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"A COMPARATIVE STUDY OF ROLE OF ORAL IRON CHELATORS AND CERTAIN ANTIOXIDANTS IN PREVENTION OF ISCHEMIC REPERFUSION MYOCARDILAL INJURY IN ALBINO RABBITS"

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ABSTRACT Coronary heart disease is one of the important causes of mortality worldwide. Though reperfuson in time reduce the infarct size and else but this reperfusion in itself sets off a chain of myocardial damage either due to oxidative damage or altered calcium metabolism. Coronary perfusion has been taken as a measure of recovery from this reperfusion injury. The hearts of albino rabbits have been perfused through Langendorff method and then artificial ischemia has been created followed by reperfusion. Thus antioxidants like oral iron chelators (deferasirox) and allopurinol have been tried for their protective role from this injury. The coronary perfusion has been observed to be enhanced in rabbits on deferasirox or allopurinol, with former having an upper hand.

KEYWORDS : Reperfusion, Deferasirox, Allopurinol, antioxidant

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.

There are four types of myocardial reperfusion injury which are-

a) Reperfusion induced arrhythmia
b) Myocardial stunning
c) Microvascular obstruction
d) Lethal myocardial reperfusion injury

Reperfusion induced arrhythmia- The sudden reperfusion of acutely ischemic myocardium in STEMI patients undergoing PPCI can be associated with arrhythmia.

Myocardial stunning- The reversible post-ischemic contractile dysfunction that occurs on reperfusing acute ischemic myocardium is myocardial stunning.

Microvascular obstruction- It is the inability to reperfuse a previously ischemic region. The major contributing factors include capillary damage with impaired vasodilatation, cadiomyocyte swelling, microembolization of friable material released from the artherosclerotic plaque, the release of soluble vasomotor and thrombogenic substances.

Lethal myocardial reperfusion injury- Reperfusion induced death of cardiomyocytes that were viable at the end of the index ischemic event is defined as lethal myocardial reperfusion injury. The major contributory factors are oxidative stress, calcium overload, mitochondrial permeability transition pore opening and hypercontracture. [2]

The chief mediators for reperfusion injury are-

- Oxygen free radicals
- Endothelial and microvascular dysfunction
- Intracellular calcium overload
- Altered myocardial metabolism

1. Oxygen free radicals- Due to unpaired/odd number of electrons free radicals are ready to participate in chain reaction. Some major oxygen radicals are- superoxide anion (O), hydroxyl radicals(OH) and alloxy or lipoxy radicals. In one of a landmark study Bolli [3] and colleagues showed that potent oxidant radicals such as superoxide anion, hydroxyl radical and peroxynitrite are produced within few minutes of reflow and play a crucial role in reperfusion injury.

2. Endothelial dysfunction and microvascular injury- Reperfusion results in marked endothelial cell dysfunction.. Endothelin-1 and oxygen free radicals increase vasoconstriction and reduce flow.

3. Alterations in Calcium Handling:
Ischemia and reperfusion are associated with an increase in intracellular calcium. This effect may be related to increased sarcolemmal calcium entry through L-type calcium channels or may be secondary to alterations in sarcoplasmic reticulum calcium cycling. [4]

4. Altered Myocardial Metabolism:
Persistent lactate production after reperfusion predicts postoperative ventricular dysfunction.(36) Likewise, the activity of mitochondrial pyruvate dehydrogenase (PDH) is inhibited by 40% after ischemia.

ANTIOXIDANTS:

Antioxidants are defined as substances which inhibit or delay the oxidative damage to sub cellular proteins, carbohydrates, lipids and DNA. [5]

They can act through several mechanisms such:

a) Scavenging ROS or their precursors.
b) Inhibiting the formation of ROS.
c) Attenuating the catalysis of ROS generation via binding to metals ions.
d) Enhancing endogenous antioxidant generation.
e) Reducing apoptotic cell death by upregulating the anti-death gene (Bc1-2). [6]

TYPES OF ANTIOXIDANTS:

Antioxidants are usually classified as-

1. **Endogenous antioxidants:** Many substances have been suggested to act as antioxidants. But superoxide dismutase (SOD), catalase, glutathione peroxidase and vitamin E, have been studied extensively.[7]
2. **Exogenous antioxidants:** Among exogenous antioxidants, some drugs have been reported to exert antioxidant action. These include thiol-containing compounds, ß- adrenergic blockers, angiotensin converting enzyme inhibitors, xanthine oxidase inhibitors, iron chelating agents and Ca2+ antagonists.

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:

Deferasirox is a tridentate oral iron chelator with high affinity for iron as Fe3+, two molecules of deferasirox bind to one Fe3+.[8] Deferasirox is capable of eliminating iron from cells (cardiac myocytes and hepatocytes) as well as iron from blood.

2. Allopurinol:

Allopurinol is a purine analog, a structural isomer of hypoxanthine (a naturally occurring purine in the body). Allopurinol and its active metabolite, oxypurinol, inhibit xanthine oxidase. Inhibition of xanthine oxidase blocks conversion of oxypurines (hypoxanthine, xanthine) to uric acid, resulting in decreased uric acid concentratio ns in serum and urine. [9]

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 were anesthetised with i.v sodium thiopentone 20mg/kg by reconstit uting in distilled water. After rabbit became unconscious and lost pedal reflex activity, heart was quickly removed from the body and placed in cold Tyrode's solution. The heart was cannulated in the aorta and perfused by Langendorff method. The perfusion was carried out at 37 0C 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 reperfusion phase was maintained for 15 minutes. Measurements were done at 5, 10 and 15 minutes by collecting perfusate.

RESULTS:

The results are discussed as follows-

Post Ischemic Coronary Flow: In group 1 (control group) the mean coronary flow at 5, 10 and 15 min were 4.08, 4.54, 4.24 respectively [Table 1]. In group 2 (deferasirox) mean post ischemic coronary flow at 5, 10,15 min were 4.5, 4.6, 4.02 respectively[Table 2] while in group 3(allopurinol group) mean coronary flow were 4.6, 4.26, 4.26 respectively [Table 3].

Table1- Post-ischemic coronary perfusion (ml/min) (control)

S. No	Post-ischemic coronary perfusion (ml/min) (control)		
	5 min	10 min	15min
1.	4.1	4.6	4.2
2.	4.1	4.7	4.3
3.	4.2	4.4	4.3
4.	4	4.5	4.2
5.	4	4.5	4.2
Mean	4.08	4.54	4.24
SE+	0.041	0.057	0.027

Table 2-Post-ischemic coronary perfusion (ml/min) (defera sirox)

S. No	Post-ischemic coronary perfusion (ml/min) (deferasirox)		
	5 min	10 min	15min
1.	4.5	4.9	4.4
2.	4.5	4.8	4.2
3.	4.5	4.5	4.1
4.	4.4	4.7	3.9
5.	4.5	4.5	3.5
Mean	4.5	4.68	4.02
SE+	0.035	0.044	0.086

Table 3- Post-ischemic coronary perfusion (ml/min) (allopuri nol)

S. No	Post-ischemic coronary perfusion (ml/min) (allopurinol)		
	5 min	10 min	15min
1.	4.6	4.5	4.4
2.	4.5	4.4	4.2
3.	4.9	4.3	4.4
4.	4.8	4.5	4.4
5.	4.2	4.6	3.9
Mean	4.6	4.46	4.26
SE+	0.136	0.057	0.109

Comparing post ischemic coronary flow of control with deferasirox it was found that though there was significant increase in post ischemic coronary perfusion in deferasirox group at 5 min ($p < .05$), there was no significant increase at 10 and 15 min($p > .05$) [Table 4].

Table 4-Statistical comparison of post-ischemic coronary perfusion level between the group receiving deferasirox and control group

Deferasirox + Standard diet(n=5)	Time interval(min)		
	5min	10 min	15 min
Mean coronary perfusion(ml/min)	4.5	4.68	4.02
SE+	0.035	0.044	0.086

Control group Common standard diet (n=5)	Time interval(min)		
	5min	10 min	15 min
Mean coronary perfusion(ml/min)	4.08	4.54	4.24
SE+	0.041	0.057	0.027

"t" value	8.57	1.48
1.42		
"P" value	<.01	>.05
>.05		

Comparing post ischemic coronary flow of control with allopurinol group it was found that though there is significant increase in post ischemic coronary flow in allopurinol group at 5 min.($p < .05$) there was no significant increase at 10 and 15 min.($p > .05$)[Table5].

Table 5- Statistical comparison of post-ischemic coronary perfusion level between the group receiving allopurinol and control group

Allopurinol + Standard diet(n=5)	Time interval(min)		
	5min	10 min	15 min
Mean coronary perfusion(ml/min)	4.6	4.46	4.26
SE+	0.136	0.057	0.109

Control group Only standard diet (n=5)	Time interval(min)		
	5min	10 min	15 min
Mean coronary perfusion(ml/min)	4.08	4.54	4.24
SE+	0.041	0.057	0.027

"t" value	4.06	1.11
0.2		
"P" value	<.01	>.05
>.05		

Comparing post ischemic coronary flow of deferasirox with allopurinol group the increase was not significant at 5, 10 and 15 min.($p > .05$)[Table 6 & figure 6].

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

Iron chelator + Standard diet(n=5)	Time interval(min)		
	5min	10 min	15 min
Mean coronary perfusion(ml/min)	4.5	4.68	4.02
SE+	0.035	0.057	0.109

Allopurinol + Standard diet(n=5)	Time interval(min)		
	5min	10 min	15 min
Mean coronary perfusion(ml/min)	4.6	4.46	4.26
SE+	0.136	0.057	0.109

"t" value	0.79	2.32
1.32		
"P" value	>.05	>.05
>.05		

DISCUSSION

The aim of present study was to see the preventive effect of oral iron chelator (deferasirox) and antioxidant (allopurinol) in myocardial reperfusion injury.

Iron is considered as the most important transition metal present in cardiac tissue. It indirectly causes ischemic reperfusion injury by formation of hydroxyl radical which reacts rapidly with various molecules such as lipid, proteins or DNA and destroys their structure. Thus oral iron chelator can have a protective role in protection of myocardial reperfusion injury.

Xanthine oxidase is a superoxide (O2) producing enzyme. Allopurinol being a xanthine oxidase inhibitor, can have a protective role in myocardial reperfusion injury.

In our study allopurinol gave significant protection against myocardial reperfusion injury by increasing post-ischemic coronary flow at 5 min like deferasirox. Thus our study indicates significant increase in post-ischemic coronary flow which was observed only at 5 min but not at 10 and 15 min by both deferasirox and allopurinol.

CONCLUSION

Our study shows that both deferasirox and allopurinol have protective effect in prevention of myocardial reperfusion injury in albino rabbits. Deferasirox is 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.

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