Molecular Aspects of Lipoic Acid in the Prevention of Diabetes Complications

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α-Lipoic acid (LA) and its reduced form, dihydrolipoic acid, are powerful antioxidants. LA scavenges hydroxyl radicals, hypochlorous acid, peroxynitrite, and singlet oxygen. Dihydrolipoic acid also scavenges superoxide and peroxyl radicals and can regenerate thioredoxin, vitamin C, and glutathione, which in turn can recycle vitamin E. There are several possible sources of oxidative stress in diabetes including glycation reactions, decompartmentalization of transition metals, and a shift in the reduced-oxygen status of the diabetic cells. Diabetics have increased levels of lipid hydroperoxides, DNA adducts, and protein carbonyls. Available data strongly suggest that LA, because of its antioxidant properties, is particularly suited to the prevention and/or treatment of diabetic complications that arise from an overproduction of reactive oxygen and nitrogen species. In addition to its antioxidant properties, LA increases glucose uptake through recruitment of the glucose transporter-4 to plasma membranes, a mechanism that is shared with insulin-stimulated glucose uptake. Further, recent trials have demonstrated that LA improves glucose disposal in patients with type II diabetes. In experimental and clinical studies, LA markedly reduced the symptoms of diabetic pathologies, including cataract formation, vascular damage, and polyneuropathy. To develop a better understanding of the preventative and therapeutic potentials of LA, much of the current interest is focused on elucidating its molecular mechanisms in redox dependent gene expression. *Nutrition* 2001;17:888–895. ©Elsevier Science Inc. 2001

KEY WORDS: lipoic acid, diabetes, gene expression, free radicals

PROLOGUE

Larry Machlin, a friend and colleague for more than three decades will be remembered for his contributions to the understanding of micronutrients about which he had encyclopedic knowledge. He was a group leader at Roche Vitamins and Fine chemicals division. In this capacity he was a generous supporter of academic research, sponsored and cosponsored many symposia and conferences, and contributed to numerous publications and books which were landmark contributions. He promoted the use of nutritional supplements and antioxidant vitamins for human health and was an industry pioneer. This article is dedicated to his memory.

STRUCTURE, OCCURRENCE, AND BIOAVAILABILITY

R-lipoic acid (1,2-dithiolane-3-pentanoic acid; its structure is shown in Fig. 1) was discovered in 1937 by Snell et al.[1] who found that certain bacteria needed a compound from potato extract for growth. However, it was not before 1951 that the so-called potato-growth factor was isolated and characterized by Reed and colleagues.[2,3] Initially, R-lipoic acid was tentatively regarded as a vitamin; subsequently, R-lipoic acid was found to be synthesized by plants and animals,[3,4] where it is covalently bound to the

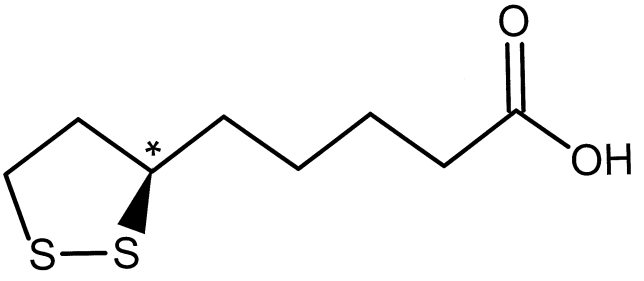

FIG. 1. Molecular structure of R-lipoic acid (1,2-dithiolane-3-pentanoic acid).

ϵ-amino group of lysine residues and functions as a cofactor of mitochondrial enzymes by catalyzing oxidative decarboxylation of pyruvate, α-ketoglutarate, and branched-chain α-keto acids.[5] Although the biosynthetic pathway of R-lipoic acid is not well understood, R-lipoic acid seems to be synthesized in mitochondria from octanoic acid and a sulfur source.[6,7] The lipoyllysine content of various plant and animal tissues has been studied by Lodge and Packer.[8] In the plant material studied, the lipoyllysine content was highest in spinach, followed by broccoli and tomato. Lower concentrations of lipoyllysine were found in garden pea, brussel sprouts, and rice bran. In animal tissues, kidney and liver had the highest contents.

Bioavailability of racemic lipoic acid has been studied extensively in humans with single-dose administrations.[9–12] Lipoic acid is absorbed with a Tmax of 0.5 to 1 h and exhibits dose proportionality between 50 and 600 mg.[12] No difference was observed

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TABLE I.

OVERVIEW OF REACTIVE OXYGEN SPECIES SCAVENGED BY LIPOIC ACID AND DIHYDROLIPOIC ACID

Oxidant	Lipoic acid	Dihydrolipoic acid
Hydrogen peroxide	Yes	Yes
Singlet oxygen	Yes	No
Hydroxyl radical	Yes	Yes
Nitric oxide radical	Yes	Yes
Superoxide radical	No	Yes
Hypochlorous acid	Yes	Yes
Peroxynitrite	Yes	Yes
Peroxyl radical	No	Yes

between R- and S-lipoic–acid concentrations in plasma after intravenous administration. However, after oral intake of the racemic mixture, significantly higher values for area under the curve and C_{max} were found for R-lipoic acid than for the S form.[13] Lipoic acid was absorbed more efficiently from an aqueous solution than from galenic preparations. Food intake reduced the bioavailability of lipoic acid[9]. Therefore, α-lipoic acid was recommended to be taken up 30 min before a meal. In insulin-dependent diabetics, who usually have delayed gastric emptying, no substantial influence on lipoic-acid bioavailability was observed.[14]

The absolute bioavailabilities (oral versus intravenous) of 200 mg of lipoic acid in aqueous solution have been estimated to be 38% for the R form and 28% for the S form.[10] Galenic forms had lower absolute bioavailabilities, with 25% for R-lipoic acid and 20% for S-lipoic acid. In a later study that did not distinguish between the enantiomers, a similar absolute bioavailability of 29% was found after ingestion of 200 mg of lipoic acid.[11] The relative low bioavailability of lipoic acid may be due to a high first-pass effect. In preclinical studies on the pharmacokinetics of lipoic acid in rats with applications of [7,8-^{14}C]rac–α-lipoic acid, the area under the curve of radioactivity in plasma averaged 66% after oral administration, as opposed to the intravenous route.[15] However, the radioactivity recovered over 168 h in the urine amounted to 93% of the administered dose. Data indicate that lipoic acid is metabolized extensively in the liver. Indeed, urinary radioactivity in rats was reported to consist mainly of shorter-chain homologues including bisnorlipoic acid, tetranorlipoic acid, and β-hydroxybisnorlipoic acid, formed through β-oxidation of lipoic acid.[16] β-Oxidation of lipoic acid also occurs in humans. After administration of a single dose of 1 g of R-lipoic acid to a male volunteer, 3-ketolipoic acid and bisnorlipoic acid were detected in his plasma.[17]

ANTIOXIDANT PROPERTIES

Radical quenching, metal chelation, amphiphilic character, bioavailability and safety, interaction with other antioxidants, and metabolic regeneration are important criteria to consider a compound as a potent antioxidant. Lipoic acid and its reduced form, dihydrolipoic acid (DHLA), meet all the criteria, making lipoic acid an ideal antioxidant. Table I presents an overview on the broad array of reactive oxygen and nitrogen species scavenged by lipoic acid and DHLA. The predominant form that interacts with reactive oxygen species is DHLA, but the oxidized form of lipoic acid also can inactivate free radicals. Transition metals such as iron, copper, mercury, and cadmium can induce free-radical damage in biological systems by catalyzing the decomposition of hydroperoxides, thus generating highly toxic hydroxyl radicals. Lipoic acid and DHLA may exhibit antioxidant activity by metal chelating,[18] which also explains the beneficial effect of lipoic acid in heavy-metal poisoning.[19–21]

An interaction of lipoic acid with other antioxidants in vivo was indicated by the observations of Rosenberg and Culik[4] who reported that lipoic acid prevents symptoms of vitamin C and vitamin E deficiency. In a study conducted in our laboratory, lipoic acid also prevented symptoms of vitamin E deficiency in hairless mice deficient in vitamin E.[22] Further, we detected free lipoic acid and its reduced form, DHLA, in various mice tissues, demonstrating that lipoic acid is reduced metabolically to DHLA in vivo. DHLA is a strong reductant regenerating oxidized antioxidants such as ascorbate, glutathione, coenzyme Q^{10}, and vitamin E.[18,23] These observations point to an interaction between various antioxidants known as the antioxidant network (Fig. 2). When vitamin E scavenges a peroxyl radical, a vitamin E radical is formed. The vitamin E radical may be reduced at the interface between lipid and water by several antioxidants such as ascorbate, ubiquinol, and reduced glutathione (GSH). DHLA can reduce all these antioxidants and be regenerated by several enzymes, including lipoamide reductase, GSH reductase, and thioredoxin reductase. Therefore, lipoic acid and DHLA take central positions in the antioxidant network. In addition, lipoic acid has water-soluble and lipid-membrane–soluble characteristics, thus enabling it to reduce oxidized antioxidants at the interphase between lipid and water.

Treatment with lipoic acid increases GSH levels in vivo and in vitro.[22–25] GSH is an important water-soluble endogenous antioxidant. It occurs in reduced thiol (GSH) and oxidized disulfide forms. GSH is linked to many physiologic processes including detoxification of xenobiotics, modulation of signal transduction, prostaglandin metabolism, regulation of immune response, and enzyme activities. Studies with human cells have provided insights into the mechanism through which lipoic acid increases GSH levels. Cysteine availability is known as the rate-limiting factor in GSH synthesis. Lipoic acid is taken up rapidly by the cell, reduced to DHLA, and secreted to the medium, where it reduces cystine to cysteine. The cell takes up cysteine about 10 times faster than cystine, resulting in enhancement of GSH biosynthesis.[26] It should be noted that the standard reduced-oxygen (redox) potential of the R-lipoic acid/DHLA pair is -320 mV. Hence, DHLA can reduce even oxidized glutathione (GSSG) chemically.[27]

OXIDATIVE STRESS AND DIABETES

Several lines of evidence underscore the benefits of lipoic acid in diabetes prevention and treatment. Oxidative stress has been observed widely in diabetes [28–35]. Diabetic patients have increased levels of lipid-peroxidation products, measured as thiobarbituric-acid–reactive substances, lipid peroxides, F_2-isoprostanes, oxidatively damaged DNA bases, and decreased levels of protective antioxidants including α-tocopherol, ascorbic acid, and reduced GSH. Oxidative stress may determine the onset and progression of late-diabetes complications.[33] Increased oxidative stress in diabetes appears to be due mainly to hyperglycemia, which results in stimulation of the polyol pathway, formation of advanced glycation endproducts (AGE), and subsequent formation of reactive oxygen radicals (Fig. 3).

Lipoic acid reduces oxidative stress in healthy adults and diabetic patients.[34] Daily supplemention of 600 mg of lipoic acid for 3 mo can significantly reduce lipid-hydroperoxide formation.[34]

CATARACT FORMATION

Under normal conditions glucose is phosphorylated by the enzyme hexokinase to glucose-6-phosphate. Glucose-6-phosphate is oxidized with glycolysis and hexosemonophosphate shunt or is used for glycogen synthesis. In hyperglycemia, where glucose-using enzymes are saturated, glucose is irreversibly reduced to sorbitol

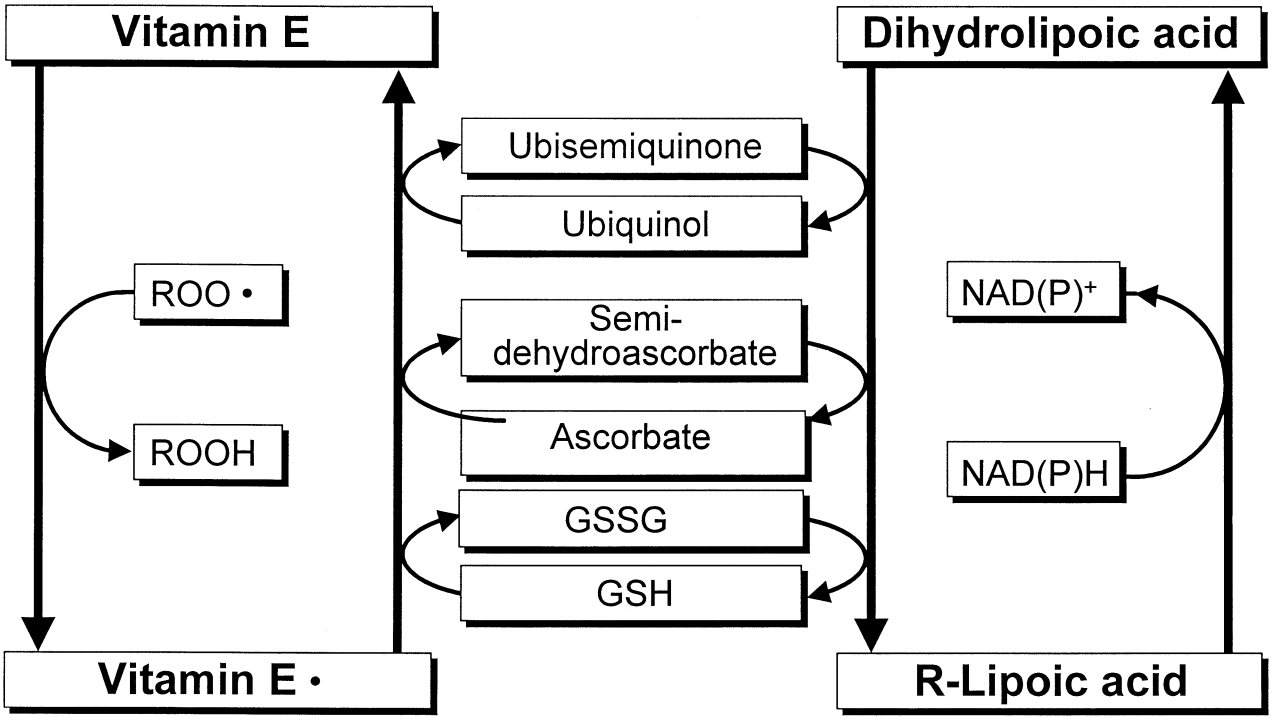

FIG. 2. The antioxidant network showing the interaction between vitamin E, ubiquinol, vitamin C, glutathione, and R-lipoic acid redox cycles.

by aldose reductase at the expense of nicotinamide adenine dinucleotide phosphate (NADPH). This reaction is called the polyol pathway. Sorbitol is then oxidized to fructose, using NAD^+ as the receptor for the reduction equivalents. The overall reaction leads to a shortage of intracellular NADPH and a surplus of NADH, i.e., a reductive imbalance. Because GSH reductase depends on NADPH, reduced GSH becomes depleted, resulting in oxidative stress. In addition, fructose and sorbitol lead to osmotic swelling of the eye lens. Further, glycosylating compounds such as sorbitol-3-phosphate or fructose-3-phophate are formed.[17]

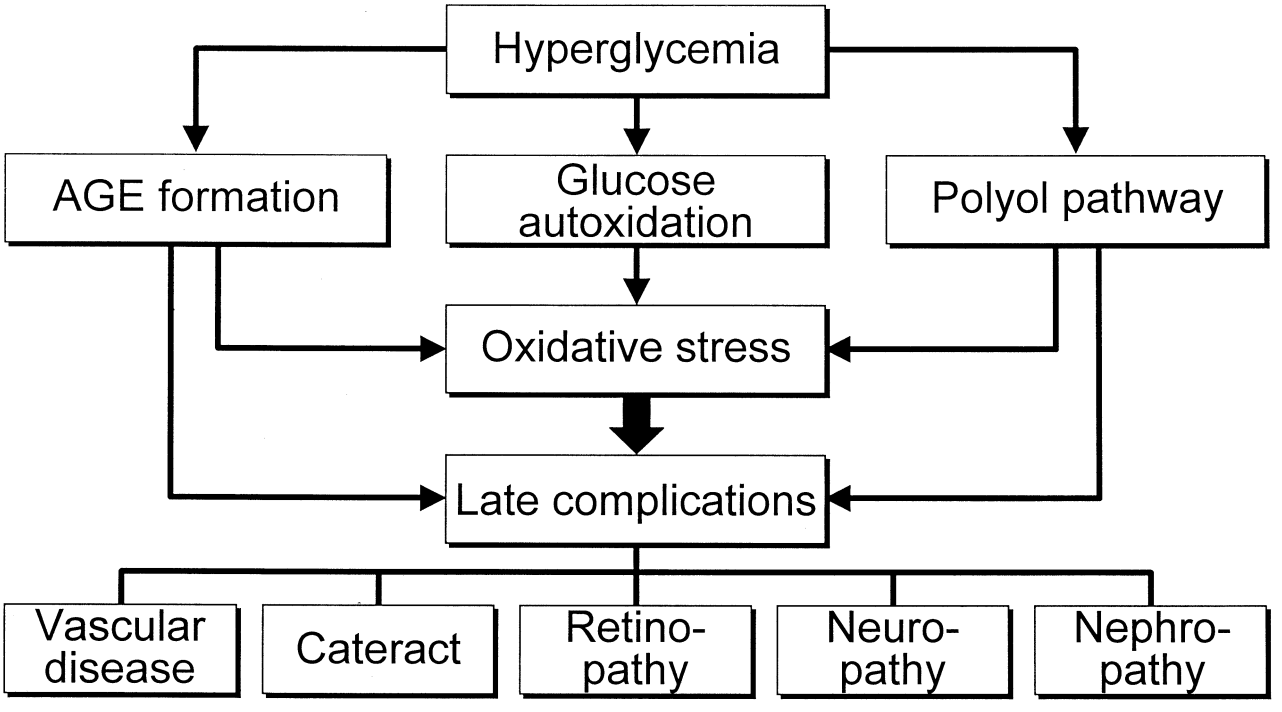

FIG. 3. Increased oxidative stress in diabetes appears to be due mainly to hyperglycemia, resulting in the stimulation of the polyol pathway and the formation of AGE and subsequent formation of reactive oxygen radicals. AGE, advanced glycation endproducts.

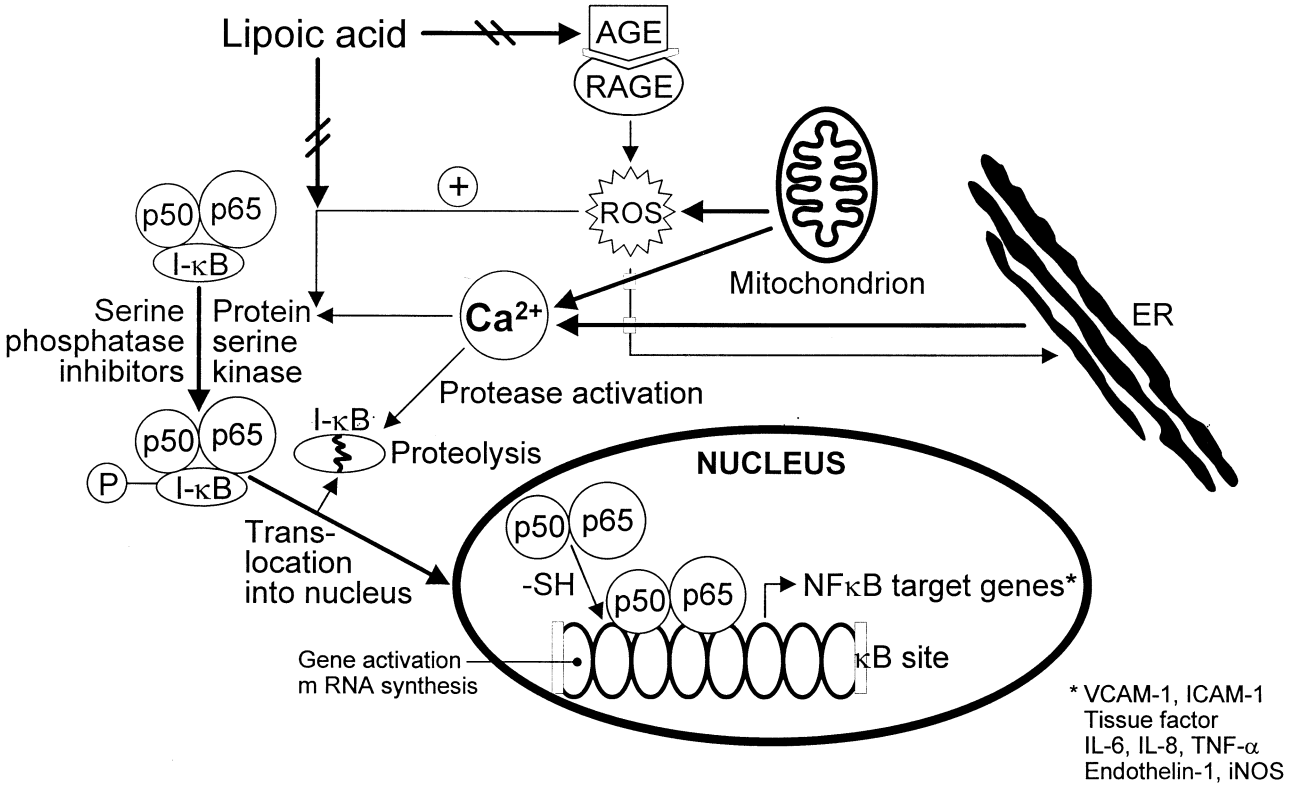

FIG. 4. The formation of AGE in vascular complications of diabetes. AGE are non-enzymatic reaction products of the aldehyde or keto group of sugars with the terminal amino group of proteins. This reaction generates superoxide and hydroxyl radicals by the autooxidation of glucose. AGE exert their damaging effects by binding to specific receptors, RAGE, on the surfaces of various cells such as endothelial cells, macrophages, neurons, and smooth muscle cells. Binding of AGE to RAGE causes oxidative stress and activates NF-κB. AGE, advanced glycation endproducts; ER, endoplasmic reticulum; ICAM, intracellular adhesion molecule; I-κB, inhibitory protein κB; iNOS, inducible nitric oxide synthase; NFκB, nuclear factor κB; IL, interleukin; RAGE, receptors of the advanced glycation endproducts; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α; VCAM, vascular cell adhesion molecule.

The polyol pathway is known as the primary cause of cataractogenesis in diabetes. Lipoic acid can exert protective effects in different ways. The reduction of R-lipoic acid by lipoamide reductase depend on NADH. Accordingly, intramitochondrial reduction of R-lipoic acid can alleviate NADH surplus in diabetes. In a model of glucose-induced lens opacity in vitro, stereospecific protection by lipoic acid was observed.[36,37] Although R-lipoic acid completely protected the lens, addition of racemic lipoic acid decreased damage only by about one-half, whereas S-lipoic acid potentiated deterioration of the lens. This result is consistent with the specific reduction of R-lipoic acid in mitochondria and its effect in enhancing GSH synthesis. In newborn rats treated with buthionine sulfoximine, a known inhibitor of GSH synthesis, lipoic acid prevented cataract formation in 60% of animals.[38]

VASCULAR DAMAGE

Endothelial cells exhibit a wide spectrum of different functions in vessel physiology and homeostasis. They regulate coagulation, leukocyte adhesion and trafficking, the tone of the vessel, and smooth-muscle growth. A series of critical cellular and molecular events occurring during the progression of vascular diseases, such as arteriosclerosis, lead to the loss of homeostatic functions of the endothelium.[39] The formation of AGE has been implicated in vascular complications of diabetes.[40] AGE are non-enzymatic reaction products of the aldehyde or keto group of sugars with the terminal amino group of proteins. This reaction generates superoxide and hydroxyl radicals by the autooxidation of glucose.[41] AGE exert their damaging effects by binding to specific receptors (RAGE) on the surfaces of various cells such as endothelial cells, macrophages, neurons, and smooth-muscle cells. Binding of AGE to RAGE causes oxidative stress and activates nuclear factor-κB (NF-κB; Fig. 4). Injury to endothelial-cell function, primarily resulting from increased oxidative stress, also leads to the activation of vascular cytokines such interleukin-1 and tumor necrosis factor α and chemoattractants such as monocyte chemoattractant protein-1 (MCP-1). These molecules in turn induce the expression of adhesion molecules on the cell surface such as vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1), which are centrally involved in the endothelial recruitment of neutrophils[42] and subsequent development or progression of atherosclerotic plaque.[43] The activation of agonist-induced ICAM-1, VCAM-1, and MCP-1 transcription in endothelial cells also depends, at least in part, on the activation of NF-κB. Importantly, activated NF-κB has been identified in situ in human atherosclerotic plaques but not in cells of normal vessels devoid of atherosclerosis.[44] Further, NF-κB activation has been shown in an arterial injury model.[45] Indeed, growing evidence indicates that NF-κB is controlled by the redox status of the cell and that generation of reactive oxygen species may be a common step in all of the signaling pathways that lead to I-κB degradation and NF-κB nuclear translocation.[46] Support for this concept comes from a variety of studies showing that the diverse agents that can activate NF-κB also elevate levels of reactive oxygen species and that chemically distinct antioxidants and overexpression of antioxidant enzymes can inhibit NF-κB activation.[47] Thus, redox regulation of signal transduction and gene expression is emerging as an important fundamental mechanism in biology. Thiol antioxidants such as

TABLE II.

CLINICAL TRIALS ON THE EFFICACY OF LIPOIC ACID IN THE TREATMENT OF DIABETIC POLYNEUROPATHY

Reference	*n* patients	Design	Dose	Treatment duration	Findings
65*	328	Parallel group	1200/600/100 mg IV	3 wk	TSS and NDS improved
66†	73	Parallel group	800 mg PO	4 mo	HRV and QTc improved
67‡	509	Parallel group	600 mg IV → 1800 mg PO 600 mg IV → placebo PO Placebo IV → placebo PO	3 wk (IV) 6 mo (PO)	TSS no change, NIS improved after 19 d, trend for improved NIS at end

* ALADIN (α-Lipoic Acid Diabetic Neuropathy Study, 1995).
† DEKAN (Deutsche Kardinale Autonome Neuropathie Studie, 1997).
‡ ALADIN III (α-Lipoic Acid Diabetic Neuropathy Study III, 1999).
HRV, heart-rate variability; IV, intravenous; NCV, nerve-conduction velocity; NDS, neuropathy disability score; NIS, neuropathy impairment score; PO, per oral; QTc, corrected QT interval; TSS, total symptom score (pain, burning, paresthesias, numbness).

GSH and lipoic acid appear to play a predominant role in the redox-dependent regulation of numerous cellular targets.[48]

In several cell lines, lipoic acid and DHLA showed a strong inhibitory effect on NF-κB-activation induced by phorbol ester, tumor necrosis factor-α, and hydrogen peroxide.[48,49] That effect seems to be mediated by a calcium-responsive element of lipoic acid. Lipoic acid at clinically relevant dosages was shown to downregulate the expression of the cell-adhesion molecules ICAM-1 and VCAM-1 in a dose-dependent manner (50 to 250 μM).[50] These observations might be of preventive and/or therapeutic benefit in arteriosclerosis and other inflammatory disorders. Moreover, lipoic acid is a potent inhibitor of AGE-induced NF-κB activation and subsequent expression of adhesion factors.[51,52] In addition to the inhibition of NF-κB activation by AGE, lipoic acid prevents protein glycation in vitro.[53] NF-κB binding activity in mononuclear cells from patients with nephropathy correlated with microalbuminuria and thrombomodulin plasma concentrations. Treatment for 3 d with 600 mg of lipoic acid improved measures of oxidative stress and reduced NF-κB activation,[54] indicating that lipoic acid is useful in the treatment of vascular dysfunction in diabetes.

POLYNEUROPATHY

Hyperglycemia and endoneural-hypoxia–causing oxidative stress have been implicated in the development of diabetic neuropathy. Hyperglycemia-induced oxidative stress was shown to induce programmed cell death of nerves and thus might contribute to the pathologies in diabetic neuropathy.[55] The roles of oxidative stress and antioxidants in nerve damage have been studied extensively in experimental diabetes and diabetic patients.[56] Motor-nerve and sensory-nerve conduction velocities are the principal endpoints in studying the therapeutic effectiveness of lipoic acid on nerve function. Lipoic acid has been reported to improve motor-nerve conduction velocity in experimental diabetic neuropathy[57,58] and protect peripheral nerves from ischemia and reperfusion injury in rats.[59] Animal data are supported by cell-culture studies in neuroblastoma cells showing that lipoic acid stimulates sprouting of neurites.[60] Diabetic neuropathy is also a major cause of male impotence. Interestingly, in a recent study, lipoic acid corrected corpus-cavernosum function in streptozotocin-induced diabetes in male rats.[61]

Patients with diabetic polyneuropathy have impaired neurovascular reflexes. That impairment is detected as a delayed decrease in microcirculation of the ipsilateral hand after a cold stimulus has been applied to the contralateral hand. In patients with diabetic polyneuropathy, capillary blood-cell flow velocity was measured by nailfold capillaroscopy before, during, and after cooling of the contralateral hand.[62] Infusion of 600 mg of lipoic acid for 3 wk significantly improved the microcirculatory response to the cold stimulus and symptoms of neuropathy decreased significantly.

Treatment of painful neuropathic symptoms and improving quality of life are of outstanding importance in the management of late-diabetic complications. Clinical trials have studied the efficacy of lipoic acid in the treatment of diabetic polyneuropathy.[62–68] In the α-Lipoic Acid Diabetic Neuropathy study (Table II), 3-wk intravenous lipoic-acid administrations of 600 and 1200 mg significantly improved clinical symptoms of neuropathy (pain, numbness, paresthesias, and burning).[65] In patients with cardiac autonomic neuropathy (the Deutsche Kardinale Autonome Neuropathie study), a daily oral dose of 800 mg of lipoic acid for 4 mo significantly improved heart-rate variability.[66] More recently, the third α-Lipoic Acid Diabetic Neuropathy study has been completed.[67] Injections with 600 mg of lipoic acid for 3 wk significantly improved the neuropathy impairment score, and there was a trend for improved neuropathy impairment scores after 7 mo in response to oral supplements of 1800 mg/d of lipoic acid. However, there was no effect on neuropathic symptoms. According to the researchers, the failing effect might have been due to intercenter variability in symptom scoring.

GLUCOSE DISPOSAL

Insulin resistance is typical for type II diabetes. Therapeutic intervention to enhance glucose uptake by skeletal muscle is potentially important for the prevention and treatment of non–insulin-dependent diabetes. As early as 1970, lipoic acid was shown to enhance glucose uptake into rat tissues.[69,70] Subsequently, obese Zucker rats, an animal model of insulin resistance, were used to investigate the effects of acute and chronic intravenous treatments with R,S-lipoic acid on glucose transport in isolated skeletal muscle.[71] Lipoic acid markedly increased net glucose uptake, which was associated with a significant enhancement of glycogen synthesis. This observation was supported by a separate experiment in vitro from the same group[13] showing an increased glucose uptake into muscle from lean (insulin-sensitive) or obese (insulin-resistant) Zucker rats. In the same model, the effect of the individual enantiomers of lipoic acid on glucose disposal, hyperinsulinemia, and dyslipidemia was studied.[72] Obese Zucker rats were treated acutely or chronically by intraperitoneal injection with R- or S-lipoic acid. Acute treatment with R-lipoic acid increased insulin-mediated glucose transport by 64%, whereas the S form showed no significant effect. Chronic R-lipoic acid administration reduced plasma insulin and free fatty acids, whereas S-lipoic acid increased insulin and had no effect on plasma free fatty acids. Further, R-lipoic acid improved insulin-stimulated glycogen syn-

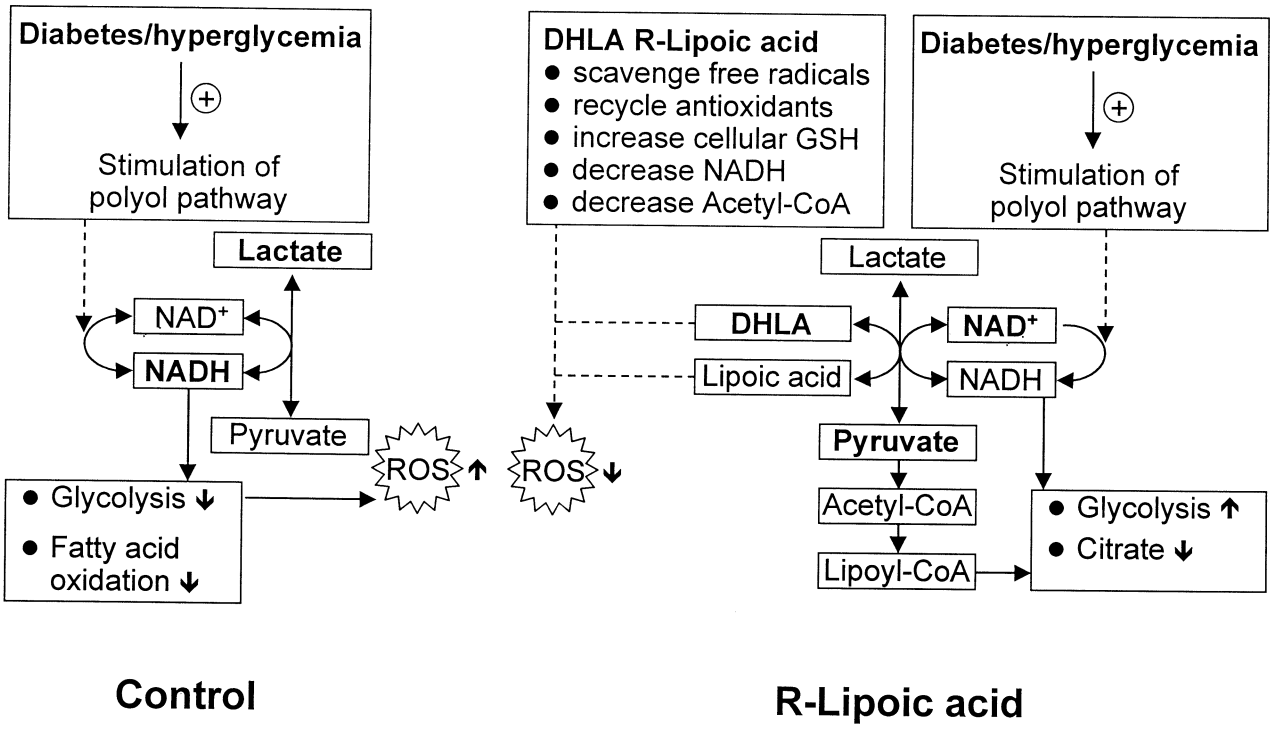

FIG. 5. NADH levels are increased in diabetes, which is attributable to a stimulation of the polyol pathway. R-Lipoic acid uses NADH for the reduction to DHLA, resulting in an increased ratio of NAD^+ to NADH and thus stimulating glycolysis. CoA, coenzyme A; DHLA, dihydrolipoic acid; GHS, glutathione; NAD, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide; ROS, reactive oxygen species.

thesis and glucose oxidation. The level of glucose transporter-4 protein was not altered after chronic treatment with R-lipoic acid but was reduced by S-lipoic acid.

More recently, in fasting non-diabetic or streptozotocin-induced diabetic rats, intravenous injection of high doses of R,S-lipoic acid rapidly reduced blood glucose at unaltered insulin levels.[73] This action was attributed to the inhibition of gluconeogenesis from alanine and pyruvate. The effect of lipoic acid on glucose uptake into heart muscle also has been investigated.[74] Glucose uptake into Langendorff hearts of insulin-resistant Zucker was measured with the [^{14}C] 3-O-methylglucose washout method. Glucose uptake rate increased 1.6-fold with R,S-lipoic acid, 1.8-fold with the R form, and was negatively influenced by the S-enantiomer (−50%).

NADH levels are increased in diabetes, which is attributable to a stimulation of the polyol pathway. R-lipoic acid uses NADH for the reduction to DHLA,[75] resulting in an increased ratio of NAD^+ to NADH and thus stimulating glycolysis (Fig. 5). Enhanced glucose uptake into tissues also might be attributed to a reduction of acetyl-CoA levels in tissues due to sequestration as lipoyl-CoA, bisnorlipoyl-CoA, or tetranorlipoyl-CoA.[76] A reduction in acetyl-CoA leads to decreased citrate levels and thus with the activation of phosphofructokinase to enhanced glycolysis. Moreover, lipoic acid has been shown to inhibit gluconeogenesis in rat liver.[76] In addition, gluconeogenesis may be reduced by an inhibitory effect of lipoic acid on biotin-dependent carboxylases such as pyruvate carboxylase, which catalyze the initial step in gluconeogenesis.[77]

Mechanistic studies in cell culture found superior glucose uptake in response to R-lipoic acid as opposed to R,S- and S-lipoic acids in L6 myotubes. R-lipoic acid restored cytokine or oxidative stress-impaired insulin-stimulated glucose uptake in L6 myotubes.[78,79] In 3T3-L1 adipocytes, R-lipoic acid translocated the glucose transporter-4 to the plasma membrane, thereby mediating the R-lipoic acid effect on glucose uptake.[80] The stimulation of glucose uptake into cells was blocked by wortmannin, an inhibitor of phosphatidylinositol-3-kinase. Phosphatidylinositol-3-kinase is also involved in insulin action, which suggests that R-lipoic acid uses at least part of the insulin-signaling cascade.

Studies on the use of R,S-lipoic acid on insulin-stimulated glucose disposal have been carried out in patients with type II diabetes. Acute intravenous administration of 1000 mg of lipoic acid significantly improved insulin-stimulated glucose disposal as assessed by the glucose clamp technique.[81] Improved insulin-stimulated glucose uptake of similar magnitude also was found in 20 patients with non–insulin-dependent diabetes after 10 d of 500-mg injections of lipoic acid.[82] In a recent multicenter trial from the same group, a 4-wk oral treatment with lipoic acid increased insulin sensitivity in patients with type II diabetes.[83] Further, a supplement of 1200 mg/d of lipoic acid over 4 wk improved glucose effectiveness after oral glucose-tolerance tests in type II diabetic patients.[84]

NATURAL VERSUS R,S- AND S-LIPOIC ACID

Until recently, chemical synthesis of lipoic acid has yielded a racemic mixture of the optically active enantiomers, the R and S forms, in a 50:50 ratio. The biopotency of the R versus S forms has not been fully established. Indepth studies addressing the exchange rate of R- versus R,S-lipoic acid are underway. However, current evidence suggests that the R form might be superior to the S form in certain model systems. In the working rat heart during reoxygenation, R-lipoic acid improved aortic flow, reaching 70% of normoxic conditions at nanomolar concentrations, whereas 1 μM of the S form was needed to achieve only 60%.[85] In the same study, R-lipoic acid added to the perfusion medium increased mitochondrial ATP synthesis of the working rat heart, whereas ATP synthesis remained unaltered in response to S-lipoic acid. Further, much higher concentrations of DHLA were detected in the effluent buffer of isolated perfused rat heart after addition of the R

form than after addition of the S form.[86] This result indicates that the high concentration of mitochondria in rat heart is responsible for reduced R-lipoic acid.

Both enantiomers of lipoic acid inhibited purified mammalian pyruvate dehydrogenase activity; in particular, the acyltransferase moiety of the enzyme complex was affected.[87] However, R-lipoic acid, as opposed to S-lipoic acid, did not inhibit pyruvate decarboxylation in HepG2 cells.

Membrane fluidity of red blood cells is reduced under hyperglycemic conditions. Lipoic acid counteracted the glucose-induced decrease in fluidity of red blood cells in vitro.[88] S-Lipoic acid increased fluidity at low concentrations. However, R-lipoic acid was more effective over a wider range of concentrations. This finding is also commensurate with the distinctive effects of the lipoic-acid enentiomers in cataractogenesis.[36,37] The favorable effects of R- versus S-lipoic acid in relation to glucose use are discussed in the section GLUCOSE DISPOSAL. Acute treatment of obese Zucker rats with R-lipoic acid increased insulin-mediated glucose transport, whereas treatment with the S form showed no significant effect. In addition, chronic treatment with R-lipoic acid reduced plasma insulin and free fatty acids, whereas treatment with S-lipoic acid increased insulin and had no effect on free fatty acids. These effects also were supported by mechanistic studies in L6 myotubes.[78]

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