Investigation of Dose-Dependent Tissue Protective Effects of Vitamin C

Doz Bağımlı Olarak C Vitamininin Doku Koruyucu Etkilerinin Araştırılması

C vitamininin Doku Koruyucu Etkileri / Tissue Protective Effects of Vitamin C

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Özet

Amaç: C vitamini biyolojik sistemlerdeki mükemmel bir antioksidandır. Ancak, C vitaminin prooksidan madde olarak kullanımı da yaygındır. Bu çelişkili olgu C vitamininin mükemmel bir indirgeyici madde olmasından kaynaklanmaktadır. Diğer yandan, Karbon tetraklorüre baktığımızda, karbon tetraklorür uygulamasına bağlı hepatotoksisite insidansında artış olduğu bildirilmektedir. Bu çalışmada, tek doz karbon tetraklorür uygulanmış erkek Wistar türü sıçanlarda farklı dozlardaki C vitamininin lipid peroksidasyonu, alanin transaminaz, aspartat transaminaz, glutatyon ve protein oksidasyon düzeylerine pozitif bir etkisinin olup olmadığı araştırıldı. Gereç ve Yöntem: İki kontrol grubu ve dört tedavi grubu olmak üzere 6 grup ve toplamda altmış Wistar albino sıçan kullanıldı. Deney gruplarına sırasıyla 4 ml distile su içinde 100 mg / kg, 200 mg / kg, 400 mg / kg ve 800 mg / kg C vitamini verilirken kontrol gruplarına oral yoldan gastrik entübasyon ile 4 ml distile su verilmiştir. Bulgular: 40 gün boyunca yapılan uygulama sonunda 100 ile 400 mg / kg arasındaki artan C vitamini konsantrasyonları, karbon tetraklorür ile muamele edilen erkek Wistar sıçanların farklı biyokimyasal parametreleri üzerinde önemli bir koruma ve azalma sağlamıştır. Ancak, aynı koruma C vitamininin 800 mg / kg konsantrasyonunda gözlenmemiştir, Üstelik bu konsantrasyondaki C vitamini karbon tetraklorür tarafından uyarılan toksik etki kadar zaralı bir etki göstermiştir. Tartışma: Bu sonuçlar, yüksek dozlardaki C vitamininin doku koruyucu bir madde olarak kullanılmasının uygun olmadığını göstermiştir. Ayrıca, sonuçlar antioksidan olduğu bilinen bir maddenin, doza bağlı olarak prooksidan madde gibi hareket edebileceğini düşündürmektedir.

Anahtar Kelimeler

C Vitamini, Protein Oksidasyonu, Lipid Peroksidasyonu, ALT, AST, Glutatyon

Abstract

Aim: Vitamin C is an excellent antioxidant in biological systems. However, It is also widely used as a pro-oxidant. This paradoxical behavior results because it is an excellent reducing agent. As for carbon tetrachloride, the incidence of carbon tetrachloride-induced hepatotoxicity is reported to be on the increase. In this study, it is aimed to test, whether additional intake of different concentrations of vitamin C improves antioxidative protection on lipid peroxidation, alanine transaminase, aspartate transaminase, glutathione and protein oxidation levels in single dose carbon tetrachloride-treated male Wistar rats. Material and Method: Two control groups and four treatment groups totaly sixty Wistar albino rats were used in this study. The control groups were fed via oral route a placebo (4 ml of distilled water), while test groups 100 mg /kg, 200 mg/kg, 400 mg/kg and 800 mg/kg body weight of vitamin C in 4ml of distilled water was given via gastric intubation. Results: The administration of vitamin C for 40 days produced a significant decrease with increasing concentrations from 100 to 400 mg/kg of vitamin C in differnt parameters carbon tetrachloride-treated rats. But, the same protection was not observed at higher concentration of vitamin C. Moreover, it has showed hazardous activity at 800 mg/kg concentration as much as carbon tetrachloride-induced toxicity. Discussion: These results showed that vitamin C had not the potential to be used as tissue protective agent at higher doses. Also, results suggest that the same substannces that optimizes antioxidant capacity may also act as prooxidant dependent on its concentration.

Keywords

Vitamin C, Protein Oxidation, Lipid Peroxidation, ALT, AST, Glutathion

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Introduction

Oxidative suppressors, commonly known as antioxidants, are compounds that retard oxidative stress. An antioxidant has been defined as any substance that, when present in low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits the oxidation of that substrate.[1] The human diet contains an array of dietary antioxidants with the most common being ascorbate (Vitamin C), tocopherols (Vitamin E), carotenoids and flavoniods. Within the body there are enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione transferase (GST) and glutathione peroxidase (GPx), which detoxify ROS (Reaktive Oxigen Species) and thus provide protection against oxidative damage. Vitamin C is a water-soluble vitamin, found naturally in many food sources such as citrus fruits and green vegetables. It structure was identified by Szent-Gyorgyi in the early 1900s.[2, 3] Most animal species especially rats which have the ability to synthesize vitamin C in the liver, can make vitamin C from glucose, with the exception of humans, primates and guinea pigs. In humans vitamin C exists in two biologically active forms, ascorbic acid and dehydroascorbic acid. The ready interconversion of these two forms gives vitamin C its antioxidant capabilities. Vitamin C is used in preventive medicine treatments such as in helping to prevent and fight the common cold and flu. Also, it is known to be involved in the metabolism of several amino acids and essential neurotransmitters, such as the formation of hydroxyproline, hydroxylysine, norepinephrine, epinephrine, serotonin, homogentistic acid, and carnitine.[4] Generally vitamin C is thought of as an excellent reducing agent; it is able to serve as a donor antioxidant in free radical-mediated oxidative processes. However, as a reducing agent it is also able to reduce redox-active metals such as copper and iron, thereby increasing the pro-oxidant chemistry of these metals. Thus vitamin C can serve both as an antioxidant and prooxidant. In general, at low ascorbate concentrations, ascorbate is prone to be a pro-oxidant, and at high concentrations, it will tend to be an antioxidant. Hence, there is a crossover effect. It is proposed that the "position" of this crossover effect is a function of the catalytic metal concentration.[5] In the light of current information the aim of this study was to test whether additional intake of different concentrations of vitamin C improves antioxidative protection on lipid peroxidation, alanine transaminase, aspartate transaminase, glutathione and protein oxidation levels in single dose carbon tetrachloride-treated offspring male rats.

Material and Method

Research proposal of this study was submitted to the Atatürk University ethics committee in February 2013, protocol no: 89. No changes were requested to the protocol, and final approval was received four weeks later. Sixty Wistar albino rats were used in this study. The rats were chosen among 3–4 weeks old. That time was especially chosen at the initiation of the study. Because that time is the starting time of rats feeding with standard rat diet. The animals were housed in stainless steel wire cages at 22 ± 1 oC, relative humidity 55 ± 5%, air change 10 times/hour and electric light between 08.00 am and 20.00 pm. Before the experiment began, the rats were fasted overnight but tap water was made available ad libitum. The rats were randomly divided into 6 groups of 10 rats per group. Control I and Control II which served as the positive and negative controls groups were fed via oral route a placebo (4 ml of distilled water), while test groups 100 mg /kg, 200 mg/kg, 400 mg/kg and 800 mg/kg body weight of vitamin C in 4ml of distilled water was given via gastric intubation for 40 days. The experiment was completed when the rats became 60 days old and rats were fasted 18 hours prior to experiments. A single dose of carbon tetrachloride (CC14) was injected intraperitoneally in the Control II and Vitamin C groups (1 ml/kg, as 20% in olive oil) and the same dose of olive oil without CC14 was administered intraperitoneally to the rats in the Control I group. Two hours later, the rats were killed humanely in accordance with sanctions approved by the Institutional Animal Care and Use Committee (IACUC) appropriate to the species. Livers of rats were quickly removed and washed in 0.9% NaCl. Liver parts were homogenized in ice-cold 0.15 M KCl (10% w/v).[6] Plasma AST and ALT activities were measured by auto-analyzer. Lipid peroxide levels in liver were measured by the thiobarbituric acid (TBA) test.[7] Briefly, 0.2 ml of 10% (w/v) tissue homogenate was added to 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with NaOH, and 1.5 ml of 0.8% aqueous solution of TBA. Distilled water was used to produce 4.0 ml of mixture. Subsequently, the mixture was heated in a water bath at 95°C for 60 minutes. After the mixtured was cooled, 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol pyridine (15:1, v/v) were added. Following centrifugation at 4,000 rpm for 10 min, the upper phase was taken and its absorbance measured at 532 nm. 1,1,3,3-Tetraethoxy-propane (TEP) was used as an external standard. Liver glutathione levels were measured by the method of Ellman.[8] Briefly, 0.5 ml of 10% (w/v) tissue homogenate was added to 1.5 ml of M KCl and 3.0 ml of the mixture of non-proteinization solution. After centrifugation, 0.5 ml of the upper phase was taken and 2 ml of 0.3 M Na2HPO4 and 0.5 ml of Ellman reagent were added. Its absorbance at 412 nm was measured. Glutathione (GSH) was used as an external standard. Protein oxidation levels in liver were determined by the methods of Levine et al.[9] and Lowry et al..[10]

Statistical Analysis

Plasma ALT and AST activities were performed by one way ANOVA and Duncan's test. Statistical analysis of liver lipid peroxide glutathione levels and protein oxidation levels were carried out by Kruskal Wallis one way ANOVA and Mann Whitney U-test.

Results

Vitamin C concentrations were compared with Control II, it was seen that the concentrations from 100 to 400 mg/kg of vitamin C significantly decreased ALT and AST activities. Moreover, the concentrations of 200 and 400 mg/kg of vitamin C decreased the ALT and AST levels previously increased by CCI4 treatment to the Control I levels. On the other hand, the same deacrising of ALT and AST levels was not seen at the 800 mg/kg concentrations of vitamin C. Moreover, viamin C inreased plasma ALT and AST levels as much as CCI4 treated Control II group (Table 1). The other parameter was liver lipid peroxide levels which were reduced with increasing concentrations from 100 to 400 mg/kg of vitamin C compared to the Control II group. On the other side, the same deacrising of liver lipid peroxide levels was

Table 1. Effect of Vitamin C concentrations on ALT, AST activities, lipid peroxide, protein oxidation and glutathione levels in male Wistar rats treated with CCl4

Parameters	Control I (n=10)	Control II (n=10)	Vitamin C 100 mg/kg (n=10)	Vitamin C 200 mg/kg (n=10)	Vitamin C 400 mg/kg (n=10)	Vitamin C 800 mg/kg (n=10)
Plasma ALT (U/L)	25.1 ± 4.3a	84.7 ± 5.2b	48.4 ± 6.2c	28.8± 6.2a	28.2 ± 6.2a	89 ± 8,2d
Plasma AST (U/L)	120.8 ± 9.6a	162.2 ± 8.4b	136.1 ± 10.8c	124 ± 7.6a	118 ± 6.6a	172 ± 9.6d
Liver glutathione (µmol GSH/ g liver)	5.1 ± 1.0a	11.5 ± 1.0b	8.5 ± 0.8c	5.0 ± 0.6a	6.2 ± 0.9a	12 ± 1.6d
Liver lipid peroxide (nmol MDA/ g liver)	111.8 ± 12.3a	184.3 ± 10.1b	138.8 ± 13.5c	116 ± 11.2a	119 ± 8.6a	194 ± 9.6d
Liver protein carbonyl content (nmol carbonyl/ mg protein)	4.8 ± 0.6a	18.7 ± 2.6b	10.4 ± 1.7c	5.1 ± 1.6a	6.1 ± 1.3a	17 ± 3.6d

The number of animals was 10 rats; different letters in the same line are statistically different, data are presented as group mean values ± SD. p< 0.05 Abbreviations; Aspartate transaminase (AST), Alanine transaminase (ALT) Malondialdehyde (MDA)

not seen at the 800 mg/kg concentrations of vitamin C (Table 1). When the liver protein carbonyl levels were evaluated, It was seen that from 100 to 400 mg/kg concentrations of vitamin C strongly inhibited protein oxidation induced by CCI4. Also itwas seen that both 200 and 400 mg/kg decreased this levels to the Control I groups level. But the same inhibition was not seen at the 800 mg/kg concentrations of vitamin C. Also, when it was compared Control II groups levels, it was seen that it strongly situmulated the protein oxidation (Table 1). The last test was the liver glutathione levels. In the vitamin C groups, the liver glutathione levels were significantly decreased compared to the Control II group except for the concentration of 800 mg/kg. 200 and 400 mg/kg of vitamin C concentrations decreased the liver glutathione levels previously increased by CCI4 treatment to the Control I levels (Table 1).

Discussion

In this study, rats were implemented different vitamin C concentrations during 40 days (from 20 to 60 days). That time was the age at puberty of rats. In general, rats reach puberty around 50 to 60 days of age.[11] This time interval was chosen on purpose in order to see the short time effecst of different vitamin C concentrations on puberty periods of rats. For this reason, Protein oxidation, Lipid peroxidation, ALT, AST, Glutathione levels in rats were evaluated in this study, the tisue protective and possible prooxidant activities of Vitamin C concentraions were evaluated. Notable correlation between ALT and AST activities and concentrations of vitamin C was observed. ALT and AST are the most frequently utilized of hepatocellular injury and represent markers of hepatocellular markers. Of these enzymes, ALT is localized primarily to the liver whereas AST is found in a wide variety of other tissues such as the cardiac and skeletal muscle, kidney, brain, etc..[12] Thus, when these enzymes levels were examined, it was seen that ncreasing in vitamin C concentrations (from 100 to 400 mg/kg) notably deacreased ALT and AST levels. As a matter of fact, studies made with animal and human[13,14] have showed that ascorbic acid to be a potent antioxidant mediating its antioxidant effect by scavenging ROS. Other studies have equally showed the protection of ascorbic acid and other vitamins in hepatic oxidative damage.[15,16] Thus, results of the present study suggest vitamin C's ameliorating effects to be possible mediated via inhibition of free radicals generation or free radical scavenging activity. On the other hand, that significant protective effect of vitamin C was not seen at higher concentration. Furthermore, the concentration of 800 mg/kg of vitamin C showed a hazardous effect on ALT and AST levels (Table 1). Because of these considerations, these results definitely demonstrate the dual effect of Vitamin C depens on the concentration. Also, these results suggest that vitamin C may act not only as an antioxidant, but also as a prooxidant effects depending on its low and high concentrations. The other parameter in the vitamin C groups, the liver glutathione levels were significantly decreased compared to the Control II group vitamin C decreased the liver glutathione levels previously increased by CCI4 treatment to the Control I levels. (Table 1). Thus, it can be concluded that vitamin C reduced the lipid peroxide levels in the liver, and because of this lowering effect the glutathione levels remained low as well. In accordance with this study, it was found compelling evidence for antioxidant protection of lipids by vitamin C in biological fluids, animals, and humans, both with and without iron cosupplementation. On the other hand, at higher dose (800 mg/kg) of vitamin C showed prooxidant effect on liver glutathione and lipid peroxide levels (Table 1). Therefore, it can be suggested that vitamin C act as a pro-oxidant under dose-dependent physiological conditions.

Although the data on protein oxidation in humans are sparse and inconclusive, the available data in animals consistently show an antioxidant role of vitamin C.

The inhibition protein oxidation was related to the concentration of vitamin C concentrations, the protective activity increased significantly as a result of increasing concentration (100 mg / kg, 200 mg/kg, 400 mg/kg). But, vitamin C didn't show inhibition effect on protein oxidation levels at higher concentration (8mg /kg). Moreover, the protein oxidation inhibition effect of vitamin C dramatically decreased at higher concentration (Table 1). This information suggests that the same substance that optimize antioxidant capacity may also acts as a prooxidant in different test systems depending on its concentration.

Finally, the results of this study confirmed that the additional intake of Vitamin C improves the liver's antioxidative defense in a dose-dependent manner. But, the same defense was not seen at higher concentrations in a dose-dependent manner. The explanation for the disproportion between doses of vitamin C and their effects on the studied parameters probably conencted with the mechanism of tissue accumulation of vitamin C. These and other important issues discussed here need to be addressed in future studies of the role of vitamin C in oxidative damage. On the other hand, further studies should be continued to get

proper information regarding the role of vitamin C as prooxidant and its involvement in the other dose depending processes.

Competing interests

The authors declare that they have no competing interests.

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