Cardioprotective effect of magnesium chloride in experimental acute myocardial infarction

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Cardioprotective role of intravenous administration of magnesium chloride was evaluated in rabbits by biochemical and histopathological parameters. Myocardial damage was induced by injecting (iv) isoprenaline 1, 2.5, 5 and 7.5 mg/kg body weight of animal. There was a dose dependent increase in the activity of cardiac enzyme creatinine kinase CK (C Max). Maximal elevation of CK (C Max) was observed with 2.5 mg isoprenaline. The mean T-max (mean of the time duration in hr at which maximum creatinine kinase activity of individual rabbit was observed in a group) shifted early, significantly with 2.5, 5 and 7.5 mg isoprenaline compared to control group. Histopathologically, myocardial damage was quite significant in 2.5 mg isoprenaline subgroup of animals. A mortality of 29% was observed in animals injected with 5 and 7.5 mg isoprenaline, whereas all animals subjected with 1 and 2.5 mg isoprenaline were alive for 72 hr. Considering the data on serial determination of cardiac enzyme CK and histopathological changes, 2.5 mg isoprenaline was chosen as standard dose to study efficacy of cardioprotection by gold standard verapamil and magnesium chloride. Verapamil (5 μM) injected prior to 2.5 mg isoprenaline administration revealed significant reduction of CK (C Max) activity (P < 0.05) compared to animals infused with isoprenaline alone. T-max value did not show any alteration in both the groups. Histopathological findings showed no areas of necrosis and cellular infiltrates in animals primed with 2.5 mg isoprenaline following verapamil. Highly significant reduction in CK (C-max) activity was observed in animals administered with 40 mg magnesium chloride prior to isoprenaline compared to animals treated with isoprenaline alone (P < 0.001). In addition to this, significant delay in T-max of CK activity was observed in group treated with 40 mg 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.

Experimental acute myocardial infarction (AMI) can be induced by coronary artery ligation, coronary occlusion by thromboembolism and subcutaneous/intramuscular/ intravenous injection of calcium or isoprenaline.

Myocardial infarct like lesions, similar to stress induced myopathy, were first produced in rats by injection (SC) of isoprenaline and injury to myocardium was confirmed histopathologically. The pathogenesis revealed characteristic myofibrillar lesions such as irregular contraction band formation and myofilament homogenization. Increased permeability of cardiac muscle cell membrane causing severe damage to cardiac myocyte was also reported under these conditions. Sympathomimetic amine, isoprenaline (dl-beta-(3, 4 dihydroxy phenyl) alpha isopropyl amino ethanol) binds to beta-adrenergic receptors and exerts its diverse hemodynamic effects. It significantly impairs both systolic and diastolic left ventricular functions. Marked decline in the endocardial to epicardial blood flow ratio causing subendocardial ischaemia was also observed. Isoprenaline causes marked increase in cardiac output, fall in diastolic and mean arterial pressure resulting in palpitation, sinus tachycardia and arrhythmias. Positive chronotropic and inotropic properties stimulate cardiac muscle cells by releasing calcium into sarcoplasm and inhibit troponin and myosin interaction. Further, activation of calcium dependent myofibrillar ATPase leading to marked reduction of intracellular ATP and creatine phosphate levels was also observed.

Extensive work has been done to evaluate biochemical and pharmacological action of various cardioprotective agents on ischaemic myocardium in experimental animals. Calcium antagonists render direct cardioprotective effect by reducing intracellular calcium overload and by inhibiting the activities of calcium activated enzymes. Their significant contribution was attributed to negative chronotropic and negative inotropic properties causing a reduction in oxygen demand. They are known to enhance oxygen supply to ischaemic area by increasing collateral as well as regional blood flow. Calcium antagonists lower the afterload and relieve coronary vasospasm. Verapamil (phenyl alkyl amine; a derivative of papaverine), a potent calcium channel blocker is a well known cardioprotective agent. It principally affects L-Ca channel, acts as putative coronary
vasodilator and possesses negative inotropic and chronotropic effects.

Magnesium, a divalent cation plays a key role in neurochemical transmission and muscular excitability. Magnesium antagonizes the effects of calcium and alters important cellular processes. Many of its cardiovascular effects are due to its physiological antagonistic actions as calcium blocker. Cardiac manifestations due to very high magnesium are similar to those of potassium. Gold et al. observed that magnesium competes with calcium and inhibits both production of endothelial derived relaxing factor (nitric oxide) and contraction of vascular smooth muscle in pulmonary artery and coronary arteries in canines. Magnesium is essential for the functioning of many key enzymes such as adenylate cyclase. Intravenous magnesium salts (chloride or sulfate) given early after AMI in humans indicated significant reduction either in mortality or in frequency of arrhythmias or both. Intravenous magnesium administration is reported to be highly effective in terminating torsades de points, a distinctive form of ventricular tachycardia. Due to its haemodynamic effect, it is known to reduce afterload and secondary rise in cardiac output without a significant alteration in cardiac function. Due to its potent vasodilator property, magnesium is known to prevent and relieve coronary artery spasms associated with AMI. Other beneficial effects of magnesium include shortening of QT intervals.

In the present study, protective effects of magnesium chloride have been evaluated in experimental rabbits after induction of myocardial damage by isoprenaline. Protective effect of magnesium chloride was compared with verapamil, a well known cardioprotective agent by using biochemical and histopathological methods.

Materials and Methods

Statistical method—To compare the differences between group, analysis of variance and Student’s t tests were employed.

Experimental animals—New Zealand white rabbits of both sexes weighing between 2 and 2.5 kg were used. They were divided into following 4 groups:

Group I (controls): This group was further divided into two subgroups A and B. A consists of 68 normal rabbits; blood was drawn prior to any intravenous injections. B had 8 rabbits. They were administered with saline (iv).

Group II (n=5): Isoprenaline: (Isoprenaline chloride; Sigma Chemical Co., USA) was administered iv through left marginal ear vein very slowly in 3-5 min. Increasing doses (mg/kg body weight) were used in subgroups C-F: C:1; D:2.5; E: 5 and F: 7.5.

Group III (n=5): Verapamil (Verapamil chloride; Sigma Chemical Co., USA) was also infused intravenously through left ear vein according to standard dose mentioned by Ghosh into animals in two subgroups: G : 5 μ mole verapamil/kg body weight and H : 5 μ mole verapamil/kg body weight followed by 2.5 mg isoprenaline/kg body weight by iv administration after 10 min.

Group IV (n=5): Magnesium chloride: (E merck, Germany) I : 20 mg magnesium chloride/kg body weight. J : 20 mg magnesium chloride/kg body weight followed by 2.5 mg isoprenaline/kg body weight after 10 minutes. K : 40 mg magnesium chloride/kg body weight. L : 40 mg magnesium chloride/kg body weight followed by 2.5 mg isoprenaline/kg body weight after 10 min.

Sample collection—Blood was drawn from right marginal ear vein and serum was separated. Samples were drawn prior to any injection at “0” hr and at 1, 2, 4, 8, 12, 24, 48 and 72 hr after iv injection of normal saline or isoprenaline or verapamil or magnesium chloride.

Estimation of creatinine phosphokinase—CK was estimated by using modified Rosalki’s Kinetic method. The rate of reduction of NADP + to NADPH and H + was measured spectrophotometrically at 340 nm.

C-max is defined as mean value of highest CK activity of individual rabbit in a group and T-Max is defined as mean of time duration in hours at which maximum CK activity of individual rabbit was observed in a group.

Histopathological analysis of myocardium—After 72 hr, the rabbits were anesthetized with ether. Hearts were immediately removed, washed with cold saline to remove blood and blood clots and fixed in 10% formaldehyde. Paraffin blocks were prepared and tissue was stained with hematoxylin and eosin, and subjected to routine histopathology.

Results

Creatinine kinase in control animals—CK activity in rabbits prior to administration of either saline or any drug was 896 ± 310 U/L. The mean level of serum CK at zero hour was 1042 ± 500 U/L. There was a gradual increase in enzyme activity at 4 hr after saline infusion (145%) giving a peak value around 12 hr (207% increase, C-max 2437 ± 946 U/L, T-max 14 ± 6 hr) followed by a steady decline at 48 hr (155% increase). The enzyme activity returned to near base value (113%) by 72 hr.

Histology of normal myocardium—The endocardial surface was trabeculated with bundles of myocardium. The myocardium was composed of branching network of muscle fiber with central elongated nuclei showing blunt edges. The chromatin was uniformly stippled. The interstitium was prominent at places, mostly in subepicardial and peripheral zones. The fibers anastomosed extensively to create slit like spaces between them. Muscle fibers also possessed cross striation and appeared as a chain of cells joined together end to end. Individual myocardial fibers were short, thick and had a single nucleus (Fig. 1).
Effect of isoprenaline

Creatinine phosphokinase—The biphasic response and peak CK showed similar trend as seen in control group except that the rise in mean CK varied from 398 to 568% with 1-7.5 mg isoprenaline compared to 207% rise observed in control animals. The activity of CK (C-max) increased significantly with 1, 2.5, 5 and 7.5 mg of isoprenaline (P < 0.05,0.002). Maximum elevation in CK activity was observed with 2.5 mg isoprenaline (C-max 7806 ± 2200 U/L). The range of C-max varied from 4627 ± 1438 U/L to 7806 ± 2200 U/L with increasing concentration of isoprenaline. In control group, mean CK activity was 2437 ± 346 U/L. The mean T-max shifted early, significantly with 2.5, 5 and 7.5 mg isoprenaline (mean ± SD) compared to control group (14 ± 6 hr). No significant change in T-max was observed with 1 mg isoprenaline (16 ± 6 hrs.) compared to controls (Table I).

Histopathological findings—Animals subjected to 1 mg isoprenaline showed patchy areas of myocardial necrosis. The myocyte loss was associated with mild inflammatory change. Myocardial damage was quite significant in 2.5 mg isoprenaline subgroup of animals. Scattered foci of necrosis were seen towards endocardial surface involving both right and left ventricles. Other additional findings were patchy vacuolations, patchy diffuse acidophilia of myocytes, interstitial edema, separation of fibers and prominent focal inflammatory changes (Fig. 2). Severe myocardial necrosis was observed with 5 mg and 7.5 mg isoprenaline (Figs 3 and 4). Randomly distributed wide areas of necrosis were seen involving mural myocardium of both the ventricles. Predominant findings included multiple small foci of myocytolysis and hyper eosinophilia of myocardial fibres with interstitial round cell infiltration. In few animals, apical infarction with myocytolysis was also observed. A mortality of 29% was observed in animals injected with 5 and 7.5 mg isoprenaline, whereas all the animals administered with 1 and 2.5 mg isoprenaline were alive for 72 hr. Keeping the data on cardiac enzyme in view, 2.5 mg isoprenaline was chosen as standard dose to study the efficacy of cardioprotection by verapamil and magnesium chloride.

Effects of verapamil

Creatinine phosphokinase—Verapamil alone, at a concentration of 5 μ mole, neither caused any significant change in CK activity (C-max 3177 ± 1160 U/L, % increase of 197%) nor changed in T-max (12 ± 2 hr) compared to control animals (T-max 14 ± 6 hr). However, verapamil administered prior to 2.5 mg isoprenaline administration produced significant reduction of CK activity (C-max 4014 ± 1002 U/L, P < 0.05) compared to animals infused with isoprenaline alone (C-max 7801 ± 2200 U/L). T-max value did not show any alterations in both the groups.

Histopathological findings—In animals injected with verapamil alone, the morphology was essentially within normal limits. Nuclear and the fibril characters were similar to controls. No areas of necrosis and cellular infiltration were seen in the animals primed with 2.5 mg isoprenaline following verapamil.

Effects of magnesium chloride

Creatinine phosphokinase: Magnesium chloride at a concentration of 20 and 40 mg/kg alone did not show any significant change in CK activity in the experimental group (C-max 1706 ± 387 U/L, 2798 ± 901 U/L) compared to controls (C-max 2437 ± 946 U/L). However, a significant reduction in T-max was noticed with both the doses (for 20 mg; 8.8 ± 3 hr; 40 mg; 10 ± 2 hr) compared to control animals (14 ± 6 hr). Maximum activity of CK was observed at 12 hr in the above three groups. The percent rise of CK at peak hours in animals administered 20 and 40 mg magnesium chloride and normal saline was 195 ± 62, 376 ± 104 and 207 ± 91 respectively. Rabbits treated with 20 mg magnesium chloride prior to isoprenaline infusion, showed significant reduction in CK activity (C-max 3223 ± 1130 U/L, P < 0.001) compared to the group treated with isoprenaline alone (C-max 7806 ± 2200 U/L). T-max in both the groups remained unchanged. Highly significant reduction in CK activity was also observed in animals administered with 40 mg magnesium chloride prior to isoprenaline (C-max 1707 ± 290 U/L, P < 0.001) compared to animals treated with isoprenaline alone (C-max 7806 ± 2200 U/L). In addition to this, significant delay in T-max was observed in the group treated with 40 mg magnesium chloride and isoprenaline (T-max 24 ± 17 hr, P < 0.01).
compared to group treated with only isoprenaline (T-max 10.4 ± 1.9 hr) (Table 1).

**Histopathological findings:** There were no structural alterations in the animals that received 20 mg magnesium chloride (Fig. 5). In animals administered 40 mg of magnesium chloride, the mural myocardium showed focal myofibrillar fragmentation and nuclear pyknosis (Fig. 6). However, no inflammatory cell infiltration was seen. There were small foci of myocardial necrosis characterised by myofibrillar fragmentation and nuclear pyknosis (Fig. 6).

**Discussion**

In the present study, acute myocardial infarction like myocardial changes were produced in rabbits by intravenous infusion of a beta adrenergic drug isoprenaline at concentrations ranging from 1-7.5 mg/kg body weight. Significant biochemical changes included profound elevation of CK with 2.5 mg isoprenaline/kg body weight. There was no mortality in this group of animals. Higher concentration of isoprenaline beyond 2.5 mg caused an appreciable mortality of 29%, associated with extensive myocardial damage and hence were excluded from cardioprotection evaluation. Infarct lesion similar to myocardial necrosis could be demonstrated histopathologically with 2.5 mg/kg drug. The subendocardial damage was quite similar to earlier reports where myocardial damage was produced by subcutaneous injection of isoprenaline at very high concentration of 10 and 85 mg/kg body weight in rats. The myofibrilal alterations such as myofibrolysis and myofibrillar degeneration were reported to occur minutes after subcutaneous injection of isoprenaline in experimental rats. It is well known that isoprenaline has cardiotoxic effects on myocardium due to its positive chronotropic and positive inotropic properties. Significantly high release and leakage of CK into serum, in the present study, may be due to increased myocardial membrane permeability and dysfunction as a result of sequence of biochemical alteration reported earlier such as increased calcium overload, activation of calcium dependent myofibrillar ATPase, phospholipase, degradation of phospholipid, ATP depletion, reduction of creatine phosphate level and perhaps free radical generation and free fatty acid release. The overall pathogenic effect of isoprenaline was myofilament hypercontraction, high energy phosphate deficiency and decreased influx of macromolecules into myocardial interstitium. Significant elevation of serum CK and histopathological confirmation of cardiotoxic damage by intravenous infusion of 2.5 mg isoprenaline per kg body weight in the present study has established the suitability of this model for experimental AMI, and for studying the cardioprotective effect of magnesium chloride.

Earlier studies on cardiac protection, mostly in rats, documented partial reversal of myocardial damage by beta blockers such as propranolol, timolol and labetolol. Propranolol prevents myocardial damage partially by inhibiting phospholipase activity, reducing myocardial oxygen consumption, obstructing sympathetic influence on heart rate and improving the metabolic indices. However, propranolol in higher concentration exhibits local anesthetic effect on the cardiac action potential. Other drugs reported to render cardioprotection in experimental animals are potassium slow channel opener along with verapamil, guggulsterone, taurine and coleonol.

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**Table 1—Effect of saline, isoprenaline, verapamil and magnesium chloride on enzyme CK activity**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T-Max (hr)</th>
<th>C-Max (U/L)</th>
<th>Increase %</th>
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<tbody>
<tr>
<td>Control (Saline)</td>
<td>14±6</td>
<td>243±946</td>
<td>208</td>
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<tr>
<td>Iso 1mg/kg</td>
<td>16±6</td>
<td>571±1555</td>
<td>652</td>
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<td>Iso 2.5 mg/kg</td>
<td>10±2d</td>
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<td>10±2d</td>
<td>462±1438</td>
<td>540</td>
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<td>10±2d</td>
<td>649±1614</td>
<td>390</td>
</tr>
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<td>Verapamil 5µ mole/kg</td>
<td>12±2</td>
<td>3177±1607</td>
<td>160</td>
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<tr>
<td>Verapamil 5µ mole/kg + 2.5 mg Iso</td>
<td>12±2</td>
<td>401±1002</td>
<td>180</td>
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<tr>
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<td>9±3d</td>
<td>1707±387</td>
<td>196</td>
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<td>1707±290</td>
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</table>

*P values compared with saline control

a ≤ 0.05; b ≤ 0.02; c ≤ 0.01; d ≤ 0.005; e ≤ 0.002; f ≤ 0.001

compared with 2.5 mg Iso: A ≤ 0.02; B ≤ 0.01

Iso = isoprenaline; MgCl2 = magnesium chloride
In the present study, cardioprotection was studied by using magnesium chloride (a physiological calcium antagonist). The results revealed significant prevention in the release of enzyme CK in animals treated with 40 mg magnesium chloride prior to infusion of 2.5 mg isoprenaline when compared to animals treated with only isoprenaline indicating a cardioprotective effect. The extent of protection in the present study was similar to that observed in animals treated with isoprenaline together with 5 μ mole of verapamil, a highly potent cardioprotective agent as evidenced by 50% reduction in the release of enzyme CK. Further, a reversal of cardiotoxicity was also confirmed by histopathological study. Animals treated with magnesium chloride (20 or 40 mg) prior to 2.5 mg isoprenaline infusion showed myocardial preservation as myofibril appeared completely normal. However, animals treated with magnesium chloride (20 or 40 mg) without isoprenaline showed myocardial edema or very mild focal necrosis inspite of reversal of CK activity.

Calcium is reported to be an essential factor for activation of phospholipase and membrane phospholipid degradation. Calcium ions play an important role in controlling myofibril excitability and maintaining contractile state of muscle tissue. The force of contraction of heart is controlled mainly by the calcium influx across the cell membrane during the cardiac action potential. In presence of ATP, this process initiates a chain of reaction resulting in the interaction between the myosin bridges and the actin filaments and subsequent myofibrillar contraction. Calcium channel blocking drugs have been reported to suppress this initiating event in myocardial muscle contraction. The observed protective effect of magnesium may be due to 1) inhibition of influx of calcium across the sarcolemma during ischaemia, 2) reducing of mitochondrial calcium overload, and 3) conservation of intracellular ATP and MgATP. Magnesium supplementation reduces mitochondrial calcium overload, preserves oxidative phosphorylation in mitochondria and facilitates the recovery of tissue ATP. In the present study, the beneficial effect of magnesium chloride as cardioprotective agent may be due to its inhibitory influence on calcium influx.

Fig. 2—Effect of isoprenaline (2.5 mg/kg, iv). Marked cytoplasmic loss with interstitial edema (H&E×100)

Fig. 3—Effect of isoprenaline (5 mg/kg, iv). Higher magnification of infarct area with myocyte necrosis and gross damaged fibre (H&E×100)

Fig. 4—Effect of isoprenaline (7.5 mg/kg, iv). Myofibrillar fragmentation associated with interstitial round cell infiltration (H&E×100)
Fig. 5—Effect of magnesium chloride alone (20 mg/kg, iv). Normal rabbit myocardial pattern (H&E×400)

Fig. 6—Effect of magnesium chloride alone (40 mg/kg, iv). Myofibrillar fragmentation, nuclear pyknosis, no inflammatory cell infiltration (H&E×100)

Fig. 7—Effect of magnesium chloride + 2.5 mg/kg isoprenaline (20 mg/kg, iv). Small foci of myofibrillar fragmentation, increased interstitial and round cell infiltration (H&E×50)

Fig. 8—Effect of magnesium chloride + 2.5 mg/kg isoprenaline (40 mg/kg, iv). Normal myocardial bronching pattern and nuclear distribution. No areas of necrosis, no cell infiltration (H&E×400)
In clinical practice magnesium salts are used to protect myocardium. During various cardiac insult it is known that there is increased release of catecholamine which is responsible for cardiac damage. Basing on present data one can investigate prophylactic role of magnesium in cardiac ischaemia and cardiac arrhythmia.

References