2013-14

Rajnibhai V. Patel
PharmInnova Award
Best M. Pharm Thesis

WINNER

Winner

Ms. Suchi Raghunathan

Research Guide

Dr. Bhoomika Patel

Subject:
Pharmacology

Thesis Title :
Investigation into the effect of Magnesium Valproate in Cardiac Hypertrophy and its related mechanism

College:
Institute of Pharmacy, Nirma University, Ahmedabad

Magnesium Valproate pioneering treatment in prevention of heart failure

Outcome of Research:

Cardiac hypertrophy is a thickening of the heart muscle (myocardium) which results in a decrease in size of the chamber of the heart. This research could bring about a shift in the treatment of cardiac hypertrophy for which currently no drugs are available to curb the disease from core. The outcome of study showed that Magnesium valproate produces beneficial effects on cardiac hypertrophy, preserves mitochondrial functioning and prevents transition to heart failure. Hence, modifying the structure of magnesium valproate to obtain new chemical entities with more selectivity towards Histone deacetylase (HDAC) enzyme in heart could be a future strategy, wherein the disease could be stalled right at its root level and prevent related complications.

Thesis Title: Investigation into the effect of Magnesium Valproate in Cardiac Hypertrophy and its related mechanism

---

ABSTRACT

Cardiovascular diseases (CVDs) are the major cause of death globally, with more people dying annually from it, rather than from any other cause. It remains one of the biggest burdens on economy despite improvements over last few decades. Identification of lead targets for cardiac hypertrophy is the need of hour. One of the important cardiac hypertrophic regulatory paradigm involves alterations in gene expression that are mediated by chromatin remodeling. Histone deacetylase (HDAC) is an important key regulator of cardiac hypertrophy and involved in cardiac remodeling and hypertrophic gene regulation. Histone acetyaltion and deacetylation is regulated by two sets of opposing enzymes - histone acetyltransferases and histone deacetylases (HDACs) respectively. Further, depletion of mitochondrial DNA (mtDNA) and its reduced replication have been identified as markers of transition from compensated hypertrophy to ventricular failure. The role of HDAC in heart is varying and has been shown to control events including hypertrophy, fibrosis, contractility, autophagy and energy metabolism. Although, class I HDAC and class II HDAC play opposing roles in regulation of cardiac hypertrophy, non-specific HDAC inhibitors (HDACi) have been found to regress hypertrophy. In lieu of this, objectives of the present study were,

1. To carry out mRNA expression studies of selected HDACs of both class I and class II HDACs in pathological hypertrophy using partial abdominal aortic constriction (PAAC) model of cardiac hypertrophy.
2. To study the effect of magnesium valproate, an HDACi, with 5 times more sensitivity to class I HDAC over class II HDAC on pathological hypertrophy.
3. To evaluate the role of mtDNA in cardiac hypertrophy and study the effect of magnesium valproate on mtDNA concentration.

In ISO induced cardiac hypertrophy, isoproterenol (5 mg/kg/day, i.p.) was administered for 10 days in healthy adult Wistar rats of either sex. Control group received normal saline and treated group received magnesium valproate (210 mg/kg/day, p.o.) for 10 days. After 10 days animals were sacrificed and various biochemical and cardiac parameters were analyzed. For PAAC induced cardiac hypertrophy, in healthy adult wistar rats, abdominal aorta was ligated by 4-0 silk thread along with 7.0 mm diameter blunt needle. The needle was then removed to leave aorta partially constricted and theyreceived magnesium valproate (210 mg/kg/day, p.o.) for 30 daysPAAC control group and sham control group received normal saline. After 30th day animals were sacrificed and various biochemical and cardiac parameters were evaluated.

Parameters evaluated were hypertrophic parameters like cardiac hypertrophy index, Left Ventricular hypertrophic index, heart weight/ body weight ratio, LV weight/ Right ventricle weight ratio, LV wall thickness, cardiomyocyte diameter, LV collagen levels, hemodynamic parameters, serum lipid profile, cardiac markers like Creatinine kinase-MB, Lactate dehydrogenase, C reactive protein, Na+K+ATPase levels, pro-oxidant and antioxidant levels and mitochondrial DNA concentration. Further, mRNA expression of HDAC 2 and HDAC 5 was carried out by Real-time polymerase chain reaction (RT-PCR).
ISO control and PAAC control rats exhibited significant increase in cardiac hypertrophy index (CHI), Left ventricular (LV) hypertrophic index (LVHI), heart weight to body weight ratio (HW/BW), left ventricular weight to right ventricular weight ratio (LVW/RVW), LV thickness and cardiomyocyte diameter. Treatment with magnesium valproate (210 mg/kg/day, p.o.) significantly reduced CHI, LVHI, HW/BW ratio, LVW/RVW, LV thickness and cardiomyocyte diameter in hypertrophic treated rats. There was significant increase in LV collagen level in the hypertrophic control rats. Treatment with magnesium valproate (210 mg/kg/day, p.o.) significantly reduced LV collagen levels in hypertrophic treated rats indicating prevention of cardiac hypertrophy.

There was significant increase in mean arterial blood pressure and heart rate in ISO control and PAAC control rats. Treatment with magnesium valproate (210 mg/kg/day, p.o.) produced significant decrease in mean arterial blood pressure and heart rate. Moreover, there was significant reduction in rate of pressure development and decay in hypertrophic control rats. Treatment with magnesium valproate (210 mg/kg/day, p.o.) significantly increased rate of pressure development and decay suggesting that magnesium valproate preserves cardiovascular functioning.
Moreover, ISO control and PAAC control rats produced significant increase in serum total cholesterol, LDL, VLDL, triglyceride and log Tg/HDL ratio while decrease in HDL levels. Treatment with magnesium valproate (210 mg/kg/day, p.o.) significantly reduced serum total cholesterol, LDL, VLDL, triglyceride and log Tg/HDL ratio and significantly increased HDL levels in hypertrophic treated rats.

There was significant increase in serum cardiac markers like Lactate dehydrogenase (LDH) and Creatinine kinase- MB (CK-MB) levels in ISO and PAAC control rats. Treatment with magnesium valproate (210 mg/kg/day, p.o.) significantly reduced serum level of LDH and CK-MB in hypertrophic treated rats. Further, hypertrophic control rats exhibited increase levels of C reactive protein (CRP) and Na+K+ATPase activity. Treatment with magnesium valproate (210 mg/kg/day, p.o.) significantly reduced CRP level and Na+K+ATPase activity in hypertrophic treated rats indicating prevention of cardiac damage.

Further, ISO control and PAAC control rats showed increase pro oxidant level i.e. malondialdehyde (MDA) and reduced antioxidant levels such as superoxide dismutase (SOD) and reduced glutathione (GSH) levels of the left ventricle. Treatment with magnesium valproate (210 mg/kg/day, p.o.) significantly reduced MDA levels and increased SOD and GSH levels in left ventricle of hypertrophic treated rats. This suggests that magnesium valproate prevents oxidative stress.

Reduction of cardiac hypertrophy with treatment of magnesium valproate (210 mg/kg/day, p.o.) in hypertrophic treated rats was further supported by histopathological studies of left ventricle, which showed marked reduction in fibrosis, increased interstitial space, reduced eosinophilia, reduced extravasated RBCs and decreased apoptosis as compared to ISO control and PAAC control rats.

There was significant decrease in mitochondrial DNA (mtDNA) concentration in LV of PAAC hypertrophic rats, which was increased significantly on treatment with magnesium valproate (210 mg/kg/day, p.o.) in hypertrophic treated rats. Improvement in mtDNA concentration is suggestive that magnesium valproate preserves mitochondrial functioning and thereby may prevent transition to heart failure.

Further, Kaplan- Meier curve indicated that administration of magnesium valproate improved the survival rate in hypertrophic treated rats as compared to hypertrophic control rats. Further, to investigate the mechanism of action of magnesium valproate, the expression of HDAC2, a class I HDAC and HDAC5- a class II HDAC was studied. A significant increase in expression levels of HDAC2 mRNA and HDAC5 mRNA was observed in the heart of PAAC hypertrophic rats compared to control animals. Treatment with magnesium valproate (210 mg/kg/day, p.o.) significantly decreased the HDAC2 mRNA levels, but had no significant effect on HDAC5 mRNA levels.

In conclusion, our data suggests that magnesium valproate (210 mg/kg/day, p.o.) produces beneficial effects on cardiac hypertrophy as is evident, specifically from reduction in hypertrophic parameters including collagen levels, improvement in mitochondrial DNA concentration and preserving of LV systolic and diastolic dysfunction. This beneficial effect of magnesium valproate is due to its HDAC inhibitory activity mediated through downregulation of class I HDACs, specifically HDAC2. Till date, the HDACi used are non-selective, differing in terms of relative selectivity. Our study is a molecular pharmacological investigation which provides an insight into newer targets for cardiac hypertrophy suggesting that selective class I HDAC inhibition is required for controlling cardiac hypertrophy. This

study can be further expanded by developing new chemical entities by modifying the structure of magnesium valproate. Focus should be laid along the lines of achieving specificity and selectivity for particular class of HDAC enzymes i.e. their major action on class I over class II HDAC. This would ensure curbing the disease at core, eliminating side-effects and associated complications, which are major drawback for existing therapies. Furthermore, it is the need of the hour to design newer HDAC inhibitors which are class I inhibitor and class II promoter to obtain a 'pan' or 'dual' natural HDAC 'regulators'.