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Effect of the Urotensin-2 Receptor Antagonist - Palosuran on Serum Electrolytes (Na^+, K^+, Ca^{++}) in Laboratory Rats with Renovascular Hypertension

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Abstract – This study was designed to assess the effect of the urotensin-2 receptor antagonist - Palosuran on blood pressure and serum electrolytes in laboratory rats with renovascular hypertension (2 kidneys + 1 clip) and determine possible changes in sodium, potassium, calcium levels.

Studies have shown that Palosuran decreases mean arterial pressure in rats with renovascular hypertension. 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. The antihypertensive effect of Palosuran was less manifested in case of relatively late onset of treatment. Supposedly, damaging effects of hypertension on blood vessels increase production of U-II and enhance the endothelium-independent vasoconstrictive effect of urotensin.

The results obtained showed a significant difference between hypertensive and nonhypertensive healthy rats for blood electrolytes. At the early stage of disease modeling Palosuran significantly decreased serum Na^+ and increased K^+ concentrations in hypertensive rats. Na^+ and K^+ concentrations were maintained within the normal range even after administration of L-NAME, except during the late-onset of treatment.

In conclusion, Palosuran might represent a new therapeutic option in individuals with hypertension disease at early stages of disease.

Keywords – Urotensin-II Receptors, Renovascular Hypertension, Na^+, K^+, Ca^{++}, Palosuran.

I. INTRODUCTION

Hypertension is a serious medical condition that significantly increases the risks of heart, brain, kidney and other diseases. High blood pressure is now recognized as the major cause of cardiovascular disease worldwide. An estimated 1.13 billion people worldwide have hypertension, most (two-thirds) living in low- and middle-income countries.

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Despite the effectiveness of the currently available anti-hypertensive medications, there is always a need for novel treatment strategies that are more effective in particular hypertensive patient groups.

The urotensin system has been hypothesized to play an important role in the pathophysiology of hypertension. Urotensin II, as the most potent vasoconstrictor known in mammals [1], [2] is upregulated in hypertension. Plasma urotensin II was raised in hypertensive patients compared to normotensive controls and was directly related to systolic blood pressure. These findings raise the possibility that urotensin II (U-II) may have an etiological role in hypertension and its complications [3].

U-II is a cyclic oligopeptide with vasoactive potential [4], [5]. By activation of the urotensin II receptor (UTR), U-II might influence different pathways, depending on the cells and vascular compartment where the receptor is located [6], [7].

U-II has shown to act as vasodilator: this action was endothelium-dependent, suggesting that vasodilatation is mediated via UTR on the endothelium whereas vasoconstriction is mediated via UTR on smooth muscle cells. Activation of the endothelial UTR leads to relaxation via NO formation, whereas activation of UTR on vascular smooth muscle cells leads to contraction via RhoA/Rho-kinase activation [8], [9].

U-II also plays a role in body fluid regulation in lower vertebrates and it now appears that this extends to mammals. The kidney appears to be a major source of U-II synthesis in mammalian species. U-II is found in both the proximal tubules and collecting ducs. The UTR localized primarily to the renal medulla, with greatest expression in the inner medullary collecting ducts.

Recent studies have shown that in the kidney, UTR influences excretion of sodium and water and glomerular filtration rate [10], [11]. U-II may function as a chronic regulator of basal vascular tone, rather than a short-term regulator of vascular resistance [12]. A number of reports have implicated U-II in the conservation of water, sodium and chloride [13] in fish species. Studies have shown that inhibition of UTR activity with the antagonist urantide resulted in an increase in GFR, dieresis and natriuresis suggesting that endogenous U-II exerts a tonic influence on basal renal function. Altered U-II expression in disease states has prompted the development of a number of UTR antagonists [14].

The most potent UTR antagonists is the Palosuran (4-ureido-quinoline derivate, ACT-058362) [15], [16]. Palosuran is selective for the human UTR. Application of these and other compounds in a variety of disease models has shown that UT antagonism has potential therapeutic benefit. In diabetic rodents and patients, Palosuran improves renal function [16]. However, possible therapeutic potential of Palosuran in the treatment of hypertension and its complications have not been studied.

Administration of Palosuran to streptozotocin-treated rats improved survival, increased insulin, and reduced the progression of renal damage [17]. In a rat model of diabetes, treatment with Palosuran increased renal blood flow and delayed the development of proteinuria and renal damage. Similar effects were not seen in preliminary trials in patients with diabetic nephropathy, but further studies are underway to refine the dose regime in an attempt to improve the outcome. The therapeutic potential of UTR antagonists

has yet to be fully realized, but it seems likely that they will be of clinical use in the treatment of hypertension, heart failure, and renal disease.

Our experiments have shown hypotensive potential of Palosuran in lab rats with renovascular hypertension [18] that could be explained by its direct effect on UTR, and we considered interesting to study influence of Palosuran on the level of serum electrolytes playing essential role in pressure regulation.

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 [19], [20], [21]. 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.

Blood pressure (systolic and diastolic) was measured once a week for 12 weeks using arterial pressure measurement system "Систола" (non-invasive tail-cuff method for BP measurement).

Blood samples were obtained by jugular venous puncture after ketamine/xylazine anesthesia. Electrolytes (Na+, K+, Ca++) were measured on a complete radiometer analyzer - ABL 80, with cartridge system. Blood was inserted into the device with a special lithium heparin tube.

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

Results of experiments (Tab. N1) have shown that in lab rats after modeling of hypertension there is progressive rise in mean arterial pressure (MAP).

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;

Administration of Palosuran in healthy rats decreased MAP by 33% ($p<0.02$). In palosuran treated rats injection of L-NAME increased MAP by 23%, but decrease of MAP by 17% was statistically unreliable compared to data from healthy rats.

In hypertensive rats after treatment with Palosuran by the 8th weeks of hypertension MAP was reduced by 32% ($p <0.001$) compared to 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.

By 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. 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

*- $p<0.05$; ** - $p<0.01$; *** - $p<0.001$

In experimental rats at different stages of the renovascular hypertension changes in serum electrolytes (serum Na+, K+, Ca++) level were detected (Tab N2, graph N1, N2, N3, N4) compared to the results of the group I animals (healthy rats).

Tab. N2 Effects of Palosuran and L-NAME on serum electrolytes (mmol/L) in healthy and hypertensive rats at different stages of hypertension.

	Groups	Without treatment			+ Palosuran			Palosuran + L-NAME		
		Na^+	K^+	Ca^{++}	Na^+	K^+	Ca^{++}	Na^+	K^+	Ca^{++}
1	Healthy rats	145,2	4,5	9,2	137,0	4,9	9,24	139,1	5,0	9,25
2	4th week of hypertension	169,1**	4,1	9,0	-	-	-	-	-	-

3	8th week of hypertension	165,0**	4,3	9,1	144,1**	4,88*	9,25	149,0*	4,6	9,22
4	12th week of hypertension	188,1***	3,0**	9,22	175,3	3,2	9,22	179,1	3,1	9,24

*- p<0.05; ** - p<0.01; *** - p<0.001

In healthy rats after administration of Palosuran decrease in serum Na+ by 6,5% was not statistically significant. The same, unreliable alterations in Na+ concentrations were detected in rats treated with Palosuran after administration of L-NAME. Serum Na+ was increased by 1,5% and compared with the results of untreated rats serum Na+ was decreased by 4,1% (p>0,05).

After 4th, 8th and 12th weeks of disease modeling in hypertensive rats there was progressive and reliable rise in serum Na+ by 17%, 14% (p<0.01) and 30% (p<0,001) compared to the data of healthy rats. Although, after 8 weeks of disease modelling serum Na+ was increased by 14% (p <0.001), it was lesser by 3% compared to the data obtained by 4th weeks of disease modelling.

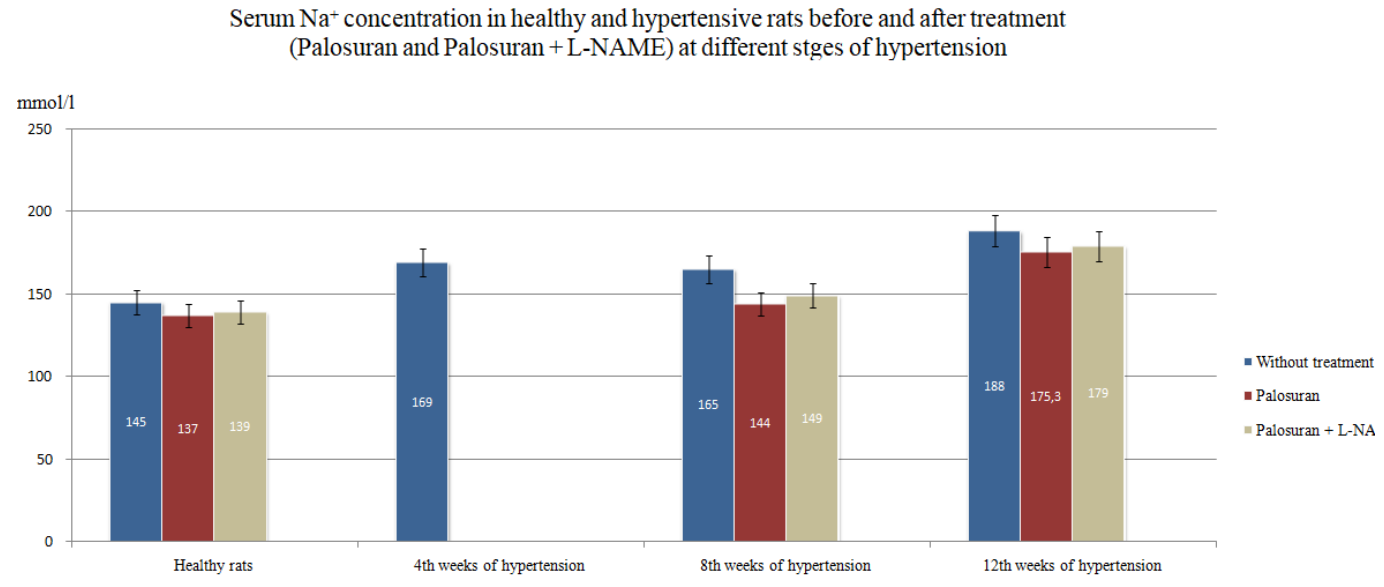
After 8th weeks of hypertension in rats treated with Palosuran serum Na + was reduced by 13% (p<0,01) and there was only a tendency of increase by 5% compared to the data of healthy rats.

Administration of L-NAME in rats treated with Palosuran have shown tendency of increase in Na+ by 3.5% and it was decreased by 10% (p<0,05) compared to untreated animals.

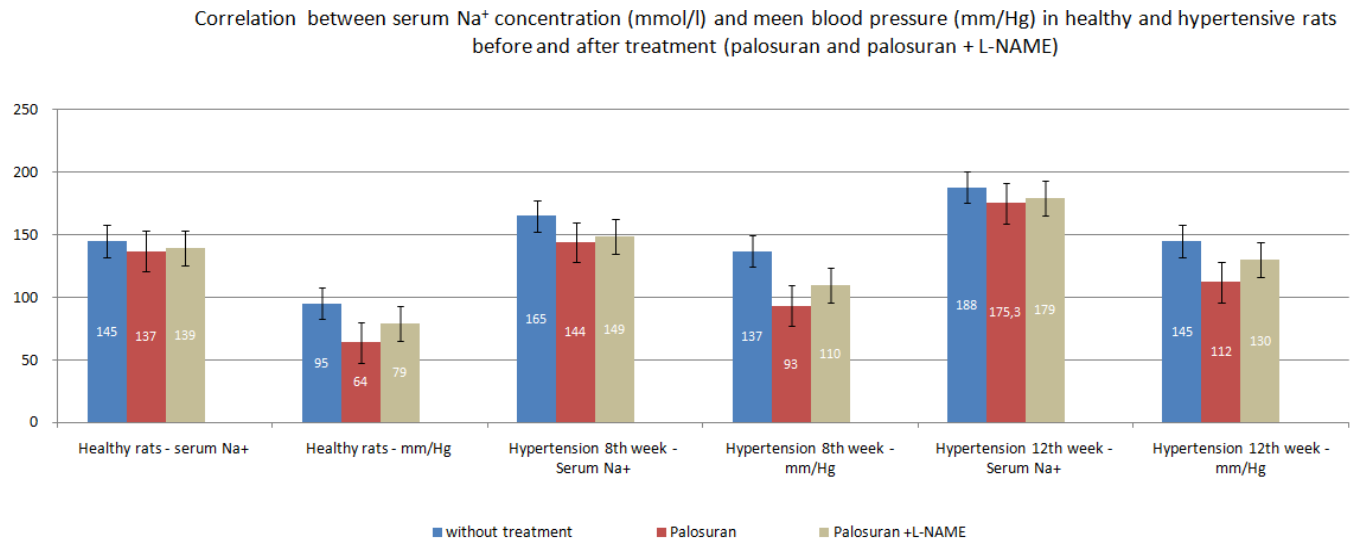
After 12 weeks of disease modeling in hypertensive rats treatment with Palosuran have shown a tendency of decrease in Na + concentration by 7% compared to the data of untreated rats. And it was increased by 21,7% (p<0,001) compared to the results obtained after 4 weeks of hypertension in treated rats.

Administration of L-NAME in Palosuran treated animals revealed tendency of increase in Na + by 2%. It was not statistically different also compared to the results of untreated rats and significantly increased by 28,8% (p<0,001) compared to the results of healthy group animals.

Graph N1

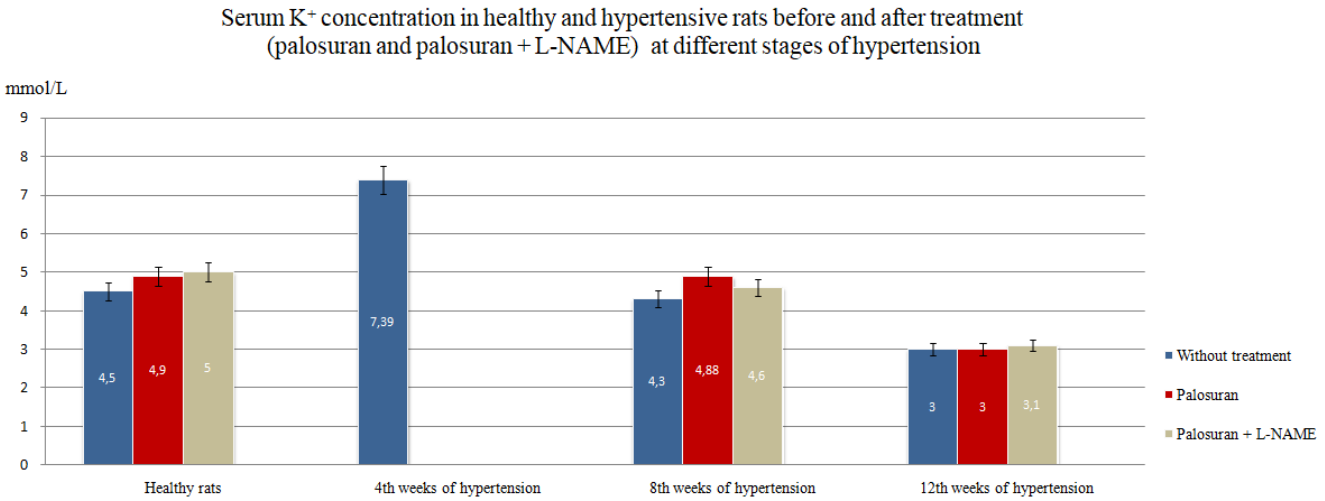


Graph N2



In healthy rats changes in serum K^+ concentration were not statistically significant neither in case of Palosuran, nor L-NAME administrations. In Palosuran treated group animals there was only a tendency of increase in serum K^+ concentration by 8,9% and in case of L-NAME injection - by 2% ($p>0,05$) compared to the initial values.

Graph N3



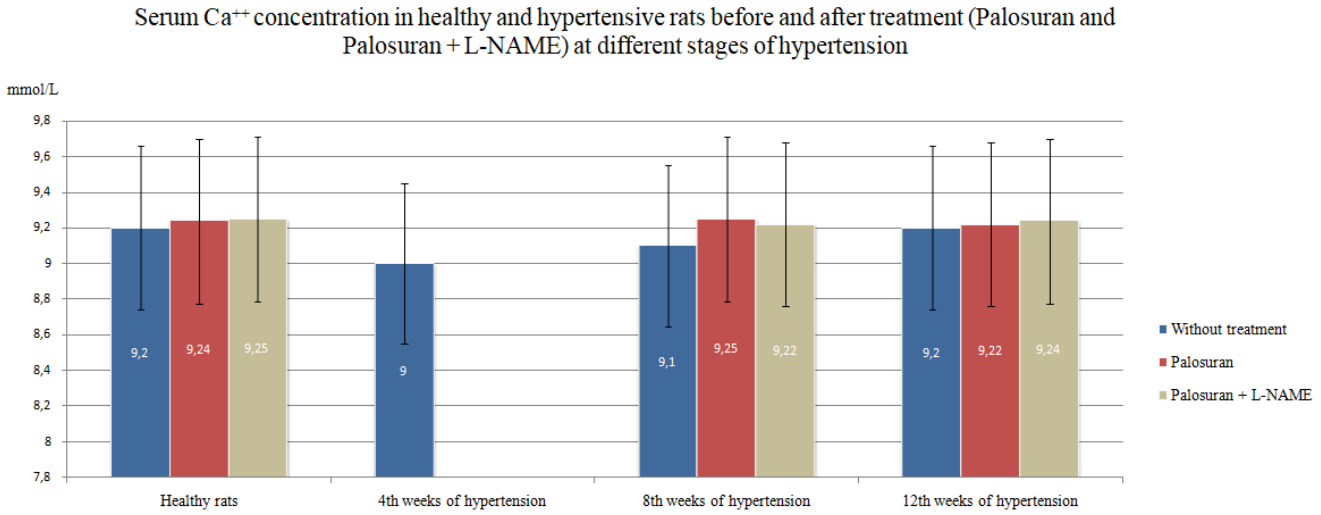
In hypertensive rats after 4 weeks of disease modeling decrease in serum K+ by 9% was not statistically significant compared to the data of healthy rats. After 8 weeks of disease modeling decrease in serum K+ by 5,5% was not statistically significant also and it was slightly increased compared to the data obtained by 4th weeks of disease modeling.

In treated with Palosuran group animals after 8 weeks of disease modeling serum K+ was increased by 13,5% ($p<0,05$). After administration of L-NAME serum K+ concentration was changed unreliably by 6% ($p>0,05$).

After 12th weeks of disease modeling in hypertensive rats serum K+ concentration was decreased by 33% ($p<0,01$). In treated group animals serum K+ was not changed significantly compared to the data of untreated group animals and administration of L-NAME have shown decrease tendency in serum K+.

Concerning the serum Ca++ levels, results have shown that serum Ca++ concentration was almost the same and there was not revealed difference between in all experimental group animals, neither before, nor after administration of Palosuran or L-NAME.

Graph N4



IV. Discussion

MAP increase in lab rats after disease modeling 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.

Treatment with Palosuran decreases MAP in both, healthy and hypertensive rats. Decline in MAP in all study groups of Palosuran treated experimental animals could be explained by antagonistic effect of Palosuran on UTR.

According to the obtained data could be said that 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.

In case of treatment started relatively later, the antihypertensive effect of Palosuran was less manifested. Supposedly, damaging effects of hypertension on blood vessels increase production of U-II and enhance the endothelium-independent vasoconstrictive effect of U-II.

As the results of study have shown, there is progressive rise in serum Na+ and decrease in K+ concentrations compared to the data of healthy rats due to a significant relationship between electrolyte levels and blood pressure [22], [23]. Notably, serum total sodium was consistently and significantly higher in hypertensive groups than in the non-hypertensive, healthy animal group, while, serum potassium was lower.

The increase in serum Na+ at renovascular hypertension develops as a result of renal artery ischemia and activation of RAAS leading to increased sodium reabsorption. By the 8th weeks of hypertension the slight decrease in serum Na+ level could be explained as compensatory reaction of kidneys. In the first, one-clip two-kidney Goldblatt hypertension, the ischemic kidney secretes renin, which leads to increased angiotensin II formation and hence elevation of blood pressure. As blood pressure rises, sodium excretion by the intact contralateral kidney increases (pressure natriuresis), therefore, there is no sodium retention.

Decreased level of serum potassium level revealed in our experimental studies at late stage of hypertensive disease could be explained by the sodium and potassium reciprocal relationship in the kidneys. Potassium levels often change with sodium levels. When sodium levels go up, potassium levels go down, and when sodium levels go down, potassium levels go up. Potassium levels are also affected by a hormone called aldosterone. In case of kidney hypoxia and activated RAAS system the hormone aldosterone acting on the distal tubules, triggers potassium excretion and resorption of sodium.

In carried out investigations we could not establish any significant differences in the serum mean levels of calcium between the healthy, control and treated group animals. It is possible that intracellular calcium level may be more important in systemic hypertension than serum calcium level, which was not measured in our study. A number of experimental studies suggest that intracellular Ca2+ concentration is abnormally increased in vascular myocytes from hypertensive animals [24] and calcium intake

may affect blood pressure by increasing intracellular calcium in vascular smooth muscle cells leading to vasoconstriction, and by increasing vascular volume through the renin–angiotensin–aldosterone system (RAAS).

Treatment with Palosuran decreased serum Na+ and increased K+ levels in rats with renovascular hypertension at early stage of hypertension. Na+ concentration in hypertensive rats correlate with the results of mean arterial pressure at different stages of hypertension. Although Palosuran reveals hypotensive effect and maintains serum Na+ concentration within the normal range, at late stages of hypertension and relatively letter onset of treatment effect of Palosuran on electrolytes was not statistically significant.

V. Conclusion

Based on the results of experiments could be concluded that at early stages of hypertension Palosuran reveals hypotensive effect in lab. rats with renovascular hypertension and result in significant alterations in serum levels of Na^+ and K^+ except serum Ca^{++} concentration.

Palosuran decreased serum Na^+ and increased K^+ concentrations in hypertensive rats significantly. Na^+ and K^+ concentrations were maintained within the normal range even after administration of L-NAME, except during the late-onset of treatment.

Effect of Palosuran on the level of serum electrolytes at the late stages of hypertension has not been observed.

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