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Effect of α-Tocopherol Acetate on Dexamethasone Induced Experimental Insulin Resistance-role of Oxidative Stress

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ABSTRACT

Dexamethasone is widely used Immunosuppressant and Anti-inflammatory agent. Its use is associated with evolution of Insulin Resistance (IR) and Hypertension these are probably attributed to oxidative stress. In the present study, effect of Antioxidant like á-Tocopherol (Vitamin E) was administered (100mg/kg /day, mixed in sesame oil and food pallets were soaked in it) through diet for Prophylactic aswell Therapeutic regimen in Experimental Induced Insulin Resistance by using Dexamethasone (20µg/kg /once daily, subcutaneously) for 14 days in male Wister rats (240-300g) and estimated plasma Insulin and TBARS level in plasma aswell in isolated Aortic tissue as a marker of oxidative stress. HOMA-IR index and MASTUDA INDEX were calculated as an Insulin Resistance/ Insulin sensitivity. From the finding of present study, Vitamin E Improved the Insulin sensitivity and prevented the Insulin Resistance probably due to its pleotropic effects other than Antioxidant property, so that indicate oxidative stress may not play the causative role in development of Dexamethasone Induce Insulin Resistance.

Key words: Insulin Resistance (IR), Oxidative Stress, Dexamethasone, 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). Insulin Resistance is a cardinal feature of type-2 diabetes and may be an Impetus for the development of Hypertension. Dexamethasone induced Insulin Resistance probably due to oxidative stress (Xi L *et al.,* 2005). Although, ROS have been proposed to have a causal role in multiple forms of Insulin Resistance. It has been reported that Crocetin and fensuccinal prevents Dexamethasone induced Insulin Resistance probably due to Antioxidant properties. (Gorbenko *et al.,* 2000). Hence, in the present study we aimed to evaluate the effect of Vitamin E (α-Tocopherol acetate, synthetic form) on Dexamethasone-Induced Experimental Insulin Resistance to explore the role of oxidative stress in Insulin Resistance.

MATERIAL 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, α-Tocopherol Acetate 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 E (100mg/kg, once daily, mixed in sesame oil and food pallets were soaked) through diet were chosen based on previous reports. (Mehta J *et al.,* 1999). The animals were divided into 4 groups containing six in each as follows.

1. Normal Control: Received normal saline (0.9% NaCl) subcutaneously for 14 days.
2. Dexamethasone: Received Dexamethasone 20µg/kg, subcutaneously, once daily for 14 days.
3. Dexamethasone and Vitamin E: Received dexamethasone 20µg/kg, subcutaneously, once daily for 14 days and fed with chow mixed with Vitamin E (100 mg/kg/day) from 1stday and continued throughout study.
4. Dexamethasone and Vitamin E: Received Dexamethasone 20µg/kg, subcutaneously, once daily for 14 days and fed with chow mixed with Vitamin E (100 mg/kg/day) from 8th day and continued throughout study.

During the experimental period 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 AND DISCUSSION

There was a significant elevation in fasting basal 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. Vitamin E significantly ($P<0.001$) prevented the Dexamethasone induced increase in blood pressure. There was no significant difference in fasting basal plasma Insulin level however Elevation of Triglycerides level induced by Dexamethasone was significantly ($P<0.05$) prevented by Vitamin E.

Table 1: Systolic blood pressure, basal insulin level and plasma triglycerides

Systolic blood	Groups Triglycerides (mg/dl)	Plasma level (mg/dl)	Basal Insulin pressure (mmHg)
Normal	58.69±12.253	13.92±1.228	130.20±1.594
Dexamethasone	131.67±16.038*	25.33±4.128*	197.9 ±5.888*
Dexamethasone and vitamin E (Preventive)	55.66±17.605*	25.83±4.347	128.44±4.651**
Dexamethasone and vitamin E (Therapeutic)	83.738±7.234*	21.35±5.713	132.2 ±6.715**

Values are expressed in Mean ± SEM; n=6.

*P<0.05 when compared with normal. **P<0.01 when compared with normal. ***P<0.001 when compared with normal.

It also prevented the elevation of malondialdehyde in plasma (P<0.01) as well as in Aortic tissue (P<0.05) when compared to Dexamethasone injected rats. Vitamin E significantly prevented the Insulin Resistance as indicated by reduction in HOMA-IR (P<0.05) index and there was a significant increase (P<0.01) in Insulin sensitivity (mastuda index) in Vitamin E treated rats when compared to Dexamethasone injected rats. Vitamin E significantly (P<0.001) reversed the Dexamethasone induced increase in systolic blood pressure. There was no significant difference in fasting basal plasma Insulin level in therapeutic regimen of vitamin E. Elevation of Triglycerides, malondialdehyde level in plasma aswell in Aortic tissue induced by Dexamethasone injected rats was significantly reversed by Vitamin E. It also significantly reversed the Insulin resistance as indicated by reduction in HOMA-IR (P<0.05) index and there was no significant difference in Insulin sensitivity (mastuda index) in Vitamin E treated rats when compared with dexamethasone injected rats.

Table 2: Insulin Resistance index, Insulin sensitivity index and Concentration of malondialdehyde in plasma and in Aortic tissue

Groups	Malondialdehyde resistance index (mg/dl)	Insulin sensitivity index (mg/dl)	Insulin concentrations in plasma. (µmol/L)	Malondialdehyde concentrations in Aorta. (µmol/L)
Normal	2.967±0.248	0.4600±0.051	0.600±0.06325	0.9500±0.4121
Dexamethasoe	8.256±1.843	0.2339±0.036	3.500±0.8062**	3.333±0.8819*
Dexamethasone and vitamin E (Preventive)	2.646± 0.7383	0.6318±0.1222	0.416±0.040**	0.466±0.084*
Dexamethasone and vitamin E (Therapeutic)	4.602± 1.943	0.4101±0.0973	0.650±0.080**	0.5833±0.054*

Values are expressed in Mean ± SEM; n=6.

*P<0.05 when compared with normal. **P<0.01 when compared with normal. ***P<0.001 when compared with normal.

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 E found to be ineffective to prevent/or reverse the insulin resistance. However, it improved the Insulin sensitivity and prevented the Insulin Resistance probably due to its pleotropic effects other than Antioxidant property.

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