THE POTENTIAL ROLE OF α-LIPOIC ACID IN THE MANAGEMENT OF DIABETES MELLITUS. POSSIBLE MOLECULAR MECHANISM

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ABSTRACT

Alpha –lipoic acid (LA) is synthesized in the liver and other tissues. In its physiological role, it acts as a co-factor in a multi-enzyme dehydrogenase complex, which generates acetyl-CoA and succinyl-CoA from pyruvate and α-ketoglutarate respectively. Acetyl-CoA and succinyl-CoA are two important compounds participating in the Krebs cycle present in mitochondria. Thus, LA is an essential compound in the generation of ATP in mitochondria. Hyperglycemia in diabetes mellitus (DM) can result in the generation of reactive oxygen species (ROS) by way of a process called glycoxidation. ROS can damage important cell components such as membrane lipids, proteins and DNA. This cell damaging action of ROS has been implicated in the development of diabetic complications such as macro-angiopathy (atherosclerosis), micro-angiopathy (retinopathy, nephropathy), neuropathy and cataract. ROS can also damage β-pancreatic cells resulting in a decrease in insulin secretion. There is also evidence that ROS can cause insulin resistance, although its exact molecular mechanism remains to be elucidated LA and its reduced form: dihydrolipoic acid (DHLA) do not appear to be present in the unbound state under normal conditions but are bound as lipoamide and dihydrolipoamide to a lysine residue present in dihydrolipoyl-transacetylase, one of the 3 subunits forming the multi-enzyme dehydrogenase complex. However, after dietary supplementation, both forms appear in various tissues in unbound forms. Exogenous LA is enzymatically reduced to DHLA. This latter compound is a strong anti-oxidant capable of scavenging ROS and regenerating endogenous anti-oxidants such as vitamin C, vitamin E and glutathione. LA has also been shown to bind Fe^{2+}, a transition metal ion required for the generation of hydroxyl radical (·OH) the most active ROS. Thus the main potential role of LA in the management of DM is to prevent the detrimental effect of ROS generated by glycoxidation including the prevention or alleviation of glucose resistance .In the case of diabetic neuropathy, other potential roles include the enhancement of ATP production since glucose uptake in neurons is not insulin dependent, and possibly also increased synthesis of the neurotransmitter acetylcholine due to the increased availability of acetyl-CoA.

***Keywords**: α-lipoic acid, diabetes mellitus, reactive oxygen species (ROS), diabetic complication*

INTRODUCTION

In 1937, a growth factor present in potato extracts was found to be necessary for the growth of Lactobacillus. Subsequently, the growth factor was isolated, purified and identified as α-lipoic acid (LA), see Figure 1

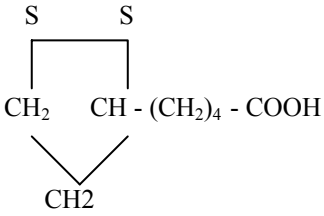


Figure 1. α – Lipoic acid (LA)

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LA can be reduced chemically or enzymatically to its dithiol form, dihydrolipoic acid (DHLA), see Figure. 2

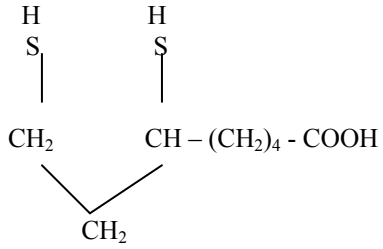


Figure 2. Dihydrolipoic acid (DHLA)

When first isolated, LA was tentatively classified as a vitamin, but it was later found to be synthesized by animals and humans. LA has been prescribed in Germany for many years to treat diabetic neuropathy, despite the fact that in the beginning the exact cause of this condition was unknown. The sound rationale behind this treatment was discovered very much later when it became known that hyperglycemia can generate reactive oxygen species (ROS), and that ROS is involved in

many diabetic complications including diabetic neuropathy

THE NORMAL PHYSIOLOGICAL ROLE OF LA

LA in humans s synthetized mainly in the liver and some other tissues. However, under normal conditions LA (and its reduced form : DHLA) is never found in its unbound form in body tissues, rather it is found in the amide form bound to a lysine residue in dihydrolipoyl - transacetylase, one of the 3 subunits of a multi-enzyme 2-oxo-acyl-dehydrogenase.complex. Only when exogenous LA is supplemented, both forms can be found as the unbound state in various tissues. The enzyme 2-oxo-acyl-dehydrogenase catalyzes the oxidative decarboxylation of pyruvate and α-ketoglutarate to acetyl-CoA and succinyl-CoA respectively. The overall reaction is as follows:

$$R\text{-}CO\text{-}COO^- + NAD^+ + CoA\text{-}SH \rightarrow CO_2 + NADH + R\text{-}CO\text{-}SCoA$$

The oxidative decarboxylation of pyruvate and α-ketoglutarate is a very complicated process catalyzed by 3 subunits of the 2-oxo-acyl-dehydrogenase multi-enzyme complex as follows:

1. $R\text{-}CO\text{-}COOH + TPP\text{-}E_1 \rightarrow R\text{-}COH\text{-}TPP\text{-}E_1 + CO_2$
   TPP = thiamin pyrophosphate
   E_1 = 2-oxo-acyl-decarboxylase
2. $R\text{-}COH\text{-}TPP\text{-}E_1 + lipoamide\text{-}E_2 \rightarrow R\text{-}CO\text{-}hydro\text{-}lipoamide\text{-}E_2$
   E2 = dihydrolipoyl-transacetylase
3. $R\text{-}CO\text{-}hydrolipoamide\text{-}E_2 + CoA\text{-}SH \rightarrow dihydrolipoamide\text{-}E_2 + R\text{-}CO\text{-}S\text{-}CoA$
4. $dihydro\text{-}lipoamide\text{-}E_2 + FAD\text{-}E_3 \rightarrow lipoamide\text{-}E_2 + FADH_2\text{-}E_3$
   E_3 = dihydrolipoyl-dehydrogenase
5. $FADH_2\text{-}E_3 + NAD^+ \rightarrow FAD\text{-}E_3 + NADH + H^+$

Both acetyl-CoA and succinyl-CoA are important components of the tricarboxylic (Krebs) cycle present in mitochondria, an essential pathway leading to the generation of ATP. Acetyl-CoA is also required for the generation of the neurotransmitter acetyl-choline, catalized by choline acetyl-transferase:

$$\underset{\text{Acetyl-CoA}}{CH_3\text{-}CO\text{-}S\text{-}CoA} + \underset{\text{choline}}{CH_2OH\text{-}(CH2)_2\text{-}N^+(CH_3)_3} \rightarrow \underset{\text{acetyl-choline}}{CH_3\text{-}CO\text{-}O\text{-}CH_2\text{-}(CH_3)_2\text{-}N^+(CH_3)} + CoASH$$

OXIDATIVE STRESS IN DIABETES MELLITUS

Diabetes can cause oxidative stress, manifested by an increase in lipid peroxidation.as shown e.g. by an increase in malon-dialdehyde (MDA) and a decrease of anti-oxidant status as shown by a decrease in vitamin C (ascorbic acid), vitamin E (tocopherol) and glutathione. The increase of lipid peroxidation and decrease in anti-oxidant status is due to the generation of reactive oxygen species (ROS) in the presence of hyperglygemia by a process called glycoxidation. Glycoxidation generates superoxide anions ($\cdot O_2^-$) which on further processing results in the formation of other ROS such as hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\cdot OH$). A simplified scheme of the production of $\cdot O2^-$ by glycoxidation of glucose is as follows:

1. R- CH - C = O → R- C = C - H
   | | | |
   OH H OH OH
   glucose enediol tautomer of glucose

   (R = CH2OH – CHOH – CHOH – CHOH -)

2. R – C = C– H + Fe^{3+} + O_2 → R – C = C –H + Fe^{2+} + $•O_2^-$ + $2H^+$

   OH OH O• O•
   enediol diradical

3. R – C = C – H → R – C – C - H

   O• O• O O
   glucosone

Other ROS can be generated :

1. $•O_2^-$ + H^+ → $•O_2H$
   peroxyl radical

2. 2 •OH → O_2 + H_2O_2
   hydrogen peroxide

3. Fe^{2+} (Cu^{+}) + H_2O_2 → Fe^{3+} (Cu^{2+}) + OH^- + •OH (Fenton reaction)
   hydroxyl radical

ROS are all oxidants capable of oxidizing and damaging important cell components such as lipids in cell membranes, proteins and DNA. Free radicals, such as hydroxyl radicals (•OH) are especially dangerous because of its reactivity and its capability of initiating chain reactions. Cell membranes contain poly-unsaturated fatty acids (PUFA),which are especially prone to attack by free radicals. The chain reaction caused by hydroxyl radicals (and other free radicals) is called lipid peroxidation and proceeds in 3 stages as follows :

1. initiation :

   •OH + L_1H → H_2O + L_1•
   lipid (PUFA) lipid radical

2. propagation :

   •L_1 + O_2 → •L_1OO
   lipid peroxy-radical

   •L_1OO + L_2H → L_1OOH + •L_2
   lipid peroxide

   •L_2 + L_3H → L_2H + •L_3 etc.

3. termination :

   •L_1 + •L_2 → L_1–L_2

   •L_2 + •L_3 → L_2–L_3

The cross-linking of the lipid chain (L_1-L_2 and L_2-L_3) will cause membrane damage and disturbs the cell osmotic balance causing water to enter the cell and eventually causing cell lysis. The lipid peroxide (LOOH) undergoes further reaction eventually causing the generation of toxic compounds such as aldehydes: malondialdehyde (MDA), 9-hydroxy-nonenal and hydrocarbons: ethane, pentane and ethylene. Thus lipid peroxidation is a very dangerous event.

Attack by ROS on proteins can cause the loss of the protein's biological functions. Thus if the protein's biological functions are important for the cell's survival (e.g. enzymes or other proteins necessary for ATP generation), the survival of the cell will be in jeopardy. Attack on DNA will cause DNA damage, which when not repaired may cause mutations and if DNA is irreparably damaged, the cell will commit suicide by apoptosis.

Under normal condition, ROS is produced daily by mitochondria as a by-product of oxidative phosphorylation (oxphos). The amount of ROS (in the form of superoxide) is quite substantial. It has been estimated that a normal average person generates 1 kg $•O_2^-$ per year which is equivalent to 1000 : 365 = 2.74 g /day. This amount is enough to cause cell damage. Fortunately our body is equipped with anti-oxidants, which under normal condition can neutralize ROS. This anti-oxidants defense system consists of metal chelating

proteins (e.g., ferritin), enzymes (superoxide dismutase, catalase, glutathione peroxidase) and native anti-oxidants (tocopherol, ascorbic acid and glutathione)

The iron binding protein ferritin binds Fe^{2+} and Cu^{+}, thus preventing the formation of ·OH from H_2O by the Fenton reaction. Accumulation of $\cdot O_2^-$ is prevented by superoxide dismutase (SOD):

$$2 \cdot O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Accumulation of H_2O_2 is prevented by 2 enzymes, catalase and glutathione peroxidase (GPx) as follows:

a. Catalase : $2H_2O_2 \rightarrow 2H_2O + O_2$
b. GPx : $2GSH + H_2O_2 \rightarrow GSSG + 2\ H_2O$
glutathione oxidized glutathione

Glutathione is restored by the action of glutathione reductase :

$$GSSG + NADPH + H^+ \rightarrow 2\ GSH + NADP^+$$

NADPH is restored by the action of glucose-6-phosphate dehydrogenase (G6PD)

Glucose-6-P + $NADP^+$ → 6-phosphogluconate + NADPH

Glutathione can also scavenge ·OH :

$$2\ GSH + 2 \cdot OH \rightarrow 2\ H_2O + GSSG$$

During hyperglycemia, ROS generation exceeds the capacity of the body anti-oxidant defences resulting in ROS accumulation and oxidative stress (see above). Oxidative stress during hyperglycemia likely play a causative role in the tissue and cellular damage associated with diabetic complications, such as damage to neurons (neuropathy), to small blood vessels in the eye (retinopathy) and kidney (nephropathy), to lens fibers (cataract), and to low density lipoprotein (atherosclerosis) Hyperglycemia via the generation of ROS and ensuing oxidative stress, may even alter or influence the clinical course of diabetes. Oxidative stress to β-pancreatic cells may damage or kill these cells, thus lowering insulin secretion. There are also enough evidences that hyperglycemia can aggravate insulin resistance, although the exact mechanism of this action is as yet not completely understood.

One important action of insulin is to increase glucose uptake in muscles and fat cells. Glucose uptake by cells is mediated by special glucose transporter proteins (GLUTs). There are a variety of GLUTs, all of which are not regulated by insulin except GLUT- 4, the specific transporter for muscles and fat cells. Insulin is able to control the uptake of glucose in these cells by triggering the translocation of this transporter from the interior to the surface of these cells upon binding to the insulin receptor, thus up-regulating the glucose uptake.

Upon binding to its receptor (insulin receptor, IR), the insulin signal is transduced by activating one after another an array of transducing molecules until finally generating the specific effect attributed to insulin. There are a number of transducing pathways activated by insulin, each generating a specific effect, most of these must go through the first transducing molecule called IRS-1 (insulin receptor substrate-1), including the signal that trigger the translocation of GLUT-4 to the surface of the cell

In normal physiologic condition, when insulin binds to IR, the receptor becomes activated and then attracts and phosphorylates IRS-1 at specific tyrosine.residues. Phosphorylation of IRS-1 activates the molecule, and it is then able to activate the next transducing molecule. In insulin resistance, insulin is unable to up-regulate glucose uptake, either because it is unable to bind to IR or because its transducing pathway is interrupted.

By a still unknown mechanism, oxidative stress can stimulate serine-threonine protein kinases belonging to the MAPK (mitogen activated protein kinase) pathway and the JNK (c-Jun kinase) pathway, also known as the SAPK (stress activated protein kinase) pathway. These serine-threonine protein kinase phosphorylate IRS-1 on specific serine and threonine residues resulting in the inability of IRS-1 to bind to IR, thus interrupting the signaling pathway that triggers the transport of GLUT-4 to the cell's surface and inability to upregulate glucose uptake

THE THERAPEUTIC ROLE OF A-LIPOIC ACID IN THE MANAGEMENT OF DIABETES

Exogenous LA is readily reduced to DHLA by lipoamide dehydrogenase. LA and DHLA forms a redox couple with a redox potential Eó = - 0.29V, thus making DHLA a strong reducing agent Considering this fact, LA should be a good candidate for the treatment of diabetic complications due to hyperglycemia induced oxidative stress, by either neutralizing or inhibiting the formation of ROS and thus preventing or alleviating the effect of ROS.

An ideal anti-oxidant should show the following characteristics:
1. It should not be toxic, carcinogenic or teratogenic
2. It should be able to scavenge ROS

3. It should be able to regenerate native anti-oxidants (glutathione, vit. C and vit E)
4. It should be both lipid and water soluble, so that it can act both within cell membranes and in aqueous phase (cytosol and extra-cellular fluid)

LA seems to fulfill all 4 criteria remarkably well and thus can be considered as an "ideal" anti-oxidant. Both LA and DHLA may play a role in alleviating the effect of hyperglycemic oxidative stress. DHLA can scavenge both $\bullet O_2^-$ and •OH as follows :

$$2\ \bullet O_2^- + 2H^+ + \underset{\text{DHLA}}{L(SH)_2} \rightarrow \underset{\text{LA}}{L(S)_2} + 2H_2O_2$$

$$2\ \bullet OH + L(SH)_2 \rightarrow L(S)_2 + H_2O$$

DHLA can also regenerate glutathione directly:

$$GSSG + L(SH)_2 \rightarrow 2\ GSH + L(S)_2$$

DHLA can also regenerate ascorbic acid (vitamin C) directly by reacting with DHAA (dehydro-ascorbic acid) :

$$2\ \underset{\text{dehydro-ascorbic acid}}{DHAA} + L(SH)_2 \rightarrow 2\ AscH_2 + \underset{\text{vit.C}}{L(S)_2}$$

DHAA results when 2 vit.C radicals (ascorbyl radical : AscH•) reacts with each other :

$$2\ AscH\bullet \rightarrow AscH_2 + DHAA$$

By its ability to regenerate glutathione, DHLA can also indirectly regenerate vit. E (tocopherol)

$$GSSG + L(SH)_2 \rightarrow 2\ GSH + L(S)_2$$

$$2\ \underset{\text{tocopheryl radical}}{Toc\bullet} + 2\ GSH \rightarrow 2\ TocH + \underset{\text{vit.E}}{GSSG}$$

Thus DHLA can either directly or indirectly regenerate glutathione, vit.C and vit.E. LA can sequester Fe^{2+} and Cu^+ ions and thus prevent the formation of •OH via the Fenton reaction. LA, like DHLA can apparently scavenge •OH although the product of its reaction has never been determined. A likely product could be either dihydroxylipoic acic or di-oxo-lipoic acid as follows:

$$L(S)_2 + 2\ \bullet OH \rightarrow \underset{\text{dihydroxy LA}}{L(SOH)_2}$$

or alternatively :

$$2\ L(S)_2 + 2 \bullet OH \rightarrow \underset{\text{di-oxo-LA}}{L(SO)_2} + L(SH)_2$$

CONCLUDING REMARKS

Considering all what have been discussed previously, LA should be a good candidate for preventing or alleviating the detrimental impact of hyperglycemia-induced oxidative stress including preventing the β-pancreatic cell damage and insulin resistance. Other benefits can be considered. For instance, in cells whose glucose uptake is not regulated by insulin, such as neurons, supplementation of exogenous LA increase the availability of acetyl-CoA. The increased availability of acetyl-CoA may enhance the production of ATP and acetyl-choline, which may well be beneficial in the treatment of diabetic neuropathy.

There are, however, some caveats to be considered:

1. Many of the beneficial effect of LA have only been shown in animal studies, whether such effects also occur in humans remain to be seen.
2. Clinical studies have shown that i.v. administration of LA appeared to be more efficacious than oral administration, including i.e. in the treatment of diabetic neuropathy and the ability to increase insulin sensitivity.
3. Diabetic complications develop over a long time, sometimes years or even decades, it will be thus unrealistic to expect dramatic improvements in only days or weeks.
4. Although in both animal and human studies, LA appears to be non-toxic (it can safely be given up to a daily dose of 1800 mg) and no evidence suggests carcinogenic or teratogenic effects, it would probably be wise to recommend that pregnant women should avoid taking supplemental LA until more data are available
5. Since adverse effects are noted in thiamin deficient rats given high dose of LA, it may be prudent that supplemental thiamin should be given in the presence of thiamine deficiency.

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