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EFFECT OF SACUBITRIL/VALSARTAN COMBINATION ON CIRCADIAN RHYTHM OF HEMODYNAMIC PARAMETERS, INFLAMMATORY BIOMARKERS AND MELATONIN SYNTHESIS IN EXPERIMENTAL ARTERIAL HYPERTENSION

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საკუბიტრილ/ვალსარტანის კომბიმაციის ეფექტი ჰემოდინამიკური პარამეტრების ცირკადულ რითმზე, ანთებით ბიომარკერებზე და მელატონინის სინთეზზე არტერიული ჰიპერტენზიის ექსპერიმენტულ მოდელში

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რეზიუმე

ბოლო პერიოდში, განსაკუთრებული ყურადღება ეთმობა არტერიული წნევის ცირკადულ რიტმს, რადგან ღამის არტერიული წნევის მატება და ადრე დილას არტერიული წნევის მომატება წარმოადგენს კარდიო-ცერებროვასკულური დაავადებების დამოუკიდებელ რისკ-ფაქტორს. რენინ-ანგიოტენზინ-ალდოსტერონის სისტემა (რაას) მონაწილეობს არტერიული წნევის ცირკადული რიტმის ფორმირებაში, ხოლო რაას ინჰიბიტორები მნიშვნელოვან როლს ასრულებენ არტერიული ჰიპერტენზიის ცირკადული რიტმის მართვაში.

ექსპერიმენტები ჩატარდა 200,0-250,0 მასის მქონე მამრ ვირთაგვებზე. ცხოველები რანდომულად დაიყვნენ შემდეგ ჯგუფებად: I – საკონტროლო ჯგუფი; II – DOCA (დეოქსიკორტიკოსტერონ აცეტატი) - მარილოვანი ჰიპერტენზიული ჯგუფი; III - საკუბიტრილი/ვალსარტანი (S/V) პერორალური დოზით 30 მგ/კგ DOCA-ს და მარილოვანი ხსნარის 4 კვირიანი მიღების შემდეგ; IV – S/V პერორალური დოზა 30 მგ/კგ DOCA-ს და მარილოვანი ხსნარის მიღების პარალელურად.

ვირთაგვების II ჯგუფში, DOCA-მარილოვანი არტერიული ჰიპერტენზიით, სისტოლური არტერიული წნევის (SBP) საშუალო მნიშვნელობები - 153,6 ± 5,4 მმ Hg, დიასტოლური არტერიული წნევა (DBP) - 67,9 ± 2,8 mmHg და გულისცემის სიხშირე (HR) - 394 ± 12/წთ იყო მნიშვნელოვნად მომატებული საკონტროლო ჯგუფთან შედარებით (123,0 ± 5,2 მმ Hg, P<0,001), (55,6 ± 3,0 mmHg, p<0,05) და (361 ± 24/წთ, p<0.001), შესაბამისად. რაც შეეხება ცხოველთა III ჯგუფს, S/V კომბინაციით მკურნალობა აღმოჩნდა ეფექტური, SBP და DBP, ისევე როგორც HR დაუბრუნდა ნორმალურ მაჩვენებლებს. IV - ექსპერიმენტულ ჯგუფში S/V კომბინაციამ გამოავლინა პრევენციული მოქმედება სისტოლური და დიასტოლური არტერიული წნევის მაჩვენებლებისა და გულის შეკუმშვათა სიხშირის შემცირებით (-50 ± 11,8 mmHg, p<0,001), (-16 ± 6,0 mmHg, p<0,001).), (- 67 ± 15,6 დარტყმა/წთ, p<0,001). ჰიპერტენზიულ ვირთაგვებში, ანთებითი მარკერების დონე მნიშვნელოვნად გაიზარდა, კერძოდ, IL-18,6 პგ/მლ და TNF-ალფა 46,8 პგ/მლ. S/V-ის გამოყენების შემდეგ III და IV ექსპერიმენტულ ჯგუფებში აღნიშნული მარკერების რაოდენობა დაუბრუნდა ნორმალურ დიაპაზონს. II ექსპერიმენტულ ჯგუფში მელატონინის დონე მნიშვნელოვნად შემცირდა საკონტროლო ჯგუფთან შედარებით (-6 პგ/მლ) და კვლავ გაიზარდა ჰიპერტენზიის პრევენციისა და S/V-ით მკურნალობის შემდეგ. შესაბამისად გამოვლინდა ჰემოდინამიკური პარამეტრების დარღვეული ცირკადული რიტმის გაუმჯობესება ცხოველთა იმავე ექსპერიმენტულ ჯგუფებში. მელატონინის რაოდენობის საპირისპიროდ, ანგიოტენზინ II-ის დონე გაიზარდა ჰიპერტენზიულ ვირთაგვებში და კვლავ შემცირდა არტერიული წნევის ნორმალიზების შემდეგ.

შეგვიძლია ვივარაუდოთ, რომ არტერიული ჰიპერტენზიის დროს მომატებული მოცირკულირე ანგიოტენზინ II კვეთს ჰემატოენცეფალურ ბარიერს და მოქმედებს მელატონინის მასინთეზირებელ ფერმენტ ტრიპტოფან ჰიდროქსილაზაზე, რასაც მოჰყვება მელატონინის დონის

დაქვეითება. ეს თავის მხრივ იწვევს ანთებითი მედიატორების, IL1 და TNF ზრდას. მიღებულ მონაცემებზე დაყრდნობით, შეგვიძლია დავასკვნათ, რომ S/V კომბინაციას აქვს მოქმედების მრავალმხრივი მექანიზმი, რომელსაც გააჩნია მნიშვნელოვანი დადებითი გავლენა არტერიული ჰიპერტენზიაზე.

Introduction. Understanding the function of innate and adaptive immunity in the pathophysiology of cardiovascular disease has improved significantly in recent years [1]. One of the most common pathological disorders is arterial hypertension (AH). AH affects one in three adults over the age of thirty. Over the previous 30 years, the overall number of diagnoses has doubled, despite the age-adjusted prevalence remaining stable [2]. The prevalence of AH rises with age; among a group of previously healthy individuals, diagnoses were detected in 0.3%, 6.5%, and 37% of cases at ages 25, 45, and 65, respectively [3].

Cardiovascular disease (CVD) is the world's leading cause of death, with 17.3 million deaths annually and a predicted rise to almost 23.6 million by 2030 [4]. As the primary risk factor for CVD morbidity and mortality worldwide, hypertension has been suggested to be responsible for half of all CVD events [4]. In order to lower the risk of CVD events and the associated healthcare burden, it is crucial to prevent, treat, and manage hypertension. angiotensin II receptor blockers (ARBs) and Angiotensin-converting enzyme inhibitors (ACEIs) are first line anti-hypertensive drug classes that are potent, effective and largely safe [5].

Hypertension has a significant impact on cardiovascular outcomes, including myocardial infarction, heart failure and stroke. As a worldwide risk factor for death, disability-adjusted life years, and loss of life years, it continues to be the most powerful predictor of mortality [7,8]. A number of sources of evidence, ranging from integrative physiology and functional genomics to molecular and genetic levels, show that the renin-angiotensin-aldosterone system RAAS, whether systemic (endocrine) or local (paracrine and autocrine), is a significant cause of hypertension and cardiovascular disease [9-11]. RAAS is an important endocrine modulator of cardiovascular homeostasis. The RAAS functions in multiple organs and systems in an endocrine, paracrine, and autocrine way, performing organ-specific activities with consequences on the cardiovascular system [9].

RAAS is triggered as part of a maladaptive response that contributes to the pathophysiology of heart failure. Vasoconstriction, hypertension, elevated aldosterone, elevated sympathetic tone, and ultimately cardiac remodeling are all consequences of RAAS activation that are harmful to the disease's course. By preventing these maladaptive components, ACEIs (angiotensin converting enzyme inhibitors) and ARBs (angiotensin receptor blockers) significantly lower the morbidity and death associated with heart failure [12]. This understanding resulted in the effective creation of RAAS-blocking medications (angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and renin inhibitors) for the treatment of hypertension and other cardiovascular disorders [13].

Angiotensin receptor neprilysin inhibitors (ARNI) are a novel class of medications, the first of which being sacubitril/valsartan (S/V). For the treatment of patients with chronic heart failure with reduced ejection fraction (HFrEF) and NYHA classes II, III, or IV, the drug has FDA approval [6]. In addition to other standard therapies for heart failure (beta-blocker, aldosterone antagonist), S/V is to be used in alternative to ACEIs or angiotensin II receptor blockers (ARBs) [14]. S/V is advised in the new AHA/ACC/HFSA guidelines (2022) for the treatment of individuals with heart failure with preserved ejection fraction (HFpEF) [15].

S/V is a combination product. Pro-drug sacubitril functions as an inhibitor of neprilysin once it is activated. The mechanism of action involves inhibiting neprilysin's ability to break down natriuretic peptides, hence prolonging the beneficial effects of these peptides [16]. Valsartan is an angiotensin receptor blocker, and it works on blocking the RAAS system. But blocking neprilysin will cause angiotensin II to build up because neprilysin breaks down angiotensin II. Because of this a neprilysin inhibitor cannot be used alone; it must always be combined with an ARB to block the effect of the excess angiotensin II [14]. The inflammatory response and immune system are important players in the aetiology of hypertension. The involvement of inflammation in the pathophysiology of hypertension is supported by the elevated levels of numerous inflammatory markers in hypertension patients [17]

These markers are linked to an increased risk of hypertension in people with normal blood pressure, and they can help predict the risk of cardiovascular events in patients with essential hypertension by indicating target organ damage [18].

Thus, understanding how inflammation contributes to hypertension opens novel options for managing the condition and its aftereffects [19]. Despite the fact that the precise pathophysiology of essential hypertension is yet unknown, endothelial dysfunction is thought to be a major factor in the development of the condition. Although it was often believed that the vascular endothelium served as an inert barrier separating the blood from the blood vessels, it is now recognized as a key hub for vascular regulation. Vascular wall tension, nutrient supply, waste elimination, inflammation, thrombosis, and coagulation are all crucial functions of the endothelium. Thus, it is claimed that hypertension is partially an inflammatory illness [20]. Oxygen metabolism produces reactive oxygen species. Reactive oxygen species are produced under oxidative stress when pro-oxidative factors exceed anti-oxidative factors [19]. According to Tenório et al. (2019) [21] oxidative stress intensifies the inflammatory response by increasing the synthesis of proinflammatory factors. Increased pro-inflammatory immune cells and cytokines and decreased regulatory immunity cells and cytokines lead to a chronic and uncontrollably inflammatory state [22]. In the vascular system, elevated levels of TNF-α and IL-1 can cause endothelial dysfunction and progressive vascular endothelial injury, which can cause widespread inflammation and cell death [23].

Melatonin is a key hormone that regulates the circadian rhythmicity of various biological systems and is mostly produced by the pineal gland at night [24]. Melatonin functions investigation has shown that it is not only a controller of the biological circadian rhythm [11], but it also has a variety of physiological functions [25]. Melatonin appears to be linked in a variety of diseases, including arterial hypertension, insomnia, dementia, mood disorders, cancer, and diabetes [26]. Although the central nervous system, immune system (IS), and endocrine system (ES) network use norepinephrine, IL-1, and cortisol, melatonin appears as a chemical capable of playing various roles in both IS and ES. Its secretion and function are regulated by norepinephrine and IL-1. Furthermore, while it was previously thought to be merely a hormone, its role as a multifunctional molecule is now being researched, and as a result, we want to think about its significance in chronic arterial hypertension [27].

Recent attention has been paid to the circadian rhythm of blood pressure (BP) because an increase in nocturnal BP and an increase in morning BP have been shown to be independent risks for cardio-cerebrovascular diseases. The renin-angiotensin-aldosterone system (RAAS) is involved in the circadian rhythm of BP, and RAAS inhibitors play a crucial role in controlling the circadian rhythm of BP [28, 29]. Nighttime administration of RAAS inhibitors is more beneficial than morning administration for lowering nocturnal and morning BP levels and converting the BP profile to a dipper pattern, a process known as chronotherapy [30]. Controlling abnormal circadian rhythms of blood pressure in addition to 24-hour BP utilizing RAAS inhibitors with optimal time dosage should be assumed for reducing cardio-cerebro-vascular events [30].

The brain produces both angiotensin and melatonin. Angiotensin, which is produced locally in the central nervous system in nuclei implicated in cardiovascular and fluid-electrolyte homeostasis, interacts with various systems, including sympathetic and vasopressinergic systems [9,31]. Furthermore, there is a local pineal RAS that regulates the production of melatonin, which is the pineal gland's major hormone secretion [32,33]. The RAS is known to be implicated in cardiovascular and metabolic disease, whereas melatonin is involved in circadian rhythms.

Research materials and methods. Experiments were carried out on male rats weighing 200,0-250,0 g. in compliance with the rules and laws developed by the Bioethical Commission of Tbilisi State Medical University (TSMU). The animals were placed in a vivarium at a temperature of 23 ± 1° C, 50 ± 5% humidity and 12 hours of light -12 hours of darkness, in terms of free access to food and water. Animals were randomly divided into the following groups: I – control group with 1% NaCl and 0.2% KCl in drinking water during 4 weeks (n=10); II – DOCA-salt hypertensive group (25 mg/kg DOCA + 1% NaCl and 0.2% KCl in drinking water) (n = 10); III - sacubitril/valsartan oral dose of 30 mg/kg per day after 28-day intake of DOCA and salt solutions administration (n = 10). IV - sacubitril/valsartan oral dose 30 mg/kg per day + DOCA + 1% NaCl + 0.2% KCl in drinking water for 4 weeks (n = 10);

To monitor the dynamics of the development of DOCA-salt hypertension, systolic, diastolic and mean blood pressure have been measured every week from the tail of non-narcotized rats in a special chamber by sphygmomanometric method (tail cuff method). For this purpose, the animals were placed in the chamber for adaptation for 2 h, after which the pressure indicators were determined 5 times, at intervals of 5-10 min, and average values were calculated. (Fig.1) For circadian rhythmicity systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart beats (HB) were measured 3 times per day, at 9:00, at 15:00 and at 21:00.

At the end of the experiment, rats were randomly divided inside the groups and were anesthetized (with pentobarbital 65 mg/kg intraperitoneally) to take samples of blood from a catheter in the carotid artery at the appropriate hours of acrophasis of blood pressure in the morning (n=5) and in the midnight (n=5).

After centrifugation the blood samples were frozen at -60◦C, and then the concentration of Melatonin (MT), Angiotensin II (Ang II), Interleukin-1 (IL-1), Tumor Necrosis Factor alpha (TNF-alpha), Endothelin-1 (ET-1) and Nuclear Factor kappa B (NFkB) were determined using the ELISA kit.

Statistical data processing revealed changes in hemodynamic parameters, blood inflammatory markers and cardiac remodeling between the groups. To compare the data of two groups t-test was used, multiple indicators from several study groups were compared by the ANOVA method.

Results. In II group of rats with DOCA-salt arterial hypertension the mean values of SBP – 153,6 ± 5,4 mmHg, DBP – 67,9 ± 2,8 mmHg and HB – 394 ± 12/min were significantly higher in comparison with I group of animals (123,0 ± 5,2 mmHg, P<0.001), (55,6 ± 3,0 mmHg, p<0.05) and (361 ± 24/min, p<0.001), respectively. In III group of animals, treatment with the S/V combination proved to be beneficial and the SBP and DBP, as well as the heart rate returned to normal values. As for IV experimental group S/V revealed preventive action regarding hemodynamic changes during development of AH by decreasing values of SBP (-50±11,8 mmHg, p<0,001), DBP (-16±6,0 mmHg, p<0,001), and HB (- 67±15,6 beat/min, p<0,001) (Fig.1, 1.2).

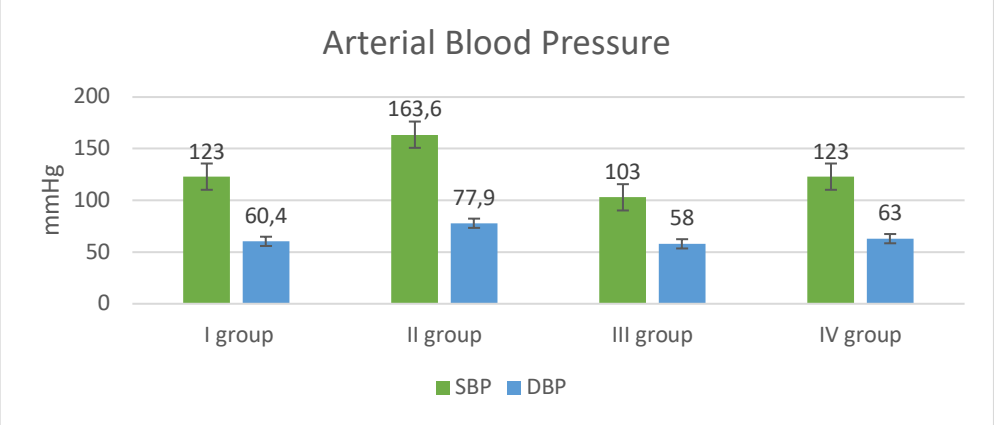

Fig.1 Systolic and diastolic blood pressure in different experimental groups of animals.

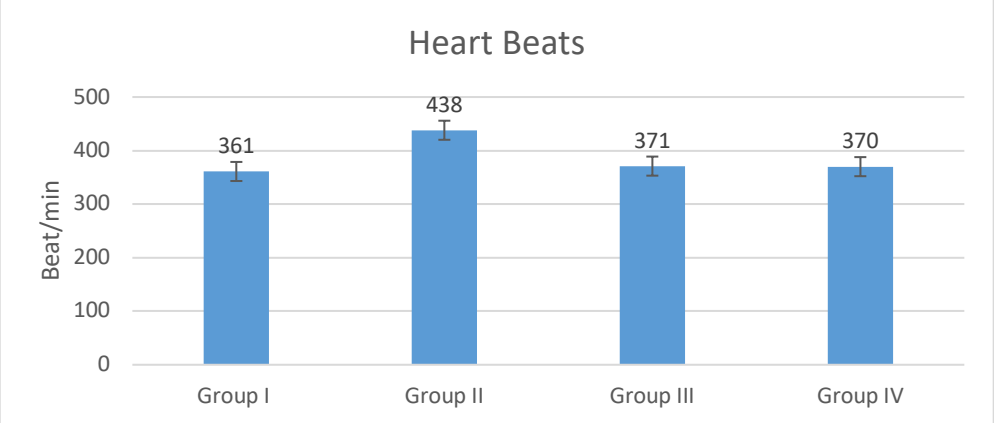

Fig. 1.2 Heart beats in different experimental groups of animals.

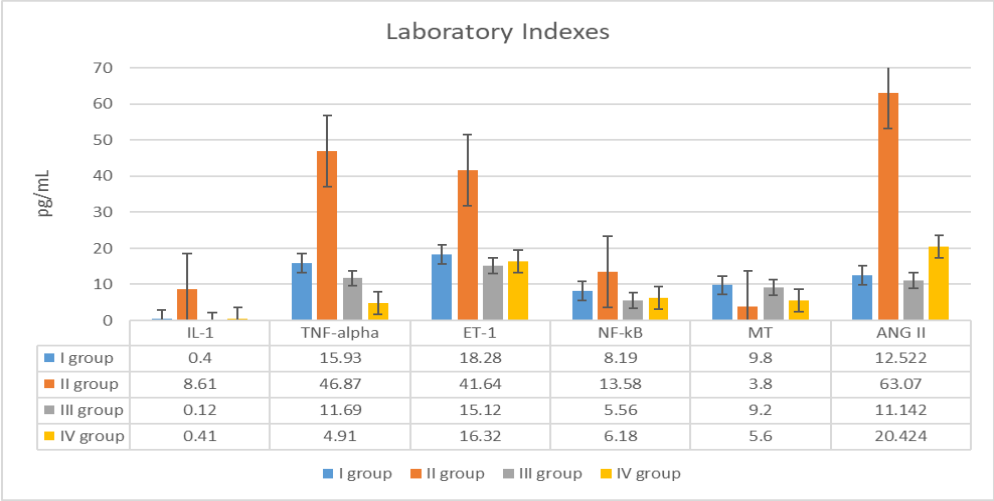

Fig.2 Laboratory indexes in different groups of animals.

Changes in hemodynamic indicators in hypertensive rats were correlated with significant increase in blood plasma levels of inflammatory biomarkers (IL-1, TNF-alpha, NF-kB) - (8,2 ± 1,7 pg/mL, p<0,001), (31,0 ± 12,7 pg/mL, p<0,001), (5,4 ± 3,8 pg/mL, p<0,05) respectively, vasoconstrictor peptides (ET-1) – (23,3 ± 3,5 pg/mL, p<0,001), angiotensin II (Ang II)– (63,070 ± 26,2 pg/mL, p<0,001), vs I group of animals. In III experimental group S/V markedly reduced plasma levels of inflammatory markers, vasoconstrictor agents in comparison with II group of animals: IL-1 (-8,2 ± 0,17 pg/mL, p<0,001), TNF-alpha (-42 ± 4,6 pg/mL, p<0,001) and NFkB (-7,4 ± 1,9 pg/mL, p<0,05), ET-1 (-16,3 ± 2,7 pg/mL, p<0,001), Ang II (-42.6 ± 4.4 pg/mL). In the IV experimental group compared to hypertensive animals, reduced indicators were also revealed: IL-1 (0.12 ± 0.21 pg/mL, p<0,001), TNF-alpha (11.69 ± 2.95 pg/mL, p<0,001) and NFkB (5.56 ± 1.52 pg/mL, p<0,05), ET-1 (15.12 ± 9.51pg/mL, p<0,001), Ang II (11,1 ± 5 pg/mL).

Significant changes were detected in the case of melatonin. In hypertensive rats vs I group, melatonin levels were decreased in hypertensive animals (3.89 ± 1.6 pg/mL, p<0,001) and in control group of animals it was remained normal range (9.8 ± 3.6 pg/mL, p<0,001). However, its index changed in the opposite way in the preventive and treated group compared to the hypertensive animals. For III group of animals (9.2 ± 2.0 pg/mL, p<0,001), and for IV group of animals (5.6 ± 1.9 pg/mL, p<0,001).

	I group	II group	III group	IV group
SBP at 09:00	109.00 ± 11.46 mmHg	144.90 ± 13.72 mmHg	110.00±11.79 mmHg	103.70 ± 4.57 mmHg
SBP at 15:00	123.00 ± 5.23 mmHg	136.20 ± 14.46 mmHg	123.20±10.84 mmHg	105.70 ± 5.57 mmHg
SBP at 21:00	105.80 ± 10.21 mmHg	153.60 ± 15.42 mmHg	108.10±9.42 mmHg	105.80 ± 8.99 mmHg
DBP at 09:00	60.100 ± 4.067 mmHg	67.900 ± 8.185 mmHg	63.500±9.992 mmHg	58.800 ± 2.486 mmHg
DBP at 15:00	60.400 ± 3.098 mmHg	57.400 ± 3.893 mmHg	59.200±4.849 mmHg	51.800 ± 6.088 mmHg
DBP at 21:00	55.600 ± 2.914 mmHg	69.500 ± 5.701 mmHg	56.500±4.275 mmHg	56.100 ± 5.065 mmHg
HB at 09:00	358.90 ± 27.30 b/min	413.90 ± 28.91 b/min	356.40±15.33 b/min	365.70 ± 11.70 b/min
HB at 15:00	361.40 ± 24.91 b/min	394.50 ± 12.47 b/min	370.20±31.00 b/min	371.10 ± 15.67 b/min
HB at 21:00	363.50 ± 30.89 b/min	438.50 ± 25.63 b/min	372.60±18.24 b/min	368.70 ± 15.39 b/min

Tab.1 Hemodynamic parameters in different experimental groups of animals.

As for circadian rhythmicity the analysis of hemodynamic parameters revealed significant differences in the baseline values of BP and HB between rats with hypertension and the control group. In hypertensive rats the highest value of SBP was 153 ± 8 mmHg in evening, which correlated with mean significances of HB – 438 ± 25 b/min at the same time. The lowest values of SBP, as well as HB were in the afternoon - 136 ± 14 mmHg, 394 ± 12 b/min, respectively. After the administration of S/V oral dose 30 mg/kg for 2 weeks the circadian rhythm of hemodynamic parameters was corrected and hemodynamic parameters measured in the morning, afternoon and evening returned to the values of the control group 110 ± 11 mmHg, 356 ± 15 b/min at 09:00h. 123 ± 10 mmHg, 370 ± 31 b/min at 15:00h. and 108 ± 9 mmHg, 372 ± 18 b/min at 21:00h. (Tab.1)

Discussion. The aim of our study was to test S/V combination for the treatment of arterial hypertension and its preventive effect on the development of arterial hypertension in DOCA-salt rats. We decided to evaluate the efficacy of this combination (sacubitril/valsartan) and its antihypertensive and preventive effects along with the level of anti-inflammatory markers, melatonin and angiotensin II because the association between arterial hypertension and inflammatory markers is well recognized [34, 35].

The renin-angiotensin-aldosterone system (RAAS) plays a crucial role in the pathogenesis of arterial hypertension [5]. The primary effector molecule of the RAAS is angiotensin II. It increases blood pressure, encourages the kidney tubules to hold on to both sodium and water, and stimulates the adrenal glands to release aldosterone. Ang II has strong vasoconstrictor properties in addition to proliferative, pro-inflammatory, and pro-fibrotic effects [36]. Angiotensin II (Ang II) is a regulatory peptide hormone that stimulates the constriction of vascular smooth muscle cells (VSMCs) [37].

In our study, the significant increase of ET-1 and Ang II in the hypertensive group emphasizes their pathogenic role in the development of arterial hypertension, which is consistent with the data in the research, and their significant reduction confirms the effectiveness of S/V in the treatment of arterial hypertension [20].

In an effort to find new pharmacological therapy targets for the treatment of hypertension, there has also been an extensive amount of study conducted to better understand the relationship between inflammation and hypertension, with a focus on the role of inflammation playing in the development of hypertension [38].

In our study, we evaluated NF-kB, TNF and IL-1 and found that the inflammatory markers were significantly increased in the hypertensive group and significantly decreased in the treated and prevention groups. Genes related to inflammation and immunological responses are regulated by nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Diabetes, atherosclerosis, and cardiovascular diseases (CVDs) may be significantly influenced by NF-κB [39], which in turn is activated by angiotensin II via AT1 and AT2 in vascular smooth muscle cells [40]. NF-κB mediates the induction of pro-inflammatory cytokines, such as TNF-α, IL-1 and IL-6, in monocytes/macrophages [41]. One of the key members of the IL-1 family of interleukins, interleukin 1 beta (IL-1β) is primarily produced by neutrophils, T cells, and monocytes. The correlation between IL-1β and hypertension has been increasing in amount of data [42]. Specifically, it has been shown that IL-1β may activate a large number of proinflammatory genes, leading to further tissue damage and inflammation-related events, such as hypertension and myocardial infarction [43]. In addition to directly initiating the inflammatory response, IL-1β also regulates the characteristics and functions of VSMCs, ultimately resulting in vascular remodeling through either independent or dependent pathways on inflammation [44]. The function of TNF-α signaling in controlling numerous secondary inflammatory processes, including the secretion of cytokines, differentiation of cells, and apoptosis [45]. TNF-α so contributes significantly to blood pressure elevation. TNF-α inhibitors are presently mostly used to treat autoimmune disorders; however, there is no proof that they can be used to lower blood pressure and improve the prognosis for cardiovascular diseases [46].

According to current findings, the pineal gland may also have an important role in regulating the immunological response [47]. Furthermore, the pineal gland and the immune system have a two-way link

as interleukins and cytokines regulate melatonin production and release [48]. Recombinant IL1 decreased blood melatonin levels in rats via a receptor mechanism, and TNF produced by pineal gland microglia hindered melatonin synthesis [49, 50]. Furthermore, through scavenging reactive oxygen species, lowering oxidative stress, reducing inflammation, delaying ageing, and activating gene damage repair pathways, melatonin plays significant roles in a number of disorders [51,52]

Our study revealed a significant decrease in melatonin in the hypertensive group and an increase in its amount in the treatment and prevention group, indicating the effect of S/V on the circadian rhythm, the disruption of which also increases cardiovascular mortality [53].

Growing data indicates that hypertension is a chronic inflammatory disease involving immune cell migration, accumulation, and activation as well as ROS generation. Because hypertension is a complex disorder, it's important to understand the pathogenic mechanisms underlying its many different stages.

We hypothesize that during arterial hypertension, elevated circulating angiotensin II that crosses the blood-brain barrier acts on the melatonin-synthesizing enzyme tryptophan hydroxylase and causes its downregulation, which is followed by a decrease in melatonin levels, which in turn causes an increase in the inflammatory mediators IL1 and TNF, because melatonin inhibits their synthesis [54], which we have shown in our research.

In our study, with the use of S/V, blood pressure was reduced and inflammatory markers were statistically significantly reduced in both the treated and prevention groups, indicating the antihypertensive and anti-inflammatory properties of the S/V combination, and therefore it can be considered as a drug for the treatment of arterial hypertension. It also has a preventive effect in the presence of risk factors and prevents the development of both hypertension and the inflammation, which subsequently leads to target organ damage and cardiovascular mortality.

Conclusion. Eventually we hypothesize that during arterial hypertension, elevated circulating angiotensin II crosses the blood-brain barrier and acts on the melatonin-synthesizing enzyme tryptophan hydroxylase causing its downregulation, which is followed by a decrease in melatonin levels. This in turn accompanied with increase in the inflammatory mediators IL1 and TNF, because melatonin inhibits their synthesis, which was shown in our research. Based on the obtained data we can conclude that the S/V has a multiple mechanism of pluripotent action that is likely provide positive effects on the long-term outcomes of hypertension and patient survival.

References:

1. Buckley LF, Abbate A. Interleukin-1 blockade in cardiovascular diseases: a clinical update. Eur Heart J. 2018 Jun 7;39(22):2063-2069. doi: 10.1093/eurheartj/ehy128. PMID: 29584915.
2. Martín-Fernández J, Alonso-Safont T, Polentinos-Castro E, et al. Impact of hypertension diagnosis on morbidity and mortality: a retrospective cohort study in primary care. BMC Prim Care. 2023 Mar 23;24(1):79. doi: 10.1186/s12875-023-02036-2.
3. Shihab HM, Meoni LA, Chu AY, Wang NY, Ford DE, Liang KY, Gallo JJ, Klag MJ. Body mass index and risk of incident hypertension over the life course: The Johns Hopkins Precursors Study. Circulation. 2012 Dec 18;126(25):2983-9. doi: 10.1161/CIRCULATIONAHA.112.117333. Epub 2012 Nov 14. PMID: 23151344; PMCID: PMC3743236.
4. Lawes CM, Vander Hoorn S, Rodgers A; International Society of Hypertension. Global burden of blood-pressure-related disease, 2001. Lancet. 2008 May 3;371(9623):1513-8. doi: 10.1016/S0140-6736(08)60655-8.
5. Bavishi C, Bangalore S, Messerli FH. Renin Angiotensin Aldosterone System Inhibitors in Hypertension: Is There Evidence for Benefit Independent of Blood Pressure Reduction? Prog Cardiovasc Dis. 2016 Nov-Dec;59(3):253-261. doi: 10.1016/j.pcad.2016.10.002. Epub 2016 Oct 21. PMID: 27777044.
6. Nicolas D, Kerndt CC, Reed M. Sacubitril-Valsartan. 2022 May 26. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan. PMID: 29939681.

7. Lawes CM, Vander Hoorn S, Rodgers A; International Society of Hypertension. Global burden of blood-pressure-related disease, 2001. Lancet. 2008 May 3;371(9623):1513-8. doi: 10.1016/S0140-6736(08)60655-8.
8. Angeli F, Reboldi G, Trapasso M, Gentile G, Pinzagli MG, Aita A, Verdecchia P. European and US guidelines for arterial hypertension: similarities and differences. Eur J Intern Med. 2019 May; 63:3-8. doi: 10.1016/j.ejim.2019.01.016. Epub 2019 Feb 4. PMID: 30732939.
9. Bader M, Peters J, Baltatu O, Müller DN, Luft FC, Ganten D. Tissue renin-angiotensin systems: new insights from experimental animal models in hypertension research. J of Molecular Medicine. 2001;79(2):76–102.
10. Campos LA, Bader M, Baltatu OC. Brain renin-angiotensin system in hypertension, cardiac hypertrophy, and heart failure. Frontiers in Physiology. 2011;2, artile 115
11. Baltatu OC, Campos LA, Bader M. Local renin-angiotensin system and the brain: a continuous quest for knowledge. Peptides. 2011;32(5):1083–1086.
12. Du AX, Westerhout CM, McAlister FA, et al. Titration and Tolerability of Sacubitril/Valsartan for Patients With Heart Failure in Clinical Practice. J Cardiovasc Pharmacol. 2019 Mar;73(3):149-154. doi: 10.1097/FJC.0000000000000643.
13. von Lueder TG, Krum H. RAAS inhibitors and cardiovascular protection in large scale trials. Cardiovasc Drugs Ther. 2013 Apr;27(2):171-9. doi: 10.1007/s10557-012-6424-y. PMID: 23224653
14. Nicolas D, Kerndt CC, Reed M. Sacubitril-Valsartan. [Updated 2022 May 26]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK507904/
15. Heidenreich PA, Bozkurt B, Aguilar D, Allen LA et al. 2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. Circulation. 2022 May 3;145(18):e895-e1032. doi: 10.1161/CIR.0000000000001063. Epub 2022 Apr 1. Erratum in: Circulation. 2022 May 3;145(18):e1033. Erratum in: Circulation. 2022 Sep 27;146(13):e185. Erratum in: Circulation. 2023 Apr 4;147(14):e674.
16. In brief: Sacubitril/valsartan (Entresto) expanded indication. Med Lett Drugs Ther. 2021 May 3;63(1623):65.
17. Pruijm M, Vollenweider P, Mooser V, et al. Inflammatory markers and blood pressure: sex differences and the effect of fat mass in the CoLaus Study. J Hum Hypertens. 2013 Mar;27(3):169-75. doi: 10.1038/jhh.2012.12. Epub 2012 Apr 12. PMID: 22495106.
18. Aydin C, Alpsoy Ş, Akyüz A, et al. Could the systemic immune-inflammation index be a predictor to estimate cerebrovascular events in hypertensive patients? Blood Press Monit. 2022 Feb 1;27(1):33-38. doi: 10.1097/MBP.0000000000000560.
19. Yihui C, Yanfeng G. Inflammatory markers in patients with hypertension. Br J Hosp Med (Lond). 2023 May 2;84(5):1-8. doi: 10.12968/hmed.2022.0531. Epub 2023 May 22. PMID: 37235676.
20. Gupta V, Sachdeva S, Khan AS, Haque SF. Endothelial dysfunction and inflammation in different stages of essential hypertension. Saudi J Kidney Dis Transpl. 2011 Jan;22(1):97-103. PMID: 21196621.
21. Tenório MB, Ferreira RC, Moura FA, Bueno NB, de Oliveira ACM, Goulart MOF. Cross-Talk between Oxidative Stress and Inflammation in Preeclampsia. Oxid Med Cell Longev. 2019 Nov 4;2019:8238727. doi: 10.1155/2019/8238727. PMID: 31781353; PMCID: PMC6875353.
22. Harmon AC, Cornelius DC, Amaral LM, Faulkner JL, Cunningham MW Jr, Wallace K, LaMarca B. The role of inflammation in the pathology of preeclampsia. Clin Sci (Lond). 2016 Mar;130(6):409-19. doi: 10.1042/CS20150702. PMID: 26846579; PMCID: PMC5484393.
23. Winklewski PJ, Radkowski M, Wszedybyl-Winklewska M, Demkow U. Brain inflammation and hypertension: the chicken or the egg? J Neuroinflammation. 2015 May 3;12:85. doi: 10.1186/s12974-015-0306-8.
24. Pevet P, Challet E. Melatonin: both master clock output and internal time-giver in the circadian clocks network. Journal of Physiology Paris. 2011;105(4-6):170–182.
25. Hardeland R. Neurobiology, pathophysiology, and treatment of melatonin deficiency and dysfunction. The Scientific World Journal. 2012;2012:18 pages.640389

26. Carpentieri A, Díaz de Barboza G, Areco V, Tolosa de Talamoni N. New perspectives in melatonin uses. Pharmacological Research. 2012;65(4):437–444.
27. Zefferino R, Di Gioia S, Conese M. Molecular links between endocrine, nervous and immune system during chronic stress. Brain Behav. 2021 Feb;11(2):e01960. doi: 10.1002/brb3.1960. Epub 2020 Dec 8. PMID: 33295155; PMCID: PMC7882157.
28. Ohkubo T., Hozawa A., Yamaguchi J., et al. Prognostic significance of the nocturnal decline in blood pressure in individuals with and without high 24-h blood pressure: The Ohasama study. J. Hypertens. 2002;20:2183–2189. doi: 10.1097/00004872-200211000-00017.
29. Virag J.A., Lust R.M. Circadian influences on myocardial infarction. Front. Physiol. 2014;5:422. doi: 10.3389/fphys.2014.00422.
30. Yagi S, Sata M. [Renin-angiotensin-aldosterone inhibitors for treatment of hypertension with abnormal circadian rhythm of blood pressure]. Nihon Rinsho. 2014 Aug;72(8):1466-71. Japanese. PMID: 25167754.
31. Campos LA, Couto AS, Iliescu R, et al. Differential regulation of central vasopressin receptors in transgenic rats with low brain angiotensinogen. Regulatory Peptides. 2004;119(3):177–182.
32. Baltatu O, Nishimura H, Hoffmann S, et al. High levels of human chymase expression in the pineal and pituitary glands. Brain Research. 1997;752(1-2):269–278.
33. Baltatu O, Lippoldt A, Hansson A, Ganten D, Bader M. Local renin-angiotensin system in the pineal gland. Molecular Brain Research. 1998;54(2):237–242.
34. Schieffer B, Schieffer E, et al. Expression of angiotensin II and interleukin 6 in human coronary atherosclerotic plaques: potential implications for inflammation and plaque instability. Circulation. 2000 Mar 28;101(12):1372-8. doi: 10.1161/01.cir.101.12.1372. PMID: 10736279.
35. Kranzhöfer R, Schmidt J, Pfeiffer CA, Hagl S, Libby P, Kübler W. Angiotensin induces inflammatory activation of human vascular smooth muscle cells. Arterioscler Thromb Vasc Biol. 1999 Jul;19(7):1623-9. doi: 10.1161/01.atv.19.7.1623. PMID: 10397679.
36. Benigni A, Cassis P, Remuzzi G. Angiotensin II revisited: new roles in inflammation, immunology and aging. EMBO Mol Med. 2010 Jul;2(7):247-57. doi: 10.1002/emmm.201000080. PMID: 20597104
37. Anderson GH Jr, Howland T, Domschek R, Streeten DH. Effect of sodium balance and calcium channel-blocking drugs on plasma aldosterone responses to infusion of angiotensin II in normal subjects and patients with essential hypertension. J Clin Endocrinol Metab. 1986 Nov;63(5):1126-35. doi: 10.1210/jcem-63-5-1126.
38. Dinh QN, Drummond GR, Sobey CG, Chrissobolis S. Roles of inflammation, oxidative stress, and vascular dysfunction in hypertension. Biomed Res Int. 2014;2014:406960. doi: 10.1155/2014/406960. Epub 2014 Jul 20. PMID: 25136585; PMCID: PMC4124649.
39. Matsumori A. Nuclear Factor-κB is a Prime Candidate for the Diagnosis and Control of Inflammatory Cardiovascular Disease. Eur Cardiol. 2023 Jun 7;18:e40. doi: 10.15420/ecr.2023.10. PMID: 37456770;
40. Ruiz-Ortega M, Lorenzo O, Rupérez M, König S, Wittig B, Egido J. Angiotensin II activates nuclear transcription factor kappaB through AT(1) and AT(2) in vascular smooth muscle cells: molecular mechanisms. Circ Res. 2000 Jun 23;86(12):1266-72. doi: 10.1161/01.res.86.12.1266. PMID: 10864918.
41. Davignon JL, Hayder M, Baron M, Boyer JF, Constantin A, Apparailly F, Poupot R, Cantagrel A. Targeting monocytes/macrophages in the treatment of rheumatoid arthritis. Rheumatology (Oxford). 2013 Apr;52(4):590-8. doi: 10.1093/rheumatology/kes304. Epub 2012 Nov 30. PMID: 23204551.
42. Krishnan SM, Ling YH, Huuskes BM, et al. Pharmacological inhibition of the NLRP3 inflammasome reduces blood pressure, renal damage, and dysfunction in salt-sensitive hypertension. Cardiovasc Res. 2019 Mar 15;115(4):776-787. doi: 10.1093/cvr/cvy252. PMID: 30357309; PMCID: PMC6432065.
43. Sims JE, Smith DE. The IL-1 family: regulators of immunity. Nat Rev Immunol. 2010 Feb;10(2):89-102. doi: 10.1038/nri2691. Epub 2010 Jan 18. PMID: 20081871.
44. Postlethwaite AE, Raghow R, Stricklin GP, et al. Modulation of fibroblast functions by interleukin 1: increased steady-state accumulation of type I procollagen messenger RNAs and stimulation of other functions but not chemotaxis by human recombinant interleukin 1 alpha and beta. J Cell Biol. 1988 Feb;106(2):311-8. doi: 10.1083/jcb.106.2.311. PMID: 2828381; PMCID: PMC2114989.

45. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol. 2003 Sep;3(9):745-56. doi: 10.1038/nri1184. PMID: 12949498.
46. Zhang Z, Zhao L, Zhou X, Meng X, Zhou X. Role of inflammation, immunity, and oxidative stress in hypertension: New insights and potential therapeutic targets. Front Immunol. 2023 Jan 10;13:1098725. doi: 10.3389/fimmu.2022.1098725. PMID: 36703963; PMCID: PMC9871625.
47. Guerrero, J. M. , & Reiter, R. J. (2002). Melatonin-immune system relationships. Current Topics in Medicinal Chemistry, 2(2), 167–179. 10.2174/1568026023394335.
48. Hardeland, R., Burkhardt, S., Antolin, I., Fuhrberg, B., & Coto-Montes, A. (1999). Melatonin and 5-methoxytryptamine in the bioluminescent dinoflagellate Gonyaulax polyedra. Restoration of the circadian glow peak after suppression of indoleamine biosynthesis or oxidative stress. Advances in Experimental Medicine and Biology, 460, 387–390.
49. Withyachumnarnkul, B., Nonaka, K. O., Santana, C., Attia, A. M., & Reiter, R. J. (1990). Interferon-gamma modulates melatonin production in rat pineal glands in organ culture. Journal of Interferon Research, 10(4), 403–411. 10.1089/jir.1990.10.403.
50. da Silveira Cruz-Machado, S., Pinato, L., Tamura, E. K., Carvalho-Sousa, C. E., & Markus, R. P. (2012). Glia-pinealocyte network: The paracrine modulation of melatonin synthesis by tumor necrosis factor (TNF). PLoS One, 7(7), e40142 10.1371/journal.pone.0040142.
51. Hardeland R. Melatonin and the theories of aging: a critical appraisal of melatonin's role in antiaging mechanisms. J Pineal Res. 2013; 55:325–56. 10.1111/jpi.12090.
52. Rezapoor S, Shirazi A, Abbasi S, et al. Modulation of Radiation-induced Base Excision Repair Pathway Gene Expression by Melatonin. J Med Phys. 2017; 42:245–50. 10.4103/jmp.JMP_9_17
53. Hermida RC, Crespo JJ, Otero A, Domínguez-Sardiña M, et al. Hygia Project Investigators. Asleep blood pressure: significant prognostic marker of vascular risk and therapeutic target for prevention. Eur Heart J. 2018 Dec 14;39(47):4159-4171. doi: 10.1093/eurheartj/ehy475. PMID: 30107515.
54. Huang CC, Chiou CH, Liu SC, Hu SL, Su CM, Tsai CH, Tang CH. Melatonin attenuates TNF-α and IL-1β expression in synovial fibroblasts and diminishes cartilage degradation: Implications for the treatment of rheumatoid arthritis. J Pineal Res. 2019 Apr;66(3):e12560. doi: 10.1111/jpi.12560.

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EFFECT OF SACUBITRIL/VALSARTAN COMBINATION ON CIRCADIAN RHYTHM OF HEMODYNAMIC PARAMETERS, INFLAMMATORY BIOMARKERS AND MELATONIN SYNTHESIS IN EXPERIMENTAL ARTERIAL HYPERTENSION

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SUMMARY

The circadian rhythm of blood pressure (BP) has been getting more attention since a rise in nocturnal BP and an increase in morning BP have been found to be independent risk factors for cardio-cerebrovascular disorders. The renin-angiotensin-aldosterone system (RAAS) is implicated in BP circadian rhythm, and RAAS inhibitors play an important role in BP circadian rhythm management.

Experiments were carried out on male rats weighing 200,0-250,0 g. Animals were randomly divided into the following groups: I – control group; II – DOCA-salt hypertensive group; III – S/V oral dose of 30 mg/kg per day after 28-day intake of DOCA and salt solutions administration. IV - sacubitril / valsartan (S/V) oral dose 30 mg/kg per day with DOCA and salt solutions as drinking water for 4 weeks;

In II group of rats with DOCA-salt arterial hypertension (AH) the mean values of systolic blood pressure (SBP) – 153,6 ± 5,4 mmHg, diastolic blood pressure DBP – 67,9 ± 2,8 mmHg and heart rate (HR) – 394 ± 12/min were significantly higher in comparison with control group of animals (123,0 ± 5,2 mmHg, $P<0.001$), (55,6 ± 3,0 mmHg, $p<0.05$) and (361 ± 24/min, $p<0.001$), respectively. As for III group of animals, treatment with the S/V combination proved to be beneficial and the SBP and DBP, as well as the HR

returned to normal values. In IV – experimental group S/V revealed preventive action regarding hemodynamic changes during development of AH by decreasing values of SBP (-50 ± 11,8 mmHg, p<0,001), DBP (-16 ± 6,0 mmHg, p<0,001), and HB (- 67 ± 15,6 beat/min, p<0,001). In hypertensive rats, the level of inflammatory markers increased significantly, namely IL-1 was 8.6 pg/ml and TNF-alpha was 46.8 pg/ml. Both values returned to normal range after the use of S/V in experimental groups III and IV. In a group of rats with DOCA-induced hypertension, melatonin levels were significantly decreased compared to the control group (-6 pg/ml) and increased again after prevention and treatment of hypertension with S/V. Respectively, an improvement in the disturbed circadian rhythm of hemodynamic parameters was revealed in the same experimental groups of animals. Contrary to melatonin, levels of angiotensin II were increased in hypertensive rats and decreased after blood pressure normalization with S/V combination.

We hypothesize that during arterial hypertension, elevated circulating angiotensin II crosses the blood-brain barrier and acts on the melatonin-synthesizing enzyme tryptophan hydroxylase causing its downregulation, which is followed by a decrease in melatonin levels. This in turn causes increase in the inflammatory mediators IL1 and TNF. Based on the data obtained as a result of our study, we can conclude that the S/V combination has a multiple mechanism of action that is likely to have significant positive effects on the long-term outcomes of hypertension and patient survival.

Keywords: Sacubitril, Valsartan, Circadian Rhythm, Melatonin, Experimental Arterial Hypertension