King Saud University

Saudi Pharmaceutical Journal

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ORIGINAL ARTICLE

The possible antianginal effect of allopurinol in vasopressin-induced ischemic model in rats

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Abstract The anti-anginal effects of allopurinol were assessed in experimental model rats of angina and their effects were evaluated with amlodipine. In the vasopressin-induced angina model, oral administration of allopurinol in dose of 10 mg/kg revealed remarkably analogous effects in comparison with amlodipine such as dose-dependent suppression of vasopressin-triggered time, duration and severity of ST depression. In addition, allopurinol produced dose dependent suppression of plasma Malondialdehyde (MDA) level, systolic blood pressure, cardiac contractility and cardiac oxygen consumption; while in contrast, amlodipine minimally suppressed the elevation of plasma MDA level. Endothelial NO synthase (eNOS) expression, serum nitrate were strikingly increased, however lipid profile was significantly reduced. Seemingly, allopurinol was found to be more potent than amlodipine – a calcium channel antagonist. To conclude, it was explicitly observed and verified that on the ischemic electrocardiography (ECG) changes in angina pectoris model in rats, allopurinol exerts a significant protective effects, reminiscent of enhancement of vascular oxidative stress, function of endothelial cells, improved coronary blood flow in addition to the potential enhancement in myocardial stress. Moreover, our findings were in conformity with several human studies.

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1. Introduction

In majority of the epidemiological studies, a striking correlation of escalating levels of uric acid in serum was observed in addition to augmented cardiovascular event rate, furthermore the rise in the serum levels of uric acid was also found to be associated with increase in the mortality in individuals with recognized hazards of vascular disorders as well as normal healthy volunteers. Nevertheless, antioxidant properties

of uric acid are well known, and few preclinical and clinical studies proposed the protective effects of uric acid in neurodegenerative disorders. In contrast, considerable data exhibit to sustain the harmful and prothrombotic effects of xanthine oxidase, and this enzyme is well recognized as a significant cause of oxidative stress in the blood vessels, in addition to the implication of high levels of serum uric acid in the progress of cardiovascular disorders. Basically, xanthine oxidase is a group of enzymes, predominantly present in the liver, gastrointestinal tract, kidney and brain. Nevertheless, its presence is revealed all through the cardiovascular system (George and Struthers, 2008). Increased levels of proinflammatory cytokines and augmentation of ischemia were revealed by expression of xanthine oxidase and uric acid, Berry and Hare (2004) suggestive of their implication in the inflammatory response which is a distinctive feature of atherosclerosis. Moreover, increased oxygenation of LDL (De scheerder et al., 1991), and augmented release of the thrombolytic components such as 5HT, ATP and ADP were also observed with uric acid (Ginsberg et al., 1977). Xanthine oxidase enzymes can stimulate or initiate oxidative stress by virtue of their property to release free radicals of hydrogen oxide and hydrogen peroxide (Hille and Massey, 1981). The significant role of uric acid to enhance in vitro production was observed in rat vascular smooth muscle (Barberi and Mene, 2006). In addition to its correlation with endothelial dysfunction in hypertensive patients by means of its enhanced impact on nitric oxide formation in the macula densa (Mazzali et al., 2002; Saito et al., 1978; Dyer et al., 1999).

Fundamentally, allopurinol has a structural resemblance with hypoxanthine and is rapid metabolism to oxypurinol, and both of them work in a similar fashion. Their preferential binding to xanthine oxidase inhibits its activity (Elion, 1966). This in turn leads to lowering of both uric acid and xanthine oxidase mediated free radical formation. All these motivating findings have focused recent clinical research on the utilization of the xanthine oxidase inhibitors allopurinol and oxypurinol in the prevention of cardiovascular disorders.

Different studies of the inhibitory effects of xanthine have revealed that, inhibition of xanthine oxidase significantly reduced the levels of oxidative stress in the circulation in individuals with heart failure (Doehner et al., 2002), diabetes (Desco et al., 2002), metabolic syndrome (Yiginer et al., 2008), obstructive sleep apnea (El Solh et al., 2006), coronary artery disease (Eskurza et al., 2006), and liver disease (Vuppalanchi et al., 2011). Furthermore, blood pressure was improved in hypertensive individuals in response to xanthine oxidase inhibition (Feig et al., 2008). A noteworthy finding on reduction of "infarct size extension" was revealed in acute coronary syndrome on treatment with allopurinol, nevertheless explanation of this finding seems to be complex in view of methodological consideration (Parmley et al., 1992). Finally, a large retrospective study recommended a protective effect of high-dose allopurinol in comparison with low-dose treatment; interestingly this study revealed that low-dose treatment is as good as no treatment (Struthers et al., 2002). Therefore, the intention of this paper is to examine the possible antianginal effects of allopurinol compared with standard antianginal drug, amlodipine, on the ST segment depression of ECG, blood pressure, myocardial function, oxidative stress, eNOS expression, serum uric acid and lipid profile using ischemic rats' model with vasopressin.

2. Materials and methods

2.1. Animal

89 Male Wistar strain rats (8–10 week old) were purchased from the faculty of pharmacy, king Abdul Aziz University, Jeddah, Saudi Arabia. The animals were housed in animal house of King Fahd Medical Research Center, King AbdulAziz University at a controlled temperature of 23–26 °C under a 12 h light–dark cycle with free access to food and water. All animals received humane care in compliance with the ethical standards.

2.2. Drugs

Allopurinol and vasopressin (VP) were purchased from Sigma (St. Louis, MO, USA). Amlodipine Tablets 5 mg were purchased from Pfizer (Norvasc®; USA). For the oral administration allopurinol and amlodipine were dissolved in sterile water to a concentration of 10 mg/ml solution. For the intravenous administration vasopressin was diluted in saline 0.9% to 1 IU/ml solution. The dose of amlodipine was 10 mg/kg selected on the basis of a study conducted by (Sasaki et al., 2005). Vasopressin dose was 2 IU/kg depended on the result of pilot study. The doses of allopurinol were 35, 70, 105 mg/kg selected according to the human therapeutic dose by using conversion formula by (Freireich et al., 1966). Rats were weighed weekly and the dose adjusted accordingly.

2.3. Induction of myocardial ischemia

Induction of ischemia was conducted based on a method of (Hirata et al., 2005). Albino male rats were anesthetized using pentobarbital (60 mg/kg), and then placed on a heating pad to maintain temperature at 37 °C with backs down. Polyethylene I.V. cannula (26G × 19 mm) was inserted in the right lateral vein of the tail. After stabilization period following the completion of the cannulation of polyethylene I.V. cannula, vasopressin (VP) was intravenously injected at a dosage of 2 IU/kg through the same cannula. The ST-segment depression $\geqslant 1$ μv was considered as an index of myocardial angina (Ikeda et al., 2006), as shown in which recorded by the Powerlab system (Model Figure). The recorded signals should be free of electrical interference and noise. When the recording is over, the rats were given time to wake up and then returned to their cages.
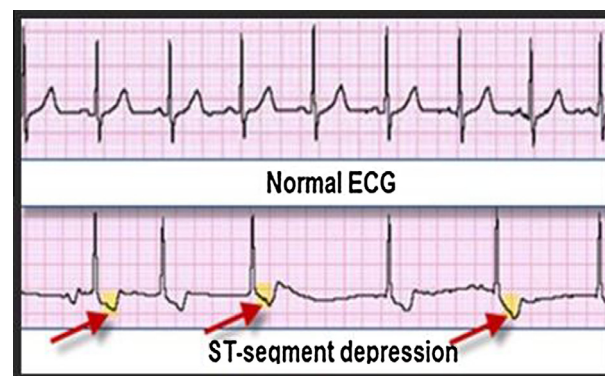

Figure 1 Induction of stable angina in rats.

Each test took about one hour including the time of anesthetic induction and recovery.

2.3.1. Measurement of the time to ST-Segment Depression, duration and severity

30 Male rats (weighing 250–350 g) grouped to 5 groups; control $n = 6$, allopurinol 35 mg/kg $n = 6$, allopurinol 70 mg/kg $n = 6$, allopurinol 105 mg/kg $n = 6$, amlodipine 10 mg/kg $n = 6$. All drugs and vehicles were administrated orally once daily for one month by gavage tube before the induction of angina by vasopressin (VP). Experiments were performed according to the method of Hirata et al. (2005)) and Yamamoto et al. (2002), using the ST-segment an index of myocardial ischemia ST-segment depression recorded by the Powerlab system, Wistar strain rats were anesthetized with pentobarbital (60 mg/kg, i.p.). Animals were placed on their backs on a heating pad maintained at 37^0C. The right lateral tail vein was cannulated with polyethylene (26G × 19 mm) I.V cannula. After an adequate stabilization period following completion of the cannulation, vasopressin (VP) was injected intravenously at the dosage of 2 IU/kg.

2.3.2. Noninvasive blood pressure (NIBP) measurement

Fifty male albino rats of average weight 250–350 g were used. Grouped into 5 groups; 10 rats each. Group I: non-treated rats, group II, III, IV: allopurinol-treated rats (31, 62, 93 mg/kg/day respectively) and group V: amlodipine-treated rats (10 mg/kg/day). All drugs and vehicles were administrated to the rats orally by gavages once daily for a period of four weeks. The rats were restrained in the MLA5024 rat Restrainer at least 5–10 min prior to obtaining pressure measurement. The tail was fully extended and exited through the rear hatch opening of the holder. Darkened nose cone into the rat restrainer to limit the animal's view and reduce the level of animal stress. Acclimatized rats will provide faster BP measurements than non-acclimatized animals. A nervous, stressed animal may have diminished circulation in the tail (Menard et al., 2010). Blood pressure was measured by Non-invasive Tail cuff method using the IN125/R NIBP System which was used in conjunction with a PowerLab system to obtain non-invasive blood pressure measurement from rats. NIBP utilizes a specialized tail cuff and pulse transducer to intermittently measure blood pressure based on the periodic occlusion of tail blood flow (Zhao et al., 2009). Normal blood pressures of all the rats were recorded as baseline blood pressure and were recorded again after drug treatment for four weeks.

2.3.3. Cardiac contractility

For this part of the experiment, twenty-eight rats grouped to 3 groups; control $n = 7$, allopurinol 70 mg/kg $n = 7$, amlodipine 10 mg/kg $n = 7$. All drugs and vehicles were administrated orally once daily for one month by gavage tube before the administration of vasopressin (VP) to induce angina as prescribed previous. Right carotid artery was catheterized with Microcatheter connected to Power-lab system to continuous record left ventricle contractility before and after vasopressin injection.

2.3.4. Plasma Malondialdehyde (MDA) level

18 Male Wistar strain rats were randomized in three experimental groups ($n = 5$ each). The first experimental group was given a small dose of allopurinol (35 mg/kg/day), second group was given intermediate dose of allopurinol (70 mg/kg/day) and the third experimental group was given a high dose of allopurinol (105 mg/kg/day). Drug treatment period for all experimental groups was 4 weeks. On 14, 21, 30 days of treatment the blood sera were obtained from the Retro orbital Puncture of rats from control group and from the three treatment groups. Plasma samples for measuring MDA were frozen immediately after sampling at −70 °C until further processing. The lipid peroxide content of the plasma studied by determining the thiobarbituric acid reactive substances (TBARS) for the estimation of malondialdehyde (MDA) content using Assay Kit (Catalog # KGE013).

2.3.5. Serum nitrate level

After four weeks of treatment blood sera from allopurinol and control groups were obtained using retro-orbital puncture in 10-ml vacationer heparinized tubes. Immediately after sampling, serum samples were frozen at a temperature of −70 °C for measuring nitrate using Colorimetric assay kit (Cayman Chemical Item Number 780001) in a simple two-step process. The first one is the conversion of nitrate to nitrite utilizing nitrate reductase. The second step is the addition of the Griess reagents which convert nitrite into a deep purple azo compound then measure the absorbance at 540 nm (Tsikas, 2007).

2.3.6. Endothelial Nitric Oxide Synthase (eNOS) Expression

In this part of experiment 20 Male Wistar strain rats were randomly assigned to a control group (vehicle, sterile water) or allopurinol group (70 mg/kg, dissolved in sterile water) administrated orally once daily for one month. Rats were sacrificed 24 h from the last dose, thoracic and abdominal aorta were

Table 1 Effect of daily oral administration of allopurinol versus amlodipine for four weeks on the onset of ST-segment depression and duration of vasopressin (2 IU/kg)-induced stable angina in an experimental albino rat.

Treated groups	Onset to ST-segment depression (seconds)		Duration of ST-segment depression (seconds)	
	Mean ± SEM	% Change	Mean ± SEM	% Change
Control (non-treated)	0	0	0	0
Control (vasopressin treated)	28.88 ± 1.302		385.38 ± 17.18	
Amlodipine 10 mg + vasopressin	48.50 ± 1.476[***]	68%	40.13 ± 6.53[***]	89.6%
Allopurinol 31 mg + vasopressin	32.75 ± 1.031[###]	13.4%	350.88 ± 15.27[###]	9%
Allopurinol 62 mg + vasopressin	39.63 ± 0.865[***,###]	37.2%	166.50 ± 6.86[***,###]	56.8%
Allopurinol 93 mg + vasopressin	43.88 ± 0.811[***,#]	52%	133.13 ± 6.44[***,###]	65.5%

Values are expressed as the mean ± SEM; $n = 10$ rats; [***]$P < 0.001$, compared with the corresponding control group values; [#]$P < 0.05$, [###]$P < 0.001$ compared with the corresponding amlodipine group values; by one-way ANOVA and Bonferroni post hoc test.

Table 2 Effect of daily oral administration of allopurinol versus amlodipine for four weeks on the severity of vasopressin (2 IU/kg)-induced angina in an experimental albino rats.

ST-segment height (μv)		Control (non-treated)	Treated groups				
			Control (vasopressin treated)	Amlodipine 10 mg + vasopressin	Allopurinol 31 mg + vasopressin	Allopurinol 62 mg + vasopressin	Allopurinol 93 mg + vasopressin
0.5 min after vasopressin injection	Mean ± SEM	0	107.4 ± 9.4	11 ± 3***	104 ± 11###	55 ± 9*,#	10.5 ± 2.5***
	% Change	0		89.7%	3.2%	48.8%	90.2%
1 min after vasopressin injection	Mean ± SEM	0	112.2 ± 12.	12.1 ± 6.3***	100 ± 5###	51.5 ± 8.5*,#	11.7 ± 6.8***
	% Change	0		89.2%	10.9%	54%	89.5%
3 min. after vasopressin injection	Mean ± SEM	0	106.4 ± 8.4	16.8 ± 6.5***	98 ± 5##	27.6 ± 12.5**	17.9 ± 7.6***
	% Change	0		84.2%	7.9%	74%	83.2%
6 min. After vasopressin injection	Mean ± SEM	0	89.5 ± 4.5	3.87 ± 2.87***	79 ± 1###	10.7 ± 1.8***	8.8 ± 4.2***
	% Change	0		95.7%	11.7%	88%	90.2%

Values are expressed as the mean ± SEM; $n = 10$ rats; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with the corresponding control group values; $^{#}P < 0.05$, $^{##}P < 0.01$, $^{###}P < 0.001$ compared with the corresponding amlodipine group values; by two-way ANOVA and Bonferroni post hoc test.

cut in segments (30 mg of each) and homogenized in liquid nitrogen using a mortar and pestle, Total RNA will be extracted by RNeasy® Fibrous Tissue Mini Kit (cat. no. 74704) QIAGEN according to manufacturer's instruction. The RNA was treated with RNase-free DNase to remove traces of genomic DNA contamination. Total RNA was then reverse-transcribed to cDNA using the High Capacity cDNA Reverse Transcription Kit and the target genes were amplified using the standard real-time PCR kit (Applied Biosystems, Foster City, CA, USA). The amplification was performed in real-time PCR system using Taqman probe. The fold induction/repression in gene expression by real-time RT-PCR was calculated after adjusting for actin using the formula $2 - \Delta\Delta Ct$. Primers used for amplification of respective gene were 5_-TTCCGGCTGCCACCTGATCCTAA-3_ and 5_ AACATGTGTCCTTGCTCGAGGCA-3_. The rat GAP-DH primers will be 5-_TCCCTCCAGATTGTCAGCAA-3_ and 5_AGATCCACAACGGATACATT-3.

2.3.7. Lipid profile

Blood samples were withdrawn at day 30 of treatment using retro-orbital puncture in 10-ml vacationer tubes. Serum was taken to determine serum uric acid level using colorimetric uric Acid Assay Kit (Catalog #K608 BioVision) according to the manufacture manual. Lipid profile (Total cholesterol, Triglyceride, High density lipoprotein and Low density lipoprotein) level was measured using an enzyme colorimetric test (Photometer BTS-303; Biosystems, Barcelona, Spain).

2.4. Statistical analysis

All data shown were representing as the mean ± S.E.M. The statistical significance of difference between mean value from control and treated groups were determined using SPSS (Statistical package of social sciences version 20). One way analysis of variance (ANOVA) and two-way analysis of variance (ANOVA) followed by Bonferroni post hoc test, were used for multiple comparisons. ($p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

3. Results

3.1. Induction of myocardial ischemia

Model Figure shows that vasopressin-induced stable angina model in an experimental albino rats based on a method of Hirata et al. (2005) (Fig. 1).

3.2. Effect of allopurinol on ST-Segment Depression time, duration and severity induced by vasopressin

Allopurinol in doses of (62 and 93 mg/kg/day) produced significant increase in the onset of ST-segment depression and significantly decreases in its duration compared to control (Table 1). Moreover, a significant decrease in the ST-segment height at time of 0.5, 1, 3 and 6 min after vasopressin injection was observed respectively (Table 2). However, a nonsignificant effect on the onset, duration and height of ST-segment depression of allopurinol dose (31 mg/kg/day) was observed compared to control group ($P > 0.05$). Furthermore, all allopurinol doses (31, 62 and 93 mg/kg/day) exhibited a significant decrease in the onset to ST-segment depression and a sig-

Table 3 Effect of daily oral administration of allopurinol versus amlodipine for four weeks on noninvasive blood pressure in an experimental albino rats.

Treated groups	Systolic blood pressure (SBP, mm Hg)	
	Mean ± SEM	% Change
Control (non-treated)	115 ± 0.55	
Control (vasopressin treated)	120 ± 0.55***	4%↑
Amlodipine 10 mg + vasopressin	104.20 ± 1.1***	9.5%↓
Allopurinol 31 mg + vasopressin	113.5 ± 0.81###	1.3%↓
Allopurinol 62 mg + vasopressin	110.4 ± 1.23**,###	4%↓
Allopurinol 93 mg + vasopressin	110.1 ± 0.9**,###	4.3%↓

Values are expressed as the mean ± SEM; n = 10 rats; **P < 0.01, ***P < 0.001, compared with the corresponding control group values; ###P < 0.001 compared with the corresponding amlodipine group values; by one-way ANOVA and Bonferroni post hoc test.

nificant increase in the duration of ST-segment depression respectively compared to amlodipine group (Tables 1 and 2). However, a nonsignificant difference except at 0.5 and 1 min after vasopressin injection was shown between allopurinol (62 and 93 mg/kg/day) and amlodipine.

3.3. Effect of allopurinol on noninvasive blood pressure (NIBP) measurement

Table 3 shows that allopurinol doses (62 and 93 mg/kg/day) produced significant decrease in systolic blood pressure compared with control. While a nonsignificant change in systolic blood pressure was observed with allopurinol (31 mg/kg/day). Moreover, systolic blood pressure was significantly decreased in amlodipine group compared with allopurinol and control groups.

3.4. Effect of allopurinol versus amlodipine on the Left Ventricular Functions (LVF) of vasopressin -induced stable angina

Administration of allopurinol (62 mg/kg/day) for four weeks significantly improved the vasopressin-induced reduction in myocardial contractility at time 0.5 and 1 min after vasopressin injection respectively and improved the vasopressin-induced reduction in max rate of LV pressure (dP/dt) 15 min after vasopressin injection (Table 4). Moreover, allopurinol produced significant decrease in heart rate at 0.5 min after vasopressin injection with% change of 17.8% versus vasopressin treated group. Furthermore, the difference in heat rate between allopurinol and amlodipine was nonsignificant (p > 0.05) at time 0.5, 1, 6, 15 min after vasopressin injection (Table 5). Finally in Table 6 allopurinol significantly decreased myocardial oxygen consumption (% change of 5.7%) and substantially increased mechanical efficiency (% change of 31.5%) compared with vasopressin and amlodipine treated group.

3.5. Effect of allopurinol on serum Malondialdehyde (MDA) level

Table 7 shows that the level of MDA significantly decreased after 21 days of treatment with allopurinol (62 and 93 mg/kg/day, p.o.) in comparison with baseline MDA level. This reduction of MDA level was extremely increased in all allopurinol treated groups (31, 62 and 93 mg/kg/day) after 30 days of the treatment.

3.6. Effect of allopurinol on serum nitrate level

We also sought to confirm that allopurinol (62 mg/kg/day) mediated vasodilatation (Table 8) demonstrates that administration of allopurinol for four weeks significantly increased serum nitrate level compared with vasopressin treated group with mean ± SEM of 14.52 ± 0.55 and mean% change of 42.6%.

3.7. Effect of allopurinol on vasopressin-induced aortic eNOS mRNA expression

Having established that allopurinol was able to regulate blood pressure and left ventricular functions we then sought to identify through which proteins this effect might be mediated (Table 9) show that the eNOS mRNA content in the aorta as expressed by $2 - \Delta\Delta Cp$ which significantly increased (% change of 71%) as compared to the vasopressin-treated groups.

3.8. Effect of allopurinol on the serum uric acid and lipid profile

We then sought after to determine whether these effects are linked also to uric acid and lipid concentration in the blood serum. Table 10 shows that a significant decrease in uric acid (UA), triglyceride (TG), Low density lipoprotein (LDL) levels respectively after four weeks of allopurinol (62 mg/kg/day) administration compared with control group with mean% change of 26.6%, 7.7%, and 51.2% respectively while no significant change in total cholesterol (TC) and high density lipoprotein (HDL) with mean% change of 0.5 and 0.9 respectively.

4. Discussion

In the present study, we have demonstrated that long-term treatment with step-dose allopurinol (31, 62 and 93 mg/kg/day) is equivalent to human therapeutics doses of 300, 600 and 900 mg/day respectively. Yet these doses, which are lower or equal a maximum dose (900 mg/day) allowed by British National Formulary (Noman et al., 2010), for more four weeks, have significantly prolonged time to develop ST – segment depression and suppressed effects on the duration and severity of ST-segment depression in vasopressin-induced ischemia model in rats compared with control group. Some of findings of our present study have been demonstrated in a few fundamental human works. In fact the American College

Table 4 Effect of daily oral administration of allopurinol versus amlodipine for four weeks on the myocardial contractility and (max dP/dt) of vasopressin induced angina.

		Control (non-treated)	Treated groups		
			Control (vasopressin-treated)	Amlodipine 10 mg + vasopressin	Allopurinol 62 mg + vasopressin
A. Myocardial contractility					
0.5 min. after vasopressin injection	Mean ± SEM	162.34 + 14	78.04 ± 12.3	130.37 ± 13	162.07 ± 15[**]
	% Change			74.4%	107.7%
1 min. after vasopressin injection	Mean ± SEM	155.14 + 11.2	71.24 ± 16.4	130.1 ± 18[*]	144.16 ± 21[*]
	% Change			82.6%	102.3%
6 min. after vasopressin injection	Mean ± SEM	160.02 + 13	117.4 ± 14.7	123 ± 16.1	154.2 ± 1
	% Change			4.8%	31%
15 min. after vasopressin injection	Mean ± SEM	148.38 + 16	117.7 ± 19.8	127.2 ± 21	143.1 ± 24.2
	% Change			8%	21.6%
B. The maximum rate of LV pressure rise (max dP/dt)					
0.5 min. after vasopressin injection	Mean ± SEM	12082.06 ± 65	8750 ± 1089	10887 ± 1098	10893 ± 1101
	% change			24.4%	24.5%
1 min. after vasopressin injection	Mean ± SEM	12104 ± 125	8584 ± 950	10684 ± 900	10801 ± 980
	% change			24.5%	25.6%
6 min. after vasopressin injection	Mean ± SEM	12263.401	11204 ± 288	11250 ± 300	11382 ± 305
	% change			0.4%	1.6%
15 min. after vasopressin injection	Mean ± SEM	12144.637	9185 ± 807	12360 ± 800	12762 ± 850*
	% change			34.6%	40%

Values are expressed as the mean ± SEM; $n = 7$ rats; [*]$P < 0.05$, [**]$P < 0.01$, compared with the corresponding control group values compared with the corresponding amlodipine group values; by two-way ANOVA and Bonferroni post hoc test.

Table 5 Effect of daily oral administration of allopurinol versus amlodipine for four weeks on the heart rate of vasopressin (2 IU/kg)-induced stable angina in an experimental albino rats.

Heart rate		Control (non-treated)	Treated groups		
			Control (vasopressin-treated)	Amlodipine 10 mg + vasopressin	Allopurinol 62 mg + vasopressin
0.5 min. after vasopressin injection	Mean ± SEM	353 ± 18	304 ± 17	300 ± 17	250 ± 28*
	% change			1.3%	17.8%
1 min. after vasopressin injection	Mean ± SEM	360 ± 14	286 ± 16	283 ± 17	252 ± 25
	% change			1%	11%
6 min. after vasopressin injection	Mean ± SEM	375 ± 14	292 ± 13	287 ± 12	251 ± 20
	% change			1.7%	14%
15 min. after vasopressin injection	Mean ± SEM	382 ± 12	296 ± 14	291 ± 13	251 ± 24
	% change			1.7% ×	15%

Values are expressed as the mean ± SEM; n = 7 rats; * $P < 0.05$, compared with the corresponding control group values compared with the corresponding amlodipine group values; by two-way ANOVA and Bonferroni post hoc test.

of Cardiology guidelines on chronic angina mention allopurinol as a possible antianginal drug based on this Lancet paper (Noman et al., 2010). Secondly, an explanation for this anti-ischemic effect in human comes from other study which shows that allopurinol reduces LV volumes especially LV end systolic volume (Halcox et al., 2002). This illustrates that allopurinol off-loads the heart and offloading the heart is a common way by which the antianginal drugs work. Furthermore, our results are in accordance with other study, which has revealed that allopurinol reduced troponin release during ST-elevation myocardial infarction (Rekhraj et al., 2013). The magnitude increase in median time to ST depression with allopurinol seems to be quite similar to that noted in other studies with other anti-anginal drugs such as amlodipine 36 s (13%) (Knight and Fox, 1998) and 60 s (11%) with nitrates (Knight and Fox, 1998), 12–47 s (4–14%) with phosphodiesterase inhibitors (Thadani et al., 2002), 46 s (13.5%) with Ivabradine (Tardif et al., 2009), and about 50 s (15%) with Atenolol and Ranolazine (Rousseau et al., 2005). In current study the absolute increase in median time to ST depression with allopurinol was 43 s (19% increases).

The potential mechanism of the antianginal effect of allopurinol needs to be explained and substantiated in the light of several relevant observations, the capability of allopurinol to reduce myocardial oxygen consumption for the stroke volume (Ekelund et al., 1999). This can be explained on the basis of decrease in oxidative stress, as xanthine oxidase is well recognized to utilize molecular oxygen to produce oxidative stress, and therefore blockade of this enzyme prevents oxygen expenditure, which in turn amplifies the molecular supply of the oxygen in ischemic myocardium (Mellin et al., 2005).

Moreover, Saavedra et al. (2002) elucidated that oxidative stress by itself could directly produce an anti- ischemic effect. Xanthine oxidase breaks down substrates for ATP, such as AMP; hence inhibition of this enzyme enhances ATP a high energy phosphate which is essential to provide energy to depleted ischemic tissues. This is suggestive of using allopurinol as a medication that can increase the oxygen and high-energy phosphates (ATP) in ischemic cardiac tissue. Besides, we have shown that allopurinol significantly decreases systolic blood pressure suggesting that the elevation of serum uric acid level contributes to vasopressin induced angina in rats.

This finding was in resemblance with other study which associates escalating levels of uric acid in the serum with concomitant augmented cardiovascular event rate and mortality in those individuals with recognized or high risk of vascular disease (Alderman, 2002; Forman et al., 2007; Stull et al., 2004; Khan et al., 2008). Remarkably several studies had linked uric acid in the genesis of hypertension and the use of allopurinol alone in recently diagnosed hypertensive adolescent has led to correction of blood pressure (Sathisha et al., 2011).

Our left ventricular function data offer interesting clues regarding the other possible mechanisms. Our study had demonstrated that, allopurinol produced a significantly improved vasopressin-induced reduction in myocardial contractility after ischemia induction, and in max dp/dt (as an indicator of LV contractile performance), decreased heart rate, decreased myocardial oxygen consumption and substantially increased mechanical efficiency (as an indicator of work done by heart). These results are consistent with the ability of XO inhibitors to improve myocardial efficiency (Max dp/dt)/MVO2) by acting as calcium sensitizers in vitro since the improvement in myo-

Table 6 Effect of daily oral administration of allopurinol versus amlodipine for four weeks on the myocardial oxygen consumption (MVO2) and cardiac efficiency in vasopressin (2 IU/kg)-induced angina in albino rats.

Treated groups	Mean ± SEM	% Change
	A. Myocardial oxygen consumption (MVO2)	
Control (non-treated)	60788 ± 112	
Control (vasopressin-treated)	61198 ± 148	
Amlodipine 10 mg + vasopressin	60792 ± 108	0.66%
Allopurinol 62 mg + vasopressin	57681 ± 257[***,###]	5.7%
	B. Cardiac efficiency	
Control (non-treated)	0.19 ± 0.102	
Control (vasopressin-treated)	0.19 ± 0.027	
Amlodipine 10 mg/kg/day	0.20 ± 0.055[**]	5.3%
Allopurinol 62 mg/kg/day	0.25 ± 0.062[***,###]	31.5%

Values are expressed as the mean ± SEM; $n = 7$ rats; [**]$P < 0.01$, [***]$P < 0.001$, compared with the corresponding control group values; [###]$P < 0.001$ compared with the corresponding amlodipine group values by two-way ANOVA and Bonferroni post hoc test.

cardial oxidative stress enhances myofilament responsiveness to activator calcium and this in turn enhances the impaired myocardial energy utilization which is a characteristic feature of heart failure (Laursen et al., 2001). It needs to be highlighted that both acute and chronic administration of allopurinol augments contractility of myocardium in experimental animals with heart failure, with simultaneous reduction in myocardial oxygen consumption (Saavedra et al., 2002).

A possible explanation to this effect, could be attributed to superoxide generated with xanthine oxidase obstructs with intracellular signals which are essential regulators of energy metabolism, a prototype depicting nitric oxide regulates the enzymes implicated in ATP formation and storage through creatine phosphokinase, and consumption of energy by cardiac myocyte calcium cycling. Furthermore, rapid reaction of superoxide (O_2-) with nitric oxide could also interrupt these signaling conduits, and eventually leads to disturbance of energy metabolism with ultimate reduction in efficiency. Moreover, nitric oxide (NO) and superoxide (O_2-) act synergistically to form peroxynitrite ($ONOO-$) (Beckman and Koppenol, 1996). Thus, it is possible that enhanced production of both NO and O_2- in angina contributes to the generation of $ONOO-$, this may explains the significant decline in cardiac contractility in control group compared with allopurinol group after ischemia induction in the present study. Another study in support of this finding, proposed that allopurinol diminishes the augmentation index indicating a reduction of left ventricular afterload (Lockette et al., 1986).

The rationalization for implication of xanthine oxidase is substantially clear by the finding of our study in which allopurinol has been shown to remarkably decreased oxidative stress, in all treated group after 30 days of treatment. These results indicate that xanthine oxidase is a major source for this oxidative stress due to decreased xanthine oxidase induced oxidative stress in treated groups by allopurinol. Vascular oxidative stress is quite distinguished as an essential pathobiological expression of Coronary artery disease. Our observation may perhaps well contribute mechanistically to the anti-ischemic action of allopurinol in pathogenesis of angina. In reality, xanthine oxidase is well recognized to utilize enormous amount of oxygen by its ability to produce superoxide anions and hydrogen peroxide in addition to the fact that the main xanthine oxidase substrate (hypoxanthine) has only one oxygen/molecule,

while on the contrary its main xanthine oxidase product, uric acid, has three oxygen/molecule (Harrison, 2002).

It is well known that in atherosclerosis, diabetes mellitus, heart failure and microthrombus formation, endothelial dysfunction is regarded as the main early characteristic feature. Therefore, the present study exclusively affords the earliest direct authentication that the chronic use of allopurinol in rats resulted in Upregulation of thoracic aortic eNOS mRNA and subsequent increases in serum nitrate bioavailability. However, the mechanisms underlying the increased expression of eNOS in response to allopurinol shown in the present study remain uncertain. Many studies have demonstrated that the degradation of NO by ROS in many animal models of hypertension, diabetes and heart failure (Harrison, 2002). There are several potential mechanisms contribute to decline in NO bioavailability e.g., reduction in eNOS expression, reduction in eNOS activity, and rapid degradation in NO by reactive oxygen species (ROS) (Tseng, 2004).

The downregulation of nitric oxide synthase is quite illustrious in ischemic tissues; allopurinol seems to be essentially beneficial due to the fact that it can satisfactorily alter both enzymes. Seemingly, the distinct effect of allopurinol to reduce the vascular oxidative stress is relatively exciting, how far this could be transformed into clinical vascular protection is still not clear, and needs further crucial studies. The correlation of raised levels of uric acid in serum and coronary artery disease is well documented in several studies (Suliman et al., 2006). Furthermore, it is also revealed that alteration in uric acid abundantly effects the lipid levels, this in turn transforms the cardiovascular outcomes attributable to deranged endothelial dependent vasodilatation by virtue of lipid oxidation.

A large number of studies have highlighted on the risk of metabolic syndrome, in contrast very few of them attempted to focus the relationship involving uric acid and lipids (Shelmadine et al., 2009). Our present study has demonstrated that the effect on serum lipid profile (SLP) on chronic administration of allopurinol for four weeks, significant fall in serum triglyceride (TG) and low density lipoprotein (LDL) was observed, whereas no significant alteration was detected in serum total cholesterol (TC) as well as high density lipoprotein (HDL).

The elucidation of the mechanism of beneficial lipid regulation by the uric acid seems to be controversial, nevertheless

Table 7 Effect of daily oral administration of allopurinol versus amlodipine for four weeks on serum malondialdehyde level in an experimental albino rats. *** $P < 0.001$, compared with the baseline values; by two -way ANOVA and Bonferroni post hoc test. Data are expressed as the mean ± SEM; n = 10 rats.

Malondialdehyde level (μm)		Treatment groups				
		Control (non-treated)	Amlodipine 10 mg/kg/day	Allopurinol 31 mg/kg/day	Allopurinol 62 mg/kg/day	Allopurinol 93 mg/kg/day
Baseline (day 0)	Mean ± SEM	0.490 ± 0.013	0.485 ± 0.01	0.494 ± 0.011	0.506 ± 0.009	0.491 ± 0.019
	% Change	→				
Fourteen days after treatment	Mean ± SEM	0.484 ± 0.009	0.493 ± 0.008	0.486 ± 0.009	0.482 ± 0.007	0.469 ± 0.016
	% Change	1.2% ↓	1.6% ↓	1.6% ↓	4.7% ↓	4.5% ↓
Twenty days after treatment	Mean ± SEM	0.488 ± 0.009	0.489 ± 0.009	0.453 ± 0.012	0.416 ± 0.008 ***	0.381 ± 0.017 ***
	% Change	0.4% ↓	1.8% ↓	8.3% ↓	17.8% ↓	22.4% ↓
Thirty days after treatment	Mean ± SEM	0.482 ± 0.012	0.493 ± 0.007	0.405 ± 0.011 ***	0.322 ± 0.010 ***	0.268 ± 0.016 ***
	% Change	1.6% ↓	1.6% ↓	18% ↓	36.4% ↓	45.4% ↓

proposed mechanism incorporates inhibition of lipid peroxidase and reduction in decisive activity of lipoprotein lipase, an essential enzyme linked together with lipolysis of triglycerides as well as oxidation of LDL-C (Sasaki et al., 2005; Jelic-Ivanovic et al., 2007). The present results are also in agreement with findings obtained by other investigators who have shown that there was no association between TC and HDL and uric acid level (Beltrame et al., 2009). Notwithstanding, there is still a dilemma, either uric acid is a cause or an outcome or merely an indicator of cardiovascular disease (Fraker et al., 2007). Further exploration is required to determine the potential role of uric acid in the genesis of ischemic heart disease. A substantial reduction in quality of life is attributed to chronic stable angina, since one out of three patients develops angina no less than once a week (Beltrame et al., 2009). Furthermore, several patients are still incapable to fulfill the guideline aspiration of the American College of Cardiology and American Heart Association of total absence of exertional angina episodes (Fraker et al., 2007).

Consequently, innovative treatments are required, allopurinol could now be considered as a safe potential alternative drug for angina. It has several advantages in comparison with other recently added drugs such as Ranolazine and Ivabradine. It has a significant safety profile of more than 40 years for the treatment of gout. In contrast to established old drugs for angina (nitrates and β receptor antagonists), allopurinol seems to be better tolerated by virtue of insignificant effect on blood pressure and heart rate in addition to lack of other collateral effect such as headache and tiredness which are frequently observed with nitrates and β blockers. Therefore, more data are needed to compare and contrast the use of allopurinol with

Table 8 Effect of daily oral administration of allopurinol (62 mg/kg/day) for four weeks on serum nitrate level in an experimental albino rats.

Treated groups	Serum Nitrate level (μmol/ml)	
	Mean ± SEM	% Change
Control (non-treated)	12.08 ± 0.53	
Control (vasopressin-treated)	10.18 ± 0.81	
Allopurinol 62 mg/kg/day	14.52 ± 0.55 ***	42.6%

Values are expressed as the mean ± SEM; n = 6 rats; ***$P < 0.001$ compared with the corresponding control group values; by unpaired t-test.

Table 9 Effect of daily oral administration of allopurinol for four weeks on the expression of endothelial nitric oxide synthase (eNOS) gene in experimental albino rats.

Treated groups	Expression of endothelial nitric oxide synthase (eNOS) $2^{-\Delta\Delta Ct}$	
	Mean ± SEM	% Change
Control (non-treated)	0.605 ± 0.05	
Control (vasopressin-treated)	0.525 ± 0.03	
Allopurinol 62 mg/kg/day	0.897 + 0.03 ***	71%

Values are expressed as the mean ± SEM; n = 10 rats; ***$P < 0.001$ compared with the corresponding control group values; by unpaired t-test.

Table 10 Effect of daily oral administration of allopurinol for four weeks on serum uric acid and lipid profile levels in an experimental albino rats.

		Treated groups		
		Control (non-treated)	Control (vasopressin-treated)	Allopurinol 62 mg/kg/day
Uric acid (mg/dl)	Mean ± SEM	1.45 ± 0.22	1.62 ± 0.28	0.79 ± 0.12[*]
	% Change			51.2% ↓
Total cholesterol (TC) (mg/dl)	Mean ± SEM	119.52 + 2.2	119.4 ± 2.36	118.8 ± 2.02
	% Change			0.5% ↓
Triglyceride (TG) (mg/dl)	Mean ± SEM	63 ± 1.23	65 ± 1.03	60 ± 1[**]
	% Change			7.7% ↓
High density lipoprotein (HDL) (mg/dl)	Mean ± SEM	54.98 ± 1.31	54.96 ± 1.28	55.46 ± 1.35
	% Change			0.9% ↑
Low density lipoprotein (LDL) (mg/dl)	Mean ± SEM	11.87 ± 1.20	13.54 ± 1.04	9.94 ± 0.60[*]
	% Change			26.6% ↓

Values are expressed as the mean ± SEM; $n = 6$ rats; [*]$P < 0.05$, [**]$P < 0.01$ compared with the corresponding control group values; by unpaired t-test.

other established treatment options in patients with angina. Moreover, potential side effects of allopurinol were not studied in the present studies and further research is needed. In view of the findings of our study, allopurinol seems to be quite valuable option for the treatment of angina, with a distinct advantages of well compliance, safety and economical. Further exploration is required for defining the status of allopurinol in the management of angina e.g., the plasma renin activity to elucidate the effect of allopurinol on blood pressure. Moreover, it may be valuable to use transgenic or knockout mice model of a xanthine oxidase to exclude the direct effect of uric acid. Moreover, molecular mechanism of action suggested in our study needs further studies for precise authentication. In order to confirm our results additional large prospective, randomized, placebo-controlled studies are required. The limitation is that the present study demonstrated only the data at the acute phase by the 15 min after vasopressin injection, therefore, the effect of allopurinol on chronic myocardial damage in rats and ST segment depression should be evaluated.

Disclosures

None.

Acknowledgment

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