

High concentrations of Vitamin C induce Oxidative Stress in Blood

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Abstract. Vitamin C is water soluble, being a potent antioxidant in organisms and also having a per-oxidant effect. In this study, the bloods of healthy animals were incubated with different concentrations of vitamin C at 4°C for 24 hours. The lipid per-oxidation and protein damage were investigated. The sample incubation with high concentrations showed significant change in the level of lipid per-oxidation and protein carbonyl. These results supported that oxidative stress may play a role in the mechanism of the toxic action of vitamin C.

Key words: Vitamin C, lipid per-oxidation, protein carbonyl.

Introduction

Vitamin C (ascorbic acid) is an important antioxidant in human plasma (Duarte et al. 2007). It is relatively nontoxic (Tripartite & Gaur 2004). Vitamin C may act as a radical scavenger (Omaye 2004). Animal studies with regards to prevention showed that the reduced form of Vitamin C is effective in blocking the formation of active forms of chemical carcinogens, or in blocking the carcinogenesis due to free-radical generation caused by radiation or by hormonal or viral carcinogens (Altug 2003). It acts as a cofactor for NADP reductase required for glutathione metabolism (Chavan et al. 2007). Moreover, vitamin C acts as a direct reducing agent for met-hemoglobin. Cyanosis results show that met-hemoglobinemia can be effectively treated with ascorbic acid (Prchal & Gregg 2005).

However, it has cytotoxic and genotoxic effects at the higher concentrations. The cytotoxicity of ascorbic acid has been associated with the formation of H₂O₂ in the extracellular environment (Duarte et al. 2007) and the genotoxicity of ascorbic acid has been estimated with the changing of the mitotic index value (Nefic 2008). In a study, the higher dosage of ascorbic acid and zinc resulted in a significant increase of the DNA damage (Harreus et al. 2005).

RBCs are particularly susceptible to oxidative damage for a number of reasons, such as high PO₂ (Boelsterli 2007) and the presence of polyunsaturated fatty acid (Mansour & Mossa 2008). Furthermore, they do not have a nucleus or endoplasmic reticulum and they are unable to replace oxidized proteins (Boelsterli 2007). In I.V. administration, vitamin C is distributed in

the blood at high concentrations. Therefore, we tested the oxidative markers in the blood as a consequence of in vitro exposure. These markers, regarded as potential laboratory monitors for xenobiotics, induce oxidative stress.

Material and methods

Lipid per oxidation

Blood samples were collected from a healthy sheep. The blood sample was centrifuged (2000xg, 5min) and the plasma and white blood cells were removed. Erythrocyte packets were prepared by washing the erythrocytes three times with isotonic saline. 1 ml of blood was exposed to different concentrations of vitamin C (0.1, 1, 10mg/ml). RBCs were incubated at 4°C for 24h. The formation of thiobarbituric acid in erythrocytes was measured for lipid per-oxidation according to the previous method (Sicinska, et al. 2006). Briefly, erythrocytes were mixed with 20% trichloroacetic acid. Samples were centrifuged. Thiobarbituric acid was added to the supernatant and the samples were heated. The absorbance of the supernatant was measured at 532 nm lipid per-oxidation was expressed in absorbance units.

Determination protein carbonyl content in plasma

For the determination of protein damage, the whole blood of a healthy dog was incubated with different concentrations of vitamin C (0.1, 1, 10mg/ml). The protein carbonyl content in the plasma was also assayed according to the previous method (Akagawa, et al. 2006). Briefly, isolated plasma was mixed with guanidinium chloride and dinitrophenylhydrazine (DNPH). Proteins were precipitated in 20% trichloroacetic acid. The protein pellets were washed with ethanol/ethyl acetate and

dissolved in guanidinium chloride. The peak absorbance was measured at 370 nm.

Results

The difference higher than 95 % ($p \leq 0.05$) was considered significant.

The level of lipid per-oxidation and protein carbonyl was significantly increased after incubation with high concentrations of vitamin C as compared to the control and other treatment groups (Table 1).

Table 1. The level of lipid per-oxidation and protein carbonyl

Concentration (mg/ml)	The absorbance unit	
	Lipid per-oxidation	Protein carbonyl
0(control)	0.004±0.001	0.58±0.01
0.1	0.0045±0.0005	0.61±0.03
1	0.009±0.01	0.75±0.006
10	0.016±0.001	1.19±0.05

Each value represents the mean ±SD per group

Discussion

The present study demonstrates that high concentrations of vitamin C are associated with lipid per-oxidation and increased protein damage. These findings are in accordance with previous experiment. It has been demonstrated that in high levels and in the presence of metals such as iron and copper, vitamin C can cause oxidative damage (Omaye 2004). Ascorbic acid is known to reduce molecular oxygen to superoxide anion leading to the formation of hydrogen peroxide (Bhat et al. 2006). Therefore, erythrocytes might be exposed to the risk of oxidation stress through the fenton reaction of hydrogen peroxide with Fe^{2+} of hemoglobin, which generates the powerful oxidant hydroxyl radical (Tavazzi et al. 2001). Moreover, biological reducing agents, such as ascorbate can promote the release of iron from ferritin, also a drop in pH may favor the detachment of iron from the protein. Collectively, iron will be available in free form to mediate oxidative damage through a Fenton reaction (Vallyathan & Shi 1997).

In conclusion, this report confirms that high vitamin C concentrations induce stress oxidative. Information about the safety and pharmacokinetics of high dose i.v. ascorbic is crucial for the proper design of clinical trials (Hoffer et al. 2008). It is possible that using a high dose or a low one in long term, intravenous administrations

of ascorbate produce large amounts in the blood concentration. Therefore, according to our results and previous findings, we anticipate the pro-oxidant effect in people, especially in those who are susceptible to oxidative stress. Some situations such as sickle cell anemia, thalassemia, vitamin E depletion, hyperglycemia, H_2O_2 , and halothane and sulphur dioxide exposure or diabetes induce stress oxidative (Kemal-Enturk et al. 2001). Especially, high intravenous administration doses should be paid attention in diabetic patients because of the considerable rate of this disease. The worldwide figure of people with diabetes is set to rise from 150 million in the year 2000 to 300 million in 2025 (Lupi, et al. 2007). Diabetic patients have significant defects of antioxidant defense elements, and the generation of reactive oxygen species is one of the major determinants of diabetic complications (Gurdol et al. 2008, Lupi et al. 2007, Wiernsperger 2003). These assessments will provide the pharmacological safety and toxicity dose in those people.

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