisomers of LA-plus. $*p < .05$, higher compared with the reduction rate of R(+) LA

Glutamate induced elevation of intracellular peroxides

In a previous report, a marked increase in intracellular peroxides in glutamate treated C6 glial cells was demonstrated [17]. Such a phenomenon was also observed in HT4 neuronal cells. In this study, the DCF data demonstrated that an increase in intracellular peroxides occurred following glutamate treatment. However, a significant DCF signal was detected in the control HT4 cells indicating steady state production of peroxides, probably as a result of mitochondrial respiration. The intensity of this signal was augmented up to 2 fold following 6 h of glutamate treatment. LA-plus was more effective than LA in protecting the cells against peroxide accumulation, and was even capable of lowering the observed DCF fluorescence signal in the cell cytosol below that of control. This observation is correlated with the capacity of these two compounds to prevent glutamate induced loss of cell viability.

Glutamate induced GSH loss and its prevention by LA-plus

Glutamate challenge induced a marked loss in cellular GSH. However, in the presence of LA-plus, the GSH level in glutamate treated HT4 cells was better maintained compared to that in cells treated with LA. The low peroxide level following exposure to glutamate in LA-plus treated cells might indicate that the remarkable sparing effect on GSH is due to scavenging of intracellular oxidants. Additionally, efflux of intracellular GSH to the cell culture medium is a typical early event in cells undergoing apoptosis [31,32], and is expected to be inhibited by LA-plus more than by LA due to improvement in HT4 cell viability.

Chemical structure and antioxidant activity

Addition of a terminal amide to LA improved efficacy of these compounds toward neuroprotection. Such effect may be attributed to a better reduction of the amide analogues than the native LA. The S(−) stereoisomer analogue of LA-plus was a strong neuroprotector, although it was not a good substrate for enzymatic reduction by lipoamide dehydrogenase. This suggests non-stereospecific reduction that may be catalyzed also by other cellular reductases. Lipoamide was found to be superior to LA in protecting the cells against glutamate challenge and LA-plus was more efficient than lipoamide against such challenge.

The increased efficiency of LA-plus, compared to lipoamide, is possibly due to higher water solubility of LA-plus. Lipoamide is not a salt and therefore has higher affinity to partition to lipophilic compartments. Compartmentalization of LA-plus in the water soluble fraction of the cell should make it accessible to (1) enzymatic reduction, (2) scavenge peroxides in the cytosol, and (3)

interact with hydroperoxide decomposing enzymes such as GPx. Consistently, lipophilic analogues of LA such as GLA-LA and cholesteryl-lipoate were ineffective as neuroprotectors in the tested model. The lipophilic esters of lipoate, racemic γ-linoleic acid α-lipoic acid conjugate, and R(+) cholesteryl-lipoate at concentrations of 25 μM and 50 μM did not provide any significant cell protection (not shown). Hence, chemically stable water soluble amide analogue of LA with the ability to (1) cross biological barriers such as membranes and (2) be reduced and retained inside cells might have better neuroprotective effects compared to native LA.

In conclusion, amide analogues of LA (lipoamide and LA-plus) were more potent than LA in protecting neuronal cells against glutamate treatment. In addition, it was noted that amide analogues of LA function as antioxidants regardless of their stereo-chemistry.

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ABBREVIATIONS

ROS—reactive oxygen species
GSH—reduced glutathione
LA—α-lipoic acid
LA-PLUS—2-(N,N-dimethylamine) ethylamido lipoate·HCl
GLA-LA—-linoleic acid-lipoic acid conjugate
GPx—glutathione peroxidase
DCFH-DA—dichlorodihydrofluorescin diacetate
DCF—dichlorofluorescein
PBS—phosphate buffered saline
LDH—lactate dehydrogenase
BSA—bovine serum albumin
HPLC—High performance liquid chromatography
DHLA—dihydrolipoic acid