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The influence of sex hormones and vitamin E on ascorbic acid concentration in rat tissues

Ascorbic acid (vitamin C) has been isolated by Albert Szent-Gyorgi from the adrenal glands of oxen (13). It functions in cells as an antioxidant, enzyme cofactor and growth modulator (8, 9). It is an electron donor for enzymes involved in collagen hydroxylation, biosynthesis of carnitine and norepinephrine, tyrosine metabolism and amidation of peptide hormones. The ability of ascorbic acid to donate electrons also makes it a potent water-soluble antioxidant that readily scavenges free radicals such as molecular oxygen, superoxide, hydroxyl radical and hypochlorous acid (13). It is considered to be the most effective antioxidant in plasma and it inhibits the oxidation of low-density lipoprotein (LDL) *in vitro*. Researchers have even suggested that only ascorbate can prevent the initiation of lipid peroxidation. Ascorbate is an important physiological antioxidant that helps to regenerate reduced antioxidative tocopherol from the tocopheroxyl radical (2, 12, 13). It is possible that vitamin C may act as a pro-oxidant in certain conditions (2). Ascorbic acid is synthesised endogenously in the liver by most species with the exception of human, guinea pigs and primates (8). L-gulono-γ-lactone oxidase is the terminal enzyme in the pathway of biosynthesis of ascorbic acid in animals (11). Thus, the rats are capable of vitamin C synthesis because of the presence of this key liver enzyme (11, 17).

Vitamin E is the most abundant lipid-soluble antioxidant. Integrated in membranes and lipoproteins, it acts as a chain breaker in the propagation of free radicals (2). Vitamin E inhibits platelet protein kinase C stimulation at physiological concentrations, which gives alpha-tocopherol the ability to control smooth muscle cell proliferation. Vitamin E leads to reduced surface expression of adhesion molecules on leukocytes and endothelial cells, resulting in reduced leukocyte-endothelium cell interactions (2, 19). *In vitro*, it has an antioxidative effect but under certain conditions at high concentration, it may be pro-oxidant (2).

The most likely point of interaction of ascorbic acid with estrogens could be antioxidation. Ascorbic acid is an effective and quantitatively the most important water-soluble antioxidant present in plasma, while estrogens are highly effective lipid-soluble phenolic antioxidants of cell structures. The metabolites of estrogens known as catecholoestrogens are even more effective antioxidants, surpassing vitamin E in potency. It has been demonstrated that ascorbic acid regenerates the oxidase form of estrogens by chemical reduction, which also restores their potential for antioxidation (3, 18). Such antioxidant "back up" is functional because estrogens inhibit LDL oxidation as catalysed by cupric ions, vascular smooth muscle cell and endothelial cells. Estrogens and vitamin C may prevent this process by reducing LDL-formation and its potential vascular cell effects (5). Estrogens prevent LDL oxidation at physiologically relevant doses only in the presence of ascorbic acid or vitamin E (3, 18). Increased uptake of LDL may contribute to the lower levels of plasma LDL in women taking estrogen replacement

therapy (5). However, Liehr and Roy (10) have suggested that while harmless at physiological levels, supra-normal levels of estrogens may induce greater tissue oxidative stress.

Based on this, the purpose of our study was to estimate the influence of sex hormones administration and vitamin E supplementation on tissue ascorbic acid concentration in female and male rats.

MATERIAL AND METHODS

The experiment was conducted on 14-month-old Wistar rats, weighting 350–400 g. The animals were divided into 12 groups, each of 10 rats. All animals were fed a standard diet (LSM dry food and redistilled drinking water). Food and water were given *ad libitum*. The groups 1–6 comprised females only and the groups 7–12 males only. The first and the seventh groups were the control groups. The other groups of animals received intragastric sex hormones suspended in a Tween 80 solution daily for 14 days. The second, third, fifth and sixth groups received *Estradiolum valerianicum*, but the third and the sixth groups at the dose of 10 $\mu g\ kg^{-1}$ of b.w., and the third and the sixth groups at the dose of 100 $\mu g\ kg^{-1}$ of b.w. The eighth, ninth, eleventh and twelfth groups received *Testosteronum propionatum*, but the eighth and the eleventh groups at the dose of 10 $\mu g\ kg^{-1}$ of b.w., and the ninth and the twelfth groups at the dose of 100 $\mu g\ kg^{-1}$ of b.w. Additionally the groups 4–6 and 10–12 obtained vitamin E at the dose of 100 $mg\ kg^{-1}$ of b.w.

After two weeks all rats were sacrificed under ketamine anaesthesia (1 ml of 5% ketamine via intraperitoneal injection). Following decapitation, liver, brain, kidneys and heart were removed and homogenised in fourfold volumes of 100 mM Tris-HCl buffer, pH 7.4. Tissue ascorbic acid concentration was determined by a modified colorimetric method of Kyaw (14). The results were submitted to statistical analysis with the Cochran-Cox test, accepting $p < 0.05$ as significant.

RESULTS

The administration of sex hormones and vitamin E altered ascorbic acid concentration in female and male rat tissues. The changes in tissue ascorbic acid concentration in female rats following *Estradiolum valerianicum* administration are shown in Table 1.

Table 1. Ascorbic acid concentration ($\mu mol\ g^{-1}$ of tissue) in female tissues receiving *Estradiolum valerianicum*

Tested tissues	Group 1 Control X ± SD	Group 2 Estradiol 10 $\mu g\ kg^{-1}$ b.w. X ± SD	Group 3 Estradiol 100 $\mu g\ kg^{-1}$ b.w. X ± SD	Group 4 Vitamin E X ± SD	Group 5 Estradiol 10 $\mu g\ kg^{-1}$ b.w. with vitamin E X ± SD	Group 6 Estradiol 100 $\mu g\ kg^{-1}$ b.w. with vitamin E X ± SD
Liver	0.45 ± 0.06	0.42 ± 0.05 ↓	0.37 ± 0.06 ↓	0.83 ± 0.07	0.52 ± 0.05 ↓*	0.43 ± 0.04 ↓*
Heart	0.21 ± 0.03	0.13 ± 0.01 ↓*	0.10 ± 0.01 ↓*	0.29 ± 0.03	0.15 ± 0.02 ↓*	0.11 ± 0.02 ↓*
Brain	0.14 ± 0.01	0.12 ± 0.01 ↓	0.11 ± 0.03 ↓	0.22 ± 0.03	0.18 ± 0.03 ↓	0.12 ± 0.02 ↓*
Kidney	0.44 ± 0.05	0.28 ± 0.03 ↓*	0.16 ± 0.03 ↓*	0.45 ± 0.07	0.35 ± 0.04 ↓*	0.20 ± 0.03 ↓*

* Statistical significance vs. control ($p < 0.05$)

Estrogen administration resulted in vitamin C loss in all tested tissues. The most significant changes were observed in the heart and kidneys from all groups of animals and in the liver from females receiving estrogen – both at the lower and the higher doses together with vitamin E. There were

statistically significant alterations, except the liver and the brain from animals obtaining estrogen solely. Moreover, significant reductions of ascorbic acid levels appeared after administration of the highest dose of estrogen together with vitamin E. The similar losses were noticed in females getting a lower dose of estrogen with vitamin E, except brain tissue; the change was not statistically significant.

Completely different shifts were observed in tissue ascorbic acid concentration in male rats after administration of *Testosteronum propionatum*. These changes are shown in Table 2.

Table 2. Ascorbic acid concentration (μmol g^{-1} of tissue) in male tissues receiving *Testosteronum propionatum*

Tested tissues	Group 1 Control X ± SD	Group 2 Testosterone 10 μg kg^{-1} b.w. X ± SD	Group 3 Testosterone 100 μg kg^{-1} b.w. X ± SD	Group 4 Vitamin E X ± SD	Group 5 Testosterone 10 μg kg^{-1} b.w. with vitamin E X ± SD	Group 6 Testosterone 100 μg kg^{-1} b.w. with vitamin E X ± SD
Liver	0.49 ± 0.04	0.57 ± 0.05 ↑	0.58 ± 0.06 ↑	0.53 ± 0.04	0.62 ± 0.06 ↑	0.77 ± 0.07 ↑ *
Heart	0.11 ± 0.01	0.13 ± 0.01 ↑	0.23 ± 0.02 ↑ *	0.21 ± 0.02	0.34 ± 0.05 ↑ *	0.23 ± 0.04 ↑
Brain	0.14 ± 0.02	0.21 ± 0.04 ↑ *	0.32 ± 0.09 ↑ *	0.13 ± 0.02	0.16 ± 0.03 ↑	0.18 ± 0.02 ↑ *
Kidney	0.20 ± 0.03	0.24 ± 0.02 ↑	0.39 ± 0.04 ↑ *	0.13 ± 0.02	0.18 ± 0.03 ↑ *	0.20 ± 0.02 ↑ *

* Statistical significance vs. control ($p < 0.05$)

Testosterone administration resulted in elevated vitamin C levels in male tissues. The largest rises were noticed in the heart, brain and kidneys tissues. The highest dose of testosterone, both with and without vitamin E, resulted in the highest increases of ascorbic acid concentrations and there were statistically significant changes.

Besides, after administration of vitamin E solely, in both females and males, ascorbic acid status was elevated in some but not all tissues as compared to controls. These increases took place in all female tested tissue and in the liver and heart from male rats. On the contrary, in male rats vitamin E administration resulted in reduced kidneys ascorbic acid levels and did not affect vitamin C status in the male brain. Apart from this, the administration of vitamin E together with sex hormone altered vitamin C status, both in females and males, in comparison to the groups receiving sex hormone only. In all tested female tissues ascorbic acid concentrations were elevated in groups receiving vitamin E additionally, in comparison to groups not obtaining vitamin E. However, in male tissues vitamin C status was increased in the case of the liver and heart, and decreased in the case of the brain and kidneys.

DISCUSSION

This study examined the effects of two weeks of daily sex hormones administration and vitamin E supplementation on tissue ascorbic acid concentration in female and male rats. Estrogen administration to females caused vitamin C loss in all the tested tissues, while the application of testosterone to males induced increase of its level. These results are similar to observations described previously (7, 15, 16, 17). In the studies conducted by Tiidus et al. (15, 16, 17) estrogen administration resulted in significant loss of vitamin C from various tissues in rats following both exercise and resting condition. The similar changes were observed in guinea pigs. There were diminished ascorbic acid concentrations in some but not all the tested tissues of both genders. Liver and heart vitamin C levels were significantly lower in estrogen injected guinea pigs as compared to controls. Plantaris muscle vitamin C levels were significantly lower in estrogen injected males but not females. Estrogen injection did not significantly affect lung vitamin C levels in male animals. However, estrogen injected females had significantly higher lung vitamin C levels than control females.

Since vitamin C is an antioxidant, its loss from tissue has been interpreted as an indicator of tissue oxidative stress (15, 17). Although estrogen has been reported to have significant antioxidant properties *in vitro* its effects, particularly in supra-physiological concentrations, on indicators of tissue oxidative stress *in vivo* have been mixed. Thus, an alternative explanation for estrogen induced reduction of tissue vitamin C level may be related to estrogen induced tissue metabolic or oxidative stress. One possible mechanism, by which tissue ascorbic acid concentrations may have been diminished in rats which were administered estrogen, is through estrogen – at higher than normal physiological levels – interfering with vitamin C synthesis in the liver. The negative effect of estrogen administration of ascorbic acid status in most tissues has been observed also in guinea pigs – unable to synthesise vitamin C due to the lack of the liver enzyme, L-gulono-γ-lactone oxidase. Hence, it suggests that the effect of estrogen administration on vitamin C metabolism in the rat was probably not manifested primarily at the synthesis site (17).

There is also evidence supporting an interaction between estrogens and ascorbic acid under physiological condition. Oral contraceptive users were found to have a lower level of plasma vitamin C compared to control women of similar age and health condition. Their ascorbic acid levels in leukocytes and platelet were also lower (8). The similar changes have been observed in female guinea pigs and rhesus monkey given contraceptive hormones (8, 9). A decrease in the intestinal ascorbic acid absorption upon oral contraceptive usage was one of the many mechanisms proposed and urinary ascorbic acid excretion was found to be lower in oral contraceptive users after a bolus dose (8). Moreover, it was proved that 17 β-estradiol and its antagonists do inhibit the accumulation of vitamin C by intestinal cells. That observation maybe the underlying cause of poor ascorbic acid status in oral contraceptive users (8, 9).

Our study has been indicated the protective effect of vitamin E supplementation on ascorbic acid concentrations in all female and some but not all male tissues. T i i d u s et al. have demonstrated the influence of estrogen administration on vitamin E levels in rats (15, 16) and in guinea pigs (17). Despite some variations, there were no significant differences in vitamin E status of any tissues of both of these species. Although vitamin C and vitamin E may act synergically as antioxidants, the reduction of tissue concentration of one does not necessarily directly affect the tissue status of the other in the short term. The differential effect of estrogen administration on two important tissue antioxidants further suggests that estrogen administration does not uniformly affect tissue oxidative status and has distinctly different effects on the metabolism of these vitamins (17).

In our study, after the application of testosterone the vitamin C status has been elevated in males. Similar results have been noticed by K u m a r i et al. (7). There were examined the effects of long-term administration of testosterone on ascorbic acid metabolism in castrated rats. It has been proved that orchidectomy caused a significant decrease in the concentration of ascorbic acid and ascorbic acid-2-sulphate in tissues, while the administration of testosterone to these animals reverses this effect. On the other hand, I t o et al. (6) have indicated that ascorbic acid-deficiency in adult ODS (+) rats (unable to synthesise ascorbic acid) causes no significant change in basal plasma level of testosterone. Moreover, there was studied the effect of ascorbic acid on *in vitro* synthesis of testosterone in rat testis. There was observed a significant stimulation of steroid dehydrogenase activity and rise in testosterone content (1). Similar studies have been conducted in alloxan diabetic rats. The prophylactic effect of ascorbic acid against alloxan-induced decrease in testosterone appeared to be related to the recovery of testicular antioxidant system and ameliorated oxidative stress through ascorbic acid administration. This is evidenced by the normal plasma testosterone level observed in alloxan-ascorbate treated rats. There was also observed a significant stimulation of steroid dehydrogenase activity and a rise in testosterone levels in testes of rats treated with ascorbic acid through controlling the testicular antioxidant (4). The supplementation of ascorbic acid had beneficial effects on serum testosterone and semen

characteristics of rabbits. The beneficial influence of vitamin C can be attributed to the fact that ascorbic acid is a very efficient antioxidant and a scavenger of oxygen free radicals, which are toxic by-products of many metabolic processes (20).

CONCLUSIONS

1. The administration of sex hormones and vitamin E altered ascorbic acid concentration in female and male rat tissues. Estrogen administration to females caused vitamin C loss in all the tested tissues, while the application of testosterone to males induced increase in this vitamin level.

2. The supplementation of vitamin E during sex hormone administration had beneficial effects on tissue ascorbic acid concentration.

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SUMMARY

Ascorbic acid concentration in a living body undergoes alterations continually. The changes in homeostasis of this important antioxidant may be induced by the effect of sex hormones for example. Based on this, the aim of our work was to estimate the influence of sex hormones administration and vitamin E supplementation on tissue ascorbic acid concentration in female and male rats. It has been showed that estrogen administration to females resulted in vitamin C loss, while testosterone administration to males elevated this level in all the tested tissues. However, the supplementation of vitamin E during sex hormones administration had beneficial effects on the tissue ascorbic acid concentration.

Wpływ hormonów płciowych i witaminy E na stężenie kwasu askorbinowego w tkankach szczurów

Stężenie kwasu askorbinowego w organizmie żywym stale podlega wahaniom pod wpływem różnych czynników. Zmiany w homeostazie tego ważnego antyoksydanta mogą być spowodowane oddziaływaniem m. in. hormonów płciowych. Celem naszej pracy było określenie wpływu hormonów płciowych i witaminy E na tkankowe stężenia kwasu askorbinowego u samic i samców szczurów. Wykazano, że podawanie szczurzycom estrogenów powoduje utratę witaminy C z tkanek, podczas gdy podawanie samcom testosteronu zwiększa poziom tej witaminy w tkankach. Dowiedziono, że podawanie witaminy E podczas aplikacji hormonów płciowych działa korzystnie na tkankowe stężenia witaminy C.