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THE EFFECT OF OZONE AND VITAMINS C AND E ON THE ACTIVITY OF 17-β-HYDROXYSTEROID DEHYDROGENASE AND ALKALINE PHOSPHATASE, AND TESTOSTERONE CONCENTRATIONS IN MALE RATS

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Key words: ozone, vitamin E, vitamin C, rats, testes.

Abstract

The objective of this study was to determine the effect of oxidative stress caused by exposure to ozone on the activity of 17-β-hydroxysteroid dehydrogenase and alkaline phosphatase, and testosterone concentrations in male rats, and to investigate the possible protective effect of easily available antioxidants such as vitamins E and C. The experiment was conducted on adult Wistar-Hannover rats. One group of animals was exposed to ozone without vitamin cover, and the remaining animals were administered vitamins E and C in various combinations and doses. Ozone exposure in the group of rats not receiving vitamin injections caused oxidative stress manifested by elevated MDA concentrations in the blood plasma and testicular tissue. An increase in MDA levels was also observed in the group of animals administered vitamins, excluding the animals receiving low- and average-dose combinations of vitamins E and C. A drop in the activity of 17-β-hydroxysteroid dehydrogenase was reported in animals exposed to ozone, but this effect was not noted in the groups exposed to ozone and receiving vitamins. The lowest blood testosterone levels were observed in rats exposed to ozone and in the groups receiving low- and average-dose combinations of vitamins E and C.

WPŁYW OZONU ORAZ WITAMINY E I C NA AKTYWNOŚĆ DEHYDROGENAZY 17-β-HYDROKSYSTEROIDOWEJ I ALKALICZNEJ FOSFATAZY ORAZ KONCENTRACJĘ TESTOSTERONU U SAMCÓW SZCZURÓW

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Słowa kluczowe: ozon, witamina E, witamina C, szczury, jądra.

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Abstrakt

Celem pracy było określenie wpływu stresu oksydacyjnego powodowanego eskpozycją na ozon na aktywność dehydrogenazy 17-β-hydroksysteroidowej i alkalicznej fosfatazy oraz koncentrację testosteronu u samców szczurów, jak również ewentualne ochronne oddziaływanie łatwo dostępnych antyoksydantów, takich jak witamina E i C. Doświadczenie przeprowadzono na dorosłych szczurach samcach szczepu Wistar/Hannover. Część zwierząt była ozonowana bez osłony witaminowej, pozostałe otrzymywały witaminy E i C w różnych kombinacjach i dawkach. Ekspozycja szczurów na ozon, bez iniekcji witamin, wywołała stan stresu oksydacyjnego, objawiający się wzrostem koncentracji MDA zarówno w osoczu, jak i w tkance jąder. Wzrost poziomu MDA nastąpił również w grupach zwierząt otrzymujących witaminy, z wyjątkiem szczurów dostających niskie i średnie dawki witaminy E i C łącznie. U zwierząt ozonowanych zaobserwowano spadek aktywności dehydrogenazy 17-β-hydroksysteroidowej, czego nie zanotowano w grupach ozonowanych z witaminami. Koncentracja testosteronu we krwi była najniższa u szczurów eksponowanych na ozon i w grupach otrzymujących średnie i wysokie dawki witaminy E i C łącznie.

Introduction

Ozone is one of the key air pollutants. Its toxicity can be attributed mainly to interactions with polyunsaturated fatty acids, which stimulates the formation of free radicals and other compounds such as hydrogen peroxide, protein oxidation products, lipid hydroperoxides and the highly toxic malondialdehyde (PRYOR et al. 1991). Ozone is a highly reactive gas that penetrates the tissue-air boundary, but ozone-induced changes have also been determined in other organs (PRYOR 1992). The above effects are attributed not only to ozone's direct oxidative activity (which is principally restricted to lungs), but also its ability to activate free radical-initiated cascade reactions and produce toxic peroxidation compounds (ESKEW et al. 1986, TOMSITS et al. 1994).

Living organisms deploy various mechanisms of protection against oxidative stress, including with the involvement of vitamins E and C. Vitamin E's protective effect on the lipid environment is manifested by two processes. The first relies on direct interactions with ozone which deprive this gas of its oxidative effects, and second involves the free radical scavenging mechanism to inhibit the autooxidation of polyunsaturated fatty acids (LIEBLER et al. 1993, ZIELIŃSKI 1997). Vitamin C is a water-soluble antioxidant. It interacts directly with peroxides, free radicals and atomic oxygen in an aquatic environment to protect low density lipoproteins (LDLs) and serum lipids against oxidative damage (PACKER et al. 1979).

There is a scarcity of published data on ozone's effects on the male reproductive system, including testes. The results of previous research have demonstrated a drop in testosterone levels and the activity of 17-β-hydroxysteroid dehydrogenase in rats subjected to long-term ozone exposure (JEDLIŃSKA-KRAKOWSKA 1998). Pathological changes in the spermatogenic epithelium were also observed (JEDLIŃSKA-KRAKOWSKA et al. 2006). In view of the mechanism of ozone-induced toxicity, the objective of this study was to investigate the effectiveness of popular antioxidants, such as vitamins E and C, in preventing the harmful consequences of oxidative stress.

Materials and Methods

The experiment was conducted on 96 Wistar-Hannover rats aged five months (at the beginning of the study), with average body weight of 370 ± 10 g. The animals were divided into 12 groups ($n = 8$ in each group):

– I (Con) – control;

– II (Con PBS) – control animals receiving PBS injections i.m.

All animals from groups 3–12 were exposed to 0.5 ppm ozone for 50 days. Daily exposure time was 5 h (8.30 a.m. to 1.30 p.m.). The air in the exposure chamber was exchanged every two hours to prevent excessive CO_2 accumulation. Ozone concentrations were restored to the original level after every exchange. The animals from groups 3–12 received vitamin E and C injections i.m. at the following doses:

III – (1.5E) – 1.5 mg vit. E; IV – (4.5E) – 4.5 mg vit. E; V – (15E) – 15 mg vit. E; VI – (3C) – 3 mg vit. C; VII – (9C) – 9 mg vit. C; VIII – (50C) – 50 mg vit. C; IX – (1.5E 3C) – 1.5 mg vit. E and 3 mg vit. C; X – (4.5E 9C) – 4.5 mg vit. E and 9 mg vit. C; XI – (15E 50C) – 15 mg vit. E and 50 mg vit. C; XII – (Oz) – ozone exposure only.

Ozone was supplied from air compressed in the IMPOZ-4 ozone generator (Institute of Precision Mechanics, Warsaw) to a chamber sealed with chemically neutral polyethylene film, where it was spontaneously mixed with air. Ozone concentrations in the exposure chamber were controlled by iodometry (SALTZMAN et al. 1959). During exposure, the animals had ad libitum access to water, but feed was not administered owing to ozone's oxidative effect. Outside the daily exposure regime of 5 h, all animals were kept under identical conditions as regards air composition, temperature and diet.

After the experiment, blood samples were obtained by heart puncture from all rats anesthetized with halothane (Narcotan, Leciva, Czech Republic) until completely bled. Blood samples were centrifuged at 4°C, 3000 rpm, for 10 minutes. Blood plasma was separated and stored at -22°C for the determination of vitamin E and C levels, testosterone and malondialdehyde (MDA) concentrations (WARD et al. 1985). Directly after bleeding, gonadal fragments were excised to measure MDA, vitamin E and C levels (RETTENMAIER et al. 1992, SANDOR et al. 1989, OMAYE et al. 1979). MDA concentrations were determined in a 20% saline homogenate of testicular tissue.

Gonadal sections were also used to prepare frozen specimens in a cryostat (CRYOCAT, Reichert Young). The activity of alkaline phosphatase (AP) was determined in the specimens by Gomori's histochemical technique, and the activity of 17-β-hydroxysteroid dehydrogenase – by the method proposed by Levy, Deane and Rubin. The activity levels of the studied enzymes were estimated using computer image analysis (System for Image Processing and Analysis LUCIA). The optical density of the image produced by the studied reaction was measured in the specimens and expressed on a scale of 0 (black – high activity) to 62 (white – low activity).

The results were processed statistically to calculate the arithmetic means, the standard error of the mean (SEM) and the significance of differences relative to the control group, using Student's *t*-test (Statgraphic application, Statistical Graphic System).

Results and Discussion

A significant increase in malondialdehyde concentrations in the blood was noted in all animals exposed to ozone, regardless of the applied vitamin dose and combination. Elevated MDA levels in testicular tissue were observed in rats exposed to ozone as well as in the animals exposed to ozone and receiving vitamin C and E injections separately, regardless of the dose. In the groups administered low-dose combinations of both vitamins, MDA concentrations were comparable to control group values. The above could be attributed to the synergistic effect of the studied antioxidants where the produced tocopheryl radicals are reduced by vitamin C, thus reinstating the original structure of vitamin E (BARTOSZ 2009). In the group of rats exposed to ozone as well as animals exposed to ozone and receiving high doses of vitamin E and C, MDA concentrations were significantly elevated. Authors differ in their opinions regarding the antioxidative and prooxidative effects of ascorbic acid. When administered at higher doses, vitamin C demonstrates prooxidative activity, it contributes to the formation of hydroxyl radicals (Fenton's reaction), oxidative stress and elevated malondialdehyde levels (CARR et al. 1999, NAIDU 2003, JEDLIŃSKA-KRAKOWSKA 2006).

Vitamin C concentrations in the blood plasma were marked by a significant increase in the group of rats receiving the highest vitamin C doses as well as average- and low-dose combinations of vitamins E and C (Table 1). Despite significant variations between groups, the increase in vitamin E levels was statistically non-significant owing to high SEM values. Ascorbic acid is a water-soluble substance that does not demonstrate a cumulative effect, nonetheless, an insignificant increase was noted in testicular tissue. A significant increase in vitamin E levels was noted in the testes of all ozone-exposed rats not receiving vitamins, in animals receiving vitamin E, animals receiving both vitamins as well as in rats administered the highest doses of vitamin C. Vitamin E may be mobilized from other parts of the body by organs that are directly exposed to ozone-induced oxidative stress, provided that it is present in sufficient quantities. The above applies mainly to lungs. The same mobilization mechanism may apply to other antioxidants, subject to the type of stress (ELSAYED et al. 1993). High levels of vitamin E in the testes of rats exposed to ozone and receiving high doses of vitamin E, high doses of vitamin C as well as high-dose combinations of both vitamins may be related to an increased demand for antioxidants (in view of the free radical cascade initiated by ozone and the properties of ascorbic acid) as well as the mutual interactions between the studied vitamins (PRYOR et al. 1991, BARTOSZ 2009).

Table 1

Concentration of vitamin E and C, and MDA in testes and blood plasma (±SEM)

Group	Vitamin C		Vitamin E		MDA	
	[μg/ml]	[μg/g]	[μg/100 ml]	[μg/100 g]	[μM/l]	
	blood plasma	testes	blood plasma	testes	blood plasma	testes
I Con	44 ± 8.9	84.5 ± 20.47	424 ± 224	70.1 ± 16.5	7.35 ± 0.84	1.88 ± 0.2
II Con PBS	34.8 ± 7.46	79.5 ± 22.87	246 ± 36.9	20.6 ± 13.1**	7.92 ± 1.02	2.15 ± 0.33
III 1.5E	44.8 ± 7.78	92.3 ± 8.62	680 ± 566	896 ± 241**	14.8 ± 1.77**	4.39 ± 0.37**
IV 4.5E	46.6 ± 4.82	98.4 ± 9.3	408 ± 65	166 ± 55**	15.46 ± 2.12**	4.47 ± 0.5**
V 15E	40.2 ± 4.66	104.9 ± 9.61	409 ± 145	225 ± 74**	12.8 ± 0.86**	2.78 ± 0.22*
VI 3C	49.1 ± 7.75	90.7 ± 8.47	237 ± 45	99 ± 24	13.4 ± 1.13**	2.98 ± 0.21*
VII 9C	50.7 ± 6.42	99.2 ± 3.51	1260 ± 1321	78 ± 17	15.4 ± 2.48**	3.53 ± 0.47**
VIII 50C	60.1 ± 4.07**	99.2 ± 9.38	2195 ± 2896	127 ± 27**	16.53 ± 1.52**	2.73 ± 0.25*
IX 1.5E 3C	38.4 ± 3.62	102.4 ± 9.24	457 ± 141	124 ± 36*	12.32 ± 1.06**	1.70 ± 0.4
X 4.5E 9C	55.7 ± 2.62**	91.2 ± 7.5	278 ± 59	743 ± 198**	11.47 ± 0.85**	1.95 ± 0.42
XI 15E 50C	58.2 ± 3.6**	90.8 ± 9.67	3696 ± 3412	843 ± 357**	12.0 ± 1.23**	4.82 ± 1.26**
XII Oz	53.4 ± 3.2	91.8 ± 10.22	359 ± 58	780 ± 152**	12.28±1.47**	4.37 ± 0.33**

** $p \leq 0.01$ * $p \leq 0.05$ significance in relation to the control group (Con)

The activity of alkaline phosphatase in testicular tissue was not marked by significant variations in comparison with the control group, and the lowest, statistically non-significant values were noted in rats exposed to ozone without vitamin cover (Table 2). Oxidative stress is generally accompanied by a drop in AP activity (OYAGBEMI et al. 2010) which it is an indicator of the quality and biological value of semen. The results of a previous study have demonstrated that although ozone exposure does not have a negative impact on sperm morphology, it reduces sperm concentrations (JEDLIŃSKA-KRAKOWSKA et al. 2006). The activity of 17-β-hydroxysteroid dehydrogenase decreased only in rats exposed to ozone and not receiving vitamins. The lowest blood testosterone levels were also noted in this group of animals. Testosterone concentrations were marked by a significant drop also in the group of rats receiving average- and low-dose combinations of vitamin E and C. The above can be attributed to the fact that ozone-induced oxidative stress disrupts steroidogenesis in testes, thus lowering testosterone levels (JEDLIŃSKA-KRAKOWSKA et al. 2007).

Table 2

The activity of alkaline phosphatase and 17-β-hydroxysteroid dehydrogenase in testes, and concentration of testosterone in blood plasma (±SEM)

Group	17-β-hydroxysteroid dehydrogenase (the optical density)*	Alkaline phosphatase (the optical density)*	Testosterone [ng ml^{-1}]
I Con	32.86 ± 1.42	21.57 ± 0.75	0.94 ± 0.12
II Con PBS	36.38 ± 1.09	23.75 ± 1.68	0.95 ± 0.08
III 1.5E	35.25 ± 0.96	17.00 ± 1.54	1.10 ± 0.11
IV 4.5E	35.12 ± 0.77	21.85 ± 1.92	0.93 ± 0.11
V 15E	36.43 ± 1.78	18.50 ± 1.48	0.87 ± 0.18
VI 3C	34.8 ± 1.12	19.25 ± 0.94	0.83 ± 0.02
VII 9C	38.14 ± 2.20	21.42 ± 1.17	0.84 ± 0.08
VIII 50C	36.13 ± 1.21	19.10 ± 1.55	0.72 ± 0.06
IX 1.5E 3C	36.75 ± 1.39	18.25 ± 1.90	0.74 ± 0.09
X 4.5E 9C	35.63 ± 0.85	20.00 ± 1.46	0.59 ± 0.09*
XI 15E 50C	35.88 ± 1.21	19.50 ± 1.43	0.69 ± 0.14*
XII Oz	37.75 ± 1.00*	22.88 ± 1.22	0.42 ± 0.07**

** $p \leq 0.01$ * $p \leq 0.05$ significance in relation to the control group (Con)

* The optical density was expressed on a scale of 0 (black – high activity) to 62 (white – low activity).

The results of this study suggest that neither vitamin E nor vitamin C effectively counteracted oxidative stress induced by ozone. The activity of 17-β-hydroxysteroid dehydrogenase in animals receiving the investigated vitamins was comparable to the levels noted in the control group, and so were testosterone concentrations (with the exception of animals receiving high-dose combinations of vitamins E and C).

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