

Urotensin II in the Pathogenesis of Atherosclerosis in Cholesterol Fed Rats

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ABSTRACT

Background: Atherosclerosis is the most common cause of ischemic heart diseases. Urotensin II is the most potent vasoactive peptide discovered to date with potency that overcomes that of angiotensin II, endothelin-1, serotonin and thromboxan A2.

Aim: This study was undertaken to evaluate the role of exogenous urotensin II in the pathogenesis of atherosclerosis and the effect of blocking the endogenous urotensin II.

Material and Methods: 32 male wistar rat were divided into 2 groups, normal control group (8 rats) and cholesterol rich diet group (24 rats). The latter is furtherly subdivided into vehicle, urotensin II and palosuran groups (each is 8 rats). In all these groups lipid profile, some inflammatory and atherosclerotic markers were measured. Histopathological examination of tissue samples from aorta and coronaries for atheromatous changes was also done in all groups. **Results:** Urotensin II produced significant hyperlipidemia, increased CRP and SVACM-1 and decreased NO. Foam cells and VSMC proliferation were evident histopathological findings. The effect of urotensin II was partially reversed by palosuran. **Conclusion:** urotensin II is an important mediator in the pathogenesis of atherosclerosis in hypercholesterolemic model. Urotensin receptor blocker may be considered as an important therapeutic target in atherosclerosis.

INTRODUCTION

Atherosclerosis, the primary cause of ischemic cardiomyopathy and ultimately a major contributing factor to death, is strongly correlated with hypertension, hyperlipidemia and type II diabetes mellitus. Urotensin II (UII) is the most potent vasoactive peptide identified to date with a potency that overcomes other vasoactive agents like endothelin-1 (ET-1), angiotensin II, serotonin and thromboxane A₂⁽¹⁾. UII is cyclic peptide of 11 amino acids sharing structural similarity to somatostatin and binds to a class of G-protein coupled receptor known as GPR14 or

recently named urotensin receptor (UT)⁽²⁾. UII/UT complex has been found to be elevated in vascular endothelial dysfunction-related diseases such as essential hypertension, atherosclerosis, coronary artery disease and congestive heart failure⁽³⁾. UII is highly expressed in endothelial cells, monocytes, macrophage-derived foam cells, myointima and vascular smooth muscle cells (VSMCs) of atherosclerotic human coronary artery using immunohistochemistry, in situ hybridization and reverse transcriptase-polymerase chain reaction (RT-PCR)⁽⁴⁾.

Palosuran (ACT-058362; 1-[2-(4-benzyl-4-hydroxy-peperidin-1-yl)-ethyl]-3-(2-methyl-ginolin-4-yl)-urea sulfate salt) is a non peptidic competitive UT receptor antagonist that binds to two populations of UII binding sites. To date, palosuran is the first UT receptor antagonist that has been tested in human⁽⁵⁾.

The aim of the present work is to study the role of chronic exogenously administered UII in the pathogenesis of atherosclerosis in high cholesterol fed rats and the effect of blocking endogenously expressed UII by palosuran.

MATERIAL & METHODS

This study was carried out on 32 male wistar rats, collected randomly weighing 200 ± 50 gm body weight. These animals were housed under 12 hours day/night cycle at room temperature. They were classified into 2 groups.

I- Control group (8 rats): Fed standard chow and tap water.

II- Cholesterol rich diet group (24 rats): fed 2% cholesterol +3% coconut oil enriched diet for 8 weeks (Winlals Company for Pharmaceuticals)⁽⁶⁾. Then this group was furtherly subdivided into 3 subgroups, each is 8 rats.

- Subgroup IIa (vehicle group): received vehicle (saline 0.9% + NH₄OH 0.01%) i.v. daily for 4 weeks.
- Subgroup IIb (urotensin II group): received a bolus daily dose of UII (3nmol/kg/day) i.v. in vehicle for 4 weeks⁽⁷⁾ (Sigma Aldrich Co.).
- Subgroup IIc (Palosuran

group): received a bolus daily dose of palosuran (10 mg/kg/day) i.v. for 4 weeks⁽⁵⁾ (Sigma Aldrich Co.)

- At the end of the experiment, all animals were deprived of food over night, sacrificed in the morning and blood samples were collected in EDTA-coated tubes and plasma was separated and stored until analysis for:

(1) Lipid profile parameters:

1. Low density lipoprotein-cholesterol (LDL-C): by method of Fruchart⁽⁸⁾.
2. High density lipoprotein-cholesterol (HDL-C) by method described by Burstein et al,⁽⁹⁾.
3. Plasma triglycerides (TG): Using the method of Fossati⁽¹⁰⁾.
4. Plasma total cholesterol (total Ch.): determined by method of Allain et al.,⁽¹¹⁾.

(2) Atherosclerotic and inflammatory markers which include:

1. Nitrite/nitrate (marker for NO) by colorimetric technique according to the method of Bartholomew,⁽¹²⁾.
2. C-reactive protein (CRP) by Kushner and Sommerville⁽¹³⁾.
3. Soluble vascular cell adhesion molecule-1 (SVCAM-1): measured by immunosorbent assay (ELISA) using commercially available kit⁽¹⁴⁾.

(3) Histopathological examination of tissue samples from aorta and coronaries for atheromatous changes by (Hx & E).

Statistical analysis:

Data were presented as mean values \pm SD and analysis of results

using one way ANOVA. Differences between individual groups were determined with Scheffe test. Results were considered significant at $P < 0.05$ using SPSS computer program version 16.

RESULTS

Table (1), Fig. (1) show that LDL-C, TG and total cholesterol significantly increased after high cholesterol diet while HDL-C significantly decreased when compared to normal control group ($P < 0.05$).

UII group showed significant increase in LDL-C, TG and total cholesterol and significant decrease of HDL-C when compared to either high cholesterol diet or UII groups ($P < 0.05$).

Palosuran group led to significant decrease of LDL-C, TG and total

cholesterol and significant increase in HDL-C when compared to high cholesterol diet rats ($P < 0.05$).

As shown in table (2), fig. (2) there is significant decrease in NO after high cholesterol diet alone or together with UII ($P < 0.05$) and significantly increased after palosuran treatment when compared to control or UII groups ($P < 0.05$).

Concerning CRP, it is significantly increased in high cholesterol fed rats and UII treatment ($P < 0.05$) but significantly decreased after palosuran treatment ($P < 0.05$) as shown in table (2), fig. (3).

As regard SVCAM-1, it is significantly increased after high cholesterol diet alone or together with UII treatment ($P < 0.05$) and significantly decreased after palosuran treatment ($P < 0.05$) as shown in table (2), fig. (4).

Table (1): Plasma level of lipid profiles among studied groups.

	<i>Control</i> (No. 8)	<i>Vehicle</i> (No. 8)	<i>Urotensin II</i> (No. 8)	<i>Palosuran</i> (No. 8)	<i>F</i>	<i>Relation</i>
LDL-C mg/dl	98.25±6.446	177.20±7.145	197.11±6.143	129.86±18.300	138.51*	Control < vehicle < urotensin II > palosuran
HDL-C mg/dl	24.86±1.35	18.85±0.805	13.74±1.13	25.41±1.67	148.97*	Control > vehicle > urotensin II < palosuran
TG mg/dl	124.05±2.63	160.79±5.20	189.68±5.76	148.43±6.09	228.93*	Control < vehicle < urotensin II > palosuran
Total Ch mg/dl	98.14±4.54	160.51±8.13	189.99±7.04	130±7.13	267.13*	Control < vehicle < urotensin II > palosuran

* Significant $P < 0.05$.

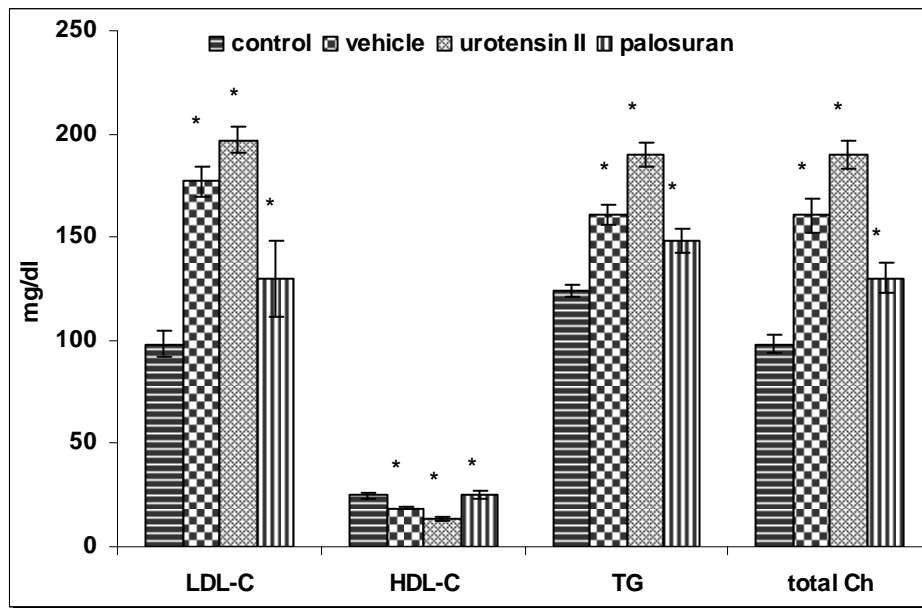


Fig. (1): Plasma level of lipid profile among studied groups.

Table (2): Plasma level of atherosclerotic and inflammatory markers among studied groups.

	Control (No. 8)	Vehicle (No. 8)	Urotensin II (No. 8)	Palosuran (No. 8)	F	Relation
Nitrite/nitrate nmol/ml	25.14±1.68	15.10±1.81	10.10±1.81	20.73±1.83	108.13*	Control > vehicle >urotensin II <palosuran
CRP mg/l	5.11±0.51	7.11±0.51	9.53±0.58	5.31±0.62	108.44*	Control < vehicle <urotensin II >palosuran
SVCAM-1 ng/ml	25.16±2.40	33.81±2.60	47.39±5.39	30.50±5.51	39.95*	Control < vehicle <urotensin II >palosuran

* Significant P<0.05.

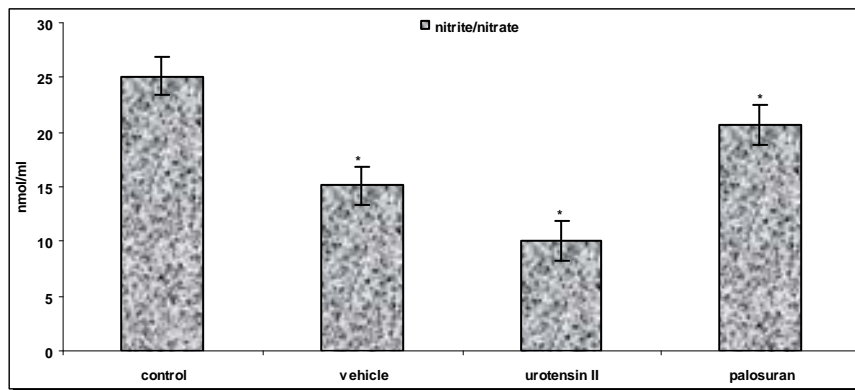


Fig. (2): Plasma level of nitrite/nitrate among studied groups

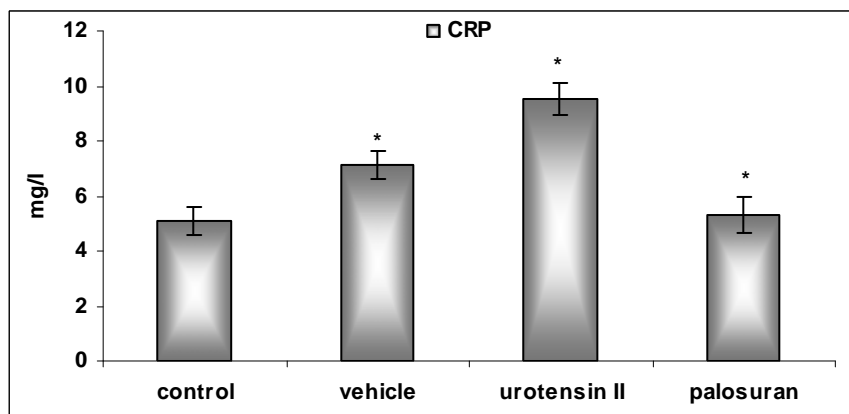


Fig. (3): Plasma level of CRP among studied groups.

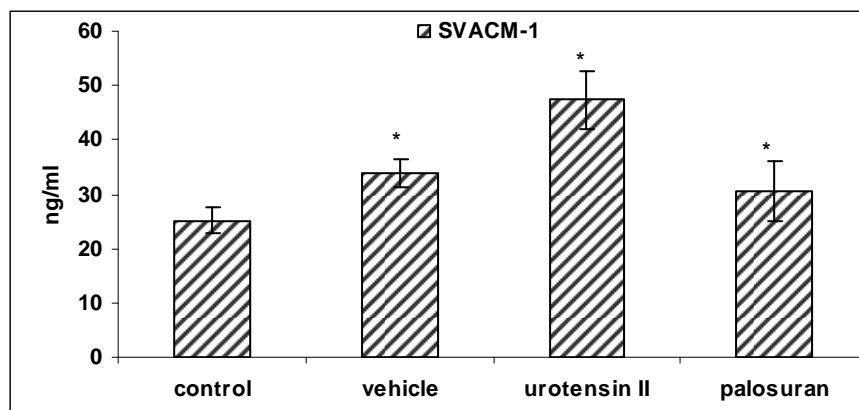


Fig. (4): Plasma level of SVACM-1 among studied groups.

Histopathological findings:

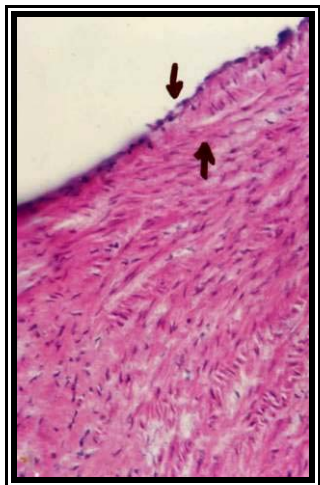


Fig. (5) Photomicrograph of section in aorta of control rat showing normal endothelial lining, normal subendothelial intima and normal musculature (Hx & E. x 250).

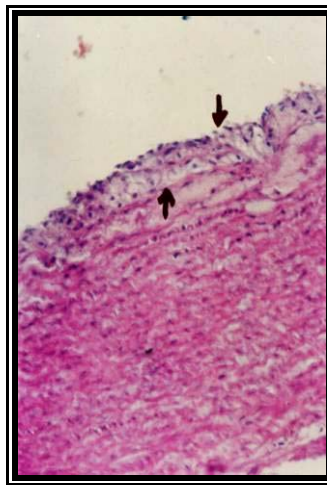


Fig. (6) Photomicrograph of section in aorta of hyperlipidemic rat showing destruction of musculo-elastic layer with multiple foci of intimal and subintimal foamy cells (Hx & E. x 250).

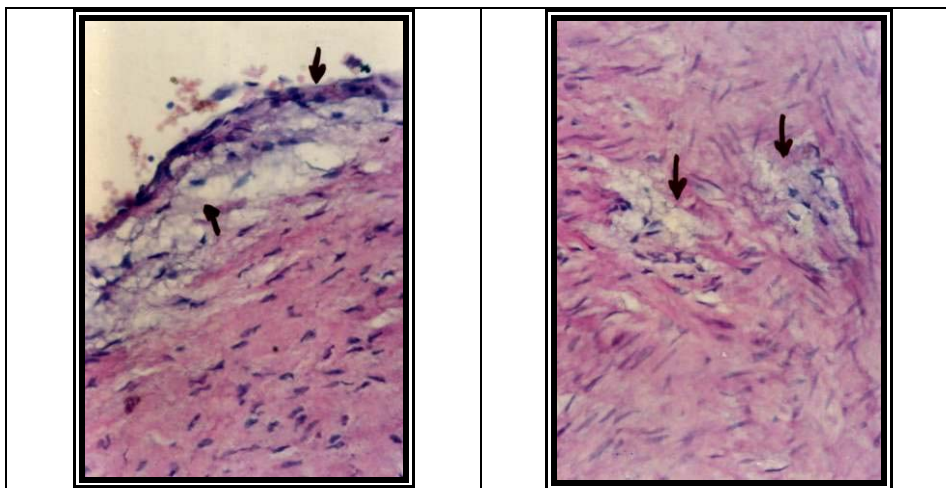


Fig. (7): Photomicrograph of sections from coronaries (A) and aorta (B) of hyperlipidemic rats treated with UII showing: (A) early atheromatous plaque showing patchy endothelial denudation, medial degeneration, with multiple foci of foamy cells (Hx & E. x 250). (B) deeper sections from the aorta, showing patchy areas of degeneration studded with foamy cells, and proliferating vascular smooth muscle cells (VSMCs) (Hx & E. x 400).

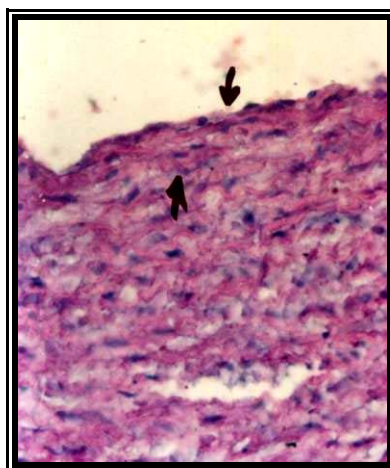


Fig. (8) Photomicrography of section in aorta of hyperlipidemic rat treated with palosuran showing apparently normal histopathological structure (non-denuded endothelium, no visible gross plaques with diffuse minute hyalinization in the intima without foam cell accumulation) (Hx & E. x 400).

DISCUSSION

The results of the present study suggest a prominent role of UII in mediating atherosclerosis in high cholesterol fed rats as evidenced from hyperlipidemia (increased LDL-C, TG and total Ch. and decreased HDL-C), inflammatory and atherosclerotic markers (decreased NO, increased CRP and SVCAM-1) and histopathological findings (atheromatous plaques of foam cells and VSMC proliferation).

UII may help induction of hyperlipidemia by enhancing the activity of depot lipase and channel of glucose to free fatty acid synthesis. Hyperinsulinemia and insulin resistance caused by UII may also be a cause of hyperlipidemia^(15,16).

Many mechanisms may serve as causal links between hyperlipidemia and many of the major pathways responsible for atherogenic disorders.

UII acts in synergy with mildly oxidized LDL (mox LDL) to promote generation of oxidative stress in vasculature via activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase⁽¹⁷⁾. Stimulation of (mox LDL) and reactive oxygen species (ROS) by UII stimulates acyl-coenzyme A: cholesterol acyl transferase-1 (ACAT-1) which stimulates esterification and storage of free cholesterol as cholesterol esters in lipid droplets and hence formation of foam cells from monocyte-derived macrophages. UII also increases expression of scavenger receptors (CD₃₆ and scavenger receptor class A)⁽¹⁸⁾.

Stimulation of monocytes with lipopolysaccharides (LPS) increases UT receptor mRNA and protein expression. There is also evidence that UII acts as chemoattractant for UT receptor-expressing monocytes via activation of Rho A/Rho kinase

signaling cascade and actin cytoskeleton reorganization. At the same time, cloning and functional characterization of the human UT receptor gene promoter revealed the presence of NF-Kappa B-binding sites involved in the stimulation of UT receptor gene expression by LPS⁽¹⁹⁾. UII also stimulates CRP, (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) expression in human endothelial and VSMCs which act as chemoattractant for UT receptor expressing monocytes⁽²⁰⁾.

Endothelial dysfunction may be due to impaired nitric oxide (NO) synthesis and/or inactivation of endothelium-derived NO by ROS⁽²¹⁾. Endothelial dysfunction complicates hypertension and is the precursor of atherosclerosis. Many studies stated the rise of UII blood levels in patients with essential hypertension and atherosclerosis and explained its pivotal role in induction of VSMC contraction and proliferation^(21,22).

The mechanism of UII-induced VSMC contraction may be increased ET-1 expression or through activation of Rho A/Rho kinase pathway and phosphorylation of myosin light chain (MLC)⁽²³⁾. Activation of phospholipase-C dependant IP₃ leading to increased cytosolic calcium ions and activation of protein kinase C (PKC) may also be involved^(24,25).

UII acts in synergy with oxLDL to induce VSMC proliferation through generation of ROS which activate mitogen activated protein kinase and protein kinase B (MAPK/PKB (Akt)), the latter activate plasminogen activator inhibitor (PAI-1) in VSMC⁽²⁶⁾. Activation of Rho A/Rho kinase pathway also stimulates

DNA synthesis in VSMCs at highest rate among other vasoactive agents assessed by measuring [3H] thymidine incorporation into DNA⁽²⁷⁾. C-Src tyrosine kinase/PKC/MAPK is another pathway⁽²⁸⁾. UII stimulates phosphorylation and hence transactivation of epidermal growth factor receptors (EGFR)⁽²⁹⁾.

UII stimulates proliferation and migratory capacity of endothelial progenitor cells (EPCs) from bone marrow via activation of Rho A/Rho kinase pathway which phosphorylates MLC of EPCs. This is supported by the high expression of UT receptors in the EPCs^(7,30). This provides new insights into role of UII in atherosclerosis not only by inducing collateral formation but also by promoting vasculogenesis and angiogenesis. UII also was found to stimulate vascular endothelial growth factor (VEGF)⁽³⁾.

In the fact of existing results showing the role of palosuran even without exogenous UII to reduce lipid profile, inflammatory and atherosclerotic markers and also improved the atherosclerotic lesions in histopathological examination. This is another evidence for the role of endogenously expressed UII in the pathogenesis of atherosclerosis. These results are agreed with many other studies^(18,31). This may provide a new therapeutic strategy for treatment of atherosclerosis.

CONCLUSION

UII/UT receptor complex is an important mediator in atherosclerosis by inducing hyperlipidemia, inflammatory and oxidative stress.

Foam cell formation and VSMC proliferation are important histopathological features. UT receptor blockers may provide a promising therapeutic strategy against atherosclerosis and ischemic heart diseases.

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اليوروتنسين-٢ كمتسبب لتصلب الشرايين في الفئران المغذاة بالكوليسترول

الهدف من البحث:

هو معرفة التأثير المحتمل لليوروتنسين-٢ في حدوث وتطور تصلب الشرايين في الفئران المغذاة بالكوليسترول وأيضاً معرفة تأثير العقار المضاد لليوروتنسين الموجود داخل الجسم.

طريقة البحث:

أجرى هذا البحث على ٣٢ من الفئران الذكور قسمت إلى مجموعتان:

- المجموعة الأولى: مجموعة ضابطة عامة (عدد ٨).
- المجموعة الثانية: المغذاة بالكوليسترول لمدة ٨ أسابيع (عدد ٢٤). ثم قسمت إلى ٣ مجموعات متساوية كل منها (عدد ٨).
- المجموعة الثانية أ: أخذت محلول ملح مع هيدروكسيد الأمونيوم لمدة ٤ أسابيع.
- المجموعة الثانية ب: أخذت اليوروتنسين -٢ لمدة ٤ أسابيع.
- المجموعة الثانية ج: أخذت بالوسران لمدة ٤ أسابيع.
- وفي نهاية التجربة أخذت البلازما لهذه الفئران وحللت لمعرفة:
 ١. نسبة الدهون (دهون ذات كثافة منخفضة وعالية وكوليسترول كلى ودهون ثلاثية).
 ٢. النيترت والنيترات.
 ٣. تفاعل البروتين - سى.
 ٤. الجزيئات الخلوية اللاصقة.
 ٥. بالإضافة لدراسة هستوباثولوجية لأنسجة الأورطى والشريان التاجى لكل المجموعات.

النتائج:

اليوروتنسين أحدث زيادة ذات دلالة إحصائية في نسبة الدهون ذات الكثافة المنخفضة والدهون الثلاثية والكوليستيرول الكلى ونقص ذو دلالة إحصائية في الدهون ذات الكثافة العالية أيضاً سبب زيادة ذات دلالة إحصائية في تفاعل البروتين سى والجزيئات الخلوية اللاصقة ونقص ذو دلالة إحصائية في النيترت والنيترات مع وجود خلايا دهنية وتكاثر في خلايا العضلات في الدراسة الهستوباثولوجية ، كل هذه التأثيرات قد عكست جزئياً بعقار البالوسران.

الاستنتاج:

ظهرت هذه النتائج أن لليوروتنسين-٢ تأثير فعال في إحداث وتطور تصلب الشرايين في الفئران المغذاة بالكوليسترول وأن عقار البالوسران له تأثير فعال في تحسين حالة تصلب الشرايين ويمكن أن يستخدم كعلاج لتصلب الشرايين في المستقبل.