

ORIGINAL RESEARCH ARTICLE

Effect of L-Ascorbate on Dexamethasone induced Experimental Insulin Resistance- Role of Oxidative stress.

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ABSTRACT

In the present study, Vitamin C (L-Ascorbate) was administered (100mg/kg /day) through Diet for Prophylactic as well Therapeutic regimen in Dexamethason (20µg/kg /once daily,s.c) Induced Experimental Insulin Resistance in male Wister (240-300g) rats. The animals were grouped into 4 groups 6 in each and estimated Plasma Insulin and Malondialdehy Level in Plasma aswell in Isolated Aortic tissue as a marker of oxidative stress. HOMA-IR index and MASTUDA INDEX were calculated as a marker of an Insulin Resistance and Insulin Sensitivity respectively. From the finding of present study, There was no significant difference in Plasma Insulin level, however significantly reversed (P<0.01) the Increase in plasma triglycerides in vitamin C treated rats when compared with dexamethasone injected rats. Vitamin C significantly prevent and reversed Malondialdehy level in plasma (P<0.01) aswell in Aortic tissue (P<0.05). However, it had no influence on Insulin Resistance and Insulin Sensitivity. In conclusion, Oxidative Stress may not be playing the initiative role in Dexamethasone induced Insulin Resistance.

Key words: Insulin Resistance (IR), Oxidative Stress, Insulin Resistance Index (HOMA- -IR) and Insulin Sensitivity Index (MASTUDA INDEX).

INTRODUCTION

Dexamethasone is widely used Therapeutic agent for Immunosuppressive and Anti-inflammatory action. Its use is associated with several adverse effects like Hypertension, Diabetes and Insulin Resistance due to over production of ROS (Bjelakovic et al., 2007). It is found that ROS levels are increased in cellular models of Insulin Resistance (Houstis N et al., 2006), suggesting increased ROS levels are an important trigger for Insulin Resistance. Although, ROS have been proposed to have a causal role in multiple forms of Insulin Resistance. It has been reported that fensuccinal prevents Dexamethasone induced Insulin Resistance probably due to Oxidative Stress (Gorbenko et al., 2000). Hence, in the present study we aimed to Evaluate the Effect of Vitamin C ((L-Ascorbate) on Dexamethasone-Induced Experimental Insulin Resistance to Explore the role of Oxidative Stress in Insulin Resistance.

MATERIALS AND METHODS

Male wistar rats weighing (240-300g) were used. They were housed in a group of six under environmentally controlled room with 12-h light/dark cycle and had free access to food and water. Dexamethasone Injection was procured from Zydus cadila, 2-Thiobarbituric acid, L-Ascorbic acid, (Vitamin C) and 1,1,3,3,-Tetramethoxy propane (Malonaldehyde bis) was procured from Himedia Laboratories Ltd, Mumbai, India, Radio Immuno Assay Kit for Insulin- RIAK-1 was procured from Board of Radiation and Isotope Technology, BARC, Mumbai, India. Dosage of the Dexamethasone phosphate administered subcutaneously was prepared in the saline (0.9 % sodium chloride saline solution). Dose of the Dexamethasone phosphate (20µg/kg, once daily, subcutaneously) for 14 days to induce Insulin Resistance were chosen based on previous reports (Rhee MS et al.,

2004). Dose of the Vitamin C (100mg/kg, once daily, mixed in water and food pallets were soaked in it) through diet were chosen based on

Previous reports (Mehta J et al). The Animals were divided into 4 groups containing six in each as follows.

(1) Normal Control (NC): Received normal saline (0.9% NaCl) subcutaneously for 14 days.

(2)Dexamethasone (DEX): Received Dexamethasone 20µg/kg, subcutaneously, once daily for 14 days.

(3) Dexamethasone and Vitamin C (VIT C-P): Received Dexamethasone 20µg/kg, subcutaneously, once daily for 14 days and fed with chow mixed with Vitamin C (100 mg/kg/day) from 1stday and continued throughout study.

(4) Dexamethasone and Vitamin C (VIT C-T): Received Dexamethasone 20µg/kg, subcutaneously, once daily for 14 days and fed with chow mixed with Vitamin C (100 mg/kg/day) from 8th day and continued throughout study.

On 13th day of Experimental period Systolic blood pressure was measured by tail cuff method. At the end of treatment period, Blood samples were collected by retro-orbital plexus puncture under light ether anesthesia after 16 h fasting (on 14th day) and then Animals were sacrificed by excess ether anesthesia and thoracic Aorta was isolated for estimation of lipid peroxides (TBARS). Systolic blood pressure was measured indirectly by the tail cuff method using noninvasive blood pressure apparatus. The Insulin estimation was done by using ImmuChem Radioimmunoassay method (Loraine JA et al., 1976). Using a standard kit obtained from BRIT, BARC, Mumbai, India. Triglyceride was estimated by method of (Buccolo G et al., 1976) using a standard kit obtained from ERBA diagnostics Manheim Ltd. Insulin Resistance index (Henry RR et al., 2003) and Insulin sensitivity Index (Albareda M et al., 2000) were calculated. The TBARS estimation is based on the reaction between Plasma Malondialdehyde (MDA), a product of lipid

peroxidation and thiobarbituric acid (TBA) and carried out as previously described (Yoshioka T

et.al., 1979). Results were expressed in (Table no. 1&2. and fig. no. 1&2). Prism 3 graph pad were used for Graphical representation. Statistical analysis was done by using unpaired student's t-test and One-way ANOVA followed by benferroni multicomparision Test.

RESULTS AND DISCUSSION

There was a Significant elevation in plasma Insulin, Triglycerides level and concentration of Malondialdehyde in Plasma (P<0.01) as well in Aortic tissue (P<0.05) in rats injected with Dexamethasone for 14 days when compared with normal rats. There was a Significant elevation of HOMA-IR value and decrease in MASTUDA INDEX in rats injected with Dexamethasone for 14 days (P<0.01) when compared with normal rats (Table no 2). There was a Significant increase in Systolic Blood pressure in rats injected with Dexamethasone for 14 days (P<0.0001) when compared with normal rats (Table no 1). Vitamin C Significantly (P<0.001) prevented the Dexamethasone induced increase in Blood pressure (Table no 1). There was no significant difference in Plasma Insulin and Triglyceride level. Vitamin C significantly prevented the elevation of Malondialdehyde in Plasma (P<0.01) but had no effect on elevation of concentration of Malondialdehyde (P<0.05) in aortic tissue when compared to Dexamethasone injected rats. Vitamin C had no effect on HOMA-IR index aswell on MASTUDA-INDEX when compared with Dexamethasone injected rats (Table no 2).Vitamin C significantly (P<0.01) reversed the Dexamethasone induced increase in Systolic Blood pressure (Table no 1). There was no significant difference in plasma Insulin level in Therapeutic regimen of Vitamin C. It also significantly reversed Elevation of Triglycerides, Malondialdehyde level in plasma aswell in Aortic tissue induced by Dexamethasone injected rats was (P<0.01, P<0.001 and P<0.005 respectively) when compared with Dexamethasone injected rats. There was no significant difference in Insulin Resistance (HOMA-IR) and Insulin sensitivity index (MASTUDA INDEX) when treated therapeutically with Vitamin C when compared with Dexamethasone injected rats.

Table 1: Systolic Blood pressure, Insulin level and Plasma Triglycerides.

Groups	Plasma Triglycerides (mg/dl)	Basal Insulin level (mg/dl)	Systolic blood pressure (mmHg)
Normal (NOR)	58.69±12.253	13.92±1.228	130.20±1.594
Dexamethasone (DEX)	131.67±16.038*	25.33±4.128*	197.9 ±5.888*
Dexamethasone and Vitamin C (VIT C-P) (Preventive)	83.86±10.804	22.16±1.195	132.66±7.601**
Dexamethasone and Vitamin C (VIT C-T) (Therapeutic)	72.655±12.544**	20.0±2.236	131.88±7.642**

Values are Expressed in Mean ± SEM; n=6. Comparison of mean values between NOR and DEX groups were performed by unpaired student's t-test. *P<0.05, **P<0.01 and **P<0.01 when compared with NOR. DEX and VIT C

treated groups were performed by One-way ANOVA followed by benferroni multicomparison Test. *P<0.01 and **P<0.001 when compared with DEX.

Table 2: Insulin Resistance Index, Insulin Sensitivity Index and Concentration of malondialdehyde in Plasma and in Aortic tissue.

Groups	Insulin resistance index (mg/dl)	Insulin sensitivity index (mg/dl)	Malondialdehyde concentrations in plasma. (µmol/L)	Malondialdehyde concentrations in Aorta.(µmol/L)
Normal (NOR)	2.967±0.248	0.4600±0.051	0.600±0.06325	0.9500±0.4121
Dexamethasone (DEX)	8.256±1.843	0.2339±0.036	3.500±0.8062**	3.333±0.8819*
Dexamethasone and Vitamin C (VIT C-P) (Preventive)	4.707±0.4840	0.2493±0.0306	0.776±0.264**	1.55±0.381
Dexamethasone and Vitamin C (VIT C-T) (Therapeutic)	3.041±0.5274	0.4813±0.1518	0.666±0.076**	0.650±0.067*

Values are expressed in Mean ± SEM; n=6. Comparison of mean values between NOR and DEX groups were performed by unpaired student's t-test. *P<0.05, **P<0.01 and **P<0.01 when compared with NOR. DEX and VIT C

treated groups were performed by One-way ANOVA followed by benferroni multicomparison Test. *P<0.01 and **P<0.001 when compared with DEX.

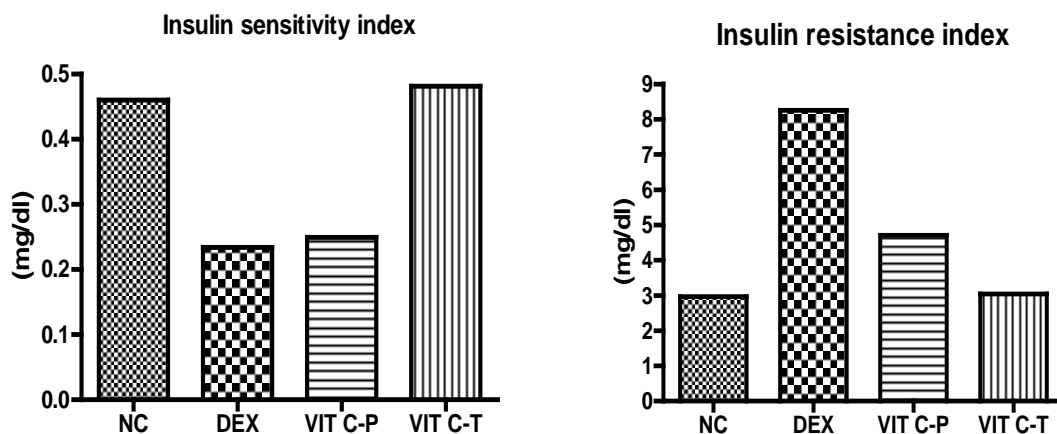


Fig. no 1: Insulin Sensitivity Index and Insulin Resistance Index.

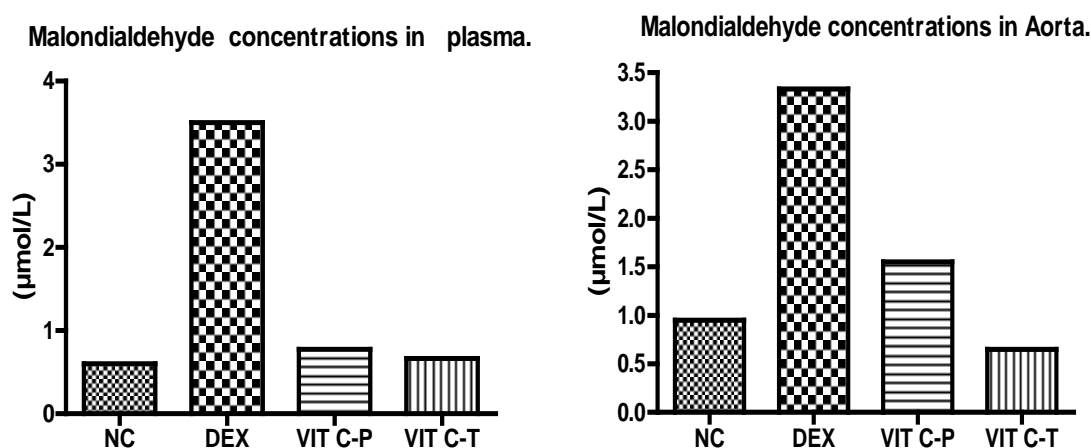


Fig.no 2: Concentration of Malondialdehyde in Plasma and in Aortic tissue.

CONCLUSION

In conclusion, Dexamethasone Induced Insulin Resistance and Hypertension accompanied by increased Oxidative Stress. However, it appears that abnormality in Insulin action and elevation of Systolic Blood Pressure occurs earlier than Oxidative Stress. Administration of Vitamin C normalized the Induced SBP and prevent aswell

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reverse the elevated Malondialdehyde in Plasma, however significantly reversed the Malondialdehyde in Aortic tissue but had no influence on markers of Insulin Resistance/ Insulin Sensitivity. Hence, it may be concluded that Oxidative Stress may not playing the initiative role in Dexamethasone Induced Insulin Resistance.

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