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Research Article

EFFECT OF VITAMIN C AND VITAMIN E ON SUCROSE INDUCED ENDOTHELIAL DAMAGE IN RATS

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ABSTRACT: Endothelial damage may contribute to the atheriosclerosis development. Chronic sucrose intake could lead to the establishment of endothelial damage due to the increase of oxygen reactive species. Although antioxidants vitamins may be useful to improve the endothelium injury, more experimental designs are required. In the current study, Vitamin C and E were used with the purpose of evaluating the regression degree of the endothelial damage produced in an induced model by a sucrose-rich diet in Wistar rats. Four groups were formed (n=6). The endothelium damage was achieved by a 35% of sucrose intake during 18 weeks. Then, the vitamins were supplied for four weeks at 10 mg/kg. Endothelial dysfuntion were assessed by two indirect methods: Histopathological evaluation of abdominal aorta and the quantification of plasmatic endothelial cells. Some biochemical and biometric parameters were also determined. It was observed that both vitamins decreased the plasmatic endothelial cells, being Vitamin C the one with the highest reduction percentage (78.7 % vs 34.8 %). Both vitamin treatments decreased hyperlypemia and the gain of body weight. The histologic analysis of the abdominal aortic fragments showed a significant regression of the endothelium injury parameters; no-organization of elastic fibers and small clear vacuoles spread or fusioned in the cellular cytoplasm.The most improved morphometric parameter was wall thickness: 67.16 ±12.34 µm for vitamin C and 76.28 ± 18.0 µm for vitamin E vs 95.16 ± 42.09 µm for group without treatment (p<0.001). It is concluded that either Vitamin C or E may improve the endothelium injury provoked by the chronic supply of sucrose.

Key words: Sucrose, Endothelium, Vitamin C, Vitamin E.

INTRODUCTION

Endothelial damage constitutes an early marker for the development of atherosclerosis by one of the three mechanisms, non enzimatic glycolization of enzymes and lipids, increase of oxygen reactive species (oxidative stress) (Muniyappa and Sowers 2013, Carvajal 2017). and Protein Kinase C (PKC) activation (Pérez-Pevida *et al.* 2016). The administration of antioxidants could inhibit or breaks the atherosclerosis significantly and normalize the endothelial function (Eichholzer *et al*. 2001, Riccioni *et al*. 2012).

Some experimental and epidemiological data have suggested that rising plasmatic levels of Vitamin E and C may reduce the incidence of atherosclerosis (Riccioni *et al*. 2012, Idris *et al*. 2014). But, However, more experimental designs are required to validate the effect of Vitamin C and E to reduce endothelial injury.

Therefore, the present investigation was designed to evaluate the effect of Vitamin C and E on sucrose induced endothelium damage in Wistar rats in terms of histopathology observation and morphometry assessment of abdominal aortic fragments, as well as the counting of the plasmatic endothelial cells (PEC).

MATERIAL AND METHODS

Animals

The experimental protocol was approved by the Animal Ethics Committee of the Experimental Toxicology Unit belonging to the Medical College of Villa Clara. All rodents were purchased at the National Centre of Animal Production Laboratory (Havana, Cuba).

Wister rats weighing 180 to 200g were housed six rats per polycarbonate cages (Tecniplast, Buguggiate, Italy) with mesh bottoms. The quarantine period was prolonged for a week. The animals were kept under free specific pathogen conditions with conventional food and water, both ad libintum. The cycles of light / darkness were 12/ 12 hours. Room temperature was 22 ± 2 °C and control relative humidity held at 50% to 70%. Experiments were carried out according to the Principles of Laboratory

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Animal Care (WHO 1985). Once the experience concluded, sacrifice by anaesthetic overdose was performed, followed by cervical dislocation (Close *et al*. 1997).

Endothelial Damage Induction and Vitamin Treatment

Wister rats were paired at a proportion of two females per male. Once pregnancy was verified, 15 ml. of sucrose drink (35 %) were daily supplied to pregnant rats during 21 days of gestation and 21 days of breast feeding. After weaning, four experimental groups were formed (n=6) with male newborns rats: Vitamin C, Vitamin E, control group without treatment (CGWT), and health (H). All these animals continued the sucrose solution intake at 35 % during 18 weeks. After this period, Vitamin C and E were administrated during four weeks by oral gavages at a dose of 10 mg/Kg. The hyperglucidic diet supply continued during the vitamin treatment in the four experimental groups.

Biometric Parameters

The body weight was weekly checked by a Sartorius BP160P balance (Sartorius, Gottingen, Germany). The body weight gain was determined at the end of the vitamin treatment.

Biochemical Parameters

The lipidic and glycemic profile was determined at 22 weeks, following the administration of vitamins. Colorimetric methods, Helfa diagnostic kit (EPB Carlos J. Finlay, Havana, Cuba) and autoanalizer Hitachi 902 (Roche Diagnostic, Tokyo, Japan), were used to perform the analysis.

Quantification of Plasmatic Endothelial Cells (PEC)

The quantification of the carcasses of the PEC was done according to the Adenosine di-Phosphate (ADP) technique (Hladovec and Rossman 1973) (Mendoza-Castaño *et al*. 2003).

Tissue Processing and evaluation of endothelial cell damage

Abdominal aorta segments of 1 cm length were processed in paraffin wax embedding. Section tissue of 5 µm of thickness were cut and stained with hematoxylin and eosin (H/E) (Presnell *et al*. 1997) and the endothelial damage were evaluated microscopically with following parameters tumefaction of endothelial cells, inflammation (Schoen *et al*. 1999), endothelial denudation and integrity of the elastic and muscular fibers (Ross 1993).

Statistical Analysis

Measurement data were processed using the SPSS 20.0 software. ANOVA - one way test was used to compare the groups after confirming the normal distribution of the data. Statistical difference among the groups was determined by Bonferroni test. The paired t-student Test was used to determine the differences in the biochemistry variables before and after the treatment. Values of $p< 0.05$ were considered significant.

Study Design.

6 Weeks mother rats		**22 Weeks male neonates rats (from all groups)**		**Sucrose intake (35%)**
Pregnant rats	Breast feeding	Experimental groups: male neonate rats	Vitamin treatment	
3 weeks	3 weeks	18 weeks	4 weeks	Entire period to all group

Table 1. Morphometric parameters of endothelial damage in abdominal aortic fragments (Mean ± SD).

	Vessel Area (mm^2)	**Lumen Area (mm^2)**	**Wall Area (mm^2)**	**Thickness Wall (ìm)**
Vit C	558.35 ±47.33[b]	352.15 ± 3.76[a,b]	206.20±14.95[a, b]	67.16± 12.34 [a]
Vit E	617.65 ±87.45[a]	407.05 ± 85.82[a]	210.60 ±34.82[a,b]	76.28 ± 18.01[a]
CGWT	527.51 ±73.12[b]	261.82 ±114.46[b]	265.69 ± 73.12[b]	95.16 ± 42.09[b]
Healthy	698.51 ±106.45	469.97 ± 95. 26	170.44 ± 101.03	74.22 ± 14.76

[a = p<0.001vs CGWT, b = p<0.001vs healthy.
Vitamin C group (Vit C), Vitamin E group (Vit E) Control Group Without Treatment (CGWT), Healthy group (H)].

Table 2. Inhibition percentage of the increment of the area and thickness of the wall of the abdominal aorta in respect to the CGWT (Mean ± SD).

	Percentage of inhibition	
	Wall Area	**Wall Thickness**
Vit C	28.81 ± 13.81	32.61 ± 17.92
Vit E	26.24 ± 13.44	54.73 ± 20.20

Vitamin C group (Vit C), Vitamin E group (Vit E).

Table 3. Glycemia and cholesterol values (mmol/L), after 28 days of vitamin treatment (Mean ± SD).

	Glycemia	Cholesterol
Vit C	10.128 ± 1.33	2.028 ± 0.22
Vit E	10.076 ± 1.6	2.214 ± 0.22
CGWT	7.965 ± 2.43	1.993 ± 0.27
Healthy	4.425 ± 0.96	1.593 ± 0.09

Vitamin C group (Vit C), Vitamin E group (Vit E) Control Group, Without Treatment (CGWT), Healthy group (H).

RESULTS AND DISCUSSION

Plasmatic Endothelial Cells count

The number of PEC in the group which receive vitamins was significantly lower compare to the CGWT (Fig. 1). The Vitamin C group achieved PEC values close to the healthy group (p= 0.13), with 78.7% of inhibition of endothelial damage. The PEC in Vitamin E group was significantly lower to the CGWT, however these results could not get closer to the healthy group, showing 34.8 % of endothelial damage inhibition.

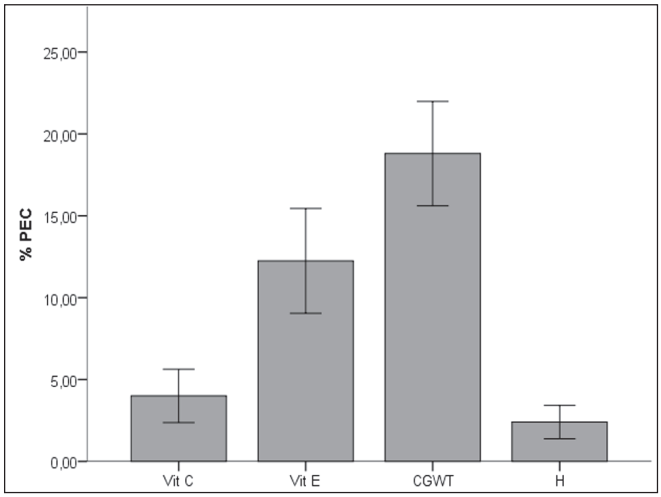

Fig. 1. Plasmatic Endothelial Cells (CEC). The bars represent the mean values expressed in percentage. Vitamin C group (Vit C), vitamin E group (Vit E) Control Group Without Treatment (CGWT), Healthy group (H).

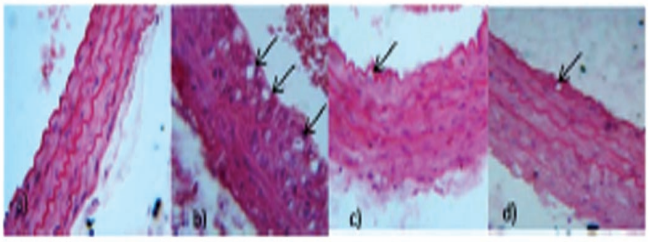

Fig. 2. Abdominal aortic segments of animals aged 22 weeks, stained with H/E magnification 400 X. a) Healthy animal: Integrity of the endothelium and the internal elastic lamina is observed, organization of elastic and smooth muscular fibers is appreciated. b) Animal of CGWT, the arrow shows spread or fusioned small clear vacuoles in the cytoplasm of the vascular smooth muscle cells (VSMC) which sometimes displace the nucleus. Wall thickness and disorganization of the elastic and muscular fibers is observed. c) Animal treated with Vitamin C, d) Animal treated with Vitamin E: A discrete sub-endothelial thickness is observed in c and d with the presence of a very reduced number of cellular cytoplasmic changes (arrow).

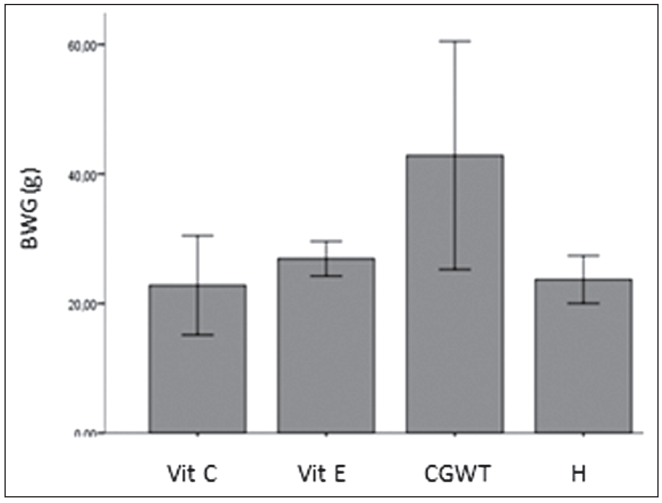

Fig. 3 Increase of Body Weight Gain (BWG) during the last four weeks of the study corresponding to Vitamin treatment.

The results are expressed as the Mean. Error bars are represented with standard deviation ($\propto < 0.05$). Vitamin C group (Vit C), Vitamin E group (Vit E) Control Group Without Treatment (CGWT), Healthy group (H).

Histological Analysis of Aortas

Figure 2 illustrates abdominal aortic segments, fixed and stained by haematoxylin/eosin technique. The histological analysis shows the presence of tumefaction in the endothelial cells in the vascular smooth muscle cells (VSMC), and marked disorganization of the muscular and elastic fibers of the media layer in animal controls without treatment. However, the animals with vitamin C treatment showed an noticeable regression of damage of the tunica layer.

Table 1 presents the result of morphometric measures, taken into account the evaluation of the endothelium

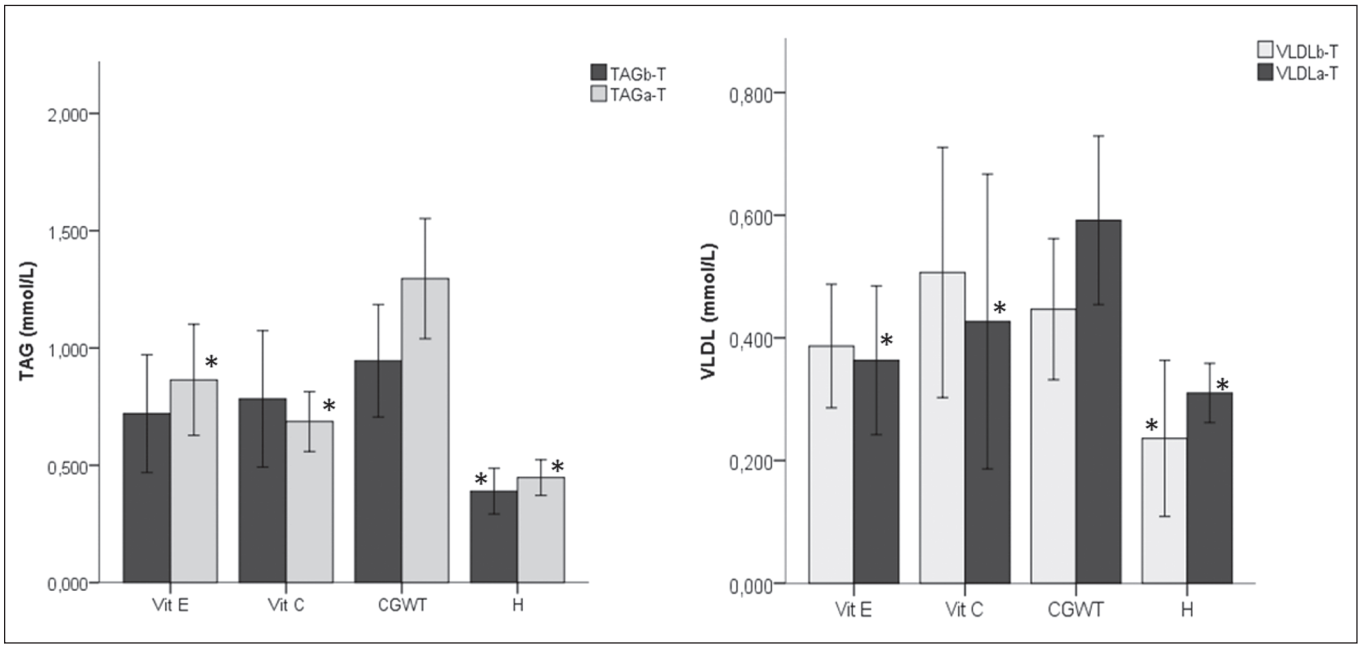

Fig. 4. TAG values before treatment (TAGb-T) and four weeks after treatment finished (TAGa-T).
The results are expressed as the Mean. Error bars are represented with standard deviation (∝< 0.05).
* p<0.05 compared with CGWT.
Vitamin C group (Vit C); Vitamin E group (Vit E); Control Group Without Treatment (CGWT); Healthy group (H).

Fig. 5. VLDL values before treatment (VLDLb-T) and four weeks after treatment finished (VLDLa-T).
The results are expressed as the Mean. Error bars are represented with standard deviation (∝< 0.05).
* p<0.05 compared with CGWT
Vitamin C group (Vit C); Vitamin E group (Vit E); Control Group Without Treatment (CGWT); Healthy group (H).

damage degree of the aortas analyzed and the inhibition degree achieved with the vitamin C treatment as compared to the CGWT.

The analysis of the evaluated parameters shows vascular remodelling which is produced by the tissue injury in a chronic hyperglucidic environment. In this model, the tissue response to a sub-endothelial hypertrophy is observed. This response determines the decrease of the vessel area at the expense of the reduction of the lumen, and an increase of the vascular wall area. Vitamin E treatment decreases the area of the vascular remodelling observed, except in the area of the arterial wall. Similar findings were exhibited in the healthy group. Vitamin C also improved the state of the vessel though in a lower magnitude than Vitamin E. It was also observed that the thickness of the arterial wall was the parameter with the best response and with a remarkable decrease compared to group without treatment and similar to the healthy group.

The percentages of inhibition for the parameter area and thickness of the arterial wall are showed in Table 2. The reduction in the area of the arterial wall was similar for both vitamin treatments. Nevertheless, the decrease of the thickness of the arterial wall was markedly greater in Vitamin C group.

Analysis of the Body Weight Gain

According to the results, the application of both vitamins retains the rhythm of gaining body weight. As can be seen in Fig. 3, the vitamin groups had a lower quantity of grams gained in comparison to CGWT ($p<0.05$), similar behaviour to the healthy animals ($p>0.05$).

Biochemistry Analysis

As Table 3 shows, vitamins had not any effect in the reduction of glycaemia, which tend to increase in sucrose supply. Values significantly higher in comparison to the healthy group ($p=0$) and similar to the CGWT were observed ($p> 0.05$). Cholesterolemia did not decrease after vitamin C treatment neither, having a similar behaviour to CGWT ($p> 0.05$) and with values statistically higher than the healthy group ($p< 0.05$).

Figure 4 and 5 illustrate the profile of triacilglycerides (TAG) and lipoprotein of very low density (VLDL) for the experimental groups. Significant reductions of TAG (32% for Vitamin E and 47% for Vitamin C) and VLDL (38% for Vitamin E and 27% for Vitamin C) were observed in each case compared to the CGWT.

Endothelial damage occurs in the first stage of the development of the atherosclerotic process and it is potentially reversible (Gimbrone and García-Cardeña 2016). The study demonstrated that rats fed with diets

rich in carbohydrates for a prolonged period of time, develop hypertriglyceridemia with an increase of oxidative stress (Selensci *et al.* 2010). The effect of free radicals over the dysfunction of the physiology mechanisms of the endothelium, specifically in the vasorelaxant function of the Nitric Oxide (NO), (Bryce *et al*. 2014) could be decreased or counteracted by antioxidant agents such as Vitamin C and E. In this case, the action of both vitamins should contribute to the regression of the vascular damage and to the recovery of the vascular endothelium. Other studies had demonstrated lipidic lipoperoxidation decrease due to Vitamin E intake in rats diagnosed with metabolic syndrome (Wallinger *et al*. 2011).

A metabolic imbalance could be achieved in groups that consumed sucrose diet. This state may contribute to the development of the endothelial damage after 18 weeks. Similar to the healthy group, significant regression of the evaluated parameters was achieved at the end of the treatment. These results differ from some reports which did not observe improvement in the lipidic profile but detected a decrease in lipidic peroxidation in the aortic tissue after the administration of Vitamin E in hypercholesterolemic rats (Oré *et al*. 2001).

The findings in the fragments of the aortas are similar to reports in literature of the first stage of endothelial dysfunction (Storino *et al*. 2014, Munari 2014). In the present study, the percentage of inhibition of the endothelial damage of Vitamin C (10 mg/kg) was higher than D-003- anti platelet which was assayed by Mendoza *et al* (2010) at a highest dose (200 mg/kg), on the other hand, the results of Vitamin E (10 mg/kg) were comparables at a dose of 25 mg/kg of D-003. These results could be influenced by the type of experimental model. In our case, the damage to endothelium from low to moderate state, was correspondingly induced to previous and incipient stage of endothelium dysfunction. However, citric acid provokes more drastic damages with desquamation of the vascular endothelium (Mendoza *et al*. 2010), which requires a more potent pharmacological effect. Therefore, it is necessary to use higher doses in order to achieve the restitution of the damage.

The treatment during four weeks with antioxidant vitamins indicates that in combination with healthy dietary, they may be an efficient alternative to decrease hyperlipemia and body weight gain which is observed in this model. They may also revert endothelial damage and consequently contribute to the recovery of the endothelial function. Further research is needed to verify the reduction of oxidative stress into endothelium after antioxidant vitamin treatment.

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