

EFFECT OF VITAMIN C , 1,2 – DIBROMOETHANE AND THEIR ASSOCIATION ON CARBONIC ANHYDRASE AND SUPEROXIDE DISMUTASE ACTIVITY IN RATS

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Abstract

The purpose of this study was to determine the effect of vitamin C on erythrocyte carbonic anhydrase (CA) and superoxide dismutase (SOD) activity in vivo. In vivo effect was followed in mice erythrocytes from Male Sprague-Dawley breed. Groups of rats were given vitamin C (1 g/kg/day, i.g.), 1,2-Dibromoethane (300 ppm/day, i.g.), Vitamin C + 1,2- Dibromoethane (1 g/kg/day + 300 ppm/day, i.g), and a Control group only Placebo. Measurement of enzymes activity was done by following the hydration reaction of CO₂ (stopped-flow method) for CA, respectively the enzymatic inhibition of oxidation of epinephrine to adrenochrome for SOD. Vitamin C produced a increase in CA I and CA II activity, and the effect is stronger on CA II isoenzyme. In vivo studies show that vitamin C decreases CA and SOD inhibition caused by Dibromoethane.

Key words: vitamin C, carbonic anhydrase, superoxide dismutase, activity

INTRODUCTION

Under physiological conditions, vitamin C functions as a potent reducing agent that efficiently quenches potentially damaging free radicals produced by normal metabolic respiration of the body (Arrigoni et al., 2002). Inability to maintain high serum levels of vitamin C may have serious health implications and is particularly relevant in the onset and progression of degenerative disease, such as cancer and cardiovascular disease, which have a strong contributing oxidative damage factor (Li et al., 2007).

The use of vitamin C in cancer treatment has been debated extensively. Proposed mechanisms of action for vitamin C in the prevention and treatment of cancer includes antioxidant as pro-oxidant properties, stimulation of the immune system, altering carcinogen metabolism, enhancement of collagen synthesis necessary for tumor encapsulation and interference with cancer cell signaling (Ullah et al., 2012).

1,2-Dibromoethane is a cytotoxic and carcinogenic agent widely used as an pesticide and additive to leaded gasoline. Is used as an intermediate in paint industry, as an industrial solvent (resins, gums, waxes), in certain

extinguishing. 1,2 - Dibromoethane causes toxicity in a variety of organs and has been shown to be genotoxic (Wormhoudt et al., 1998).

Furthermore, tumor formation has been observed in a number of studies with experimental animals, both at the site of application and at distant sites. Tumors and proliferative lesions have been found in the stomach after oral administration (Olson et al., 1973), in the nasal cavities and lungs after inhalation (Stinson et al., 1981), and in the skin after dermal application (Van Duuren et al., 1979). Based on animal studies, 1,2-DBE is suspected to be a carcinogen in humans.

Carbonic anhydrase (CA) (E.C. 4.2.1.1.) is a Zn containing metalloenzyme that catalyzes the reversible hydration of carbondioxide. CAI is found primarily in erythrocytes. The human CA-II isozyme is widely distributed. It has been identified in erythrocytes, brain, eye, kidney, etc. Normally CA-I and CA-II each contribute about 50 percent of the total activity (Yılmaz et al., 2000). The maintenance of appropriate acid-base homeostasis is a prerequisite for normal cell growth. In literature, recent studies suggest that acid-base state may play an important role in tumorigenesis (Chegwidden et al., 2000). Tumor growth is generally known to involve complex interactions between cells and their microenvironment characterized at least in part by an acidic extracellular pH (Kato et al., 2013).

The genetic polymorphism of SOD isoenzymes and changes in their activity are associated with oxidative damage of DNA involved in carcinogenesis, and the subsequent risk of increased susceptibility to cancer, in particular lung, gastric, colorectal, cervical, breast cancer and myelodysplastic syndrome (Marta D.S., 2011).

Because of the possible involvement of 1,2-dibromoethane in carcinogenesis and oxidative metabolism, our study followed the effects of administration of Vitamin C, 1,2-dibromoethane and their association on the activity of CA isozymes, especially CA II involved in the carcinogenic process, and SOD, enzyme with antioxidant role.

MATERIAL AND METHOD

We conducted an experimental study in laboratory animals in which we explore the new aspects of the relationship between vitamin C and CA and SOD activity.

Study protocol was approved by the Ethics Committee of the Research and Nursing Center, Șimleul Silvaniei, Sălaj.

In this study was followed the effect of carcinogens in experimental animals (alone and in combination with vitamin C) on CA isoenzymes and SOD activity.

Forty male rats from Male Sprague-Dawley breed, weighing 260 ± 20 g were used in the study. During the study the animals were kept under standard conditions, in isolated room with constant temperature of $23 \pm 2^\circ\text{C}$, with constant access to water, but fed with standard laboratory chow, the same for all and a day-night circadian cycle of 12 hours. They were randomly divided into 4 groups (10 individuals each) and placed in separate cages during the study. They were treated by gavage for 10 days as follows:

Group 1 - Control group that received Placebo

Group 2 - Vitamin C, 1g/day

Group 3 - 1,2- Dibromoethane, 300 ppm/day;

Group 4 - Vitamina C, 1g/day + 1,2- Dibromoethane 300 ppm/day.

Blood samples have been taken after 10 days and the activity of CA I, CA II and SOD from erythrocyte hemolysate was measured.

CA activity was assayed by Stopped-flow method following the hydration reaction of CO_2 (Khalifah R.G., 1971) with a rapid kinetic spectrophotometer model SF-51 HI-TECH MX (England).

For CA, enzyme activity expressed as enzyme units (EU/ml) was calculated using the equation $\frac{t_0 - t_E}{t_E}$, where t_0 and t_E are the times for pH change (from 7,5 to 6,5) of the nonenzymatic and the enzymatic reaction catalyzed by erythrocyte CA, respectively. Differentiation of CA I from CA II activity was done using Nicosilvanil Test (Puşcas et al., 1999).

SOD activity was assessed according to the method that follows enzymatic inhibition of oxidation of epinephrine to adrenochrome (Misra et al., 1972) with a rapid kinetic spectrophotometer model SF-51MX HI-TECH (England) at a wavelength of 480 nm and the results are expressed as enzyme units (EU).

Changes of enzyme activity are presented as mean \pm standard deviation.

For statistical processing of data we used the Student's test. The level of statistical significance was set at $p < 0.05$.

RESULTS AND DISSCUSIONS

Erythrocyte CA I activity in the Control group was $0,344 \pm 0,111$ EU/ml and erythrocyte CA II activity was $1,399 \pm 0,202$ EU/ml. Results for groups that received vitamin C, 1,2 - Dibromoethane and their association are presented in Table 1, Fig. 1 and Fig. 2.

Table 1

Enzymology results for CA at experimental animals treated with vitamin C, 1,2 - Dibromoethane and their association compared with the Control group

*statistically significant difference compared with Control group (p<0.05)

Group	Treatment	Erythrocyte CA I (EU/ml)	Erythrocyte CA II (EU/ml)
1.	Control	0,344 ± 0,111	1,399 ± 0,202
2.	Vitamin C	0,578 ± 0,149	2,908 ± 0,273
3.	1,2-Dibromoethane	0,165 ± 0,053	0,347 ± 0,069
4.	Vitamin C + 1,2-Dibromoethane	0,491 ± 0,144	2,392 ± 0,241

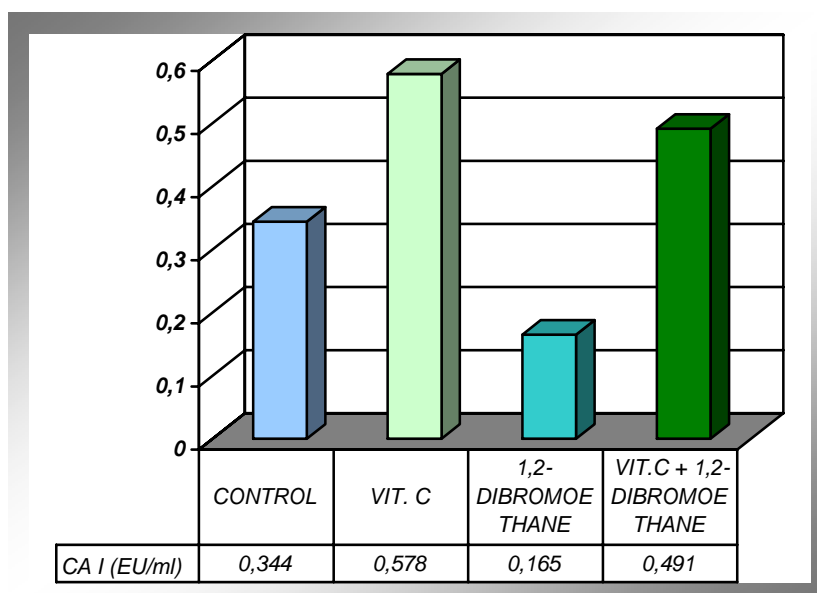


Fig. 1. Changes in erythrocyte CA I activity at experimental animals treated with Vitamin C, 1,2-Dibromoethane and their association, compared with the Control group

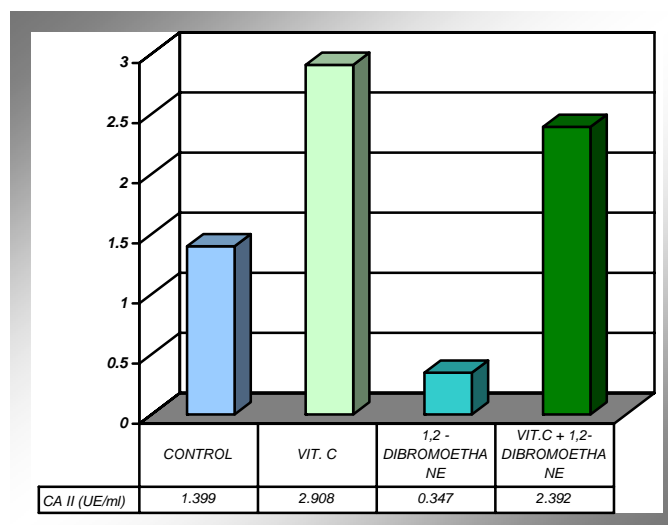


Fig. 2. Changes in erythrocyte CA II activity at experimental animals treated with Vitamin C, 1,2-Dibromoethane and their association, compared with the Control group

The results reveal that Vitamin C administered as monotherapy increases the activity of CA I and II, and the effect is stronger on CA II isoenzyme.

1,2 -Dibromoethane produces a strong decrease of CA isoenzymes, reaching over 75% inhibition of CA II, isoenzymes involved in carcinogenesis.

The association Vitamin C + 1,2 -Dibromoethane completely antagonize the inhibitory effect of carcinogenic compounds on CA isozymes, even producing their activation, but lower than that achieved by vitamin C administered as monotherapy.

With regard to SOD activity, in the Control group, its value was 6.84 ± 0.13 EU. Results for groups that received Vitamin C, 1,2 -Dibromoethane and their association are presented in Table 2 and Fig. 3.

Table 2
Enzymology results for SOD at experimental animals treated with vitamin C, 1,2 -Dibromoethane and their association compared with the Control group

Group	Treatment	SOD activity (EU/ml)
1.	Control	$6,84 \pm 0,13$
2.	Vitamin C	$8,17 \pm 0,34$
3.	1,2-Dibromoethane	$3,61 \pm 0,42$
4.	Vitamin C + 1,2-Dibromoethane	$4,93 \pm 0,18$

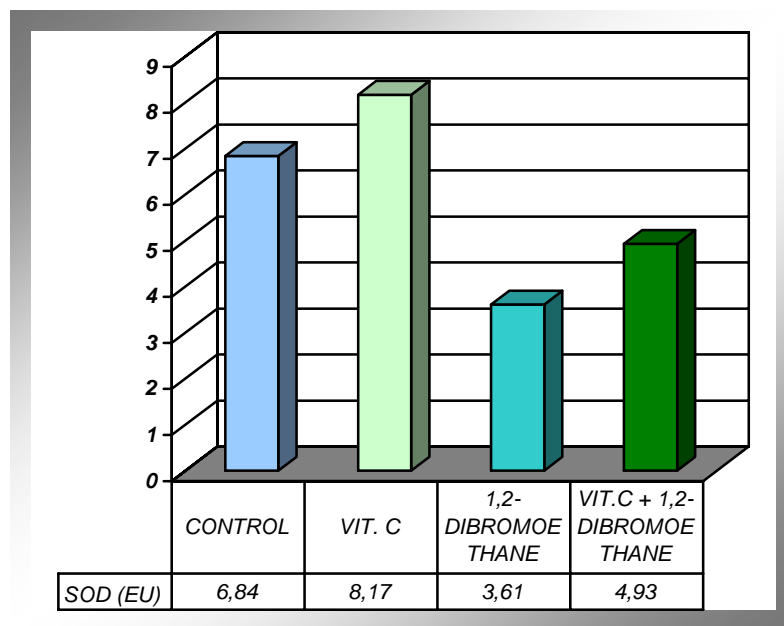


Fig. 3. Changes in erythrocyte SOD activity at experimental animals treated with Vitamin C, 1,2-Dibromoethane and their association, compared with the Control group

The study shows a slight increase of SOD activity in the group receiving vitamin C as monotherapy, results that are in accordance with literature data (Khassaf et al., 2003). For group who received 1,2 - Dibromoethane, a substance known to have a powerful carcinogenic potential, SOD activity was decreased by approximately 48%, probably due to modification of antioxidant systems caused by reactive oxygen species that result from chronic exposure to carcinogenic action (Kong W., 2012). For group that received Vitamin C + 1,2 - Dibromoethane association SOD activity reduction is not so marked as in Group 3. Administration of the vitamin C invariably eliminated such alterations and consequently spare endogenous primary antioxidant enzymes reserves, conclusion that is in accordance with other researches (Wang et al., 2012; Suhail et al., 2012).

CONCLUSIONS

These studies reveal that vitamin C increase CA activity in vivo and the effect is stronger on CA II isoenzyme. 1,2 - Dibromoethane markedly inhibited CA activity but this inhibitory effect was completely antagonize by vitamin C. In vivo, vitamin C cause a slight increase of SOD activity and 1,2 - Dibromoethane cause a decrease in SOD activity by approximately 48%. Concomitant administration of vitamin C partially attenuated the

inhibitory effect of Dibromoethane at group that received Vitamin C + 1,2 -
Dibromoethane association.

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