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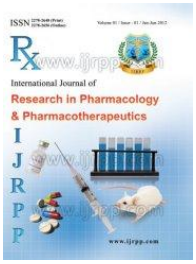

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Role of allopurinol as an oxygen free radical scavanger in prevention of ischemic reperfusion myocardial injury in albino rabbits

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ABSTRACT

Aim

To study the role of allopurinol as an oxygen free radical scavenger, in prevention of myocardial ischemic reperfusion injury in albino rabbits.

Materials and Methods

An experimental analytical study was carried out after getting approval from institutional animal ethical committee. Albino Rabbits of either sex were divided into two groups control (n=5) and test group (n=5), receiving antioxidant allopurinol. Experiments were done using isolated heart perfusion apparatus (Langendorff apparatus). The experiment was divided into three phases perfusion (15 min) followed by ischemia (5 min) followed by reperfusion (15 min). LDH level of perfusate and coronary flow were taken as biochemical and physiological marker of myocardial reperfusion injury respectively were measured at 5, 10 and 15 min of post ischemic period. Control and test groups were compared.

Result

The result shows that in the group receiving allopurinol the decrease in post ischemic LDH level was significant ($p<0.01$) at 5,10 and 15 min while there was significant increase in post ischemic coronary flow at 5 min ($p<0.05$) as compared with control.

Conclusion

Antioxidant allopurinol has protective role in prevention of myocardial reperfusion injury in albino rabbits.

Keywords: Allopurinol, Langendorff apparatus, LDH (lactate dehydrogenase), Reperfusion injury.

INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death and disability worldwide. According to WHO 7,254,000 deaths worldwide resulted from CHD in 2013[1]. The effects of CHD are usually attributable to the detrimental effects of acute myocardial ischemia- reperfusion injury (IRI). IRI typically arises in patients presenting with acute ST-segment elevation myocardial infarction (STEMI)[1].

The most effective therapeutic intervention for reducing acute myocardial ischemic injury is timely and effective myocardial reperfusion by thrombolysis or primary percutaneous coronary intervention (PCCI). However, the process of myocardial reperfusion can itself induce further myocardial damage, which is called myocardial reperfusion injury[2].

Although the process of myocardial reperfusion continues to improve with more timely and effective reperfusion and with advances in primary percutaneous coronary intervention (PPCI) technology, thrombolytic and antiplatelet drugs for maintaining the patency of the infart related coronary artery, there is still no effective therapy for prevention of myocardial reperfusion injury[3].

Oxygen derived free radicals are important in pathogenesis of myocardial damage during ischemia and reperfusion[4-7] and is it based on the studies that have reported protective effects of superoxide dismutase, mannitol and other free radical scavangers.[8-11] The generation of superoxide ion or hydrogen peroxide produced during ischemia and reperfusion damages myocardial tissue.[12, 13] Xanthine oxidase is a superoxide ($O_2^{\cdot}$) producing enzyme.

There has been very few studies on Allopurinol in prevention of myocardial reperfusion injury. Our aim was to study the protective effect of free radical scavanger allopurinol in protection of myocardial reperfusion injury.

MATERIALS AND METHODS

Present study was conducted on healthy albino rabbits of either sex weighting 1.5-2.0 Kg. from October 2014 to November 2015 after getting approval from institutional ethical animal committee. The animals were made available in the animal house of Department of Pharmacology, GSVM Medical College, Kanpur. They were maintained on Standard husbandry conditions (room temperature 27 ± 3^{o}C, relative humidity 65 ± 10 % and 12 hours light /dark cycle) and standard diet ad libitum. Animals were divided into two groups consisting of five animals in each group. Control group was given no drug and was maintained on standard diet ad libitum for 7 days and test group received allopurinol 75mg/kg/day and standard diet ad libitum for 7 days. Experiments were done using isolated heart perfusion apparatus (Langendorff apparatus). Rabbit was given Heparin i.v 750IU/kg via marginal ear vein, after 40 minutes of heparinization rabbit was anesthesized with i.v sodium thiopentone 20mg/kg by reconstituting in distilled water.[14]

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 langendorff apparatus. The perfusion was carried out at 37^{0}C and pH 7.4 with modified tyrode buffer and aeration was maintained.[15]

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 15 minutes. After 15 min of ischemia, reperfusion was started by opening tap between perfusion apparatus and heart. This was called post-ischemic or reperfusion phase. This phase was maintained for 15 minutes. Measurements (LDH levels in IU/L and coronary flow in ml/min) in post ischemic phase were done at 5, 10 and 15 minutes after collecting perfusate. Data was presented as mean±SEM (standard error of mean). Coronary flow and LDH level were compared in post ischemic phase between control and test group using student t- test for independent variable. p value was calculated. $p < 0.05$ was considered as significant.

RESULTS

Post-ischemic LDH Levels

In control group the mean post ischemic LDH level at 5, 10 and 15 min were 147.6, 223, 184.8 respectively, while in allopurinol group LDH levels at 5, 10 and 15 min were 49.2, 65.2 and 55.8 respectively [Table 1]. Comparing observations of control vs allopurinol [Table 1 and Figure 1] we found that there was significant ($p < .01$) decrease in post ischemic LDH level at 5, 10 and 15 minutes. Observation indicates that antioxidant allopurinol

caused significant decrease in post-ischemic LDH level as compared to control.

Post Ischemic Coronary Flow

In control group the mean coronary flow at 5, 10 and 15 min were 4.08, 4.54, 4.24 respectively [Table 2]. In allopurinol mean post ischemic coronary flow at 5, 10 and 15 min were 4.6, 4.26, 4.26 respectively [Table 2]. Comparing post ischemic coronary flow of control with allopurinol it was found that though there was significant increase in post ischemic coronary perfusion in allopurinol group at 5 min ($p < 0.01$), there was no significant difference at 10 and 15 min. [Table 2 & Figure 2].

Table 1: Statistical comparison of mean post-ischemic ldh (iu/l) levels of perfusate between group receiving allopurinol and control group**.**

Allopurinol + Standard diet (n=5)	**Time interval (min)**		
	5min	**10min**	**15min**
Mean LDH Level (IU/L)	49.2	65.2	55.8
SE±	5.36	4.89	5.98

Control group	**Time interval (min)**		
	5min	**10min**	**15min**
Mean LDH Level (IU/L)	147.6	223	184.8
SE±	4.30	16.24	14.21

't' value	**15.88**	**10.40**	**9.88**
'P' value	**<0.01**	**<0.01**	**<0.01**

Table 2: Statistical comparison of post-ischemic coronary perfusion (ml/min) levels between group receiving allopurinol and control group.

TABLE 2

Allopurinol + Standard diet (n=5)	**Time interval (min)**		
	5min	**10min**	**15min**
Mean coronary perfusion (ml/min)	4.6	4.46	4.26
SE±	.136	.057	.109

Control group	**Time interval (min)**		
	5min	**10min**	**15min**
Mean coronary perfusion (ml/min)	4.08	4.54	4.24
SE±	.041	.057	.027

't' value	**4.06**	**1.11**	**0.2**
'P' value	**<.01**	**>.05**	**>.05**

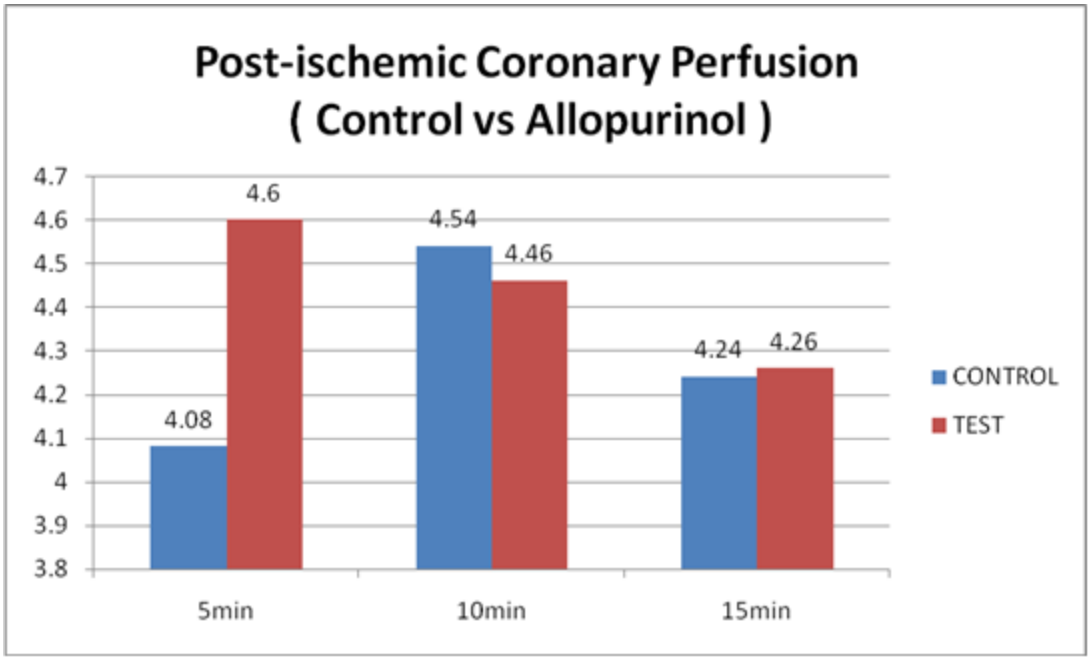

Fig.1: Comparision of mean post-ischemic ldh(iu/l) level between allopurinol and control at 5 min, 10 min and 15 min.

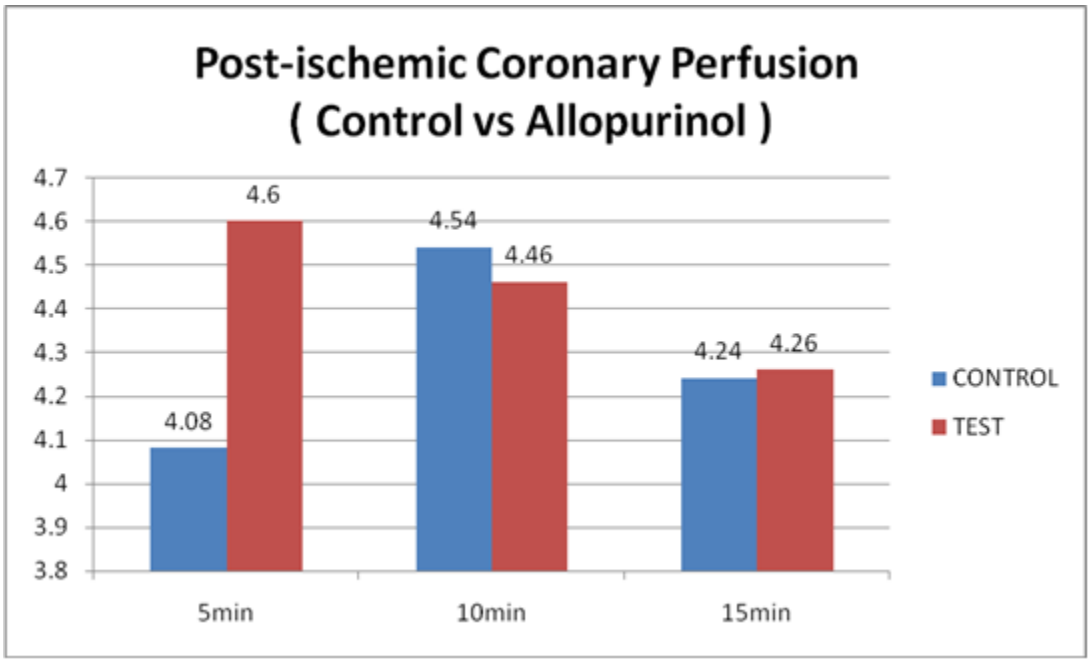

Fig.1: Comparision of mean post-ischemic coronary perfusion (ml/min) between allopurinol and control at 5 min, 10 min and 15 min.

Discussion

The aim of present study was to see the preventive effect of allopurinol in myocardial reperfusion injury.

LDH is an important biochemical marker of myocardial ischemia as well as myocardial reperfusion injury. Free radical damage caused during ischemia and reperfusion leads to cell necrosis and release of LDH. Coronary perfusion can be altered in reperfusion injury due to endothelial cell dysfunction. Endothelium dependent vasodilation is

impaired, whereas responses to endothelium dependent vasoconstrictors are exaggerated. Increased production of potent vasoconstrictors such as endothelin-1 and oxygen free radicals increases coronary vasoconstriction and reduces blood flow.[16]

Oxidative stress resulting from the generation of excessive oxygen free radicals from molecular oxygen entering the ischemic myocardium and via enzymatic pathways (for example, xanthine oxidase), accompanied by a reduction in the activity of endogenous free radical scavenging pathways (for example, superoxide dismutase), likely represents an important mechanism for vascular and myocardial injury.[17]

Xanthine oxidase is a superoxide ($O_2^{\cdot}$) producing enzyme. Superoxide ion is an important free radical. So it is a mediator of myocardial reperfusion injury. Being reactive oxygen species it leads to peroxidation 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.

There have been few studies on allopurinol for its protective role in myocardial reperfusion injury. Joke A. Gimpel (1995) evaluated the usefulness of allopurinol for prevention of myocardial reperfusion injury in open heart surgery. His result indicate that allopurinol reduced ischemia reperfusion injury during open heart surgery.[18]

Our study indicated significant protection by allopurinol in myocardial ischemic reperfusion injury in albino rabbits as it significantly reduced post-ischemic LDH level during 15 min phase of reperfusion and there was significant increase in post-ischemic coronary perfusion at 5 min of reperfusion.

CONCLUSION

Our study indicates that allopurinol provides significant protection against myocardial reperfusion injury in albino rabbits in isolated heart perfusion experiment in post ischemic phase by significantly reducing post-ischemic LDH level at 5, 10 and 15 min and significantly increasing post ischemic coronary flow at 5 min of reperfusion.

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