Lipoic Acid in the Prevention of Acute Kidney Injury

Jun Zhang^a Peter A. McCullough^{a–d}

^aBaylor Heart and Vascular Institute, ^bBaylor University Medical Center, and ^cBaylor Jack and Jane Hamilton Heart and Vascular Hospital, Dallas, Tex., and ^dThe Heart Hospital Baylor, Plano, Tex., USA

Key Words

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-dithiolane-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-

protection, with emphasis on antioxidant functions of LA and multiple signaling pathways underlying protective effects of LA on CI-AKI as well as cardiac and renal IRI.

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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-

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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 vascular cell adhesion molecule-1 through repression of nuclear factor- κ B (NF- κ B) [17]. In addition, animal studies have revealed that myocardial IRI [18–23] and renal IRI [24–27] could be protected by a naturally occurring cellular 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 α 1-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 decrease 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 ability 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.

Fig. 3. A schematic diagram illustrating cardioprotective effects exerted by LA.

ductive 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 de-

creased 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- κ B, the mRNA expression of cyclooxygenase-2 and the expression of intercellular adhesion molecule-1 were decreased [21]. An in vivo study with dietary supplementation of LA to rat has shown that the thioctic LA protected against IRI in the isolated perfused Langendorff heart.

Moreover, thioctic 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

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

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 recover 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 PKC ϵ 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 cardiomy-

ocytes 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-

tenuation 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-1 β , 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 vacuolation, 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 3 β (GSK3 β) in response to phosphorylation increased by IPC [37]. The IPC-mediated cardioprotection is primarily via the activation of the PI3K-PKB/Akt reperfusion

injury salvage kinase pathway, in which enhanced phosphorylation of Akt/PKB and GSK3 β exert better functional recovery and reduces cell death [37, 40].

The Role of PI3K/Akt Pathway in Myocardial Contractility

PI3K α (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 PI3K γ exhibits an increase in cardiac contractility. Loss of PI3K γ is associated with substantial increases in contractility and relaxation, and thus PI3K γ 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 cardiomyocytes 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-1 β 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-1 β , and IL-6, IL-1 β and TNF- α). The administration of wortmannin (a PI3K/Akt inhibitor) reduced Akt expression, NF- κ B 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- κ B 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-AKI and IRI in the heart and the kidney.

Disclosure Statement

There are no conflicts of interest to disclose.

References

- McCullough PA: Contrast-induced acute kidney injury. *J Am Coll Cardiol* 2008;51:1419–1428.
- Ball T, McCullough PA: Statins for the prevention of contrast-induced acute kidney injury. *Nephron Clin Pract* 2014;127:165–171.
- Heyman SN, Rosen S, Khamaisi M, Idée JM, Rosenberger C: Reactive oxygen species and the pathogenesis of radiocontrast-induced nephropathy. *Invest Radiol* 2010;45:188–195.
- Tasanarong A, Kongkham S, Itharat A: Antioxidant effect of phyllanthus emblica extract prevents contrast-induced acute kidney injury. *BMC Complementary Altern Med* 2014;14:138.
- Brezis M, Rosen S: Hypoxia of the renal medulla – its implication for disease. *N Engl J Med* 1995;332:647–655.
- Tumlin J, Stacul F, Adam A, Becker CR, Davidson C, Lameire N, McCullough PA: Pathophysiology of contrast-induced nephropathy. *Am J Cardiol* 2006;98:14K–20K.
- Yang Y, Yang D, Yang D, Jia R, Ding G: Role of reactive oxygen species-mediated endoplasmic reticulum stress in contrast-induced renal tubular cell apoptosis. *Nephron Exp Nephrol* 2014;128:30–36.
- Packer L, Witt EH, Tritschler HJ: Alpha-lipoic acid as a biological antioxidant. *Free Radic Biol Med* 1995;19:227–250.
- Whiteman M, Tritschler H, Halliwell B: Protection against peroxynitrite-dependent tyrosine nitration and alpha 1-antiproteinase inactivation by oxidized and reduced lipoic acid. *FEBS Lett* 1996;379:74–76.
- Biewenga GP, Haenen GR, Bast A: The pharmacology of the antioxidant lipoic acid. *Gen Pharmacol* 1997;29:315–331.
- Moini H, Packer L, Saris NE: Antioxidant and prooxidant activities of alpha-lipoic acid and dihydrolipoic acid. *Toxicol Appl Pharmacol* 2002;182:84–90.
- Trujillo M, Radi R: Peroxynitrite reaction with the reduced and the oxidized forms of lipoic acid: new insights into the reaction of peroxynitrite with thiois. *Arch Biochem Biophys* 2002;397:91–98.
- Rezk BM, Haenen GR, van der Vijgh WJ, Bast A: Lipoic acid protects efficiently only against a specific form of peroxynitrite-induced damage. *J Biol Chem* 2004;279:9693–9697.
- Trujillo M, Folkes L, Bartsaghi S, Kalyanaram B, Wardman P, Radi R: Peroxynitrite-derived carbonate and nitrogen dioxide radicals readily react with lipoic and dihydrolipoic acid. *Free Radic Biol Med* 2005;39:279–288.
- Dhalla NS, Elmoselhi AB, Hata T, Makino N: Status of myocardial antioxidants in ischemia-reperfusion injury. *Cardiovasc Res* 2000;47:446–456.
- Sahna E, Parlakpınar H, Turkoz Y, Acet A: Protective effects of melatonin on myocardial ischemia/reperfusion induced infarct size and oxidative changes. *Physiol Res* 2005;54:491–495.
- Freisleben HJ: Lipoic acid reduces ischemia-reperfusion injury in animal models. *Toxicology* 2000;148:159–171.
- Shay KP, Moreau RF, Smith EJ, Smith AR, Hagen TM: Alpha-lipoic acid as a dietary supplement: molecular mechanisms and therapeutic potential. *Biochim Biophys Acta* 2009;1790:1149–1160.

- 19 Ko KM, Yiu HY: Schisandrin B modulates the ischemia-reperfusion induced changes in non-enzymatic antioxidant levels in isolated-perfused rat hearts. *Mol Cell Biochem* 2001; 220:141–147.
- 20 Oh SK, Yun KH, Yoo NJ, Kim NH, Kim MS, Park BR, Jeong JW: Cardioprotective effects of alpha-lipoic acid on myocardial reperfusion injury: suppression of reactive oxygen species generation and activation of mitogen-activated protein kinase. *Korean Circ J* 2000; 39:359–366.
- 21 Wang X, Yu Y, Ji L, Liang X, Zhang T, Hai CX: Alpha-lipoic acid protects against myocardial ischemia/reperfusion injury via multiple target effects. *Food Chem Toxicol* 2011;49: 2750–2757.
- 22 He L, Liu B, Dai Z, Zhang HF, Zhang YS, Luo XJ, Ma QL, Peng J: Alpha lipoic acid protects heart against myocardial ischemia-reperfusion injury through a mechanism involving aldehyde dehydrogenase 2 activation. *Eur J Pharmacol* 2012;678:32–38.
- 23 Deng C, Sun Z, Tong G, Yi W, Ma L, Zhao B, Cheng L, Zhang J, Cao F, Yi D: α -Lipoic acid reduces infarct size and preserves cardiac function in rat myocardial ischemia/reperfusion injury through activation of PI3K/Akt/Nrf2 pathway. *PLoS One* 2013;8:e58371.
- 24 Takaoka M, Ohkita M, Kobayashi Y, Yuba M, Matsumura Y: Protective effect of alpha-lipoic acid against ischaemic acute renal failure in rats. *Clin Exp Pharmacol Physiol* 2002;29: 189–194.
- 25 Sehirli O, Sener E, Cetinel S, Yüksel M, Gedik N, Sener G: Alpha-lipoic acid protects against renal ischaemia-reperfusion injury in rats. *Clin Exp Pharmacol Physiol* 2008;35:249–255.
- 26 Bae EH, Lee KS, Lee J, Ma SK, Kim NH, Choi KC, Frøkiaer J, Nielsen S, Kim SY, Kim SZ, Kim SH, Kim SW: Effects of alpha-lipoic acid on ischemia-reperfusion-induced renal dysfunction in rats. *Am J Physiol Renal Physiol* 2008;294:F272–F280.
- 27 Koga H, Hagiwara S, Kusaka J, Goto K, Uchino T, Shingu C, Kai S, Noguchi T: New α -lipoic acid derivative, DHL-HisZn, ameliorates renal ischemia-reperfusion injury in rats. *J Surg Res* 2012;174:352–358.
- 28 Cremer DR, Rabeler R, Roberts A, Lynch B: Safety evaluation of alpha-lipoic acid (ALA). *Regul Toxicol Pharmacol* 2006;46:29–41.
- 29 Cremer DR, Rabeler R, Roberts A, Lynch B: Long-term safety of alpha-lipoic acid (ALA) consumption: a 2-year study. *Regul Toxicol Pharmacol* 2006;46:193–201.
- 30 Müller C, Dünschede F, Koch E, Vollmar AM, Kiemer AK: Alpha-lipoic acid preconditioning reduces ischemia-reperfusion injury of the rat liver via the PI3-kinase/Akt pathway. *Am J Physiol Gastrointest Liver Physiol* 2003; 285:G769–G778.
- 31 Serbinova E, Khwaja S, Reznick AZ, Packer L: Thiocetic acid protects against ischemia-reperfusion injury in the isolated perfused Langendorff heart. *Free Radic Res Commun* 1992;17: 49–58.
- 32 Haramaki N, Packer L, Assadnazari H, Zimmer G: Cardiac recovery during post-ischemic reperfusion is improved by combination of vitamin E with dihydrolipoic acid. *Biochem Biophys Res Commun* 1993;196:1101–1107.
- 33 Scheer B, Zimmer G: Dihydrolipoic acid prevents hypoxic/reoxygenation and peroxidative damage in rat heart mitochondria. *Arch Biochem Biophys* 1993;302:385–390.
- 34 Somani SM, Husain K, Whitworth C, Trammel GL, Malafa M, Rybak LP: Dose-dependent protection by lipoic acid against cisplatin-induced nephrotoxicity in rats: antioxidant defense system. *Pharmacol Toxicol* 2000;86:234–241.
- 35 Oymak O: Contrast media induced irreversible acute renal failure in a patient treated with intraperitoneal cisplatin. *Clin Nephrol* 1995; 44:135–136.
- 36 Packer L, Kraemer K, Rimbach G: Molecular aspects of lipoic acid in the prevention of diabetes complication. *Nutrition* 2001;17:888–895.
- 37 Oudit GY, Penninger JM: Cardiac regulation by phosphoinositide 3-kinases and PTEN. *Cardiovasc Res* 2009;82:250–260.
- 38 Duronio V: The life of a cell: apoptosis regulation by the PI3K/PKB pathway. *Biochem J* 2008;415:333–344.
- 39 Sayed D, He M, Hong C, Gao S, Rane S, Yang Z, Abdellatif M: MicroRNA-21 is a downstream effector of AKT that mediates its anti-apoptotic effects via suppression of Fas ligand. *J Biol Chem* 2010;285:20281–20290.
- 40 Zhu M, Feng J, Lucchinetti E, Fischer G, Xu L, Pedrazzini T, Schaub MC, Zaugg M: Ischemic postconditioning protects remodeled myocardium via the PI3K-PKB/Akt reperfusion injury salvage kinase pathway. *Cardiovasc Res* 2006;72:152–162.
- 41 Crackower MA, Oudit GY, Kozieradzki I, Sarao R, Sun H, Sasaki T, Hirsch E, Suzuki A, Shioi T, Irie-Sasaki J, Sah R, Cheng HY, Rybin VO, Lembo G, Fratta L, Oliveira-dos-Santos AJ, Benovic JL, Kahn CR, Izumo S, Steinberg SF, Wymann MP, Backx PH, Penninger JM: Regulation of myocardial contractility and cell size by distinct PI3K-PTEN signaling pathways. *Cell* 2002;110:737–749.
- 42 Xu P, Wang J, Yang ZW, Lou X-L, Chen C: Regulatory roles of the PI3K/Akt signaling pathway in rats with severe acute pancreatitis. *PLoS One* 2013;8:e81767.
- 43 Rong R, Xijun X: Erythropoietin pretreatment suppresses inflammation by activating the PI3K/Akt signaling pathway in myocardial ischemia-reperfusion injury. *Exp Ther Med* 2015;10:413–418.