*Polish Journal of Environmental Studies Vol. 14, No 5 (2005), 599-604*

# Vitamins E and C Concentrations in the Liver and Kidney of Rats Exposed to Cadmium and Ethanol

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*Received: December 1, 2004*
*Accepted: January 19, 2005*

## Abstract

In the present study, the effect of co-exposure to cadmium (Cd) and ethanol (EtOH) on concentrations of vitamins E and C were evaluated in the liver and kidney homogenates of rats which were administered, singly or in combination, Cd (50 mg Cd/dm$^3$ in drinking water) and EtOH (5 g EtOH/kg b. wt./24 h, intragastrically) for 12 weeks.

The exposure to Cd caused a decrease in the concentration of vitamins E and C in the liver, whereas the concentration of vitamin E increased in the kidney, compared to control group. EtOH administration led to a decrease in vitamins E and C concentrations in the liver. Exposure to Cd alone enhanced the concentration of vitamin C in the kidney. In the co-exposed group, the concentration of vitamin E decreased in the liver as compared to control and Cd group but increased in comparison to EtOH group. The concentration of vitamin C in this organ decreased compared to control and Cd- or EtOH-exposed groups. The co-exposure to both substances caused a rise in vitamin E concentration in the kidney compared to control and EtOH groups, whereas the concentration of vitamin C increased compared to control and Cd group. In the liver positive correlation was noted between vitamin E or C concentrations and glutathione (GSH) concentration. In the kidney, negative correlation was found between the concentration of vitamin E and GSH. No correlation was observed between the concentration of vitamin C and GSH in this organ.

The results obtained suggest that the change in vitamin E concentration in the liver of rats co-exposed to Cd and EtOH resulted from an independent action of EtOH and its interaction with Cd, whereas in the kidney it was due to an independent Cd effect. In the animals co-exposed to Cd and EtOH, the change in vitamin C detected in the liver was the result of an independent action of either Cd or EtOH. In the kidney, the concentration of vitamin C was only EtOH-dependent. The results of this study together with our previous finding in the model used suggest the involvement of vitamins E and C in the development of Cd- and/or EtOH-induced oxidative stress.

**Keywords**: cadmium, ethanol, vitamin E, vitamin C

## Introduction

Oxidative stress, which involves disorders in the balance between prooxidant (free radicals) and antioxidant agents (antioxidant barrier components), is an important toxic mechanism of the action of a number of xenobiotics. Our studies [1, 2] as well as those of other authors [3, 4, 5, 6] have shown that cadmium (Cd) and ethanol (EtOH) can induce redox processes.

Cd involvement in these processes is based mainly on disturbances in the antioxidative barrier in the organism [3, 4]. EtOH is a direct inducer of free radicals [5, 6]. It

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can also, like Cd, disturb the antioxidative barrier [7, 8]. We have revealed that both Cd and EtOH, administered singly and in combination, alter the activity of superoxide dysmutase (SOD) and catalase (CAT) in the liver and kidney, leading to lipid peroxidation [2]. We have also found that the Cd and/or EtOH-caused decrease in glutathione (GSH) concentration has a substantial contribution to the induction of this process [9].

Despite a number of research studies, the mechanism of lipid peroxidation following Cd and EtOH administration, especially in conditions of co-exposure, has not been fully elucidated. The enzymatic antioxidant system that includes SOD and CAT is supplemented with non-enzymatic antioxidants, such as vitamin E, vitamin C and GSH [10, 11, 12].

Vitamin E (tocopherol) is the major lipid-soluble antioxidant, present in all cell membranes, which protects cells against lipid peroxidation [13, 14]. Its reaction with active radicals produces tocopheroxyl radicals, being further reduced to tocopherol via vitamin C or GSH [15, 16].

Vitamin C (ascorbic acid) is a low-molecular antioxidant which protects cells against water-soluble oxygen and nitric radicals [12, 15]. Ascorbic acid can scavange radicals generated during lipid peroxidation or reduce tocopheroxyl radicals [15, 16]. Dehydroascorbic acid produced in these reactions can be reduced by GSH [16].

Experimental studies have proved the occurrence of interactions between Cd and vitamins E and C [17, 18, 19]. The concentrations of these vitamins can also be influenced by EtOH [20, 21, 22]. However, no literature data are available on the effect of combined exposure to Cd and EtOH on the concentrations of vitamins E and C. Knowledge of the effect of this co-exposure on the concentrations of the antioxidative vitamins may contribute to further elucidation of the mechanisms of Cd and EtOH interaction in the organism.

Therefore, the aim of the present study was to assess the concentrations of vitamins E and C in the liver and kidney in an experimental model of rats' co-exposure to Cd and EtOH in which we have revealed a significant interactive effect of these substances on GSH concentration and lipid peroxidation [2, 9].

## Experimental Procedures

The study was conducted on 32 adult (8-week-old) male Wistar rats, initial body weight 170 g. The animals were kept in standard laboratory conditions: temperature 22 ± 2°C, relative humidity 50 ± 10% and natural day/night cycle. They had unlimited access to drinking water and LSM diet (Agropol, Motycz, Poland). The animals were randomly divided into 4 groups of 8 rats each:

– Control group was divided into two subgroups; rats of the one were given redistilled water ad libitum, animals of the other additionally received physiological saline (0.9% NaCl) by intragastric tube in a similar way to EtOH;
– Cd group, which received cadmium chloride ($CdCl_2$) in drinking water at a concentration of 50 mg Cd/$dm^3$;
– EtOH group, which received redistilled water to drink and 5 g EtOH/kg b. wt./24 h by intragastric tube in two equal doses for 5 days a week;
– Cd + EtOH group, co-exposed to Cd (like the Cd group) and EtOH (like the EtOH group).

The experiment lasted for 12 weeks. After its termination, the rats were anaesthetized (by intraperitoneal administration of Vetbutal, 30 mg/kg b. wt.) and liver and kidneys were collected for analysis. The organs were rinsed in cold 0.9% NaCl and weighed. The biological material that was not used directly for analysis was frozen at -80 $^0$C.

The liver and kidney were homogenized in cold 0.9% NaCl (for determination of vitamin E) or in 50 mM perchloric acid (for determination of vitamin C) to obtain 10% homogenates. The homogenates were centrifuged at 10,000 x g for 15 min at 4°C and the supernatant was used for analyses. High performance liquid chromatography method (HPLC) was applied to assess the concentrations of vitamins E and C. The concentration of vitamin E was determined according to Leenher et al. [23] and vitamin C following Barja et al. [24].

The research was approved by the Local Ethics Committee for Animal Experiments in Białystok.

Results were presented as the arithmetic means ± SEM and subjected to one-way analysis of variance (ANOVA) with the use of non-parametric Kruskal-Wallis test. Differences were considered to be statistically significant at $p<0.05$. Two-way analysis of variance ANOVA/MANOVA (with F test) was used to assess the interactive effect of Cd and EtOH on vitamin E or C concentrations. F values were considered statistically significant at $p<0.05$. Spearman's correlation analysis was applied to detect any correlations among the two vitamins. The relationship between vitamin E and C concentrations and previously reported in those animals GSH concentration were also investigated [9]. Statistical calculations were performed using a computer programme Statistica 5.0 (StatSoft, Tulsa, OK, USA).

## Results

### Vitamin E and Vitamin C Concentrations in the Liver (Table 1)

Exposure to Cd caused a reduction in the concentrations of vitamins E and C as compared to control group, respectively, by 9.7% ($p<0.05$) and 11.5% ($p<0.01$). A reduction in the concentration of vitamin E (by 39.1%, $p<0.001$) and vitamin C (by 5.8%, $p<0.05$) was also noted in the rats exposed to EtOH, as compared to control animals. The co-exposure to Cd and EtOH resulted in a decrease in vitamin E concentration, compared to control (by 20.3%, $p<0.01$) and Cd group (by 11.8%,

Table 1. Effects of cadmium (Cd), ethanol (EtOH) and their combination on vitamin E and C concentration in the liver.

| Group | Vitamin E (μg/g tissue) | Vitamin C (μg/g tissue) |
|---|---|---|
| Control | 20.50 ± 0.74 | 236.0 ± 3.3 |
| Cd | 18.51 ± 0.44 * | 208.8 ± 7.3 * |
| EtOH | 12.49 ± 0.613 * | 222.3 ± 3.8 * |
| Cd + EtOH | 16.33 ± 0.38 *†‡ | 188.7 ± 4.2 *†‡ |
| [a] Main effect of Cd<br>[a] Main effect of EtOH<br>[a] Interactive effect of Cd and EtOH | NS<br>F = 64.7, p = 0.000<br>F = 12.6, p = 0.001 | F = 16.9, p = 0.000<br>F = 5.4, p = 0.027<br>NS |

Values are means ± SEM of 8 rats. Values are significantly different (ANOVA + Kruskal-Wallis ranks test) compared to: *control, † Cd and ‡ EtOH groups. NS, not statistically significantly different. [a] Two-way analysis of variance (ANOVA/MANOVA, test F).

Table 2. Effects of cadmium (Cd), ethanol (EtOH) and their combination on vitamin E and C concentration in the kidney.

| Group | Vitamin E (μg/g tissue) | Vitamin C (μg/g tissue) |
|---|---|---|
| Control | 16.40 ± 0.89 | 172.3 ± 3.2 |
| Cd | 20.10 ± 0.58 * | 175.4 ± 2.6 |
| EtOH | 17.46 ± 0.93 | 189.1 ± 5.5 * |
| Cd + EtOH | 21.14 ± 0.91 *‡ | 198.7 ± 3.5 *† |
| [a] Main effect of Cd<br>[a] Main effect of EtOH<br>[a] Interactive effect of Cd and EtOH | F = 16.8, p = 0.001<br>NS<br>NS | NS<br>F = 23.0, p = 0.000<br>NS |

Values are means ± SEM of 8 rats. Values are significantly different (ANOVA + Kruskal-Wallis ranks test) compared to: *control, † Cd and ‡ EtOH groups. NS, not statistically significantly different. [a] Two-way analysis of variance (ANOVA/MANOVA, test F).

$p<0.05$) and an increase compared to EtOH group (by 30.7%, $p<0.01$). The concentration of vitamin C in rats co-exposed to Cd and EtOH decreased in comparison to control group and the animals administered Cd and EtOH singly, respectively by 20.0% ($p<0.001$), 9.6% ($p<0.05$) and 15.1% ($p<0.001$).

The ANOVA/MANOVA analysis revealed that the changes in vitamin E concentration in the liver of rats co-exposed to Cd and EtOH were due to an independent action of EtOH and its interaction with Cd, whereas the concentration of vitamin C was determined by an independent effect of Cd and EtOH.

## Vitamin E and Vitamin C Concentrations in the Kidney (Table 2)

In Cd-exposed rats an increase was noted in vitamin E concentration by 22.6% ($p<0.05$), as compared to control animals. Vitamin C concentration ranged within control values. The exposure of animals to EtOH caused an increase in vitamin C concentration (by 9.7%, $p<0.05$) as compared to control group, although it had no effect on vitamin E concentration. Co-administration of Cd and EtOH caused an increase in vitamin E concentration in comparison to control (by 28.9%, $p<0.01$) and EtOH group (by 21.1%, $p<0.05$). Vitamin C concentration in these animals increased in comparison to control and Cd group, respectively by 15.3% ($p<0.01$) and 13.3% ($p<0.001$).

Two-way analysis of variance ANOVA/MANOVA revealed that the change in the concentration of vitamin E in the kidney of rats co-exposed to Cd and EtOH was the main effect of Cd, whereas the concentration of vitamin C was mainly influenced by EtOH.

## Correlations Between GSH and Vitamins E and C Concentration in the Liver and Kidney (Table 3)

Positive correlation was noted between GSH concentration and the concentrations of vitamins E and C in the liver. No statistically significant correlation was found between the concentrations of these two vitamins in this organ.

In the kidney, the concentration of vitamin E negatively correlated with GSH concentration. No statistically significant correlation was noted between the kidney GSH and vitamin C concentrations and between the two vitamins.

Table 3. Correlation between GSH concentration and vitamin E and C concentrations in liver and kidney.

| Parameters | | Liver | Kidney |
|---|---|---|---|
| Vitamin E | Vitamin C | r = 0.291<br>p = 0.106 | r = 0.132<br>p = 0.470 |
| Vitamin E | GSH | r = 0.711<br>p = 0.000 | r = -0.438<br>p = 0.012 |
| Vitamin C | GSH | r = 0.528<br>p = 0.002 | r = -0.235<br>p = 0.196 |

Data are presented as correlation coefficients (r) and level of statistical (p). Correlation was considered as statistically significant at $p<0.05$.

## Discussion

In this study, the influence of exposure to Cd and/or EtOH on the concentrations of vitamins E and C in the liver and kidney was investigated on a rat model. As it is believed that vitamins E and C as well as GSH can exert a synergistic effect in peroxidative processes [15, 16], the analysis of correlation between the concentrations of vitamin E and C and the concentrations of the both vitamins and GSH concentration previously determined in those animals [9] was conducted.

It has been found that the changes in the levels of vitamins E and C are significantly correlated with the Cd and EtOH exposure mode. The reduction in vitamin E concentration in the liver following both separate and combined exposure to Cd and EtOH may result from its utilization in the processes of free radicals scavenge [7, 19, 25, 26]. Vitamin E effectively reacts with organic lipid radicals produced in the process of lipid peroxidation [27, 28]. In our previous studies [2, 9], it has been shown that Cd, through its effect on the activity of antioxidative enzymes and GSH concentration, can induce lipid peroxidation. Therefore, it can be assumed that the reduction in vitamin E concentration in the liver after Cd exposure is due to the interaction of this vitamin with radicals generated during lipid peroxidation. Vitamin E can also act as a scavenger of radicals that originate during EtOH biotransformation, particularly of hydroxyethyl radical (HER) generated by CYP 2E1, which – like vitamin E – is located in cell membranes [21, 29]. This close location of vitamin E with the site of HER production, as well as greater ability of EtOH than Cd to induce free radicals, might be the cause of the main effect of EtOH regarding the vitamin E concentration in the liver of rats co-exposed to Cd and EtOH revealed by ANOVA/MANOVA analysis. The increase in vitamin E concentration in the kidney of rats exposed to Cd, both alone and in combination with EtOH, can be a defensive response of this organ to lipid peroxidation recently reported in these animals [2]. An increase in vitamin E concentration in the kidney after exposure to this heavy metal has also been reported by Stajan et al. [30]. In the kidney, which is a critical organ for Cd, the change in vitamin E concentration after co-exposure of Cd and EtOH was due to an independent Cd action.

The reduction in vitamin C concentration in the liver of rats exposed to Cd and EtOH, singly or in combination, can result from its utilization in reactions with radicals generated by these substances. Vitamin C protects cells against various water-soluble radicals [12, 15, 18]. Vitamin C, like vitamin E, also can react with HER [21, 29]. Positive correlation between vitamin C and GSH concentrations in the liver allows an assumption that the decrease in the vitamin concentration may result from deficiency of GSH, which is necessary for regeneration of dehydroascorbic acid produced during vitamin C reaction with free radicals. The ANOVA/MANOVA analysis shows that in the rats co-exposed to Cd and EtOH the change noted in vitamin C concentration in the liver is due to independent actions of either Cd or EtOH; however, the analysis of F values exhibits greater Cd involvement. Since EtOH and its metabolites have been reported to induce the synthesis of vitamin C under conditions of oxidative stress [31-33], it is possible that the increased kidney vitamin C concentration in the rats exposed to EtOH alone and in conjunction with Cd resulted from an enhanced production of this vitamin.

It should also be taken into consideration that Cd and EtOH might influence the concentration of antioxidative vitamins in the organism via pathways not related with induction of oxidative stress. Both Cd and EtOH can change the metabolism of these vitamins via influencing their absorption from digestive tract, biosynthesis and urinary excretion [31, 33-36].

Until now, the majority of studies on the vitamin C – vitamin E system have been performed using in vitro models. In those studies, the synergistic action of the two vitamins has been noted [16, 37-39]. The present study, conducted on an in vivo model, gives evidence that these vitamins may act independently. Positive correlation noted in the liver between GSH and vitamin C or E levels seems to indicate an important role of GSH in the regeneration of both vitamins, particularly vitamin C.

The present study has revealed that exposure to Cd and EtOH alone and in combination influence the liver and kidney concentrations of the non-enzymatic antioxidants such as vitamins E and C. Disorders in vitamin E and C concentrations at co-exposure to Cd and EtOH may, dependent on the kind of tissue, be an independent effect of Cd and/or EtOH or their interactive effect. The results of this study, together with our previous finding in the model used, suggest the involvement of vitamins E and C in the development of Cd- and/or EtOH-induced oxidative stress.

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