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Verapamil Ameliorates Cardioprotective Potential of Vitamin E in Myocardial Oxidative Damage Induced by Isoproterenol: A Biochemical Study

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Abstract: Cardiotoxicity resulted from exposure to the environmental toxicants, drugs, pesticides, and even food additives and pollutants have been known for a long time. Chemicals can induce persistent molecular changes with functional consequences without leading to morphologically detectable alterations. Depending on dosage and duration of the exposure to toxic substances, functional changes may be less serious and of transient nature, but they may also irreversible. Since functional effects are frequently of acute nature, they may quickly lead to abnormal heart function, which may be fatal..Present study aimed to evaluate amelioration of cardioprotective effect of Vitamin E by Verapamil. Albino rats were orally treated with different combinations of Verapamil and Vitamin E orally for 30 days. On the day 29th and 30th, Isoproterenol was administered S.C. Different biochemical parameters were screened from serum and heart preparation. The present study concluded that the subtherapeutic doses combinations of Verapamil and vitamin E prevent cardiac damage significantely.

Keywords:- *Isoprotereno, Verapamil, Vitamin E, Cardiotoxicity.*

INTRODUCTION

β-adrenoreceptor agonists have been widely used in the treatment of bronchoconstriction in patient with asthma or chronic pulmonary disease because of their ability to relax smooth muscle. In addition to this action, they also cause cardiac hypertrophy and oxidative stress in the myocardium[1].

Myocardial infarction is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand. There is substantial evidence that ischemic tissue generate oxygen derived free radicals. Free radicals and reactive oxygen species have been implicated in cardiac disease and metabolic disorders, which results due to exposure to chemicals and environmental agents[2]. Substances can also cause structural, more specifically degenerative and inflammatory changes in the heart and blood vessels. These in turn lead to functional abnormalities[3]

Isoprenaline a synthetic catecholamine and beta adrenergic agonist has been found to cause a severe stress in the myocardium resulting in infarct like necrosis of the heart muscle and is also well known to generate free radicals and stimulate lipid peroxidation[2].

However, cardiotoxicity was not a defined discipline in the past. An advance in biochemical research and practice has made possible an urgent need for cardiotoxicological studies.

In present investigation the combined treatment of calcium channel blocker (verapamil) and antioxidant (vitamin E) for treating cardiotoxicity induced by isoprenaline was studied.

Isoprenaline metabolism produces quinines, which react with oxygen to produce superoxide anion and hydrogen peroxide leading to oxidative stress and depletion of the endogenous antioxidant system. Isoprenaline caused oxidative stress evidenced by the reduction in myocardial SOD and catalase activity. Production of superoxide radical and hydrogen peroxide inactivates SOD and catalase resulting in loss of activity and accumulation of superoxide anions and H_2O_2 thus damaging the myocardial cell[14, 15].

MATERIAL METHOD

Animals

Male Albino wistar rats weighing 150-200 g were used in this study. All rats were procured from animal house of Appasaheb Birnale College of Pharmacy; Sangli All animal procedures were performed in accordance with institutional guidelines approved by local authorities.

Animals were kept for one week to acclimatize to laboratory conditions before starting the experiment; they were given free access to

water and standard rat feed. The study was approved and carried out as per the guidelines of the Institutional Animal care Ethics Committee.

Drugs and chemicals

Isoproterenol hydrochloride was purchased from Ozone international, Mumbai. Vitamin E was purchased from E Merck Mumbai. Verapamil was received as gift sample from Cipla pharmaceuticals, Goa. The all other chemicals were of analytical grade.

Induction of myocardial oxidative damage[4,5]

Isoproterenol was dissolved in normal saline and injected subcutaneously (85 mg/kg). On 29th and 30th day at an interval of 24 hrs. ISO-induced cardiac damage was confirmed by abnormal changes in different enzyme levels in blood and tissue on day 31st

Group (III-VI) received Verapamil (5,10,15,20 mg/kg), Group VII-IX received vitamin E (50,75,100 mg/kg) , Group X-XI received different combinations of verapamil and vitamin E. Verapamil was dissolved in water and administered once daily for thirty days Vit E (100 mg/kg) suspended in normal saline with the help of carboxy methyl cellulose (CMC) once daily for 1 month and in addition challenged with ISP (85 mg/kg, *sc*) on 29th and 30th day, at an interval of 24 h. Vitamin E and different combinations were also administered in same manner once daily for 30 consecutive days. On 29th and 30th day Isoproterenol 85 mg/kg was given by s.c route. On day 31st different biochemical parameters were recorded.

Measurment of serum biomarkers and tissue enzyme activity:-

After completion of the treatment the animal were mildly anesthetize with ether and blood was withdrawn from retro orbital plexus, then animals were sacrificed with spinal dislocation hearts were removed rapidly and a 10% homogenate was prepared in ice-cold phosphate buffer (pH 7.4, 0.05M). Serum separated by centrifugation was used for estimation of Serum Glutamate Oxaloacetate Transaminase(SGOT)/ Aspartase aminotransaminase(AST) by DNPH method using diagnostic kits (Beacon diagnostics Pvt. Ltd. Navsari.)

Determination of level of MDA (a product of lipid peroxidation) from heart tissue homogenate by Method of Ohkawa et al[6,7] Determination of reduced glutathine from heart tissue homogenate by Ellmans method[8], Determination of superoxide dismutase enzyme activity from heart tissue homogenate by the method of Marklund et al[9]. Determination of catalase enzyme activity from heart tissue homogenate by the method of Aebi[10] Determination of total protein from heart tissue homogenate by the method of Lawry[11]

Statistical analysis

Descriptive studies such as mean and SEM were calculated for all variables for each group Values are expressed as mean± SEM; Statistical analysis was performed using Dunnet's t-test. **p<0.01 was taken as the criterion of statistical significance.

RESULT AND DISCUSSION

Effect of Verapamil, vitamin E, and co-administered Verapamil and vitamin E on serum markers

Supramaximal doses of isoprenaline induced subendocardial myocardial ischemia, hypoxia, necrosis and finally fibroblastic hyperplasia with decreased myocardial compliance and inhibition of diastolic and systolic function[12].

Isoprenaline induces morphological and functional alterations in the heart leading to myocardial necrosis. It also produces excessive production of free radicals resulting from oxidative metabolism of catecholamines. Although cardiotoxicity occurs primarily via adrenoceptor activation, there is increasing evidence that it may also occur through oxidative mechanisms. It has been reported that excess catecholamines affect calcium transport mechanism primarily via oxidation reactions involving free radical mediated damage[13].

Cytosolic enzymes ALT (alanine aminotransferase) and AST (aspartase aminotransferase) which serve as the diagnostic markers of myocardial tissue damage, leak out from the damaged tissues into the blood stream when the cell membrane becomes permeable or ruptured. The amount of these cellular enzymes presented in plasma reflects the alterations in plasma membrane integrity and/or permeability[14]. The isoprenaline control group shows significant elevation in the level of these serum markers, which confirmed the cardiac damage.

Isoprenaline induced elevation ($p<0.01$) in the mean activity of SGOT enzyme was found to be decreased and the level was significantly restored ($p<0.01$) near to normal on combined treatment of verapamil (15 mg/kg) with vitamin E (75 mg/kg) (49.64% reduction) followed by the groups which received verapamil (15 mg/kg) + vitamin E (50 mg/kg) and verapamil (10 mg/kg) with vitamin E (75 mg/kg) (48.27% and. 45.63% reduction respectively).

The combined treatment of Verapamil (15 mg/kg) + (Vitamin E 75 mg/kg) attenuate the isoprenaline induced average increase ($p<0.01$) in SGPT level which was close to the mean value of normal control (66.86 % reduction). The groups treated with combination of verapamil (10 mg/kg) + vitamin E (75 mg/kg) and verapamil (15 mg/kg)+ vitamin E (50 mg/kg) also maintain this level. This might be due to the action of these combinations on maintaining membrane integrity thereby restricting the leakage of these enzymes. Hence present results suggested that these sub therapeutic regimens of verapamil and vitamin E inhibits myocardial damage.

Table No. 1 Alteration in Serum Enzyme Activities by Isoprenaline and Effect of Different Treatments

Group	SGOT Unit/ml	SGPT Unit/ml
Control (Normal saline 5ml/kg)	91.60 ± 0.3330	33.6± 0.2108
ISP 85mg/kg	182.66± 0.9098$^{\#\#}$	115± 1.078$^{\#\#}$
Verapamil 5 mg/kg	180.66± 0.7014	115± 0.3651
Verapamil 10 mg/kg	179.33± 0.9545	114.16± 0.2001
Verapamil 15 mg/kg	125.00± 1.693	66.6± 0.333
Verapamil 20 mg/kg	96.166± 0.7032**	41± 0.3651**
Vitamin E 50 mg/kg	136± 0.5831	99.166± 0.4773
Vitamin E 75 mg/kg	119.53± 0.4944**	60.83± 0.4014**
Vitamin E 100 mg/kg	101.5± 0.4282**	35.83± 0.83**
Vitamin E 50 mg/kg +Verapamil 5 mg/kg	120.5± 0.8466**	42.42± 0.6851**
Vitamin E 50 mg/kg + Verapamil 10 mg/kg	96± 1.155**	42.33± 0.2108**
Vitamin E 50 mg/kg + Verapamil 15 mg/kg	94.5± 0.3416**	36± 0.4472**
Vitamin E 75 mg/kg +Verapamil 5 mg/kg	105.5± 0.7638**	39± 0.8162**
Vitamin E 75 mg/kg + Verapamil 10 mg/kg	99.33± 0.4944**	36.48± 0.6585**
Vitamin E 75 mg/kg + Verapamil 15 mg/kg	92.66± 0.4944**	34.66± 0.33**

Values are expressed as mean ± SEM; n= 5. Statistical analysis was performed using Dunnet's test.
**$p<0.01$ was taken as the criterion of significance.
**$p<0.01$ when compared to ISP control group,
$^{\#\#}p<0.01$ when compared to control group.

Effect of Verapamil, vitamin E, and co-administered Verapamil and vitamin E on antioxidant markers of tissue.

Administration of high concentration of isoprenaline has been reported to induce severe oxidative stress and result in necrotic lesions in the myocardium of rats. The increased generation of reactive oxygen species and/or depletion of the antioxidants in the defense system may contribute to oxidative stress and affect the pathogenesis of myocardial injury. Lipid peroxidation is an indication of the severity of isoprenaline induced necrotic damage of the heart, and has been linked with altered membrane structure and enzyme inactivation[15]. Isoprenaline treated rats showed marked increase (approx. 3 fold) in lipid peroxidation in myocardium, measured as MDA content.

Table No. 2 Effect of Isoprenaline and Different Treatments on antioxidant Markers MDA and Reduced Glutathione

Group	Malondialdehyde (nmoles/g heart)	Reduced glutathione μmoles/mg of protein	Superoxide dismutase (SOD) unit/mg of protein	Catalase μmoles/mg of protein
Control (Normal saline 5ml/kg)	483.65± 3.989	15.28± 0.5612	17.378±0.1148	65.876±0.4554
ISP 85mg/kg	1277.42± 3.11##	11.064±0. 3578##	8.454±0.3118##	34.03±0.2166##
Verapamil 5 mg/kg	1165.49± 1.95**	10.484±0.1294	8.526±0.1328	33.544±0.3197
Verapamil 10 mg/kg	846.32± 1.641**	11.856±0.086	11.34±0.2636**	36.892±0.2956**
Verapamil 15mg/kg	785.85± 2.28**	11.97±0.02429	13.434±0.1691**	38.75±0.3782**
Verapamil 20 mg/kg	667.254± 2.052**	12.472±0.2031**	15.088±0.1890**	52.474±0.2611**
Vitamin E 50 mg/kg	1030.32± 3.15**	10.77±0.0926	9.172±0.1742	34.144±0.1907
Vitamin E 75 mg/kg	846.806± 2.364**	11.45±0.1082	12.648±0.1148**	42.89±0.2627**
Vitamin E 100 mg/kg	595.26± 1.565**	12.132±0.2894**	13.858±0.0778**	49.07±0.2627**
Vitamin E 50 mg/kg +Verapamil 5 mg/kg	738.1576± 1.3718**	11.57±0.1195**	11.742±0.1333**	48.012±0.6005**
Vitamin E 50 mg/kg + Verapamil 10 mg/kg	704.042± 4.983**	12.242±0.08777**	14.466±0.1136**	54.03±0.4311**
Vitamin E 50 mg/kg + Verapamil 15 mg/kg	577.7248± 1.830**	12.24±0.0877**	15.024±0.07414**	53.66±0.3767**
Vitamin E 75 mg/kg +Verapamil 5 mg/kg	732.06± 2.257**	13.208±0.099**	13.784±0.1187**	56.62±0.2575**
Vitamin E 75 mg/kg + Verapamil 10 mg/kg	658.094± 3.151**	14.566±0.1006**	17.344±0.1951**	64.916±0.2356**
Vitamin E 75 mg/kg + Verapamil 15 mg/kg	592.362± 5.939**	15.122±0.09708**	17.538±0.1328**	65.15±0.2917**

Values are expressed as mean± SEM; n= 5. Statistical analysis was performed using Dunnet's test.
**p<0.01 was taken as the criterion of significance.
**p<0.01 when compared to ISP control group,
##p<0.01 when compared to control group.

Administration of vitamin E (50 mg/kg) with verapamil (15 mg/kg) markedly reduced lipid peroxidation as evidenced by average reduction (54.75%) in myocardial MDA level in comparison to isoprenaline treated group. The group treated with the combination of vitamin E (75 mg/kg) + verapamil (15 mg/kg) also retained (i.e.53.64%) the mean MDA content near normal.

The GSH and GSH dependent antioxidant enzymes protect the cellular and subcellular membrane from peroxidative damage by eliminating hydrogen peroxide and lipid peroxide. Decreased activities of these enzymes leads to accumulation of these oxidants and make myocardial cell membrane more susceptible to oxidative damage[14,15]. In present study, a significant decline ($p<0.01$) in the mean concentration of GSH (reduced glutathione) in the heart was observed in isoprenaline induced rats when compared with the mean value of normal control indicating the depletion of GSH resulting in enhanced lipid peroxidation and excessive lipid peroxidation causing increased GSH consumption[14].

Treatment of vitamin E (50 mg/kg, 75 mg/kg) and verapamil (5 mg/kg,10 mg/kg and 15 mg/kg) alone did not improved the activity of reduced glutathione, but the combination of vitamin E (75 mg/kg) with verapamil (15 mg/kg) retained the average values which was close to normal value.

The average SOD activity was significantly decreased in isoprenaline control group compared to normal control. The activity of this enzyme was significantly ($p<0.01$) restored by the groups, which received verapamil (15 mg/kg), and (10 mg/kg) in combination with vitamin E (75 mg/kg). The % increase in activity shown by groups receiving verapamil (15 mg/kg) + vitamin E (75 mg/kg) was 107.45 % and verapamil (15 mg/kg) + vitamin E (75 mg/kg) was 105.11%.

Significantly decreased activity ($p<0.01$) of catalase was observed in the heart of isoprenaline-induced rat when compared with normal control rats.

The treated group with the combination of verapamil (10 mg/kg) + vitamin E (75 mg/kg), and verapamil (5 mg/kg) + vitamin E (75 mg/kg) showed significant increase in the activity of catalase, (% increase 91.76%. & 91.44%) respectively.

This finding proved the beneficial cardioprotective effect of these combinations against cardiac stress, in which oxidative stress was long known to contribute to the pathogenesis

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REFERENCES

1. Garg A, Sharma S. Isoproterenol ameliorates workstress-induced rat skeletal muscle degeneration. Indian J of Biochem Biophys 2006 April; 43:82-7.
2. Prabhu S, Jainu M, Sabitha KE, Shyamaladevi CS. Cardioprotective effect of mangiferin on isoproterenol induced myocardial infarction in rats. Indian J of Exp Biol 2006 March; 44:209-15
3. Raymond NJM, John DE, Uries MA, Hollinger. Toxicology:Principle and Aplication, CRC press;1996.p.788-815.
4. Rajadurai M, Mainzen PS. Preventive effect of naringin on isoproterenol-induced cardiotoxicity in Wistar rats: an in vivo and in vitro study. Toxicology 2007; 232: 216–25.
5. Nandave M, Mohanty I, Nag TC, Ojha SK, Mittal R, Kumari S et.al.. Cardioprotective response to chronic administration of vitamin E in isoproterenol induced myocardial necrosis: Hemodynamic, Biochemical and ultrastructural studies. Indian J Clin Biochem 2007; 22(1):22-8.
6. Okhawa H, Ohishi N, Yaki K. Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal biochem 1979; 95:351.
7. Ojha SK, Nandave M, Kumari S, Arya DS. Antilipidperoxidative and free radical scavenging activity of *Tribulus terrestris*. Indian drugs 2006 Feb; 43(2):136-40.
8. Gupta M. Majumdar UK, Kumar TS, Gomathi P, Kumar RS. Antioxidant and hepatoprotective effect of Bauhinia racemosa against paracetamol and carbon tetrachloride induced liver damage. Iranian J. Pharmacol and Therapeutics 2004; 3:12-20.
9. Marklund Sl. Pyrogallol autooxidation. In: greenwald RA, editor. Handbook of methods for

oxygen radical research. Boca raton: CRC press 1985.p.243-247.

10. Aebi H. Catalase. In: Bergmeyer HV, editor. Methods in enzymatic analysis. New york: Acadamic press 1974; 2:.674-684.
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ Protein measurement with the Folins-phenol reagent. J boil Chem 1951; 193:265-75.
12. Zhou R , Qingbin X , Zheng P, Yan L , Zheng J, Dai G. Cardioprotective effect of fluvastatin on isoproterenol-induced myocardial infarction in rat. European J of Pharmacol. In press. 2008
13. Rajadurai M, Mainzen PS. Preventive effect of naringin on isoproterenol-induced cardiotoxicity in Wistar rats: an in vivo and in vitro study. Toxicology 2007; 232: 216–25.
14. Devika PT, Prince PSM. Protective effevt of (-)-epigallocatechin-gallate (EGCG) on lipid metabolism in Isoproterenol induced myocardial infarction on male wistar rats: A histopathological study. Biomed & pharmacotherapy. In press. 2007
15. Gutteridge JMC. Lipid peroxidation and Antioxidant as Biomarkers of tissue Damage.Clinical Chem 1995; 41(12):1819-28.