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COMPARABLE EFFECTIVENESS OF DIFFERENT ANTIHYPERTENSIVE AGENTS IN THE TREATMENT OF EXPERIMENTAL HYPERTENSION IN RATS

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სხვადასხვა ანტიჰიპერტენზიული აგენტის შედარებითი ეფექტურობა ექსპერიმენტული ჰიპერტენზიის მკურნალობის დროს ვირთხებში

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი

რეზიუმე

დღეისათვის ჰიპერტენზიის ეტიოლოგია და მისი კავშირი NO-დამოკიდებულ ენდოთელურ დისფუნქციასთან გაურკვეველი რჩება.

არტერიული ჰიპერტენზია გამოწვეული იყო მამრობითი სქესის ვირთაგვებში (წონა 200-220 გრამი) L-NAME (SIGMA-ALDRICH) ინტრაპერიტონეალური შეყვანით 4 და 7 კვირის განმავლობაში. ბოლო 3 კვირის განმავლობაში ცხოველთა ცალკეულ ჯგუფში ერთდროულად შეყვანილი იყო L-NAME, L-არგინინი (SIGMA-ALDRICH) და სხვადასხვა ანტიჰიპერტენზიული საშუალებები (ნებილეტი+ (ნებივოლოლი (ბეტა-ბლოკერი) + ჰიდროქლორთიაზიდი), ლოზაპი + (ანგიოტენზინის სპეციფიკური ანტაგონისტი II რეცეპტორები), ჰიდროქლორთიაზიდი (დიურეზული წამალი) და ნორვასკის (კალციუმის არხის ბლოკატორი)+ ჰიდროქლორთიაზიდის კომბინაცია. ცხოველებში ვსაზღვრავდით სისტოლურ და დიასტოლურ წნევას. NO-ს დონე სისხლის ნიმუშებში განისაზღვრა სპექტრო-ფოტომეტრიულად, გრისის რეაგენტის გამოყენებით.

კვლევის შედეგები აჩვენებს, რომ ჰიპერტენზიის პათოგენეზი ძალიან რთულია და ვაზოდილატაციის ნაერთების ნაკლებობის გარდა მოიცავს ფაქტორთა რიგს, ამიტომ, ანტიჰიპერტენზიული პრეპარატების მოქმედება დაკავშირებულია სხვადასხვა მექანიზმებთან და მოითხოვს არა მხოლოდ NO-ს სუბსტრატის არსებობას. ამ მექანიზმების შესწავლა დაგვეხმარება ჰიპერტენზიის მკურნალობის ეფექტური მეთოდების შემუშავებაში.

L-NAME is widely used in both acute and long-term in vitro and in vivo experiments when the effects of the restriction of NO production are investigated. It is commonly used for the induction of NO-deficient hypertension. Literature data testify that chronic treatment of L-NAME led to hypertension, decreased NO synthase (NOS) activity, myocardial hypertrophy and fibrosis [1,2,3], suppressed angiogenesis by growth factors stimulation [4,5], reduced vasorelaxation, and also induced thickening of the vascular wall [6,7], that indicates the significant role of NO in these processes.

The aetiology and relationship between the NO content and the mechanisms of development of hypertension, whether dysfunction of the endothelium and a decrease in NO production have occurred before or after the development of hypertension, remains unclear. The dissolution of this problem is very important for the development of effective methods of treating hypertension.

We investigated the competitive interaction of different antihypertensive drugs (Nebilet+ (Nebivolol (beta-blocker) + hydrochlorothiazide), Lozap + (Specific antagonist of angiotensin II receptors), Hydrochlorothiazide (diuretic medication) and a combination of Norvasc (calcium channel blocker)+ Hydrochlorothiazide) and the substrate of NO synthase, L-arginine, and its analogue, N-nitro-methyl ether-L-arginine (L-NAME), which is considered a non-selective inhibitor of NO synthase in experimental rats [8-11].

Materials and methods. The study was conducted on 49 experimental white Wistar rats (200-220g), taking into consideration international laws of animal protection. All animals were kept in acrylic cages with wood shavings in an acclimatized room (12/12 h light/dark cycle; 22±3°C) with free access to food and water. All animal procedures were approved by the Animal Care and Use Committee of the Tbilisi State Medical University and were conducted in accordance with the “Guide for the Care and Use of Laboratory Animals” (NIH Publication No. 85–23, revised 1996). All efforts were made to minimize the

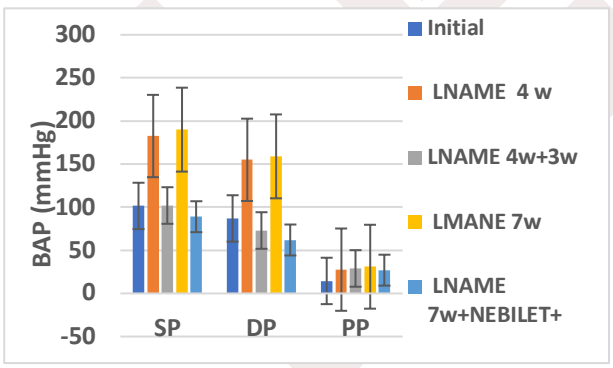
number of animals and their suffering throughout the experiment. Hypertension was induced with intraperitoneal administration of L-NAME (SIGMA-ALDRICH) (40 mg/kg).

Animals were divided into three groups. I group – of intact rats; II group – of rats with an experimental model of hypertension. Group II consists of two subgroups: IIa subgroup – rats were treated by L-NAME (nonselected inhibitor of NO-synthase) intraperitoneal injection (40 mg/kg) during 4 weeks; IIb subgroup - rats were treated by L-NAME intraperitoneal injection (40 mg/kg) during 4 weeks and then following 3 weeks were not treated (spontaneous lavage [7, 10]. Group III rats were treated with L-NAME (40 mg.kg) for 7 weeks, from the beginning of the 5th week (week 5, 6, 7) additionally with L-NAME antihypertensive drugs were added: IIIa subgroup - L-NAME + Nebilet+ (10/25mg/kg); IIIc subgroup - L-NAME + Hydrochlorothiazide (50mg/kg); IIId subgroup - L-NAME + Norvasc + Hydrochlorothiazide (10/12.5mg/kg); IIIe subgroup - L-NAME + L-Arginine (300mg/kg);

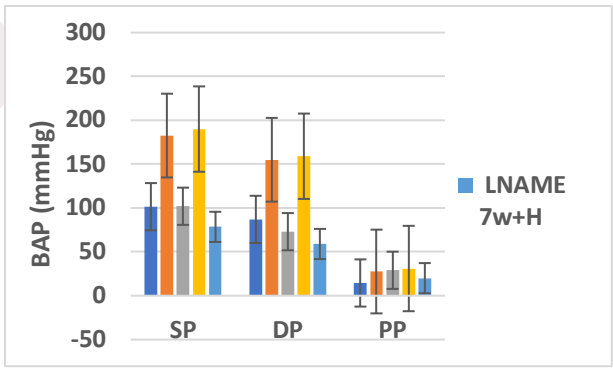
2.1 Blood pressure measurement. The blood pressure (systolic (SP) and (DP) diastolic) of the rats was measured every second day by the tail-cuff method by equipment „Systola“ and oscillograph, made by Russia, LLC “Neurobotics”, according to conscious blood pressure (systolic pressure (SP), Diastolic pressure (DP) and pulse pressure (PP)) monitoring, following the manufacturer's protocol.

2.3 Measurement of Total NO Level in blood. The level of NO_x in blood samples was determined by a modified method of Miranda et al. [9]. As the first step, blood serum sample deproteinization was achieved by adding equal volumes of 0.3 M NaOH to 1000µl of blood serum, it was mixed well and incubated for 5 min at room temperature; then 100µl of 5% $ZnSO_4$ was added, mixed well and incubated for additional 5 min at room temperature. After the incubation, the mixture was centrifuged at 3000 rpm at 4°C for 15 min. An aliquot of 100µl of the clear supernatant was then mixed with 200µl of Griess Reagent Sigma-Aldrich. Griess Reagent was prepared just prior to the assay and contained 0.25% VCl_3, 0.1% sulfanilamide and 0.05% N-(1-Naphthyl)-ethylenediamine (NED) (SIGMA-ALDRICH) in 0.5 M HCl. The reagent blank was the same but contained 100µl of distilled water instead of the blood serum sample. The mixture was incubated for 30 min at 37°C and absorbance was measured at 540 nm with a microplate reader (Multiscan GO, Thermo Fischer Scientific, Finland). The standard curve for $NaNO_2$ was used to calculate the total NO_x concentration in the samples [12].

2.4 Statistical analysis. Statistical analysis of obtained results was performed by the use of the SPSS statistical analysis program package (version 10.0). The average parameters and their statistical derivations were analyzed. The difference between groups was evaluated by Student t-criterium. In all cases, statistical significance was obtained at $p < 0,05$.



A



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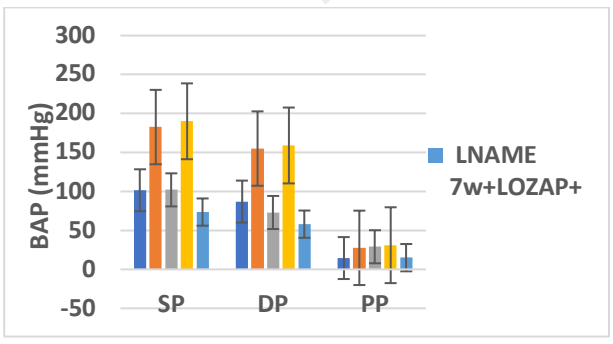

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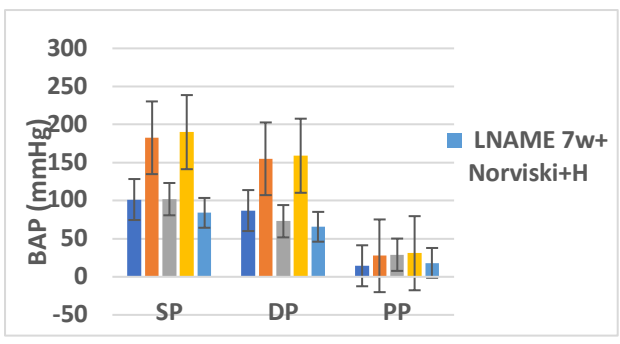

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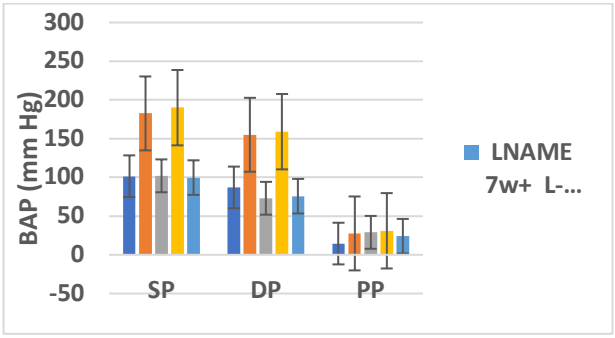

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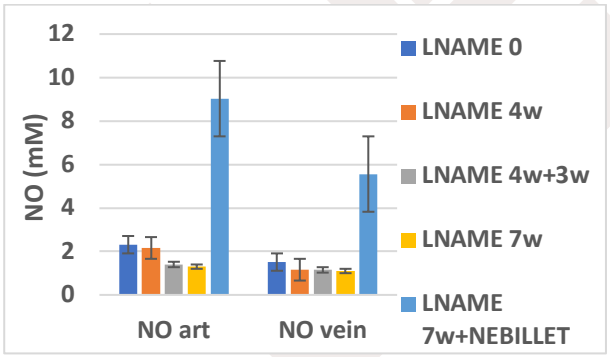
Fig. 1. Arterial pressure (SP, DP) and pulse pressure (PP) of hypertensive rats under the treatment with Nebilet+, Lozap+, Hydrochlorothiazide (H), Norvasc+H and L-Arginene

Results and Discussion. On Figure 1 the means of systolic and diastolic pressure in rats under the 4 and 7-week influence of NO-synthase inhibitor, and 3 weeks of treatment with different antihypertensive drugs. As it seems from the results of the study, after 4 weeks injection of the L-NAME (subgroup IIa) systolic pressure increased by 110% and diastolic – by 66%, in comparison to the initial level. After 3 weeks in rats, treated with L-NAME for 4 weeks, (subgroup IIb) systolic and diastolic pressure decreased by 55%/54% and returned to the initial level.

Treatment of hypertensive rats during the last 3 weeks with Nebilet+ (Nebivolol (beta-blocker) + hydrochlorothiazide), Lozap + (Specific antagonist of angiotensin II receptors), Hydrochlorothiazide (diuretic medication) and a combination of Norvasc (calcium channel blocker)+ Hydrochlorothiazide (Subgroups IIIa, b, c, d) induced decrease of arterial pressure and pulse pressure (by 53%,/61%, 13%; 61%64%, 52%; 59%/70%, 35 %; 66%/59%, 41%, correspondingly) and it becomes close to the initial level (Fig. 1A-D). L-Arginine administration induced decrease of arterial pressure and pulse pressure by 48%/52%, 23% (Fig. 1E).

The level of NO in arterial and vein blood under the influence of Nebilet+, Hydrochlorothiazide (H) and Lozap+ 7,4; 5,5 fold increased (Fig. 2A, C), the effect of Hydrochlorothiazide combination with Norvasci was higher (12,3; 8,2) (Fig. 2D) but Hydrochlorothiazide only was less effective (5,6; 5.2 fold) (Fig. 2B); under the administration of L-Arginine level of NO in arterial blood increased 1,4 fold and didn't change in venous blood (Fig. 2E).



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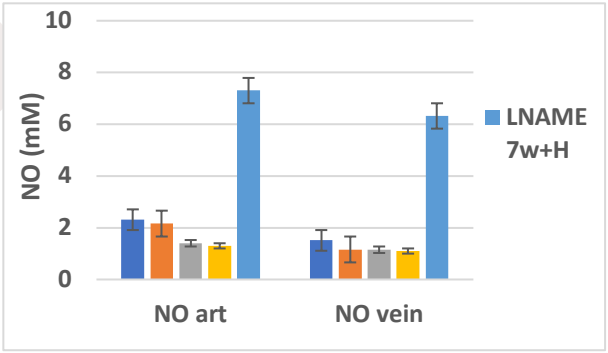

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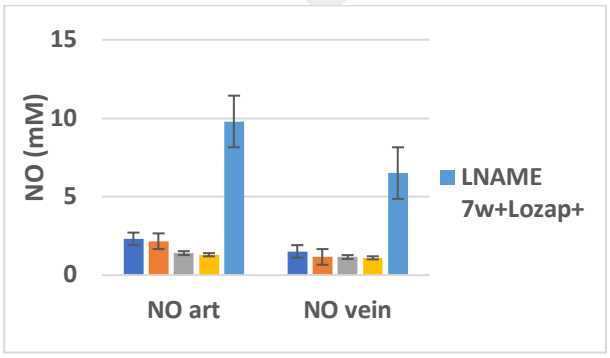

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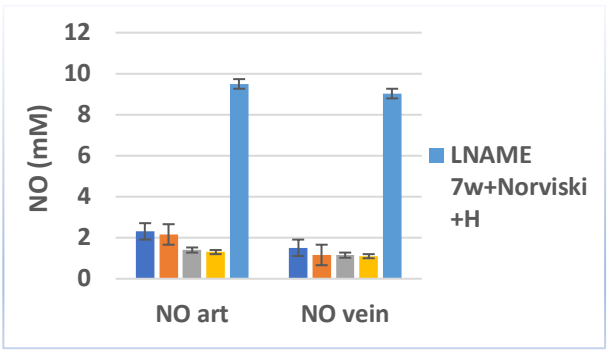

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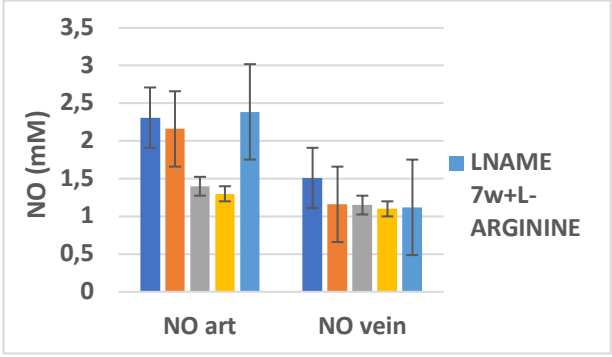

E

Fig. 2. NO content in hypertensive rats arterial and venous blood under the treatment with Nebilet+, Lozap+, Hydrochlorothiazide (H), Norvasc+H and L-Arginene

The results of our experiments indicate after L-NAME administration increase of SP and DP was reversible whereas the increase of PP and decrease of NO content in rats' blood was irreversible. These data indicate the irreversible character of the alterations of vascular elasticity (stiffening) and endothelial dysfunction (decreased bioavailability of NO) [13].

As shown in experiments results, treatment of the hypertensive rats (under continuous administration of LNAME for 7 weeks) during the last 3 weeks with Nebilet+, Lozap, Hydrochlorothiazide and a combination of Norvasc with Hydrochlorothiazide effectively decreased the SP and DP; concerning the normalisation of PP, the most effective were Lozap (reduced PP by 52%), and the combination of calcium channels blocker, Norvasc, with diuretic Hydrochlorothiazide (reduced PP by 41%); the Hydrochlorothiazide and Nebilet+ had no effects on PP. It must be noted, that the combined effect of Norvasc with Hydrochlorothiazide was exspessialy high concerning increased NO levels in arterial and venous blood, whereas Hydrochlorothiazide alone had a very low effect. The administration of the NO donor L-arginine, although it caused the normalization of blood pressure (BP, BP), did not importantly affect the level of PP and the content of NO in the blood. These data indicate that hypertension pathogenesis is very complex and includes a series of factors, additionally to the lack of vasodilatation agents [14, 15, 16, 17, 18].

The effects of antihypertensive drugs are related to various mechanisms and require the presence of not only an additional substrate necessary for the synthesis of NO. Studying these mechanisms will help us to develop effective methods of treating hypertension.

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КЕТЕВАН КАКАБАДЗЕ, ИРАКЛИЙ МЕГРЕЛАДЗЕ, НИНО КИПИАНИ, ТАМАР САНИКИДЗЕ

СРАВНИТЕЛЬНАЯ ЭФФЕКТИВНОСТЬ РАЗЛИЧНЫХ АНТИГИПЕРТЕНЗИВНЫХ СРЕДСТВ ПРИ ЛЕЧЕНИИ ЭКСПЕРИМЕНТАЛЬНОЙ ГИПЕРТЕНЗИИ У КРЫС

Тбилисский Государственный Медицинский Университет

РЕЗЮМЕ

Этиология гипертензии и ее связь с NO-зависимой дисфункцией эндотелия и снижением продукции NO до сих пор не установлены.

Артериальную гипертензию индуцировали у самцов крыс линии Вистар (массой 200-220г) внутрибрюшинным введением L-NAME (SIGMA-ALDRICH) в течение 4 и 7 недель. В течение последних 3 недель отдельной группе животных одновременно вводили L-NAME, L-аргинин (SIGMA-ALDRICH) и различные антигипертензивные препараты (Небилет+ (Небиволол (бета-адреноблокатор) + гидрохлоротиазид), Лозап + (Специфический антагонист ангиотензина). II), гидрохлоротиазид (диуретическое лекарство) и комбинацию норваск (блокатор кальциевых каналов) + гидрохлоротиазид). У животных контролировали систолическое и диастолическое давление. Уровень NO в образцах крови определяли спектрофотометрически с использованием реактива Грисса.

Результаты исследования показывают, что патогенез артериальной гипертензии очень сложен и зависит от ряда факторов, помимо отсутствия вазодилататоров. Поэтому эффекты антигипертензивных средств связаны с различными механизмами и требуют наличия не только дополнительного субстрата, необходимого для синтеза NO. Изучение этих механизмов необходимо для разработки эффективных методов лечения гипертензии.

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COMPARABLE EFFECTIVENESS OF DIFFERENT ANTIHYPERTENSIVE AGENTS IN THE TREATMENT OF EXPERIMENTAL HYPERTENSION IN RATS

Tbilisi State Medical University

SUMMARY

The aetiology and relationship between the NO content and the mechanisms of development of hypertension, whether dysfunction of the endothelium and a decrease in NO production have occurred before or after the development of hypertension, remains unclear.

Arterial hypertension was induced in male Wistar rats (weighing 200-220 grams) by intraperitoneal administration of L-NAME (SIGMA-ALDRICH) for 4 and 7 weeks. During the last 3 weeks, to a separate group of animals simultaneously with L-NAME, L-arginine (SIGMA-ALDRICH) and different antihypertensive drugs (Nebilet+ (Nebivolol (beta-blocker) + hydrochlorothiazide), Lozap + (Specific antagonist of angiotensin II receptors), Hydrochlorothiazide (diuretic medication) and a combination of Norvasc (calcium channel blocker) + Hydrochlorothiazide) were administered. The animals were monitored for systolic and diastolic pressure. The level of NO in blood samples was determined spectrophotometrically using a Griess Reagent.

The results of the study show that hypertension pathogenesis is very complex and includes a series of factors, additionally to the lack of vasodilatation compounds. Therefore, the effects of antihypertensive drugs are related to various mechanisms and require the presence of not only an additional substrate necessary for the synthesis of NO. Studying these mechanisms will help us to develop effective methods of treating hypertension.

Keywords: hypertension, nitric oxide, antihypertensive drug