Anti-dyslipidemic effect of zinc oxide nanoparticles against cyclophosphamide induced dyslipidemia in male albino rats

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ABSTRACT

Background: Cyclophosphamide (CP) is used for the treatment of ovarian cancer, breast cancer, chronic lymphocytic leukemia, non-Hodgkin’s lymphoma, neuroblastoma, soft tissue sarcoma, rhabdomyosarcoma, and Wilms’ tumor. It causes dyslipidemia in experimental animals. Zinc oxide is the most widely used nanoparticles among various nanomaterials. Zinc oxide nanoparticles were ameliorated hepatotoxicity, hematotoxicity, oxidative stress, and disturbance in the antioxidant defense system induced by cyclophosphamide in experimental animals.

Objectives: The present study aimed to evaluate the anti-dyslipidemic effect of zinc oxide nanoparticles against cyclophosphamide-induced dyslipidemia in male albino rats.

Materials and Methods: Twenty-four adult male rats (Sprague Dawley) were grouped randomly into four groups of six rats each. Group I: Control group: Received 0.2 ml saline / day i.p. injection for 14 days (day by day), group II (nZnO group): Received nZnO (5 mg/kg/day b.w., intraperitoneally for 14 days), Group III (CP group): Received CP 20 mg/kg/day body weight (b.w.) day by day for 14 days by intraperitoneal injection, Group IV (CP + nZnO NPs group): Received nZnO group: Received nZnO (5 mg/kg/day) b.w., intraperitoneally for 14 days, plus CP 20 mg/kg/day body weight (b.w.) day by day for 14 days by intraperitoneal injection. At the end of the experimental period, rats were anesthetized using light ether. Blood samples were taken and prepared for biochemical measurements.

Results: Administration of CP to rats resulted in a significant increase (p<0.05) in the levels of serum total cholesterol, triglycerides, and LDL-cholesterol and a significant decrease (p<0.05) in serum HDL-cholesterol, as compared to control group. Conversely, the groups treated with nZnO showed a significant decrease (p<0.05) in serum total cholesterol, triglycerides, and LDL-cholesterol and a significant increase (p<0.05) in serum HDL-cholesterol, as compared to CP group.

Conclusion: It can be concluded that exposure of rats to CP induced dyslipidemia and treatment of rats with zinc oxide nanoparticles and CP together prevented dyslipidemia induced by CP. These results may provide further visions into the proper treatment of patients by improving the side effects of chemotherapy. However further studies are necessary to establish optimal doses of nZnO and receive the best safety profile.

Keywords: Zinc oxide nanoparticles, Anti-dyslipidemia, Anti-hyperlipidemia, Cyclophosphamide, Lipid profile, Albino rats.

1. Introduction

Cyclophosphamide (CP) is a drug used as an anticancer and immunosuppressant agent. CP induces myocardium damage and dyslipidemia [1]. It is used for the treatment of ovarian cancer, breast cancer, chronic lymphocytic leukemia, non-Hodgkin’s lymphoma, neuroblastoma, soft tissue sarcoma, rhabdomyosarcoma, and Wilms’ tumor [1, 2].
CP is activated by hepatic cytochrome P450 mixed-function oxidase system and converted to 4-hydroxycyclophosphamide, which is in equilibrium with aldophosphamide. Aldophosphamide, delivered to both tumour and normal tissue is converted to cytotoxic form phosphoramido mustard and acrolein by non-enzymatically [1, 2].

Acrolein damages many cell types by producing reactive oxygen species and lipid peroxidation. Oxidative stress is associated with the pathogenesis of cardiovascular disorder including ischemic heart disease, heart failure, and atherosclerosis. Acrolein also decreases cholesterol clearance by reducing hepatic lipase activity. Reduced cholesterol clearance is associated with dyslipidemia. It induced dyslipidemia is characterized by changes in the lipoprotein composition especially in VLDL cholesterol and triglycerides and induces pro-atherogenic changes in lipoproteins. It was found that, acrolein can induce apoptosis by activation of the mitochondrial pathway or death receptor signaling. In some studies, it was proved that acrolein can exert detrimental effects in adult mice heart tissues through apoptotic cell death [1, 3].

CP administration to albino rats caused a significant increase in the levels of total cholesterol, LDL-cholesterol, triglyceride, and decrease HDL-cholesterol level in the serum compared to the control group [1].

The development of atherosclerosis depends on the balance between pro-inflammatory; anti-inflammatory, and anti-oxidative defense mechanisms [5]. Vascular proliferation and inflammation are closely linked [6], and excessive proliferation of vascular cells plays an important role in the pathology of vascular occlusive disease. Free radicals are considered to play a causal role in this process [7], and ROS lead to the oxidation of low-density lipoprotein (LDL), and this accumulates within plaques, and contributes to the inflammatory state of atherosclerosis and plays a key role in its pathogenesis [8]. Oxidized-LDL leads to endothelial dysfunction, and can result in either cell growth or apoptotic cell death. Free radicals have also been implicated in congestive heart failure, the annual incidence of which is one to five per 1000 person, and the relative incidence doubles for each decade of life after the age of 45. Experimental evidence suggests a direct link between free radical production and CHF [9, 10], and the presence of ROS in circulating blood is also the key intermediary related to vascular injury and organ dysfunction [10-12].

The use of nanoscale materials is growing exponentially as concerns rise about the human hazards to it. It is assumed that living beings are coevolved with nanoparticles ever since the origin of life on earth and therefore, they must have developed the defense and toxicity mitigating mechanisms for nanoparticles. Although having peculiar properties these new materials also present new health risks upon interacting with biological systems. Zinc oxide is the most widely used nanoparticles among various nanomaterials. Recently, these nanoparticles have been shown to specifically kill cancerous cells; therefore, it is believed that these nanoparticles may be used as an alternative anti-tumor agent. However, it is also known that these nanoparticles pose several deleterious effects on living beings [13].

Previous studies reported that zinc oxide nanoparticles were ameliorated hepatotoxicity, hematotoxicity oxidative stress, and disturbance in the antioxidant defense system induced by cyclophosphamide in male albino rats [14-16].

2. Objectives

The present study aimed to evaluate the anti-dyslipidemic effect of zinc oxide nanoparticles against cyclophosphamide induced dyslipidemia in male albino rats.

3. Material and methods

Experiment was carried out at the Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University laboratories.

3.1. Chemicals

All reagents were of the highest quality. Zinc oxide as nanoparticles with an average size of 67 nm is a gift from Dr. Eman El-Trass. Cyclophosphamide (200 mg/ampoule) was purchased from Sigma-Aldrich.

3.2. Animals

Twenty four male rats with an average body weight of 150±30 g were obtained from the faculty of agriculture, Alexandria University, and acclimatized for two weeks before starting the experiment. The rats were maintained under temperature (25oC). They were fed with standard food and had free access to water. Animals were randomly divided into 4 groups and housed in Universal galvanized wire cages.
3.3. Experimental design

Adult male rats (Sprague Dawley) were randomly divided into four groups (six rats each) as follows:

- **Group I. Control group**: Received 0.2 ml/day saline i.p. injection for 14 days (day by day).
- **Group II. nZnO group**: Received nZnO (5 mg/kg/day) b.w., intraperitoneally for 14 days [17].
- **Group III. CP group**: Received CP 20 mg/kg/day body weight (b.w.) (i.p. injection of saline) day by day for 14 days by intraperitoneal injection [18].
- **Group IV. CP + nZnO group**: Received nZnO (5 mg/kg/day) b.w., intraperitoneally for 14 days, plus CP 20 mg/kg/day b.w., day by day for 14 days by intraperitoneal injection.

After 24-hr from the last treatments all the animals were anesthetized using light ether. Blood samples were taken from the heart within 1 min. Tubes were used to compile blood drawn from the heart directly; 3 ml was collected in glass tubes for coagulation and serum formation, blood was left for 30 min at 4°C to clot, then centrifuged for 5 minutes at 1000 x g. Packed cells were discarded and the supernatant serum samples were decanted and stored into capped sterile polyethylene tubes at -20°C until used (within 24 hours).

3.4. Biochemical analysis

Cholesterol was determined after enzymatic hydrolysis and oxidation according to the method described by Richmond [19]. Triglycerides were determined according to the method described by Carr et al. [20].

LDL Cholesterol test results are based on a reading of light reflected off a test strip that has changed color after blood is applied. The intensity of the color is directly proportional to the concentration of LDL cholesterol in the sample. The analyzer converts this reading into LDL cholesterol result and displays it. This test, which selectively measures LDL cholesterol, is an enzymatic colorimetric test based on the "Trinder Method" for the determination of cholesterol. In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to cholesterol-4-en-one and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with 4-aminoantipyrine and N,N-disubstituted aniline to form a blue dye [21].

Phosphotungstic acid and magnesium ions selectively precipitating all lipoproteins except the HDL fraction-cholesterol present in the supernatant can be determined by the same method used for total cholesterol [22].

3.5. Statistical Analysis

Values obtained as Mean±SE were subjected to one-way analysis of variance (ANOVA) followed by Tukey test using GraphPad Prism version 5.0 for windows from GraphPad Software, San Diego, California, USA). Values of P < .05 were considered significant.

4. Results and Discussion

Administration of CP to rats resulted in a significant increase (p<0.05) in the levels of serum total cholesterol, triglycerides, and LDL-cholesterol and a significant decrease (p<0.05) in serum HDL-cholesterol, as compared to control group. Conversely, the groups treated with nZnO showed a significant decrease (p<0.05) in serum total cholesterol, triglycerides, and LDL-cholesterol and a significant increase (p<0.05) in serum HDL-cholesterol, as compared to CP group (Table 1, Figures 1-4).

### Table (1). Effects of treatment of rats with zinc oxide nanoparticles and/or Cyclophosphamide on serum cholesterol, triglycerides, HDL, and LDL.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Control Mean±SE</th>
<th>nZnO Mean±SE</th>
<th>CP Mean±SE</th>
<th>nZnO + CP Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum Cholesterol (mg/dl)</td>
<td>52.54 ± 3.86bcd</td>
<td>68.12 ± 3.81ac</td>
<td>83.72 ± 6.17abcd</td>
<td>63.72 ± 3.86ac</td>
</tr>
<tr>
<td></td>
<td>Serum Triglycerides (mg/dl)</td>
<td>62.38 ± 5.12ad</td>
<td>66.46 ± 4.52ad</td>
<td>102.8 ± 10.04abcd</td>
<td>80.90 ± 4.91abc</td>
</tr>
<tr>
<td></td>
<td>Serum LDL (mg/dl)</td>
<td>13.67 ± 2.05bcd</td>
<td>28.56 ± 2.92ad</td>
<td>32.22 ± 4.91ad</td>
<td>18.02 ± 1.26abc</td>
</tr>
<tr>
<td></td>
<td>Serum HDL (mg/dl)</td>
<td>37.28 ± 1.44bc</td>
<td>29.30 ± 1.41ac</td>
<td>18.50 ± 7.42abcd</td>
<td>31.50 ± 6.10c</td>
</tr>
</tbody>
</table>

Significance at P < 0.05. a Comparison of control and other groups; b Comparison of nZnO and other groups; c Comparison of CP and other groups; d Comparison of nZnO + CP and other groups.
Figure 1: Effects of treatment of rats with zinc oxide nanoparticles and/or cyclophosphamide on serum Cholesterol concentration. Significance at \( P < 0.05 \). \(^a\) Comparison of control and other groups; \(^b\) Comparison of nZnO and other groups; \(^c\) Comparison of CP and other groups; \(^d\) Comparison of nZnO + CP and other groups.

Figure 2: Effects of treatment of rats with zinc oxide nanoparticles and/or cyclophosphamide on serum Triglycerides concentration. Significance at \( P < 0.05 \). \(^a\) Comparison of control and other groups; \(^b\) Comparison of nZnO and other groups; \(^c\) Comparison of CP and other groups; \(^d\) Comparison of nZnO + CP and other groups.
Figure 3: Effects of treatment of rats with zinc oxide nanoparticles and/or cyclophosphamide on serum LDL concentration. Significance at $P < 0.05$. a Comparison of control and other groups; b Comparison of nZnO and other groups; c Comparison of CP and other groups; d Comparison of nZnO + CP and other groups.

Figure 4: Effects of treatment of rats with zinc oxide nanoparticles and/or cyclophosphamide on serum HDL concentration. Significance at $P < 0.05$. a Comparison of control and other groups; b Comparison of nZnO and other groups; c Comparison of CP and other groups; d Comparison of nZnO + CP and other groups.
Acute cardiotoxicity such as cardiac decompensation and cardiomyopathy has been associated with high dose cyclophosphamide therapy. The pathogenesis of acute cardiotoxicity may be attributed to an increase in free oxygen radicals in oxazaphosphorine induced cardiotoxicity [23].

Serum lipids and lipoprotein abnormalities are regarded as a highly modifiable risk factor for cardiovascular diseases [1, 24]. Hypercholesterolemia, hypertriglyceridemia and impaired secretion of heart lipoprotein lipase have been reported in cyclophosphamide treated rabbits [25, 26]. Perturbation of energy and lipid homeostasis plays a central role in the pathogenesis of heart disease. Primary and/or secondary alterations of lipid metabolism pathways in various conditions lead to myocardial lipid accumulation and lipotoxic cardiomyopathy. Defects in fatty acid uptake, secretion, and oxidation can disrupt myocardial energy and lipid homeostasis [27]. Treatment of cultured vascular smooth muscle cells with free radicals resulted in cholesterol accumulation [28].

The metabolism and physiology of lipids and lipoproteins is a dynamic integrated process. Lipoprotein abnormalities resulting in the disruption of serum and cellular lipid levels account for the genesis of vascular diseases. The acrolein-lsine adducts detected in the aorta and plasma LDL of cyclophosphamide treated animals suggest that these adducts wherein acrolein is a metabolite of cyclophosphamide, may play a role in the development of atherosclerosis or atherogenesis [29].

Cyclophosphamide is known to result in hypertriglyceridemia and hypercholesterolemia, which are well-known risk factors in cardiovascular diseases [25].

Cyclophosphamide induces oxidative stress through its toxic metabolite acrolein. It can cause dyslipidemia by increasing cholesterol biosynthesis, decreasing cholesteryl ester hydrolysis, and reducing cholesterol efflux [1, 30, 31]. Cyclophosphamide induces free radicals [32], which may cause cellular cholesterol accumulation, (a) by increasing cholesterol biosynthesis and its esterification, (b) by decreasing cholesteryl ester hydrolysis and (c) by reducing cholesterol efflux [1, 28, 30, 31]. The conversion of cholesterol to bile acids is quantitatively the most important mechanism for the degradation of cholesterol. However, McClure and Stupans [33] previously reported that after 7 days following a single dose of cyclophosphamide (200 mg/kg body weight) there was a decrease in cytochrome P450 activity in male rats, which may, in turn, depress cholesterol 7-hydroxylase activity, the key enzyme in the conversion of cholesterol to bile acids. The decline in the cardiac phospholipid content with a concomitant increase in the serum could be due to the peroxidation of unsaturated membrane lipids by free radicals in biomembranes and tissues causing the leakage of these lipids into circulation [34].

Cholesterol and phospholipids are carried in plasma by lipoproteins, which are synthesized and secreted by the intestine and liver. VLDL and HDL are secreted from the liver into the bloodstream. Boren et al. [35] have indicated that cardiac apoB enables the heart to secrete excess lipids in lipoproteins. VLDL is degraded into IDL and LDL by the action of the enzyme LPL and through the exchange reactions with HDL. LDL serves as a major carrier of cholesterol to extrahepatic tissues. High levels of LDL are associated with an increased risk of cardiovascular disease whereas high levels of HDL afford protection by reverse cholesterol transport to the liver. Previously in cyclophosphamide treated rats, lipid composition showed that HDL cholesterol was very low compared to a high VLDL cholesterol [25].

In these animals VLDL was larger than normal, corresponding to triglyceride enrichment [36].

Triglycerides are degraded by the lipoprotein lipase to fatty acids. Alterations in lipoprotein lipase activity induced by cyclophosphamide may increase triglycerides level [1, 30, 31]. Lipoprotein lipase (LPL) is predominantly present in the skeletal muscle, cardiac muscle, and adipose tissue. Defective secretion of LPL may contribute to the poor expression of lipolytic activity in the vascular bed and to the occurrence of hypertriglyceridemia during cyclophosphamide treatment. Simultaneously heart LPL activity was also decreased in fasted animals [37]. Due to the alterations in LPL activity, an increase in triglycerides was associated with a drop in fatty acid levels in the heart of group II cyclophosphamide treated rats. The moderate increase in the rate of triacylglycerol synthesis by the liver contributes to the occurrence of hypertriglyceridaemia in cyclophosphamide treated rats [37]. Hypercholesterolemic changes in these rats may be explained by a marked reduction in the activities of fat splitting enzymes, such as plasma LCAT and cardiac LPL. LCAT is secreted by the hepatocytes and released into the plasma. It converts cholesterol into long-chain cholesteryl ester on HDL and favours reverse cholesterol transport from tissues to the liver. The esterification of cholesterol by LCAT leads to the remodeling of the lipoprotein HDL, and results in the formation of large HDL particles that are known to offer protection against coronary artery disease [38]. Cholesterol esterification in the tissue is reported to be mediated through cholesterol ester Synthetase.

The present study proved that treatment of the CP-treated rats with ZnONPs prevented changes in serum lipids profile; this may be due to the antioxidant characters of ZnONPs [39]. It is well known that Zn is a powerful antioxidant metal; it is the core constituent of antioxidant enzymes such as SOD and a recognized protector of sulphydryl groups; it is also thought to impair lipid peroxidation by displacing transition metals such as iron and copper from catalytic sites [40]. nZnO can protect cell membrane integrity against oxidative stress damage, increase antioxidant enzyme levels, and decrease MDA level. It can improve antioxidant activity, enhance the activities of antioxidant enzymes, and decrease the levels of free radicals [41]. The authors concluded that zinc oxide nanoparticles improved the lipid profile when it administered in combination with CP.
5. Conclusion

It can be concluded that exposure of rats to CP induced dyslipidemia and treatment of rats with zinc oxide nanoparticles and CP together prevented dyslipidemia induced by CP. These results may provide further visions into the proper treatment of patients by improving the side effects of chemotherapy. However further studies are necessary to establish optimal doses of nZnO and receive the best safety profile.

References


