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SHORT TERM EFFECTS OF FENOTEROL ON CARDIAC MUSCLE OF MICE

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ABSTRACT

Beta- adrenergic agonists (Fenoterol, Clenbuterol) are sympathomimetic substances which are capable of activating both β_1 and β_2 adrenoceptors in vivo. These drugs produce effects equivalent to those produced as a result of impulses transmitted from postaganglionic fibres of central nervous system. Fenoterol treatment causes a small increase in fatigability due to decrease in oxidative metabolism with some cardiac hypertrophy. Major side effects of fenoterol can be derived from their action at β_2 adrenoceptor and loss of selectivity at higher doses. Thus, tachycardia, tremor, palpitations and nervousness are among the most common adverse events cardiac connective tissue is composed of collagen with small amount of elastin, laminin, fibronectin. Myocardial injury is accompanied by reprative fibrosis. Accumulation of collagen is a characteristic of hypertrophied hearts. The present investigation is an attempt to examine short term effects of fenoterol on major non- contractile apparatus which mainly comprises of collagen in left ventricle. Our present study is carried on short term effect of fenoterol on cardiac muscle of mice. These effects are studied between 2-72 hrs of drug administration. Histopathological examinations have shown the – ve effects of fenoterol on non-contractile machinary of heart. Single dose of fenoterol (2.5mg/kg body wt) has resulted a severe collagen infilteration in the myocardium of left ventricle. These effects results in to disruption of supporting extracellular matrix framework, thereby decreasing ventricular compliance. The myocardial fibrosis observed in left ventricular tissue possibly shows the state of reprative fibrosis, a pathophysiological state.

KEYWORDS: Fenoterol, Collagen, Ventricular tissue, Van Gieson staining, ECM, Apoptosis.

INTRODUCTION

Cardiac muscle is an involuntary, striated muscle that is found in the walls and histological foundation of heart, specifically myocardium. Cardiac muscle cells, unlike most other tissues in the body, rely on an available blood and electrical supply to deliver oxygen and nutrients and remove waste products such as Co_2. The coronary arteries help fulfill this function. Intercalated disc are part of cardiac muscle sarcolemma and they contain gap junctions and desmosomes. Cardiomyocytes regeneration occurs through the division of pre-existing cardio-myocytes during the normal aging process (Senyo *et al*., 2013). The division process of pre- existing cardiomyocytes has also been shown to increase in areas adjacent to site of myocardial injury. Certain growth factor promote the self- renewal of endogeneous cardiomyocytes and cardiac stem cells. Swiss albino mice are increasingly used to study cardiac disease development, heart muscle repair and regeneration after experimental injury and and in pharmacological studies. Our present study is carried on short term effect of fenoterol on cardiac muscle of mice. These effects are studied between 2-72 hrs of drug administration. Cellular architecture showed significant deterioration. Apoptotic markers within the myocardium became increasingly frequent by 20 hrs of drug treatment. Significant deterioration in ventricle function and heart rate accompanied by significant histological changes consistent with cell death and loss of cardiomyocyte cell integrity. Further studies are needed to assess whether this preparation can be optimised for longer term survival. In the present investigation, a remarkable increase in the non- contractile element is recorded in the left ventricular tissue of heart after 4 hrs of single dose of fenoterol administration. Any increase in distribution of collagen is likely to reduce left ventricular compliance and hence a reduced hyperemic flow (Mc Anish *et al*., 1995). Qualitative distribution of collagen in histopathological studies involving Van Gieson stain revealed localized masses of densely stained areas indicating collagen accumulation. Histopathological examinations have clearly shown the negative effects of fenoterol on the non contractile machinery of heart. Here

single dose of fenoterol (2.5 mg/kg body wt) has resulted a severe collagen infilteration in the myocardium of left ventricle. Collagen infilteration is followed by apoptotic lesion in cardiac tissue. These effects ultimately result in to disruption of supporting ECM framework and thereby decreasing ventricular compliance. The myocardial fibrosis observed in left ventricular tissue possibly shows the state of reparative fibrosis, pathophysiological state.

MATERIALS AND METHODS

Adult Swiss albino female mice weighing 22-25g were procured from the Central Research Institute (CRI), Kasauli (H.P) and were maintained in the animal house of Department of Biosciences, HP University under suitable hygienic conditions with 16 hrs day light and temprature of 24 $\pm 2^0$C. The mice were provided feed Hindustan lever Ltd. And water *ad libitum*. In order to study the short term effects of fenoterol on cardiac muscles, the animals were divided in to two main groups:

1) Animals of first group served as control and received equal volume of saline water.
2) Animals in group two received equal volume of single oral dose of fenoterol (2.5 mg/ kg of body wt). Mice were sacrificed after 2 hrs, 4, 10, 20 and 72 hrs. For each experiment, left ventricular tissue of heart was taken for histological studies.

HISTOLOGICAL STUDIES

Left ventricular tissue was processed to determine the proliferation of collagen. In each set of experiment heart was excised from the dissected animal and washed in normal saline to make it free from any type of fat or connective tissue. Left ventricle was separated from heart and fixed in Bouin's fixative for 24 hrs. Tissue blocks were then thoroughly washed in running tap water to make them free from fixative. After complete dehydration through different alcoholic grades, the tissue blocks were embedded in paraffin wax. The distribution of collagen was confirmed on 6µm thick sections of left ventricle by employing Van Gieson stain for 5 min. Excess stain was rinsed off before rapid dehydration by immersing in ascending alcohol grade series, cleared in xylene and permanently mounted in DPX.

RESULTS

a) Normal cardiac muscle: Clearly intact muscle fibres with well defined linear arrangement are seen in the longitudinal section of left ventricular tissue. Van Gieson staining is used to study the distribution of collagen in the myocardium of left ventricle. Collagen (Pink and dark red) is in general either not present in ventricular myocardium or exhibit an association with negligible amount of collagen, therefore the, tissue section is showing uniform yellow colour throughout (Fig.1).

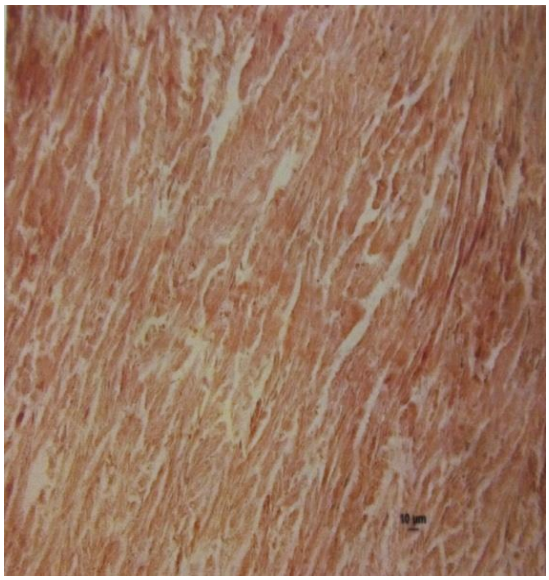

Fig. 1

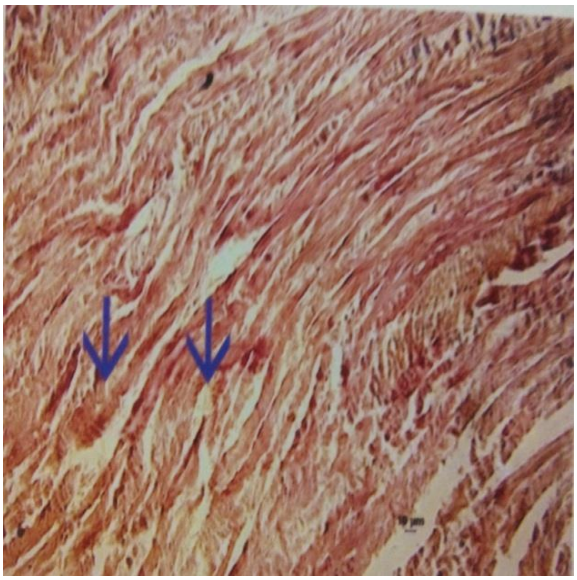

Fig. 2

L.S of left ventricular muscle showing no collagen After 2hrs of drug slight collagen infiltration infilteration.

b) Drug treated: Fenoterol administration has resulted in slight infilteration of collagen in myocardium of ventricular wall just after 2hrs of drug administration. It is observed through collagen specific staining (red color) in the intercellular spaces of myocytes (Fig.2). The shape of the myocytes is also affected to some extent by this infilteration. After 4 hrs of drug administration collagen infilteration is increased to significant level which is indicated by the extent of staining. Here red stained area is more widespread (Fig.3).

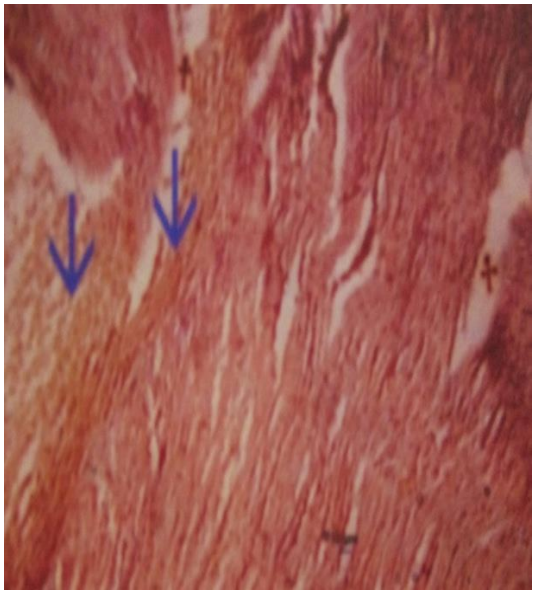

Fig. 3

After 4hrs of drug reasonable collagen infiltration And slight apoptotic lesions (+)

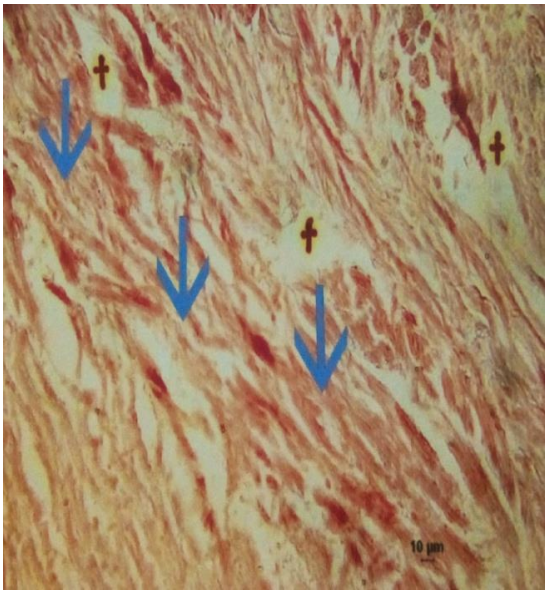

Fig. 4

After 10hrs heavy collagen infiltration and apoptosis

Effects are more pronounced with high collagen infilteration and considerable apoptotic lesions in the myocardial region of left ventricle after 10hrs of drug administration. Apoptosis initially is observed as small gaps between myofibres (Fig.4). Heavy collagen infiltration demonstrated by red staining areas everywhere in tissue section and severe apoptotic lesion shown by larger gaps, devoid of myocytes are observed after 20hrs of drug administration (Fig.5).

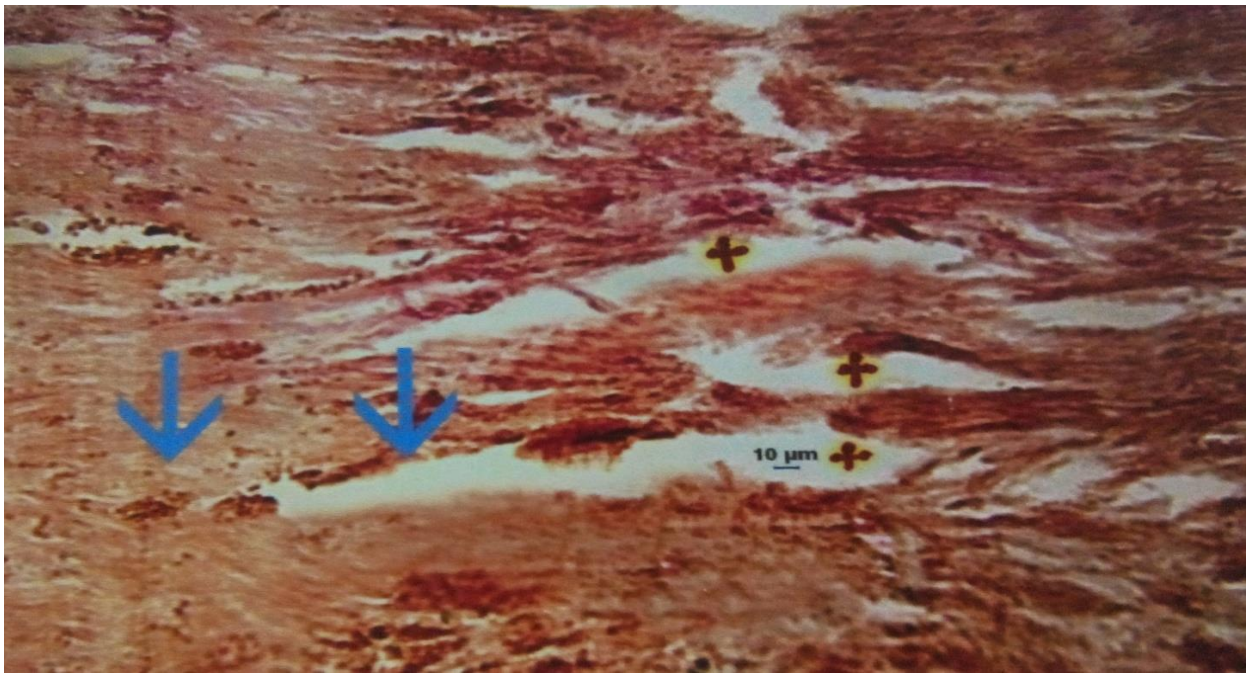

Fig. 5: After 20 hrs of drug treatment heavy collagen infiltration and severe apoptotic lesions in L.S of ventricular myocardium.

At 72 hrs, both the negative effects of fenoterol i.e collagen infilteration and myonecrosis are observed to recover, in which the stained areas in the ventricular waals here show less stain in comparision to earlier stages of 10 and 20 hrs. Apoptotic lesions are also seen to recover as the gap between myocytes get shortened in this stage and arranged as that of normal mice ventricular tissue (Fig.6).

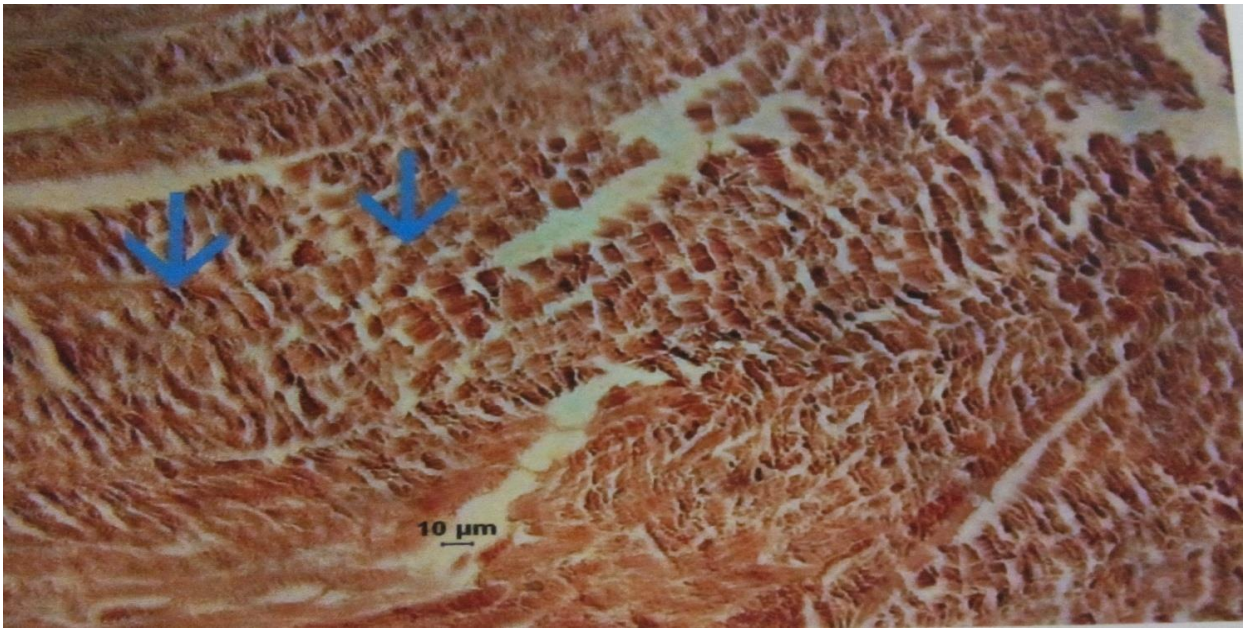

Fig. 6: L.S through left ventricular myocardium exhibiting a recovery from collagen infiltration and apoptosis after 72hrs of drug administration.

From the above observations it appeared that negative effects of fenoterol in left ventricular tissue start appearing immediately after drug administration i.e nearly 2hrs and keep on increasing up to 20-40 hrs after which the tissue start recovering from deleterious effects, which means that drug has been metabolised totally and being excreted in the urine. Like all other β- agonists fenoterol is also found to disrupt the ECM structure and myocytes disorganization in the heart tissue. ECM disruption directly affects the functional efficiency of heart because they provide a firm support to the contractile machinary in the heart.

DISCUSSION

β- adrenergic agonists were synthesized mainly as therapeutic agents for muscular disorder. But later on unfortunately these were found to have many of side effects and come in to drug abuse category. Beta agonists like clenbuterol, cimeterol and isoproteronol are widely known to produce muscle specific anabolic effects and general lipolytic effects (Choo *et al.*, 1992; Katoch *et al.*, 2000; Kumar *et al.*, 2003). In the present investigation, a remarkable increase in the non contractile element is recorded in the left ventricular tissue of heart after 4hrs of single dose of fenoterol administration. Even a small rise in collagen content is significant in affecting contractile properties of heart tissue. Any increase in distribution of non contractile element are likely to reduce left ventricular compliance and hence a reduced hypermic flow (Mc Anish *et al.*, 1995).

Qualitative distribution of collagen in histopathological studies involving Van Gieson Stain revealed localized masses of densely stained areas indicating collagen accumulation.Collagen distribution is rare in the myocardial region of left ventricle in normal control mice, whereas fenoterol treated animals showed collagen infilteration, redistribution and increased deposition in the wall of ventricle. Our observation of single dose of fenoterol administration is in agreement with findings that β_1 adrenoceptor signalling induce cardiomyopathy (Engelhardt *et al.*, 1999). On the other hand overexpression of β_2 adrenoceptor is not deterimental (Liggett *et al.*, 2000). A significant myonecrosis in left ventricular tissue in response to a single dose of significant myonecrosis in left ventricular tissue in response to a single dose of β- agonist clenbuterol is reported by Burniston *et al.*, 2002. Collagen content is greatly increased after clenbuterol treatment (Burniston *et al.*, 2006), which may be indicative of reparative fibrosis. Fenoterol being stimulator of both β_1 and β_2 adrenoceptor is found to be more myotoxic (Burniston *et al.*, 2006; Patiyal and Sharma, 2007).

In the present study, a collagen build up in the myocardium of left ventricle after 20 hrs of fenoterol administration also indicate reparative fibrosis, a pathophysiological condition which ultimately affect the normal heart functioning. Toffukkji *et al.*, 2000, have reported hetrrogeneity in β-adrenoceptor distribution where they found an abundance of β- adrenoceptors in the papillary muscles and subendocardium of rat in comparision to other areas. This is consistent with pattern of damage found in our present investigation.

Our present study is carried on short term effects of fenoterol on cardiac muscle of mice. Histopathological examinations have clearly shown the negative effects of fenoterol on the non contractile machinary of heart. Here single dose of fenoterol (2.5mg/kg body wt) has resulted a severe collagen infilteration in the myocardium of left ventricle. Collagen infilteration is followed by apoptotic lesions in cardiac tissue. These effects ultimately result

in to disruption of supporting ECM framework and thereby decreasing ventricular compliance. The myocardial fibrosis observed in left ventricular tissue possibly shows the state of reparative fibrosis, a pathophysiological state. There is significant deterioration in ventricle function and heart rate accompanied by significant histological changes consistent with cell death and loss of cardiomyocyte cell integrity. Further studies are needed to assess whether this preparation can be optimised for longer term survival.

CONCLUSION

It appears likely that the mitogen-activated protein kinase superfamily plays a key role in mediating the actions of adrenergic pathways on myocyte apoptosis. Histological approach could allow the assessment of short to medium term responses of heart to Fenoterol and various pharmacological, genetic and molecular manipulations. These observations suggest that the adrenergic nervous system plays an important role in the regulation of myocyte apoptosis, and may thus contribute to the development of myocardial failure. Greater understanding of the mechanism by which the adrenergic nervous system regulates myocyte apoptosis will lead to even further improvement in the treatment of heart failure.

ACKNOWLEDGEMENTS

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