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EFFECTS OF ORAL COENZYME Q10 SUPPLEMENTATION ON SODIUM NITRITE-INDUCED LIPID PEROXIDATION IN RATS

WPŁYW KOENZYMU Q10 NA PEROKSYDACJĘ LIPIDOWĄ INDUKOWANĄ AZOTYNEM SODOWYM U SZCZURÓW

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Present studies elucidate the anti-oxidative effectiveness of oral coenzyme Q10 supplementation in rats poisoned per os with sodium nitrite for two weeks. The anti-oxidant agent has been found to mitigate sodium nitrite-induced lipid peroxidation in the small intestine and liver of rats, and it increased the total anti-oxidant status of rat blood.

INTRODUCTION

Coenzyme Q10 (2,3-dimethoxy-5-methyl-6-multiprenyl-1,4-benzoquinones) is well-known as a component of the mitochondrial electron transport system [20]. A good deal of experimental evidences also exists that coenzyme Q10 acts as a powerful anti-oxidant in blood and tissues. For example, ubiquinol, a reduced form of coenzyme Q10, has been found to play an anti-oxidative role in selenium-deficient plasma membranes, and the agent protected serum low-density lipoproteins from peroxidation processes [18, 23]. In our previous experiments, coenzyme Q10 has been found to decrease the exercise-induced lipid peroxidation of rat muscles [7, 8], and it increased the anti-oxidative effect(s) of diallyl sulfide in gamma irradiated rats [10]. It should be emphasized that coenzyme Q10 was beneficially used as an anti-oxidant drug-candidate for the treatment of a variety of pathologies including breast cancer [25], Huntington's disease [9], coronary heart failure [19, 30], hyperthyroidism [4], and/or carbon tetrachloride-induced hepatotoxicity [29].

In recent years, the pro-oxidant properties of sodium nitrite have received much attention as these may contribute its pathological role in the gastrointestinal tract of laboratory rodents [11]. Although sodium nitrite has been found to increase lipid peroxidation [13] and the agent decreased ATP production in rat small intestinal mitochondria [12], the anti-oxidant action(s) of coenzyme Q10 was not studied in nitrite-poisoned rats. We therefore performed this pilot study to elucidate whether a short-term oral coenzyme Q10 supplementation could mitigate sodium nitrite-induced lipid peroxidation and/or pro-oxidant shift(s). The total anti-oxidant status of rat blood was also investigated.

MATERIALS AND METHODS

Male *Wistar* rats (220 ± 20 g) were used in the studies. Before the experiment, the animals were acclimatized for two weeks under standard conditions. Throughout the experiment, the rats were given a standard laboratory chow (Murigran pellet, Motycz, Poland) and water *ad libitum*. The animals were divided into 2 groups of 14–16 rats in each group, and they were treated *per os* with either an aqueous solution of sodium nitrite (10 mg/kg body weight) or normal saline (control) daily for 14 days. On day 7^{th} of the experimental period, the half of randomly selected nitrite-or saline-treated rats was pretreated *per os* with coenzyme Q10 (10 mg/kg body weight) for 7 days only. The agent was dissolved in corn oil, and it was daily dosed to rats at 3–4 hr post-nitrite and/or post-saline pretreatment. The animals were sacrificed by cervical dislocation at 24 hr after the last nitrite and/or saline dosage (day 15), and thiobarbituric-acid reactive substances (TBARS) were determined in rat blood and the small intestinal mucosa and/or liver homogenates by the method of *Ohkawa* et al. [24]. Briefly, thiobarbituric acid test was performed using 100 µl of rat serum and/or 100 ml of 10% tissue homogenates prepared in 1,15% KCl, which was added to 100 µl of 8,1% SDS. Thereafter, 20% glacial acetic acid and 2-thiobarbituric acid (v/v) were added to the reaction mixture. To start the reaction, the samples were heated for one hour at $95^{o}C$, and then were cooled in a water bath. The mixtures were extracted with a spectral pure *n*-butanol and centrifuged (4000,0 x g) for 10 min at $4^{o}C$. All butanol extracts were measured spectrophotometrically at 532 nm. Standard samples contained 1,1,3,3-tetraethoxypropane instead of homogenate. The total antioxidant status (TAS) of rat blood was determined using the Randox^{TM} assay kit (TAS, Randox^{TM}, 1993, pp. 1–6, Radnox Laboratories Ltd., Antrim, UK). In this procedure, the azo-compound ABTS® (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) was incubated with a peroxidase (metmyoglobin) and hydrogen peroxide (H_2O_2) to produce the radical cation $ABTS^{\cdot+}$. The cation has had a relatively stable blue-green colour, which it was measured spectrophotometrically at 600 nm. Antioxidants in the added sample caused suppression of this colour production to a degree, which was proportional to their concentration. In the present assay, TMCA® (6-hydroxy-2,5,7,8-teramethylchroman-2-carboxylic acid) was used as a standard. Protein content was measured by the method of *Lowry* et al. [21] with bovine serum albumin as a standard. All reagents were of the highest quality available from Randox Laboratories Ltd., and Sigma Chemical Company (St. Louis, MO, USA).

The results were subjected to statistical analysis by *Student's t*-test for unpaired samples. Differences were considered significant when probability (p) values were less than 0.05.

RESULTS AND DISCUSSION

In the present studies, sodium nitrite has been found to increase thiobarbituric-acid reactive substances (TBARS) in the small intestinal mucosa and liver of rats, however, the agent did not have any effect(s) on the total anti-oxidant status and lipid peroxidation of rat blood (Fig. 1). Our results were found in accordance with those reported by *Mansouri* [22], who showed that sodium nitrite acts as an oxidative stress producer in red cells. It should be noted that sodium sodium nitrite increased lipid peroxidation in the small intestinal mucosa of rats, and it reduced ATP-ase activity in brush border membranes [13]. As evidenced by *Grudziński* and *Szymański* [14], sodium nitrite also decreased activities of succinate and lactate dehydrogenases in the pyloric stomach of rats, and the agent lowered alkaline phosphatase activity in both rat serum and small intestinal mucosa [15]. Sodium nitrite has been found to increase the activity of ornithine decarboxylase, a first-step enzyme in putrescine biosynthesis in gastric epithelium [16], and it also decreased oxygen consumption and ATP production in murine

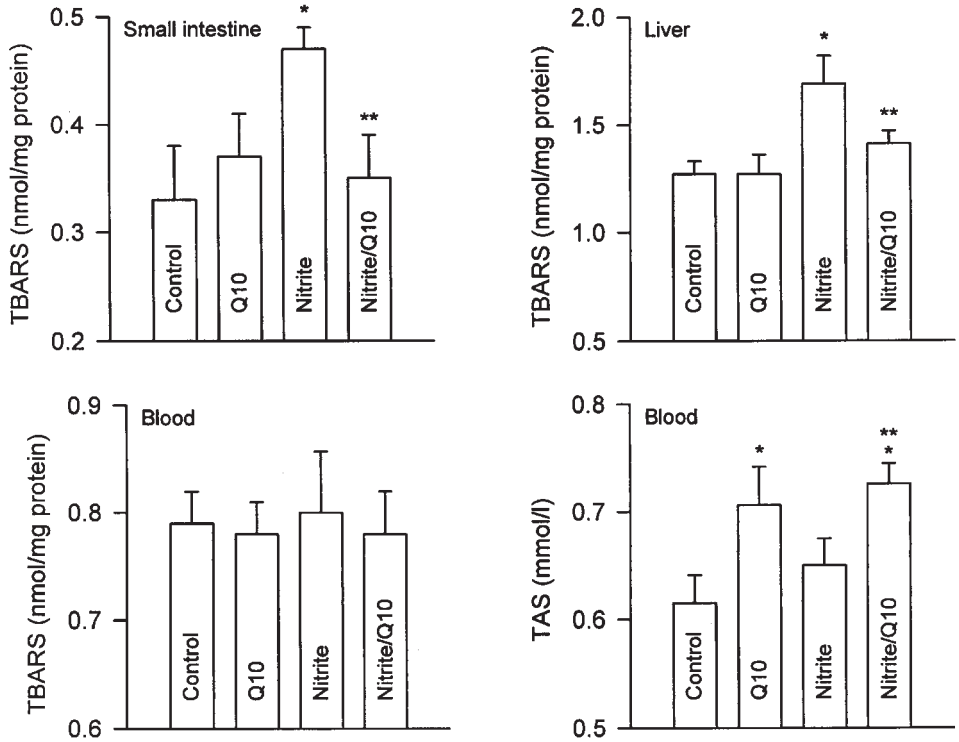

Fig. 1. The effect of oral coenzyme Q10 supplementation on the level of thiobarbituric acid reactive substances (TBARS) in the small intestine, liver and serum, and the total anti-oxidant status of blood in Wistar rats pretreated with or without sodium nitrite. Values are mean ± SEM, n= 7–8. * $P < 0.05$, sodium nitrite or coenzyme Q10 *vs.* saline (control), ** $P < 0.05$, sodium nitrite plus coenzyme Q10 *vs.* sodium nitrite.

mitochondria [12]. Since water- and/or food-born inorganic nitrite has been classified as a risk factor(s) of gastric and/or colorectal cancers in humans [6], we have decided to investigate whether an oral coenzyme Q10 supplementation influences sodium nitrite-induced lipid peroxidation and/or pro-oxidant shift(s).

Pretreatment of sodium nitrite-poisoned rats with coenzyme Q10 significantly ($p<0.05$) decreased the amount of TBARS in examined tissues (Fig. 1). Interestingly, coenzyme Q10 increased the level of the total anti-oxidant status of blood in animals treated with or without sodium nitrite. In accordance with our results, several studies have reported on decreased susceptibility to TBARS production after supplementation of coenzyme Q10 *in vivo*. For example, statistically significant decrease in lipid peroxidation products such as malonyldialdehyde and 4-hydroxynonenal was observed in the paint and lacquer industry workers orally supplemented with coenzyme Q10 following inhalation expose to organic solvents [5]. In other recent studies, an antioxidant role of coenzyme Q10 against the ischemia and reperfusion-induced lipid peroxidation in fetal rat brain was evidenced [32]. Coenzyme Q10 was found to reduce acetaminophen-induced hepatic injury and lipid peroxidation in mice [1], and the agent

preserved the normal cellular defenses against the oxidative stress induced by adriamycin [27]. As evidenced by *Kagan* [17], coenzyme Q10 was enable to block apoptosis and the agent decreased fumonisin B1-induced oxidative DNA damage in both rat liver [2] and human lymphocytes, respectively [31]. It should be noted that coenzyme Q10 mitigated iron-induced liver toxicity [28], and it decreased lipid peroxidation and free radicals in ethanol-fed animals [3]. Since free radicals are well established promoters of platelet activation [26], diminished vitronectin-receptor expression and reduced platelet size resulting from coenzyme Q10 therapy has been recently published.

Beside the preliminary-established an anti-oxidant role of coenzyme Q10 in sodium nitrite-treated rats, the results of this experiment cannot be discussed beyond the model studied here. Since we did not examine any further biochemical and/or cellular mechanim(s) by which coenzyme Q10 supplementation affects nitrite-induced lipid peroxidation, our present observation should be re-tested in further controlled experiments with a variety of lipid peroxidation biomarkers and/or anti-oxidant enzyme measurements.

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Summary

Studies were carried out to examine the anti-oxidative effect(s) of oral coenzyme Q10 supplementation (10 mg/kg b.w./day) in rats treated *per os* with either sodium nitrite (10 mg/kg b.w./day) or saline (control) for 14 days. Results showed that sodium nitrite increases thiobarbituric-acid reactive substances (TBARS in rat small intestinal mucosa and liver, and the agent did not have any effect(s) on the total anti-oxidant status (TAS) and lipid peroxidation of rat blood. Pretreatment of nitrite-poisoned rats with coenzyme Q10 mitigated TBARS and increased TAS in animal blood. Coenzyme Q10 has been found to be a promising anti-oxidant agent in sodium nitrite-induced lipid peroxidation.

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Streszczenie

Badano antyoksydacyjne właściwości koenzymu Q10, który podawano dożołądkowo (10 mg/kg m.c./dzień) szczurom zatruwanym *per os* azotynem sodowym (10 mg/kg m.c./dzień) przez okres 14 dni. Azotyn sodowy zwiększał poziom substancji reagujących z kwasem tiobarbiturowym (TBARS) w błonie śluzowej jelita cienkiego i wątrobie szczurów nie wpływając na poziom peroksydacji lipidowej oraz całkowity status anty-oksydacyjny krwi. Koenzym Q10 obniżał azotynowo-indukowaną peroksydację lipidową oraz zwiększał poziom TAS u szczurów zatruwanych azotynem sodowym. Przeprowadzone badania dowodzą, że koenzym Q10 posiada antyoksydacyjne właściwości mogące obniżać peroksydację lipidową indukowaną azotynem sodowym.

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