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Review

Lipoic acid – biological activity and therapeutic potential

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Abstract:
α-Lipoic acid (LA; 5-(1,2-dithiolan-3-yl)pentanoic acid) was originally isolated from bovine liver by Reed et al. in 1951. LA was once considered a vitamin. Subsequently, it was found that LA is not a vitamin and is synthesized by plants and animals. LA is covalently bound to the ε-amino group of lysine residues and functions as a cofactor for mitochondrial enzymes by catalyzing the oxidative decarboxylation of pyruvate, α-ketoglutarate and branched-chain α-keto acids. LA and its reduced form – dihydrolipoic acid (DHLA), meet all the criteria for an ideal antioxidant because they can easily quench radicals, can chelate metals, have an amphiphlic character and they do not exhibit any serious side effects. They interact with other antioxidants and can regenerate them. For this reason, LA is called an antioxidant of antioxidants. LA has an influence on the second messenger nuclear factor κB (NF-κB) and attenuates the release of free radicals and cytotoxic cytokines. The therapeutic action of LA is based on its antioxidant properties. Current studies support its use in the ancillary treatment of many diseases, such as diabetes, cardiovascular, neurodegenerative, autoimmune diseases, cancer and AIDS. This review was undertaken to gather the most recent information regarding the therapeutic properties of LA and its possible utility in disease treatment.

Key words:
lipoic acid, antioxidant activity, therapeutic application

Abbreviations: DHLA – dihydrolipoic acid, GSH – reduced glutathione, GSH/GSSG – redox potential of reduced glutathione/glutathione disulfide, GSSG – glutathione disulfide, LA – α-lipoic acid, NADPH – nicotinamide adenine dinucleotide phosphate-oxidase, NF-κB – nuclear factor κB, ROS – reactive oxygen species, TNF-α – tumor necrosis factor α

Introduction

α-Lipoic acid (LA) (IUPAC name: 5-(1,2-dithiolan-3-yl)pentanoic acid) was first isolated from bovine liver in 1951 [62]. Other names for LA include thioctic

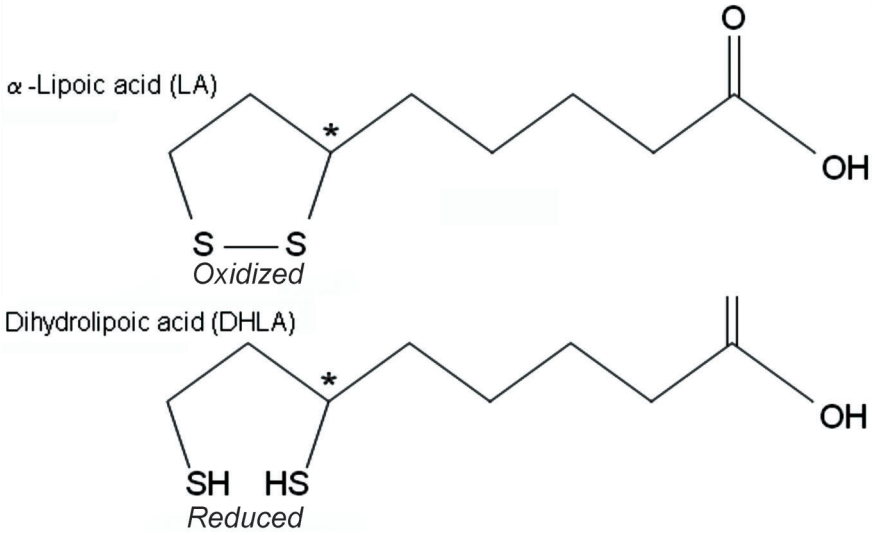

Fig. 1. The oxidized and reduced forms of lipoic acid [68]

acid, 6,8-thioctic acid, 6,8-dithioctane acid and 1,2-dithiol-3-valeric acid. LA is an eight-carbon disulfide that contains a single chiral center (Fig. 1) and an asymmetric carbon, thus resulting in two possible optical isomers: R-LA and S-LA. The R-isomer is synthesized endogenously and binds to proteins. For therapeutic purposes, the R form is usually administered as a racemic mixture of R-LA and S-LA [68]. The absolute bioavailabilities (oral *vs.* intravenous in humans) of 200 mg of LA in aqueous solution have been estimated to be 38% for the R form and 28% for the S form [35]. However, no difference between the plasma concentrations of R- and S-LA was observed following intravenous administration.

LA is a small molecule that contains two oxidized or reduced thiol groups. Its oxidized form is usually defined as α-lipoic acid or just lipoic acid, and the reduced form of LA is known as dihydrolipoic acid (DHLA). DHLA is the predominant form that interacts with reactive oxygen species (ROS), but the oxidized form of LA can also inactivate free radicals [59]. LA is found naturally in mitochondria where it is bound to the subunit E2 and where it acts as the coenzyme for pyruvate dehydrogenase and α-ketoglutarate dehydrogenase [60]. It is highly reactive because of the tension of the S-S-C bond in the heterocyclic disulfide circle. LA is relatively stable as a solid, but it polymerizes when heated above its melting point (47.5°C) or under the influence of light when it is dissolved in a neutral solution.

Humans can synthesize LA *de novo* from fatty acids and cysteine, but only in very small amounts. Therefore, LA needs to be absorbed from exogenous sources [18]. LA and DHLA are amphipathic molecules that possess both hydrophilic and hydrophobic properties [58]. The biological effects of LA are primarily associated with its antioxidant properties. LA also exhibits antimutagenic and anticarcinogenic activities [22, 51, 55]. Bilska et al. [10] suggest that the biological action of LA may contribute to its influences on sulfane sulfur metabolism and the activity of the mitochondrial enzyme rodanase.

Bioavailability and safety of oral supplementation of LA

Food sources

Dietary LA is obtained from both animal and plant sources. LA is found primarily in animal-derived foods, such as red meat and liver, heart and kidney. The most abundant plant sources of LA are spinach, broccoli, tomatoes, brussel sprouts, potatoes, garden peas and rice bran [40, 46]. It has also been suggested that food intake reduces the bioavailability of LA. Therefore, it is recommended that LA be taken 30 min before or 2 h after eating [29].

Absorption and LA concentration in plasma

LA administered as single-dose tablets (from 50 to 600 mg) was entirely absorbed after 30 min to 1 h [14]. LA absorption when prepared as an aqueous solution rather than in a gelatin has been shown to be more effective. The half-life of LA in plasma is 30 min, and the endogenous plasma levels of LA and DHLA are 1–25 × 10^9 and 30–140 × 10^9 g/ml, respectively [79].

Safety and toxicity

LA exhibits low toxicity in low doses. Treatment of male or female Wistar rats with LA administered at the doses of 31.6 or 61.9 mg/kg/d for four weeks did not cause any adverse effects [22]. In addition, the long-term (two-year) administration of up to 60 mg/kg/day did not cause any adverse effects [23]. Higher doses of LA (121 mg/kg) caused significant changes in liver enzyme activities. After four weeks of administration, the concentrations of alanine aminotransferase (ALAT) and glutamate dehydrogenase (GLDH) were increased, and some histopathological changes in the liver and mammary glands were observed [22].

Following the oral administration of LA to rats, the measured LD_{50} value was 2,000 mg/kg of body weight, indicating that this antioxidant has very low acute toxicity. Doses of 2,000 mg/kg produced sedation, apathy, piloerection, hunched posture and/or eye closure in some rats [23].

In clinical trials in which LA supplementation up to 2,400 mg/day was used, no adverse effects compared with the placebo were observed [28, 66]. Similarly, LA administered intravenously at the doses of 600 mg/day for three weeks did not cause serious side effects [13]. Oral administration of 1,800 mg LA for 6 months also did not bring about significant adverse effects as compared with the placebo [88]. However, the intraperitoneal administration of racemic LA at a high chronic dose (100 mg/kg/day for 2 weeks) in aged rats caused an increase in plasma lipid hydroperoxide level and oxidative protein damage in the heart and brain [16, 41]. One possible explanation for these effects is that DHLA, a derivate of LA, is capable of removing the Fe^{2+} from ferritin and reducing ferric to ferrous increasing the possibility of oxidative damage [16].

Lipoic acid availability and supplements

LA in humans is synthesized in the liver and other tissues. This antioxidant is readily absorbed from the diet and is converted to DHLA by reduced nicotinamide adenine dinucleotide or by reduced nicotinamide adenine. The mitochondrial reduced form of nicotinamide adenine dinucleotide-dependent dihydrolipoamide dehydrogenase demonstrates a marked preference for R-LA, whereas the cytosolic reduced form of nicotinamide adenine dinucleotide-dependent glutathione reductase shows greater activity toward the (S)-(+)-LA stereoisomer. The activity of this reductase is particularly important in the heart, kidney and liver. The amount of LA available in dietary supplements (200–600 mg) is likely to be up to 1,000 times greater than the amount that could be obtained from diet alone [68].

In other studies, it was also reported that the dietary supplementation of LA induced a decrease in oxidative stress while restoring reduced levels of other antioxidants. Supplementation of 600 mg/d "per os" LA for 2 months decreased urinary F2-isoprostane concentration (a biomarker of lipid peroxidation) and increased the lag time of LDL oxidation in healthy men [49].

Metabolism of LA

LA is primarily metabolized in the liver through mitochondrial β-oxidation. Following the oral administration of a single dose of 1 g of R-LA to a male volunteer, 3-ketolipoic acid and the dimethylated products 2,4-bismethylmercapto-butanoic acid and 4,6-bismethylmercapto-hexanoic acid were detected in his plasma [9, 60].

The liver is the main detoxifying organ for many toxic substances and drugs that contribute to oxidative stress. The increased ROS production makes mitochondrial membranes highly susceptible to oxidative damage [17, 25]. It is now widely accepted that ROS play a critical role in the development of endothelial injury and hepatic fibrosis [38, 76]. LA can scavenge a number of free radicals, and it can therefore be used in the prevention or treatment of several pathological conditions that are mediated by oxidative stress. LA has been shown to have a protective effect against the hepatotoxic effects of antitubercular drugs that produce many metabolic and morphological aberrations in liver [64]. This antioxidant may be effective in preventing the development of hepatic steatosis and he-

patic fibrosis [53, 61]. Tabassum et al. [77] showed that LA reduced mitochondrial oxidative stress in liver tissue to which methotrexate (a folic acid antagonist used as a cytotoxic chemotherapeutic agent) had been administered. The mice that were exposed to methotrexate followed by LA showed a decrease in lipid peroxidation, and the detoxification of free radicals by antioxidants was enhanced.

Antioxidant action of LA and DHLA

LA as a biological antioxidant

According to Packer et al. [60], a therapeutic antioxidant should be absorbed from the diet and easily converted by cells and tissues into a usable form. It should also possess a variety of antioxidant characteristics, including interactions with other antioxidants, in both the membrane and aqueous phase and low toxicity. LA fulfills all of these requirements, making it a potentially highly effective therapeutic antioxidant. This characteristic makes DHLA one of the most potent naturally occurring antioxidants. LA as an antioxidant is able to directly scavenge ROS, regenerate endogenous antioxidants, such as glutathione, and vitamins E and C, and possess metal chelating activity [6, 9, 11].

LA and DHLA create a potent redox couple that has a standard reduction potential of 0.32 V, whereas the redox potential of reduced glutathione/glutathione disulfide (GSH/GSSG) is 0.24 V. Therefore, LA/DHLA is often called the "universal antioxidant", and it seems that LA/DHLA redox might regenerate several other antioxidants, such as vitamin C and vitamin E [28]. DHLA is not destroyed while quenching free radicals, but it can be recycled from LA [65]. It has been shown that DHLA is an essential cofactor for mitochondrial bioenergetic enzymes, including pyruvate dehydrogenase and α-ketoglutarate dehydrogenase. LA is primarily catabolized through mitochondrial β-oxidation.

Moreover, the beneficial action of LA may result in its ability to reduce nicotinamide adenine dinucleotide phosphate (NADPH) oxidase/endothelial cell-mediated ROS generation, restore GSH/GSSG contents and enhance the mitochondrial expression of key antioxidant enzymes, including glutathione reductase [12]. Furthermore, the beneficial action of LA was implicated during lipopolysaccharide (LPS)-induced oxidative stress [30, 31, 69].

Scavenging actions of LA and DHLA

LA is a unique endogenous and exogenous antioxidant because it is a direct ROS quencher in its oxidized and reduced form. Lipoic acid is both water and fat soluble and therefore elicits its antioxidant action in the cytosol as well as in the plasma membrane, serum and lipoproteins (the water and lipid components of blood) in contrast to vitamin C, which is hydrophilic, and vitamin E, which is hydrophobic. LA may scavenge hydroxyl radicals, hypochlorous acid and oxygen singlets [75, 84]. DHLA scavenges superoxide radicals and peroxyl radicals, thereby preventing the free radical-mediated peroxidation of proteins [60]. LA and DHLA are not active against hydrogen peroxide. DHLA has the salubrious property of neutralizing free radicals without becoming involved in the process.

Regeneration of other antioxidants

An antioxidant is a compound that scavenges free radicals. It becomes oxidized and is not able to scavenge additional ROS until it has been reduced. DHLA is a potent reducing agent that has more antioxidant activity than LA and that has the capacity to reduce the oxidized forms of several important antioxidants, including vitamin C and glutathione [73]. DHLA can regenerate vitamin C and vitamin E from their oxidized forms [6, 9, 45]. DHLA has been shown to be more potent in vitamin C regeneration than the regeneration of reduced glutathione; however, glutathione has twice the chemical reactivity in its thiol group [73]. DHLA may reduce the α-tocopheryl radical (the oxidized form of tocopherol) directly or indirectly by reducing the oxidized form of vitamin C (dehydroascorbate), which is able to reduce this radical. Moreover, DHLA can also reduce the oxidized forms of coenzyme Q10, which may additionally reduce the α-tocopheryl radical [6]. Coenzyme Q10 is an important component of the mitochondrial electron transport chain that has antioxidant activity as well. LA directly recycles and prolongs the metabolic lifespan of vitamin C, glutathione and coenzyme Q10, and it indirectly renews vitamin E.

LA as a metal chelator

LA and DHLA, as direct reactive oxygen species scavengers, may chelate redox-active metals, such as free

iron, copper, manganese and zinc both *in vitro* and *in vivo*. These metals can induce oxidative damage by catalyzing reactions that generate highly reactive free radicals [80]. Compounds that chelate (bind) free metal ions in a way that prevents them from generating free radicals might be a step toward the treatment of chronic diseases in which metal-induced oxidative damage may play a role [26]. LA preferentially chelates Cu^{2+}, Zn^{2+} and Pb^{2+} but cannot chelate Fe^{3+}, while DHLA forms complexes with Cu^{2+}, Zn^{2+}, Pb^{2+}, Hg^{2+} and Fe^{3+} [56]. Suh et al. showed that DHLA, but not LA, in *in vitro s*tudies strongly inhibited Cu(II)-mediated ascorbate oxidation in a concentration-dependent manner [66, 72]. In addition, other authors have emphasized that only DHLA prevented the Cu(II)-mediated oxidation of low density lipoproteins (LDL) *in vitro* [48]. Bush et al. [15] found that the DHLA-mediated chelation of iron and copper in the brain had a positive effect on the pathobiology of Alzheimer's disease by lowering free radical damage. Preliminary studies have shown that DHLA is a potent chelator for transition metals in a redox-inactive manner and therefore mitigates metal-catalyzed free radical reactions in conditions in which they accumulate [66].

Role of LA on nuclear factor κB (NF-κB) pathway

The changes in intracellular thiol redox status and the protein structures of signaling molecules may result in the alteration of transcription factor activities. LA may oxidize sulfhydryl groups or form mixed disulfides on proteins, thus influencing the changes in the thiol redox status of signaling molecules. NF-κB is a redox-sensitive transcription factor. This factor plays a role in the expressions of a variety of genes that are involved in inflammatory responses and in apoptosis in multiple tissues and cell types [5]. LA causes a down-regulation of NF-κB in the monocytes of diabetes patients [36]. NF-κB was a potent inhibitor of NF-κB activation in human T cells and osteoclast precursor cells and was useful in inhibiting NF-κB translocation into the nucleus in renal tubular epithelial cells and thoracic artery cells [42, 44, 74, 85]. Consistent with these effects on NF-κB, acute treatment with LA inhibited the adhesion of leukocytes in the cremasteric circulation when acutely administered to mice 24 h prior to an inflammatory challenge with tumor necrosis factor (TNF-α) [85]. LA inhibited the TNF-α-induced degradation of inhibitor of κB (IκB) and the activation of NF-κB expression in ovarian epithelial cells [74, 82, 86]. The suppression of the NF-κB pathway by LA under conditions of elevated TNF-α and glucose levels can suppress the migration of smooth muscle cells (SMC) and inhibits the expression of matrix metalloproteinase-9 (MMP-9) in SMCs from the thoracic aorta [43]. LA has also been shown to suppress the NF-κB-dependent up-regulation of intracellular adhesion molecules (ICAM), TNF-α and monocyte chemoattractant protein (MCP-1) *in vitro* and *in vivo* [37]. Other authors were unable to prove that LA pretreatment inhibited the increase in the nuclear translocation of NF-κB caused by endotoxin [24]. Recently, it was shown that LA also attenuated the cisplatin-induced increases in the phosphorylation and nuclear translocation of the NF-κB p65 subunit in kidney tissue [1].

Therapeutic usage of lipoic acid

LA in aging

Mitochondria provide energy for basic metabolic processes, and their decay with age impairs cellular metabolism and leads to cellular decline. Mitochondrial mass is reduced with age, which leads to a defective energy homeostasis. Oxidative mitochondrial decay and the production of damaging free radicals is a major contributor to aging [87]. Aged rats given LA supplements showed a decrease in lipid peroxidation and an increase in the activities of mitochondrial enzymes, such as isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, NADPH dehydrogenase and cytochrome C oxidase. Moreover, no significant changes in mitochondrial enzyme activity were found in young rats treated with LA [3]. The authors conclude that LA reverses the age-associated decline in mitochondrial enzymes and, therefore, may lower the increased risk of oxidative damage that occurs during the aging process [3, 4].

It was also shown that a decline in the mitochondrial oxidative capacity of skeletal muscle may contribute to the whole-body aging process [67]. Animal studies have found that LA protects the heart mitochondria against aging effects [39]. LA corrected the age-related increase in oxidative stress and the age-

related decline in mitochondrial enzyme activity of the citric acid cycle and respiratory chain in the liver and kidney of aging rats [3, 50]. Some of this decay can be reversed in old rats by feeding them normal mitochondrial metabolites, such as acetylcarnitine, and high doses of LA. The study showed that in aged rodents and dogs, reducing oxidative damage by providing an antioxidant either in supplements or in fruit and vegetables can improve learning, memory and motor functions [8, 63]. This decline has been connected with the observation that dogs develop cognitive impairments in measures of learning and memory as they age [19, 71, 78]. Similarly, other authors showed that treatment with LA improved cognitive function through increased mitochondrial function in dogs [33]. This work suggested a link between oxidative damage to mitochondrial dysfunction and cognitive decline [87]. Furthermore, LA was reported to be effective in protecting the brain from oxidative damage and memory loss [70]. Recently, it has been shown that the anti-inflammatory and antiapoptotic actions of LA in aged diabetic rats occur through a PI3K/Akt signaling pathway [12].

Lipoic acid, diabetes and neuropathy

Diabetes is a major factor for the development of atherosclerosis and hypertension, which may lead to the heart failure, myocardial infarction, neuropathic pain and stroke [7]. Hyperglycemia leads to oxidative stress, which is connected with the overproduction of free oxygen radicals in mitochondria that in turn activates intracellular pathways (e.g., protein kinase C and hexosamine pathways) and causes neuronal and endothelial damage [20].

Excessive oxidative stress in endothelial cells has been shown to ameliorate proinflammatory cytokine levels (e.g., TNF-α, IL-1β) and to mediate the degradation of insulin receptors *via* MAPK cascades [2].

Both, LA and DHLA take part in insulin production. It has been shown that LA ameliorates glucose uptake in both insulin-resistant and insulin-sensitive muscle tissues [27, 28]. LA also decreased the diabetes-associated up-regulation of $p22_{phox}$ and $p47_{phox}$ expression of NADPH oxidase [28]. Because LA enhances glucose utilization and improves glycemic control, it plays a key role in the detoxification mechanisms that take place in the liver.

Recently, Bitar and coworkers [12] showed that 50 mg/kg LA administered to rats for 30 days, prevented diabetes-mediated mitochondrial and endothelial dysfunction *via* a PI3K/Akt-dependent signaling pathway. Moreover, LA was shown to reduce NADPH oxidase/ETC-mediated ROS generation and increase GSH/GSSG redox status in aortic tissue.

Recently, it was shown that intravenous LA treatment improves endothelium-dependent vasodilatation of the vasculature in patients with type-2 diabetes [34].

Although LA seems to be effective in improving glucose utilization, it was shown to be ineffective in decreasing neuropathic pain in diabetic patients [52]. However, in animal models of diabetes, LA supplementation was reported to improve neural blood flow and nerve conduction [21].

Lipoic acid and cancer

LA has been shown to protect mitochondria against respiration-linked oxidative stress and to augment the functional life span and preserve the genomic and structural integrity of these organelles [32]. Moreover, LA acts as an important coenzyme for enzymes inside the mitochondria, including dehydrogenase and α-ketoglutarate dehydrogenase [54]. LA has also been reported to suppress the inflammatory response by inhibiting molecular signaling pathways activated by proinflammatory cytokines, such as TNF-α. TNF-α is a key activator of the NF-κB pathway, which mediates inflammatory responses and regulates the expression of several inflammatory mediators, including chemokines, cytokines and cytokine receptors [9, 28, 82]. NF-κB also activates genes encoding enzymes involved in inflammatory process, such as cyclooxygenase 2 (COX2) and inducible nitric oxide synthase (iNOS). Vig-Varga et al. [82] demonstrated that the TNF-α-induced NF-κB activity was inhibited following LA administration in both mouse and human tumorigenic cells. It was also shown that LA causes a significant depletion in TNF-α-induced caspase activation [55] and increases the activation of caspase-3-like activity connected to DNA fragmentation in HT-29 human colon cancer cells [83], Jurkat and FaDu cell lines, and Ki-v-Ras-transformed Balb/c 3T3 murine mesenchymal cells [24, 81]. The anticancer effects of α-lipoic acid were also seen in B16F10 murine melanoma cells and ovarian epithelial cancer cells [58, 82]. On the other hand, the apoptotic effect of LA was not seen in non-transformed human cells [57, 83].

LA exhibits antimutagenic and anticlastogenic activities and thus belongs to the group of natural antimutagenes [55]. It has recently been shown that the application of LA to a human breast cancer cell line inhibits cancer metastasis, and this inhibition is likely due to a decrease in the activity and mRNA expression levels of MMP-2 and MMP-9 caused by LA [47].

Conclusion

LA is a natural compound, chemically named 5-(1,2-dithiolan-3-yl)pentanoic acid, that is also known as thioctic acid. In humans, LA is synthesized in the liver and other tissues with high metabolic activity, such as the heart and kidney. It is also synthesized in small amounts by plants.

LA is both water and fat soluble and therefore cross biological membranes easily, thus reaching all the compartments of the cell. Recently, a great deal of attention has been paid to the antioxidant function of LA and its reduced form DHLA because they effectively protect cells against ROS-induced damage.

LA is an ideal antioxidant that can scavenge a number of free radicals, such as hydroxyl radicals, hypochlorous acid and oxygen singlets. It may also exert antioxidant effects in biological systems by transitional metal chelation. The LA/DHLA complex has the capacity to recycle endogenous antioxidants, such as vitamins E and C. A number of experimental as well as clinical studies emphasize the usefulness of LA as a therapeutic agent for diverse conditions, including diabetes, atherosclerosis, insulin resistance, neuropathy, neurodegenerative diseases and ischemia-reperfusion injury. Moreover, LA represents a potential therapeutic agent for the vascular endothelium. The LA/DHLA redox couple is recognized as one of the most powerful biological antioxidant systems. Although LA is currently used in clinical practice, long-term and thorough clinical studies are needed to confirm the function of LA as a natural therapeutic remedy for prophylaxis of the liver and kidney, as well as dysfunctions and diseases of other organs.

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