VIA MEDICA

ORIGINAL ARTICLE

Larysa Borysivna Bondarenko, Ganna Mykhailivna Shayakhmetova, Tetiana Anatoliivna Karatsuba, Anatoliy Vasyliovych Matvienko, Valentyna Mykolaivna Kovalenko

SI "Institute of Pharmacology & Toxicology National Academy of Medical Sciences of Ukraine", Kyiv, Ukraine

Metabolic syndrome and losartan treatment effects in adult and pubertal rats

Corresponding author:

Larysa Borysivna Bondarenko, SI "Institute of Pharmacology & Toxicology National Academy of Medical Sciences of Ukraine", 14 Antona Tzedika St, 03680 Kyiv, Ukraine; e-mail: larabon04@yahoo.com

Medical Research Journal 2022; Volume 7, Number 1, 66–73
DOI: 10.5603/MRJ.a2022.0011

ISSN 2451-2591
e-ISSN 2451-4101

ABSTRACT

Introduction: Comparative estimation of losartan treatment effects in adult and pubertal rats with metabolic syndrome (MS).

Material and methods: MS model was induced by full replacement of drinking water with 20% fructose solution at Wistar male rats of two age categories: young animals of 21–23 days age (50–70 g) and adults (160–180 g). Clinical hematology and biochemistry tests, blood pressure measurement, chromatin DNA fragmentation, and liver morphological investigations were carried out after 60 days of MS modeling and losartan treatment.

Results: Effects of losartan on blood clotting time, lipid metabolism, and DNA fragmentation were more pronounced in pubertal rats, while more profound influence on high-density lipoprotein (HDL) contents, pancreas, and visceral fat relative weights was found in adults.

Conclusions: In pubertal and adult rats with MS, losartan effects were age-dependent for lipid metabolism indices, blood clotting time, nuclear DNA fragmentation, relative organs weights, and liver morphologic structure. Losartan treatment normalized blood pressure independently of age, while its effects on other parameters in adults and young rats differed not only in their degree of manifestation but also in their very nature.

Key words: metabolic syndrome, losartan, pubertal, adult, rats

Med Res J 2022; 7 (1): 66–73

Introduction

The constant increase in the incidence of metabolic syndrome (MS) in the most diverse age groups of the population makes the problem of its optimal pharmacotherapy extremely topical [1]. This is especially important when the onset of metabolic disorders lies in childhood and adolescence [2, 3] as MS development in adults and children greatly differs. Studies exploring the features of MS pharmacotherapy in children and adolescents are only fragmentary and completely insufficient.

One of the few antihypertensive drugs used in children with MS is losartan [4, 5]. Preliminary studies [6–8] indicate that its in-vivo metabolism (involving cytochrome CYP3A, CYP2C, and CYP2E1) varies significantly with age. Thus, losartan can have unexpected effects on young organisms, different from its action in adults. Its in-vivo effect seems to be much more diverse and stronger than previously thought. It remains completely unclear how and to what extent the biological effects of losartan will manifest in a young growing organism with MS, nor will it lead to an increase in adverse effects of losartan and the occurrence of new long-term effects of its use in children and adolescents. Comparative investigation of losartan biological effects in different ages could be of great importance for the understanding of its side effects mechanisms, Losartan safety, and efficacy prediction and monitoring should also take into account possible age differences.

Our present study aimed to provide a comprehensive estimation of MS and losartan-mediated age-dependent changes in rats.

Material and methods

A total of 36 Wistar male rats of two age categories [young animals of 21–23 days age (50–70 g) and adults

(160–180 g)] were used in the study. They were kept under a controlled temperature (from 22°C to 24°C), relative humidity of 40% to 70%, lighting (12 hours light-dark cycle), and on a standard pellet feed diet ("Phoenix" Ltd., Ukraine). The study was performed in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and approved by the Institutional Animal Care and Use Committee. The model of MS was reproduced according to the protocol of Abdulla et al. [9]. Young and adult animals were divided into 6 groups (6 animals in each group): 1 — Control 1 (intact young rats), 2 — Control 2 (intact adult rats), 3 — MS1 (young rats with MS), 4 — MS2 (adult rats with MS), 5 — MS1+losartan (young rats with MS and losartan treatment [4.43 mg/kg of body weight (b.w.), *per os*, 60 days)], 6 — MS2 + losartan (adult rats with MS and losartan treatment). MS was induced by full replacement of drinking water with 20% fructose solution (200 g/L).

Using crystalline D-fructose > 99% (Khimlaborreactiv, Ukraine, series 072000897834, batch XW 130105), 20% fructose solution was prepared daily and given every day for two months *ad libitum*. In our experiments, losartan (losartan potassium, manufactured by LLC "KUSUM PHARM", Sumy, Ukraine) was used.

After 60 days of 20%, fructose solution consumption and losartan treatment rats were sacrificed under mild ether anesthesia by decapitation.

A comprehensive assessment including determination of hematological and clinical biochemistry parameters, hepatocytes' chromatin DNA fragmentation (as an apoptosis marker) and liver morphological macroscopic and microscopic studies, was carried out after 60 days of MS modeling. The blood, serum, and liver tissue were used for investigation. Blood samples were studied with the hematology analyzer Mythic 22 (Switzerland), blood clotting time — by standard clinical Burker's method. Serum levels of total bilirubin, total cholesterol, low-density lipoproteins (LDL), and high-density lipoproteins (HDL) were measured with a fully automatic biochemical analyzer Prestige 24i (Japan) using kits supplied by "P. Z. Cormay" (Poland).

Changes in rats' blood pressure were investigated as described by Khromov et al. [10]. Noninvasive investigation of all animals' blood pressure (96–72 hours before euthanasia) was carried out on a tail artery with an ultrasound sensor of vascular pulsation using a SPHYGMOMANOMETER S-2 (HSE, Germany). Vascular pulsation monitoring was carried out with oscilloscope HM 303-4 (HAMEG GmbH, Germany). Data were analyzed using "Chart 5" (AD Instruments, Australia).

Abdominal fat, liver, kidneys, and pancreas of all animals were extracted, weighed, and used for morphologic investigation. Relative organ weights were calculated per 100 g of body weight. Pieces of extracted organs were fixed in 10% solution of neutral formalin, dehydrated in ethanol solutions of graded concentrations, and embedded in paraffin wax. Histological cross-sections (6 mm) were made and stained with hematoxilyn and eosine. Histological examination was performed under light microscope (100 x, 200 x, and 400 x). In frozen (–20°C) slices of organs, neutral fat was determined by Sudan black B staining [11]. Nucleic acids content (DNA and RNA) was determined histochemically by Shubich method [11], glycogen — by Periodic acid-Schiff (PAS)-reaction [12], succinate dehydrogenase activity — by the method of Nachlas et al. [12], and lactate dehydrogenase activity — by the method of Hess et al. [11]. Microscopic studies were carried out with a Cytophan microscope (Leica Microsystems Wetzlar GmbH).

Hepatic DNA was isolated as previously described [13]. Chromatin DNA fragmentation evaluation (as an apoptosis marker) was carried out according to Bondarenko et al. [14]. Briefly, tissue was homogenized and digested in digestion buffer [100 mM NaCl; 10 mM Tris-HCl; 25 mM ethylenediaminetetraacetic acid (EDTA), pH 8; and 0.5% sodium dodecyl sulfate (SDS)] and freshly added 0.1 mg/mL proteinase K (Sigma-Aldrich, Ink., USA) (1:1.2 mg/mL) with shaking at 50°C for 15 h. RNA was degraded by incubation of the samples with 1–100 mg/mL thermostable RNase H for 1.5 h at 37°C. DNA was extracted with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) and centrifuged for 10 minutes at 1700 × g. The DNA was precipitated by adding 0.5 vol. 7.5 M ammonium acetate and 2 vol. 100% ethanol to the aqueous layer; samples were separated by centrifugation at 1,700 × g for 5 minutes, rinsed with 70% ethanol, and air-dried. The pellets were dissolved in TBE buffer (10 mm Tris-HCl and 1 mm EDTA, pH 8); and then were fractionated through 2% agarose gels (50–60 V; 3.5 h). After electrophoresis, gels were stained with ethidium bromide and visualized under a UV transilluminator (BIORAD, USA). Electrophoresis data analysis was carried out with Quantity One Software (USA).

Statistical analysis

The obtained data were expressed as the mean ± standard error of the mean (M ± SEM) and analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test using OriginPro 7.5 Software. Differences were considered to be statistically significant at $p < 0.05$.

Results

The results of pubertal and adult rats' blood pressure investigation with MS and losartan treatment are given in Figure 1.

As one would expect, losartan effectively reduced blood pressure in MS-animals of both age groups. At the same time, in young rats, this decrease was significant not only in comparison with the MS group but also with the controls.

The results of pubertal and adult rats' serum biochemical parameters investigation with MS and losartan treatment are given in Table 1.

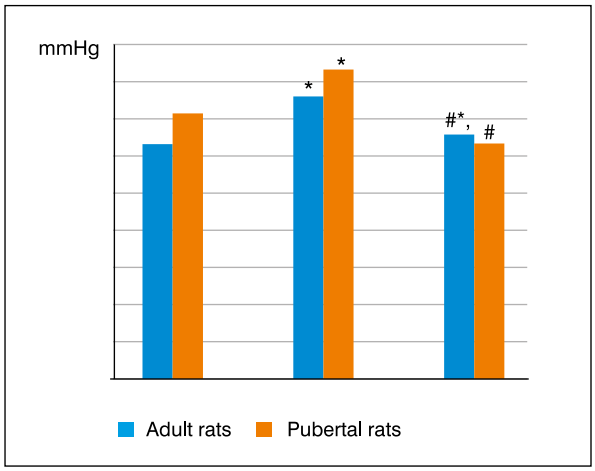

Figure 1. Blood pressure levels in adult and pubertal rats with MS on losartan treatment, M ± n, n = 6, mmHg *p < 0.05 in comparison with control; #p < 0.05 in comparison with MS

The levels of total cholesterol, HDL, LDL, and HDL/LDL ratio were increased in all experimental groups regardless of age (Tab. 1). It should be noted that among the MS animals, changes in LDL levels and HDL/LDL ratio were more profound in the adult group. Lowering of LDL levels and HDL/LDL ratio with losartan treatment was found only in adult rats.

The investigation of the hematological parameters in rats with MS of both age groups (Tab. 2) showed no changes in the number of platelets, erythrocytes, nor in total leukocytes and their subtypes count.

However, in adult rats with MS and pubertal animals there was a decrease in hemoglobin concentration and hematocrit. These findings might indicate a change in rats' hemorheological profile. In pubertal rats, losartan treatment led only to normalization of hemoglobin concentration, while in adult animals with MS we noticed normalization of hematocrit during losartan treatment, with a simultaneous decrease in mean corpuscular volume and mature red blood cells (RBC) cellular hemoglobin content indices.

We also found a significant shortening of blood clotting time, both in pubertal and adult rats with MS: 1.7-fold in adult and 2.2-fold in pubertal animals, without normalization on losartan treatment. The effect of losartan on blood clotting was age-dependent, leading to a slight increase in blood clotting times in pubertal rats (as compared with the MS group) and a decrease in adult animals.

Previously, we noted neutral fat accumulation, necrobiotic changes in hepatocytes, β–cells number decrease in Langerhans islets, accompanied by patchy congestion of glomerular capillaries, partial detachment of nephrocytes distal parts in proximal tubules, reduced quantities of neutral mucopolysaccharides in proximal tubules, the scalloped structure of some nephrocytes

Table 1. Serum biochemical indices in adult and pubertal animals with MS and losartan treatment, (M ± m; n = 6)

Indices	Pubertal animals			Adult animals		
	Control 1	MS1	MS1 + losartan	Control 2	MS2	MS2 + losartan
Glycosylated hemoglobin [mmoles/L]	2.66 ± 0.32	2.65 ± 0.25	2.19 ± 0.51	3.66 ± 0.30	3.42 ± 0.26	3.76 ± 0.26
Glucose [mmoles/L]	7.53 ± 0.19	7.36 ± 0.39	6.70 ± 0.47	7.07 ± 0.39	6.56 ± 0.50	7.44 ± 0.41
Triglycerides [mmoles/L]	0.40 ± 0.03	0.48 ± 0.13	0.38 ± 0.05	0.65 ± 0.08	0.52 ± 0.03	0.83 ± 0.23
Total bilirubine [mmoles/L]	0.47 ± 0.16	0.76 ± 0.25	0.77 ± 0.28	0.73 ± 0.19	0.93 ± 0.28	0.96 ± 0.27
Total cholesterol [mmoles/L]	1.03 ± 0.11	1.45 ± 0.24*	1.77 ± 0.14*	1.19 ± 0.11	1.66 ± 0.09*	1.76 ± 0.07*
HDL [moles/L]	0.88 ± 0.03	1.13 ± 0.01*	1.35 ± 0.07*	0.92 ± 0.05	1.13 ± 0.09	1.21 ± 0.09*
LDL [moles/L]	0.16 ± 0.003	0.25 ± 0.01*	0.20 ± 0.02	0.16 ± 0.06	0.36 ± 0.04*	0.25 ± 0.03#
Ratio HDL/LDL	0.19 ± 0.01	0.22 ± 0.016*	0.16 ± 0.27*	0.17 ± 0.05	0.33 ± 0.04*	0.21 ± 0.02#

*p < 0.05 in comparison with control; #p < 0.05 in comparison with MS

Table 2. Venous blood hematological indices in adult and pubertal animals with MS and losartan treatment, (M ± m; n = 6)

Indices	Pubertal animals			Adult animals		
	Control 1	MS1	MS1 + losartan	Control 2	MS2	MS2 + losartan
Leukocytes [10^3μL]	2.97 ± 0.55	2.67 ± 0.22	3.00 ± 0.33	4.45 ± 0.48	5.15 ± 0.64	4.05 ± 0.38
Lympho cytes (%)	69.52 ± 2.96	73.47 ± 1.38	68.62 ± 2.13	65.95 ± 2.40	68.33 ± 4.37	67.30 ± 2.00
Monocytes (%)	2.93 ± 0.34	3.10 ± 0.25	3.07 ± 0.50	4.10 ± 0.45	4.42 ± 0.68	3.33 ± 0.27
Neutrophils (%)	22.60 ± 2.26	18.97 ± 1.39	22.27 ± 2.17	25.13 ± 2.35	22.33 ± 3.02	24.07 ± 1.51
Eosinophils (%)	0.97 ± 0.37	0.60 ± 0.34	0.67 ± 0.18	0.40 ± 0.10	0.42 ± 0.21	0.67 ± 0.50
Basophils (%)	3.98 ± 0.44	3.87 ± 0.30	4.55 ± 0.46	4.42 ± 0.39	5.50 ± 1.10	4.63 ± 0.50
Erythrocytes [10^6μL]	8.54 ± 0.11	7.98 ± 0.28	8.15 ± 0.16	8.24 ± 0.19	8.43 ± 0.13	8.59 ± 0.07
Hemoglobin [g/dL]	15.82 ± 0.27	14.23 ± 0.51*	14.71 ± 0.17	14.88 ± 0.29	14.88 ± 0.17	14.85 ± 0.12
Hematocrit (%)	43.12 ± 0.60	39.82 ± 0.94*	40.85 ± 0.38*	41.03 ± 0.78	38.45 ± 0.63*	41.15 ± 0.36
Mean corpuscular volume [fL]	50.55 ± 0.76	50.08 ± 0.99	50.23 ± 1.02	49.82 ± 0.31	48.92 ± 0.90	47.92 ± 0.34*
Mature red blood cells (RBC) cellular hemoglobin content [pg]	18.57 ± 0.41	17.85 ± 0.16	18.08 ± 0.26	18.05 ± 0.11	17.67 ± 0.30	17.28±0.13*
Platelets [10^3 μL]	549.8 ± 50.1	858.2 ± 318.4	663.5 ± 90.8	592.3 ± 34.0	543.7 ± 28.5	522.0 ± 19.9
Peripheral blood clotting time (sec)	311.4 ± 6.1	141.1 ± 10.5*	223.1 ± 7.8*,#	444.5 ± 9.3	260.0 ± 7.2*	222.2 ± 8.8*,#

*p < 0.05 in comparison with control; #p < 0.05 in comparison with MS

Table 3. Relative organs weights in adult and pubertal animals with MS and losartan treatment, g/100 g b.w. (M ± m; n = 6)

Organs	Pubertal animals			Adult animals		
	Control 1	MS1	MS1 + losartan	Control 2	MS2	MS2 + losartan
Liver	3.33 ± 0.18	3.18 ± 0.10	3.20 ± 0.14	3.33 ± 0.18	3.18 ± 0.10	3.20 ± 0.14
Pancreas	3.17 ± 0.10	3.46 ± 0.07*	3.45 ± 0.13	0.21 ± 0.01	0.17 ± 0.009*	0.16 ± 0.01*
Kidneys	0.18 ± 0.007	0.16 ± 0.004*	0.15 ± 0.009*	0.61 ± 0.022	0.63 ± 0.015	0.67 ± 0.03
Visceral fat	0.59 ± 0.091	0.65 ± 0.044	0.62 ± 0.05	3.58 ± 0.25	4.81 ± 0.40*	4.09 ± 0.41

*p < 0.05 in comparison with control.

tubules apical parts in kidney tissues in animals with MS, which were more pronounced in adults [14]. The relative weight of visceral fat in rats with MS increased independently of age (Tab. 3).

Simultaneously, in pubertal animals, the relative weights of the liver were increased, while the pancreas weight was decreased. In adult rats, only pancreas relative weight decrease was noted. Regardless of age, losartan treatment did not correct the changes in the liver weight.

The condition of the hepatic tissues in pubertal animals during losartan administration was largely similar to animals with MS (Fig. 2).

Losartan treatment did not greatly improve the pubertal rats' liver tissue architectonics in comparison to MS animals. Isolated acidophilic necrosis, lymphohistiocytic infiltrates of varying degrees of severity, and activated stellate reticuloendoteliocytes with hyperchromic nuclei were observed. Decrease in liver glycogen content decrease was demonstrated histochemically. Along with normal hepatocytes, dystrophic cells could also be encountered occasionally. On the periphery of the liver lobules, hepatocytes contained foci of medium and small vacuoles demonstrating a positive reaction to fat (Sudan black B). In adult animals with MS treated with losartan (Fig. 3), similar, but less profound changes were present as in pubertal animals. The level of glycogen was not normalized. There was some isolated acidophilic necrosis and some dystrophic cells could be seen. In general, the effect of losartan treatment was more pronounced in young animals.

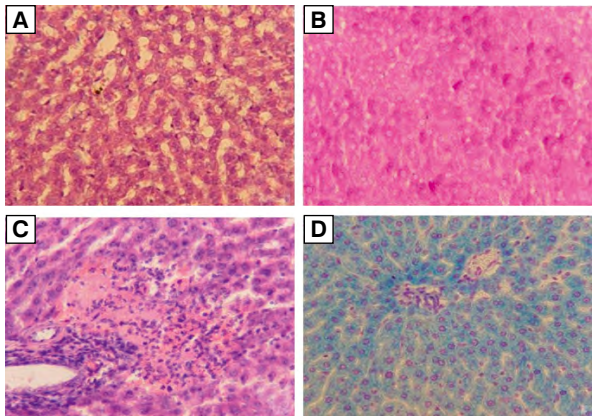

Figure 2. Pubertal rat liver with MS and losartan treatment; **A.** Focal liver sinusoids plethora (hematoxylin-eosine staining, × 400); **B.** Focal decreasing of liver glycogen accumulation (MacManus Method, × 400); **C.** Hepatocytes acidophilic necrosis (hematoxylin-eosine staining, × 400; **D.** Histochemical reaction on nucleic acids in rat liver (Shubich Method, × 400)

In our experiments, MS development caused an intensification of DNA fragmentation in the liver of adult rats in comparison with controls (Fig. 4, Tab. 4).

In the group of adult animals with MS, 9 fractions of DNA low molecular weight fragments were detected, whereas in the control group — only two. In adult rats with MS, the percentage of DNA fragmentation grew almost 5.8-fold. The majority of fractions (8 out of 9) were fragments with masses of 20–250 base pairs (b.p.), whereas relatively longer fragments were represented only by 1 fraction (750–800 b.p.). With losartan treatment, a marked increase in DNA fragmentation was also observed — 10 fractions (from 20 to 1000 b.p.), however, the percentage of DNA fragmentation was slightly lower in this group in comparison with MS animals.

Also in the liver of pubertal rats with MS, DNA fragmentation significantly increased in comparison with controls (Fig. 4, Tab. 4) — as many as 13 fractions of low molecular weight fragments could be identified. The percentage of DNA fragmentation increased almost

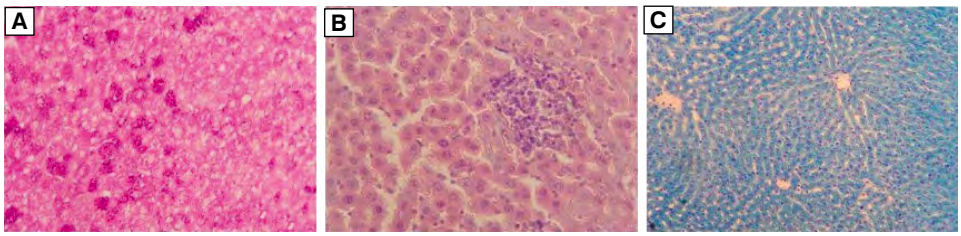

Figure 3. Adult rat liver with MS and losartan treatment; **A**. Unequal content of glycogen in liver cells (MacManus Method, × 400); **B**. Focal coagulation necrosis with mononuclear infiltrate, along with unchanged hepatocytes (hematoxylin-eosine staining, × 400); **C**. The histochemical reaction on nucleic acids (DNA, RNA) is unchanged (Shubich Method, × 400)

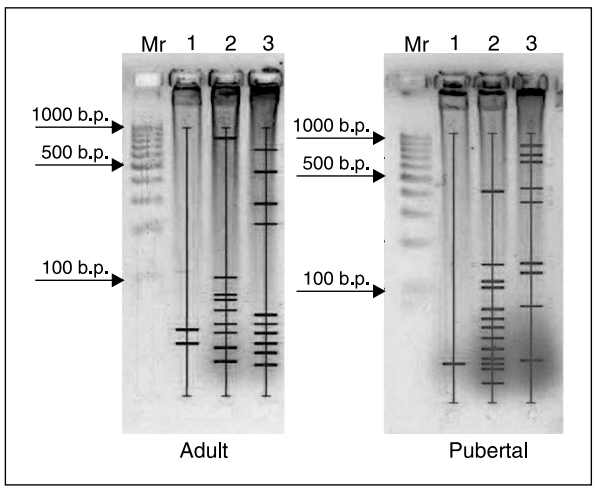

Figure 4. DNA fragmentation in adult and pubertal rats' hepatocytes with MS and losartan treatment; **1.** control; **2.** MS; **3.** MS+ losartan

Table 4. The relative proportion of rat liver DNA fragmentation in adult and pubertal animals with MS and losartan treatment

Experimental groups	% of DNA fragmentation	
	Pubertal animals	Adult animals
Control	4.42	5.57
MS	35.21	32.50
MS+ losartan	45.08	29.15

8-fold. Most of the fractions (11 out of 13) contained small fragments with weights 20–100 b.p., whereas relatively longer fragments were represented by only 2 fractions (450 and 250 b.p.).

With losartan treatment, a profound increase in DNA fragmentation was also observed: 9 fragments with masses 40–900 b.p. were formed. Unlike MS

animals, in this group, 5 fractions were represented by fragments with 400–900 b.p., whereas relatively shorter fragments — by 4 fractions (220, 200, 75, and 40 b.p.). The percentage of DNA fragmentation increased in this group both in comparison with the control and MS rats.

Discussion

Since MS is a complex combination of risk factors for the development of cardiovascular pathology and type 2 diabetes, it is important that the pharmacological correction of any of its components does not aggravate others and doesn't cause long-term undesirable consequences, especially in children during the period of intensive growth and development. In our experiments, losartan effectively corrected levels of blood pressure independently of age. However, the hypotensive effect was accompanied by an increase in serum total cholesterol and HDL, particularly in the pubertal animals. LDL level decreased simultaneously and accordingly, we observed only a partial correction of the HDL/LDL ratio.

Similar results were obtained in experiments with diabetic rodents [15]. Losartan, when administered alone or in combination with repaglinide or gliclazide in diabetic rats caused a significant increase in serum HDL with a simultaneous significant decrease in LDL [15].

Our data on the losartan effect on LDL also were in good correspondence with the results of other authors [16]. In these experiments, losartan demonstrated anti-atherosclerotic potential, despite the fact that serum levels of total cholesterol, HDL- and LDL-fractions remained unchanged.

On the other hand, our hematological investigations are in good agreement with the data of other researchers who noted that losartan significantly increased hematocrit (Hct), platelets (Plts), and HDL in rats [17, 18]. With regard to the blood clotting time, losartan treatment in pubertal rats partially improved this index in comparison with MS animals. Similar effects of losartan were found in other experiments on hypertensive rats [19]. The opposing effect of losartan treatment in adult rats could be associated with different angiotensin AT2 and AT1 receptor interactions during losartan antithrombotic activity realization in young and adult animals [19].

Our conclusions concerning the effect of losartan on biochemical and hematological parameters were confirmed by the results of morphological investigations. Losartan did not fully normalize the morphology of the liver, kidneys, and pancreas, neither in the young nor adult rats. As for relative masses of organs, the losartan effect on the young animals was significantly weaker than in the case of adults, possibly due to the higher rates of transformation of this drug preparation in young organisms and the higher speed of all metabolic processes in pubertal animals. Also, direct losartan-induced inhibition of growth and development processes in young rats could not be excluded [20].

To clarify to what extent such losartan effect is associated with changes in epigenetic mechanisms such as chromatin components stability, we investigated losartan influence on DNA fragmentation in liver cells.

Apoptotic changes are accompanied by violations in DNA fragmentation processes [21]. With MS development and losartan treatment, such changes could be caused by violations in the functioning of NEIL1 endonuclease (involved in DNA repair processes in mammalian cells [22]), as well as by processes that accompany MS [23, 24] such as mitochondrial dysfunctions, vascular wall pathologies, and neurodegeneration. In our previous research, we demonstrated a significant increase in liver DNA fragmentation in adult and pubertal rats with MS and metformin treatment [14, 25]. Just like in our present investigation, also now these changes, caused by MS or pharmacological intervention, were more pronounced in pubertal rats.

Presently, we have found that losartan treatment decreased the rate of DNA fragmentation in the liver of adult rats. That is fully consistent with reports from other researchers, who associated losartan anti-apoptotic effects with the involvement of Angiotensin II in mediating apoptosis in diabetics [21, 26].

Reduced stability of DNA could significantly change cell viability by increasing the pool of damaged and dead cells (as confirmed by our results of morphological studies). According to other literature reports, systemic structural changes in DNA molecules directly correlate with the rates of MS development [27] and thus, apoptosis violations may inhibit tissue regeneration and lead to deepening of pathological changes [27, 28]. Close relationships between DNA repair processes distributions, apoptosis, and MS development were also noted in reports from other authors [23, 24].

The differences in the nature and intensity of DNA fragmentation processes can be explained by age-related changes in the levels of activity of various enzymes that ensure the stability of the DNA structure [29] as well as by the successful inclusion of different sets of such nucleases in ontogeny [30].

Our data indicate that changes in physiological, biochemical, and hematological indices, as well as in DNA fragmentation rates precede changes at the level of whole cells, tissues, and organs (liver necrotic transformations). This observation completely coincides with our previous results and data of other authors who showed that DNA structure disturbances play important role in triggering liver cell necrosis [28]. As it was previously demonstrated for MS and metformin treatment [14, 25], in the case of losartan administration indices of young animals with MS also changed more definitely in comparison with adults.

Our experimental data (regarding losartan effect on total cholesterol, HDL, blood clotting time, visceral fat relative weight, and DNA fragmentation) evidence that losartan pharmacotherapy of hypertension in adolescents requires special caution. Further detailed clinical studies of losartan treatment benefits and undesirable side effects in children and adolescents are required.

Conclusions

In experiments with pubertal and adult rats with MS and losartan treatment, we established age-dependent effects of this medication for lipid metabolism indices, blood clotting time, DNA fragmentation parameters, relative organs weights, and target organs morphological changes. Losartan treatment normalized blood pressure independently of age. Simultaneously, in adult rats partial correction of serum levels of LDL and HDL/LDL ratio, hematocrit, DNA fragmentation rates, with a concomitant increase in serum total cholesterol and HDL concentration, blood clotting time, and pancreas relative weights were noted. In pubertal animals, partial correction of serum levels of LDL, HDL/LDL ratio, and blood clotting time, with simultaneous increase in serum total cholesterol and HDL concentration, hematocrit, visceral fat, and pancreas relative weights were present. The effects of losartan on a number of parameters (blood clotting time, DNA fragmentation, the relative weight of visceral fat) in adult and young rats differed not only in the degree of manifestation but also in their very nature.

Conflict of Interests: *None.*

Acknowledgements: *The authors gratefully acknowledge the financial support of this study by the National Academy of Medical Sciences of Ukraine.*

References

1. Swislocki ALM, Siegel D, Jialal I. Pharmacotherapy for the metabolic syndrome. Curr Vasc Pharmacol. 2012; 10(2): 187–205, doi: 10.2174/157016112799305003, indexed in Pubmed: 22022773.
2. Weiss R, Dziura J, Burgert TS, et al. Obesity and the metabolic syndrome in children and adolescents. N Engl J Med. 2004; 350(23): 2362–2374, doi: 10.1056/NEJMoa031049, indexed in Pubmed: 15175438.
3. Zimmet P, Alberti G, Kaufman F, et al. International Diabetes Federation Task Force on Epidemiology and Prevention of Diabetes. Global and societal implications of the diabetes epidemic. Lancet. 2007; 369(9579): 2059–2061, doi: 10.1016/S0140-6736(07)60958-1, indexed in Pubmed: 17586288.
4. Chu PY, Campbell MJ, Miller SG, et al. Anti-hypertensive drugs in children and adolescents. World J Cardiol. 2014; 6(5): 234–244, doi: 10.4330/wjc.v6.i5.234, indexed in Pubmed: 24944754.
5. Webb NJA, Wells TG, Shahinfar S, et al. A randomized, open-label, dose-response study of losartan in hypertensive children. Clin J Am Soc Nephrol. 2014; 9(8): 1441–1448, doi: 10.2215/CJN.11111113, indexed in Pubmed: 24875194.
6. Bondarenko LB, Shayakhmetova G, Voronina A, et al. Losartan effects on liver cytochromes CYP3A, CYP2C and CYP2E1 functioning at metabolic syndrome in young and adult rats. Medical Research Journal. 2019; 4(2): 72–79, doi: 10.5603/mrj.a2019.0009.
7. Iwamura A, Fukami T, Hosomi H, et al. CYP2C9-mediated metabolic activation of losartan detected by a highly sensitive cell-based screening assay. Drug Metab Dispos. 2011; 39(5): 838–846, doi: 10.1124/dmd.110.037259, indexed in Pubmed: 21321060.
8. Choi DH, Li C, Choi JS. Effects of myricetin, an antioxidant, on the pharmacokinetics of losartan and its active metabolite, EXP-3174, in rats: possible role of cytochrome P450 3A4, cytochrome P450 2C9 and P-glycoprotein inhibition by myricetin. J Pharm Pharmacol. 2010; 62(7): 908–914, doi: 10.1211/jpp.62.07.0012, indexed in Pubmed: 20636879.
9. Abdulla MH, Sattar MA, Abdullah NA, et al. The contribution of α1B-adrenoceptor subtype in the renal vasculature of fructose-fed Sprague-Dawley rats. Eur J Nutr. 2011; 50(4): 251–260, doi: 10.1007/s00394-010-0133-8, indexed in Pubmed: 20882287.
10. Khromov OS, Dobrelya NV. Experimental study of phosphatidyl choline liposomes as new hipotensive. Bulletin of Dnipropetrovsk University, 2008; 1(16): 197–203.
11. Romeis B. Mikroskopische Technik. Foreign Literature. Inostrannaya literatura. 1953: 248–252, doi: 10.1515/9783486777031.
12. Piers E. Histochemistry. Foreign Literature. Inostrannaya literatura. 1962: 962.
13. Mannervik B, Jemth P. The Glutathione Pathway. Measurement of Glutathione Transferases. Costa LG, Hodgson E, Lawrence DA, Ozolins TR, Reed DJ, Greenlee WF, editors. Current Protocols in Toxicology. N. In: Costa LG, Hodgson E, Lawrence DA, Ozolins TR, Reed DJ, Greenlee WF. ed. Current Protocols in Toxicology. John Wiley & Sons Inc, New York 2005: 2758.
14. Bondarenko LB, Karatzuba T, Shayakhmetova G, et al. Specificity of metabolic syndrome model reproduction at pubertal and adult male rats. Romanian Journal of Diabetes Nutrition and Metabolic Diseases. 2015; 22(3): 251–260, doi: 10.1515/rjdnmd-2015-0031.
15. el-Batran SA, el-Shenawy SM, Nofal SM, et al. Studies on the glycemic and lipidemic effect of monopril and losartan in normal and diabetic rats. Pharmacol Res. 2004; 50(2): 131–136, doi: 10.1016/j.phrs.2003.12.019, indexed in Pubmed: 15177300.
16. Luchtefeld M, Bandlow N, Tietge UJF, et al. Angiotensin II type 1-receptor antagonism prevents type IIA secretory phospholipase A2-dependent lipid peroxidation. Atherosclerosis. 2007; 194(1): 62–70, doi: 10.1016/j.atherosclerosis.2006.09.024, indexed in Pubmed: 17069818.
17. Gaafar AG, Abo-Youssef AM, Khalaf MM, et al. Protective effects of vitamin D and losartan in complete Freund's adjuvant-induced arthritis in rats. Pak J Pharm Sci. 2019; 32(2): 593–600, indexed in Pubmed: 31081771.
18. Abdel El-Gaphar OA, Abo-Youssef AM, Abo-Saif AA. Effect of losartan in complete freund's adjuvant-induced arthritis in rats. Iran J Pharm Res. 2018; 17(4): 1420–1430, indexed in Pubmed: 30568700.
19. Kamińska M, Mogielnicki A, Stankiewicz A, et al. Angiotensin II via AT1 receptor accelerates arterial thrombosis in renovascular hypertensive rats. J Physiol Pharmacol. 2005; 56(4): 571–585, indexed in Pubmed: 16391415.
20. Spence SG, Cukierski MA, Manson JM, et al. Defining the susceptible period of developmental toxicity for the AT1-selective angiotensin II receptor antagonist losartan in rats. Teratology. 1995; 51(6): 367–382, doi: 10.1002/tera.1420510603, indexed in Pubmed: 7502237.
21. Wang X, Lu Y, Cederbaum AI. Induction of cytochrome P450 2E1 increases hepatotoxicity caused by Fas agonistic Jo2 antibody in mice. Hepatology. 2005; 42(2): 400–410, doi: 10.1002/hep.20792, indexed in Pubmed: 16025513.
22. Vartanian V, Lowell B, Minko IG, et al. The metabolic syndrome resulting from a knockout of the NEIL1 DNA glycosylase. Proc Natl Acad Sci U S A. 2006; 103(6): 1864–1869, doi: 10.1073/pnas.0507444103, indexed in Pubmed: 16446448.
23. Mercer JR, Cheng KK, Figg N, et al. DNA damage links mitochondrial dysfunction to atherosclerosis and the metabolic syndrome. Circ Res. 2010; 107(8): 1021–1031, doi: 10.1161/CIRCRESAHA.110.218966, indexed in Pubmed: 20705925.
24. Mattson MP. Roles of the lipid peroxidation product 4-hydroxynonenal in obesity, the metabolic syndrome, and associated vascular and neurodegenerative disorders. Exp Gerontol. 2009; 44(10): 625–633, doi: 10.1016/j.exger.2009.07.003, indexed in Pubmed: 19622391.
25. Bondarenko L, Shayakhmetova G, Karatsuba T, et al. Age dependent effects of metformin in Wistar albino male rats with metabolic syndrome. Rom J Diabetes Nutr Metab Dis. 2018; 25: 47–58

26. Kumar D, Zimpelmann J, Robertson S, et al. Tubular and interstitial cell apoptosis in the streptozotocin-diabetic rat kidney. Nephron Exp Nephrol. 2004; 96(3): e77–e88, doi: 10.1159/000076749, indexed in Pubmed: 15056984.
27. Erol A, Erol A. Metabolic syndrome is a real disease and premalignant state induced by oncogenic stresses to block malignant transformation. Med Hypotheses. 2010; 74(6): 1038–1043, doi: 10.1016/j.mehy.2010.01.001, indexed in Pubmed: 20097480.
28. Ray SD, Kamendulis LM, Gurule MW, et al. Ca2+ antagonists inhibit DNA fragmentation and toxic cell death induced by acetaminophen. FASEB J. 1993; 7(5): 453–463, doi: 10.1096/fasebj.7.5.8462787, indexed in Pubmed: 8462787.
29. Singh DK, Ahn B, Bohr VA. Roles of RECQ helicases in recombination based DNA repair, genomic stability and aging. Biogerontology. 2009; 10(3): 235–252, doi: 10.1007/s10522-008-9205-z, indexed in Pubmed: 19083132.
30. Håkansson A, Zhivotovsky B, Orrenius S, et al. Apoptosis induced by a human milk protein. Proc Natl Acad Sci U S A. 1995; 92(17): 8064–8068, doi: 10.1073/pnas.92.17.8064, indexed in Pubmed: 7644538.