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Mini-review

The disulfide compound α-lipoic acid and its derivatives: A novel class of anticancer agents targeting mitochondria

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ABSTRACT

The endogenous disulfide α-lipoic acid (LA) is an essential mitochondrial co-factor. In addition, LA and its reduced counterpart dihydro lipoic acid form a potent redox couple with anti-oxidative functions, for which it is used as dietary supplement and therapeutic. Recently, it has gained attention due to its cytotoxic effects in cancer cells, which is the key aspect of this review. We initially recapitulate the dietary occurrence, gastrointestinal absorption and pharmacokinetics of LA, illustrating its diverse anti-oxidative mechanisms. We then focus on its mode of action in cancer cells, in which it triggers primarily the mitochondrial pathway of apoptosis, whereas non-transformed primary cells are hardly affected. Furthermore, LA impairs oncogenic signaling and displays anti-metastatic potential. Novel LA derivatives such as CPI-613, which target mitochondrial energy metabolism, are described and recent pre-clinical studies are presented, which demonstrate that LA and its derivatives exert antitumor activity *in vivo*. Finally, we highlight clinical studies currently performed with the LA analog CPI-613. In summary, LA and its derivatives are promising candidates to complement the arsenal of established anticancer drugs due to their mitochondria-targeted mode of action and non-genotoxic properties.

Introduction

α-Lipoic acid (LA) is an endogenous disulfide compound synthesized *de novo* in mitochondria, where it is covalently bound to multi-enzyme complexes such as pyruvate dehydrogenase, representing a pivotal co-factor for decarboxylation of α-keto acids. Besides its important role in mitochondrial energy metabolism, it is also known for its powerful anti-oxidative effects and has been used for the treatment of chronic diseases associated with high levels of oxidative stress, such as diabetic polyneuropathy and Alzheimer's disease. A growing number of studies have demonstrated that LA and derivatives thereof are capable of suppressing growth of various cancer cell lines, while non-transformed primary cells were hardly affected. The mode of action of LA and its derivatives in cancer cells are described in detail and promising results from recent preclinical studies are presented.

De novo synthesis and dietary occurrence of LA

LA is a disulfide-containing substance termed 1,2-dithiolane-3-pentanoic, which was firstly isolated over 60 years ago [1]. It bears a chiral carbon atom (Fig. 1A) and therefore occurs as R- and S-enantiomers. However, only the R-enantiomer is covalently linked to conserved lysine residues in mitochondrial enzymes such as pyruvate dehydrogenase and displays biological activity. LA is synthesized *de novo* in mitochondria by the action of lipoyl synthase [2,3]. This enzyme contains an auxiliary $[4Fe\text{-}4S]^{2+}$ cluster and catalyzes the incorporation of two sulfur atoms at positions 6 and 8 into protein-bound octanoic acid, which requires S-adenosylmethionine (SAM) as co-substrate [4,5]. The reaction is proposed to occur in a sequential manner via formation of a C6-octanoyl radical, which reacts with the auxiliary $[4Fe\text{-}4S]^{2+}$ cluster. Another sulfur atom is then introduced at the C8-position and the remaining FeS cluster is released, giving rise to a lipoylated protein, *e.g.* E2 subunit of pyruvate dehydrogenase complex (Fig. 1B) [6].

LA is found in various dietary sources at the low μg/g range. Animal tissues with the highest lipoate levels include kidney, heart and liver [7], while it also occurs in vegetables at comparable amounts, as shown for spinach (4 μg/g) [8]. The highest lipoyllysine content was detected in spinach, followed by broccoli and tomato [9]. The dietary uptake from these natural sources is yet considered to be insufficient, not providing substantial amounts of LA in the bloodstream [10]. Commercially available dietary supplements typically contain between 50 and 600 mg of racemic LA, which are therefore the main sources of free LA.

Gastrointestinal absorption, cellular uptake and metabolism of LA

Oral supplementation with LA results in its gastrointestinal absorption and is enhanced if LA is administered as sodium salt [11,12].

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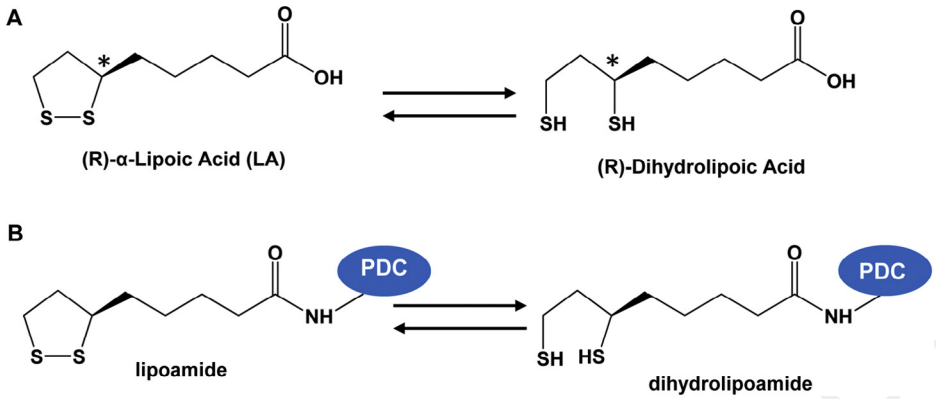

Fig. 1. Chemical structure of free and bound LA. (A) α-lipoic acid (LA) and its reduced counterpart dihydro lipoic acid, which form a potent redox couple. The asterisk indicates the chiral carbon atom. (B) LA is covalently bound to pyruvate dehydrogenase complex as lipoamide and converted into dihydrolipoamide during decarboxylation of pyruvate to acetyl-CoA.

Upon oral administration of 200 mg LA, a mean bioavailability of 30% was observed [11]. Furthermore, LA is usually given as racemic mixture (R,S-LA), which results in approximately 50% higher plasma concentrations of R-LA as compared to S-LA [13]. The cellular uptake of LA was shown to be mediated by two carrier proteins, the monocarboxylate transporter (MCT) [14] and Na^+-dependent multivitamin transporter (SMVT) [15]. Using a Caco-2 cell transwell model, the uptake of LA was found to occur in a pH-dependent manner, which was inhibited in the presence of monocarboxylic acids such as benzoic acid and octanoic acid, suggesting MCT-dependent internalization [14]. LA was further identified as a substrate of SMVT together with biotin and pantothenate, inhibiting the internalization of both vitamins at low μM concentrations [16]. A recent study revealed R-LA as physiological substrate of human SMVT. Binding of R-LA to SMVT occurs only in the presence of Na^+, with two R-LA molecules binding simultaneously to the transporter [15]. Upon internalization, LA is converted into its reduced form DHLA, which was confirmed in different cell models including Jurkat T-cells, fibroblasts, erythrocytes and endothelial cells [17–20]. The conversion is an NADPH-dependent process, which is most likely catalyzed by glutathione reductase, thioredoxin reductase and dihydrolipoamide dehydrogenase [17–19].

LA is rapidly absorbed and has a mean plasma elimination half-life of 0.5 hours [21]. Studies in rodents have shown that over 50% of LA is excreted within the first 24 h following oral administration and underwent massive biotransformation mainly via mitochondrial ß-oxidation, resulting in the generation of more than 10 different metabolites [22]. In humans, LA is primarily metabolized by S-methylation and ß-oxidation [21]. The most frequent metabolites were identified as bisnorlipoate, tetranorlipoate and their bis-methylated mercapto derivatives. However, in contrast to rodents, only 13% of the administered dose was recovered in the urine after 24 h, which points to the involvement of another excretion route or additional degradation pathways in humans [21].

Both oral administration and intravenous (i.v.) injection of LA were reported in humans (for review see [10]). 600 mg LA given orally resulted in a mean maximal plasma concentration (C_{max}) of 80 μM, while i.v. injection of 300 mg racemic LA yielded a C_{max} of 110 μM [12]. Importantly, no significant adverse effects were observed in clinical trials, when LA was administered at doses up to 1200 mg i.v. and up to 2400 mg orally per day [23–25]. Building on these studies, an estimated C_{max} value of up to 400 μM may be reached, specifically in a setting of i.v. administration.

Antioxidant properties of LA and DHLA

Owing to the chemical reactivity of its dithiolane ring, LA and its reduced counterpart DHLA form a potent redox couple and display an impressive array of antioxidant functions (Fig. 2) (for review see [26]). Both compounds were reported to directly scavenge reactive oxygen species (ROS), including hypochlorous acid and hydroxyl radicals, whereas superoxide radicals are eliminated only by DHLA [27,28]. Furthermore, LA and DHLA form complexes with the transition metal ions Mn^{2+}, Cu^{2+} and Zn^{2+} [29] and block the Cu^{2+}-mediated oxidation of ascorbic acid [30]. DHLA was also described to remove ferric (III) iron from ferritin followed by its chelation [31]. In line with this finding, an animal study with aged rats reported that dietary supplementation with LA reduces the cortical iron content [32]. The redox couple LA/DHLA is further capable of regenerating cellular antioxidants. It was shown that DHLA, but not LA, reduced ascorbyl radicals to ascorbate, which subsequently recycled chromanoxyl radicals to vitamin E [33]. LA was demonstrated to increase the level of reduced glutathione (GSH) in lymphocytes, erythrocytes and neuronal cells by reduction of cystine to cysteine, which is then available for GSH synthesis [34]. This finding was confirmed in aged rats, in which LA treatment caused an increase in cysteine tissue content and improved cerebral GSH levels [35]. Further work illustrated that LA stimulates the *de novo* synthesis of GSH by inducing the expression of γ-glutamylcysteine ligase, which catalyzed the rate-limiting step in GSH synthesis [36]. This

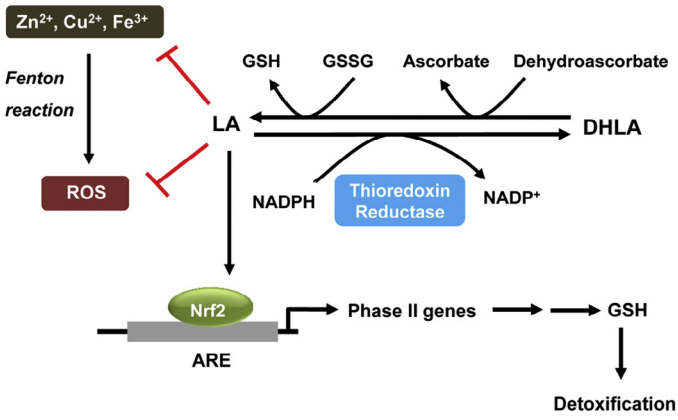

Fig. 2. Antioxidative functions of LA. Both LA and its reduced form DHLA exert antioxidative activity by chelating redox-active metals (*e.g.* Cu^{2+}), directly scavenging of reactive oxygen species (ROS) and by regeneration of cellular antioxidants (Vitamin C, GSH). LA is converted into DHLA in an NADPH-dependent manner by thioredoxin reductase and also glutathione reductase. Moreover, LA is able to induce the *de novo* synthesis of GSH by activating the transcription factor Nrf2, which binds to antioxidant-response elements (ARE) in the promoter region of phase II detoxification genes, including γ-glutamylcysteine ligase, and drives their expression.

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