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EFFECT OF MAGNESIUM CHLORIDE IN ISOPRENALINE INDUCED MYOCARDIAL INFARCTION IN RATS

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ABSTRACT

Cardioprotective role of intravenous administration of magnesium chloride was evaluated in albino rats by biochemical and histopathological parameters. Myocardial damage was induced by injecting (i.v.) isoprenaline 2 mg/kg body weight of animal. There was a dose dependent increase in the activity of cardiac enzymes CPK, LDH, SGOT and serum cholesterol level. Histopathologically, myocardial damage was quite significant in 2 mg/kg body weight isoprenaline induced group of animals. Considering the data on determination of cardiac enzymes and histopathological changes, 2 mg isoprenaline was chosen as standard dose to study efficacy of cardioprotection by gold standard, Verapamil and Magnesium chloride. Verapamil (5 microM) injected(i.v) prior to 2 mg isoprenaline administration revealed significant reduction of cardiac enzymatic activity ($P < 0.01$) compared to animals infused with isoprenaline alone. Histopathological findings showed no areas of necrosis and cellular infiltrates in animals primed with 2 mg isoprenaline following verapamil. Highly significant reduction in CPK, LDH, SGOT activity and Cholesterol levels was observed in animals administered with $MgCl_2$ (i.v) at 40 mg/kg body weight. In addition to this, significant delay of enzymatic activity was observed in group treated with 40 mg/kg body weight magnesium chloride and isoprenaline compared to group treated with only isoprenaline ($P < 0.01$). The study clearly highlighted and confirmed the valuable role of magnesium chloride as cardioprotective agent.

INTRODUCTION:

Magnesium chloride is a simple salt that exists abundantly in both of the world's oceans and all of its salt water seas. The hydrated magnesium chloride can be extracted from brine or sea water. Anhydrous magnesium chloride is the principal precursor to magnesium metal, which is produced on a large scale[1]. $MgCl_2$ have beneficial hemodynamic effects in Pre-eclampsia[2], Tocolysis[3], eclampsia[4], gall stone disease[5], ADHD[6], Asthma[7]. However Magnesium had been significantly used in acute myocardial infarction[8]. The present study was carried out to investigate the possible and efficient protective effect of magnesium chloride on isoprenaline induced myocardial infarction.

MATERIALS AND METHODS:

DRUGS AND CHEMICALS:

Isoprenaline used as an inducing agent purchased from Sigma Aldrich, Bangalore. Verapamil purchased from SISCO chemicals, Hyderabad. All other chemicals used in the study were of analytical grades.

The intravenous injection of Isoprenaline at 2 mg/kg bodyweight of rat was employed for inducing myocardial infarction[9].

Magnesium chloride, purchased from Merck Company, India were subjected for the cardioprotective studies.

ANIMALS:

Albino rats (Wistar strain) of male sex weighing about 150-200 g obtained from King Institute, Chennai were used in the present investigations. The animals were grouped and housed in polyacrylic cages with not more than 6 animals per cage and maintained under standard laboratory conditions (Temperature 25±2 °C) with dark and light cycle (12/12h) with a relative humidity of 45% to 65%. Animals were allowed to acclimatize for at least 7 days before each experiment and were observed daily or more frequently for signs of ill health; body weights were determined at appropriate times. They were allowed free access to standard dry pellets diet-containing protein-21%, lipids-5%, crude fibre-4%, ash-8%, calcium-1%, phosphorus-0.6%, nitrogen free extract-55% and provided with metabolizable energy at 3600K cal/Kg and also enriched with vitamins and minerals. It was supplied by the "Hindustan Lever, Kolkata", India marketed under the name, "Gold Mohr Feeds" & water *ad libitum*.

Acute toxicity test: Acute toxicity tests were performed according[10] to OECD-423 guidelines (acute toxic class method). Male albino rats were selected by random sampling technique were employed in this study. The animals were fasted for 12 hours with free access to water only. Administration of initially 20 mg/kg bodyweight of $MgCl_2$ was given intravenously and observed for 24 hours for mortality, if any. If no mortality was observed then the higher doses (20, 30, 40 mg/kg body weight) were performed. The doses were selected for the evaluation of cardioprotective activity was 40 mg/kg bodyweight.

EXPERIMENTAL DESIGN:

Assessment of the cardioprotective activity was done by Verapamil as a standard drug. Animals were divided into four groups with 6 animals each.

Group I: Received balanced food and water alone.

Group II: Received Isoprenaline HCl (2 mg/kg body wt) as a single dose intravenously.

Group III: Received $MgCl_2$ (40 mg/kg body wt) as a single dose followed by Isoprenaline HCl (2 mg/kg body wt) after 10 mins of administration intravenously.

Group IV: Received the standard drug, Verapamil (5 µmole/kg body wt) as a single dose followed by Isoprenaline HCl (2 mg/kg body wt) after 10 mins of administration intravenously.

Autopsy procedures and Sample collection:

Rats were sacrificed after 48 hours of administration of drugs from each group by anaesthesia with chloroform and the blood was removed from the carotid vein and collected into serum separator tube and clotted at room temperature for 2 hours and centrifuged at 2500 rpm for 10 mins and the serum was harvested and the biochemical estimations of cardiac enzymes like LDH, CPK, SGOT and Cholesterol were done with semi-autoanalyser kits. The heart was harvested, washed in normal saline & placed in 10% formalin solution. It was subjected to histopathological examinations.

HISTOPATHOLOGICAL EXAMINATION:

One animal from the treated groups showing maximal activity as indicated by improved biochemical parameters from control, isoprenaline induced, standard verapamil and $MgCl_2$ were utilized for this purpose. The animals were sacrificed and the heart was removed. Then, 5 mm thick pieces of heart were fixed in appropriate fixative for 12 hrs and then embedded in paraffin, using conventional methods, and cut into 5 mm thick sections and stained using H&E dye and

finally mounted in diphenyl xylene. Then, the sections were observed under microscope for histopathological architecture and their photomicrographs were taken.

STATISTICAL ANALYSIS:

The different of biochemical parameters were measured using the statistical method of Analysis of Variance (ANOVA). Analysis of variance refers to the examination of differences among the samples. It is a statistical tech. specially designed to test whether the means of more than the quantities population are equal. All data were expressed as Mean ± Std deviation of the no. of experiments. The statistical significant was evaluated by One-way analysis of variance (ANOVA) using SPSS Version 16.0 obtained the individual comparisons.

RESULTS

The results of isoprenaline induced myocardial infarction and pre-treatment with $MgCl_2$ are shown in table-1 and graphical representation of results in Figure-1.

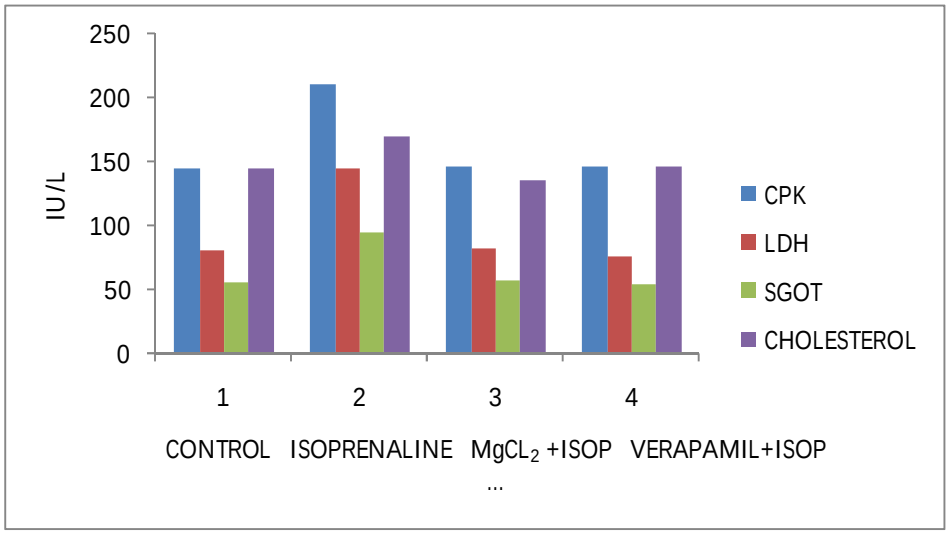
TABLE 1:

GROUPS	CPK(IU/L)	LDH(IU/L)	SGOT(IU/L)	CHOLESTEROL (mg/dl)
Control (Vehicle treated)	145.42±0.34	80.64±0.23	55.53±0.28	145.40±0.33
Isoprenaline induced (2mg/kg body weight)	210.60±0.77	145.69±0.36	95.55±0.37	170.70±1.11
$MgCl_2$ (40mg/kg bodywt)+ Isoprenaline(2mg/ kg body wt)	146.81±1.05	81.92±0.95	56.55±0.94	135.51±0.35
Verapamil(5µmole/kg body wt) + Isoprenaline(2mg/ kg body wt)	146.85±0.97	75.54±0.31	53.51±0.35	146.90±1.31

Results are expressed as Mean±SD. Level of significance is $p<0.01$ when compared with control for CPK and LDH and $p<0.05$ when compared with control for SGOT and Cholesterol. A single dose of administration of isoprenaline produced a marked elevation of the serum levels of CPK, LDH, SGOT and Cholesterol levels in experimental rats which indicates the myocardial damage and myofibrillar obstructions in Group-II animals alone. Pre-treatment with $MgCl_2$ at a dose of 40 mg/kg body wt in Group-III rats significantly prevents the increased levels of enzymes towards the respective normal value, which is an indication of stabilization of plasma membrane as well as repair of the myocardial tissue damage caused by isoprenaline administered after 10 mins of $MgCl_2$ infusion.

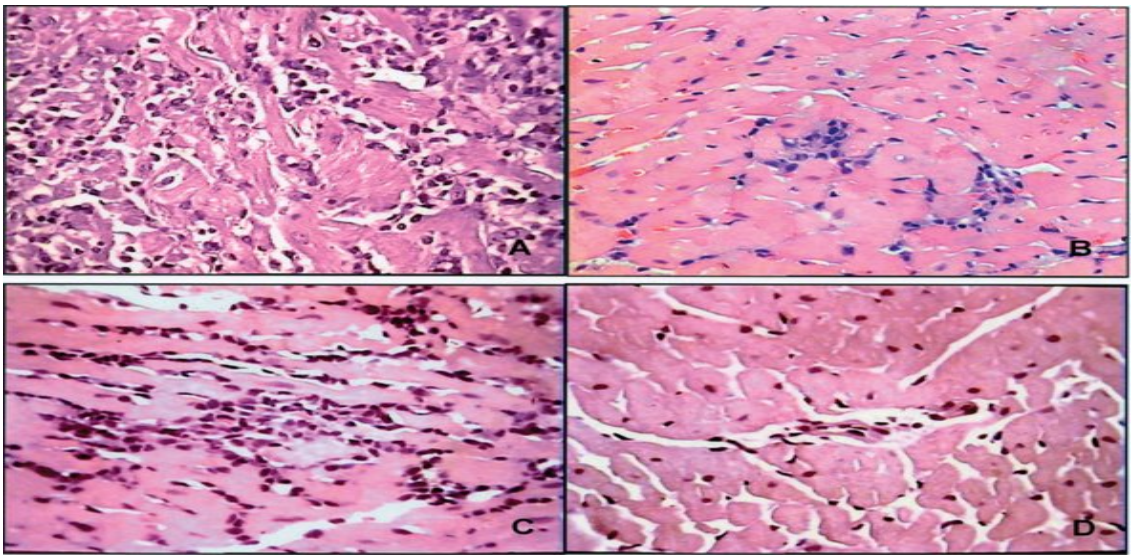
The results obtained are statistically significant and comparable to the Verapamil treated groups.

FIGURE-1: Effect of $MgCl_2$ on serum CPK, LDH, SGOT and Cholesterol.



Isoprenaline induction shows marked increase in myocardial membrane permeability and dysfunction as a result of sequence of biochemical alteration. Histopathological examination in Figure-1 showed a protective effect of $MgCl_2$ on isoprenaline induced myocardial infarction, as compared to the standard Verapamil.

HISTOPATHOLOGICAL CHANGES IN EXPERIMENTAL RAT HEART:



A. NORMAL HEART- Normal myocytes are seen.
B. ISOPRENALINE INDUCED- Lesions and degradation of cells due to toxic drug.
C. $MgCl_2$ + ISOPRENALINE- Protection of cells from isoprenaline action by $MgCl_2$.
D. VERAPAMIL+ISOPRENALINE- Standard drug action.

DISCUSSION:

It is well established that when Isoprenaline, β-adrenergic agonist binds to its receptor on the sarcolemma, adenylate cyclase is activated via GTP binding protein, which in turn causes cAMP formation that binds to protein kinase with subsequent release of a catalytic subunit which phosphorylates the calcium channel. The net result is increased in Ca^{2+} transient leading to augmented force of contraction. This leads to increased intracellular calcium and a positive inotropic action[11]. This is evidenced by the elevation of serum marker enzymes CPK, LDH and SGOT levels. Pre-treatment of $MgCl_2$ prevents myocardial damage partially by inhibiting phospholipase activity, reducing myocardial O_2 consumption and improves the metabolic indices[12, 13]. $MgCl_2$ infusion reduces the influx of Ca^{2+} across the sarcolemma during ischemia and preserves the oxidative phosphorylation in mitochondria[14, 15]. Magnesium salts have prophylactic role in cardiac ischemia and cardiac arrhythmia and enhance the oxygen supply to ischemic area by increasing collateral production of endothelial derived relaxing factor (Nitric oxide) and contraction of vascular smooth muscle in pulmonary artery and coronary arteries[16].

Heart is the vital organ which plays essential role in the purification of blood all over the body through the circulatory system. Drugs such as Isoprenaline, a potent β-blocker is used which releases the calcium from the myocardial cells and damage the myocardium may leading to elevated levels of serum enzymes. $MgCl_2$ is naturally available and has been used by mankind since before recorded history, which is safe, effective and affordable. Magnesium antagonizes the effect of calcium and alters important cellular processes. The results of cardioprotective activity are expressed in terms of the serum enzymes such as CPK, LDH and SGOT and the serum cholesterol levels. Magnesium chloride antagonizes the effect of calcium and alters the important cellular processes. Due to its potent vasodilator property, magnesium is known to prevent and relieve coronary artery spasms associated with acute myocardial infarction. The present study shows the effect of magnesium chloride on isoprenaline induced myocardial infarction in rats significantly.

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REFERENCES:

1. Ferrari R., Hearse DJ. Reperfusion does it exist & does it have clinical relevance. *J. Thromb. Thrombolysis,* 1997; 4, 25-34.
2. Scardo JA, Hogg BB and etal. Favorable hemodynamic effects of magnesium sulfate in pre-eclampsia. *Am J Obstet Gyanaecol* 1995; 177: 1249-53.
3. Witlin AG, Friedman SA, Sibai BM. The effect of magnesium sulfate therapy on the duration of labor in women with mild Pre-eclampsia at term: a randomized double-blind, place bo- controlled trial. *Am J Obstet Gynaecol* 1995; 173:1249-53.
4. Mason BA, Standley CA & etal. Magnesium is more efficacious than phenytoin in reducing N-methyl-D-aspartate seizures in rats. *Am J Obstet Gynaecol* 1994; 171: 999-1002.
5. Chung-JyiTsai M, SCD, Michael F Leitzmann Dr. pH and Edward, L Giovannucci MD, SCD, *The American Journal of Gastroenterology* (2008), 103, 375-382.

6. Chase, Brad "How magnesium affects ADHD. May 2008, Ezine Articles.com
7. Alter, HJ. Koespell, TD. Hilty, WM. Intravenous Mg as an adjuvant in acute bronchospasm; a meta analysis. *Ann Emerg Med* 2000; 36:191-7
8. Woods KL, Fletcher S and etal. Intravenous magnesium sulfate in suspected acute myocardial infarction results of the second Leicestor intravenous magnesium intervention Trial (Limit 2). *Lancet* 1992; 339:1553-9.
9. Naik P, Malati T, Ratnakar KS and etal. Cardioprotective effect of magnesium chloride in experimental acute myocardial infarction. Indian J Exp. Biol. 1999. Feb;37(2):131-7
10. Ecobichon, D.J., (1977): The Basis of Toxicology Testing. CRC Press, New York, pp. 43-86
11. Axel rod, J., Whit by, L.G., Hertting, G., & Kopin, I.J (1961). Studies on the metabolism of catecholamines. *Circulation Res.,* 9, 715-719.
12. Taizo K, Ogawa Y, Sugiyama S, Tatsuo T & Ozawa T, *Cardiovasc res,* 21 (1987) 248.
13. Kloner RA & Brauwald E, *Cardiovasc Res.;* 14 (1980) 371.
14. Agus, ZS. Kelepouries, E., Duke, I and Morad, M., *Am J Physiol,* 256 (1989) 462.
15. Ferrari R, Albertim A, Curelb S, Cecons C, Dihinsa F, Raddino R & Visiolio J.*Mol.cell cardiol,*18 (1986) 487.
16. Gold ME, Buga GM, Woods KL, Byrns RE, Chaudhuri G & Ignarro LJ, *Circulation Res,* 66, (1990). 355.