ORIGINAL RESEARCH PAPER

Pharmacology

"A COMPARATIVE STUDY OF ROLE OF DEFERASIROX AND ALLOPURINOL IN PREVENTION OF ISCHEMIC REPERFUSION MYOCARDIAL INJURY IN ALBINO RABBITS"

KEY WORDS: Deferasirox, Allopurinol, Ischemic myocardial reperfusion injury, Rabbits

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ABSTRACT

Coronary heart disease is one of the leading causes of death and disability worldwide. Timely reperfusion using thrombolytic therapy or primary percutaneous coronary intervention can limit the size of myocardial infarction but process of myocardial reperfusion can itself induce cardiomyocyte death, which is referred as myocardial reperfusion injury. Antioxidants and iron chelators aid heart to overcome the ischemic-reperfusion injury. Effect of deferasirox and allopurinol was studied in prevention of myocardial reperfusion injury in albino rabbits using isolated heart perfusion. Post ischemic LDH level was measured at 5, 10 and 15 min during reperfusion period. Deferasirox and allopurinol reduced post ischemic LDH level at 5, 10 and 15 min as compared with control. Deferasirox reduced post ischemic LDH levels more significantly as compared to allopurinol at 15 min. Deferasirox and allopurinol have protective effect in prevention of myocardial reperfusion injury in albino rabbits with an upper hand of oral iron chelator.

INTRODUCTION

Coronary heart disease is one of the leading causes of death and disability worldwide. As per WHO (2013) almost 13% of the total deaths worldwide resulted due to coronary heart diseases.[1] Though timely and effective myocardial reperfusion using thrombolytic therapy or primary percutaneous coronary intervention(PPCI) can limit the size of myocardial infarction, but still the process of myocardial reperfusion can in itself induce cardiomyocyte death, which can be referred as myocardial reperfusion injury. The myocardial reperfusion injury is highly accountable for the deaths due to coronary heart disease.

Antioxidants and iron chelators aid heart to overcome the ischemic-reperfusion injury. [2] It has been proposed that oxidative stress and calcium overload can be the etiologic factors behind this injury. Oxidative stress gets usually associated with formation of reactive oxygen species, modification of phospholipids and protein leading to lipid peroxidation and oxidation of thiol groups leading to alteration in membrane permeability and configuration.

This oxidative stress induced changes in the sarcoplasmic reticulum calcium pump as well as sarcolemmal Na^+-K^+ pump are not just limited to the cardiac cells but can also proceed to the coronary artery smooth muscle cell.[3] These damages caused by these reactive oxygen species has been shown to be reduced antioxidants such as catalase and superoxide dismutase.

Xanthine dehydrogenase was not found in human and rabbit hearts, a considerable amount of the enzyme was detected in vascular endothelial cells by histochemical methods.[4] Allopurinol, an inhibitor of xanthine oxidase (XO), has also been shown to be beneficial in protecting myocardium from ischemia—reperfusion injury.

Various studies have reported the beneficial effects of antioxidants as these agents render resistance to the heart against the ischemic—reperfusion injury. However, some investigators have failed to observe such results.[5] Several factors can contribute to this discrepancy such as species difference, various experimental methods and techniques applied, different time periods of ischemia—reperfusion insults, as well as different types of antioxidants with different properties. There is a clear need for a systematic study to determine the exact role of antioxidants in protection against the ischemic—reperfusion injury.

Thus here the aim of the study was to find the effect of the iron chelators- deferoxamine (DFO) and Allopurinol over LDH level.

MATERIAL AND METHODS

Present study was conducted on healthy albino rabbits of either sex weighting 1.5-2.0 Kg. The animals were made available in the animal house of Department of Pharmacology. They were maintained on Standard husbandry conditions (room temperature 27 ± 30C, relative humidity 65 ± 10 % and 12 hours light / dark cycle) and standard diet ad libitum.

DRUGS USED IN THE EXPERIMENT

1. DEFERASIROX
2. ALLOPURINOL

STUDY DESIGN:

For this study 15 albino rabbits of either sex weighing 1.5- 2.0 Kg were divided into three groups consisting of five rabbits in each group.

1. **Control group** : It was given no drug but maintained on standard diet ad libitum for 7 days.
2. **Oral deferasirox group**: It received oral deferasirox 50mg/kg/day and standard diet ad libitum for 7 days.
3. **Oral allopurinol group**: It received oral Allopurinol 75mg/kg/day for 7 days and standard diet ad libitum.

PROCEDURE:

Experiments were done using isolated heart perfusion apparatus (Langendorff apparatus). Rabbits were given heparin i.v 750 IU/kg via marginal ear vein. After 40 minutes of heparinisation rabbit was anesthetised with i.v sodium thiopentone 20mg/kg by reconstituting in distilled water. After rabbit became unconscious and lost pedal reflex activity, heart was quickly removed from the body and placed in cold tyrode solution. The heart was cannulated in the aorta and perfused by method of Langendorff. The perfusion was carried out at 37 ^{0}C and pH 7.4 with modified tyrode buffer and aeration was maintained. The perfusion was maintained for 15 minutes after 15 minutes of perfusion total ischemia was created by closing the tap between perfusion apparatus and heart. Ischemia was maintained for 10 minutes. After 10 min of ischemia reperfusion was started by opening tap between perfusion apparatus and heart. This was called as post-ischemic or reperfusion phase. This phase was maintained for 15

minutes. Measurements were done at 5, 10 and 15 minutes by collecting perfusate.

MEASUREMENTS

LDH level : Sample were collected at 5, 10,15 min in post ischemic phase(reperfusion phase) and LDH was measured using tranasia biomedicals Ltd autoanalyser anq Liquixx LDH-p kit by erba diagnostics, Mannheim. LDH levels of perfusate were expressed in IU (International Units) per litre (L).

CALCULATIONS

Data was presented as mean ± SEM (standard error of mean). LDH was compared in reperfusion phase between control and test groups using student t- test for independent variable. P value was calculated. P < .05 was considered significant.

OBSERVATION AND RESULTS

In group 1 (control group) the mean post ischemic LDH level at 5, 10 and 15 min were 147.6, 223, 184.8 respectively [Table 1], while in group 2 (deferasirox group) mean post ischemic LDH levels at 5, 10 and 15 min were 45.6, 54.6 and 45 respectively [Table 2] and in group 3(allopurinol group) it was 49.2, 65.2 and 55.8 respectively [Table 3].

Table1

S. No	Post-ischemic LDH levels (IU/L) (control)		
	5 min	10 min	15min
1.	139	278	147
2.	140	210	170
3.	148	202	222
4.	151	199	186
5.	160	226	199
Mean	147	223	184.8
SE+	4.30	16.24	14.21

Table 2

S. No	Post-ischemic LDH level (IU/L) (deferasirox)		
	5 min	10 min	15min
1.	49	58	49
2.	44	50	46
3.	44	57	42
4.	41	49	40
5.	50	59	48
Mean	45.6	54.6	45
SE+	1.39	2.36	1.93

Table 3

S. No	Post-ischemic LDH levels (IU/L) (allopurinol)		
	5 min	10 min	15min
1.	32	49	49
2.	48	66	52
3.	50	66	58
4.	56	70	66
5.	60	75	54
Mean	49.2	65.2	55.8
SE+	5.36	4.89	5.98

Comparing control vs deferasirox [Table 4] we found that there was significant (p < .01) decrease in post ischemic LDH level at 5, 10 and 15 minutes. Observations indicate that there was significant decrease in post-ischemic LDH level in group that received deferasirox.

Table 4 Statistical comparison of post-ischemic LDH levels between the group receiving deferasirox and control group

Deferasirox + Standard diet (n=5)	Time interval (min)		
	5min	10 min	15 min
Mean LDH level(IU/L)	45.6	54.6	45
SE+	1.39	2.36	1.93
Control group Only standard diet (n=5)	**Time interval(min)**		
	5min	10 min	15 min
Mean LDH level(IU/L)	147.6	223	184.8
SE+	4.30	16.24	14.21
"t" value	24.23	11.47	10.89
"P" value	<.01	<.01	<.01

Comparing control group with allopurinol group [Table 5] we found that there was significant (p < .01) decrease in post ischemic LDH level at 5, 10, and 15 min in group that received allopurinol. Thus allopurinol caused a significant decrease in post ischemic LDH level.

Table 5 Statistical comparison of post-ischemic LDH levels between the group receiving allopurinol and control group

Allopurinol + Standard diet(n=5)	Time interval (min)		
	5min	10 min	15 min
Mean LDH level(IU/L)	49.2	65.2	55.8
SE+	5.36	4.89	5.98
Control group Only standard diet (n=5)	**Time interval (min)**		
	5min	10 min	15 min
Mean LDH level(IU/L)	147.6	223	184.8
SE+	4.30	16.24	14.21
"t" value	15.88	10.40	10.89
"P" value	<.01	<.01	<.01

Comparing deferasirox group with allopurinol group [Table 6] we found that decrease in post-ischemic LDH level was comparable at 5 and 10 min but oral iron chelator caused a more significant (p < .05) decrease in post-ischemic LDH level at 15 min as compared to allopurinol.

Table 6 Statistical comparison of post-ischemic LDH level between the group receiving deferasirox and allopurinol

Deferasirox + Standard diet (n=5)	Time interval (min)		
	5min	10 min	15 min
Mean LDH level(IU/L)	45.6	54.6	45
SE+	1.39	2.36	1.93
Allopurinol + Standard diet(n=5)	**Time interval (min)**		
	5min	10 min	15 min
Mean LDH level(IU/L)	49.2	65.2	55.8
SE+	15.36	4.89	5.98
"t" value	0.71	2.18	3.17
"P" value	<.05	<.05	<.05

DISCUSSION

LDH levels were measured in IU (international units) per litre (L). These values were used as parameters in our study to evaluate myocardial reperfusion injury. LDH is an important biochemical marker of myocardial ischemia as well myocardial reperfusion injury. Free radical damage caused during ischemia and reperfusion leads to cell necrosis and release of LDH.

Thus oral iron chelator can have a protective role in protection of myocardial reperfusion injury. Till date as per web research data there have been studies on parenetral iron chelator such as desferrioxamine in preventing ischemic and reperfusion injury in experimental models , but pubmed research has scarce data on oral iron chelator, in prevention of myocardial reperfusion injury. Our study indicated significant protection by oral iron chelator deferasirox in myocardial ischemic reperfusion injury as it significantly reduced post-ischemic LDH level during 15 min phase of reperfusion.

Xanthine oxidase is a superoxide (O_2) producing enzyme. Super oxide ion is an important free radical . So it is a mediator of myocardial reperfusion injury. Being reactive oxygen species it leads to of cellular and subcellular membrane causing cell necrosis. Allopurinol is xanthine oxidase inhibitor, so it is an important antioxidant. Thus allopurinol can have protective role in myocardial reperfusion injury. In our study allopurinol gave

significant protection against myocardial reperfusion injury by decreasing post-ischemic LDH level during 15 min phase of reperfusion injury.

Thus our study shows that both deferasirox and allopurinol has protective effect in prevention of myocardial reperfusion injury in albino rabbits and also deferasirox is shown to be significantly superior in preventing myocardial reperfusion injury as compared to allopurinol. However further multicentric studies and clinical trials are warranted to establish the efficacy and safety of deferasirox and allopurinol to be used in prevention of myocardial reperfusion injury. It is prudent, also to validate the findings by employing more markers of ischemic reperfusion injury.

CONCLUSION

Deferasirox and allopurinol have protective effect in prevention of myocardial reperfusion injury in albino rabbits with an upper hand of oral iron chelator. However further multi centric experimental studies using more biochemical injury markers and clinical trials are required to validate its efficacy and safety, before it can be advocated to be used in prevention of myocardial reperfusion injury along with morbidity and mortality in ischemic heart diseases.

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