

Urotensin-2 Receptor Antagonist - Palosuran Decreases Blood Pressure And Plasma Renin Concentration In Laboratory Rats With Renovascular Hypertension

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Abstract – We were aimed to study effects of the Urotensin-2 receptor antagonist - Palosuran on blood pressure and plasma rennin concentration in laboratory rats with renovascular hypertension (2 kidneys + 1 clip). Blood pressure was measured using noninvasive “tail cuff” method.

Studies have shown that in experimental rats palosuran (10 mg/kg/daily, during 4 weeks) reveal hypotensive effect. Blood pressure was decreased even after administration NO-synthase inhibitor - L-NAME (10 mg/kg, single dose), supposedly due to its urotensin-2 receptor antagonistic properties.

In hypertensive rats plasma renin concentration was increased progressively compared to the data of healthy rats. In palosuran-treated hypertensive rats renin concentration was significantly lower than in untreated hypertensive rats. Decreased renin concentration was maintained after administration of L-NAME, except during the late-onset of treatment.

It could be concluded that in experimental rats with renovascular hypertension the vasodilatory effect of palosuran outweighs the inhibitory effect of L-NAME on NO production and urotensin-induced endothelium-independent vasoconstrictive effect, especially at the early stages and early onset of treatment of hypertension. Palosuran might represent a new therapeutic option in individuals with hypertension disease.

Keywords – Renovascular hypertension, urotensin-II receptors, renin, palosuran, L-NAME.

I. INTRODUCTION

According to the World Health Organization, arterial hypertension is a serious medical condition that can increase the risk of heart, brain, kidney and other diseases. About 26% of the population dies each year due to arterial hypertension. It is a major cause of premature death worldwide. A review of current trends shows that the number of adults with hypertension increased from 594 million in 1975 to 1.13 billion in 2015. WHO estimates that 54% of strokes and 47% of cases of ischemic heart disease are the direct consequence of high blood pressure, which thus takes its place among the main risk factors for cardiovascular morbidity and mortality [1].

The objectives of antihypertensive treatment are to prevent the occurrence/progression/recurrence of cardiovascular disease related to sustained high blood pressure, reduce mortality and help patients with hypertension lead their lives as normally as do healthy people [2]. The prescription of antihypertensive drugs to achieve the recommended target blood pressures remains the main step of the management of hypertensive patients. Drugs, targeting BP must be well tolerated, economically affordable, and simple to take, thus supporting long-term persistence [3].

Nowadays, four major classes of antihypertensive drugs are available: diuretics, calcium antagonists, blockers of the renin-angiotensin system (RAS), and beta blockers. Despite their ability to lower blood pressure, significantly improve the long-term prognosis of patients and reduce cardiovascular outcomes, it is important to consider the tolerability profile of antihypertensive drugs as tolerability is the major determinant of the long-term persistence on therapy and side effects. For instance, Diuretics/Thiazides may produce hyponatraemia, hypokalaemia, hyperuricaemia, increase in cholesterol and LDL, serum creatinine/urea, risk of diabetes. Patients may suffer with weakness, muscle cramps, impotence, gout attacks. Antialdosterone diuretics may lead to dizziness, drowsiness, allergic reactions, sexual disturbances, nausea, vomiting, hyperkalaemia. ACE inhibitors result in persistent dry cough, angio-oedema, dry mouth, nausea, rash, hyperkalemia, increased serum creatinine. Hyperkalaemia, increased serum creatinine, nausea, dry mouth, abdominal pain are common manifestations of Angiotensin receptor blockers. Calcium antagonists/dihydropyridines result in peripheral edema, headache, flushing, palpitations, constipation, nausea, gingival hyperplasia. Beta blockers Increase risk of diabetes, increase triglycerides, decrease HDL, aggravate asthma, produce fatigue, insomnia, nightmares, reduced ability to exercise, rash, weight gain [4]. Thus, could be said that the problem of effective treatment of arterial hypertension has not lost its importance and its solution in a particular clinical situation often remains very difficult.

In recent years, the interest of researchers and scientists in cyclic vasoactive neuropeptide urotensin-2 has increased significantly. The role of the UII system in human pathophysiology is not yet fully understood. Urotensin-II (U-II), as a regulator of vascular tone, is found in the cardiovascular and central nervous systems, kidneys, lungs, liver, ovaries, endocrine glands and is involved in many physiological and pathological processes [5], [6], [7], [8], [9]. Circulating blood levels of human UR-II, the most potent vasoconstrictor peptide identified to date, are increased in patients with essential hypertension. U-II binds to the U-receptor, activates the Gq-protein, and induces activation of the inositol-triphosphate cycle by phospholipase-C activation [10], [11]. U-II is a more potent vasoconstrictor than endothelin-1, vasopressin, and vasoconstrictor prostaglandins. U-II acts as an endothelium-independent vasoconstrictor and endothelium-dependent vasodilator [12], [13], [14], [15], [16].

Vasoconstriction is mediated by receptors on smooth muscle cells (SMCs) and appears to be variable and highly dependent on the vascular bed, whereas vasodilation is endothelium-mediated [17]. However, in a disease state of chronic heart failure or essential hypertension, U-II loses its dilatory ability [18]. It is understood that such a loss and dysfunction of endothelial cells would favor a contractile response over a relaxant one [19].

Increased levels of U-II and over expression of urotensin receptors (UTR) detected during hypertension, heart failure, diabetes, portal hypertension, and renal failure, suggest that U-II/UTR system may play a crucial role in the development of these diseases [20], [21]. In this regard, investigation of UTR antagonists seems interesting and prospective in treatment of hypertension and other diseases accompanying hypertension.

Palosuran is a non-peptide UTR antagonist with promise in drug development has been developed to inhibit the accumulation of calcium by U-II and the phosphorylation of mitogen-activated protein kinase. Data in the literature on the use of palosuran in hypertensive individuals are scarce and mutually exclusive [22], [23], [24], [25]. In rat models of acute renal failure and diabetes,

palosuran significantly improved renal function, decreased the number of tubular and tubulointerstitial lesions and improved survival [26].

Based on all of the above, it is interesting to study the effect of urotensin receptor antagonist - Palosuran on blood pressure in laboratory rats with experimental arterial hypertension.

II. MATERIALS AND METHODS

The study was performed on 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 [27], [28], [29]. Under general anaesthesia (Nembutal - 50 mg / kg), after separation of the renal artery from the vein and nerve, the silver clip (0.2 mm internal diameter) was placed on the left renal artery close to the aorta.

The experimental animals were divided into 3 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.

In the groups II and III rats, NO-synthase inhibitor - L-NAME (10 mg/kg, single dose) was administered intraperitoneally also after completion of the treatment with palosuran.

Systemic arterial pressure (systolic pressure, diastolic pressure) was measured once a week for 12 weeks using arterial pressure measurement system "Систола" (non-invasive tail-cuff method for BP measurement). The mean arterial pressure was calculated. Plasma renin concentration was determined using ELISA (HumaStar HS).

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 $P < 0.05$.

III. RESULTS

In experimental rats at different stages of the renovascular hypertension changes in mean arterial pressure (MAP) was detected compared to MAP of the group 1 animals (healthy rats).

Results of experiment (Tab. N1, N2) 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 1 animals;

In healthy rats, after administration of palosuran the MAP reduced by 33% ($p < 0.02$). On the background of palosuran after injection of L-NAME, there was a 23% increase in MAP compared to palosuran-treated rats, and a statistically unreliable decrease of MAP by 17% compared to data from healthy rats.

In hypertensive rats after treatment with palosuran started on the 4th weeks of disease modelling on the 8th weeks of hypertension MAP was reduced by 32% ($p < 0.001$) compared to control, untreated hypertensive rats.

L-NAME on the background of palosuran have shown increase tendency of MAP by 18% compared to palosuran-treated rats and statistically significant reduction of MAP by 20% ($p < 0.02$) compared to MAP of the group I animals.

In hypertensive rats, after treatment with palosuran started on the 8th weeks of disease modelling on the 12th weeks 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).

After administration of L-NAME on the background of palosuran there was a 16% increase tendency in MAP compared to palosuran-treated rats and compared to untreated rats, the decrease in MAP by 10% was not statistically significant also.

Table N1. Systolic and diastolic blood pressure in healthy and hypertensive rats after treatment with Palosuran and L-NAME injections at different stages of renovascular hypertension.

N	Groups		Systemic Blood Pressure (mm/Hg)		
			Without treatment	Palosuran	Palosuran + L-NAME
1	Healthy rats	Systole	110 ± 3,4	81 ± 4,1**	98± 3,2
		Diastole	87± 4,8	55 ± 3,1**	69± 4,2*
2	1 week after hypertension modeling	Systole	114± 4,1	-	-
		Diastole	91± 4,5	-	-
3	2 weeks after hypertension modeling	Systole	159 ± 2,7**	-	-
		Diastole	98 ± 4,2*	-	-
4	3 weeks after hypertension modeling	Systole	117± 5,7	-	-
		Diastole	93 ± 4,5	-	-
5	4 weeks after hypertension modeling	Systole	185 ± 9,3**	-	-
		Diastole	110 ± 5,4**	-	-
6	Treatment started after 4 weeks of hypertension modeling - 8 th week	Systole	192 ± 9,3**	134 ± 5,7***	143 ± 11,3**
		Diastole	110 ± 5,4**	72 ± 3,1**	94± 4,3**
7	Treatment started after 8 weeks of hypertension modeling - 12 th week	Systole	205 ± 10,1***	149± 8,1**	181± 10,7
		Diastole	115 ± 7,1***	94± 5,2**	104± 6,1

*- p<0.02; **- p<0.01; ***- p<0.001

Table N2. Mean arterial pressure (MAP) in healthy and hypertensive rats after treatment with Palosuran and L-NAME injections at different stages of renovascular hypertension.

N	Groups		Mean Arterial Pressure – MAP (mm/Hg)		
			Before treatment	Palosuran	Palosuran + L-NAME
1	Healthy rats		95 ± 3,1	64±3,0**	79± 2,5

2	1 week after modeling hypertension	97±3,5	-	-
3	2 weeks after modeling hypertension	118±4,1*	-	-
4	3 weeks after modeling hypertension	101 ±9,2	-	-
5	4 weeks after modeling of hypertension	135 ± 10,0**	-	-
6	8 weeks after modeling of hypertension	137± 8,3**	93 ± 5,5***	110± 8,2**
7	12 weeks after modeling of hypertension	145 ± 10,0***	112± 7,2**	130±9,5

*- $p<0.05$; ** - $p<0.01$; *** - $p<0.00$

IV. DISCUSSION

As the results of study have shown, 1 week after hypertension modeling, only a tendency for an increase in MAP was observed, while a 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 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.

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

Palosuran is known to have an antagonistic effect on U-II receptors, thereby reducing the vasoconstrictive effect of U-II. 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. This phenomenon can explain the decline in MAP in all study groups of experimental animals [30].

After administration of L-NAME there was not a statistically significant increase in MAP compared to animals treated with palosuran, while MAP was decreased compared to control, untreated hypertensive rats, but this decrease was statistically significant only in group of rats, where treatment was started earlier.

After administration of L-NAME, as NO-synthase inhibitor, a significant increase in blood pressure was expected compared to the data of the control group animals. But, experiments revealed just the opposite reaction in palosuran-treated rats, especially in case of the early-onset of treatment. This may be explained by the fact that palosuran inhibiting the effect of urotensin is likely increased NO production thereby inhibiting the vasoconstrictive effect of L-NAME.

In case of treatment started relatively later, the antihypertensive effect of palosuran was less manifested. We suppose that damaging effects of hypertension on blood vessels increase production of U-II and enhance the endothelium-independent vasoconstrictive effect of urotensin [31].

In experimental rats the plasma rennin concentration (PR) at different stages of modelling of renovascular hypertension was changed compared to the norm (tab N3). In particular, after 1 week of disease modelling, there was a tendency of increase in PR by 4%; On 2nd week, PR was increased significantly by 45% ($p < 0.01$); In the 3rd weeks of hypertension increase in PR was relatively less - 42% ($p < 0.01$). By the 4th week PR decreased and it was not statistically different compared to the norm; After 8 weeks, the PR increased by 162% ($p < 0.001$) and after 12 weeks the PR was increased extremely by 234% ($p < 0.001$).

In healthy rats after administration of palosuran decrease in PR by 12% was not statistically significant. Administration of L-NAME in rats treated with palosuran showed only a tendency of increase in PR by 9% also, and compared to untreated hypertensive rats, decrease in PR by 4% PR was not statistically significant as well.

In hypertensive rats treated with palosuran started 4 weeks after disease modelling, by the 8th week of hypertension, palosuran induced a statistically significant decrease in PR by 33% ($p < 0.01$) compared to data from hypertensive, untreated rats. Administration of L-NAME in rats treated with palosuran showed a tendency of increase in PR by 9%. PR was reduced by 26% ($p < 0.05$) compared to data of untreated hypertensive rats.

In hypertensive rats subjected to treatment with palosuran, started after 8 weeks of disease modelling, on the 12th week of hypertension PR was decreased by 24% ($p < 0.01$) compared to control, untreated rats. In treated rats injection of L-NAME increased PR by 30%. Compared to the control, untreated rats, effect of L-NAME on PR was not statistically significant.

Table N3 Plasma Renin concentration (PR) in healthy and hypertensive rats at different stages of hypertension after treatment with palosuran and injections of L-NAME.

N	Groups	Renin – PR (ng / ml)		
		Before treatment	+ Palosuran	Palosuran + L-NAME
1	Healthy rats	1,72 ± 0,5	1,52 ± 0,3	1,65 ± 0,4
2	1 week after hypertension modeling	1,79 ± 0,3	-	-
3	2 weeks after hypertension modeling	2,49 ± 0,4**	-	-
4	3 weeks after hypertension modeling	2,45 ± 1,3**	-	-
5	4 weeks after hypertension modeling	1,94 ± 0,1	-	-
6	8 weeks after hypertension modeling	4,5 ± 1,4***	3,02 ± 0,9**	3,32 ± 0,5*
7	12 weeks after hypertension modeling	5,75 ± 1,5***	4,39 ± 1,5**	5,71 ± 1,2

* – $p < 0,05$, ** – $p < 0,01$, *** – $p < 0,001$

Thus, the experiment revealed that changes in PR were observed at different stages of modelling of renovascular arterial hypertension, compared to PR in hypertensive rats. In particular, by the 2nd and 3rd weeks after disease modelling, PR was increased almost uniformly compared to the norm, 1.45-fold and 1.42-fold, most likely due to renal ischemia.

4 weeks after modelling of hypertension PR decreased and it was not different from the PR of healthy rats. Although PR was within the normal range that could be explained by a second, intact kidney-compensatory mechanism decreasing renin production [32], [33], the BP was remained at high levels.

High arterial pressure which was revealed by experiment 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 production of antidiuretic hormone, stimulating secretion of adrenocorticotrophic hormone and potentiating peripheral vasoconstriction [34], [35].

The increase in basal tone supposedly is caused by an increase in the amount of sodium in the blood vessel walls, leading to the water retention causing their swelling and thickening. In addition, sodium increases the sensitivity of α -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 also [36], [37].

By the 8th week of disease modelling, PR was increased 2,6-fold compared to the norm, and 3.34-fold by the 12th 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. It should also be noted that in healthy rats, both palosuran and L-NAME were administered at a single dose and samples were taken 2 hours after injection of preparations. The hypotensive effect of palosuran supposedly develops due to its vasodilatory effect, which later is reflected on renin production. Probably, this short period of time (2 hours) at single administration of the drugs is not sufficient for the reliable changes in PR. The same could be said for the tendency of increase in PR after injection of L-NAME in treated rats.

The decreasing effect of palosuran on PR was revealed in both, early-onset and late-onset of treatment by 8th and 12th weeks of hypertension, but PR was significantly lower at early onset of treatment than at late-onset of treatment.

V. CONCLUSION:

Based on the results of experiments could be concluded that palosuran reveals the hypotensive effect in both, healthy and hypertensive rats. The vasodilating effect of palosuran exceeds the inhibitory effect of L-NAME on NO and the urotensin-induced endothelium-independent vasoconstrictive effect, especially in the early stages of hypertension. At stable hypertension the PR progressively increases compared to the norm. Palosuran significantly decreases PR compared to untreated, hypertensive rats, which is maintained in case of administration of L-NAME, except for late-onset of treatment. Palosuran might represent a new therapeutic option in individuals with hypertension disease.

REFERENCES

- [1] Lawes CM, Vander Hoorn S, Rodgers A. Global burden of blood-pressure-related disease, 2001. *Lancet*. 2008;371:1513–1518;
- [2] *Hypertension Research* 2014;37:279–285;
- [3] Burnier M., Kjeldsen S., Heagerty A., Williams B. Drug treatment of hypertension Oxford Medicine Online <https://oxfordmedicine.com/view/10.1093/med/9780198784906.001.0001/med-9780198784906-chapter-569>;
- [4] Podzolkov V., Tarzimanova A., Georgadze Z. Modern Principles of Treatment of Uncontrolled Hypertension. *Rational Pharmacotherapy in Cardiology Home*. 2019; 15(5): 736-741;

- [5] Suguro T, Watanabe T, Ban Y, et al. Increased human urotensin II levels are correlated with carotid atherosclerosis in essential hypertension. *Am J Hypertens*. 2007 Feb; 20(2):211-7;
- [6] Totsune K, Takahashi K, Arihara Z, et al. Elevated plasma levels of immunoreactive urotensin II and its increased urinary excretion in patients with Type 2 diabetes mellitus: association with progress of diabetic nephropathy. *Peptides*. 2004 Oct; 25(10):1809-14;
- [7] Bousette N., Hu F., Ohlstein E. H., Dhanak D., Douglas S. A., Giaid A. Urotensin-II blockade with SB-611812 attenuates cardiac dysfunction in a rat model of coronary artery ligation. *J. Mol. Cell. (2006). Cardiol.* 41, 285–295.10.1016/j.yjmcc.2006.05.008;
- [8] Desai N, Sajjad J, Frishman WH. Urotensin II: a new pharmacologic target in the treatment of cardiovascular disease. *Cardiol Rev*. 2008 May-Jun; 16(3):142-53;
- [9] Suguro T, Watanabe T, Ban Y, et al. Increased human urotensin II levels are correlated with carotid atherosclerosis in essential hypertension. *Am J Hypertens*. 2007 Feb; 20(2):211-7;
- [10] Douglas SA, Ohlstein EH. Human urotensin-II, the most potent mammalian vasoconstrictor identified to date, as a therapeutic target for the management of cardiovascular disease., *Trends Cardiovasc Med*. 2000 Aug; 10(6):229-37;
- [11] Gardiner SM, March JE, Kemp PA, et al. Regional heterogeneity in the haemodynamic responses to urotensin II infusion in relation to UT receptor localisation. *J Pharmacol*. 2006 Mar; 147(6):612-21;
- [12] Lapp H, Boerrigter G, Costello-Boerrigter LC, et al. Elevated plasma human urotensin-II-like immunoreactivity in ischemic cardiomyopathy. *Int J Cardiol*. 2004 Mar; 94(1):93-7;
- [13] Bottrill FE, Douglas SA, Hiley CR, White R, Br. Human urotensin-II is an endothelium-dependent vasodilator in rat small arteries. *J Pharmacol*. 2000 Aug; 130(8):1865-70;
- [14] Matsushita M, Shichiri M, Imai T, et al. Co-expression of urotensin II and its receptor (GPR14) in human cardiovascular and renal tissues. *J Hypertens*. 2001 Dec; 19(12):2185-90;
- [15] Maguire JJ, Davenport AP. Is urotensin-II the new endothelin? *Br J Pharmacol* 137: 579–588, 2002;
- [16] Douglas S. A., Dhanak D., Johns D. G. From “gills to pills”: urotensin-II as a regulator of mammalian cardiorenal function. *Trends Pharmacol. Sci*. 2004; 25:76–85.10.1016/j.tips.2003.12.005.
- [17] Sondermeijer B., Komp A., Komesaroff P., Krum H. Effects of exogenous urotensin-II on vascular tone in skin microcirculation of patients with essential hypertension. *Am.J. Hypertens*. 2005; 22:1341–1344
- [18] Lim et al., 2004 Lim M., Honisett S., Sparkes C. D., Komesaroff P., Komp A., Krum H. Differential effect of urotensin II on vascular tone in normal subjects and patients with chronic heart failure. *Circulation*. 2004; 109, 1212–1214;
- [19] American Journal of Hypertension. *Am J Hypertens* 2007;20: 211–217;
- [20] Lim M, Honisett S, Sparkes CD, et al. Differential effect of urotensin II on vascular tone in normal subjects and patients with chronic heart failure. *Circulation*. 2004 Mar 16; 109(10):1212-4;
- [21] Clozel M, Hess P, Qiu C, Ding SS, Rey M J. The urotensin-II receptor antagonist palosuran improves pancreatic and renal function in diabetic rats. *Pharmacol Exp Ther*. 2006 Mar; 316(3):1115-21;
- [22] Pehlivan Y, Turkbeyler IH, et al. Urotensin inhibition with palosuran could be a promising alternative in pulmonary arterial hypertension. *Onat AM1, Hypertension*. 2010; 55 (5):1206-9;
- [23] Sidharta PN, Wagner FD, Bohnemeier H, et al. Pharmacodynamics and pharmacokinetics of the urotensin II receptor antagonist palosuran in macroalbuminuric, diabetic patients. *Clin Pharmacol Ther*. 2006 Sep; 80(3):246-56;
- [24] Vogt L1, Chiurchiu C, Chadha-Boreham H. et al. Effect of the urotensin receptor antagonist palosuran in hypertensive patients. *Trends Pharmacol Sci*. 2004 Feb; 25(2):76-85;

- [25] Clozel M, Binkert C, Birker-Robaczewska M, et al. Pharmacology of the urotensin-II receptor antagonist palosuran (ACT-058362; 1-[2-(4-benzyl-4-hydroxy-piperidin-1-yl)-ethyl]-3-(2-methyl-quinolin-4-yl)-urea sulfate salt): first demonstration of a pathophysiological role of the urotensin system. *J Pharmacol Exp Ther*. 2004; 311: 204–212;
- [26] Clozel M, Hess P, Qiu C, Ding SS, Rey M. The urotensin-II receptor antagonist palosuran improves pancreatic and renal function in diabetic rats. *J Pharmacol Exp Ther*. 2006; 316: 1115–1121;
- [27] Goldblatt P. The Goldblatt experiment: a conceptual paradigm. In: *Hypertension: Pathophysiology, Diagnosis and Management*, edited by J. H. Laragh and B. M. Brenner. New York: Raven, 1995; pp. 23–35;
- [28] Mitchell K., Navar L.. Intrarenal actions of angiotensin II in the pathogenesis of experimental hypertension. In: *Hypertension: Pathophysiology, Diagnosis, and Management*, edited by J. H. Laragh and B. M. Brenner. New York: Raven, 1995, pp.1437–1450;
- [29] Ploth D. Angiotensin-dependent renal mechanisms in two-kidney one-clip renal vascular hypertension. *Am. J. Physiol*. 245 (Renal Fluid Electrolyte Physiol. 14): F131–F141, 1983).
- [30] Ishihata A, Ogaki T, Aita T, Katano Y. Role of prostaglandins in urotensin II-induced vasodilatation in the coronary arteries of aged rats. *Eur J Pharmacol* 523: 119–126, 2005;
- [31] Lin L., Ding WH., Jiang W., Zhang YG., Qi YF., Yuan WJ., Tang CS. Urotensin-II activates L-arginine/nitric oxide pathway in isolated rat aortic adventitia. *Peptides* 25: 1977–1984, 2004;
- [32] Katano Y, Ishihata A, Aita T, Ogaki T, Horie T. Vasodilator effect of urotensin II, one of the most potent vasoconstricting factors, on rat coronary arteries. *Eur J Pharmacol* 402: 209–211, 2000;
- [33] Gibson A. Complex effects of Gillichthys urotensin II on rat aortic strips. *Br J Pharmacol* 91: 205–212, 1987;
- [34] Kimiko Ishiguro, Hiroyuki Sasamura, Yusuke Sakamaki, Hiroshi Itoh, Takao Saruta. Developmental Activity of the Renin-Angiotensin System during the “Critical Period” Modulates Later L-NAME–Induced Hypertension and Renal Injury. *Hypertension Research*. 2007, p.30–75;
- [35] Liffert Vogt, Carlos Chiurchiu, Harbajan Chadha-Boreham, et al. Effect of the Urotensin Receptor Antagonist Palosuran in Hypertensive Patients With Type 2 Diabetic Nephropathy. *Hypertension*. 2010;55:1206-1209;
- [36] Wendy J. Bottinor, Megan M. Shuey, Ali Manouchehri, et al. Renin-Angiotensin-Aldosterone System Modulates Blood Pressure Response During Vascular Endothelial Growth Factor Receptor Inhibition. *JACC: CARDIOONCOLOGY*, 2019;1(1); <http://creativecommons.org/licenses/by-nc-nd/4.0/>;
- [37] Marisa K. Ames, Clarke E. Atkins, Bertram Pitt. The renin-angiotensin-aldosterone system and its suppression. *J Vet Intern Med*. 2019;33(2):