REVIEW ON THE SYNTHESIS OF ALPHA-LIPOIC ACID AND ITS THERAPEUTIC POTENTIAL IN EXPERIMENTAL MODEL OF DEMENTIA

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Received: 09 January 2024
Accepted: 06 March 2024
DOI: 10.59957/jctm.v59.i3.2024.8

ABSTRACT

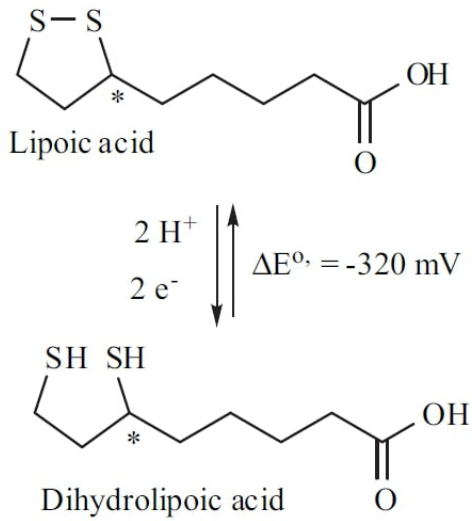
LA or (±)-α-Lipoic acid (6,8-thioctic acid, 5-(1,2-dithiolan-3-yl)pentanoic acid) (LA) is a heterocyclic thia fatty acid consisting of pentanoic acid and a 1,2-dithiolan-3-yl group at the 5-position. LA is an amphiphilic pharmacophore with excellent antioxidant, anti-inflammatory and neuroprotective effects attested by numerous studies for its potential utility in both the treatment and diverse forms of prophylaxis of neurodegenerative disorders, especially Alzheimer's disease. This article aims to assess the differences in neuroprotective effect after short-term (11 days) and long-term (51 days) LA administration in an experimental rat model of scopolamine-induced dementia by correlating biochemical data on acetylcholinesterase activity with behavioural data. Behaviourally appraised changes in learning and memory appear to correlate with biochemical changes in AChE activity. The neuroprotective effect of LA, as ascertained by its beneficial effects on learning and memory in a scopolamine animal model, appears to be associated with cholinergic mechanisms (a decrease in AChE activity), and to be larger after short-term administration rather than long-term administration. These results further underscore the candidacy of LA as a viable drug candidate suitable for continued investigation and derivate synthesis endeavours.

Keywords: thioctic acid, neurodegeneration, memory, scopolamine, acetylcholinesterase, passive avoidance task.

INTRODUCTION

LA or (±)-α-Lipoic acid (6,8-thioctic acid, 5-(1,2-dithiolan-3-yl)pentanoic acid) is a heterocyclic thia fatty acid consisting of pentanoic acid and a 1,2-dithiolan-3-yl group at the 5-position [1]: a disulfide five-membered ring, a C2-C5 linkage, and carboxylic acid. It forms a redox couple together with its active metabolite: its reduced form dihydrolipoic acid (DHLA) (Fig. 1) [2]. LA has one chiral center, located at carbon six and exists as two different enantiomers: R-(+)-LA and S-(−)-LA (Fig. 1). R-(+)-LA is found in nature and S-(−)-LA is synthetic. LA can be found both as a drug and a food supplement, most often as a racemic mixture because S-(−)-LA does not appear to show significant biological side effects [3 - 5].

LA's considerable medicinal significance (outlined below) and market size (USD 106.89 million in 2022, expected to expand to USD 134.15 million by 2028) have garnered substantial interest from synthetic chemists in both academic and industrial realms. Tens of synthetic approaches have been reported in a rough



Properties	LA	DHLA
pKa (COOH)	4.76-5.3	4.85
pKa (SH)	--	10.7
λmax (nM)	333	--

Fig. 1. Chemical structures and properties of LA and DHLA. The chiral center is denoted by an asterisk [5].

span of 70 years (1952 - present) [6 - 8]. Those relating to the synthesis of racemic LA can be summarized as: functional modification, fragment assembly, and ring opening. Fragment assembly and ring opening are newer approaches that converge towards the older functional modification approach; therein, LA is synthesized from precursors featuring an intrinsic C2-C5 linkage and two terminal functional groups. Other approaches are stereoselective with the specific goal of introducing chirality into the molecule and synthesizing the biologically active enantiomer of natural origin: R-(+)-LA. They can be classified as chiral resolution (chemical and enzymatic), chiral pool, chiral auxiliary, and asymmetric catalysis (chemical and enzymatic) (Fig. 2) [8].

The industrial-scale syntheses of LA and R-(+)-LA are summarized below.

LA is synthesized in a three-stage process. In the first stage, monomethyl or monoethyl adipate is converted into the corresponding acid chloride by reacting with thionyl chloride. This is followed by a treatment with ethylene and anhydrous aluminium chloride, yielding 8-chloro-6-ketooctanoate. In the second stage, the resultant ester is reduced to 8-chloro-6-hydroxyoctanoate by thionyl chloride in pyridine. In the third stage, sodium disulfide is used to replace the chlorine atoms with sulfur atoms. The resulting dithionooctyl ester is hydrolysed with alcoholic potassium hydroxide, yielding LA [9].

R-(+)-LA synthesis involves the direct chemical resolution of racemic LA, utilizing R-(+)-methylbenzylamine (RAMBA) as the chiral resolution reagent. RAMBA is added to a racemic LA solution to form S-(−)-LA and a key intermediate. The latter is separated by crystallization and converted into R-(+)-LA via acid hydrolysis [8].

LA is a molecule that has been attested by numerous studies for its potential utility in both the treatment and diverse forms of prophylaxis of neurodegenerative disorders, especially Alzheimer's disease (AD). It is an amphiphilic pharmacophore with excellent antioxidant, anti-inflammatory and neuroprotective effects able to cross the blood-brain barrier and distribute in various

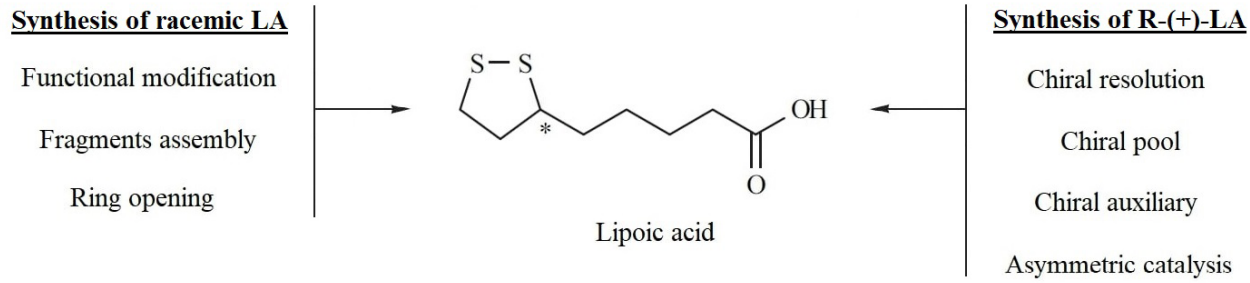


Fig. 2. Synthetic approaches to the synthesis of racemic LA and R-(+)-LA. The chiral center is denoted by an asterisk (adapted from Wang et al. [8]).

locations in the brain [10 - 19].

In our previous studies we noticed differences in the effects of short-term and long-term LA administration in rat dementia model [20, 21]. This could be due to changes in oxidative stress levels over time, in accordance with our previous data [13], but also to other mechanisms affecting, e.g. brain acetylcholinesterase (AChE) activity, which are not as well documented.

This article aims to comparatively assess the differences in neuroprotective effect after short-term and long-term LA administration in an experimental rat model of scopolamine-induced dementia by correlating biochemical data on AChE activity with behavioural data.

EXPERIMENTAL

Experimental Animals

All experimental procedures were conducted on adult male Wistar rats weighing 160 - 180 g from Bulgarian Academy of Sciences' Laboratory Animal Resource Center in Sofia, Bulgaria. The animals were group-housed, accommodating five per cage, in a laboratory environment. They were allowed to adapt to its 12-hour light/dark cycle, temperature, humidity, and ventilation. Continuous access to food and water was provided *ad libitum*.

Experimental procedures were conducted in accordance with national and international regulations: *Directive 2010/63/EU of the European Parliament and of the Council* of 22 September 2010, *Guide for the Care and Use of Laboratory Animals* (NIH Publication Number 85 - 23), *Ordinance No. 20 of the Ministry of Food and Agriculture* of 1 November 2012.

Experimental Design

LA-induced changes in AChE activity and behavior were assessed in an experimental rat model of scopolamine-induced dementia, generated by injecting 2 mg kg^{-1} of scopolamine hydrobromide (0.5 mL/100 g, *i.p.*) every day for 11 consecutive days, in concordance with our previous studies [20, 22] and literature data [23 - 25].

The rats were segregated into the following types of experimental groups, each comprising ten to twelve rats:

- 'Control' group (CTRL) receiving saline (0.5 mL/100 g, *i.p.*) every day for 11 consecutive days.
- 'Scopolamine' group (SCO) receiving scopolamine hydrobromide (2 mg kg^{-1}, *i.p.*) every day for 11 consecutive days.
- 'Lipoic acid' group (SCO+LA) receiving scopolamine hydrobromide (2 mg kg^{-1}, *i.p.*) every day for 11 consecutive days, together with LA. LA administration was either short-term or long-term. Short-term, LA (30 mg kg^{-1}, *i.p*) was administered for 11 days, concomitantly with the scopolamine injections. Long-term, LA (100 mg kg^{-1}, *p.o.*) was administered for 40 days before the scopolamine injections and after that for 11 days more - concomitantly.

Behavioural phenotyping was done for 12 days - in conjunction with the 11-day injection period - using a Passive Avoidance task to evaluate changes in learning and memory. On the day following the final day of testing, the rats were euthanized by CO_2 inhalation. Their brains were then quickly removed, and two brain structures related to memory - the hippocampus and prefrontal cortex - were dissected on ice. They were subsequently analyzed biochemically to determine AChE activity.

Behavioral Test

The Passive Avoidance task (Step-through Passive Avoidance Test) is a fear-aggravated test used to evaluate the effect of novel chemical entities on learning and memory in rodent models of central nervous system disorders. In this neuropharmacological task, subjects learn to avoid an environment where an aversive stimulus (e.g. a foot shock) was previously delivered [26, 27].

Its two-chambered testing apparatus divides into a lit chamber and a dark chamber, with a gate in between. The floor of the dark chamber has a steel grid through which a mild electric foot shock can be given. If done so, the rodents will learn to associate certain properties of the dark chamber with the foot shock. Rodents with normal learning and memory will then avoid entering the dark chamber. This is measured by recording the latency to cross through the gate between the chambers.

The experimental procedure was two-phased: training and testing [28]. The training phase was on the day preceding the scopolamine injection period. Each rat was placed in the lit chamber, and after a one-minute habituation period, the gate was opened. That allowed the rat to enter the dark chamber whereafter it received a one-time electric foot shock (0.7 mA, 3 s). The time it took the rat to enter the dark chamber with all four paws was written down as initial latency (IL). The testing phase was during the scopolamine injection period.

One hour, twenty-four hours, and twelve days, respectively, after being injected with scopolamine, each rat was placed again in the lit chamber and allowed to enter the dark chamber, but without receiving a foot shock. The time it took the rat to enter the dark chamber with all four paws was written down as step-through latency (STL). The cut-off time of IL and STL was 180 s, and they were used to evaluate changes in the short-term and long-term memory of the rats in the different experimental groups.

Biochemical Analysis

The Ellman method was used for biochemical analysis of AChE levels. It is a spectrophotometry-based assay in which thiocholine, produced by AChE, reacts with 5,5'-Dithiobis (2-nitrobenzoic acid) (Ellman's reagent) to form a yellow-coloured product, proportional to the AChE activity present [29].

AChE activity of two brain structures related to memory - hippocampus and prefrontal cortex - was determined. Both structures were frozen at −25°C, cut into smaller fragments and weighed. The fragments were mixed with PBS (0.01 mol L^{-1}, pH=7.4) and homogenized (tissue weight (g): PBS (mL), volume = 1:9) with a Potter-Elvehjem Glass Homogeniser with a Teflon pestle. The homogenate was then centrifugated at 5000 × g for 5 min, and the resultant supernatant was used to determine AChE activity. All procedures were kept under temperature control, between 0°C and 4°C. 100 μL of supernatant were incubated with the Ellman reagent: 2.9 mL of 0.1 M phosphate buffer (pH = 8), 100 μL of 0.1 M Ellman reagent, and 20 μL of 0.075 M freshly prepared acetylthiocholine iodide. 500 μL of the reaction mixture were then analyzed with a semi-automatic biochemistry analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzen, China) and reaction kinetics were monitored for 3 min at 405 nm. Probe protein concentration was measured according to Lowry et al. [30].

Statistical Analysis

Statistical analysis was conducted using a one-way analysis of variance (ANOVA) and Dunnett's as the post hoc comparison test, two-way ANOVA and Tukey's multiple comparisons test. Student's t test was used for statistical analysis of unpaired data. The results are expressed as mean ± SEM. Statistically significant differences were marked with asterisks and hashtags above bars: *P < 0.05, **P < 0.01, ***P < 0.001; $^{\#}$P < 0.05, $^{\#\#}$P < 0.01, $^{\#\#\#}$P < 0.001. Statistical analysis was made using the GraphPad Prism program.

RESULTS AND DISCUSSION

Scopolamine is a muscarinic receptor antagonist, which, when given *i.p.* to rats, is able to induce neurodegenerative brain changes similar to ones found in other animal models of dementia (mice, monkeys, etc.) and in patients with AD. It does so by increasing oxidative stress levels, by causing neuroinflammation, apoptosis and mitochondrial damage as well as through a variety of other mechanisms. It can also adversely affect the cholinergic system by increasing the activity of AChE, thus decreasing the levels of the neuromediator acetylcholine involved in memory and other cognitive processes [19 - 21].

LA and DHLA are versatile molecules, soluble in both polar and nonpolar environments. Their hydrophilic properties are due to their carboxylic acid groups and their hydrophobic properties to their hydrocarbon tails [10, 31, 32]. This amphiphilicity allows them to cross the blood-brain barrier and distribute in various locations in the brain. LA and DHLA are pharmacophores with excellent antioxidant, anti-inflammatory and neuroprotective effects. Both LA and DHLA can scavenge reactive oxygen and nitrogen species, and chelate redox-active metal ions [2]. Metals such as manganese, zinc, cadmium, lead, cobalt, nickel, iron and copper are known to form complexes with LA and DHLA [33]. LA can increase levels of the antioxidant glutathione and change brain antioxidant enzyme activity, as shown in one of our previous studies and in literature [6, 13, 16]. LA can also reduce the levels of pro-inflammatory enzymes like iNOS, COX-2 and caspase-3 and inhibit TNF-α-induced NF-κB activation [2, 34, 35]. DHLA has more antioxidant properties than LA and is capable of reducing the oxidized forms of several important antioxidants: coenzyme Q10, vitamin C, vitamin E and glutathione (a key regulator of intrinsic redox homeostasis) [36 - 38]. It can also alleviate inflammation via the LAMP-1/CaMKII/TAK1 pathway [39].

The aforementioned presents research rationale for conducting the experiments and the present comparison. To our knowledge, this is the first time that LA-induced changes in AChE activity in prefrontal cortex and hippocampus (brain structures related to learning and memory) have been compared with behaviourally-appraised changes in memory in short-term and long-term experimental designs in an experimental rat model of scopolamine-induced dementia.

Results from the statistical analysis of behavioural and biochemical data are presented in Fig. 3 and Fig. 4 and discussed below.

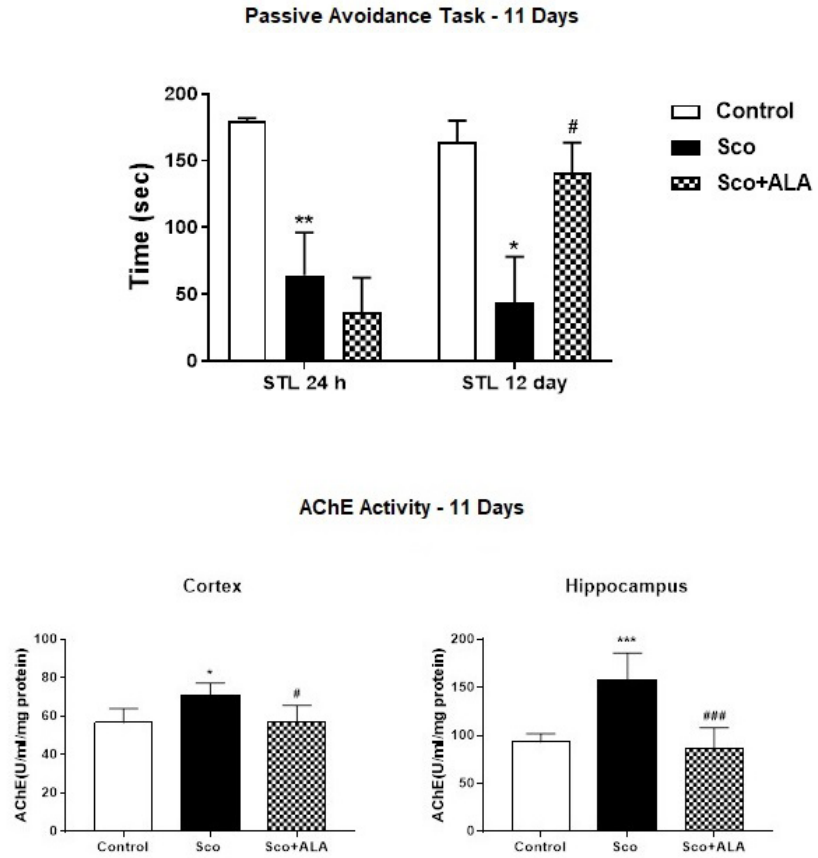


Fig. 3. Statistical analysis of behavioural and biochemical data at 11 days.

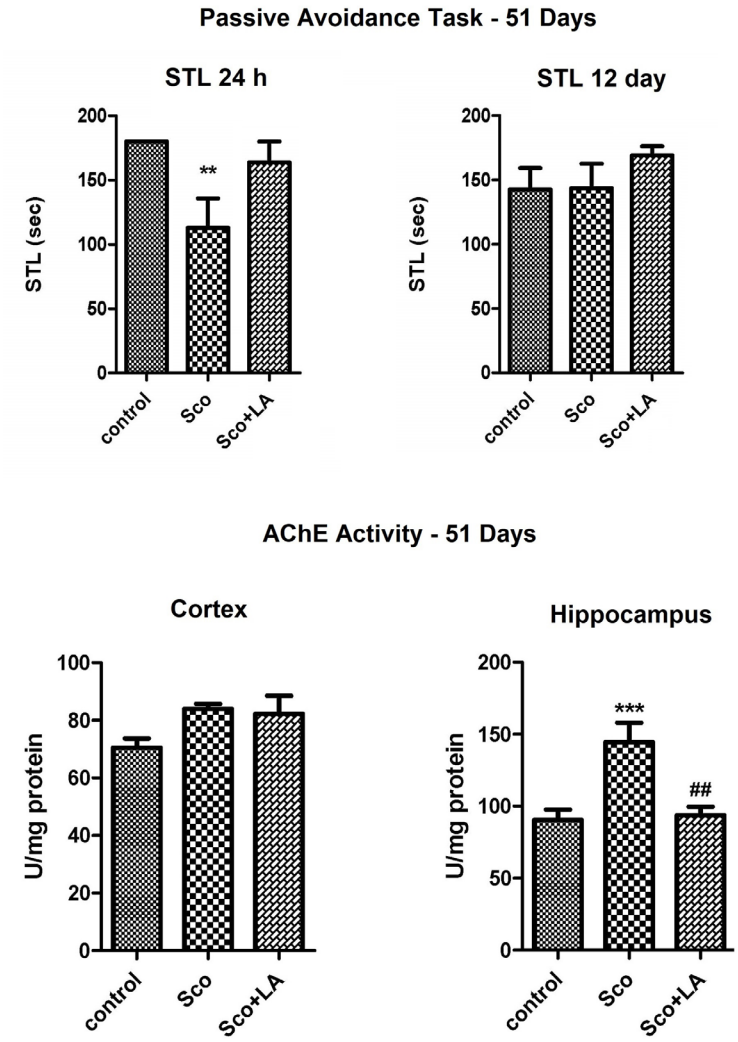


Fig. 4. Statistical analysis of behavioural and biochemical data at 51 days.

Scopolamine administration induces a significant memory impairment as measured in the SCO groups. The decrease in short-term memory (STL 24 h) was larger at 11 days (64 %) than at 51 days (37 %). Long-term memory (STL 12 day) was significantly decreased at 11 days by 71 % and nonsignificantly at 51 days. These results are accompanied by an increase in AChE activity, measured on the 12th day: 25 % (cortex) and 40 % (hippocampus) at 11 days, and 19 % (cortex) and 60 % (hippocampus) at 51 days. Thus, the Sco-induced increase in AChE activity was more pronounced in the hippocampus than in the prefrontal cortex in both experiments but similar between experiments.

LA administration induces a significant memory improvement as measured in the SCO + LA groups. The increase in long-term memory (STL 12 day) was larger at 11 days (69 %) than at 51 days (18 %). These results are accompanied by a decrease in AChE activity, measured on the 12th day: 20 % (cortex) and 44 % (hippocampus) at 11 days, and 2 % (cortex) and 35 % (hippocampus) at 51 days. Thus, the LA-induced decrease in AChE activity was more pronounced in the hippocampus than in the prefrontal cortex in both experiments, and in the cortex at 11 days.

By decreasing the activity of AChE, LA increases the levels of the neuromediator acetylcholine (ACh), which plays a pivotal role in learning and memory. LA can increase ACh levels also by increasing glucose uptake-

Table 1. Changes in short-term and long-term memory (STL in Passive Avoidance task) after 11 days or 51 days of LA treatment.

Treatment Duration	SCO *vs.* CTRL		SCO + LA *vs.* SCO
	STL 24 hr	STL 12 day	STL 12 day
11 Days	64 % ↓	73 % ↓	69 % ↑
51 Days	37 % ↓	1 % ↑	18 % ↑

Table 2. Changes in brain AChE activity after 11 days or 51 days of LA treatment.

Treatment Duration	SCO *vs.* CTRL		SCO + LA *vs.* SCO	
	AChE Activity (Cortex)	AChE Activity (Hippocampus)	AChE Activity (Cortex)	AChE Activity (Hippocampus)
11 Days	25 % ↑	40 % ↑	20 % ↓	44 % ↓
51 Days	19 % ↑	60 % ↑	2 % ↓	35 % ↓

thus supplying more acety l- CoA for its production - and activating choline acetyltransferase, which catalyzes ACh resynthesis. Our recently published data shows that LA can also upregulate BDNF and pCREB levels [20, 21].

We found that the behaviourally appraised changes in learning and memory correlated well with the biochemical changes in AChE activity. The neuroprotective effect of LA, as ascertained by its beneficial effects on learning and memory in a scopolamine animal model, appears to be associated with cholinergic mechanisms (a decrease in AChE activity), and to be larger after short-term administration (11 days) rather than long-term administration (51 days). A detailed comparison is presented in Table 1 and Table 2.

CONCLUSIONS

The beneficial effects of LA on learning and memory, when assessed from a behavioural standpoint, correlate to a large extent with changes in AChE activity at the biochemical level. The observed neuroprotective effects of LA appear to be linked to its cholinergic mechanisms and appear more pronounced following short-term administration as opposed to long-term administration. These results further underscore the candidacy of LA as a viable drug candidate suitable for continued investigation and derivate synthesis endeavors.

Acknowledgements

This research is supported by the Bulgarian Ministry of Education and Science under the National Program "Young Scientists and Postdoctoral Students - 2".

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