ALLOPURINOL AS A PROMISING ANTI-ANGINAL AGENT: AN OLD DRUG WITH NEW INDICATION

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ABSTRACT

Objective: To investigate protective role of Allopurinol (xantine oxidase inhibitor) in myocardial injury in patients with effort induced stable angina pectoris. Design: Prospective open label study. Setting: Tertiary care cardiac center. Patients: 14 Diagnosed cases of effort induced stable angina & 11 healthy volunteers for comparison. Interventions: Trade mill test (TMT) was performed in 14 patients of effort induced stable angina before allopurinol therapy and in 10 patients after two weeks allopurinol therapy (4 dropouts). Main outcome measures: Pre and post therapy total exercise duration, estimated maximum metabolic equivalents tasks (METs), % of target heart rate (THR) achieved and maximum ST depression. Pre and post therapy mean plasma levels of Malonyldialdehyde (MDA), Superoxide-dismutase (SOD), and Catalase were compared mean levels of healthy volunteers.

Results: In patients of effort induced angina, after receiving allopurinol mean walking time (Pre-therapy 235.57±20.25; vs Post-therapy 368.3±22.65; p <0.001) and mean estimated
maximum METs (5.71±0.21; vs 7.0±0.25; p <0.001) increased significantly, mean maximum ST depression (322.0±26.25; vs 229.0±19.33; p <0.05) significantly reduced, while no significant change was observed (97.56±16.9; vs 94.8±16.1; p >0.05) in mean of % THR in comparison to pre-therapy values. After allopurinol therapy MDA (Healthy volunteer 1.85±0.30; vs 3.8 ± 0.47; & 1.91 ± 0.41; p < 0.01) and catalase (Healthy volunteer 371.8±52.3; vs 387.35±36.5 ; & 246.53±27.51; p < 0.01) decreased while SOD (Healthy volunteer 25.8±2.85; vs 15.67±1.66; & 24.60 ± 1.52; p <0.001) increased significantly.

**Conclusion:** Allopurinol has potential to ameliorate free radical induced myocardial injury and improve exercise tolerance in patient of exercise induced stable angina.

**KEYWORDS:** Allopurinol; Stable angina; MDA; SOD; Catalase.

**INTRODUCTION**

In recent times, important milestones have been reached with the availability of more overt evidence which shows that cardiovascular disease mechanisms are strongly linked to the production of reactive oxidant species and the dysregulation of oxidant-antioxidants pathways. In this regard, the oxidation and nitration of cellular proteins, lipids and nucleic acids, and the formation of aggregates of oxidized molecules underlie the loss of cellular function, cellular ageing and the inability of cells to withstand physiological stresses. In addition, reactive oxidant species modulate signal transduction processes and energy metabolism in response to conditions of oxidative/nitrosative stress.\(^1\)

Biochemical, molecular and pharmacological studies further implicates xanthine oxidoreductase (XOR) as a source of reactive oxygen species (ROS) in cardiovascular system (CVS). XOR is a member of molybdoenzyme family and it is best known for catalytic role in purine degradation, metabolizing hypoxanthine and xanthine in uric acid with concomitant generation of superoxide. A role for XOR beyond purine metabolism was first suggested in ischemia-reperfusion-injury. Now there is growing awareness that it also participate in endothelial dysfunction, hypertension and heart failure.\(^2\) In last two decades various clinical studies in human established role of xanthine oxidase inhibitors (allopurinol and oxypurinol) in ischemia- reperfusion- injury,\(^3-8\) chronic heart failure\(^9-14\) and endothelial dysfunction.\(^11, 12, 15, 16\)

Allopurinol has been shown to improve mechano-energetic uncoupling in the myocardium during heart failure,\(^10,17-18\) which indicates that allopurinol reduces myocardial oxygen demand per unit of cardiac output. This suggests that xanthine oxidase inhibitors which
reduce oxygen consumption might be proved beneficial in patients with angina pectoris, but there are very few clinical studies in which this possibility has been investigated. In a recent clinical trial high-dose allopurinol has recently been shown to markedly prolong the time to appear chest pain and to ST-segment depression during exercise in patients with chronic stable angina.\textsuperscript{[19]} This might be associated to earlier experimental work directing that allopurinol somehow cuts down the myocardial oxygen consumption for a given stroke volume.\textsuperscript{[10,17-18]} Our study is an attempt to investigate whether allopurinol has any protective role in myocardial injury in patients with effort induced stable angina pectoris or not.

**MATERIAL AND METHODS**

Present study was conducted in L.P.S. Institute of Cardiology in collaboration with Department of Pharmacology & Therapeutics and Department of Biochemistry, GSVM Medical College, Kanpur after approval from institutional ethics review board. Present study carried out in accordance with the guidelines provided by Declaration of Helsinki. In this study we recruited 14 patients advised the treadmill test (TMT) from the outpatient department (OPD) as study group and 11 healthy volunteers as age-sex matched control group after obtaining proper informed consent from patients.

**Study group**

This group comprised of patients with a history of new onset effort angina and chest discomfort during TMT along with significant ST changes (horizontal or down sloping ST segment depression of 0.01 mV or more for 0.08 seconds).

**Inclusion criteria**

1. 35-60 years of age.
2. New onset effort angina.
3. Exercise stress test positive to ischemia (Angina plus significant ST depression).

**Exclusion criteria**

**Pertaining to TMT**

1. Acute myocardial infarction (within 3-5 days).
2. Unstable angina.
3. Infective endocarditis.
4. Symptomatic severe aortic stenosis.
5. Uncontrolled symptomatic heart failure.
6. Acute pulmonary emboli or pulmonary infarction.
7. Acute non-cardiac disorder that may affect exercise performance or be aggravated by exercise (e.g. infection; renal failure; thyrotoxicosis).
8. Physical disability that would preclude safe and adequate test performance.
9. Changes on resting ECG, precluding diagnosis of ischemia (e.g. left bundle branch block; Wolff-Parkinson-White syndrome).

Pertaining to alter free radical status
1. Hypertension
2. Diabetes mellitus
3. Drugs (e.g. propranolol; captopril; paracetamol)
4. Infections
5. Other conditions like chronic obstructive airway disease, hepatic disorders etc.

Control group
In this group we have included age and sex matched healthy subjects from medical, non-medical, and other staff.

All the cases and controls in the study were subjected to complete clinical examinations and routine investigations (Complete haemogram; Fasting and postprandial blood sugar; Serum urea and creatinine; Lipid profile; Thyroid profile; Liver function test and Resting 12 lead ECG).

Study design
Three group of population were studied:
1. Control group— included age-sex matched 11 healthy subjects. Blood sample was drawn for the estimation of Malonyldialdehyde (MDA), Super-oxide-dismutase (SOD), and Catalase.
2. Pre-therapy group— included 14 patients with new onset effort angina who had developed chest discomfort and significant ST depression during TMT and subjected to be treated with allopurinol. Total exercise duration, estimated maximum metabolic equivalents tasks (METs), percentage of target heart rate (THR) achieved and maximum ST depression has been noted. As soon as the ST segment started normalizing blood sample was drawn for the estimation of MDA, SOD and Catalase.
3. Post-therapy group— included 10 patients (as 4 patients were dropout from study group) of pre-therapy group in whom a repeat TMT has been performed after 2 weeks of oral allopurinol therapy (100 mg once a day for first week and 200 mg once a day for another week). Total exercise duration, METs, % THR achieved and maximum ST depression has been estimated and blood sample was drawn just after TMT for the assay of MDA, SOD and Catalase.

All patients were treated with recommended standard therapy for stable angina and allopurinol was used as ad-on therapy in test group.

**Exercise stress test**

A maximal treadmill exercise test was performed according to the standard Bruce-protocol. Exercise duration is usually symptom limited, and the exercise tests is discontinued upon appearance chest discomfort, ST segment ischemic depression >0.2 mV (2 mm) from the base line, or other generally accepted criteria like—

1. Drop in systolic blood pressure (>10 mmHg) despite an increase in workload.
2. Central nervous system symptoms (e.g. ataxia; dizziness or near syncope).
3. Signs of poor perfusion (cyanosis or pallor).
4. Serious arrhythmias.
5. Technical difficulties in monitoring the ECG or systolic BP.
6. Subject’s request to stop.

The positive ischemic ST-segment response generally is defined as flat or downsloping depression of the ST segment >0.1 mV (1 mm) below baseline (i.e., the PR segment) and lasting longer than 0.08 s. Upsloping or junctional ST-segment changes are not considered characteristic of ischemia and do not constitute a positive test. Although T-wave abnormalities, conduction disturbances, and ventricular arrhythmias that develop during exercise should be noted, they are also not diagnostic. Negative exercise tests in which the target heart rate (85% of maximal predicted heart rate for age and sex) is not achieved are considered nondiagnostic.\(^{[20]}\)

**Collection of blood sample and estimation of biochemical parameters**

The samples of 4.5 ml venous blood were drawn in disposable syringes containing 0.5 ml of 3.8 % sodium citrate solution. Samples were immediately transferred to test tubes and centrifuged at 800 rpm for 10 minutes. The supernatant plasma was separated and used for the estimation of Malonyldialdehyde (MDA). The packed RBCs were washed twice with
equal volume of normal saline and then centrifuged at 4000 rpm for 10 minutes at 4C. The washed RBCs were used for the estimation of Super-oxide-dismutase (SOD) and Catalase. Assay of MDA, SOD and Catalase was estimated with standard biochemical procedures.

**Statistical analysis**

Results were presented as Mean ± SEM and statistical significance of observed differences in the parameters between the various groups was determined by paired and unpaired student ‘t’ tests. p values < 0.05; p < 0.01 and p < 0.001 were considered respectively as significant; moderately significant and highly significant.

**OBSERVATIONS AND RESULTS**

In present study mean age of control and study group was 47.73 ± 5.18 and 48.28 ± 4.06 years respectively. Majority of subjects in both control (M=8; F=3) and study group (M=10; F=4) were males. Thus the both control and study group were comparable (age-sex matched).

**Biochemical parameters**

Mean plasma MDA levels in patients of angina before receiving allopurinol were found higher (p < 0.01; moderately significant) than healthy volunteers and after allopurinol therapy, moderately significant (p < 0.01) decrease in mean plasma MDA level was observed in same patients. (Table-1)

Mean SOD level observed before allopurinol therapy group was lower in comparison to SOD level observed in control group and the difference of means is highly significant (p < 0.001). In same patients after two weeks allopurinol therapy a highly significant (p < 0.001) increment in mean SOD level was observed in comparison to their pre-allopurinol-therapy mean SOD level. (Table-1)

There was no significant (p > 0.05) statistical difference noted in mean catalase levels of pre-therapy study group and control group. While after allopurinol therapy, moderately significant (p < 0.01) decline was observed in same angina patients. (Table-1)

**Exercise stress test related parameters**

In patients of effort induced angina, after receiving allopurinol mean walking time and mean estimated maximum METs increased significantly in comparison to pre-therapy values (p < 0.001; highly significant). (Table-2)
In post-therapy group mean maximum ST depression significantly (p < 0.05) reduced in comparison to pre-therapy one while no significant (p > 0.05) change was observed in mean of % THR after allopurinol therapy. (Table-2)

No adverse drug reaction was reported in study groups during study period.

**DISCUSSION**

On exercise stress test in patients of effort induced stable angina after allopurinol therapy we observed significant increase in mean walking time and mean estimated maximum metabolic equivalent of task (METs), significant decrease in mean maximum ST depression and no significant change in mean of % target heart rate (THR) in comparison to pre-therapy values. These observations indicate that endogenous xanthine oxidase activity imparts somehow to exercise-induced myocardial ischaemia (MI). These results also depict that allopurinol prolongs exercise tolerance in patients of stable angina pectoris.

It is widely evident that inadequate perfusion of a tissue/organ leads to oxygen (O₂) and adenosine triphosphate (ATP) depletion, and the accumulation of toxic metabolites. Another effect of hypoperfusion is the conversion of xanthine dehydrogenase to xanthine oxidase.
(XO), which upon reperfusion, catalyzes the conversion of hypoxanthine to xanthine with the concomitant production of ROS.\textsuperscript{[24]} XO is a source of superoxide, and although there are conflicting reports in the literature, XO activity has been demonstrated in human myocardium.\textsuperscript{[25]} Superoxide produced by XO interferes with intracellular signals that are important regulators of energy metabolism. For example, nitric oxide regulates enzymes involved in ATP production,\textsuperscript{[26]} high-energy phosphate storage via creatine phosphokinase,\textsuperscript{[27]} and energy consumption by cardiac myocyte calcium cycling.\textsuperscript{[28,29]} Superoxide can react rapidly with nitric oxide and may disrupt these signaling pathways, resulting in dysregulation of energy metabolism and a decrease in myocardial efficiency. In animal models of heart failure, various studies have shown that XO inhibition improves myocardial efficiency\textsuperscript{[17,30]} and enhances the contractile response of failing myocardium to dobutamine and to exercise.\textsuperscript{[18, 31]}

Biochemical parameters observed in this study suggest a role of endogenous xanthine oxidase in pathogenesis of exercise-induced myocardial injury. The level of MDA, which was used as the indicator of oxidative-stress, was found to be significantly elevated in patient of effort-induced stable angina as compared to healthy controls. This reflect that episodes of ischemia and reperfusion leads to generation of reactive oxygen species (ROS) which react with unsaturated lipids of cell-membrane ensuing membrane-lipido-peroxidation (MLP) to produce highly cytotoxic endoperoxides. As MDA is an intermediary product of MLP so increase in MDA level denotes enhanced MLP and oxidative-stress. In present study we also measured levels of SOD and catalase which served as parameters for antioxidant-defence. The level of SOD was found significantly decreased in patients of angina in comparison to healthy volunteers this might be due to consumption of SOD during the process of scavenging the ROS in settings of ischemic insult in patients of angina. But no significant change in level of catalase in patients of angina in comparison to healthy controls was found, which could be because (a) the degree of ischemic insult in stable angina is less than that in those of unstable angina or MI or (b) the level of catalase alter at a later period consequent to ischemia/reperfusion than do levels of MDA and SOD. These biochemical investigations establish that in patients of effort-induced stable angina, endogenous xanthine oxidase activity contributes to myocardial ischemic injury.

Allopurinol inhibits XO-derived reactive oxygen species generation that has been proposed to contribute to ischemic injury via ATP catabolism during hypoxia.\textsuperscript{[3,5,8]} Other proposed
mechanisms have been inhibition of lipid peroxidation,[6] heat shock factor expression,[32] calcium sensitizing[30] and the effect on the antioxidant status of the cells.[33]

So, a xanthin-oxidase-inhibitor (allopurinol and oxypurinol) may prove beneficial in settings of present study in exercise-induced stable angina. In present study on comparison of pre and post-allopurinol-therapy levels of biochemical markers just after exercise-stress-test, we noticed significantly decreased levels of MDA and catalase and significantly increased level of SOD in post-therapy patients than pre-therapy one. Reduced MDA level indicates decreased levels of ROS due to anti-oxidant effect of allopurinol and increased level of SOD suggests that allopurinol is successfully prevented the consumption of SOD in free radicals scavenging, by pulling down generation of ROS and/or by scavenging them. At first glance the significant reductions in post-therapy levels of catalase does not seems to be in synchrony with the anticipated finding that level of catalase should have increased, because an anti-oxidant (allopurinol) should enhance the levels of anti-oxidant enzymes by precluding their consumptions. The decreased activity of post-therapy catalase level (which was almost equal in pre-therapy patients as compared to healthy controls) could be due to its down-regulation because of enhanced protection against ROS by allopurinol and oxypurinol. Another remote possibility is that allopurinol and/or oxypurinol somehow inhibit catalase production. Above findings suggests antioxidant effect of allopurinol significantly decrease the oxidative stress in myocardium.

Allopurinol has been shown to improve endothelial dysfunction, reduce oxidative stress burden and improve myocardial efficiency by reducing oxygen consumption in smaller mechanistic studies.[34] Similarly our results also suggest that antioxidant properties of allopurinol may lead to significant increase in exercise tolerance and significant decrease in ST depression in post-allopurinol-therapy patients of stable angina. These results are explainable by the following possible mechanisms:

1. Reduced myocardial oxygen demand

Previous studies show that allopurinol can decrease myocardial oxygen expenditure for a particular stroke volume.[10,17,30] Because xanthine oxidase is known to use molecular oxygen to produce oxidative stress, and hence blocking the enzyme might prevent oxygen wastage and thereby increase the supply of molecular oxygen in ischemic tissue.[35-37]
2. Enhanced availability of ATP in ischemic myocardium

Substrates for ATP, such as AMP, are broken down ultimately by xanthine oxidase, and, as a result, inhibition of this enzyme augments high-energy phosphates, such as ATP, which should supply essential energy to tissues that are depleted of energy by ischaemia.\cite{37,38} Therefore, allopurinol could enhance oxygen and energy (ATP) within ischemic tissue.

3. Positive inotropic effect of allopurinol (without increasing O$_2$ demand)

Notably, short-term allopurinol administration exerted a positive inotropic effect without increasing myocardial oxygen consumption, indicating improved myocardial efficiency after allopurinol.\cite{39} Allopurinol improved myofilament calcium sensitivity as contraction force increased without a concomitant rise in systolic Ca$^{2+}$ influx. The effects were not seen in endothelial NOS deficient mice, suggesting a role for neuronal NOS preventing XO inhibition of cardiac excitation-contraction coupling.\cite{40} The finding that allopurinol is a potent myofilament Ca$^{2+}$ sensitizer, particularly in the setting of ischemia, is thought to be due to the inhibition of basal XO production. A previous study found an almost exclusive increase in force generation without a lowering of inward transient Ca$^{2+}$.\cite{30}

4. Decreased NOS-XO mediated myocardial ischemic injury and improved energetics

Under ischemic conditions, xanthine oxidase can reduce nitrite to generate NO. NO and peroxynitrite can inhibit pathways of oxygen radical generation, and, in turn, oxidants can inhibit NO synthesis from NOS. oxidants and free radicals are generated in the ischemic and reperfused heart, and are important mediators of postischemic injury. Both oxygen radical and NO formation are greatly increased through a series of interacting enzymes and cellular pathways. Nitric oxide generation is increased in the ischemic heart through both NOS dependent formation and NOS-independent nitrite reduction. It has become clear that there is a critical balance of oxygen radical formation and NO formation and their catabolism in normal myocardial physiology and signaling. In the setting of myocardial ischemia and reperfusion with the marked increases in oxygen radical and NO generation, this balance is disrupted resulting in oxidative injury.\cite{41} Moreover, the effect of allopurinol on oxygen and energy could be interlinked since increased oxygen consumption seems to occur especially when xanthine oxide is upregulated and nitric oxide synthase is downregulated.\cite{42} Nitric oxide synthase downregulation is a common situation within ischemic tissues and also one in
which allopurinol is liable to be particularly beneficial because it can favourably alter both enzymes.

5. **Improved coronary blood flow & reduced left ventricular afterload**

In the vascular endothelium, the xanthine oxidase (XO) system is one of the main producers of superoxide anions,\(^{[43]}\) since superoxide anions have been shown to inactivate nitric oxide\(^{[44]}\) and inhibit endothelium-dependent vasorelaxation.\(^{[45]}\) Therefore, XO inhibition with allopurinol prevents the formation of superoxide free radicals, which could lead to better endothelial function.\(^{[46]}\) Improved peripheral endothelial function,\(^{[11, 12, 16, 35, 47-50]}\) has two consequences. First, it might be matched by an improvement in coronary endothelial function, meaning that coronary microvascular flow might improve.\(^{[51]}\) Second, it is matched by a reduction in augmentation index, which means the left ventricular afterload is reduced.\(^{[14,52]}\) Unloading the heart is a common mechanism of action for antianginal drugs. Thus, the anti-ischaemic effect of allopurinol could be supplemented with improved coronary blood flow and reduced left ventricular afterload.

6. **Improved vascular endothelial function**

In the vascular walls, the enzyme systems involved in the production of free radicals include xanthine oxidase, NADPH oxidase and the endothelial nitric oxide (NO) synthase (eNOS). Because of its location, the endothelium is probably the prime target for ROS damage. The endothelium-dependent vasorelaxation activity is highly sensitive to IR injury. Elevated levels of ROS reduce the bioavailability of NO through reacting with NO to form peroxynitrite.\(^{[53]}\) The reduced NO availability aggravates local oxidative stress by the formation of peroxynitrite and a reduced blood flow due to decreased NO availability. ROS have also shown to disrupt the integrity of the endothelial cell junctions leading to increased endothelial permeability, tissue edema and protein leakage.\(^{[54]}\) Additional endothelial dysfunction related to ROS include production of proinflammatory cytokines, activation of complement system, decreased production of prostacyclin (PGI\(_2\)), increased production of platelet activation factor (PAF), and thromboxane A\(_2\) (TXA\(_2\)) and increased expression of adhesion molecules.\(^{[55]}\) Vascular oxidative stress is also a fundamental pathobiological manifestation of CAD, and allopurinol has been shown to improve vascular oxidative stress and endothelial function in patients with CAD.\(^{[56]}\)

Allopurinol might now be considered as a potential agent for angina. Beside its antianginal effect it has many advantages on other available antianginal drugs. Allopurinol is inexpensive
in comparison to newer antianginal agents like ranolazine and ivabradine. It is better tolerated
than older antianginal drugs (nitrates, β blockers) because it does not reduce the blood
pressure or the heart rate, and does not cause many side-effects, such as headaches and has a
favourable long-term (>40 years) safety data. More data are needed to compare and contrast
the use of allopurinol with other treatment options in patients with angina.\[^{19}\]

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**Competing interests:** No such.

**CONCLUSION**

Present study indicates towards potential of allopurinol in ameliorating free radical induced
myocardial injury and improving exercise tolerance in patient of ischemic heart disease. The
results of this study suggests that allopurinol is a viable alternative as an adjuvant therapy in
patients with effort induced stable angina with the advantage of being well tolerated, safe in
the long term, and inexpensive. Allopurinol may improve outcome in patients of ischemic
heart disease when used as ad-on therapy with existing standard anti-anginal treatment. Role
of allopurinol as anti-anginal agent needs to be probed further in larger clinical trials.

**REFERENCES**

1. Elahi MM, Kong YX, Matata BM. Oxidative stress as a mediator of cardiovascular


36. Mellin V, Isabelle M, Oudot A. Transient reduction in myocardial free oxygen radical levels is involved in the improved cardiac function and structure after long-term allopurinol treatment initiated in established chronic heart failure. Eur Heart J, 2005; 26: 1544–1550.


