Lipoic Acid in the Prevention of Acute Kidney Injury

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\textsuperscript{α}-Lipoic acid · Antioxidant · Hypoxia · Cardioprotection · Renoprotection · Reactive oxygen species · Oxidative stress · Ischemia-reperfusion injury · Dihydrolipoic acid · Phosphatidylinositol 3-kinase/Akt pathway

Abstract
Hypoxia, reactive oxygen species (ROS) and oxidative stress contribute to contrast-induced acute kidney injury (CI-AKI) and ischemic reperfusion injury (IRI) in the kidney and heart. Imbalance between the increased formation of ROS by hypoxia in the cardiac and renal tissue and the low availability of endogenous antioxidants is a common cause of cellular and tissue damage. Therefore, a strategy to inhibit ROS generation or to scavenger free radicals becomes an important intervention to prevent CI-AKI and myocardial IRI. Evidence has shown that a naturally occurring cellular antioxidant lipoic acid (LA) (1,2-dithilane-3-pentanoic acid) acts as a free radical scavenger of ROS and reactive nitrogen oxide species for cardioprotection and renoprotection. The mechanisms whereby LA exerts its protective effects are not entirely understood, but may be related to the phosphatidylinositol 3-kinase/Akt/Nrf2 pathway and the PI3-kinase/Akt pathways. This review will provide the current information of LA as an exogenous antioxidant for cardioprotection and reno-

Introduction
Contrast-induced acute kidney injury (CI-AKI) is a common cause of hospital-acquired renal failure, with substantial morbidity, prolonged lengths of stay, higher hospitalization costs and increased mortality [1]. The incidence of CI-AKI varies greatly in patients from <1 to 10–20\%, depending on risk factors [2]. Renal hypoxia leading to reactive oxygen species (ROS) and oxidative stress have been proposed to contribute to CI-AKI [3, 4]. Renal hypoxia promotes further ischemic renal injury by the increase of free radicals through oxidative stress [5]. Imbalance between decreased antioxidant reservation in the kidney tissue and increased formation of ROS by hy-
poxia gives rise to renal injury [6]. In the rat renal tubular cell line (NRK-52E) exposed to iopromide, it was found that ROS-mediated endoplasmic reticulum stress is involved in contrast-induced renal tubular cell apoptosis [7]. Therefore, a strategy to inhibit ROS generation or scavenger free radicals becomes an important intervention to prevent CI-AKI [3]. Interestingly, a naturally occurring cellular antioxidant lipoic acid (LA) (1,2-dithiolane-3-pentanoic acid) was reported to have potent antioxidant property, and it can serve as a free radical scavenger of ROS and reactive nitrogen oxide species (RNOS) [8–14].

In terms of organ interplay between the kidney and heart, hypoxia, ROS and oxidative stress contribute not only to CI-AKI, but also to myocardial ischemic reperfusion injury (IRI) [15, 16]. In this context, LA has been shown to improve glucose and ascorbate handling, increase endothelial nitric oxide synthase activity, activate phase II detoxification via the transcription factor Nrf2 and lower expression of matrix metalloproteinase-9 and thus the biological antioxidant LA, without being toxic to rats [28, 29]. The mechanisms whereby LA exerts its protective effects are not entirely understood, but may be related to the phosphatidylinositol 3-kinase (PI3K)/Akt/Nrf2 pathway [23] and the PI3-kinase/Akt pathway, as ischemic preconditioning (IPC) reduces IRI of the rat liver via these pathways [30].

This review will provide the current information of LA as an exogenous antioxidant for cardioprotection and renoprotection, with emphasis on antioxidant functions of LA and multiple signaling pathways underlying the protective effects of LA on CI-AKI as well as cardiac and renal IRI.

**Chemical Structures of LA**

A naturally occurring LA, in the form of lipoyllysine, is extensively present in vegetables and in animal tissue. Based on lipoyllysine concentration, the decreased order of its appearance in vegetables and in animal tissues are spinach, broccoli and tomatoes and the kidney, heart and liver, respectively [11]. Normal mammalian cells are capable of taking up α-lipoic acid (α-LA), reducing it to dihydrolipoic acid (DHLA) and releasing DHLA. Therefore, if α-LA is administered extracellularly, the effects of α-LA and DHLA may be present both intracellularly and extracellularly [8]. Chemically, α-LA is a disulfide derivative of octanoic acid that forms an intramolecular disulfide bond in its oxidized form. The 2 sulfur atoms in the 1,2-dithiolane ring confers upon LA a high tendency for reduction of other redox-sensitive molecules. In contrast, the presence of 2 – SH groups in DHLA makes it more effective, on a molar basis, than glutathione (GSH) or N-acetylcysteine in protecting against α1-antiproteinase [8–14].

**Antioxidant Functions of LA**

Although the question of whether reduced form DHLA acts as pro-oxidant in biological system remains, the overwhelming evidence showed that both α-LA and DHLA have potent antioxidant functions, and thus they have been referred to as a 'universal antioxidant' [8]. As amphipathic molecules, they may act as antioxidants both in hydrophilic and lipophilic environments.

Both α-LA and DHLA act as a free radical scavenger of ROS and RNOS (e.g., peroxynitrite, nitroxyl and nitrogen dioxide). These reactive species are by-products of oxidative metabolism. An increase in these species levels can damage macromolecules (e.g., lipids, proteins and DNA). Scavenging activity of LA thus can decreases oxidative stress (an imbalance of oxidant production and the antioxidant capacity) and protect against oxidative injury. The molecule α-LA (1,2-dithiolane-3-pentanoic acid) naturally exists in both prokaryotic and eukaryotic cells. Studies have shown that both α-LA and DHLA efficiently protect against cellular damage by peroxynitrite [9, 12, 13]. Moreover, peroxynitrite-derived carbonate and nitrogen dioxide radicals readily react with α-LA and DHLA [14]. The protection against peroxynitrite-mediated cellular damage is particularly furnished by oxidized and reduced forms of LA, and thus the biological antioxidant effects of LA may act as a scavenging agent for ROS and RNOS [8, 9]. In addition, α-LA and DHLA have an activity to chelate metals, are capable of interacting with other oxidants, have significant effect on gene expression and inhibit apoptosis [8, 10–12] (fig. 1, 2).

**Cardioprotection of LA**

The antioxidant functions of LA have important implications in the prevention and treatment of oxidation-related damage. In addition to anti-ROS and anti-RNOS generation involving hypoxic/reoxygenation and peroxi-
Fig. 1. A schematic diagram illustrating the antioxidant functions of α-LA.

Fig. 2. A schematic diagram illustrating the antioxidant functions of DHLA.
dative damage, multiple beneficial effects (e.g., anti-IRI, anti-apoptotic, anti-inflammatory and anti-enzyme effects; fig. 3) have been reported in animal models associated with LA administration [18–23, 31, 32]. These results indicate that LA therapy may serve as a novel treatment modality in clinical setting.

Treatment of rats with DHLA resulted in the recovery of the contractile function, significantly changed mitochondrial parameters (reduced ATPase activity, increased ATP synthesis) and decreased the release of creatine kinase (CK) [18]. Pretreatment of rats with schisandrin B (a Chinese herb as an antioxidant) and α-LA recovered the contractile force, inhibited the leakage of lactate dehydrogenase (LDH) and increased myocardial levels of cellular non-enzymatic antioxidants, such as GSH, vitamin C (ascorbic acid) and vitamin E (α-tocopherol) [19]. Pretreatment of myocardial IRI rat model with α-LA also reduced the infarct size, and the effect may be attributed to decreased myocardial apoptosis via suppression of ROS generation and mitogen-activated protein kinase (MAPK) activity (increased activity of pERK1/2 and decreased activity of pJNK1/2) [20]. Administration of LA before reperfusion in rats has shown to protect against myocardial IRI via antioxidant, anti-apoptotic, anti-inflammatory and antioxidant enzyme effects [21]. The activities of CK and antioxidant enzymes, myocardial apoptosis, oxidant stress-related parameters, such as malondialdehyde (MDA) contents, superoxide dismutase (SOD) and catalase (CAT) were attenuated, but GSH and total antioxidant activity significantly increased [21]. DNA fragmentation in apoptosis was blocked [21]. The protein expression of NF-κB, the mRNA expression of cyclooxygenase-2 (COX-2) and the expression of intercellular adhesion molecule-1 (ICAM-1) were decreased [21]. An in vivo study with dietary supplementation of LA to rat has shown that the thiocyst LA protected against IRI in the isolated perfused Langendorff heart.

Moreover, thiocyst LA decreased the appearance of fluorescent lipid peroxidation products after ischemia reperfusion and lowered the rate of lipid peroxidation in heart homogenates [31]. The protection may be attributed to the antioxidant mechanism resulting from the couple of
thioctic LA and DHLA [31]. DHLA has been reported to prevent hypoxic/reoxygenation and peroxidative damage in rat heart mitochondria [31]. Other rodent studies also demonstrated that the combination of exogenous DHLA with high endogenous vitamin E highly improved cardiac recovery during post-ischemic reperfusion [32], and DHLA prevented hypoxic/reoxygenation and peroxidative damage in the mitochondria of the heart [33].

**Mechanisms of LA Cardioprotection**

The mechanisms by which LA protects against myocardial IRI are multifactorial. In a Langendorff model of IRI in rats and in cultured cardiomyocytes, the cardioprotective effects of LA are demonstrated to involve in aldehyde dehydrogenase 2 (ALDH2) activation, and the regulatory effect on ALDH2 activity further depends on PKCe signaling pathway [22]. In rat hearts subjected to myocardial IRI, LA reduced the release of LDH and CK, attenuated the size of infarction, reduced necrosis and apoptosis of cardiomyocytes and inflammation, and recovered cardiac function [23]. In addition, LA increased the heme oxygenase (HO-1) gene transcription and expression and the redox-sensitive transcription factor 2 (Nrf2) nuclear translocation [23]. The increased expression of HO-1 has a capacity to respond to oxidative stress, hypoxia, cytokines, etc., while Nrf2 is capable of surviving cells due to IRI, thus both of them are believed to be cytoprotective genes [23]. Therefore, it is suggested that the cardioprotection is via activating the PI3K/Akt pathway as well as subsequent Nrf2 nuclear translocation and induction of HO-2 [23]. The PI3-kinase/Akt pathway in rat IRI model is consistent with that in rat liver IRI model [30]. Mechanisms involving MAPK [20], ALDH2 activation [22], mitochondrial damage of cardiomyocytes (mitochondria, a preferable site for the action of DHLA) [33] were also proposed.

**Renoprotection of LA**

Figure 4 illustrates the renoprotective effects exerted by LA. An animal model has shown that LA protects against ischemic acute renal failure, as exemplified by at-

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**Fig. 4.** A schematic diagram illustrating renoprotective effects exerted by LA.

Potential renoprotection from LA

- **Anti-oxidative functions**
  - Restore diminished activities of SOD GSH peroxide, CAT GSH reductase
  - Decrease iNOS and lipid peroxidase
  - Increase GSH and total antioxidant activity (T-AOC)

- **Anti-ischemic reperfusion-induced injury**
  - Attenuate BUN, plasma concentration of creatinine (PcR), urinary osmolality, Scr, fractional excretion of Na+
  - Ameliorate tissue damage: tubular necrosis, tubular dilatation, proteinaceous casts, medullary congestion
  - Regenerate tubular epithelium
  - Increase Na+/K+-ATPase activity

- **Anti-inflammatory effects**
  - Decrease ET-1 content
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tension of blood urea nitrogen (BUN), plasma concentration of creatinine, urinary osmolality, creatinine clearance (SCr), and fractional excretion of Na+, as well as attenuation of tubular necrosis, proteinaceous casts and medullary congestion in the renal tissue [24]. The protective effects may relate in part to decreased content of endothelin-1 (ET-1) in the kidney [24]. Pretreatment of IRI rat model with α-LA led to decreases in SCr, BUN, LDH, IL-1β, IL-6, TNF-α, 8-hydroxydeoxyguanosine, MDA, myeloperoxidase, collagen levels and chemiluminescence levels, whereas α-LA resulted in increases in total antioxidant capacity and reduced GSH and Na+/K+-ATPase activity [25]. Histopathology revealed that α-LA regenerated and reduced tubular dilation and regenerated tubular epithelium [25]. In a rat model with IRI induced renal dysfunction, intrarenal vasoconstriction, related to a shift in the balance between ET-1 and nitric oxide attracted attention was detected [26]. It was also found that α-LA prevented eNOS and neuronal NOS, but decreased inducible NOS [26]. Meanwhile, α-LA decreased expression levels of ET-1 [26]. Both in vitro and in vivo studies revealed that a new α-LA (DHL-HisZn, sodium zinc histidine dithiooctanamide) reduced serum levels of BUN and SCr, decreased MDA levels and ROS levels as well as alleviated the severity of kidney lesions (tubular cell necrosis, cytoplasmic vacoulation, hemorrhage and tubular dilatation) [27]. No acute, subacute and long-term toxicity by α-LA were found in both in vitro and in vivo studies [28, 29]. LA was also shown to protect cisplatin-induced nephrotoxicity via oxidant defense system [29, 34]. This is of particular interest because it was reported that in a patient treated with cisplatin, contrast media induced irreversible acute renal failure [35].

**Mechanisms of LA Renoprotection**

Mechanisms by which LA exhibits renoprotective effects may be multifactorial as well. The renoprotection by LA may be due to the suppression of overproduction of ET-1 [24], or may be partially attributed to inhibit neutrophil infiltration and to balance oxidant–antioxidant status and regulate the generation of inflammatory mediators [25], or preservation of normal activities of local vasopressin/cAMP, nitric oxide/cGMP and ET systems [26]. Another proposed mechanism seems likely to involve restoration of diminished activities of renal SOD, CAT, GSH peroxidase and GSH reductase and to suppress elevated lipid peroxidation [11].

**Clinical Implications of LA**

DHLA cannot be used as a drug because it is unstable and oxidizes rapidly. In contrast, α-LA stabilizes the major intracellular antioxidant system. Furthermore, the administration of the oxidized form of LA to the body can produce the reduced form DHLA via enzymatic and non-enzymatic equilibrium mechanisms [18]. Since diabetics have increased levels of lipid hydroperoxidase, DNA adducts and protein carbonyls and since there are several possible sources of oxidative stress in diabetes, α-LA markedly reduced the symptoms of diabetic pathology in clinical studies, thus preventing diabetes complication [36].

**PI3K/Akt Pathway**

It has been postulated that all of these protective effects are mediated by activating PI3K-Akt pathway. In this context, the PI3K/Akt pathway plays important roles in cellular survival, myocardial preconditioning, IRI, myocardial contractility and local inflammation [37–39].

*The Role of PI3K/Akt Pathway in Cellular Survival*

Activation of PI3K enhances cell survival and antagonizes apoptosis in cardiomyocytes, cardiac fibroblasts, vascular smooth muscle cells and endothelial cells. The anti-apoptotic action of Akt/protein kinase B (PKB) involves both cytoplasmic and nuclear compartments via negative regulation of pro-apoptotic proteins, Bad, caspase-3, etc. [37, 38]. In addition, the hypoxia-regulated Akt pathway also plays a role in anti-apoptotic effect via microRNA-21 (miR-21)-dependent suppression of Fas ligand [39]. It was found that prolonged hypoxia depressed miR-21, which regulates phosphatase and tensin homologue deleted on chromosome 10 (PTEN) and targets the Fas ligand. Importantly, hypoxia-induced down-regulation of miR-21 and upregulation of Fas ligand and PTEN can be reversed by activated Akt [39].

*The Role of PI3K/Akt Pathway in Myocardial Preconditioning and IRI*

In acute IPC, Akt/PKB activation resulting in cardioprotection by IPC may be attributed to enhancement of cellular survival and metabolic shifts by Akt/PKB stimulation and/or inhibition of glycogen synthase kinase 3β (GSK3β) in response to phosphorylation increased by IPC [37]. The IPC-mediated cardioprotection is primarily via the activation of the PI3K-PKB/Akt reperfusion

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injury salvage kinase pathway, in which enhanced phosphorylation of Akt/PKB and GSK3β exert better functional recovery and reduces cell death [37, 40].

The Role of PI3K/Akt Pathway in Myocardial Contractility

PI3Kα (and Akt/PKB) can influence myocardial contractile strength due to increased expression of multiple Ca2+-regulating proteins [37]. In the PTEN/PI3K signaling pathway, PTEN results in a dramatic decrease in cardiac contractility, while PI3Kγ exhibits an increase in cardiac contractility. Loss of PI3Kγ is associated with substantial increases in contractility and relaxation, and thus PI3Kγ acts as a negative regulation of cardiac contractility, and it can modulate contractility in the absence of exogenous agonist [41]. In addition, Akt (a serine-threonine kinase) regulates the contractile function in myocardocytes and increases inotropism through the functional regulation of Ca++-handling proteins [37].

The Role of PI3K/Akt Pathway in Anti-Inflammation

The PI3K/Akt pathway plays a key role in inflammation. In animal models of IRI of the kidney and intestine, erythropoietin (a glycoprotein cytokine) has been shown to have significant protective effects of IRI. In a rat model of myocardial IRI, pretreatment of erythropoietin led to significant decrease in the levels of proinflammatory cytokines (IL-6, IL-1β and TNF-α). The effects of EPO were found to be associated with the activation of PI3K/Akt signaling, which suppressed the inflammatory responses [42]. Similarly, in a rat model with severe acute pancreatitis, Akt expression in pancreas was significantly increased, along with the activation of cytokines (TNF-α, IL-1β, and IL-6, IL-1β and TNF-α). The administration of wortmannin (a PI3K/Akt inhibitor) reduced Akt expression, NF-κB and p38MAPK expression associated with attenuation of the level of inflammation factor, suggesting that the anti-inflammation property of the PI3K/Akt pathway results in the suppression on NF-κB and p38MAPK activity [43].

Summary and Future Perspectives

A naturally occurring cellular antioxidant LA has potent antioxidant property, and it can serve as a free radical scavenger of ROS and RNOS. Therefore, it is a reasonable therapeutic intervention in the prevention of AKI, particularly CI-ARI and IRI in the heart and the kidney.

Disclosure Statement

There are no conflicts of interest to disclose.


