

# Potential Therapeutic Value of Palosuran in Renovascular Hypertension and Associated Cardio-vascular and Renal Complications

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## Abstract

Hypertension is the most common chronic disease in the world and produces substantial morbidity and mortality. Individuals with uncontrolled hypertension are at high risk of stroke, coronary artery disease leading to myocardial infarction, or acute renal failure. Nowadays, treatment of hypertension still remains a topical problem due to the side effects of existing antihypertensive drugs. In recent years, interest in the cyclic vasoactive neuropeptide urotensin-2 (U-II), which is characterized by strong vasoconstrictor properties, has been increased. Increased secretion of U-II and excessive expression of UT-II receptors have been identified in several pathologies, including hypertension. Using U-II/UTR antagonists remain an attractive target in cardiovascular and renal system pathology. We were aimed to study effect of the UTR antagonist - Palosuran on blood pressure, cardio-vascular system and renal tissue in rats with hypertension (2 kidneys+1 clip). Palosuran was administered intraperitoneally (10 mg/kg once daily, during 4 weeks). Blood pressure was measured non-invasively, using “tail cuff” method. Studies have shown that in hypertensive rats Palosuran reveals hypotensive effect, decreases workload on the myocardium and reduces risk of complications. Effect of treatment is better expressed at early stage of hypertension.

**Keywords:** Hypertension; Palosuran; cardio-vascular system; kidney.

## 1. Introduction

Hypertension is the most common chronic disease in the world and produces substantial morbidity and mortality.

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Individuals with uncontrolled hypertension are at high risk of stroke, coronary artery disease leading to myocardial infarction, or acute renal failure. Patients with hypertension and heart failure (HF) frequently have reduced kidney function. The links between the kidneys and cardiovascular system are being elucidated, with blood pressure (BP) being a key risk factor. In large HF registries, 20–68% of patients with hypertension and associated HF have moderate to severe kidney disease. The main goal of studying hypertension etio-pathogenesis in animal models is to foster the development of improved approaches to preventing and treating high blood pressure and its complications.

Urotensin II (U-II) is a cyclic oligopeptide with vasoactive potential. It is currently one of the most potent vasoconstrictor known in mammals. The kidney is the major source of U-II in humans and animal species. The role of the U-II system in human physiology/pathophysiology is not yet fully understood. Increased plasma U-II concentrations have been associated with hypertensive disease, chronic heart failure and diabetes. Elevation in U-II endogenous tone has also been recently demonstrated to contribute to the deterioration of renal function in rats [1].

Because of its potent vasoconstrictor effect, U-II attracted the interest of researchers in general hemodynamic. In fact, hemodynamic responses to U-II show regional heterogeneity in relation to its receptor localization, even in the differences of functional state of the endothelium.

U-II is a potent vasoconstrictor, but has some vasodilating properties in specific vascular beds, notably in nondiseased blood vessels via enhanced NO activity. The central action of U-II, in part mediated via the autonomic nervous system, leads to increased cardiac output and resultant higher BP. U-II is a constrictor of large resistive vessels. Conversely, it relaxes mesenteric vessels. U-II also plays a role in body fluid regulation, decreases glomerular filtration and increases renal sodium retention.

U-II action is brought about via activation of G-protein-coupled receptor 14 (GPR14). U-II increases inositol phosphate turnover and intracellular  $Ca^{++}$ . By activation of UTR, U-II might influence different pathways, depending on the cells and vascular compartment where the receptor is located.

UTR is expressed within the renal tubules, cardio vasculature (vascular smooth muscle, endothelium, myocardium etc.) and may, therefore, contribute to the physiological and pathophysiological regulation of cardiovascular homeostasis in humans. UTR are located peripherally on vascular smooth muscle and endothelial cells. Activation of UTR on vascular smooth muscle cells leads to contraction via RhoA/Rho-kinase activation (contractile responses) and activation of the endothelial UTR, leads to relaxation via NO formation (dilatory responses). In the kidney, UTR influences sodium and water homeostasis and glomerular filtration rate [2].

Studies have shown that U-II is upregulated in hypertension and UTR stimulation in the kidney, influences sodium and water homeostasis and glomerular filtration rate. U-II produces mesenteric vasodilation and portal hypertension in rats with secondary biliary cirrhosis [3]. Iontophoresis of U-II in healthy volunteers produces vasodilation (of the forearm), while in patients with heart failure or hypertension a constriction is observed.

Increased U-II expression in disease states has prompted the development of a number of UTR antagonists. There are preclinical and some clinical studies showing potential benefits of inhibiting U-II/UTR function in hypertension, heart failure, renal disease and diabetes that could be achieved using UTR antagonists.

One of the UTR antagonists – Palosuran (ACT-058362; 1-[2-(4-benzyl-4-hydroxy-piperidin-1-yl)-ethyl]-3-(2-methyl-quinolin-4-yl)-urea sulfate salt), in experimental studies revealed cardio-renal and metabolic protection in rat models of both ischemic and diabetic nephropathy [4]. Palosuran increased renal perfusion, glomerular filtration rate, renal sodium excretion, and improved renal function in cirrhotic rats supposedly due to direct effect of UTR blockade at the tubules and glomeruli. It lowered portal pressure via splanchnic vasoconstriction in these rats via activation of mesenteric vascular Rho-kinase and an inhibition of NO/cGMP-dependent PKG signaling.

Studies in other disease conditions are eagerly awaited. There are epidemiologic data to support a relationship between U-II and human essential hypertension. In a study of 62 individuals with hypertension and 62 age-matched normotensive controls, plasma U-II levels were significantly higher in the hypertensive group and correlated positively with the systolic blood pressure. In addition, U-II excreted in urine was significantly higher in patients with essential hypertension than in normotensives and in patients with hypertensive renal disease compared to normotensive patients with renal disease [5]. In our preliminary studies conducted on rats with renovascular hypertension Palosuran revealed antihypertensive effect compared to control.

Because the U-II/UTR is involved in cardiovascular system regulation UTR antagonism remains an attractive target in cardiovascular and renal system pathology and using U-II/UTR antagonists supposedly could have clinical potential and therapeutic benefit in patients with hypertensive disease and associated complications.

Coming from the aforesaid, we considered interesting to investigate effects of UTR antagonist - Palosuran on blood pressure, heart, kidney and mesenteric blood vessels in laboratory rats with renovascular hypertension.

## **2. Material and methods**

The study was performed on 32 male Wistar rats weighing 200-250 g. after an adaptation period of at least 1 week. All rats were housed in the lab as a group of eight per cage in climate-controlled conditions with a 12-h light/dark cycle and free access to normal pelleted rat chow and drinking water.

The protocol used in this study for the use of rats as the animal model for research was overseen and approved by the Tbilisi State Medical University Animal Welfare and Use Ethics Committee (N39 - 17/08/2019).

For experimental modelling of hypertension we used the reno-vascular (the two-kidney, one-clip - 2K1C) H. Goldblatt model. After separation of the renal artery from the vein and nerve the clip was placed on the left renal artery close to the aorta under general anaesthesia (Nembutal - 50 mg / kg).

The experimental animals were divided into 4 groups: Group I - healthy, intact rats; Group II - hypertensive rats; Group III - hypertensive rats, subjected to treatment with palosuran, started after 4 weeks of disease modelling;

Group IV - hypertensive rats, subjected to treatment with palosuran, started after 8 weeks of disease modelling. Palosuran was injected intraperitoneally with the dose of 10 mg/kg, daily, during 4 weeks.

Blood pressure was measured non-invasively, using the systolic blood pressure measurement system ("tail cuff" method) once a week for 12 weeks.

Blood samples were obtained by jugular venous puncture after ketamine/xylazine anesthesia. Serum creatinine was measured using spectrophotometric method. The method explores the oxidation of p-methylamino phenol sulfate (Metol) in the presence of copper sulfate and creatinine which yields an intense violet colored species with maximum absorbance at 530 nm. Plasma renin concentration (PR) was measured using ELISA.

For evaluation of mesenteric arteries anaesthetized rats were placed on a heating plate at 37°C and a lateral laparotomy was performed. A short segment (2 cm) of the proximal part of the small intestine and mesentery was carefully pulled out. A segment of a branch of the mesenteric artery with the corresponding vein was placed into the reservoir filled with a physiological solution (PS). The other exteriorized tissue was kept moist using bandages soaked in PS. The arterial diameter was evaluated using microscopy.

Heart and kidney samples for morphological investigations were stained with hematoxylin-eosin dye. All statistical tests were conducted using IBM SPSS Statistics. Differences between control and treated animals were determined by using the Independent-Samples T test. The criterion for significance was set to  $p < 0.05$ .

### **3. Results**

In experimental rats at different stages of the reno-vascular hypertension changes in mean arterial pressure (MAP) was detected compared to MAP of the group I animals (intact, healthy rats).

Results of experiment (Table 1) have shown that after 1 week of disease modelling, MAP was not increased significantly, after 2 weeks - MAP increased by 24% ( $p < 0.05$ ), after 4 weeks, MAP increased by 42% ( $p < 0.02$ ), after 8 weeks there was a significant increase in MAP by 44% ( $p < 0.02$ ) and after 12 weeks of disease modelling, MAP was increased by 53% ( $p < 0.001$ ) compared to MAP of the group I animals;

In healthy rats after administration of Palosuran MAP decreased by 33% ( $p < 0.02$ ). In hypertensive rats on the 8<sup>th</sup> week of hypertension MAP was reduced by 32% ( $p < 0.001$ ) compared to the control, untreated hypertensive rats and on the 12<sup>th</sup> week of hypertension, Palosuran revealed relatively less effect on MAP than at treatment started earlier. However, MAP was still reduced significantly by 23% ( $p < 0.02$ ) compared to control group (untreated, hypertensive rats).

**Table 1:** Mean arterial pressure in healthy, hypertensive untreated and Palosuran-treated rats.

Rats	Mean arterial pressure (mm/Hg)	
	Without treatment	+ Palosuran
Intact, healthy rats	95±3,1	64±3,0**
1st week after renovascular hypertension	97±3,5	-
2nd week after renovascular hypertension	118±4,1*	-
3rd week after renovascular hypertension	101±9,2	-
4th week after renovascular hypertension	135±10,0**	-
8th week after renovascular hypertension	137±8,3**	93±5,5***
12th week after renovascular hypertension	145±10,0***	112±7,2**

\*p<0.02, \*\*p<0.05, \*\*\*p<0.001

In hypertensive rats plasma renin (PR) concentration was increased progressively compared to the data of healthy rats. In palosuran-treated hypertensive rats PR was significantly lower than in untreated hypertensive rats. After 1 week of disease modeling increase in PR by 4% was not statistically significant. After 2 weeks, PR was increased by 45% (p<0,01); After 3 weeks it was increased by 42% (p<0,01); After 4 weeks PR decreased and it was not statistically different compared to the norm, but after 8 weeks, PR was increased by 162% (p<0,001) and after 12 weeks, it was sharply increased by 234% compared to data of healthy rats.

Palosuran administration in healthy rats slightly decreased PR by 12% (p>0,05). After 8 weeks of disease modeling in hypertensive rats treatment with palosuran decreased PR by 33% (p<0,01) and after 12 weeks, PR was decreased by 24% (p<0,01) compared to data of untreated hypertensive rats.

The serum creatinine level (SC) in rats after 4 weeks of disease modeling was not changed. SC on 8<sup>th</sup> week of hypertension was increased by 22,7% (p<0,05) and on 12<sup>th</sup> week of hypertension - by 45,4% (p<0,01).

In healthy rats after administration of Palosuran changes in SC were not statistically significant. In treated with Palosuran group animals after 8 weeks of disease modeling SC was decreased by 26% (p<0,05) and after 12 weeks of disease modeling, in palosuran-treated hypertensive rats, decrease in SC by 9,4% was not statistically significant (Table 2).

**Table 2:** Renin concentration and creatinine in healthy, hypertensive untreated and Palosuran-treated rats.

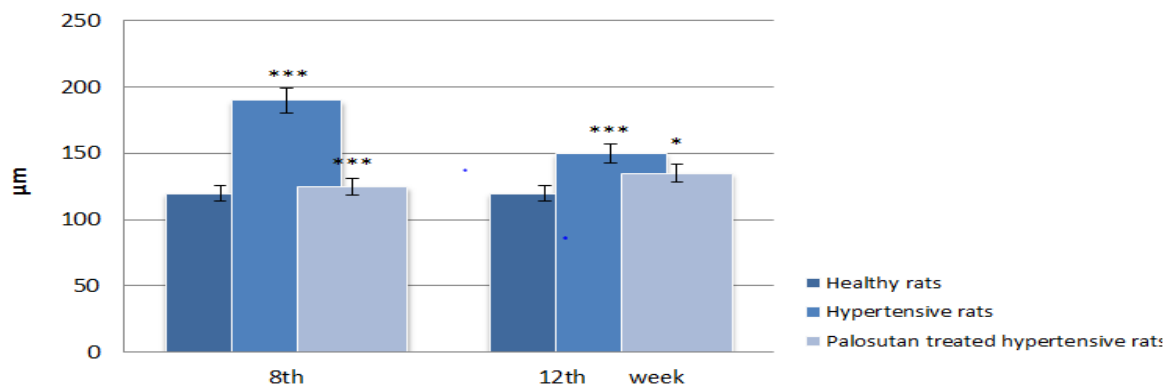
Rats	Without treatment		+ Palosuran	
	Renin(ng/ml)	Creatinine (mg/dL)	Renin (ng/ml)	Creatinine (mg/dL)
Intact, healthy rats	1,72±0,5	0,22±0.03	1,52±0,3	0,21±0.01
1st week of hypertension	1,79±0,3	-	-	-
2nd week of hypertension	2,49±0,4**	-	-	-
3rd week of hypertension	2,45±1,3**	-	-	-
4th week of hypertension	1,94±0,1	0,23±0.05	-	-
8th week of hypertension	4,5±1,4***	0,27±0.04*	3,02±0,9**	0,20±0.01*
12th week of hypertension	5,75±1,5***	0,32±0.03**	4,39±1,5**	0,29±0.02

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001

The diameter of mesenteric arteries (MA) in untreated hypertensive rats after 8 weeks of disease modeling were increased by 58% (p<0,001) and by 25% (p<0,01) on 12<sup>th</sup> week of hypertension compared to data of healthy rats.

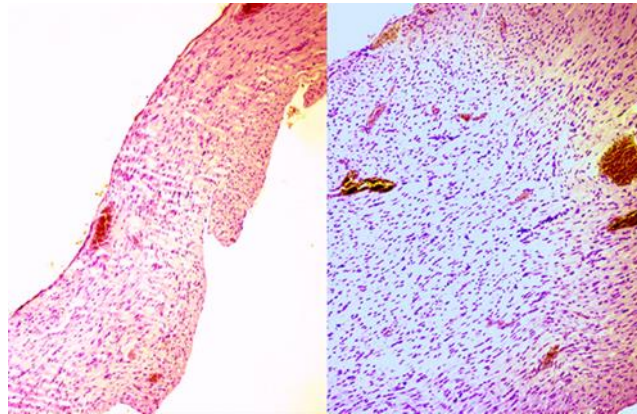
The diameter of MA in Palosuran treated hypertensive rats after 8 weeks of disease modeling were decreased by 34,2% (p<0,001) and by 23% (p<0,05) on 12<sup>th</sup> week of hypertension compared to data of untreated hypertensive rats.

In Palosuran-treated hypertensive rats diameter of MA were not changed significantly compared to data of healthy rats. By the 8<sup>th</sup> week of disease modeling increase in MA by 4% and on 12<sup>th</sup> week of hypertension decrease by 5% was not significant.



**Figure 1:** Diameter of mesenteric arteries in healthy, untreated and Palosuran-treated rats at different stages of reno-vascular hypertension.

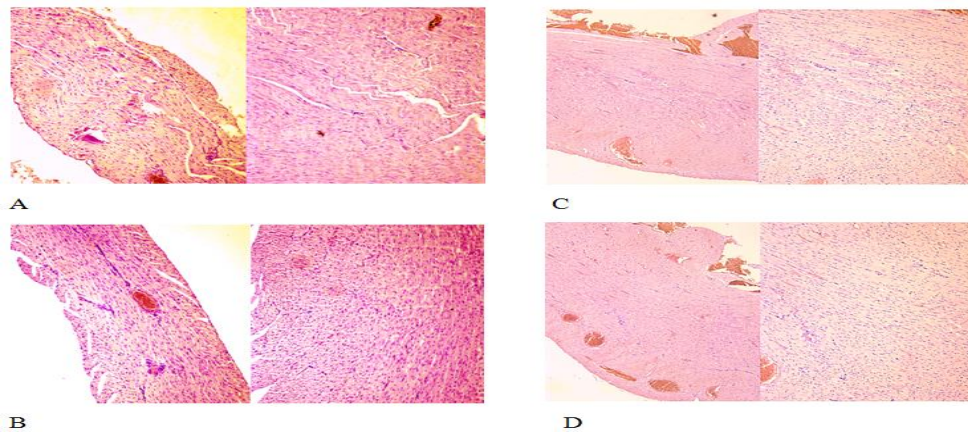
According to the results of morphological investigations the healthy rats left ventricular size was 1,0mm, right - 0,2 mm; cardiomyocytes were within the norm (picture 1).



**Figure 2:** Myocardial tissue of healthy rat. Staining H&E; x150. x250

After 8 weeks of disease modeling, in untreated hypertensive rats there was a slightly expressed left ventricular hypertrophy. Left ventricular size was 1,4mm, right - 0,21mm. Cardiomyocytes were within the norm. After 12 weeks of hypertension there was a well-expressed left ventricular hypertrophy. Left ventricular size was 2.0mm, right - 0,22mm.

In palosuran-treated rats by 8 weeks of hypertension myocardial hypertrophy was not detected. Left ventricular size was 1,07mm, right - 0,19mm. Slightly expressed hypertrophy was observed by 12<sup>th</sup> week of hypertension. Left ventricular size was 1,12mm and right - 0,21mm (picture 2).



**Figure 3:** Myocardial tissue of healthy, untreated and Palosuran-treated rat at different stages of renovascular hypertension. H&E, x150. x250

**A-** Myocardial tissue of hypertensive rat (8<sup>th</sup> week);

**B -** Myocardial tissue of hypertensive rat (12<sup>th</sup> week);

**C -** Myocardial tissue of Palosuran-treated hypertensive rat (8<sup>th</sup> week);

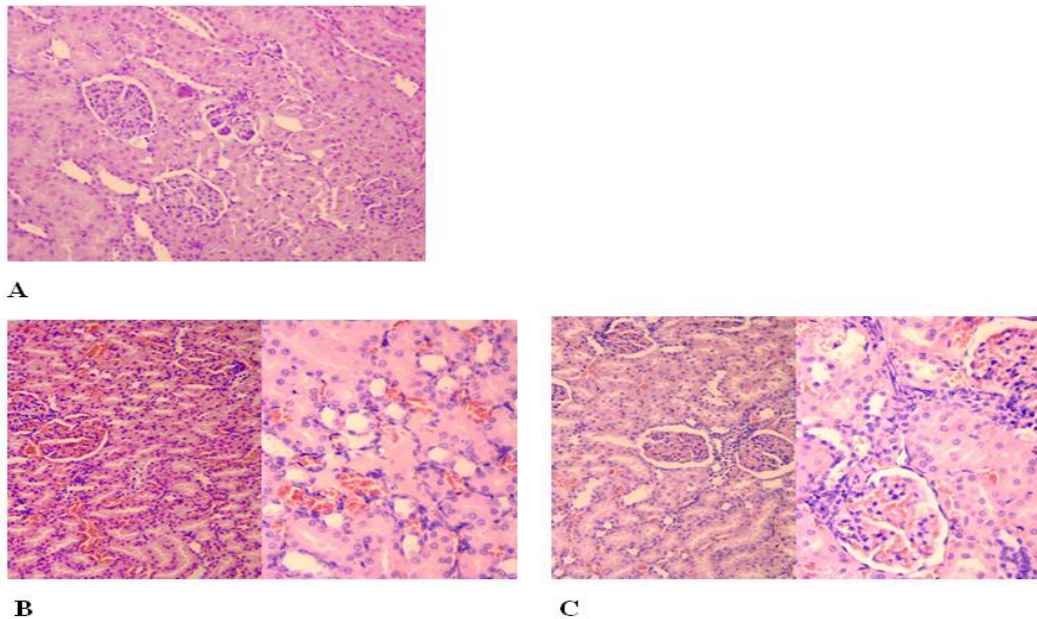


**D - Myocardial tissue of Palosuran-treated hypertensive rat (12<sup>th</sup> week)**

Morphological investigations of renal tissue in healthy rats have shown normal renal histoarchitecture with pronounced mild hyperemia. Glomerular and tubular apparatus was without pathology.

In hypertensive rats after 12 weeks of disease modeling there was intraglomerular hyperemia with RBC diapedesis, tubular dilatation and severe hyperemia.

In Palosuran-treated hypertensive rats 12 weeks after disease modeling tubular dilatation was detected without glomerular hyperemia and RBC diapedesis (picture 3).



**Figure 4:** Renal tissue of healthy, untreated and Palosuran-treated rat at 12<sup>th</sup> week of renovascular hypertension.

**A** -Healthy rat (H&E, x250); **B** - Rat with renovascular hypertension (12<sup>th</sup> week. H&E, x250, x300); **C** - Palosuran-treated rat with renovascular hypertension (12<sup>th</sup> week. H&E, x250, x300).

#### 4. Discussion

As the results of study have shown, statistically significant increase in blood pressure was created after 2 weeks of disease modeling. After 4 weeks, progressive increase in blood pressure was reliable and statistically significant. The increase in blood pressure at renovascular hypertension first of all develops due to the renal artery ischemia in the clipped kidney leading to ischemia, hypoxia, activation of the renin-angiotensin-aldosterone system (RAAS), total peripheral vasoconstriction and water retention.

3 weeks after modelling of hypertension, reduction in MAP could be explained by the compensatory reaction of the second, intact kidney, decreasing rennin production and inhibiting RAAS system to restore homeostasis. However, on the 4 weeks of renovascular hypertension, compensatory reaction of the intact kidney fades away,



pressure regulatory system unable to maintain the blood pressure within the normal range and it increases significantly. At this stage of hypertension, increased blood pressure and MAP manifested in experimental animals supposedly is caused due to the complex action of RAAS and activated sympathetic nervous system. The latter, results in further increase in renin production and peripheral vasoconstriction.

Intraperitoneal treatment with Palosuran decreased blood pressure significantly in all study groups. The antihypertensive effect of palosuran was demonstrated in both cases, at early treatment (started after 4 weeks of hypertension modeling) and at relatively late onset of treatment (started after 8 weeks of hypertension modeling).

Decreased MAP in all study groups of experimental animals could be explained by URT antagonistic effects of Palosuran [6]. However, it must be mentioned that at relatively late onset of treatment, the antihypertensive effect of Palosuran was lesser. According to the literature, U-II in a small doses induce the active production of NO (by activating NO-synthase) and consequently, the dilation of blood vessels as an endothelium-dependent vasodilator, but damaging effects of hypertension on blood vessels supposedly increases production of U-II and enhance the endothelium-independent vasoconstrictive effect of urotensin [27].

In hypertensive rats PR was increased progressively compared to the data of healthy rats. In palosuran-treated hypertensive rats PR was significantly lower than in untreated hypertensive rats.

In hypertensive rats by 2<sup>nd</sup> and 3<sup>rd</sup> weeks after disease modelling increase in PR 1.45- and 1.42-fold along with increased MAP develops due to renal ischemia. 4 weeks later, decrease in PR could be explained by a second, intact kidney-compensatory mechanism decreasing renin production.

High BP at the same period of hypertension in the presence of relatively low PR could be explained by increase in blood osmotic pressure, increase in circulating blood volume and increase in vascular basal tone due to hyperproduction of aldosterone, leading to the increased sodium reabsorption with further increase in blood osmolality and increased secretion of antidiuretic hormone, stimulating release of adrenocorticotrophic hormone and potentiating peripheral vasoconstriction [8].

The increase in basal tone supposedly is caused by an increase in the amount of Na<sup>+</sup> in the blood vessel walls, leading to the water retention causing their swelling and thickening. In addition, Na<sup>+</sup> increases sensitivity of  $\alpha$ -adrenoceptors in blood vessel walls in response to catecholamine. Aldosterone also facilitates the release of norepinephrine from the sympathetic nerve endings and as a result, increases vascular neurogenic tone as well [9].

By the 8<sup>th</sup> week of disease modelling PR was increased 2,6-fold compared to the norm, and 3.34-fold by the 12<sup>th</sup> week of hypertension correlating with the data of systemic blood pressure and MAP.

Palosuran produced significant decrease in PR in all study group animals compared to control (especially in case of early onset of treatment), except in healthy rats, where only a tendency of decrease in PR was observed.

In healthy rats after administration of Palosuran arterial pressure and PR were not changed significantly that could be explained by the fact that urotensin production is relatively low in healthy rats, hence the effects of the palosuran is less respectively.

Increased SC in rats by 12<sup>th</sup> week of hypertension supposedly points on kidney malfunctioning. Although, Palosuran decreased SC in hypertensive rats at early onset of treatment the kidney sparing effect was not obvious at late stages of hypertension and late onset of treatment. Morphological investigations confirmed the renal and cardiac complications developed due to hypertension. Increased cardiac workload was reflected on myocardial hypertrophy and renal malfunctioning was manifested by intraglomerular hyperemia with RBC diapedesis, tubular dilatation and severe hyperemia.

In Palosuran-treated rats due to normalization of renal hemocirculation, reduced MAP and decreased workload on heart muscle all investigated parameters were normalized, but only at the early stage of disease and early onset of treatment of hypertension.

Investigation of mesenteric arteries (MA) have shown that in hypertensive rats MA at early stage of disease was more dilated than later (by the 12<sup>th</sup> week of hypertension).

There is some evidence that U-II upregulates human and rat eNOS, which would suggest a hypothesis that U-II can cause arterial vasodilatation via NO, but causes vasoconstriction in diseased vessels without an intact endothelium. The activity of endothelial NO synthase (eNOS) has been shown to increase in hypertensive rats with elevated NO and cGMP production [10].

Nevertheless, reduced NO bioavailability has been established in hypertensive individuals, depending on the duration and severity of arterial hypertension. Indeed, in hypertensive rats, endothelium-derived constrictor factors (EDCFs) are produced, including angiotensin II, thromboxane A<sub>2</sub>, and endothelin-1. The net result of EDCFs, reactive oxygen species (ROS) and NO production by endothelial cells in hypertensive rats is an impaired endothelial function and vasodilatation compared to normotensive rats.

Because at hypertension U-II concentration is increased we suppose that high concentration of U-II causes relaxation of mesenteric arteries through its endothelin-dependent vasodilatory properties and later, due to arterial damage eNOS upregulation vasodilatation via NO fades away and predominates endothelin-independent vasoconstriction. In mesenteric vessels, Palosuran supposedly upregulated expression of RhoA and Rho-kinase, increased Rho-kinase-activity, diminished nitric oxide (NO)/cyclic guanosine 3',5'-monophosphate (cGMP) signaling and produced vasoconstriction.

## **5. Conclusion**

In summary, treatment with Palosuran, along with a decrease in blood pressure in rats, significantly reduces the workload on the myocardium and, therefore, reduces the risk of complications. Effect of treatment is better expressed at early stage of hypertension.

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