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THE EFFECTS OF COENZYME Q10 MICELLAR SOLUTION AND NANOLIPOSOMES ON SUPEROXIDE DISMUTASE (SOD) ACTIVITY IN CISPLATIN-INDUCED OXIDATIVE STRESS IN RATS

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ABSTRACT

Introduction: CoenzymeQ10 (CoQ10) is a lipid-soluble antioxidant that plays a key role in the mitochondria respiratory chain in the synthesis of adenosine triphosphate (ATP). It combats the oxidative stress in the body via increasing endogenous cellular defense system represented by superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) activity. Cisplatin is an antineoplastic drug used for treatment of various human malignances but because of its cytotoxicity, the therapeutic outcome of this drug is limited. Namely, it causes oxidative stress in the body by reducing the levels for glutathione (GSH), SOD, GpX, CAT and GR. Therefore, the aim of this study was to evaluate the influence of the CoQ10 supplementation (in a form of micellar solution or encapsulated into nanoliposomes) on SOD activity in oxidative stress, induced by the treatment with Cisplatin on rats.

Materials and methods: 90 normotensive Wistar rats (250-300 g) were included in this study. The animals were divided in 6 groups, each consisting of 15 rats. Cisplatin (5 mg/kg) and different formulations/combinations with CoQ10 (micellar solution or nanoliposomes dispersion, 10 mg/kg) were administrated i.p.

After 12 days, both kidneys were removed for measuring of SOD activity in the tissue. SOD activity was determined by the autoxidation of pyrogallol spectrophotometrically at 420 nm.

Statistical analysis was performed using Statistica 7.1 for Windows. Significance was determined at $p<0.05$

Results: CoQ10 nanoliposome treated group showed significantly increased SOD activity, compared to all other five groups. CoQ10 nanoliposome/Cisplatin treated group showed significantly increased SOD activity compared to the Cisplatin group. Additionally, CoQ10/Cisplatin group showed increased kidney SOD activity, compared to the Cisplatin group.

Conclusion: According to these results, CoQ10, as a potent antioxidant and encapsulated into nanoliposomes could be one of the possible solutions to reduce the oxidative stress and nephrotoxicity caused by the cisplatin treatment as a side effect, which is a common reason for reducing or discontinuing therapy.

Keywords: SOD activity, oxidative stress, cisplatin, coenzyme Q10, micellar solution, nanoliposomes.

INTRODUCTION

Cisplatin is one of the most effective chemotherapeutic agents, widely used in the treatment of several malignant diseases of the testes (1), head and neck (2), esophagus (3), bladder (4), ovaries (5), uterus (6), breast (7), small cell lung cancer (8), as monotherapy or in combination with other chemotherapeutic agents. However, it has limited use in clinical practice due to severe side effects, particularly nephrotoxicity, which causes acute renal failure in 20 to 35% of cisplatin-treated patients (9). Cisplatin-induced nephrotoxicity is dose-dependent and involves necrosis and apoptosis of cells in the renal tubules. In vitro studies have shown that apoptosis is caused by lower concentrations of Cisplatin, while cell necrosis is caused by higher concentrations of Cisplatin (10, 11). An increased concentration of TNFα has been observed, accompanied by an increase in IL-8, IL-1β, and IL-18 in the renal parenchyma of Cisplatin-treated animals. The high concentration of these cytokines further exacerbates the inflammation caused by TNF< and by the use of Cisplatin (12). Cisplatin nephrotoxicity is associated with structural and functional impairment of mitochondria (13). Cisplatin accumulates in the mitochondria of renal cells, inducing the production of reactive oxygen species (ROS), and at the same time, reducing the absorption of Calcium in the mitochondria, thus resulting in the release of pro-apoptotic factors that ultimately lead to tubular renal cell death (14). Literature data show that mitochondrial DNA is more affected by cisplatin damage than nuclear DNA. Cisplatin generates positively charged metabolites that accumulate predominantly in negatively charged mitochondria. Consequently, the sensitivity of cells to Cisplatin correlates with the number of mitochondria as well as the potential of the mitochondrial membrane. This explains the toxicity of Cisplatin predominantly on cells in the proximal renal tubules, relative to other parts of the body, mainly because the kidneys are the site of a large number of mitochondria, the so-called mitochondrial density (15).

Oxidative stress, i.e., ROS production and accumulation of lipid peroxidation products, causes Cisplatin nephrotoxicity and myelosuppression (16). Mitochondrial activation activates oxidative metabolism by producing ROS (superoxide anion O2), hydrogen peroxide H2O2, and hydroxyl radicals-OH), which reduce the defense capacity of antioxidants such as GSH, SOD, CAT, and GPx. The cellular effect of ROS is enhanced by high levels of nitric oxide, which in turn is produced by the activation of nitric oxide synthetase (iNOS). This leads to forming nitrogen metabolites that react with free oxygen anions, contributing to even more significant deterioration of cisplatin nephrotoxicity. The production of free oxygen and nitrogen radicals causes enzyme inactivation, lipid peroxidation, and irreversible DNA damage (17). Recently, a series of research have been done on the topic of herbal products, etc. dietary supplements that would act to reduce oxidative stress in the body during treatment with antineoplastic drugs that cause nephrotoxicity. One of them is Coenzyme Q10 (CoQ10), a liposoluble provitamin, which is present in many eukaryotic cells, mainly in the mitochondria of cells and is responsible for energy production, as well as for the exchange of electrons in redox processes. This substance in nutritional supplements, as monotherapy or in combination with others, is a potential solution for dealing with nephrotoxicity caused by antineoplastic drugs (18, 19). Wide spectrum of pathogenic processes in the body are closely linked to free radicals. SOD has attracted a lot of interest for therapeutic usage because of its capacity for scavenging (20). Therefore, the aim of this study was to evaluate the influence of the supplementation with CoQ10 prepared as micellar solution or as nanoliposomal dispersion on SOD activity in oxidative stress, induced by the treatment with Cisplatin on rats.

MATERIALS AND METHODS

MATERIALS

A "ready to use" Cisplatin solution (1 mg/mL) was purchased from Accord, Latvia and used as received. CoQ10 standard was supplied from Sigma-Aldrich, Germany. Tween 80 and ascorbic acid were obtained from Merck, Germany. The anesthetic agent (thiopental sodium 500 mg) was purchased from Ciron Drugs & Pharmaceuticals, India.

All other chemicals were of pharmaceutical/chemical grade and were used without further modifications.

Preparation of CoQ10 micellar solution for i.p. administration

The weight of each rat was reevaluated before preparing the CoQ10 micellar solution and the dose was adjusted accordingly. CoQ10 powder was solubilized in saline solution (0.9% NaCl) containing 1% Tween 80 in order to prepare CoQ10 micellar solution (5 mg/ml). Final micellar solution was obtained after continuous mixing and

heating (200 rpm, 65 ºC, 15 min). Afterwards, ascorbic acid (0.1% w/v) was added to maintain the antioxidant capacity of the prepared solution.

Preparation of nanoliposomes with CoQ10 for i.p administration

Nanoliposomal formulation with CoQ10 (final conc. 5 mg CoQ10/ml of liposomal dispersion) was prepared by a modified dry lipid film hydration method (21), previously designed and optimized at the Center for Pharmaceutical Nanotechnology, Faculty of Pharmacy, UKIM, Skopje. Prepared nanoliposomes were of uniform size distribution (PDI 0.26), with average size of 125 nm and prolonged release of the encapsulated CoQ10 (~50% within 24 hours (data not presented) (21).

For the control group of rats, saline solution (0.9% NaCl) containing 1% Tween 80 and 0.1% ascorbic acid was administered to rats every day of the study.

METHODS

This experiment involved ninety male, normotensive Wistar rats (250-300 g), obtained from the animal house of Faculty of Natural Science and Mathematics, UKIM Skopje and kept in wire polypropylene cages under typical laboratory environment (temperature 25±2 °C and artificial 12 h light/12 h dark cycle). The rats were fed with animal standard pellet diet and water ad libitum and allowed to acclimatize 1 week prior to the study. The animals were divided into 6 groups, each consisting of of 15 rats. Rats in group 0 (control group) received a physiological solution containing Tween 80 and ascorbic acid, i.p. (22). Rats in group 1 (Cisplatin treatment) received Cisplatin 5 mg/kg i.p, on the 4-th day of the experiment. Group 2 (CoQ10 treatment) included rats that received only CoQ10, 10 mg/kg i.p for 11 days, each day of the experiment. The rats in group 3 (Cisplatin/ CoQ10 treatment) received Cisplatin 5 mg/kg i.p, on the 4th day and CoQ10 10 mg/kg i.p, for 11 days, each day of the experiment. In the nanoliposome-treated groups, the dosage regimen was reduced considering the results of the in vitro release rate of CoQ10 (~50% during 24 hours) (21). Namely, the rats in group 4 (nanoliposomal CoQ10 treatment) received dispersion of nanoliposomes with encapsulated CoQ10 (equivalent to 10 mg/kg/ tt CoQ10 micellar solution) i.p. on days: 1, 3, 5, 7, 9, 11 of the study. The rats in group 5 (Cisplatin/nanoliposomal CoQ10 treatment), received Cisplatin 5 mg/kg i.p. on the 4-th day and nanoliposomal CoQ10 i.p. on days: 1, 3, 5, 7, 9, 11 of the study (equivalent to 10 mg/ kg/ tt CoQ10 micellar solution).

After the completion of the treatment of all groups, on the 12th day, under general anesthesia with thiopental, the abdomen of the rats was opened and both kidneys were removed for examination of SOD activity in kidney tissue by the method of Marklund and Marklund (23). A 3 ml assay mixture containing 0.2 mM of pyrogallol, 1 mM of EDTA and 50 mM of Tris-HCL buffer pH 8.2 was used for SOD evaluation. Pyrogallol autoxidation was monitored at 420 nm for 3 min spectrophotometrically (Cary 60 UV-VIS, Agilent Instruments, Germany), with or without the enzyme. The inhibition of pyrogallol oxidation was linear with the activity of the enzyme present. Inhibition of 50% in pyrogallol autoxidation/mg protein/min is expressed as one unit of the enzyme activity (24).

The data analysis was performed in a statistical program Statistica 7.1 for Windows. Significance was determined at $p<0.05$. The following methods were applied: 1. Descriptive statistics (Mean; Std. Deviation; ±95.00% CI; Median; Minimum; Maximum) were made for the series with numerical marks (kidney protein activity/ day 12) and 2. The difference in kidney protein activity/ day 12 between the six groups of experimental rats using Analysis of variance (F / p) /Post-hoc/LSD Test.

All obtained data are tabulated and graphically presented.

RESULTS

Table 1 and Figure 1 show descriptive statistics of kidney protein activity on day 12 in the six groups of experimental rats. The activity of proteins in the kidney/day 12 in the control group (0) varies in the interval 6413.54±857.72 kU/ mg; ±95.00% CI:5918.31-6908.78; the median is 6398.30 kU/mg, the minimum value is 4487.10 kU/mg and the maximum value is 7826.65 kU/mg.

Protein activity in kidney/day 12 in group 1 varies in the interval 1492.62±377.92 kU/mg; ±95.00% CI:1238.73-1746.52; the median is 1512.10 kU/mg, the minimum value is 1011.55 kU/mg and the maximum value is 1919.23 kU/ mg.

Protein activity in kidney/day 12 in group 2 varies in the interval 8144.22±1480.56 kU/mg; ±95.00% CI:7149.57-9138.88; the median is 8293.31 kU/mg, the minimum value is 5931.29 kU/mg and the maximum value is 11170.72 kU/mg.

Protein activity in kidney/day 12 in group 3 varies in

the interval 2397.38±611.18 kU/mg; ±95.00% CI:1960.17-2834.59; the median is 2563.45 kU/mg, the minimum value is 1221.73 kU/mg and the maximum value is 3212.45 kU/mg.

Protein activity in kidney/day 12 in group 4 varies in the interval 8882.46±1477.01 kU/mg; ±95.00% CI:7647.65-10117.28; the median is 9325.18 kU/mg, the minimum value is 5571.45 kU/mg and the maximum value is 10252.19 kU/mg.

Protein activity in kidney/day 12 in group 5 varies in the interval 3583.33±360.52 kU/mg; ±95.00% CI:3281.93-3884.73; the median is 3642.02 kU/mg, the minimum value is 2985.76 kU/mg and the maximum value is 4191.05 kU/mg.

Table 1. Protein activity in kidney/day 12 in different treatment groups

Variable	Valid N	Mean	Confidence -95.00%	Confidence +95.00%	Median	Minimum	Maximum	Std.Dev.
SOD (0) day 12 kidney	14	6413.54	5918.31	6908.78	6398.30	4487.10	7826.65	857.72
SOD (1) day 12 kidney	11	1492.62	1238.73	1746.52	1512.10	1011.55	1919.23	377.92
SOD (2) day 12 kidney	11	8144.22	7149.57	9138.88	8293.31	5931.29	11170.72	1480.56
SOD (3) day 12 kidney	10	2397.38	1960.17	2834.59	2563.45	1221.73	3212.45	611.18
SOD (4) day 12 kidney	8	8882.46	7647.65	10117.28	9325.18	5571.45	10252.19	1477.01
SOD (5) day 12 kidney	8	3583.33	3281.93	3884.73	3642.02	2985.76	4191.05	360.52

*Control group (0); Treatment groups: (1(Cisplatin); 2(micellar solution CoQ10); 3(Cisplatin+ micellar solution CoQ10); 4(nanoliposomal CoQ10); 5(Cisplatin+nanoliposomal CoeQ10))

Table 2. Protein activity in kidney (day 12)/Difference between groups

Variable	SS Effect	Df Effect	MS Effect	SS Error	Df Error	MS Error	F	p
Protein activity / day 12 kidney	474965493	5	94993099	52455470	56	936704.8	101.41	0.000

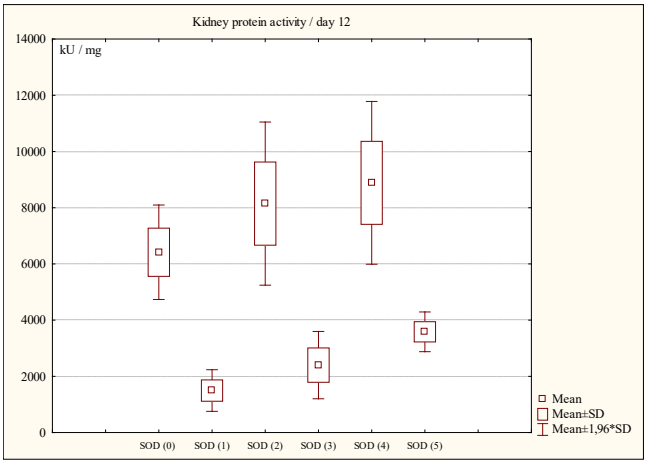

Fig. 1. Graphical presentation of descriptive statistics of kidney protein activity on day 12 in the six groups of experimental rats.

For F = 101.41 and p<0.001(p=0.000) there is a significant difference in protein activity in kidney (day 12) between the six groups of experimental rats (Table 2).

The results shown in Table 3 refer to the Post-hoc/LSD Test analysis of protein activity in the kidney (day 12) between the six groups of experimental rats.

The highest protein activity in the kidney (day 12) was observed in group 4 (experimental group of animals treated with nanoliposomal dispersion of CoQ10)/M=8882.5 kU/mg, which for p<0.001 (p=0.000) is significantly higher than the activity of proteins in kidney (day 12) in control group 0 (M=6413.5 kU/mg), in group 1 (M=1492.6 kU/mg), in group 3 (M=2397.4 kU/mg), in group 5 (M=3583.3 kU/mg) and for p>0.05 (p=0.11) it is insignificantly higher than the activity of proteins in the kidney (day 12) in group 2 (M=8144.2 kU/mg) (p<0.001 (p=0.000)).

The lowest protein activity in the kidney (day 12) was observed in group 1 (experimental group of animals treated with Cisplatin)/M=1492.6 kU/mg, which for p<0.001 (p=0.000) is significantly lower than the activity of proteins in kidney (day 12) in control group 0 (M=6413.5 kU/mg), in group 2 (M=8144.2 kU/mg), in group 4

(M=8882.5 kU/ mg), in group 5 (M=3583.3 kU/mg.), and for $p<0.05$ ($p=0.03$) it is significantly lower than the activity of proteins in the kidney (day 12) in group 3 (M=2397.4 kU/ mg).

Table 3. Protein activity in kidney (day 12)/Post-hoc/LSD Test

Group	{1} M=6413,5	{2} M1492,6	{3} M=8144,2	{4} M=2397,4	{5} M=8882,5	{6} M=3583,3
0 {1}		0.000	0.000	0.000	0.000	0.000
1 {2}	0.000		0.000	0.03	0.000	0.000
2 {3}	0.000	0.000		0.000	0.11	0.000
3 {4}	0.000	0.03	0.000		0.000	0.01
4 {5}	0.000	0.000	0.11	0.000		0.000
5 {6}	0.000	0.000	0.000	0.01	0.000	

*Control group (0); Treatment groups: (1(Cisplatin); 2(micellar solution CoQ10); 3(Cisplatin+ micellar solution CoQ10); 4(nanoliposomal CoQ10); 5(Cisplatin+nanoliposomal CoeQ10))

DISCUSSION

Oxidative stress is a serious pathological process and plays an important role in Cisplatin- induced nephrotoxicity. According to experimental data, cisplatin- induced nephrotoxicity is caused by increased ROS and MDA levels as well as decreased activity of the antioxidant enzymes SOD and CAT (25-27). These findings agreed with the findings of the current investigation.

CoQ10 serves as an endogenous antioxidant as well as an important cofactor of the electron transport chain (28, 29).

CoQ10 increases the SOD activity in kidney tissue in rats. While SOD neutralizes superoxide radicals produced in tissues, glutathione prevents the production of ROS by scavenging superoxide radicals inside tissue (30). SOD, as an antioxidant enzyme, removes superoxide anions by converting it to oxygen and hydrogen peroxide. This stops the generation of peroxynitrite and other damage-causing processes. In a study performed on experimental animals where the administration of cisplatin-induced nephrotoxicity, co-administration of CoQ10 decreased serum urea (BUN) and creatinine levels, increased glutathione GSH and SOD dismutase activity, followed by reduced lipid peroxide and of TNF< concentrations (22). CoQ10 applied as a monotherapy or combined with other supplements significantly reduces Malondialdehyde (MDA) levels and raises SOD, CAT, and GSH concentrations in diabetic nephropathy (31). In an experimental study with rats (Wistar strain), where lead-induced nephrotoxicity was induced by administration of 20 mg/kg/tt., lead, subsequent co-administration of CoQ10 at a dose of 10 mg/ kg/tt. had shown an improvement in all blood parameters and a reduction in values of proinflammatory cytokines TNF< and IL-1< Oxidative status (SOD, CAT, GPx) was significantly improved in the group where CoQ10 was given compared to the group in which led (PbAc) was administered without the presence of the supplement (32). Nevertheless, this supplement is a potential solution to improve oxidative stress due to many pathological conditions such as ischemic retinal injury and coronary artery disease. The supplement's effects are directly proportional to the dose of CoQ10 applied (33, 34).

Concerning the treatment with CoQ10 nanoliposomes, the current study demonstrated that CoQ10 prepared as nanoliposomal dispersion administrated at a dose of 10 mg CoQ10/kg, significantly improves the oxidative stress caused by the treatment with cisplatin than the micellar solution. This might be owing to the extended circulation duration of nanoliposome-encapsulated CoQ10 in the blood and increased supplement distribution in the kidney with prolonged release of CoQ10. Namely, liposomal carrier systems have many advantages in the direction of their potential to increase pharmacological features such as stability (by encapsulation), bioavailability, tissue targeting and facilitating the intracellular uptake of different actives (35). So far, liposomes with encapsulated CoQ10 had shown improving IL-6, CRP and phospho-NF-<B after the acute exposure to paracetamol overdose, improving oxidative enzyme SOD, CAT, GPx in diabetic rats and improving the morphological and histopathological kidney tissue in diabetic rats (36, 37) probably due to the fact that liposomes can penetrate the membrane easily and transport CoQ10 directly to the mitochondria thanks to their unique bilayer membrane structure (38).

CONCLUSION

Coenzyme Q10 shows vigorous antioxidant activity in many diseases in which oxidative stress is the cause of the illness or is a consequence of toxicity caused using certain antineoplastic drugs, such as Cisplatin. Despite its nephrotoxicity as a side effect, Cisplatin remains at the forefront in treating many malignancies due to its efficacy. Coenzyme Q10 designed as micellar solution or nanoliposome dispersion, as a potent antioxidant, could be one of the possible solutions to reduce the oxidative stress and nephrotoxicity caused by the cisplatin treatment as a side effect, which is a common reason for

reducing or discontinuing therapy.

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