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Cytoprotective effects of DL-alpha-lipoic acid or squalene on cyclophosphamide-induced oxidative injury: An experimental study on rat myocardium, testicles and urinary bladder

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ABSTRACT

The present study aimed to evaluate the role of DL-alpha-lipoic acid (LA) and squalene (SQ) on oxidative cardiac, testicular and urotoxic damage induced by cyclophosphamide (CP). Male Wistar rats were divided into four groups; three groups received a single intraperitoneal injection of CP (200 mg/kg BW) to induce toxicity, and two of these groups received either LA (35 mg/kg BW) or SQ (0.4 ml/rat) orally 7 days before and 7 days after CP injection. A vehicle-treated control group was also included. Oxidative damage was observed by decreased serum total antioxidant capacity (TAC) level and abnormal alterations in glutathione peroxidase (GPx) and glutathione reductase (GR) activities, levels of glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO) and calcium (Ca^{+2}) in the heart, testes and urinary bladder of CP-administered rats. Cardiac marker enzyme activities; creatine phosphokinase (CPK), lactate dehydrogenase (LDH), and aspartate transaminase (AST) showed severe declines whereas testicular markers; sorbitol dehydrogenase (SDH), γ-glutamyl transferase (γ-GT), acid and alkaline phosphatases (ACP and ALP), serum testosterone (T) level and haemoglobin (Hb) absorbance were abnormal. Histopathological observations were also altered. These CP-induced pathological alterations were attenuated by treatment with LA or SQ. These findings highlight the efficacy of LA and SQ as cytoprotectants in CP-induced toxicity.

1. Introduction

Cyclophosphamide (CP) is a cytotoxic alkylating agent that has been widely used in the acute treatment of various neoplastic diseases and in the chronic treatment of autoimmune disorders. The major limitation of CP chemotherapy is the injury of normal tissue, leading to multiple organ toxicity mainly in the heart, testes and urinary bladder (Fraiser et al., 1991). The main mechanism of both the therapeutic and the toxic effects of CP is the requirement of the metabolic activation by the hepatic microsomal cytochrome P450 mixed functional oxidase system (Sladek, 1988). Phosphoramide mustard and acrolein are the two active metabolites of CP (Ludeman, 1999). Cyclophosphamide's antineoplastic and immunosuppressive effects are associated with phosphoramide mustard, while acrolein is linked with its toxic side effects (Kern and Kehrer, 2002; Angulo et al., 2002).

Phosphoramide mustard acts by causing a loss of two chlorine atoms and the formation of a positively charged reactive intermediate that irreversibly binds to a nucleophilic site (alkylation). While positively charged reactive intermediates will covalently bind to a variety of molecules including water, amino acids, and proteins, the most important site of binding is DNA. Reactive intermediate binding to DNA causes intra- and interstrand cross-linkages to occur, leading to DNA strand breaks, the inability to synthesise DNA, and ultimately cell death, which is a major anticancer mechanism of CP (Moore, 1991). In addition, acrolein, which inactivates the DNA repair protein O6-methylguanine-DNA methyltransferase (Lee et al., 1992), also partially contributes to the cellular toxicity of CP. The precise mechanism by which CP causes toxicity is unknown; however, numerous studies have shown that CP exposure can disrupt the redox balance of tissues,

Abbreviations: ACP, acid phosphatase; ALP, alkaline phosphatase; AST, aspartate transaminase; CPK, creatine phosphokinase; CP, cyclophosphamide; DHLA, dihydrolipoic acid; FRAP, ferric reducing antioxidant power; γ-GT, gamma glutamyl transferase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; Hb, haemoglobin; HC, haemorrhagic cystitis; (3β-HSD), 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; iNOS, inducible nitric oxide synthase; LDH, lactate dehydrogenase; LA, lipoic acid; LPO, lipid peroxidation; MDA, malondialdehyde; NO, nitric oxide; GSSG, oxidized glutathione; ROS, reactive oxygen species; RNS, reactive nitrogen species; SDH, sorbitol dehydrogenase; SQ, squalene; T, testosterone; TAC, total antioxidant capacity.

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suggesting that biochemical and physiological disturbances may result from oxidative stress (Das et al., 2002; Ghosh et al., 2002; Selvakumar et al., 2006).

The high therapeutic doses of CP can cause lethal cardiotoxicity, with symptoms and signs of the myopericarditis leading to fatal complications such as congestive heart failure, arrhythmias and myocardial depression (Shanholtz, 2001). The cardiotoxic effects of CP consist of acute, dose-dependent cardiac damage, which is morphologically characterized by necrosis, haemorrhage and later fibrosis (Mills and Roberts, 1979; Goldberg et al., 1986). The acrolein-lysine adducts detected in the aorta and plasma LDL of CP-treated animals suggests that these adducts may play a role in the development of atherosclerosis or atherogenesis (Arikketh et al., 2004).

A number of reports have indicated that CP alters human fertility (Howell and Shalet, 1998). CP is cytotoxic to rapidly dividing cells, which makes the highly proliferative testes a target for the damaging effects of this drug. Use of CP for the treatment of cancer in male patients increases the incidence of oligo- and azoospermia and results in male infertility (Howell and Shalet, 1998). Previous studies have demonstrated that chronic administration of CP to male rats/mice leads to decreased testicular weight, transitory oligospermia, decreased DNA synthesis in spermatogonia and protein synthesis in spermatids (Anderson et al., 1995).

Haemorrhagic cystitis (HC) is a common and distressing complication of CP. HC is the major dose-limiting side effect of CP or ifosfamide treatment, a synthetic analogue of CP (Levine and Richie, 1989). The incidence of this side effect is dose-dependent and can be as high as 75% in patients receiving a high intravenous CP dose. These effects include transient irritative voiding symptoms such as dypsia, haemorrhagic cystitis, bladder fibrosis, necrosis, contracture and vesicometral flux (Levine and Richie, 1989). The urotoxicity of CP is mainly attributable to the renal excretion of acrolein (Gray et al., 1986). It has been proposed that urothelial damage occurs with direct contact to acrolein, which causes oedema, haemorrhage, ulceration, leukocyte infiltration and in severe cases, fibrosis and necrosis of the bladder (Bishel, 1979). Because the bladder is the storage organ for urine, the content of these metabolites is higher than in other areas of the urinary tract, which increases the sensitivity of the bladder to damage.

In addition, CP-induced immunosuppression is reported to prompt various types of infection (Angulo et al., 2002). Some of the infectious agents have glutathione (GSH)-depleting effects (Hung and Wang, 2004; Rydkinaa et al., 2004). Thus, CP treatment may decrease GSH content itself, but the associated secondary infections can cause an additional decrease in the GSH level. Therefore, a patient undergoing CP chemotherapy needs excessive supply of GSH restoring antioxidants or compounds that induce GSH production. For that reason, GSH-inducing compounds have been found to be effective in reducing CP toxicity in animals (Manesh and Kuttan, 2005; Bhatia et al., 2006).

To prevent these toxic side effects, antioxidants should be able to detoxify the acrolein during CP chemotherapy. Therefore, there is a need for novel agents that would protect the normal tissue from chemotherapy-induced toxicity without tumour protection and tumour growth stimulation properties.

Lipoic acid (LA), a naturally occurring nutraceutical, functions as an essential cofactor in metabolic reactions involved in energy utilization. It is a disulfide compound that is found naturally in mitochondria as the coenzyme for mitochondrial dehydrogenase multienzyme complexes. The best food sources of LA are believed to be those foods rich in mitochondria such as red meat (skeletal muscle, heart, liver, and kidney). Other sources are yeast, spinach, and broccoli. LA and its reduced form, dihydropolic (DHLA) acid, are effective against conditions in which oxidative stress plays a role (Packer et al., 1995). It shows beneficial effects in oxidative stress conditions because both of its isoforms act as antioxidants directly through free radical quenching and metal chelation and indirectly through recycling of other cellular antioxidants (Navari-Izzo et al., 2002). LA, which is a universal antioxidant, functions in both the aqueous and membrane phases (Kagan et al., 1992).

Squalene (SQ), the intermediate of cholesterol metabolism, is an isoprenoid compound with six isoprene units. SQ occurs widely in nature in both plants and animals. It is an oily substance that easily absorbs oxygen and facilitates delivery of oxygen throughout the body to the cell tissues that require it. As such, SQ exerts stimulating and strengthening effects on the immune system. SQ can be found in olives, green leafy vegetables, and wheat germ, but it is only found in minute quantities that are insufficient to replenish the much needed isoprenoids as a person ages. An abundant source of SQ is the livers of the deep-sea dogfish, which are encapsulated and taken as dietary supplements. SQ has been reported to possess antioxidant and membrane stabilizing properties (Ko et al., 2002). *In vitro* evidence indicates that SQ is a highly effective singlet oxygen-scavenging agent (Saint-Leger et al., 1986). SQ is secreted in human sebum, where it may protect the skin from ultraviolet radiation (Kohno et al., 1995).

Several experimental models have demonstrated the detoxifying activities of SQ against a wide range of chemicals such as hexachlorobiphenyl, hexachlorobenzene, arsenic, theophylline, phenobarbital and strychnine. Experimental evidence suggests that SQ might act as a sink for highly lipophilic xenobiotics, assisting in their elimination from the body (Fan et al., 1996; Senthilkumar et al., 2006a). SQ has also been found to have protective activity against several carcinogens, including azoxymethane-induced colon cancer (Rao et al., 1998) and nicotine-derived nitrosaminoketone (NMK)-induced lung carcinogenesis (Smith et al., 1998). SQ is also capable of suppressing the growth of tumour cells (Tomita, 1983). Studies have shown that 60–85% of SQ is absorbed when administered orally and is then distributed to various tissues. However, no signs of toxicity have been reported with an increased intake of SQ (Miettinen and Vanhanen, 1994). Such protective, anticarcinogenic and differential activities in normal versus tumour tissue suggest that SQ may have cytoprotective potential for normal tissue against chemotherapeutic agents (Senthilkumar et al., 2006a). In the present study, an attempt has been made to assess the preventive effects of dl-LA and SQ against CP-induced toxicities in the heart, testes and urinary bladder of rats.

2. Materials and methods

2.1. Drugs and chemicals

Cyclophosphamide (Endoxan®) was purchased from Baxter Oncology GmbH, Frankfurt, Germany. LA was kindly received as a gift from EVA Pharma for pharmaceutical and medical appliances, Egypt. SQ (⩾97% by GC) was purchased from Sigma Chemicals Company, St. Louis, MO, USA. All other chemicals used were of the highest purity and analytical grade.

2.2. Experimental design

Male Wistar albino rats (170–200 g) were used for the study. Rats were allowed free access to food and water throughout the experimental period. After one week of acclimatisation, rats were randomly divided into four groups. Group I ($n = 18$) served as the vehicle-treated control; Group II animals ($n = 20$) were injected intraperitoneally with a single dose of CP (200 mg/kg BW) dissolved in saline (Mythili et al., 2005a). In Groups III ($n = 20$) and IV ($n = 17$), rats were treated with CP (as in Group II) and LA (35 mg/kg BW, dissolved in saline at alkaline pH 7.8) or SQ (0.4 ml/rat). LA and SQ were administered orally for 7 days prior to CP administration and were followed continuously daily for 7 days up to the end of the experimental period. These doses of LA and SQ have been previously shown to have protective effects in CP-induced toxicity in rats (Selvakumar et al., 2005; Senthilkumar et al., 2006a).