



Review

Lipoic acid – the drug of the future?

Anna Bilska, Lidia Włodek

Institute of Medical Biochemistry, Medical College, Jagiellonian University, Kopernika 7, PL 31-034 Kraków, Poland

Correspondence: Lidia Włodek, e-mail: mbwlodek@cyf-kr.edu.pl

Abstract:

Numerous experimental and clinical studies proved efficiency of treatment with lipoic acid-containing drugs in diseases, in which pro- and antioxidant balance is disrupted (diabetes, neurodegenerative diseases, acquired immune deficiency syndrome (AIDS), tumors, etc.). Efficiency of lipoate has been attributed to unique antioxidant properties of lipoate/dihydrolipoate system, its reactive oxygen species (ROS) scavenging ability and significant effect on the tissue concentrations of reduced forms of other antioxidants, including one of the most powerful, glutathione (thus lipoate is called an antioxidant of antioxidants). Moreover, analysis of literature data suggests participation of lipoic acid in processes of cell growth and differentiation. This fact can be crucial to clinical practice, however, this problem requires further studies.

Key words:

lipoic acid, diabetes, neurodegenerative diseases, tumors, signal transduction

Abbreviations: AGEs – advanced glycation end products, CML – N-ε-carboxymethyllysine, DA – dopamine, L-Dopa – L-dihydroxyphenylalanine, DOPAC – aldehyde of dihydroxyphenylacetic acid, GSH – glutathione, ROS – reactive oxygen species, β-APP – β-amyloid precursor protein

Introduction

α-Lipoic acid was isolated from bovine liver in 1950 [34]. In the next years, its chemical structure was established and confirmed by synthesis. α-Lipoic acid is 6,8-dithio-octanoic acid. Due to the presence of asymmetrical carbon atom, it is an optically active compound. Naturally occurring α-lipoic acid is in R configuration and is dextrorotary (Fig. 1). Amide of lipoic acid (Fig. 2) is a coenzyme of E₂ (dihydrolipoate acyltransferase) subunit of multienzymatic mitochon-

drial complexes catalyzing oxidative decarboxylation of pyruvate, α-ketoglutarate and branched-chain α-ketoacids, formed during transamination of leucine, isoleucine and valine. These reactions share the mechanism consisting in α-ketonic group transfer onto coenzyme A. Lipoic acid is also an essential element of mitochondrial complex of four proteins participating in glycine synthesis and degradation (glycine cleavage system).

Considering its role in biochemical processes, lipoic acid was initially included into vitamin B complex. However, at present, a majority of researchers believe that lipoic acid is not a vitamin. It is supposed that it is synthesized in human and animal body in mitochondria, where, similarly as in bacterial and plant cells, octanoic acid and cysteine, which is the source of sulfur, are direct precursors of lipoic acid [31].

In the last decade, we have witnessed a surge of interest in pharmacological properties of lipoic acid

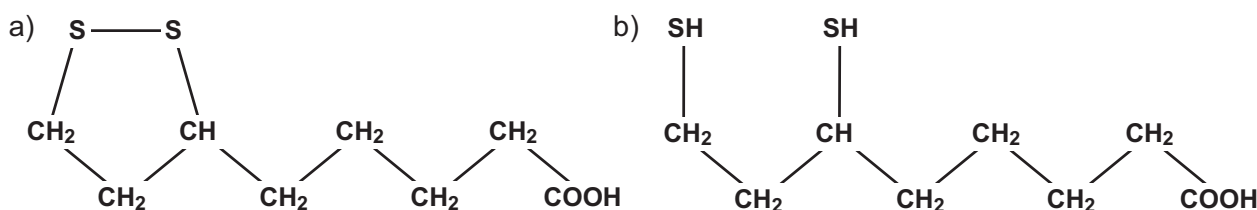


Fig. 1. Lipoic acid (a) and its reduced form – dihydrolipoic acid (b)

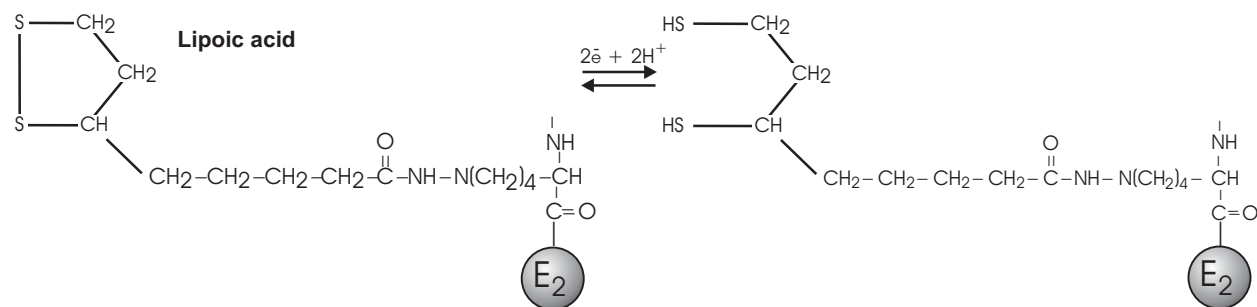


Fig. 2 Lipoamide and its reduced form-dihydrolipoamide

[29]. There is an avalanche rise in the number of publications confirming beneficial effect of lipoic acid in therapy of many diseases, including diabetes, atherosclerosis, degenerative processes in neurons, diseases of joints, or acquired immune deficiency syndrome (AIDS). An interest of contemporary medicine in this compound results principally from unique reductive power of lipoic acid. Due to low redox potential of lipoate/dihydrolipoate system (Tab. 1), reduced lipoic acid participates as well in reactions neutralizing reactive oxygen species (ROS scavenger) as in reduction of the oxidized forms of other antioxidants. The more so that lipoic acid is soluble as well in water as in fats, which is a unique feature among antioxidants [35]. For this reason, it is called an antioxidant of antioxidants.

Already dozens of years ago Linus Pauling stated that progress in medicine will be decided by so-called “orthomolecular chemistry” [32]. This concept assumed that the most important goal of medical assistance would involve aiding the cellular metabolism with natural compounds, which administered at appropriate dose and time would maintain or restore proper bodily functions. Considering almost countless publications devoted to lipoic acid pharmacology, one can conclude that, in line with the Pauling’s idea, lip-

oic acid will appear in the future as a key component of practically all drug formulations at amounts corresponding to a contribution of oxidative stress to ethiopathogenesis of a given disease. At present, not only the role of ROS in pathomechanism of many diseases

Tab.1. The values of standard biological redox potentials E_0' of reactive oxygen species (ROS) and selected redox systems

System	E_0' (V)
$\text{NAD}^+ + 2 \bar{e} + 2\text{H}^+ \Leftrightarrow \text{NADH} + \text{H}^+$	-0.32
$\text{lipoate} + 2 \bar{e} + 2\text{H}^+ \Leftrightarrow \text{dihydrolipoate}$	-0.29
$\text{GSSG} + 2\text{H}^+ + 2 \bar{e} \Leftrightarrow 2\text{GSH}$	-0.23
$\text{FAD} + 2 \bar{e} + 2\text{H} \Leftrightarrow \text{FADH}_2$	-0.06
$\text{dehydroascorbate} + 2 \bar{e} + 2\text{H}^+ \Leftrightarrow \text{ascorbate}$	-0.058
$\text{TO}^\cdot + \text{H}^+ + \bar{e} \Leftrightarrow \text{TOH}$ (chromanoxyl radical of tocopherol)	0.48
$1/2\text{O}_2 + 2 \bar{e} + 2\text{H}^+ \Leftrightarrow \text{H}_2\text{O}$	0.82
$\text{H}_2\text{O}_2 + 2 \bar{e} + 2\text{H}^+ \Leftrightarrow \text{O}_2^{\cdot-}$	0.87
$\text{H}_2\text{O}_2/\text{H}_2\text{O}$	1.32
$^\cdot\text{OH} + \text{H}^+ + \bar{e} \Leftrightarrow \text{H}_2\text{O}$	2.31

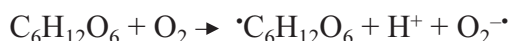
is not questioned but compounds endowed with antioxidant properties and able to aid maintaining high level of the reduced form of glutathione (GSH) gain greater and greater significance in studies focused on search for new drugs. Already first successes have been achieved.

- Lipoic acid is under intensive preclinical and clinical trials for therapy of diabetes and diabetic neuropathy [41, 44].
- This compound has gained also attention of cosmetologists and dermatologists since available data indicate positive effect of lipoic acid-containing preparations (creams, ointments, etc.) on the skin [4]. Creams containing lipoic acid are presently available on the market (also in Poland).
- Sportsmen in many sport clubs receive lipoic acid-containing preparations. This compound alleviates effects of oxidative stress caused by exercise and accelerates muscle regeneration [website www.apz.pl]. They are marketed in Poland as well.

Lipoic acid in the therapy of some diseases accompanied by oxidative stress

Diabetes and its complications

The reaction of glucose with oxygen leads to formation of superoxide radical anion and glucose radical.



This reaction proceeds in healthy people as well but due to high glucose level in diabetic patients, superoxide radical anion is formed in dramatically large quantities. This leads to oxidation of hemoglobin to methemoglobin, cysteine to cysteinic acid and methionine to sulfoxides. It is also known that in the presence of transition metal ions (e.g. copper ions), glucose stimulates lipid peroxidation, biodegeneration of proteins and modification of amino acid residues, which frequently change activity of enzymes. Horowitz et al. [13] demonstrated that incubation of rhodanase with reducing sugars (fructose, glucose and mannose) inactivated this enzyme, while catalase and superoxide dismutase protected it from loss of activity under these conditions. The presence of oxidative

stress in diabetes is corroborated by the fact that diabetic patients have significantly lower cellular and plasma levels of antioxidants, particularly GSH, ascorbate (vitamin C) and tocopherol (vitamin E) in comparison with healthy people [31]. The elevated level of N-ε-carboxymethyllysine (CML) and pentosidine, the products of nonenzymatic glycosylation (glycation) of proteins is also one of proofs of systemic oxidative stress in diabetes. Neuropathic changes, cataract, neurological disturbances, degenerative joint disease belong to frequent complications of diabetes, while atherosclerosis, cardiovascular diseases (heart attack, embolism, stroke) are the main causes of death of diabetic patients. Many of these problems are connected with glycation of three long-lived proteins: myelin, collagen and crystallin.

Studies on an influence of lipoic acid on glycation of animal proteins was first conducted in the laboratory of Packer [40]. They showed that formation of advanced glycation end products (AGEs) was markedly reduced by lipoic acid and other antioxidants. These studies were later confirmed by human studies. It was demonstrated that lipoic acid lowered protein glycation rate and decreased CML level [36].

Cataract is one of complications of diabetes mellitus, in which AGEs play a certain role. Cells of eye lenses maintain high level of GSH to protect themselves from glycation and reactions related to free radicals. However, in diabetes, glutathione concentration in eye lenses is considerably reduced. Research conducted on an animal model of cataract produced by the use of a known GSH synthesis inhibitor, L-buthionine-(S,R)-sulfoximine (BSO), revealed that administration of lipoic acid to animals caused a rise in cellular level of GSH, vitamin C and vitamin E, and contributed to an increase in activity of glutathione peroxidase, catalase and ascorbate reductase in the lenses [23]. These results prompted Kilic et al. to make an attempt to evaluate efficiency of lipoic acid in cataract induced by high glucose level [21]. The experiments were carried out on normal rat lenses at normal and elevated glucose concentration. Opacification of the lenses at high glucose level was significantly weaker in the presence of lipoic acid in comparison with opacification observed in lenses in the absence of lipoate.

At present, medications preventing chronic complications of diabetes, like neuropathy and nephropathy, are intensively searched for because currently used therapies are not satisfactorily efficient in combating

these complaints. These complications develop not only when diabetes is treated too late but also when the disease is managed from the beginning (e.g. type 1 diabetes). Patients were randomly divided into 4 groups (including one control group which did not take the drug), who were administered 600 mg of α -lipoic acid (group 1), 100 mg of selenium (sodium salt, group 2) or 1200 units of D- α -tocopherol (group 3) once a day for 3 months. The patients receiving antioxidants were observed to have significantly lower level of thiobarbituric acid-reactive substances and statistically significantly lower albumin excretion with urine. An improvement of sensibility in lower limbs [19] was also noted.

One of the first controlled clinical trials of lipoic acid (without supplementation of other antioxidants) was the program ALADIN, in which, apart from random assignment of subjects to experimental and control group, double-blind, placebo-controlled procedure was implemented [43]. A total of 328 patients with type 2 diabetes and symptoms of peripheral neuropathy participated in this study. The patients received *iv* lipoic acid at doses of 1200, 600 and 100 mg or placebo for 3 weeks. Response rates (lowered blood glucose level, improvement of sensibility in lower limbs and reduction of plasma concentration of lipid peroxidation products) in treatment groups were 24, 26 and 8%, respectively, in comparison with the control group given placebo.

Administration of lipoic acid in experimental model of diabetes induced by administration of streptozotocin, which destroys pancreatic β cells

In this model, impairment of glucose utilization is manifested by a 50% drop in glucose uptake and an increase in lactate and pyruvate concentrations, while lipoic acid administration normalizes these parameters [39]. In cyclophosphamide-induced diabetes in experimental animals, concomitant administration of lipoic acid markedly decreased inflammatory process of pancreatic islands and percentage of animals developing the disease (by about 30%) [10]. Moreover, the studies on genetically obese animals with diabetes and muscle resistance to insulin showed that lipoic acid improved glucose uptake by muscle tissue by 62% and accelerated glucose oxidation in glycolysis by over 30%. Furthermore, lipoic acid administration increased glycogen concentration in muscles (21%) and lowered (by 15–17%) plasma concentration of

free fatty acids. It was also shown that α -lipoic acid considerably stimulated glucose transport systems and aerobic and anaerobic glycolysis rate in insulin-resistant skeletal muscles in rats [17]. This indicates protective action of lipoate on β cells of pancreatic islands. Similar results were obtained in a trial involving 20 patients with type 2 diabetes, who received *iv* injections of lipoic acid at a dose of 500 mg/day for 10 days, *viz.* acceleration of glucose removal was documented [15]. Preliminary studies have shown that oral administration of lipoic acid to patients with type 2 diabetes increased tissue sensitivity to insulin and lowered glycemia level [16].

Neurodegenerative diseases

Although investigations of an influence of lipoic acid on preservation and/or improvement of the central nervous system function are only in their initial phase, the results are promising [28]. A potential of lipoic acid to exert protective effect on nervous cells was first mentioned in a comprehensive review on oxygen toxicity published as early as in 1968 [12].

Brain damage due to stroke, heart arrest, hemorrhage or head injury is a result of sudden reoxygenation of tissues (reperfusion) after a period of more or less enhanced hypoxia. Administration of lipoic acid to animals subjected to ischemia-reperfusion (e.g. by occlusion of cerebral artery) alleviated effects of reperfusion, *viz.* level of ROS in the brain cells was lower, extent of the damage was reduced and survival time of animals was decidedly longer in comparison with the control group [30, 33, 42].

Byrd from Medical Research Center from San Bruno suggests on the website www.lipoic.com that administration of lipoic acid to pregnant women, particularly during parturition can be advisable. He supports his view by the fact that childbirth is almost always connected with weaker or stronger hypoxia and then reperfusion and sudden oxygenation of the newborn. Since cytoprotective action of lipoic acid in reperfusion has been proven, it is conceivable that administration of this compound to women in labor could prevent quite high loss of neurons in a child and could have beneficial effect on health and intelligence of a newborn. This is only a hypothesis, nevertheless, pediatricians should promptly try to verify it.

A confirmation of the mutual relationship between oxidative stress level and redox state of cells, and progression of neurodegenerative processes comes from

studies showing the lowered level of the reduced GSH in neurons of the substantia nigra of patients who died of Parkinson's disease [1, 2]. *Post mortem* studies of the brains of Parkinson's disease patients demonstrated an elevated dopamine (DA) turnover in the extrapyramidal system [1, 2]. The increased DA turnover results in the enhanced production of H₂O₂, thereby increasing concentration of the neurotoxic hydroxyl radical ·OH. Monoamine oxidase type B (MAO-B) catalyzes the following reaction with DA as a substrate:



where: DOPAC – aldehyde of dihydroxyphenylacetic acid.

At present, the role of free radicals and mitochondrial disturbances in pathogenesis of Parkinson's disease not only is not questioned but just the opposite, in view of these data, a problem has emerged with the use of a direct DA precursor L-DOPA (3,4-dihydroxyphenyl-L-alanine) in the Parkinson's disease therapy, hitherto considered to be a golden standard of the treatment. Namely, it was shown that production of hydrogen peroxide, and consequently, a rise in hydroxyl radical concentration results not only from oxidation of DA but also from oxidation of its precursor, L-DOPA [18].

Therefore, currently new drugs and substances with neuroprotective potential are intensely searched for, among which antioxidants and compounds aiding preservation of high GSH level in the cells of the CNS, are seriously taken into consideration. In animal model of Parkinson's disease induced by intraventricular administration of glutathione synthase inhibitor, BSO and 6-hydroxydopamine, which lowers DA level, lipoic acid administration did not cause significant increase in the reduced GSH in the substantia nigra neurons of experimental animals. Lipoic acid also did not increase DA level in the striatum, but turnover of this neurotransmitter was increased in the extrapyramidal system. Furthermore, a rise in 5-hydroxyindoleacetic acid (5-HIAA), a product of serotonin (5-hydroxytryptamine, 5-HT) metabolism was observed [37].

Post mortem studies in Parkinson's disease patients showed the presence of adducts of free-radical forms of DA, L-DOPA and DOPAC with GSH and cysteine. Particularly high level of these adducts was noted in

the substantia nigra and in the shell. Dihydrolipoic acid was demonstrated to suppress formation of these conjugates under *in vitro* conditions, but lipoic acid was ineffective [38].

Alzheimer's disease is a progressive disease of the CNS, whose symptoms include loss of memory and cognitive capacities, accompanied by personality disintegration. It is believed that ROS also contribute to the course of this disease.

Histopathological hallmark of this disease is the presence of extracellular deposits of β-amyloid (amyloid plaques, senile plaques), which is formed from a precursor called β-amyloid precursor protein (β-APP). Under physiological conditions, β-APP is proteolytically split by several types of secretases yielding soluble amyloid-β peptide composed of 40 amino acids (βA₄₀). A change in activity of secretases leads to formation of insoluble peptides containing 42 (βA₄₂) and 43 (βA₄₃) amino acids. These peptides are the main constituents of senile plaques in Alzheimer's disease, and, as suggested by some studies, are a source of ROS [6, 22].

Lipoic acid applied in patients with the diagnosed dementia inhibited progress of the disease, which was confirmed by two neuropsychological tests, minimal mental state examination (MMSE) and Alzheimer's disease assessment scale, cognitive subscale (ASAScog) [11]. Although these studies are preliminary, and, as underscored by the authors, they do not comply with methodology of a controlled trial, they indicate a potential neuroprotective effect of lipoic acid in the course of Alzheimer's disease.

Is lipoic acid only an antioxidant?

Authors attribute efficiency of therapy with lipoic acid in diabetes mellitus and neurodegenerative diseases to unique antioxidant properties of lipoate/dihydrolipoate system, i.e. ROS scavenging ability and significant influence on tissue concentrations of the reduced forms of other antioxidants, including one of the most powerful, GSH (lipoate as an antioxidant of antioxidants).

It is known that oxygen shock can trigger binding of signal molecules, like cytokines, to membrane receptors. In this situation, ROS are considered to be second messengers within the cell [7, 8]. Binding of cytokines (TNF, IL-1β) to a specific receptor triggers oxygen shock in the cell, resulting in a rise in cellular

level of ROS, which transmit the signal to transcription factors, that activate expression of specific genes [20]. In mammalian cells, response to oxygen shock includes activation of heme oxidase and tyrosine phosphatase genes and AP-1 and NF- κ B-dependent promoters [24]. Several studies demonstrated that lipoic acid counteracted NF- κ B activation [27], which could be significant in the therapy of AIDS, since active NF- κ B was shown to be able to bind to certain DNA sequences of provirus HIV, which led to acceleration of virus replication in the infected T_H lymphocytes, culminating in their apoptosis [26].

NF- κ B is also responsible for proproliferative action in many tumor cells. Preliminary results of our studies indicate that lipoic acid at doses between 10–30 μ M slightly suppressed proliferation of WM35 melanoma cell line derived from radial growth phase with clear tendency to a drop in proliferation with increasing concentrations of lipoic acid. It was also shown using the method of BrdU incorporation that lipoic acid at a concentration of 15 mM inhibited proliferation of both, cells derived from primary focus of WM35 tumor growth and cells originating from fully developed metastasizing A350 melanoma [unpublished data]. Analysis of these results suggests that this property can be accounted for by completely different mechanism of action of lipoic acid, not connected with NF- κ B and ROS. Tumor tissues are characterized by high concentration of the reduced GSH [14, 25] and strong activity of enzymes involved in its metabolism and antioxidant action. In addition, melanin present in the cells shows antioxidant properties. High level of the reduced GSH in tumor cells indicates that the intensively proliferating cells are distinguished by the elevated demand for this peptide. Prostaglandins PGA and PGJ suppress growth of tumor cells, but they lose these potential upon conjugation with GSH. Antiproliferative action of PGJ₂ strongly rises with a drop in GSH level in the cells [3].

It may be expected that lipoic acid, whose stimulatory effect on GSH level was unequivocally proven, not only will not block proliferation of tumor cells but will even increase it. Literature data also indicate that lipoic acid effect depends on its dose. At low lipoate concentrations (1 μ mol/l), the authors observed an increased cell proliferation rate (lipoate acted as a growth factor). At high concentrations (100 μ mol/l), lipoic acid exhibited distinct antiproliferative effect [9]. It is not excluded that lipoic acid indeed has a dual nature, and its mechanism of action is more

complex than first expected. A property of lipoic acid to act as growth factor or to stimulate growth factors and/or receptors is corroborated by results of therapy in patients with posthepatic cirrhosis (after *hepatic viralis C*). These patients received lipoic acid, and selenium preparations and silymarin. After a one year long therapy, their liver function was fully normalized [5].

Concluding remarks

Redox reactions belong to the basic metabolic pathways of all cells. Displacement of a balance between pro- and antioxidants can account for mechanisms involved in etiopathogenesis and/or progression of many, apparently unconnected pathological states, like tumors, AIDS, neurodegenerative diseases, diabetes, etc. This underlines significance of antioxidants in the therapy of these diseases. It seems that lipoic acid is the most efficient drug of all antioxidants, which is confirmed by the following data.

- Low potential of lipoate/dihydrolipoate system is decisive for strong antioxidant properties of dihydrolipoate. Therefore, lipoic acid is characterized by high reactivity towards free radicals and is capable of regeneration of vitamin C and E, in addition, it elevates tissue level of GSH.
- Lipoic acid can be administered perorally since it is easily absorbed in the stomach. It crosses blood-brain barrier and does not show toxic actions at doses used for prophylactic and therapeutic purposes.
- Many experimental and clinical studies proved beneficial effect of lipoic acid in such diseases as diabetes, atherosclerosis and heart diseases, cataract, neurodegenerative diseases, liver diseases and AIDS.
- Preliminary results on lipoic acid effect on alleviation of old age-related disorders and adverse influence of exercise are promising.

Hence, it seems that beneficial properties of lipoic acid should not be questioned. However, very interesting problems related to involvement of lipoic acid in cell growth and differentiation require further intense studies.

References:

1. Antkiewicz-Michaluk L: Perspectives in antiparkinsonian medicines research. In: Neuropsychopharmacology. To-

- day and Tomorrow (Polish). Ed. Bijak M, Lasoń W, Kraków, 2000, 235–262.
2. Antkiewicz-Michaluk L, Krygowska-Wajs A, Michaluk J, Romańska I, Szczudlik A, Vetulani J: Plasticity of extrapyramidal dopamine system in Parkinson's disease – *post mortem* study. *Neurosci Res Commun*, 1999, 25, 97–109.
 3. Astmon J, Freeman MJ, Meredith MJ, Sweetman BJ, Roberts JI: Conjugation of 9-deoxy- Δ^9 ,- Δ^{12} , (E) prostaglandin D₂ with intracellular glutathione enhancement of its antiproliferative activity by glutathione depletion. *Cancer Res*, 1990, 50, 1879–1885.
 4. Beitner H: Randomized, placebo-controlled, double blind study on the clinical efficacy of a cream containing 5% α -lipoic acid related to photoageing of facial skin. *Br J Dermatol*, 2003, 149, 841–849.
 5. Berkson BM: A conservative triple antioxidant approach to the treatment of hepatitis C. Combination of α -lipoic acid (thioctic acid), silymarin, and selenium: three case histories. *Med Klin*, 1999, 94, Suppl 3, 84–89.
 6. Butterfield DA, Hensley K, Harris M, Mattson M, Carney J: β -Amyloid peptide free radical fragments initiate synaptosomal lipoperoxidation in a sequence-specific fashion: implications to Alzheimer's disease. *Biochem Biophys Res Commun*, 1994, 200, 710–715.
 7. Buttke TM, Sandstrom PA: Redox regulation of programmed cell death in lymphocytes. *Free Radic Res*, 1995, 22, 389–397.
 8. Deneke SM: Thiol-based antioxidants. *Curr Top Cell Regul*, 2000, 36, 151–180.
 9. Dovinova I, Novotny L, Rauko P, Kvasnicka P: Combined effect of lipoic acid and doxorubicin in murine leukemia. *Neoplasma*, 1999, 46, 237–241.
 10. Faust A, Burkart V, Ulrich H, Weischer CH, Kolb H: Effect of lipoic acid on cyclophosphamide-induced diabetes and insulinitis in non-obese diabetic mice. *Int J Immunopharmacol*, 1994, 16, 61–66.
 11. Hager K, Marahrens A, Kenkies M, Riederer P, Munch G: α -Lipoic acid as a new treatment option for Alzheimer type dementia. *Arch Gerontol Geriatr*, 2001, 32, 275–282.
 12. Haugaard N: Cellular mechanisms of oxygen toxicity. *Physiol Rev*, 1968, 48, 311–373.
 13. Horowitz PM, Butler M, McClure GD Jr: Reducing sugars can induce the oxidative inactivation of rhodanese. *J Biol Chem*, 1992, 267, 23596–23600.
 14. Huang ZZ, Chen C, Zeng Z, Yang H, Oh J, Chen L, Lu SC: Mechanism and significance of increased glutathione level in human hepatocellular carcinoma and liver regeneration. *FASEB J*, 2001, 15, 19–21.
 15. Jacob S, Henriksen EJ, Tritschler HJ, Augustin HJ, Dietze GJ: Improvement of insulin-stimulated glucose-disposal in type 2 diabetes after repeated parenteral administration of thioctic acid. *Exp Clin Endocrinol Diabetes*, 1996, 104, 284–288.
 16. Jacob S, Ruus P, Hermann R, Tritschler HJ, Maerker E, Renn W, Augustin HJ et al.: Oral administration of RAC- α -lipoic acid modulates insulin sensitivity in patients with type-2 diabetes mellitus: a placebo-controlled pilot trial. *Free Radic Biol Med*, 1999, 27, 309–314.
 17. Jacob S, Streeper RS, Fogt DL, Hokama JY, Tritschler HJ, Dietze GJ, Henriksen EJ: The antioxidant α -lipoic acid enhances insulin-stimulated glucose metabolism in insulin-resistant rat skeletal muscle. *Diabetes*, 1996, 45, 1024–1029.
 18. Jenner P, Brin MF: Levodopa neurotoxicity: experimental studies versus clinical relevance. *Neurology*, 1998, 50, 39–43.
 19. Kahler W, Kukliński B, Ruhlmann C, Plotz C: Diabetes mellitus – a free radical-associated disease. Results of adjuvant antioxidant supplementation (German). *Z Gesamte Inn Med*, 1993, 48, 223–232.
 20. Keyse SM, Tyrrel RM: Heme oxygenase is the major 32-kDa stress protein induced in human skin fibroblasts by UVA radiation, hydrogen peroxide, and sodium arsenite. *Proc Natl Acad Sci USA*, 1989, 86, 99–103.
 21. Kilic F, Handelman GJ, Serbinova E, Packer L, Trevithick JR: Modelling cortical cataractogenesis 17: *in vitro* effect of α -lipoic acid on glucose-induced lens membrane damage, a model of diabetic cataractogenesis. *Biochem Mol Biol Int*, 1995, 37, 361–370.
 22. Kowalska A: Genetic basis of neurodegeneration in familial Alzheimer's disease. *Pol J Pharmacol*, 2004, 56, 171–178.
 23. Maitra I, Serbinova E, Trischler H, Packer L: α -Lipoic acid prevents buthionine sulfoximine-induced cataract formation in newborn rats. *Free Radic Biol Med*, 1995, 18, 823–829.
 24. Meyer M, Schreck R, Baeuerle PA: H₂O₂ and antioxidants have opposite effects on activation of NF- κ B and AP-1 in intact cells: AP-1 as secondary antioxidant-responsive factor. *EMBO J*, 1993, 12, 2005–2015.
 25. Montironi R, Mazzucchelli R, Stramazzotti D, Pomante R, Thompson D, Barlel PH: Expression of pi-class glutathione S-transferase: two populations of high grade prostatic intraepithelial neoplasia with different relations to carcinoma. *Mol Pathol*, 2000, 53, 122–128.
 26. Pace GW, Leaf CD: The role of oxidative stress in HIV disease. *Free Radic Biol Med*, 1995, 9, 523–528.
 27. Packer L: α -Lipoic acid: a metabolic antioxidant which regulates NF- κ B signal transduction and protects oxidative injury. *Drug Metab Rev*, 1998, 30, 245–275.
 28. Packer L, Tritschler HJ, Wessel K: Neuroprotection by the metabolic antioxidant α -lipoic acid. *Free Radic Biol Med*, 1997, 22, 359–378.
 29. Packer L, Witt EH, Tritschler HJ: α -Lipoic acid as a biological antioxidant. *Free Radic Biol Med*, 1995, 19, 227–250.
 30. Panigrahi M, Sadguna Y, Shivakumar BR, Kolluri SV, Roy S, Packer L, Ravindranath V: α -Lipoic acid protects against reperfusion injury following cerebral ischemia in rats. *Brain Res*, 1996, 717, 184–188.
 31. Patel MS, Vettakkorumakankav NN: Lipoic acid-requiring proteins: recent advances. In: *Biothiols in Health and Disease*. Ed. Packer L, Cadenas E, Marcel Dekker Inc., New York, 1995, 373–388.
 32. Pauling L: Orthomolecular psychiatry. Varying the concentrations of substances normally present in the human body may control mental disease. *Science*, 1968, 160, 265–271.
 33. Prehn JH, Karkoutly C, Nuglisch J, Peruche B, Krieglstein J: Dihydrolipoate reduces neuronal injury after cerebral ischemia. *Cereb Blood Flow Metab*, 1992, 12, 78–87.

34. Reed LJ, DeBusk BG, Gansalus IC, Hornberger JrCS: Crystalline α -lipoic acid: a catalytic agent associated with pyruvate dehydrogenase. *Science*, 1951, 114, 93–94.
35. Roy S, Packer L: Redox regulation of cell functions by α -lipoate. *Biofactors*, 1998, 8, 17–21.
36. Schleicher E, Wagner E, Nehrlich AG: Increased accumulation of the glyoxidation product N-epsilon-(carboxymethyl)-lysine in human tissues in diabetes and aging. *J Clin Invest*, 1997, 99, 457–468.
37. Seaton TA, Jenner P, Marsden CD: Thioctic acid does not restore glutathione levels or protect against the potentiation of 6-hydroxydopamine toxicity induced by glutathione depletion in rat brain. *J Neural Transm*, 1996, 103, 315–329.
38. Spencer JP, Jenner P, Daniel SE, Lees AJ, Marsden DC, Halliwell B: Conjugates of catecholamines with cysteine and GSH in Parkinson's disease: possible mechanisms of formation involving reactive oxygen species. *J Neurochem*, 1998, 7, 2112–2122.
39. Strodter D, Lehmann E, Lehmann U, Tritschler HJ, Bretzel RG, Federlin K: The influence of thioctic acid on metabolism and function of the diabetic heart. *Diabetes Res Clin Pract*, 1995, 29, 19–26.
40. Suzuki YJ, Tsuchiya M, Packer L: Lipoate prevents glucose-induced protein modifications. *Free Radic Res Commun*, 1992, 17, 211–217.
41. Vinik AI, Park TS, Stansberry KB, Pittenger GL: Diabetic neuropathies. *Diabetologia*, 2000, 43, 957–973.
42. Wolz P, Krieglstein J: Neuroprotective effects of alpha-lipoic acid and its enantiomers demonstrated in rodent models of focal cerebral ischemia. *Neuropharmacology*, 1996, 35, 369–375.
43. Ziegler D, Hanefeld M, Ruhnau KJ, Meissner HP, Lobisch M, Schutte K, Gries FA: Treatment of symptomatic diabetic peripheral neuropathy with the anti-oxidant α -lipoic acid. A 3-week multicentre randomized controlled trial (ALADIN Study). *Diabetologia*, 1995, 38, 1425–1433.
44. Ziegler D, Nowak H, Kempfer P, Vargha P, Low PA: Treatment of symptomatic diabetic polyneuropathy with the antioxidant α -lipoic acid: a meta-analysis. *Diabet Med*, 2004, 21, 114–121.

Received:

January 31, 2005; in revised form: July 7, 2005.