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CONCOMITANT ADMINISTRATION OF GLIBENCLAMIDE AND METOPROLOL ATTENUATES DOXORUBICIN INDUCED CARDIAC INFARCTION/TOXICITY VIA ELECTROPHYSIOLOGICAL INHOMOGENEITY DURING MYOCARDIAL ISCHEMIA

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ABSTRACT

In the present study, attenuation effect of concomitant administration of glibenclamide and metoprolol on cardiomyopathy / cardiotoxicity induced by doxorubicin via electrophysiological inhomogeneity during myocardial ischemia in albino wistar rats**.** Various parameters such as measurement of body and organ weight measurement, estimation of serum biomarkers, estimation of oxidative stress and histopathology were studied in two different models i.e., preventive and curative model. In this study we used Doxorubicin 2.5mg/kg body weight i.p. in six equal injection is for two weeks for a total cumulative dose of 15mg/kg body weight for cardiotoxicity and treatment with combination of glibenclamide and metoprolol. This treatment served as preventive model and curative model.

KEYWORDS: Concomitant, Glibenclamide, Metoprolol, Doxorubicin.

INTRODUCTION

Doxorubicin is an anthracycline anti-cancer chemotherapeutic agent widely used in the treatment of soft tissue sarcoma[1], small cells carcinoma of the lung[2], acute leukaemia[3], breast cancer[4] etc. Due to its severe cardio toxic effects the use of doxorubicin is limited. Though several mechanisms have been suggested to explain cardio toxic effects of

doxorubicin but none of them completely understood. [5] Several mechanisms via oxidative stress[6], alteration of myocardial energy metabolism[7], altered molecular signalling[8], programmed cell death[9], and iron dependent oxidative damage to biological macromolecules[10] have been explained. Role of reactive oxygen species (ROS) [11] were proposed as a contributing factor to the deterioration of cardiac functions along with ischemic and non ischemic path. [12] In a study it is reported that perfusion of mouse heart with 5µM doxorubicin for 60 min led to a 50% reduction of coronary flow due to increase the resistance at coronary arteries.

It is also reported that doxorubicin induces its acute toxicity secondary to its increasing gene expression (transforming growth factor)TGF-β/Smad pathway. [13] However, involvement of TGF-β1 gene expression is increased in the left ventricular myocardium of patients with idiopathic hypertrophic cardiomyopathy or dilated cardiomyopathy and in animals after myocardial infarction. [14]

The combination of glibenclamide and metoprolol resulted in a significant improvement in recovery from ventricular fibrillation and in a higher survival rate during the acute phase of myocardial infarction. This beneficial drug interaction might be the consequence of the synergistic effect in inhibiting the development of electrophysiologic inhomogeneity during myocardial ischaemia. Dispersion of the refractory period due to nonuniform shortening of the action potential may have a significant role in the development of fatal ventricular arrhythmias during acute myocardial infarction. Glibenclamide, as an inhibitor of KATP channels, may prevent the shortening of the action potential duration. This effect could be specific for the ischaemic cells, where these channels open due to the loss of intracellular ATP. Metoprolol, an β-adrenoceptor blocker, inhibits sympathetic excitation during the acute phase of myocardial infarction, which would otherwise also result in shortening of the action potential duration in myocardial ischaemia. Thereby both agents, glibenclamide and metoprolol, act against the development of regional differences in the refractory period and their combination decreases the incidence of fatal arrhythmias. Furthermore, treatment with glibenclamide produces a fall in blood glucose concentration, which may result in a reflex activation of the sympathetic autonomic nervous system. Such an effect on the heart is not desirable during acute myocardial infarction and its inhibition by metoprolol could also contribute to the significant protection afforded by the combination treatment in the present experiments of doxorubicin cardiotoxicity. [15]

MATERIALS AND METHODS

Animals

Wistar rats of either sex weighing 150 to 200 g were used (n=6 in each group). Animals were acclimatized for one week to laboratory conditions (temperature, 23 ± 2˚C, humidity, 50 ± 5% and 12 hour light dark cycle) before study. The animal care and handling was carried out according to the guidelines set by CPCSEA. Animals were provided free access to food and water and ad *libitum*. The study was approved by the institutional Animal Ethical Committee.

Drug Preparation and Mode of Administration

Doxorubicin was purchased from market and animals were administered with 2.5mg/kg body weight i.p. glibenclamide and metoprolol was purchased from market.

Experimental protocol

Animals were devided in to five groups of six animals each. Group I served as Normal received saline 5ml/kg/day i.p. for a period of fifteen days. Group II animals received doxorubicin 2.5mg/kg body weight i.p. in six equal injection is for two weeks for a total cumulative dose of 15mg/kg body weight. Group III animals received combination of glibenclamide and metoprolol for seven days. Group IV animals received doxorubicin 2.5 mg/kg body weight i.p. in six equal injection is for two weeks for a total cumulative dose of 15mg/kg body weight and after fifteenth day same group received combination of glibenclamid (5mg/kg i.p.) and metoprolol (2mg/kg i.p.) for fifteen days as a curative model (CM). Group V received combination of glibenclamid and metoprolol for fifteen days and after this received doxorubicin 2.5 mg/kg body weight i.p. in six equal injection is for two weeks for a total cumulative dose of 15mg/kg body weight as a preventive model (PM). After performing the above procedure the various studies were gone.

At the end of the study animals were weighed and sacrificed. Determination of Body Weight, Heart Weight, Kidney Weight and Liver was carried out. Samples were withdrawn for further histological analysis.

Estimation of Serum Biomarkers – After experimental protocol blood was collected by retero orbital puncture under light anaesthesia using heparinised micro capillaries. The blood collected was divided in to two parts, I part was used to measure cardiac biomarkers like creatine phosphokinase (CPK) and lactate dehydrogenase (LDH), whereas, II part of a blood

sample was subjected to centrifugation to collect serum for estimation of marker enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Estimation of oxidative stress & Histopathological Studies – After completion of treatment protocol heart was isolated and cut in to two parts. A portion of heart from all the groups was crushed to get in and a 30% w/v homogenate was prepared in 0.9% buffered KCL (pH 7.4) for the estimation of glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA). The remaining portion of the heart tissue was used for histopathological studies.

– Myocardial tissue from all the groups was subjected to histopathological studies. The tissue was fixed using 10% formalin solution in phosphate buffer and sections were prepared using paraffin blocks and stained with hematoxylin and eosin after dewaxing. The sections were observed for histopathological changes.

Statistical Analysis – The results were expressed as the mean ± SEM and analyzed using one way ANOVA followed by Dunnett's multiple comparison tests. Data were computed for statistical analysis using the Graph Pad Prism Software.

RESULTS AND DISCUSSION

Body Weight, Heart Weight, and Liver Weight and Ratio of Heart weight to Body weight, Ratio of Liver weight to Body weight.

The changes in the heart weight, body weight, liver weight and the ratio of Heart weight to Body weight and f Liver weight to Body weight are shown in Table 1. The heart weight and the liver weight in doxorubicin treated was significantly increased with compare to normal rats. The heart weight and liver weight in glibenclamide and metoprolol combination treated rats was not changed significantly as compare with the normal rats. The heart weight and the liver weight in PM and CM groups was significantly decreased as compared with doxorubicin treated rats. The body weight in doxorubicin treated rats was significantly decreased compared with the normal rats. The body weight in PM and CM groups was significantly increased to be nearly the same as in normal rats.

Serum Enzyme Levels

Rats administered with doxorubicin had significantly increased levels of CPK, LDH, AST and ALT as compared with normal rats (Table 2). Whereas in PM and CM groups there was significant decrease in the levels of these enzymes.

Oxidative Stress

The effect of doxorubicin on oxidative stress is shown in Table 3. The MDA levels was increased whereas GSH, SOD, CAT levels were significantly decreased in doxorubicin treated groups as compared with normal rats. In PM and CM groups, there was a significant increase in the levels of GSH, SOD and CAT as compared with doxorubicin, whereas a significant decrease in the MDA level.

Histopathological Observation

The histology of the heart tissue from the normal group animals showed normal morphological appearance (Figure 1), whereas in doxorubicin group, disruption or loss of myofibrils and vacuolization of the cytoplasm, enlarged swollen mitochondria, patchy necrosis and inflammatory cells were observed (Figure 2). The histology of heart tissue from group PM showed a lesser loss of myofibrils, Vacuolization of the cytoplasm and inflammatory cells (Figure 3). The histology of heart tissues from group CM showed moderate loss of myofibrils and vacuolization of the cytoplasm.

Table No. 1: Effect of Combination (Glibenclamide and Metoprolol) on Heart weight, Body weight, Liver weight and ratio of heart weight to body weight and liver weight to body weight.

Treatment	Body Weight (g)	Heart Weight (g)	Liver Weight (g)	Heart/Body ratio ($x10^3$)	Liver/Body ratio ($x10^3$)
Normal	185 ± 0.5774	0.623 ± 0.0520	8.59 ± 0.757	3.36	46.43
Doxorubicin	160 ± 5.774**	0.837 ±0.0352*	11.75 ± 0.7254**	5.23	73.4
Combination	188 ± 3.215	0.636 ± 0.0491 ns	8.77 ± 0.2186 ns	3.38	46.64
Comb + Dox	182 ± 5.132ns	0.716 ± 0.0491 ns	8.38 ± 0.0723 ns	3.9	46.04
Dox + Comb	180 ± 1.732ns	0.723 ± 0.0145 ns	8.10 ± 0.3215 ns	4.01	45.0

Data were analyzed by ANNOVA followed by Dunnett's test. Each values is mean ± SEM; n=6 in each group,*P<0.05, **P<0.001 when compared to normal, ns= not significant, Dox= doxorubicin, Comb= combination(Glibenclamide and Metoprolol)

Table No. 2. Effect of Combination (Glibenclamide and Metoprolol) on serum biomarkers in doxorubicin exposed rats.

Treatment	CPK (IU/L)	LDH (IU/L)	ALT (IU/L)	AST (IU/L)
Normal	75.76±9.689	52.9±1.823	41.40±1.332	44.50 ±5.415
Doxorubicin	136.4±10.05**	67.63±1.330***	63.30±1.652***	64.87±3.014**
Combination	58.20±0.3786 ^{ns}	58.60±2.730 ^{ns}	23.37±4.279**	40.17±1.077^{ns}
Comb + DOX	65.87±4.405 ^{ns}	48.96±1.564 ^{ns}	41.63±1.906^{ns}	39.11±2.106^{ns}
DOX + Comb	30.57±1.955**	43.67±1.346*	59.36±2.747**	42.93±1.271^{ns}

Data were analyzed by ANNOVA followed by Dunnett's test. Each values is mean ± SEM; n=6 in each group,*P<0.05, **P<0.001 , ***P<0.001 when compared to normal, ns= not significant, Dox= doxorubicin, Comb= combination(Glibenclamide and Metoprolol), CPK = creatine phosphokinase, LDH = lactate dehydrogenase, ALT = alanine amino transferase, AST = aspartate aminotransferase.

Table No. 3. Effect of Combination (Glibenclamide and Metoprolol) on glutathione, malondialdehyde, catalase, superoxide dismutase in doxorubicin-treated rat hearts.

Treatment	GSH (n mole/min/mg of wet tissue)	Lipid peroxidation (n mol of MDA/min/mg of wet tissue)	CAT ((Unit^{x}/mg protein))	SOD ((Unit^{y}/mg protein))
Normal	2.91±1.825	18.29±3.785	49.54±1.540	36.73±1.48
Doxorubicin	1.31±0.240**	49.28±0.925**	11.29±2.45***	21.60±1.30 **
Combination	3.05±0.315^{ns}	19.12±3.415^{ns}	62.03±4.285^{ns}	35.12±2.53 ^{ns}
Comb + DOX	2.19±0.350*	35.45±3.090*	48.71±1.705 ^{ns}	40.04±0.645 ^{ns}
DOX + Comb	1.93±0.270*	25.63±2.375^{ns}	41.46±3.69 ^{ns}	39.27±0.950 ^{ns}

Data were analyzed by ANNOVA followed by Dunnett's test. Each values is mean ± SEM; n=6 in each group,*P<0.05, **P<0.001 , ***P<0.001 when compared to normal, ns= not significant, Dox= doxorubicin, Comb= combination(Glibenclamide and Metoprolol), x = µmole of H2O2 consumed/min, y = one unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in min.

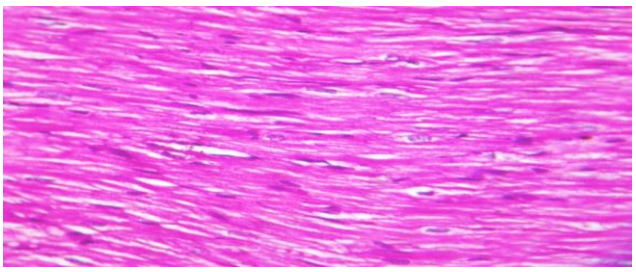

Figure 1(a): Photomicrograph of rat heart of normal showing regular morphology.

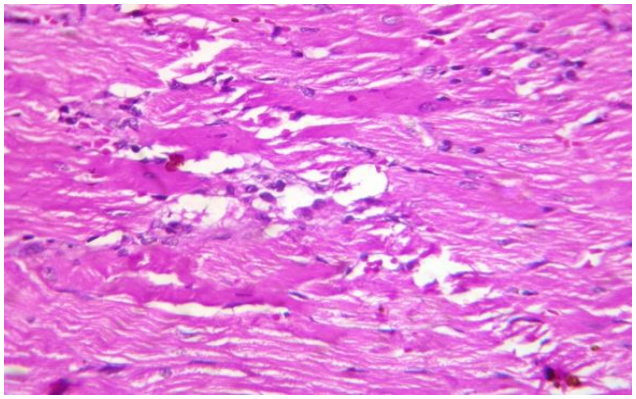

Figure 1(b): Photomicrograph of rat heart treated with doxorubicin showing extensive vacuolization and myofibril loss.

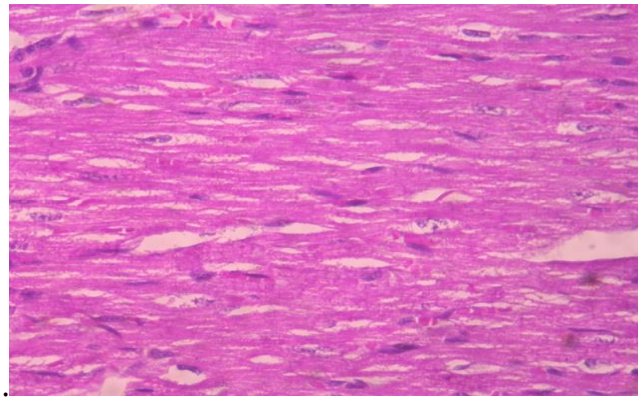

Figure 1(c): Photomicrograph of rat heart treated with combination (glibenclamide+metoprolol) + doxorubicin (PM) showing less extensive vacuolization and no myofibril loss.

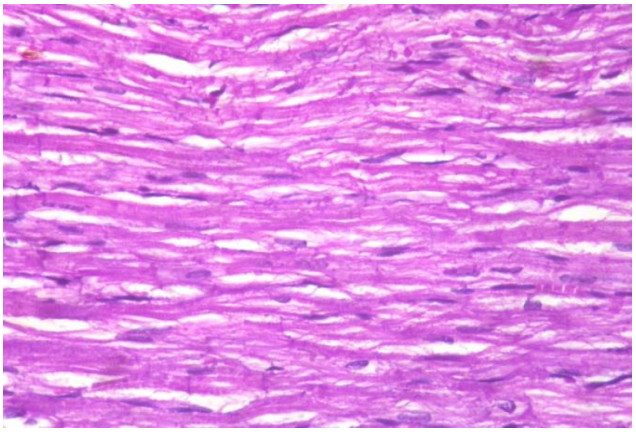

Figure 1(d): Photomicrograph of rat heart treated with doxorubicin + combination (glibenclamide+metoprolol) (CM) showing less extensive vacuolization and myofibril loss as compared with doxorubicin treated rats.

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