α-Lipoic acid – the potential for use in cancer therapy
Minireview

L. NOVOTNY[1*] , P. RAUKO[2], C. COJOCEL[3]

[1] Faculty of Pharmacy, Kuwait University, Kuwait; e-mail: novotny@hsc.edu.kw, [2] Cancer Research Institute, Slovak Academy of Sciences, Bratislava, Slovak Republic; [3] Faculty of Medicine, Kuwait University, Kuwait

Received June 18, 2007

This review deals with alpha-lipoic acid (LA) from the point of its chemical and biological characteristics affecting enzymatic activities that are part of cellular biochemical processes in normal and cancer cells. This includes attributes of LA that are related to its ability to act as a free-radicals scavenger and also as a radical generator. LA is discussed in the light of its physico-chemical features, toxicity, biochemical bases of LA biological activities, and mechanisms of action. Additionally, it is discussed how these properties of LA are reflected by results of *in vivo* experiments with cancer cells and in experimental cancer chemotherapy. Finally, the results of LA use in human cancer chemotherapy and as chemopreventive agent are discussed in the light of LA future inclusion into chemotherapeutic protocols.

Key words: Lipoic acid, cancer therapy, mechanism of action, biological activity, cancer prevention

Lipoic acid (LA) is a small molecule with only two kinds of functional groups. It is a naturally-occurring co-factor present in many multi-enzyme complexes regulating metabolism. It is present in human body in two forms (Fig. 1): lipoic acid, which is an oxidized form, or as the reduced form dihydrolipoic acid. Lipoic acid or more specifically α-lipoic acid (alpha-lipoic acid) is chemically 5-(1,2-dithiolan-3-yl)pentanoic acid (Fig. 1A, $C_8H_{14}S_2O_2$). The other name of LA is thioctic acid and some other name were in use, i.e. 6,8-thioctic acid, 6,8-dithioctane acid, 1,2-tithiol-3-valeric acid or 1,2-ditiol-3-pentanoic acid [1]. The formula of its reduced form, dihydrolipoic acid or 6,8-disulfanyloctanoic acid. (Fig. 1B) is $C_8H_{16}S_2O_2$. LA exists in the form of two enantiomers, R or S. In physiological condition, LA is present in the form of lipoate with the proton of the hydroxyl functional group substituted by remains of an organic alcohol or with an inorganic ion. LA (in the form of lipoate) acts as a cofactor in reactions of aerobic metabolism. It participates in transfers of acyl and methylamine groups. It is essential for aerobic processes of life and serves as a coenzyme in the Krebs cycle [2].

LA was discovered as a growth factor in some microorganisms [3]. Presence of LA was then confirmed in mammals where it participates in many reduction-oxidation reactions catalyzed by cellular dehydrogenases: pyruvate dehydrogenase complex (PDG) and α-ketoglutarate dehydrogenase complex (KGDG) [4]. Both enzyme complexes are necessary for proper functioning of the citrate cycle. The presence of LA as a cofactor was also shown in H-protein glycine-utilizing system [5,6]. It was shown that LA helps in protecting the body against free radicals [7,8].

Physico-chemical properties and toxicity. Alpha-LA is a sulfurous fatty acid. It would be recognized as a vitamin. However, the human body is able to synthesize it. As a potent antioxidant, LA quenches free radicals, inhibits reactive oxygen-generators and regenerates other antioxidants [9]. Elucidation of the specific cellular target(s) for LA is in need of more research for better identification. However, it is well established that on cellular level, R-LA is the most efficient oxidative-stress protector [10] and is stimulating increase in mitochondrial metabolism.

In the organism, LA is bonded to various proteins and, consequently, there is not possible to extract it by water or non-polar solvents. However, LA is soluble in both water and lipids [11]. LA is highly reactive due to 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

* Corresponding author

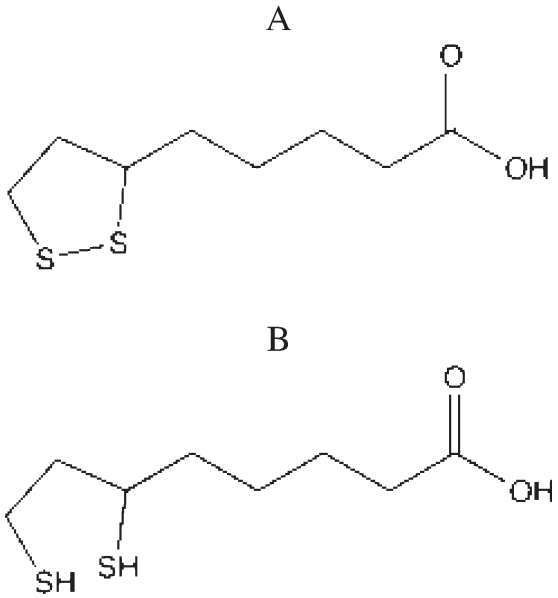

Fig. 1. Chemical structure of lipoic (A) and dihydrolipoic (B) acids.

a light, when it is dissolved in a neutral solution. Photolysis in an acidic environment does not result in polymerization but in an opening of the disulfide ring [3]. To possess a free-radical scavenging activity, LA has to be a redox-active molecule. Consequently, its ΔE = -0.288 makes it possible to undergo thiol-disulfide exchange [9] and participate in maintaining optimal cellular redox status [12]. As an antioxidant, LA is able of bringing other antioxidants to their active antioxidant state. These effects were reported for an interaction with ascorbic acid, vitamin E, coenzyme Q10 and glutathione [13-16].

Toxicity of LA is low but very large doses are toxic. When applied orally to rats, the LD_{50} value established for LA was >2000 mg/kg of body weight and LA had no acute toxicity to the experimental animals [17]. The high doses were associated with small changes of liver enzymes activities. Additionally, some histopathological effects were observed on the liver and mammary glands. LA does not possess any mutagenic or genotoxic activities [17]. On the other hand, LA exhibits antimutagenic and anticlastogenic activities and as such belongs to the group of natural antimutagens [18]. The no-observed-adverse-effect level (NOAEL) dose reported is 61.9 mg/kg of body weight per day [17].

Biochemical bases of LA biological activities and mechanisms of action. The interaction of LA with various biological molecules and macromolecules and with various cellular targets was investigated on different levels. It demonstrated that LA or lipoate participates in many biochemical reactions affecting cellular processes.

LA as an essential cofactor of various enzymes is requiring formation of covalent bonding to the enzyme for demonstration of biological activity. The main lipoylated proteins are enzymes of central metabolism. These are the pyruvate and alpha-ketoglutarate dehydrogenase complexes. Normally, the lipoate ligase activates the LA carboxyl group using ATP and this activation is followed by attachment of LA. However, there are some exceptions to this [19]. In Escherichia coli lipoylated proteins are formed in the absence of ATP-dependent ligase activity [19].

The cleavage of LA from proteins and small molecules is accomplished through the action of lipoamidase [20]. Lipoamidase belongs among amidohydrolases and it removes not only LA but also biotin from 2-oxoacid dehydrogenases [21].

LA's R-isomer lowers glucose and lactase levels in diabetic subjects. This is probably due to LA ability to inhibit mammalian pyruvate dehydrogenase kinase. Out of 4 pyruvate dehydrogenase kinase (PDK and two pyruvate dehydrogenase phosphatase (PDP) isoenzymes, LA affects only PDKs but not PDPs. It was shown in purified proteins system that LA directly regulates activity of the pyruvate dehydrogenase complex (PDC) through phosphorylation/dephosphorylation of its pyruvate dehydrogenase (E1) component. The inhibition of PDKs by LA leads to decreased phosphorylation and, consequently, toward increased activity of PDC [22].

LA takes part in complex enzymatic reactions/systems, i.e. the 2-oxo acid dehydrogenase multienzyme complexes. In some cases, the LA catalytic site is very similar to the site for biotin [23]. It was shown experimentally that cellular dihydro-LA catalyzes the denitrosation of S-nitrosoglutathione, S-nitrosocaspase 3, S-nitrosoalbumin, and S-nitrosometallothionenin to their reduced state with concomitant generation of nitroxyl (HNO) [24]. In this sense, this activity is the same as of other cellular dithiol thioredoxin. The denitrosation of S-nitrosocaspase 3 is in an agreement with findings in HT-29 human colon cancer cells that exposure to LA dose dependently increases caspase-3-like activity associated with DNA-fragmentation [25]. These effects were accompanied by cytosolic oxygen-radical scavenging and by increase oxygen-radical generation inside mitochondria resulting in the down-regulation of the anti-apoptotic protein bcl-X(L). However, when LA was examined in non-transformed human colonocytes, it did not induce any apoptotic processes. The induction of apoptosis in colon cancer cells relates to an increased uptake of oxidizable substances/substrates into mitochondria. Additionally, when cellular antioxidant status of cultured HL-60 cell was improved through pre-incubation with LA [26], the protection against caspase-3 activation and apoptosis induced by an addition of 200 μM hydrogen peroxide was observed.

LA in in vitro experiments. *In vitro* experiments involving LA are usually reporting on the free-radical scavenging effects or on the processes that are mediated by free radicals or on the processes related to glutathione synthesis. Additionally, some authors speculate that cytotoxic effects of LA occur because of the similarity of the LA structure to the structure of fatty acids, i.e. octanoic acid [27]. This makes it possible for LA to correct

the deficient thiol status of cells, which can be used to the advantage of patients in clinical situations and therefore highlights clinical relevance of LA applications. When tested *in vitro* in human Jurkat T cells, human erythrocytes, C6 glial cells, NB41A3 neuroblastoma cells and peripheral blood lymphocytes [28], LA mediated an increase in reduced cellular glutathione. This was caused through reduction of LA to its dihydro form that, after being released into the medium, reduced cystine to cysteine. Cysteine was than transported back into cells and used in the synthesis of glutathione. LA is essential in helping cystine to overcome the problem with its low transport as xc-transport system. It is expressed weakly in some cells and it is also inhibited by glutamate. Consequently, LA makes it possible for gamma-glutamylcysteine synthetase to work as needed. By this mechanism LA normalizes cell subpopulations with compromised thiol status.

It was reported that, by an unknown mechanism, combination of vitamin D3 with LA be useful in overcoming the differentiation block present in acute promyelocytic leukemia cells [29]. Additionally, some other vitamin D-related substances, i.e. calcitrol, are capable of increasing the activity of tumor necrosis factor alpha (TNFalpha) [30,31]. However, LA was demonstrated to cause significant reduction of this enhancing effect of calcitrol on TNF-alpha-induced caspase activation [30,31].

It was shown that the human tumor cell lines FaDu and Jurkat and in the Ki-v-Ras-transformed Balb/c-3T3 murine mesenchymal cell line that LA induces hyperacetylation of histones in cultured cells. The presence of LA in medium has different effect on the growth and viability of normal and transformed cells [32]. Non-transformed cell lines treated with LA responded to the treatment only through reversible arrest of cell cycle in G0/G1. LA induces a post-translational increase of the cyclin-dependent kinase inhibitor p27Kip1 levels. This inhibitor is necessary for the LA-mediated arrest of cell cycle. On the other hand, the pro-apoptotic effect of LA in transformed cells seems to beneficial only for the LA-use in cancer chemotherapy.

Cultivation of HT-29 cells with either form of LA (oxidized or reduced) leads to the dose dependent increase of caspase-3-like activity. The LA effects were associated with DNA fragmentation [25] and dihydro-LA was acting as a scavenger of cytosolic oxygen radicals. Interestingly, both forms of LA were reported to increase formation of oxygen radicals in mitochondria. However, this is preceded by an increased influx of lactate or pyruvate into mitochondria and, consequently, the anti-apoptotic protein bcl-X(L) is down-regulated. Apoptosis that is induced by LA (or its dihydro form) can be prevented by the free radical scavenger benzoquinone. In the contrast, this apoptotic effect of LA was not seen in normal human colonocytes [25]. This seems to be an additional indication of the benefits of LA inclusion in cancer therapy, especially because some other reports [33] indicate that LA also mediates upregulation of phase II detoxication enzymes, i.e. NAD(P)H:quinone oxidoreductase (NQO1) and glutathione-S-transferase (GST). A preferential cytotoxicity of LA toward the leukemic cell lines compared to mitogen-stimulated normal peripheral blood lymphocytes was also reported [34]. This is in the line of the work including normal human colonocytes [25]. LA was found to induce apoptosis in leukemia cells but not in normal cells. This was documented as substantial but opposite changes of interleukin-2 (IL-2) concentrations in normal and leukemia cells [34]. Favorable effects of LA on some important T-cell functions (*in vitro*) were also reported in patients in advanced-stage of the disease [35]. LA induced generation of reactive oxygen species (ROS) is accompanied by increase of apoptotic cells in human lung epithelial cancer H460 [36]. On the other hand, inhibition of ROS formation or overexpression of glutathione peroxidase and superoxide dismutase that in fact functions as antioxidant enzyme inhibits this LA-induced apoptosis. LA-induced apoptosis is the result of the activation of the mitochondrial death pathways. This requires caspase-9 activation. Consequently, this apoptosis is fully inhibited by caspase inhibitors [36]. Additionally, LA induces down-regulation of mitochondrial Bcl-2 protein while its overexpression prevents the apoptotic effect of LA [36].

LA in experimental cancer therapy and cancer chemotherapy in human. In experimental cancer therapy, LA was tested in combination with doxorubicin for its effect on experimental murine leukemia L1210. These experiments tested the combination of the drug (doxorubicin) that is known for high rate of free-radical formation and free-radicals scavenging antioxidant LA [37]. The experiments were performed with the idea that LA would decrease the toxicities of doxorubicin. However, the selection of an appropriate dose of the antioxidant was shown to be crucial in designing therapeutic protocols. At low concentration of 1 μmol/l, LA acted as a growth factor while it functioned as an antiproliferation agent at concentration 100 μmol/l [38]. It was observed (*in vitro*) at the majority of doxorubicin and LA combination that the effect of LA on doxorubicin was antagonistic. Synergistic effect of doxorubicin and LA was observed only at the relatively high concentrations of both drugs. However, the use of doxorubicin and LA *in vivo* produced an increase in survival of experimental mice. The combination of a single dose of 5mg/kg of doxorubicin and 16 mg/kg of LA resulted in super-additive effect on survival of leukemia-bearing mice thus confirming that the proper scheduling of anticancer drugs in therapy is highly important for achieving desired therapeutic outcome. On the other hand, it indicates that insufficient dosage may result in adverse effects in patients.

There is only one report on the use of LA in human patients. A rare but interesting case of a patient with pancreatic cancer without any LA toxic or other adverse effect during a long-time survival was reported [39]. The patient was on the intravenous LA and low-dose naltrexone (LA-N) protocol. This together with a healthy lifestyle possibly extended the life of the otherwise terminal patient for several years.

Because of this, other patients were put on the LA-N protocol and they were reported to benefit from this treatment [38].

LA prevention of cancer-chemotherapy toxicity. LA's ability to prevent toxicities related to cancer therapy is arising from its ability to be a scavenger of free-radicals. Consequently, any toxicity caused by a formation of free radical during cancer therapy can be potentially alleviated by an administration of LA as already shown in several scientific publications.

LA in the form of a racemate demonstrated protective effect on cyclophosphamide induced hyperlipidemic cardiomyopathy [39]. It was shown that 200 mg cyclophosphamide per kg of body weight induces abnormal elevation of serum lipids, levels of free and esterified cholesterol and triglycerides in cardiac muscle and in serum. This mirrored the abnormal distortion in the activity of lipid-metabolizing enzymes that followed cyclophosphamide administration. The addition of racemic lipoic acid in the dose of 25 mg/kg of body weight for 10 days resulted in normalization of the lipid levels and activity of the lipid-metabolizing enzymes. LA was also shown to induce lysosomal changes in cases oxidative cardiotoxicity [40].

As previously reported [41], LA is also capable of influencing the nephrotoxicity potential of adriamycin. This study was performed with Wistar strain adult male albino rats receiving intravenous adriamycin (1 mg/kg of body weight. once a week. for the period of 12 weeks) without or with LA (35 mg/kg of body weight per day for the period of 12 weeks, once a week, 24 h prior to the administration of adriamycin). An administration of adriamycin led to the decrease in activities of the glycolytic enzymes in the rat renal tissue. The gluconeogenic enzymes, glucose-6-phosphatase and fructose-1,6-diphosphatase, also showed a decline in their activities on adriamycin administration. Decreased activities of the TCA cycle enzymes isocitrate dehydrogenase, succinate dehydrogenase and malate dehydrogenase, suggested a loss in mitochondrial function and integrity. Nephrotoxicity was evident from the increased excretions of N-acetyl-beta-D-glucosaminidase and gamma-glutamyl transferase in the urine of adriamycin treated rats. These biochemical disturbances were effectively counteracted by a pre-treatment with lipoic acid that resulted in an increase in activities of glycolytic enzymes, ATPases and the TCA cycle enzymes. Other reports from the same research team deal with the influence of LA on adriamycin-induced hyperlipidemic nephrotoxicity [42] and adriamycin-induced lipid peroxidation [43,44] in rat kidney, and also with protective role of racemic LA against adriamycin-induced cardiac lipid peroxidation [45]. All these studies report on the beneficial effects of LA when applied in situations when free-radical related toxicity appears. Consequently, LA administration leads to an improvement in parameters reflecting oxidative stress induced by an anticancer agent.

Similar findings were reported when LA was used to prevent cardiotoxicity induced by doxorubicin (15 mg/kg, i.p.) [46]. Doxorubicin cardiotoxicity was reflected by a significant elevation of serum creatine phosphokinase and lactate dehydrogenase and by the significant increase in lipid peroxides 48 hours after doxorubicin administration. Protein thiols in heart muscle were decreased. Orally administered LA (100 mg/kg, 5 days prior doxorubicin and 2 days after doxorubicin administration) resulted in a significant protection against cardiotoxicity mirrored by an improvement of the biochemical parameters. The results suggest that LA may be useful in increasing therapeutic index of doxorubicin. The same therapeutic-index improvement would probably be observed when LA is included in therapeutic regimes of any free radical-producing anticancer agent, for which the dose administration is limited by toxicity based on the oxidative stress in human tissue.

Clinical experience shows that the LA can be used to treat effectively oxaliplatin-induced cumulative polyneuropathy [47] that is, very probably, also caused by excessive production of free radicals.

The free-radical scavenging is not the only way, by which LA decreases toxicity. It was shown that LA can be used to prevent glutamate cytotoxicity [48]. This kind of toxicity occurs through an inhibition of cystine transport as glutamate and cystine share the same transporter. Consequently, elevated extracellular glutamate competitively inhibits cystine transport and, therefore causes depletion of intracellular glutathione. An impairment of cellular antioxidant defenses and oxidative stress occur. The addition of LA increases supply of cysteine to cells from their surrounding environment. As cysteine is the reduced form of cystine that is transported into the cell by a glutamate-insensitive transport mechanism, the glutamate cytotoxicity can be prevented. The LA protection corresponds with the intensity of glutathione protection. However, it was shown experimentally [48] that doses of LA smaller than 100 μM do not protect cells against glutamate-induced cytotoxicity and that protection against glutamate cytotoxicity even in glutathione synthesis-arrested cells occurs only at concentrations of LA above 500 μM. This indicates that the primary mechanism of LA protection at low concentrations is mediated by a pro-glutathione quality rather than direct scavenging of reactive oxygen. A direct antioxidant effect of LA takes place rather at high concentrations.

An interesting open, non-randomized phase II study was performed [49] that included patients with advanced solid tumors. The patients received a maintenance treatment that included recombinant interleukin-2 (rIL-2; 1.8 MIU; 3 times/week subcutaneously on alternate days for the first two weeks of every month), medroxyprogesterone acetate (MPA; 500 mg/day at alternate days without interruption; orally) and two antioxidants, LA (300 mg/day orally; continuously) and N-acetyl cysteine (NAC; 1800 mg/day orally; continuously). The median duration of this maintenance treatment in 28 patients was 10 months (6-30+). Eleven patients achieved complete remission. No significant toxicity was reported. The conclusion of this study is that this protocol has "a very low toxicity and results in the improvement of biological markers which are predictive for patient outcome" [50].

Conclusion

LA is a very interesting substance that is involved in many important biological and biochemical cellular processes. It has the ability to influence activities of some specific enzymes with acting role in those processes. LA with its free-radical-scavenging capacity has the potential to become a very useful substance for interfering with processes within malignant cells. However, as many clinically-used anticancer drugs act through generating various radical species, it is quite possible that some of them (i.e. alkylating agents) may demonstrate decreased therapeutic effect as their active radicals may be eliminated by LA. On the other hand, it was already demonstrated that toxicity of some anticancer drugs is related to the formation of free radicals and may be decreased by LA. Because of this, inclusion of LA in therapeutic protocols or its use in chemoprevention of cancer may be beneficial. Furthermore, the research findings published so far warranty future investigations on this very interesting compound

This work was supported by Kuwait University grant No. MR01/05.

References

[1] DOVINOVA I. α-Lipoic acid – natural disulfidic cofactor and antioxidant with anticarcinogenic activities. Cs Farmacie 1996; 45: 237–241 (In Slovak).

[2] PERHAM RN. Swinging arms and swinging domains in multifunctional enzymes: catalytic machines for multistep reactions. Ann Rev Biochem 2000; 69: 961–1004.

[3] REED LJ, DEBUSK BG, GUNSALUS IC, et al. Crystalline alpha-lipoic acid; a catalytic agent associated with pyruvate dehydrogenase. Science 1951; 114:93–94.

[4] MILNE JL, WU X, BORGNIA MJ, et al, Molecular structure of a 9-MDa icosahedral pyruvate dehydrogenase subcomplex containing the E2 and E3 enzymes using cryo-electron microscopy. J Biol Chem 2006; 281: 4364–4370.

[5] FUJIWARA K, OKAMURA-IKEDA K, MOTOKAWA Y. Chicken liver H-protein, a component of the glycine cleavage system. Amino acid sequence and identification of the N epsilon-lipoyllysine residue. J Biol Chem 1986; 261: 8836–8841.

[6] VANDEN BOOM TJ, REED KE, CRONAN JE JR. Lipoic acid metabolism in Escherichia coli: isolation of null mutants defective in lipoic acid biosynthesis, molecular cloning and characterization of the E. coli lip locus, and identification of the lipoylated protein of the glycine cleavage system. J Bacteriol 1991; 173: 6411–6420.

[7] PEINADO J, SIES H, AKERBOOM TP. Hepatic lipoate uptake. Arch Biochem Biophys 1989; 173: 389–395.

[8] KAGAN VE, SHVEDOVA A. Dihydrolipoic acid- a universal antioxidant both in the membrane and in the aqueous phase. Reduction of peroxyl, ascorbyl and chromanoxyl radicals. Biochem Pharmacol 1992; 44: 1637–1649.

[9] PACKER L, WITT EH, TRITSCHLER HJ. alpha-Lipoic acid as a biological antioxidant. Free Radic Biol Med 1995; 19: 227–250.

[10] SMITH JR, THIAGARAJ HV, SEAVER B, et al.. Differential activity of lipoic acid enantiomers in cell culture. J Herb Pharmacother 2005; 5: 43–54.

[11] KAGAN VE, SHVEDOVA A, SERBINOVA E, et al. Dihydrolipoic acid – universal antioxidant both in the membrane and in the aqueous phase. Reduction of peroxyl, ascorbyl and chromanoxyl radicals. Biochem Pharmacol 1992; 44: 1637–1649.

[12] HENRIKSEN EJ. Exercise training and the antioxidant alpha-lipoic acid in the treatment of insulin resistance and type 2 diabetes. Free Radic Biol Med 2006; 40: 3–12.

[13] LYKKESFELDT J, HAGEN TM, VINARSKY V, et al. Age-associated decline in ascorbic acid concentration, recycling, and biosynthesis in rat hepatocytes – reversal with (R)-alpha-lipoic acid supplementation. FASEB J 1998; 12: 1183–1189.

[14] SCHOLICH H, MURPHY ME, SIES H. Antioxidant activity of dihydrolipoate against microsomal lipid peroxidation and its dependence on alpha-tocopherol. Biochem Biophys Acta 1989; 1001: 256–261.

[15] BUSSE E, ZIMMER G, SCHORPOHL B, et al.. Influence of alpha-lipoic acid on intracellular glutathione in vitro and in vivo. Arzneimittelforschung 1992; 42: 829–831.

[16] KAGAN V, SERBINOVA E, PACKER L. Antioxidant effects of ubiquinones in microsomes and mitochondria are mediated by tocopherol recycling. Biochem Biophys Res Commun 1990; 169: 851–857.

[17] CREMER DR, RABELER R, ROBERTS A, et al. Safety evaluation of alpha-lipoic acid (ALA). Regul Toxicol Pharmacol 2006; 46: 29–41.

[18] MIADOKOVA E, VLCKOVA V, DUHOVA V. Antimutagenic effect of alpha-lipoic acid on three model test systems. Pharmazie 2000; 55: 862–863.

[19] JORDAN SW, CRONAN JE JR. A new metabolic link. The acyl carrier protein of lipid synthesis donates lipoic acid to the pyruvate dehydrogenase complex in Escherichia coli and mitochondria. J Biol Chem 1997; 272: 17903–17906.

[20] REED LJ, KOIKE M, LEVITCH ME, et al. Studies on the nature and reactions of protein-bound lipoic acid. J Biol Chem 1958; 232: 143–158.

[21] JIANG Y, CRONAN JE. Expression cloning and demonstration of Enterococcus faecalis lipoamidase (pyruvate dehydrogenase inactivase) as a Ser-Ser-Lys triad amidohydrolase. J Biol Chem 2005; 280: 2244–2256.

[22] KOROTCHKINA LG, SIDHU S, PATEL MS. R-lipoic acid inhibits mammalian pyruvate dehydrogenase kinase. Free Radic Res 2004; 38: 1083–1092.

[23] MCMANUS E, LUISI BF, PERHAM RN. Structure of a putative lipoate protein ligase from Thermoplasma acidophilum and the mechanism of target selection for post-translational modification. J Mol Biol 2006; 356: 625–637.

[24] STOYANOVSKY DA, TYURINA YY, TYURIN VA, et al. Thioredoxin and lipoic acid catalyze the denitrosation of low molecular weight and protein S-nitrosothiols. J Am Chem Soc 2005; 127: 15815–15823.

[25] WENZEL U, NICKEL A, DANIEL H. alpha-Lipoic acid induces apoptosis in human colon cancer cells by increasing

mitochondrial respiration with a concomitant O2-*-generation. Apoptosis 2005; 10: 359–368.

[26] MYZAK MC, CARR AC. Myeloperoxidase-dependent caspase-3 activation and apoptosis in HL-60 cells: protection by the antioxidants ascorbate and (dihydro)lipoic acid. Redox Rep 2002; 7: 47–53.

[27] SEN CK, ROY S, HAN D, PACKER L. Regulation of cellular thiols in human lymphocytes by alpha-lipoic acid: a flow cytometric analysis. Free Radic Biol Med 1997; 22: 1241–1257.

[28] HAN D, HANDELMAN G, MARCOCCI L, et al. Lipoic acid increases de novo synthesis of cellular glutathione by improving cystine utilization. Biofactors 1997; 6: 321–338.

[29] SOKOLOSKI JA, HODNICK WF, MAYNE ST, et al. Induction of the differentiation of HL-60 promyelocytic leukemia cells by vitamin E and other antioxidants in combination with low levels of vitamin D3: possible relationship to NF-kappaB. Leukemia 1997; 11: 1546–1553.

[30] WEITSMAN GE, RAVID A, LIBERMAN UA, et al. Vitamin D enhances caspase-dependent and -independent TNFalpha-induced breast cancer cell death: The role of reactive oxygen species and mitochondria. Int J Cancer 2003; 106: 178–186.

[31] WEITSMAN GE, RAVID A, LIBERMAN UA, et al. Vitamin D enhances caspase-dependent and independent TNF-induced breast cancer cell death: the role of reactive oxygen species. Ann NY Acad Sci 2003; 1010: 437–440.

[32] VAN DE MARK K, CHEN JS, STELIOU K, et al. Alpha-lipoic acid induces p27Kip-dependent cell cycle arrest in non-transformed cell lines and apoptosis in tumor cell lines. J Cell Physiol 2003; 194: 325–340.

[33] FLIER J, VAN MUISWINKEL FL, JONGENELEN CA, et al. The neuroprotective antioxidant alpha-lipoic acid induces detoxication enzymes in cultured astroglial cells. Free Radic Res 2002; 36: 695–699.

[34] PACK RA, HARDY K, MADIGAN MC, et al. Differential effects of the antioxidant alpha-lipoic acid on the proliferation of mitogen-stimulated peripheral blood lymphocytes and leukaemic T cells. Mol Immunol 2002; 38: 733–745.

[35] MANTOVANI G, MACCIO A, MELIS G, et al.. Restoration of functional defects in peripheral blood mononuclear cells isolated from cancer patients by thiol antioxidants alpha-lipoic acid and N-acetyl cysteine. Int J Cancer 2000; 86: 842–847.

[36] MOUNGJAROEN J, NIMMANNIT U, CALLERY PS, et al. Reactive oxygen species mediate caspase activation and apoptosis induced by lipoic acid in human lung epithelial cancer cells through Bcl-2 down-regulation. J Pharmacol Exp Ther 2006; 319: 1062–1069.

[37] DOVINOVA I, NOVOTNY L, RAUKO P, et al. Combined effect of lipoic acid and doxorubicin in murine leukemia. Neoplasma 1999; 46: 237–241.

[38] BERKSON BM, RUBIN DM, BERKSON AJ. The long-term survival of a patient with pancreatic cancer with metastases to the liver after treatment with the intravenous alpha-lipoic acid/low-dose naltrexone protocol. Integr Cancer Ther 2006; 5: 83–89.

[39] MYTHILI Y, SUDHARSAN PT, SUDHAHAR V, et al. Protective effect of DL-alpha-lipoic acid on cyclophosphamide induced hyperlipidemic cardiomyopathy. Eur J Pharmacol 2006; 543: 92–96.

[40] MYTHILI Y, SUDHARSAN PT, AMUDHA G, et al.Effect of dl-alpha-lipoic acid on cyclophosphamide induced lysosomal changes in oxidative cardiotoxicity. Life Sci 2007; 80: 1993–1998.

[41] MALARKODI KP, BALACHANDAR AV, VARALAKSHMI P. The influence of lipoic acid on adriamycin induced nephrotoxicity in rats. Mol Cell Biochem. 2003; 247: 15–22.

[42] MALARKODI KP, BALACHANDAR AV, VARLAKSHMI P. The influence of lipoic acid on adriamycin-induced hyperlipidemic nephrotoxicity in rats. Mol Cell Biochem 2003; 247: 139–145.

[43] MALARKODI KP, BALACHANDAR AV, VARALAKSHMI P. Protective effect of lipoic acid on adriamycin-induced lipid peroxidation in rat kidney. Mol Cell Biochem 2003; 247: 9–13.

[44] MALARKODI KP, BALACHANDAR AV, SIVAPRASAD R, et al. Prophylactic effect of lipoic acid against adriamycin-induced peroxidative damages in rat kidney. Ren Fail 2003; 25: 367–377.

[45] BALACHANDAR AV, MALARKODI KP, VARALAKSHMI P. Protective role of DLalpha-lipoic acid against adriamycin-induced cardiac lipid peroxidation. Hum Exp Toxicol 2003; 22: 249–254.

[46] AL-MAJED AA, GDO AM, AL-SHABANAH OA, et al.Alpha-lipoic acid ameliorates myocardial toxicity induced by doxorubicin. Pharmacol Res 2002; 46: 499–503.

[47] BEDLIKE C, SCHEITHAUER W, SCHULL B et al. Effective treatment of oxaliplatin-induced cumulative polyneuropathy with alpha-lipoic acid. J Clin Oncol 2002; 20: 3359–3361.

[48] HAN D, SEN CK, ROY S, et al.. Protection against glutamate-induced cytotoxicity in C6 glial cells by thiol antioxidants. Am J Physiol 1997; 273: R1771–1778.

[49] MANTOVANI G, MACCIO A, MADEDDU C, et al.Phase II study of subcutaneously administered interleukin-2 in combination with medroxyprogesterone acetate and antioxidant agents as maintenance treatment in advanced cancer responders to previous chemotherapy. Oncol Rep 2002; 9: 887–896.

[50] MANTOVANI G, MADEDDU C, GRAMIGNANO G, et al. Subcutaneous interleukin-2 in combination with medroxyprogesterone acetate and antioxidants in advanced cancer responders to previous chemotherapy: phase II study evaluating clinical, quality of life, and laboratory parameters. J Exp Ther Oncol 2003; 3: 205–219.