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Research Paper

Hypolipidaemic activity of orally administered diphenyl diselenide in Triton WR-1339-induced hyperlipidaemia in mice

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Abstract

Objectives A significant association between the trace element selenium and hypercholesterolaemia has been reported. This study was designed to investigate a potential hypolipidaemic effect of diphenyl diselenide ($(PhSe)_2$) in Triton WR-1339-induced hyperlipidaemia in mice.

Methods Triton was administered intraperitoneally (400 mg/kg) to overnight-fasted mice to develop acute hyperlipidaemia. $(PhSe)_2$ was administered orally (10 mg/kg) 30 min before Triton. At 24 h after Triton injection, blood samples were collected to measure plasma lipid levels. The hepatic thiobarbituric acid reactive substances and ascorbic acid levels as well as catalase and glutathione peroxidase activity were recorded.

Key findings $(PhSe)_2$ administration significantly lowered total cholesterol, non-high-density lipoprotein-cholesterol and triglycerides, whilst it increased high-density lipoprotein-cholesterol levels in plasma of hyperlipidaemic mice. Neither oxidative stress nor the antioxidant effect of $(PhSe)_2$ was observed in the mouse liver in this experimental protocol.

Conclusions These findings indicated that $(PhSe)_2$ was able to lower plasma lipid concentrations. Further studies are needed to elucidate the exact mechanism by which $(PhSe)_2$ exerted its hypolipidaemic effect in the management of hyperlipidaemia and atherosclerosis.

Keywords diphenyl diselenide; hypercholesterolaemia; oxidative stress; selenium; Triton WR-1339

Introduction

Plasma lipid levels are determined by exogenous lipid absorption and endogenous lipid synthesis and metabolism in the body.[1] The majority of research on hyperlipidaemia has focused on the effects of a high cholesterol diet on atherosclerosis and coronary heart disease. However, hypercholesterolaemia could also lead to the accumulation of lipid droplets in the liver, resulting in hepatic damage, such as steatosis.[2]

The liver is a key organ for lipid metabolism since hepatic cholesterol uptake from serum, coupled with intracellular processing and biliary excretion are important features in the removal of excess cholesterol from the body.[3] The transport of excessive cholesterol from the periphery to the liver by high-density lipoprotein (HDL) (reverse cholesterol transport) alleviates the accumulation of cholesterol in the arterial wall, a critical step in atherogenesis.[4]

Taking into account that the liver plays a central role in the maintenance of systemic lipid homeostasis, it is especially susceptible to damage by reactive oxygen species (ROS).[5] Studies have demonstrated that hyperlipidaemia reduces the hepatic antioxidant defence system.[6,7] Thus, there is a relationship between hyperlipidaemia and hepatic oxidative damage.

The biochemistry and pharmacology of the trace element selenium (Se) is a subject of intense current interest, particularly from the viewpoint of public health.[8] Se is largely known to develop its biological activity as an integral part of several peroxidases and redox enzyme systems, which protect cells from oxidative stress.[9,10] Some studies have related the selenium status to increased plasma cholesterol concentration.[11–13] Dhingra and Bansal[14] reported the therapeutic potential of Se supplementation in lipid metabolism, by

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demonstrating that 1 ppm Se supplementation down regulated apolipoprotein B and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase expression, a rate controlling enzyme in the cholesterol biosynthesis in animals, during hypercholesterolaemia in rats.

The interest in organoselenium pharmacology has increased in the last decade due to a variety of organoselenium compounds that possess biological activity.[15] In accordance, a number of novel pharmaceutical agents which are selenium-based or which target specific aspects of Se metabolism are under development.[16] In addition to their antioxidant property, Se compounds have been found to have neuroprotective, antinociceptive and anti-inflammatory properties.[16–18]

Diphenyl diselenide $(PhSe)_2$, an organoselenium compound, has been reported as a potential pharmacological and antioxidant agent.[15] Of particular importance, $(PhSe)_2$ inhibits lipid peroxidation in human isolated LDL *in vitro*. The protective effect of $(PhSe)_2$ against Cu^{2+}-induced loss of tryptophan fluorescence indicated that, besides its beneficial effects against oxidation of lipid moieties of LDL, this organoselenium compound prevented the oxidation of protein moieties of human LDL, pointing to an additional mechanism that could contribute to the inhibition of the atherogenic process.[19]

Triton WR-1339 (an oxyethylated tertiary octyl phenol formaldehyde polymer), one of the well known nonionic detergents, has been used widely to produce acute hyperlipidaemia in animal models.[20,21] Thus, this study was designed to investigate a potential hypolipidaemic effect of $(PhSe)_2$ on Triton WR-1339-induced acute hyperlipidaemia in mice.

An important property of a compound that may affect hyperlipidaemia is its antioxidant capacity. Considering that liver is the major organ responsible for cholesterol transport, metabolism and excretion, it is reasonable to study hepatic lipaemic-oxidative disturbances in hypercholesterolaemia. Kumar *et al.*[7] demonstrated that there was a significant increase in hepatic markers of oxidative damage, such as lipid peroxidation, accompanied by deteriorating enzymatic and nonenzymatic antioxidant status in rats fed a high cholesterol diet for 30 days. These findings were suggestive of signifying heightened oxidative injury in the liver. Thus, another objective of this study was to evaluate whether Triton WR-1339-induced acute hyperlipidaemia in mice altered some parameters of oxidative damage in the hepatic tissue and to assess whether the antioxidant effect of $(PhSe)_2$ was related to this process.

Materials and Methods

Chemicals

$(PhSe)_2$ was prepared and characterized in our laboratory by the method previously described by Paulmier.[22] Analyses of the ^1H nuclear magnetic resonance (NMR) and ^{13}C NMR spectrum showed that $(PhSe)_2$ obtained presented analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of the compound (99.9%) was determined by gas chromatography/high-performance liquid chromatography. $(PhSe)_2$ is a solid compound, very stable and can be stored in the laboratory in a simple flask for a long time. $(PhSe)_2$ was dissolved in canola oil. Triton WR-1339 (Tyloxapol) was purchased from Sigma-Aldrich (St Louis, MO, USA) and was dissolved in saline solution (pH 7.4). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Experimental animals

Male adult Swiss mice (25–35 g) from our own breeding colony were used. The animals were kept on a 12 h light/dark cycle, at room temperature (22 ± 2°C), with free access to food and water. All experiments were approved by and supervised under 'The Ethic Committee for Animal and Human Experiment' according to the declaration of Helsinki 1975. All efforts were made to minimize suffering and to reduce the number of animals used in the experiments.

Experimental design

Mice were fasted for 12 h and then divided into four groups. Group 1, control ($n = 6$): mice received canola oil (10 ml/kg, p.o.) 30 min before saline (2.5 ml/kg, i.p.). Group 2, $(PhSe)_2$ ($n = 6$): mice received $(PhSe)_2$ (10 mg/kg; 10 ml/kg, p.o.) 30 min before saline (2.5 ml/kg, i.p.). Group 3, Triton ($n = 5$): mice received canola oil (10 ml/kg, p.o.) 30 min before Triton WR-1339 (400 mg/kg, 2.5 ml/kg, i.p.).[6] Group 4, $(PhSe)_2$ + Triton ($n = 6$): mice received $(PhSe)_2$ (10 mg/kg; 10 ml/kg, p.o.) 30 min before Triton WR-1339 (400 mg/kg, 2.5 ml/kg, i.p.).

All animals remained in a fasted state for the duration of the experiment (36 h). At 24 h after the Triton injection, blood samples were collected directly from the ventricle of the heart in anaesthetized animals, using heparin as the anticoagulant, and plasma was separated by centrifugation (2400*g*) for 15 min. Subsequently mice were killed by decapitation. The livers were quickly removed and homogenized in 50 mM Tris-HCl, pH 7.4 (1/10, w/v). The homogenate was centrifuged at 2400*g* at 4°C for 15 min and a low-speed supernatant fraction (S_1) was used for assays.

Measurement of plasma lipid

Plasma total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglycerides were determined by enzymatic colorimetric methods using commercial kits (Labtest Diagnostica, MG, Brazil). Non-HDL values were obtained by the difference between total cholesterol and HDL-cholesterol levels. Plasma lipid levels were expressed as mg/dl.

Determination of hepatic oxidative stress parameters

Thiobarbituric acid reactive species

Thiobarbituric acid reactive species (TBARS), a measure of lipid peroxidation, were determined as described by Ohkawa *et al.*[23] A sample of S_1 was incubated with 0.8% thiobarbituric acid (TBA), acetic acid buffer pH 3.4 and 8.1% sodium dodecyl sulfate for 2 h at 95°C. The colour reaction was measured at 532 nm. TBARS level was expressed as nmol malondialdehyde (MDA)/mg protein.

Nonenzymatic antioxidant defence

Ascorbic acid determination was performed as described by Jacques-Silva *et al*.[24] S_1 was precipitated in 10 vol cold 4% trichloroacetic acid solution. A portion of the sample in a final volume of 1 ml of the solution was incubated at 38°C for 3 h, then 65% H_2SO_4 (v/v) was added to the medium. The reaction product was determined using colour reagent containing 4.5 mg/ml dinitrophenyl hydrazine and $CuSO_4$ (0.075 mg/ml). The colour reaction was measured spectrophotometrically at 520 nm. Ascorbic acid content was expressed as μg ascorbic acid/g tissue.

Antioxidant enzymatic defences

The activity of catalase and glutathione peroxidase (GPx) was determined. Catalase activity in S_1 was assayed spectrophotometrically by the method of Aebi[25], which involved monitoring the disappearance of H_2O_2 in the presence of S_1 at 240 nm. A sample of S_1 was added to 50 mM potassium phosphate buffer pH 7.0 and the enzymatic reaction was initiated by adding H_2O_2. One unit of enzyme was defined as the amount of enzyme required for monitoring the disappearance of H_2O_2. The enzymatic activity was expressed as Unit (U)/mg protein (1 U decomposes 1 μmol H_2O_2/min at pH 7 at 25°C).

GPx activity in S_1 was assayed spectrophotometrically by the method of Wendel[26], through the glutathione/NADPH/glutathione reductase system, by the dismutation of H_2O_2 at 340 nm. S_1 was added in the glutathione/NADPH/glutathione reductase system and the enzymatic reaction was initiated by adding H_2O_2. In this assay, the enzyme activity is indirectly measured by means of NADPH decay. H_2O_2 is decomposed, generating glutathione disulfide (GSSG) from glutathione. GSSG is regenerated back to glutathione by glutathione reductase present in the assay medium at the expense of NADPH. The enzymatic activity was expressed as nmol NADPH/min per mg protein.

Protein determination

Protein concentration was measured by the method of Bradford[27], using bovine serum albumin as the standard.

Statistical analysis

The results were presented as the means ± SEM. The statistically significant difference between groups was calculated by means of two-way analysis of variance followed by Duncan's test when necessary. Probability values less than 0.05 ($P < 0.05$) were considered as statistically significant.

Results

Measurement of plasma lipid

Two-way analysis of variance of cholesterol total data yielded a significant Triton WR-1339 × $(PhSe)_2$ interaction ($F_{1,19} = 6.200$; $P < 0.05$). Post-hoc comparisons demonstrated that Triton WR-1339 increased plasmatic cholesterol total levels in mice (2.4-times higher than the control group). $(PhSe)_2$ pretreatment was effective in preventing the increase of cholesterol total levels caused by Triton WR-1339 injection in mice (e.g. Figure 1a).

Two-way analysis of variance of HDL-cholesterol levels showed a significant Triton WR-1339 × $(PhSe)_2$ interaction ($F_{1,19} = 4.585$; $P < 0.05$). Post-hoc comparisons demonstrated that Triton WR-1339 decreased plasmatic HDL-cholesterol levels in mice (2.0-times lower than the control group). $(PhSe)_2$ pretreatment increased HDL-cholesterol levels reduced by Triton WR-1339 injection in mice (e.g. Figure 1b).

Two-way analysis of variance of non-HDL-cholesterol levels yielded a significant Triton WR-1339 × $(PhSe)_2$ interaction ($F_{1,19} = 12.523$; $P < 0.05$). Post-hoc comparisons demonstrated that Triton WR-1339 increased plasma non-HDL-cholesterol levels in mice (6.3-times higher than the control group). $(PhSe)_2$ pretreatment was effective in preventing the increase of non-HDL-cholesterol levels caused by Triton WR-1339 injection in mice (e.g. Figure 1c).

Two-way analysis of variance of triglyceride data revealed a significant Triton WR-1339 × $(PhSe)_2$ interaction ($F_{1,19} = 5.606$; $P < 0.05$). Post-hoc comparisons demonstrated that Triton WR-1339 increased the plasma triglycerides levels in mice (19.7-times higher than the control group). Oral administration of $(PhSe)_2$ in mice avoided the increase of triglyceride levels induced by Triton WR-1339 injection (e.g. Figure 1d).

Determination of hepatic oxidative stress parameters

Two-way analysis of variance of TBARS, ascorbic acid, catalase and GPx data showed that neither $(PhSe)_2$ nor Triton WR-1339 changed any of these parameters in livers of tested mice (Table 1).

Discussion

The purpose of this study was to demonstrate the hypolipidaemic effect of oral administration of $(PhSe)_2$ on Triton WR-1339-induced hyperlipidaemia in mice. A single oral dose of $(PhSe)_2$ was able to prevent the augmentation of total cholesterol, non-HDL-cholesterol and triglyceride levels as well as to increase HDL-cholesterol levels in hyperlipidaemic mice.

The nonionic detergent, Triton WR-1339, has been used widely to block the uptake of triacylglycerol-rich lipoproteins from plasma by peripheral tissues to produce acute hyperlipidaemia in animal models, which are often used for a number of objectives, in particular for screening natural or chemical hypolipidaemic drugs.[1,20,21,28] Experimental evidence supports the concept that Triton WR-1339 physically alters very low density lipoproteins (VLDL), rendering them refractive to the action of lipolytic enzymes of blood and tissue.[29] This prevents or delays their removal from blood and secondarily stimulates the hepatic cholesterol biosynthesis, enhancing the hyperlipidaemia.[30]

In this study, the levels of total cholesterol, non-HDL-cholesterol and triglycerides were increased 24 h after a single Triton WR-1339 injection in mice. The results demonstrated here were in accordance with those reported by others.[1,21] Moreover, $(PhSe)_2$, given by the oral route to mice, presented a hypolipidaemic effect by preventing

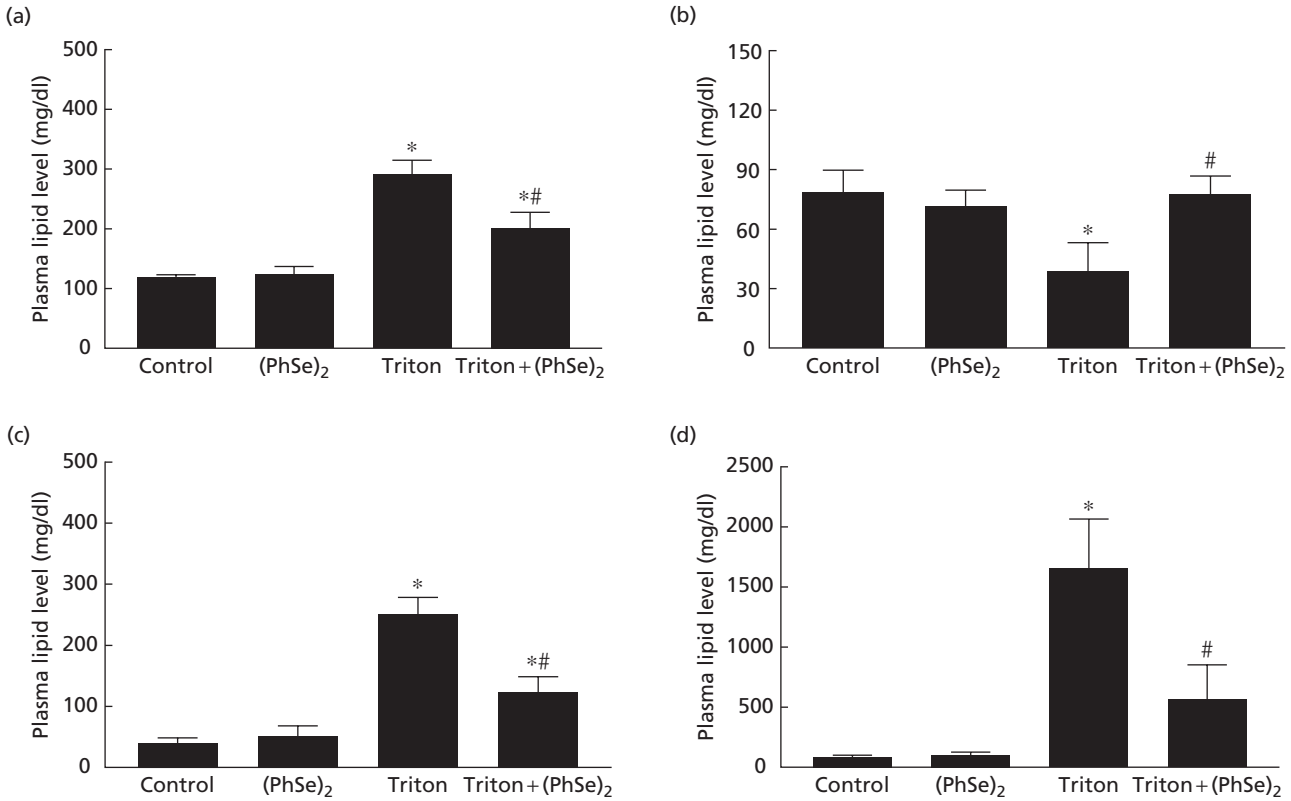

Figure 1 Effect of Triton WR-1339 and diphenyl diselenide on plasma lipid levels in mice. (a) Total cholesterol, (b) high-density lipoprotein (HDL)-cholesterol, (c) non-HDL-cholesterol, and (d) triglyceride levels from plasma of male adult mice. $(PhSe)_2$, diphenyl diselenide. Data are reported as means ± SEM for five to six animals per group. *Compared with control group; #compared with Triton group, $P < 0.05$ (two-way analysis of variance/Duncan).

Table 1 Effect of Triton WR-1339 and diphenyl diselenide on parameters of oxidative stress from liver of male adult mice

Treatment group	TBARS (nmol MDA/mg protein)	Ascorbic acid (μg ascorbic acid/g tissue)	Catalase (U/mg protein)	Glutathione peroxidase (nmol NADPH/min per mg protein)
Control	33.11 ± 4.71	328.17 ± 10.78	29.75 ± 5.68	108.23 ± 5.26
$(PhSe)_2$	34.11 ± 7.88	341.24 ± 22.90	29.03 ± 3.90	114.13 ± 8.01
Triton	40.18 ± 6.44	324.69 ± 26.82	33.59 ± 6.15	111.14 ± 7.33
$(PhSe)_2$ + Triton	37.18 ± 8.75	364.05 ± 11.28	27.65 ± 4.45	106.92 ± 12.87

$(PhSe)_2$, diphenyl diselenide; TBARS, thiobarbituric acid reactive substances. Data are reported as mean ± SEM for five to six animals per group.

the augmentation of total cholesterol, non-HDL-cholesterol and triglyceride levels in a Triton WR-1339-induced hyperlipidaemic model.

It has been postulated that high levels of HDL-cholesterol are associated with a reduced amount of atherosclerotic disease.[31] The HDL-cholesterol fraction may down regulate the total cholesterol via reverse cholesterol transport to the liver, i.e. the HDL fraction increases the cholesterol elimination from tissues and facilitates the cholesterol transport to the liver. Accordingly, Wójcicki *et al*.[32] reported an increase in HDL-cholesterol fraction on Se supplementation. In this way, it should be plausible to expect that $(PhSe)_2$ could augment HDL-cholesterol levels. Despite this, our data revealed that $(PhSe)_2$ itself was not capable of increasing significantly HDL-cholesterol levels, although it had raised HDL-cholesterol to control levels in Triton WR-1339-induced hyperlipidaemia in mice.

Oxygen free radicals or, more generally, reactive oxygen species (ROS), are the products of normal metabolic and signal-transduction events within a cell but free radical oxidation is responsible for the degradation of fatty acids and their esters in biological membranes and lipoproteins.[33] Consequently, this oxidation may also play a role in pathologic processes. Stokes *et al*.[34] reported that ROS levels in hypercholesterolaemia were higher than in the normal state. In agreement with this, Oh *et al*.[6] found that 18 h after Triton WR-1339 administration to mice, the level of plasma TBARS was increased and the activity of two hepatic detoxicating enzymes, catalase and GPx, were decreased compared with the control group. Conversely, in

this study the hepatic markers of oxidative stress were not altered in mice treated with Triton WR-1339. The unconformity between results presented herein and the data from Oh *et al.*[6] could be attributed to differences in the protocol of exposure, in other words, the different time of exposure to Triton WR-1339. In fact, in the experimental protocol carried out in this study, blood was collected from mice 24 h after Triton WR-1339 administration.

Although the mechanism involved in the hypolipidaemic effect of $(PhSe)_2$ has yet to be fully elucidated, its antioxidant property in liver was not demonstrated in this experimental protocol, since results from this study did not show any oxidative alteration in hepatic parameters tested. It is important to consider that liver TBARS and antioxidant defences are likely to have minimal effects on the cholesterol formation or tissue/vascular uptake of cholesterol because these are essentially two separate phenotypes with little correlation to the other. Therefore, we should not rule out that the antioxidant effect plays a role in $(PhSe)_2$-reduced hyperlipidaemia. In fact, the experimental design assesses no correlation of liver TBARS/antioxidant defences with altered blood lipids, indicating that the antioxidant action of $(PhSe)_2$ could be discharged in liver, but not necessarily in blood or other tissues.

$(PhSe)_2$ has been reported as an organoselenium compound, which presents thiol peroxidase-like activity *in vitro*.[16] In-vivo, $(PhSe)_2$ increased the levels of ascorbic acid and glutathione and the activity of glutathione S-transferase in lungs of rats.[35,36] However, in this study the $(PhSe)_2$ effect on the basal levels of ascorbic acid were not demonstrated. This lack of $(PhSe)_2$ effect could be explained by a possible tissue specific action, but this study is in accordance with Santos *et al.*[37] who verified no changes on ascorbic acid content in liver of mice after $(PhSe)_2$ treatment.

Literature data have reported that chalcogen compounds, containing selenium or tellurium, inhibit human squalene monooxygenase, the second enzyme in the downstream pathway for cholesterol biosynthesis.[38,39] The inhibition of squalene monooxygenase by selenium results from binding to one or more critical sulfhydryl groups on the enzyme.[39] Since the mechanism by which Triton WR-1339 caused hyperlipidaemia in mice involved the prevention or delay of VLDL removal from blood and secondarily the stimulation of hepatic cholesterol biosynthesis, it could be speculated that the inhibition of squalene monooxygenase, which resulted in reduction of cholesterol biosynthesis, by $(PhSe)_2$ could have been involved in its hypolipidaemic effect.[29,30] In accordance, we have reported that a structural analogue of $(PhSe)_2$, $(PhTe)_2$, produced a significant decrease in the plasma cholesterol levels in rats.[40]

Cholesterol homeostasis in the body is maintained primarily by the liver through the regulation of LDL receptor (LDL-R) and HMG-CoA reductase that affect exogenous uptake and endogenous synthesis of cholesterol, respectively.[14] So, another possible explanation for the hypolipidaemic action of $(PhSe)_2$ is related to HMG-CoA reductase activity. In fact, HMG-CoA reductase, a rate-limiting step in cholesterol biosynthesis, possesses cysteine residues and is subject to regulation by thiol–disulfide exchange. Nassir *et al.*[41] reported that Se deficiency led to increased HMG-CoA reductase activity in rats, that in turn resulted in increased endogenous cholesterol synthesis. In agreement, Dhingra and Bansal[14] demonstrated that 1 ppm Se supplementation was responsible for downregulation of HMG-CoA reductase expression during hypercholesterolaemia.

A balance between therapeutic versus toxicological effects of a compound is an important parameter when evaluating its usefulness as a pharmacological drug. The increased selenium bioaccumulation may be related to its ability to substitute sulfur in methionine to form the analogue, selenomethionine; the latter is directly incorporated into protein and so enhances the chance of its tissue accumulation.[42] Moreover, at the molecular level, selenium toxicity has been attributed to the oxidation of thiols of biological importance.[43] In this sense, it is important to mention that the liver is the major target organ of selenium toxicity.[44] Presumably this chronic type of poisoning is the result of prolonged ingestion of 10–30 ppm selenium, but in this study, $(PhSe)_2$ was used in a subtoxic dose (10 mg/kg e.g. 5.1 ppm Se).[43]

Furthermore, it has been known for a long time that inorganic selenium is highly toxic to several species of mammals.[45] However the methylated selenium is more easily excreted than inorganic selenium, resulting in lower tissue selenium levels and subsequent lower toxicity.[46] In line with this, previous results from our research group have demonstrated that $(PhSe)_2$, an organoselenium compound, has potential toxic effects only when animals are exposed to high doses, affecting the central nervous system of mice and causing impairment of glutamatergic transmission as well as liver and renal toxicity.[47] Reinforcing this idea, results from Meotti *et al.*[48] indicated that subchronic exposure to $(PhSe)_2$ at high doses (94 mg/kg) induced minor toxicological effects in rats. In addition, it is very important to mention that $(PhSe)_2$ does not induce toxic effects at doses in which it has pharmacological effects.

In this context, the toxicity of selenium compounds not only depends on the chemical form and the quantity of the element consumed, but also on a variety of other factors including species, age, physiological state, nutrition and dietary interactions, and the route of administration.[49]

Conclusions

A single oral administration of $(PhSe)_2$ was able to prevent the augmentation of plasma total cholesterol, non-HDL-cholesterol and triglyceride levels as well as to restore the HDL-cholesterol level in a Triton WR-1339-induced model of hyperlipidaemia in mice. The antioxidant effect of $(PhSe)_2$ in liver was not observed in this experimental protocol. Further studies are needed to elucidate the exact mechanism by which $(PhSe)_2$ exerted its hypolipidaemic effect, and its effect on atherosclerosis, since this organochalcogen has the potential to be a candidate for the development of a new hypolipidaemic drug.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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References

1. Xie W *et al*. Hypolipidemic mechanisms of *Ananas comosus* L. leaves in mice: different from fibrates but similar to statins. *J Pharmacol Sci* 2007; 103: 267–274.
2. Jeong WI *et al*. Mild hepatic fibrosis in cholesterol and sodium cholate diet-fed rats. *J Med Vet Sci* 2005; 67: 235–242.
3. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science* 1986; 232: 34–47.
4. Fielding CJ, Fielding FE. Molecular physiology of reverse cholesterol transport. *J Lipid Res* 1995; 36: 211–228.
5. Hamelet J *et al*. Hyperhomocysteinemia due to cystathionine beta synthase deficiency induces dysregulation of genes involved in hepatic lipid homeostasis in mice. *J Hepatol* 2007; 46: 151–159.
6. Oh PS *et al*. Hypolipidemic and antioxidative effects of the plant glycoprotein (36 kDa) from *Rhus verniciflua* stokes fruit in Triton WR-1339-induced hyperlipidemic mice. *Biosci Biotechnol Biochem* 2006; 70: 447–456.
7. Kumar SA *et al*. Protective role of eicosapentaenoate-lipoate (EPA-LA) derivative in combating oxidative hepatocellular injury in hypercholesterolemic atherogenesis. *Atherosclerosis* 2006; 189: 115–122.
8. May SW. Selenium-based pharmacological agents: an up date. *Exp Opin Invest Drugs* 2002; 11: 1261–1269.
9. Huang K *et al*. Role of selenium in cytoprotection against cholesterol oxide-induced vascular damage in rats. *Atherosclerosis* 2002; 162: 137–144.
10. Papp LV *et al*. From selenium to selenoproteins: synthesis, identity, and their role in human health. *Antioxid Redox Signal* 2007; 9: 775–806.
11. Suadicani P *et al*. Serum selenium concentration and risk of ischaemic heart disease in a prospective cohort study of 3000 males. *Atherosclerosis* 1992; 96: 33–42.
12. Thomas JP *et al*. Lethal damage to endothelial cells by oxidized low density lipoprotein: role of selenoperoxidases in cytoprotection against lipid hydroperoxide- and iron-mediated reactions. *J Lipid Res* 1993; 34: 479–490.
13. Lee O *et al*. The relationship between serum selenium levels and lipid profiles in adult women. *J Nutr Sci Vitaminol* 2003; 49: 397–404.
14. Dhingra S, Bansal MP. Modulation of hypercholesterolemia-induced alterations in apolipoprotein B and HMG-CoA reductase expression by selenium supplementation. *Chem Biol Interac* 2006; 161: 49–56.
15. Nogueira CW *et al*. Organoselenium and organotellurium compounds: toxicology and pharmacology. *Chem Rev* 2004; 104: 6255–6286.
16. Meotti FC *et al*. Protective role of aryl and alkyl diselenide on lipid peroxidation. *Environ Res* 2004; 94: 276–282.
17. Porciuncula LO *et al*. Ebselen prevents excitotoxicity provoked by glutamate in rat cerebellar granule neurons. *Neurosci Lett* 2001; 299: 217–220.
18. Nogueira CW *et al*. Anti-inflammatory and antinociceptive activity of diphenyl diselenide. *Inflamm Res* 2003; 52: 56–63.
19. de Bem A *et al*. Diphenyl diselenide, a simple glutathione peroxidase mimetic, inhibits human LDL oxidation in vitro. *Atherosclerosis* 2008; 201: 92–100.
20. Pérez-Pastén R *et al*. Hypolipidaemic and antiplatelet activity of phenoxyacetic acid derivatives related to α-ascarone. *J Pharm Pharmacol* 2006; 58: 1343–1349.
21. Harnafi H *et al*. Hypolipemic activity of polyphenol-rich extracts from *Ocimum basilicum* in Triton WR-1339-induced hyperlipidemic mice. *Food Chem* 2008; 108: 205–212.
22. Paulmier C. *Selenium Reagents and Intermediates*. *Organic Synthesis*. Oxford: Pergamon, 1986.
23. Ohkawa H *et al*. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95: 351–358.
24. Jacques-Silva MC *et al*. Diphenyl diselenide and ascorbic acid changes deposition of selenium and ascorbic acid in liver and brain of mice. *Toxicol Pharm* 2001; 88: 119–125.
25. Aebi H. Catalase in vitro. *Methods Enzymol* 1984; 105: 121–126.
26. Wendel A. Glutathione peroxidase. *Methods Enzymol* 1981; 77: 325–333.
27. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. *Anal Biochem* 1976; 72: 248–254.
28. Schurr PE *et al*. Triton induced hyperlipidaemia in rats as an animal model for screening hypolipidaemic drugs. *Lipids* 1972; 7: 69–74.
29. Friedman M, Byers SO. The mechanism responsible for the hypercholesteremia induced by Triton WR-1339. *J Exp Med* 1953; 97: 117–130.
30. Goldfarb S. Rapid increase in hepatic HMG-CoA reductase activity and in vivo cholesterol synthesis after Triton WR 1339 injection. *J Lipid Res* 1978; 19: 489–494.
31. Von Eckardstein A *et al*. High density lipoproteins and atherosclerosis: role of cholesterol efflux and reverse cholesterol transport. *Arterioscler Thromb Vasc Biol* 2001; 21: 13–27.
32. Wójcicki J. Effect of selenium and vitamin E on the development of experimental atherosclerosis in rabbits. *Atherosclerosis* 1991; 87: 9–16.
33. Morgan MJ *et al*. Lipid rafts and oxidative stress-induced cell death. *Antioxid Redox Signal* 2007; 9: 1471–1483.
34. Stokes KY *et al*. Hypocholesterolemia promotes inflammation and microvascular dysfunction: role of nitric oxide and superoxide. *Free Radic Biol Med* 2002; 33: 1026–1036.
35. Luchese C *et al*. Antioxidant effect of diphenyl diselenide on oxidative damage induced by smoke in rats: involvement of glutathione. *Ecotoxicol Environ Saf* 2009; 72: 248–254.
36. Luchese C *et al*. Brain and lungs of rats are differently affected by cigarette smoke exposure: antioxidant effect of an organoselenium compound. *Pharmacol Res* 2009; 59: 194–201.
37. Santos FW *et al*. Diphenyl diselenide reverses cadmium-induced oxidative damage on mice tissues. *Chem Biol Interac* 2005; 151: 159–165.
38. Landen BP, Porter TD. Inhibition of human squalene monooxygenase by tellurium compounds: evidence of interaction with vicinal sulfhydryls. *J Lipid Res* 2001; 42: 235–240.
39. Gupta N, Porter TD. Inhibition of human squalene monooxygenase by selenium compounds. *J Biochem Mol Toxicol* 2002; 16: 18–23.
40. Borges VC *et al*. Repeated administration of diphenyl ditelluride induces hematological disorders in rats. *Food Chem Toxicol* 2007; 45: 1453–1458.
41. Nassir F *et al*. Effect of selenium deficiency on hepatic lipid and lipoprotein metabolism in the rat. *Br J Nutr* 1997; 78: 493–500.

42. Panter KE *et al*. Comparative toxicity of selenium from seleno-DL-methionine sodium selenate, and *Astragalus bisulcatus* in pigs. *Fundam Appl Toxicol* 1996; 32: 217–223.
43. Painter EP. The chemistry and toxicity of selenium compounds, with special reference to the selenium problem. *Chem Rev* 1941; 28: 179–213.
44. Diskin CJ *et al*. Long-term selenium exposure. *Arch Intern Med* 1979; 139: 824–826.
45. Penrith ML. Acute selenium toxicosis as a cause of paralysis in pigs. *J S Afr Vet Assoc* 1995; 66: 47–48.
46. Chen CL *et al*. Effect of vitamin B12 on performance and tissue selenium content in rats fed sub-toxic levels of selenite. *Toxicology* 1993; 85: 101–115.
47. Nogueira CW *et al*. Investigations into the potential neurotoxicity induced by diselenides in mice and rats. *Toxicology* 2003; 183: 29–37.
48. Meotti FC *et al*. Toxicological evaluation of subchronic exposure to diphenyl diselenide in rats. *J Appl Toxicol* 2008; 28: 638–644.
49. Tinggi U. Essentiality and toxicity of selenium and its status in Australia: a review. *Toxicol Lett* 2003; 137: 103–110.