

EFFECTS OF L-ASCORBIC ACID AND ALPHA-TOCOPHEROL ON BIOCHEMICAL PARAMETERS OF SWIMMING-INDUCED OXIDATIVE STRESS IN SERUM OF GUINEA PIGS

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Abstract

Background: The purpose of this study is to determine the effect of L-ascorbic acid and alpha-tocopherol as well as combination of these vitamins with or without exposure to physical exercise on intensity of lipid peroxidation, activity of xanthine oxidase, activity of total antioxidative system, concentration of glutathione, and activity of catalase in the serum of guinea pigs.

Materials and Methods: The experimental measurements of intensity of lipid peroxidation, activity of xanthine oxidase, activity of total antioxidative system, concentration of glutathione, and activity of catalase were done in the serum of guinea pigs. The animals were exposed to the test load to achieve exhaustion and the test was terminated when the animal for the third time to sink into the water.

Results: The results of this study demonstrated that endurance exercise of guinea pigs induced oxidative stress response in terms of increased lipid peroxidation and activity of xanthine oxidase in the serum of experimental animals. Our study investigated the antioxidant activity of L-ascorbic acid and alpha-tocopherol also measuring three protective markers in the serum: total antioxidant activity, content of glutathione and activity of catalase. The results obtained show that the vitamins influence the concentrations of above mentioned biochemical parameters, which points out their protective effect of swimming-induced oxidative stress.

Conclusion: Single or combined administration of L-ascorbic acid and alpha-tocopherol caused significant inhibition of these markers indicating the important antioxidant activity of the vitamins. Results lead to conclude that the combined treatments with vitamins with or without exposure to physical exercise showed the clear synergistic effect..

Key words: L-ascorbic acid, alpha-tocopherol, guinea pigs, oxidative stress, biochemical parameters

Introduction

The term oxidative stress was first defined in 1985 as “a disturbance in the pro-oxidant-antioxidant balance in favor of the former” (Sies, 1985; Sies et al., 1985). Dean Jones has proposed that the term “oxidative stress” should be redefined as “a disruption of redox signaling and control” and this definition is gaining widespread acceptance (Jones, 2006). Practically, under physiological conditions (endogenous sources) as well as under some adverse events (exogenous sources), in biological systems there is continuous production of free radicals i.e. ions, molecules and compounds that have unpaired electron and are highly reactive with biological molecules (Halliwell and Gutteridge, 1999). As a result of their reaction with lipids, proteins or DNA, oxidized products are made (Halliwell and Whiteman, 2004). Cells are equipped with enzymatic and nonenzymatic antioxidant system to eliminate free radicals and to maintain redox homeostasis (Valko et al., 2007). In oxidative stress assessment, instead of measuring free radicals themselves (which have very short half-life) we can rely on measurement of products of biological damage as well as on measurement of antioxidative capacity (enzymatic and nonenzymatic components). Vitamin C and Vitamin E play pivotal role in cellular protection against reactive oxygen species (Halliwell and Whiteman, 2004).

Vitamin E (alpha-tocopherol) as a low-molecular-weight agent is the primary chain-breaking lipid-soluble antioxidant located primarily in the membranes of tissues and it is capable of scavenging reactive oxygen species (Janero, 1991; Packer, 1991). Ascorbic acid (vitamin C) is found in two chemical forms, as reduced (L-ascorbic acid, and its ionized form of L-ascorbate) and oxidized form as (L-dehydroascorbic acid; and its ionized form of L-dehydroascorbate). Ascorbic acid is hydrophilic compound and functions better in an aqueous environment. The ascorbate anion is the predominant form existing at physiological pH (pKa of ascorbic acid is 4.25) (Powers and Jackson, 2008; Yu, 1994). The most striking chemical activity of ascorbic acid is its ability to act as a reducing agent, implicated in detoxifying various oxygen radicals *in vivo*. The speed of conversion of ascorbic acid into dehydroascorbic acid in aerobic conditions is facilitated by the higher pH values, compared to the acidic environment (Carr and Frei, 1999). Mechanism of antioxidant activity involves the conversion of ascorbic acid into its oxidized form (dehydro-ascorbic acid) by donating two electrons to reactive oxygen species (Fischer et al., 2004). Ascorbic acid can act directly scavenging lipid hydroperoxide, superoxide and hydroxyl radicals or, indirectly, playing an important role in recycling of tocopherol, a process that results in the conversion of ascorbic acid into semiascorbyl radical (Packer et al., 1979). Some authors have shown the influence of physical exercise on oxidative stress and changes in sleep pattern (Esteves et al., 2014).

The aim of this work was to study the effect of L-ascorbic acid and alpha-tocopherol on some biochemical parameters of oxidative stress in the serum of guinea pigs as no previous study known to us investigated their effects on the experimental model exploited in our research. We hypothesized that the combination of two vitamins would achieve significant synergistic effects in the terms of oxidative protection.

Materials and methods

Chemicals

All the chemicals were purchased from Sigma-Aldrich, Germany. Analytical grade chemicals vitamin C (L-Ascorbic acid, ampule 1000 mg) and vitamin E ((+/-)-alpha-tocopherol, ampule 100 mg) were used.

Study animals

Forty guinea pigs were used during the experiment. The animals were obtained from VMA-Department for breeding laboratory and experimental animals, Belgrade, Serbia). At the start of the experiment, the both sexes of guinea pigs weighed 450 g \pm 50 g and were kept under constant environmental conditions (temperature 25°C \pm 2°C; humidity 60 \pm 1.5 %, with 12 h light period, 10 days before the beginning experiment and during the experiment). All the animals received human care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. The ethic regulations have been followed in accordance with National and institutional guidelines for the protection of animal welfare during experiments.

Experimental design

The animals were randomly assigned into eight groups consisting five animals for each experiment. The first experimental group was the control group. The second experimental group is exposed to a test of excessive physical activity swimming to exhaustion. The third group was administered with 20 mg/kg, L-ascorbic acid (Vitamin C) intraperitoneally (ip), without exposing test physical load. The test substances (alpha-tocopherol and L-ascorbic acid) were administered intraperitoneally in one of the quadrants lower abdominal guinea pigs lateral to the midline, taking care to avoid injury of the bladder, liver or intestines. After the needle stick, before the injection of the substance required aspirated content. The fourth experimental group is exposed to a test of physical load and supplementation of L-ascorbic acid (20 mg/kg). The fifth group was administered with 25 mg/kg, alpha-tocopherol (vitamin E) intraperitoneally (ip), without exposure to physical stress test. The sixth group experimental group is exposed to a test of physical load and supplementation of alpha-tocopherol (25 mg/kg). The seventh group was administered with a combination of 20 mg/kg of L-ascorbic acid and 25 mg/kg alpha-tocopherol ip, without exposure to a test of the physical load. The eighth experimental group is exposed to a test of physical load and supplementation of combination of L-ascorbic acid (20 mg/kg) and alpha-tocopherol (25 mg/kg). The test load was determined by swimming to exhaustion. The experimental animals were swimming around in the water tank depth of 60 cm, with the average amount of water and 20 dm³, a temperature of 32 °C. The animals were exposed to the test load to achieve exhaustion and the test was terminated when the animal for the third time to sink into the water.

Biochemical evaluation

The extent of lipid peroxidation (LPx) was determined after Buege and Aust (1988), activities of xanthine oxidase (XOD) after Bergmayer (1976), catalase (CAT) after Beers and Sizer (1952), the content of reduced glutathione (GSH) after Beuthler et al. (1983) and total antioxidant status (TAS) after Miller et al. (1993). The experimental measurements of above-mentioned parameters were done in the serum of guinea pigs. The results of biochemical analysis are presented as mean value \pm standard deviation. The difference between the control and test groups was analyzed by the last significant difference at the $p \leq 0.005$ confidence levels.

Statistical analysis

The results obtained from 5 replicates (n = 5) of each experiment. The descriptive values are presented as the mean, standard deviation (SD), minimal (min) and maximal (max) values, standard error (SE) and coefficient of variation (CV%). The significance of the difference between the experimental groups was tested two-sided using Student's t-test or Mann-Whitney test depending on the data distribution pattern (normal or non-parametric). SPSS version 19.0 was employed to analyze data. The level of probability of statistical significance was set up at $p \leq 0.05$.

Results and Discussion

The results of the influence of L-ascorbic acid and alpha-tocopherol and the combination of these vitamins with or without exposure to physical stress on the intensity of lipid peroxidation, the activity of xanthine oxidase, the activity of total antioxidative system, content of glutathione, and the activity of catalase in guinea pigs are given in Tables 1-5. The experimental measurements of above-mentioned parameters were done in the serum of guinea pigs.

The endurance exercise of guinea pigs significantly increased the lipid peroxidation values (LPx) in their serum (Table 1). Single administration of L-ascorbic acid and alpha-tocopherol decreased significantly activity of this pro-oxidative enzyme in both the control and the endurance-exercise group of experimental animals.

The endurance exercise of guinea pigs significantly increased the activity of xanthine oxidase (XOD) in their serum (Table 2). Single administration of L-ascorbic acid and alpha-tocopherol gave significantly lower increase in comparison with both the

control and the endurance-exercise group of experimental animals. In addition, the combination of the vitamins showed the clear synergistic effect.

The endurance exercise of guinea pigs slightly increased the activity of total antioxidant system (TAS) in their serum but the effect was not statistically significant (Table 3). Single administration of L-ascorbic acid and alpha-tocopherol slightly reduced the activity in the comparison with both the control and the endurance-exercise group of experimental animals but the effect was not significant.

The endurance exercise of guinea pigs very slightly increased the content of glutathione (GSH) in their serum but the effect was not statistically significant (Table 4). Single administration of L-ascorbic acid and alpha-tocopherol slightly reduced the activity in comparison with the control group but the effects were not significant. On the other side, the combination of the vitamins had variable effects on glutathione content and the oscillation which they induced was not significantly different from both the control and the endurance activity groups.

The endurance exercise of guinea pigs significantly increased the activity catalase (CAT) in their serum (Table 5). In comparison with the control, single administration of L-ascorbic acid and alpha-tocopherol increased catalase activity but only in the case of L-ascorbic acid the effect was statistically significant. The combination of L-ascorbic acid and alpha-tocopherol had the effect of lesser magnitude than the individual vitamins and in comparison with the control the difference was not statistically significant. In comparison with endurance-exercise group, single administration of L-ascorbic acid and alpha-tocopherol decreased serum catalase activity and their combination increased it but only in the case of L-ascorbic acid the difference was statistically significant.

The results of our study showed that the endurance exercise of guinea pigs induced oxidative stress response in terms of increased lipid peroxidation and activity of xanthine oxidase in the serum of experimental animals. Single or combined administration of L-ascorbic acid and alpha-tocopherol caused significant inhibition of both markers indicating the important antioxidant effects of the vitamins.

It is well-known that exercise induced metabolic stress by the generation of wide range of reactive oxygen species (Mason et al., 2014). Reactive oxygen species, in turn, react with cellular lipids producing lipid peroxides within tissues (Zsolt et al., 2013). Vitamin C and vitamin E are among the most widely studied dietary antioxidants. Both, vitamin E and vitamin C, react with and scavenge various reactive oxygen species providing protection from oxidative stress induced by different triggers (Janero, 1991; Packer, 1991; Fischer et al., 2004; Wu et al., 2014). Finally, vitamin C has been cited as being capable of regenerating vitamin E (Krishnamurthy and Wadhvani, 2012). This all explain our results about synergistic effects of vitamin E and vitamin C in diminishing lipid peroxidation and it support our hypothesis of usefulness of the vitamin combination for protection from exercise-induced oxidative stress damage.

In our study, administration of vitamin E and vitamin C suppressed the activity of xanthine oxidase, an enzyme having pro-oxidant nature. The effect of vitamin E was of greater magnitude and the most inhibition was achieved with their combination. Previous investigators proved the inhibitor effect of both vitamin E and, in some lesser extent of vitamin C on xanthine oxidase activity supporting the results of our study (Mohd et al. 2012, Dwenger et al., 1992).

To our knowledge, no previous study investigated the effects of L-ascorbic acid and alpha-tocopherol combination in similar settings neither in the same or other mammal species, making our results the novelty in the available published literature. Our study investigated the possible mechanism of antioxidant activity of L-ascorbic acid and alpha-tocopherol measuring three protective markers in the serum samples taken from experimental animals: total antioxidant activity, activity of glutathione and activity of catalase. The major finding was the increase of catalase activity, induced with endurance exercise and L-ascorbic acid but not with alpha tocopherol. The absence of significant changes of total antioxidant capacity and glutathione activity in our study could be, at least partly, explained with methodological issues. Researchers reported that different assays used for detection of these antioxidative markers (including those used in our research) could have different diagnostic performances (Cighetti et al. 2015, Gungör et al., 2011). Consequently, we could not exclude the possibility that the laboratory assays used in our study for these two analytes gave false-negative results, particularly if their changes were of small magnitude.

L-ascorbic acid and alpha-tocopherol had triggered different molecules of oxidative stress defense which played the role in the protection from swimming-induced oxidative stress response. A recent research tracing several biochemical and molecular markers of oxidative damage and antioxidative defense reported dual effects of vitamin C and vitamin E combination, pro-oxidant and antioxidant, depending on the doses and other experimental conditions (de Oliveira et al., 2013). Therefore, the future research with more detailed and focused designs, using various laboratory assays and different experimental conditions, are necessary in order to clarify the exact mechanisms of action of vitamin C and vitamin E on oxidative stress response during endurance exercise.

Table 1: The intensity of lipid peroxidation (LPx) in the serum of experimental guinea pigs in different study groups

LPx	Serum							
	C	E	A	E+A	T	E+T	A+T	E+A+T
Mean	2.110	3.178	1.788	2.377	1.496	1.852	1.008	1.198
SD	0.047	0.048	0.158	0.095	0.029	0.013	0.037	0.012
Min	2.03	3.11	1.51	2.29	1.46	1.83	0.96	1.18
Max	2.15	3.25	1.90	2.52	1.53	1.86	1.05	1.21
SE	0.02	0.02	0.08	0.04	0.01	0.01	0.02	0.01
CV%	2.13	1.52	8.84	4.01	1.93	0.70	3.67	0.98
Test		T=30.91; p<0.001*	T=4.07; p<0.001*	T=18.39; p<0.001*	T=20.88; p<0.001*	T=59.1; p<0.001*	T=32.78; p<0.001*	T=97.52; p<0.001*

C = control; E = endurance exercise; A = L-ascorbic acid; T = alpha-tocopherol;
 *compared with the control group; **compared with the endurance exercise group
 Content of LPx is expressed in nmol malondialdehyde/mg protein

Table 2: The activity of (XOD) in the serum of experimental guinea pigs in different study groups

XOD	Serum							
	C	E	A	E+A	T	E+T	A+T	E+A+T
Mean	1.123	2.263	0.810	1.438	0.512	0.834	0.240	0.338
SD	0.185	0.464	0.267	0.061	0.205	0.338	0.176	0.189
Min	0.83	1.76	0.52	1.36	0.32	0.32	0.10	0.18
Max	1.36	2.83	1.12	1.53	0.78	1.21	0.53	0.68
SE	0.08	0.21	0.13	0.03	0.10	0.17	0.09	0.08
CV%	16.45	20.51	32.92	4.25	40.08	40.56	73.54	55.87
Test		T=5.59; p<0.001*	T=2.3; p<0.05*	T=4.32; p<0.01**	T=5.2; p<0.001*	T=5.71; p<0.001*	T=8.05; p<0.001*	T=9.41; p<0.001*

C = control; E = endurance exercise; A = L-ascorbic acid; T = alpha-tocopherol;
*compared with the control group; **compared with the endurance exercise group
Content of XOD is expressed in nmol/mg protein·min⁻¹

Table 3: The total antioxidant status (TAS) in the serum of experimental guinea pigs in different study groups

TAS	Serum							
	C	E	A	E+A	T	E+T	A+T	E+A+T
Mean	1.175	1.478	1.122	0.988	0.814	0.864	0.904	0.918
SD	0.422	0.963	0.470	0.420	0.134	0.156	0.108	0.223
Min	0.71	0.67	0.60	0.68	0.72	0.66	0.75	0.64
Max	1.76	3.28	1.57	1.79	1.02	1.09	1.04	1.20
SE	0.19	0.43	0.24	0.19	0.07	0.08	0.05	0.10
CV%	35.94	65.17	41.91	42.54	16.43	18.05	11.93	24.26
test		T=0.71; ns*	T=0.20; ns*	T=1.14; ns**	T=1.82; ns*	T=1.4; ns**	T=1.39; ns*	T=1.39; ns**

C = control; E = endurance exercise; A = L-ascorbic acid; T = alpha-tocopherol;
*compared with the control group; **compared with the endurance exercise group
Content of TAS is expressed in mmol/l

Table 4: The glutathione (GSH) content in the serum of experimental guinea pigs in different study groups

GSH	Serum							
	C	E	A	E+A	T	E+T	A+T	E+A+T
Mean	0.307	0.338	0.257	0.262	0.264	0.387	0.222	0.293
SD	0.067	0.092	0.012	0.082	0.076	0.114	0.059	0.038
Min	0.218	0.247	0.24	0.15	0.21	0.26	0.14	0.26
Max	0.418	0.508	0.27	0.34	0.39	0.55	0.28	0.36
SE	0.03	0.04	0.01	0.04	0.04	0.06	0.03	0.02
CV%	21.87	27.24	4.84	31.46	28.83	29.50	26.43	12.99
Test		T=0.66; ns*	T=1.62; ns*	T=1.51; ns**	T=0.99; ns*	T=0.8; ns**	T=2.21; ns*	T=1.1; ns**

C = control; E = endurance exercise; A = L-ascorbic acid; T = alpha-tocopherol;
*compared with the control group; **compared with the endurance exercise group
Content of GSH is expressed in nmol GSH/mg protein

Table 5: The activity of catalase (CAT) in the serum of experimental guinea pigs in different study groups

CAT	Serum							
	C	E	A	E+A	T	E+T	A+T	E+A+T
Mean	77.826	168.912	148.494	123.629	105.829	139.600	90.403	178.756
SD	15.388	47.175	53.294	7.492	60.091	82.048	29.382	49.899
Min	47.60	125.77	60.11	113.96	26.06	71.22	60.80	108.40
Max	87.90	247.03	204.29	132.03	183.45	230.00	139.67	247.72
SE	6.88	21.10	26.65	3.35	30.05	41.02	14.69	22.32
CV%	19.77	27.93	35.89	6.06	56.78	58.77	32.50	27.91
Test		T=4.5; p<0.01*	T=3.13; p<0.05*	T=2.32; p<0.05**	T=1.11; ns*	T=0.74; ns**	T=0.92; ns*	T=0.35; ns**

C = control; E = endurance exercise; A = L-ascorbic acid; T = alpha-tocopherol;
*compared with the control group; **compared with the endurance exercise group
Content of CAT is expressed in nmol/mg protein·min⁻¹

Conclusion

L-ascorbic acid and alpha-tocopherol and the combination of these vitamins with or without exposure to physical exercise influence on the changes of important biochemical parameters in the serum of guinea pigs. The combined treatments with vitamins has a protective effect on forced swimming-induced oxidative stress and show the clear synergistic effect.

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