EFFECT OF NICORANDIL AND AMLODIPINE ON BIO-CHEMICAL PARAMETERS DURING ISOPROTERENOL INDUCED MYOCARDIAL NECROSIS IN RATS.

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RUNNING TITLE: Nicorandil and amlodipine in experimentally induced myocardial infarction

ABSTRACT

Objective: To investigate the protective effect of nicorandil and amlodipine in isoproterenol induced myocardial necrosis in rats.

Methods: The effect of nicorandil and amlodipine on bio-chemical parameters during isoproterenol induced myocardial necrosis in rats was examined by determining the activity of creatine phosphokinase (CK), lactate dehydrogenase (LDH), and transaminases (AST, ALT) in the serum of the animals.

Results: Isoproterenol (150mg/kg/day) administered rats showed statistically significant rise in activities of LDH(1.02 ± 0.19 to 1.85 ± 0.05), CK(4.3 ± 0.19 to $7.37 \pm .27$), AST($0.38 \pm .03$ to $0.78 \pm .05$) and ALT($0.19 \pm .017$ to $0.346 \pm .027$) in the serum. Pre-co-treatment of rats with nicorandil and amlodipine significantly lowered the raised levels of these enzymes and thereby restoring the enzyme activity to near normal as was clear from the chart i.e. LDH was 1.10 ± 0.04 , CK was 4.37 ± 0.19 , AST and ALT were 0.39 ± 0.028 and 0.199 ± 0.04 respectively.

Conclusion: It is demonstrated that pre-co-treatment with nicorandil and amlodipine either alone or in combination help in providing protective effect on isoproterenol induced myocardial necrosis.

Key words: creatine phosphokinase, lactate dehydrogenase, transaminases, myocardial necrosis, amlodipine, nicorandil

INTRODUCTION

Amlodipine, a calcium channel blocker has been used as an effective anti-hypertensive agent. The inhibition of lipoprotein oxidation is considered as an important aspect of its mechanism of action thereby preventing the formation of potentially atherogenic lipoproteins(1). Nicorandil is an unique drug with a dual mode of action that acts not only as an ATP-sensitive potassium channel opener, but also as a nitrate by activating guanylate cyclase(2). It is currently used for the treatment of angina pectoris. Nicorandil act by closing the voltage operated calcium channels as a result of cellular hyperpolarisation(3). Nicorandil also inhibits

superoxide ion formation in neutrophils *in vitro*(4).

Isoproterenol is a synthetic β -agonist that leads on to severe stress in the myocardium, resulting in infarct like necrosis of heart muscles(5).

Myocardial infarction induced by Isoproterenol results in increased activity of creatine phosphokinase, lactate dehydrogenase and transaminases(6).

MATERIALS AND METHODS

Animals

Adult male albino rats of wistar strain weighing about 120-140gm were obtained from S.S. Parmar, Amritsar. They were fed commercial rat chow and water was given *ad libitum*. This study was conducted as per the guidelines of human/animal ethical committee.

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Drugs

Amlodipine and nicorandil were obtained from Cipla limited, Isoproterenol hydrochloride, α -ketoglutaric acid and ATP were obtained from Sigma Chemical Co., U.S.A. All the reagents of analytical grade were used in the study.

Experimentation

30 rats were divided into five groups of 6 each.

Group1 : Control

Group2 : Isoproterenol administered rats (in 0.9% NaCl as vehicle given i.p.)

Group3 : Nicorandil+isoproterenol administered rats

Group4 : Amlodipine+isoproterenol administered rats

Group5 : Nicorandil+amlodipine+isoproterenol administered rats.

Drugs were administered as follows: isoproterenol was given in a dose of 150mg/kg per day intraperitoneally for 2 days(7); nicorandil in a dose of 2.5mg/kg/day given orally with distilled water as vehicle for 3 days and amlodipine given orally in distilled water as vehicle in a dose of 5mg/kg/day for 3 days(8). After the experimentation the blood was collected from the rat tail vein, and serum separated immediately which is then stored in refrigerator and used for enzyme assays afterwards. The activity of creatine phosphokinase(9)(by using UV-Kinetic method) lactate dehydrogenase(10) (King's colorimetric method) and transaminases (AST and ALT)(11) by UV-Kinetic method in the serum were assayed.

Statistical Analysis

The results were recorded as mean $\pm$ S.D. The data were analysed using one way ANOVA

The Tukey multiple comparison method(12) was used for post-hoc test. Significance was determined at $p < 0.05$.

RESULTS

The activity of creatine phosphokinase, lactate dehydrogenase and transaminases in serum are presented in the table. The values of creatine phosphokinase (4.3 ± 0.19 to 7.37 ± 0.27), lactate dehydrogenase (1.02 ± 0.19 to 1.85 ± 0.05) and transaminases, AST (0.38 ± 0.03 to 0.78 ± 0.05) and ALT (0.19 ± 0.017 to 0.346 ± 0.027) were elevated

significantly ($p < 0.001$) following isoproterenol induced myocardial infarction. Pre-co-treatment with nicorandil and amlodipine resulted in a significant ($p < 0.01$) reduction in the levels of these enzymes. In nicorandil treated rats the value of CK was 4.6 ± 1.16 , LDH was 1.14 ± 0.03 , AST was 0.43 ± 0.02 and ALT was 0.20 ± 0.02 and in amlodipine treated rats these values were CK 4.67 ± 0.14 , LDH 1.16 ± 0.09 , AST 0.45 ± 0.04 and ALT 0.23 ± 0.03 . When both nicorandil and amlodipine were given simultaneously they produced even more reduction in levels of these enzymes which is statistically significant ($p < 0.05$) when compared with the enzymatic activity of rats given amlodipine and nicorandil individually.

DISCUSSION

The release of cellular enzymes reflect a non-specific alteration in the plasma membrane integrity and permeability as a response to β -adrenergic stimulation(13). Serum levels of creatine kinase, lactate dehydrogenase and transaminases are the diagnostic indicators of myocardial injury(6). An increase in activity of these enzymes in serum is due to leakage of enzymes from the heart as a result of necrosis induced by isoproterenol(14). This is in agreement with our findings showing that in isoproterenol administered rats the activity of serum LDH, CK and transaminases (AST, ALT) were increased.

Serum transaminases, lactate dehydrogenase and creatine phosphokinase are important mediators of acute myocardial infarction and their release into the cytoplasm stimulate the formation of certain inflammatory mediators. It has been suggested that the oxygen free radicals generated during ischemia in addition to direct myocardial damaging effect, may also be responsible for the cardiac damage through the release of lysosomal enzymes(15). The activity of serum creatine kinase, lactate dehydrogenase and transaminases significantly increased in myocardial infarcted rats as compared with those in control. Nicorandil plus amlodipine reversed the increased enzymatic activity by their inhibiting effect on lipid peroxidation thereby reducing extent of lysosomal damage (16). Mason *et al* (17) reported that amlodipine inhibits lipid peroxidation in cellular membranes as a result of modulation of physicochemical properties of lipid membrane independent of calcium channel blockade.

Nicorandil produces the antioxidant action through forcing structural interactions with lipid membranes(18). Nicorandil and amlodipine treatment in isoproterenol administered rats may inhibit the release of enzymes, which may be due to the stabilizing action of these drugs on the lipid membranes because amlodipine, as a calcium channel blocker and nicorandil, by hyperpolarizing the membrane and reducing the membrane excitability, reduced Ca^{2+} influx and inhibited Ca^{2+} overload mediated cell injury.

The result of the present study indicates that creatine kinase, lactate dehydrogenase and transaminases play an important role in isoproterenol induced

myocardial infarction. The combination therapy with nicorandil and amlodipine proved to be more cardio-protective than the therapy with individual drugs. It is clear from the study that amlodipine and nicorandil are very useful drugs to prevent myocardial infarction when used alone but their combination proved to be synergistic to prevent the attack of infarction in predisposed patients.

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Table showing the activity of CK,LDH and transaminases in serum of rats.

Control(treatment)	Transaminase AST	Transaminase ALT	Creatine kinase	Lactate dehydrogenase
1. Control	0.38±0.03	0.190±0.017	4.30±0.19	1.02±0.05
2. Isoproterenol	0.78±0.05 ^s	0.346±0.027 ^s	7.37±0.27 ^s	1.85±0.05 ^s
3. Nicorandil+Isoproterenol	0.43±0.027 ^s	0.202±0.028 ^s	4.60±0.16 ^s	1.14±0.035 ^s
4. Amlodipine+Isoproterenol	0.45±0.046 ^s	0.230±0.03 ^s	4.67±0.14 ^s	1.16±0.09 ^s
5. Nicorandil+Isoproterenol+ Amlodipine.	0.39±0.028 [*]	0.199±0.04 [*]	4.37±0.19 [*]	1.10±0.04 [*]

Results are Mean±S.D.(n=6). * $p < 0.01$ and s means $p < 0.001$. Group 1 is control group and is compared with group 2, group 3 and 4 are compared with group 2 and group 5 is compared with groups 3 and 4. Enzyme activity is expressed as $\mu\text{cat/litre}$.

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