Innov Biosyst Bioeng, 2025, vol. 9, no. 3, 33–43
doi: 10.20535/ibb.2025.9.3.337336

Original article

EFFECTS OF METFORMIN AND LOSARTANE ON HEPATIC CYTOCHROMES *CYP3A*, *CYP2C* AND *CYP2E1* FUNCTIONING AT METABOLIC SYNDROME IN RATS

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Received 19 March 2025; Accepted 30 June 2025

Background. The study of drugs' possible metabolic and biological interactions in preclinical models is a necessary condition for improving the development of most combinations of medicines.
Objective. The aim of our present study was to study the joint effects of metformin and losartan on hepatic *CYP3A*, *CYP2C*, and *CYP2E1* mRNA expression, their marker enzymes, liver antioxidant system and lipid peroxidation of adult rats with metabolic syndrome.
Methods. Wistar albino male rats were divided into 5 groups (8 animals in each group): 1 – Control (intact rats), 2 – MS (rats with MS), 3 – MS + metformin (rats with MS and metformin (266.0 mg/kg of body weight, *per os*, 60 days)), 4 – MS + losartan (rats with MS and losartan (4.43 mg/kg of body weight, *per os*, 60 days)), 5 – MS + metformin + losartan (rats with MS and metformin and losartan treatment). MS was induced by full replacement of drinking water with 20% fructose solution (200 g/l). Metformin and losartan doses were calculated based on the species sensitivity coefficient. After 60 days of MS modeling, investigation of rat liver CYP3A, CYP2C and CYP2E1 mRNA expression, their marker enzymes activities, as well as lipid peroxidation parameters were carried out.
Results. It was demonstrated that combined administration of metformin and losartan affects the levels of *CYP2E1*, *CYP2C23* and *CYP3A2* genes expression, diclofenac hydroxylase activity, reduced glutathione contents, and the activity of lipid peroxidation processes.
Conclusions. Our experiments showed that the noted changes were not the simple summation of the effects of metformin and losartan administered separately, but in most cases were determined only by losartan. Obtained results indicate the need for caution in the simultaneous prescription of metformin with losartan.
Keywords: metabolic syndrome; metformin; losartan; CYP450.

Abbreviations

MS – metabolic syndrome
CYP – cytochrome P450
PNP – *p*-Nitrophenol hydroxylase
LPO – lipid peroxidation
TBARS – thiobarbituric acid reactive substance

Introduction

The biological effect of the medicines combined use is one of modern pharmacology and drug toxicology most important problems. Various combinations are widely used because different drugs have their own targets and affect specific cell subpopulations. When combining drugs, the main goal is to mutually enhance their therapeutic effects and reduce side effects.

But the matter is that the vast majority of combination regimens are developed empirically, and there are very few systematic experimental studies aimed at thoroughly elucidating various combinations of drugs' biological effects using appropriate methods of analysis [1].

However, the study of drugs' possible metabolic and biological interactions in preclinical models is a necessary condition for improving the development of most combinations of medicines.

The situation is aggravated by the fact that in addition to prescribing several drugs of various groups at the same time, almost half of all marketed drugs are fixed combination preparations. It is also common to use empirically selected combinations of over-the-counter drugs, which can have a variety of unknown side effects [2]. Even if each component of the combination is necessary to achieve the desired therapeutic effect and the benefits outweigh the additional risk of using two or more drugs, a thorough preclinical study of drug combination metabolism in the organism is necessary before prescribing each combination [3–5].

Monitoring of adverse reactions to drug combinations is a problem in all countries, both developed and developing [6]. However, compared with

the huge number of drugs and their combinations used, the scale of the study of the biological consequences of the combined use of drugs is completely insufficient. This is especially true of the impact on the system of cytochromes P-450 (CYP450), which are involved in the processes of biotransformation of xenobiotics (including drugs). The accumulated experimental data indicate the possibility of the presence of multidirectional effects on different cytochromes of this family at the same chemical compound [7–9]. The combination of several substances can lead to the appearance of qualitatively different total effects on these enzymes.

The unhealthy lifestyles widespread have led to the facts that among the most widely prescribed drugs according to the WHO are losartan and metformin [10]. Both losartan and metformin are broadly used for metabolic syndrome (MS) treatment. Series of experiments with different CYP450 isoforms inducers and inhibitors allowed clarifying their roles in metformin and losartan metabolisms in rats [7–9]. According to obtained data metformin in rats affected mainly hepatic CYP2C11, 2D1, and 3A1/2, while losartan is metabolized via CYP3A and CYP2C isoforms of CYP450. Estimation of complex effects of MS and medications combination for its treatment on different CYP450 isoforms could be of special interest, as it allows defining additional modifying components for metabolic interactions of preparations.

The aim of this work was to study the joint effects of metformin and losartan on hepatic *CYP3A*, *CYP2C*, and *CYP2E1* mRNA expression, their marker enzymes, liver antioxidant system, and lipid peroxidation of adult rats with metabolic syndrome.

Materials and Methods

Study design. A total of 40 adult Wistar albino male rats (160–180 g) were used in the study. They were kept under a controlled temperature (from 22 to 24 °C), relative humidity of 40% to 70%, lighting (12 h 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 metabolic syndrome was reproduced according to the protocol of Abdulla *et al.* [11]. Animals were divided into 5 groups (8 animals in each group): 1 – Control (intact rats), 2 – MS (rats with MS), 3 – MS + metformin (rats with MS and metformin (266.0 mg/kg of body weight, *per os*, 60 days)), 4 – MS + losartan (rats with MS and losartan (4.43 mg/kg of body weight, *per os*, 60 days)), 5 – MS + metformin + losartan (rats with MS and metformin and losartan treatment). MS was induced by full replacement of drinking water with 20% fructose solution (200 g/l). Metformin and losartan doses were calculated based on coefficient for conversion of human doses to animal equivalent doses based on body surface area [12].

Crystalline D-fructose >99% (Khimlaborreactiv, Ukraine, series 072000897834, batch XW 130105) was used in experiments. 20% fructose was prepared daily and given every day for two month *ad libitum*. In our experiments, metformin (metformin hydrochloride, manufactured by SANDOZ, Lek C.A., Poland, Series CN8407) and losartan (potassium losartan, manufactured by LLC "KUSUM PHARM", Sumy, Ukraine) were used.

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

Post mitochondrial and microsomal fractions of livers were obtained by the method of Kamath *et al.* [13], and aliquots were kept frozen at –70 °C until needed.

Enzymes activities study. We investigated changes in rat orthologs of human cytochromes P-450: CYP2E1, CYP3A2 instead of CYP3A4 [14] and CYP2C23 instead of CYP2C9 and CYP2C19 [15]. *p*-Nitrophenol hydroxylase (PNP-hydroxylase) activity (a selective enzyme marker for CYP2E1) was determined in microsomal fraction of the liver according to the method of Koop *et al.* [16]. Erythromycin N-demethylase activity (a selective enzyme marker for CYP3A) was determined in liver microsomal fraction according to the method of Wang *et al.* [17], diclofenac hydroxylase activity (a selective enzyme marker for CYP2C) – according to the method of Necrasova *et al.* [18]. Glutathione-S-transferase activity was determined in liver post mitochondrial fraction according to the method of Habig *et al.* [19], glutathione reductase activity – in microsomes in accordance with *Current Protocols in Toxicology* [20], reduced glutathione and proteins SH-groups contents – in liver homogenates with Ellman's reagent by the method of Sedlak [21]. Protein contents were determined with Total Protein Kit, Micro Lowry, Onishi & Barr Modification (Sigma-Aldrich, USA).

Cytochrome P-450 isoforms study. The rats' livers were used for the investigation of cytochrome

P-450 isoforms mRNA expression rates by the method of reversed transcriptase polymerase chain reaction (rPCR). Isolation of total mRNA was carried out with TRI-Reagent (Sigma, USA). Synthesis of cDNA was carried out with reagents and protocol of Fermentas (Germany). rPCR reaction mixture contents, specific primers for *CYP2E1* gene amplification (forward 5′-CTTCGGGCCAGTGTTCAC-3′ and reverse 5′-CCCATATCTCAGAGTTGTGC-3′), as well as amplification protocol, were chosen according to Lankford *et al.* [22]. rPCR reaction mixture, amplification protocol, and following specific primers – forward 5′-TACTACAAGGGCTTAGGGAG-3′ and reverse 5′-CTTGCCTGTCTCCGCCTCTT-3′ were used for *CYP3A2* gene amplification according to Jager *et al.* [14]. rPCR reaction mixture, amplification protocol and following specific primers – forward 5′-GATGCTGTCTTCCGTCATGC-3′ and reverse 5′-GTAATAGGCTTGATGTCAAG-3′ were used for *CYP2C23* gene amplification according to Imaoka *et al.* [15]. rPCR with primers of β-actin (sense 5′-GCTCGTCGTCGACAACGGCTC-3′ and antisense 5′-CAAACATGATCTGGGTCATCTTCT-3′) was carried out for internal control. All primers were synthesized by "Metabion" (Germany). Thermocycler MyCycler (BioRaD, USA) was used for amplification. Electrophoresis of PCR products (*CYP2E1*-744 b.p., *CYP2C23*-252 b.p., *CYP3A2* – 349 b.p. and β-actin-353 b.p.) was carried out in 2% agarose gels (80 V; 1.5 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) [23].

Lipids peroxidation study. The levels of lipid peroxidation (LPO) in liver microsomes were investigated as the rates of NADPH-dependent thiobarbituric acid reactive substances (TBARS) formation [24].

Statistical analysis. The obtained data were expressed as the mean ± standard error of the mean (M ± SEM) and by one-way analysis of variance (ANOVA) followed by Tukey's test using OriginPro 7.5 Software [25]. Differences were considered to be statistically significant at $p < 0.05$.

Results

Results of CYP2E1 mRNA expression comparative study in the livers of rats with MS and metformin and losartan administration are shown in Figs. 1 and 2.

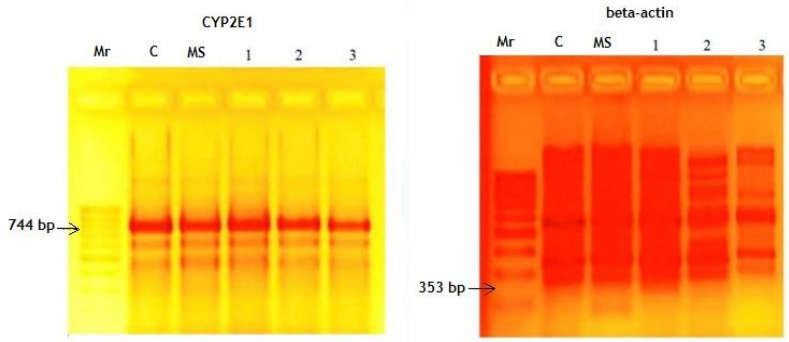

Figure 1: Electrophorogram of *CYP2E1* gene RT-PCR products (744 p.p.) and reference-gene β-actin (353 b.p.) RT-PCR products in the liver of rats with MS and metformin and losartan administration (Mr – DNA marker; C – control; MS – model of MS; 1 – MS + metformin; 2 – MS + losartan; 3 – MS + metformin + losartan, $n = 8$)

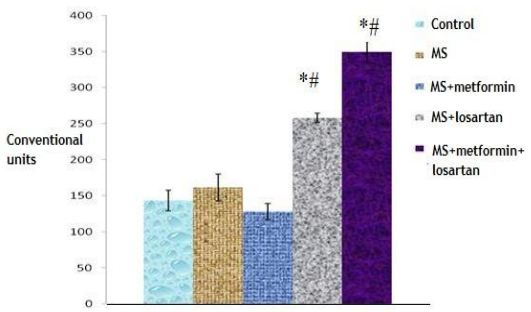

Figure 2: Average rate of *CYP2E1* mRNA expression in the liver of rats with MS and metformin and losartan administration (Mr – DNA marker; C – control; MS – model of MS; 1 – MS + metformin; 2 – MS + losartan; 3 – MS + metformin + losartan, $n = 8$, signal intensity of β-actin was taken as 100%); * – $p < 0.05$ in comparison with control, # – $p < 0.05$ in comparison with MS

Under the conditions of MS, no probable changes in CYP2E1 gene expression were noted (Figs. 1 and 2). Metformin (administered separately) also did not cause any shifts compared to the control level, unlike losartan. Joint administration of metformin and losartan to animals with MS led to a pronounced increase in the expression of the CYP2E1 gene by 2–2.5 times as compared with control, as well as with the MS group.

We noted statistically significant decrease in *CYP2C23* isoform mRNA expression level in the liver of rats with MS (Figs. 3 and 4). Metformin (administered separately) against the background of MS, normalized this indicator: no significant changes in the expression level of *CYP2C23* mRNA were detected compared to the control. Losartan (administered separately) against the background of MS, as in the case of *CYP2E1* mRNA expression, significantly increased the level of *CYP2C23* mRNA expression. Co-administration of metformin and losartan also resulted in an increase in *CYP2C23* mRNA expression compared to both control (by 1.5 times) and MS (by 2.5 times).

At the same time, no significant changes were detected in the level of mRNA expression of the *CYP3A2* gene in the liver of rats both with MS and with MS and metformin and losartan administration (Figs. 5 and 6).

We investigated the activity of CYP2E1 marker enzyme – *p*-nitrophenol-hydroxylase in liver microsomes of rats with MS and metformin and losartan administration. Statistically significant growth of PNP-hydroxylase activity was detected both with MS (1.38 fold) and metformin administration (1.73 fold) (Table 1).

The administration of losartan alone and concomitantly with metformin against the background of MS did not lead to a significant disturbance of this enzyme activity in comparison with the control (Table 1). In this case, intensification of CYP2E1 expression was not accompanied by stimulation of the corresponding enzymatic activity, as it was also noted previously [26, 27].

Investigation of CYP2C23 marker enzyme – diclofenac-hydroxylase activity rates (see Table 1) demonstrated its reduction with MS. Under the

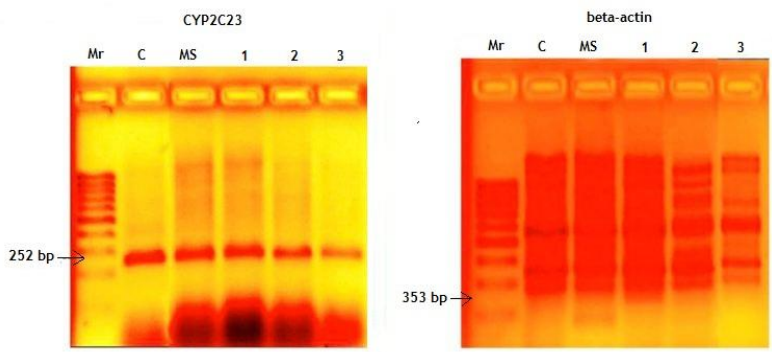

Figure 3: Electrophorogram of *CYP2C23* gene RT-PCR products (252 p.p.) and reference-gene β-actin (353 b.p.) RT-PCR products in the liver of rats with MS and metformin and losartan administration (Mr – DNA marker; C – control; MS – model of MS; 1 – MS + metformin; 2 – MS + losartan; 3 – MS + metformin + losartan, $n = 8$)

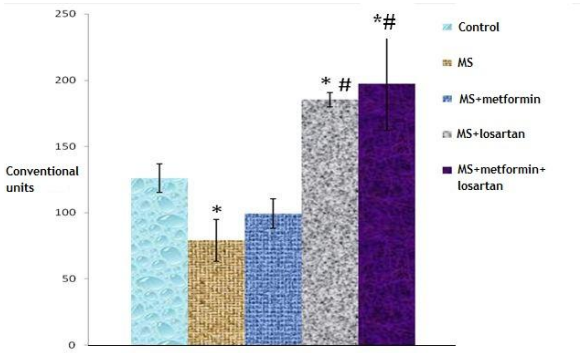

Figure 4: Average rate of *CYP2C23* mRNA expression in the liver of rats with MS and metformin and losartan administration (Mr – DNA marker; C – control; MS – model of MS; 1 – MS + metformin; 2 – MS + losartan; 3 – MS + metformin + losartan, $n = 8$, signal intensity of β-actin was taken as 100%); * – $p < 0.05$ in comparison with control, # – $p < 0.05$ in comparison with MS

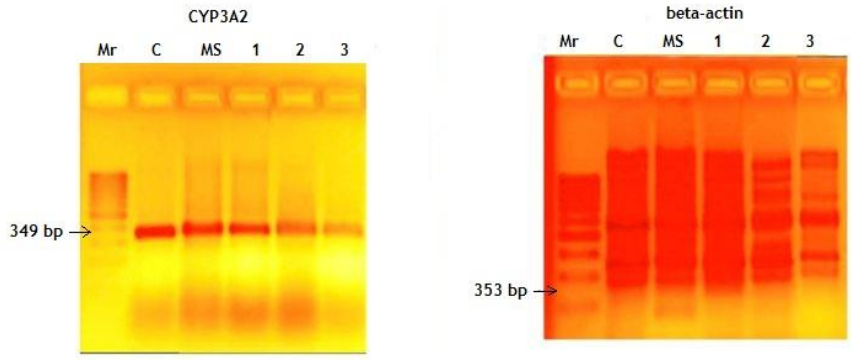

Figure 5: Electrophorogram of *CYP3A2* gene RT-PCR products (349 p.p.) and reference-gene β-actin (353 b.p.) RT-PCR products in the liver of rats with MS and metformin and losartan administration (Mr – DNA marker; C – control; MS – model of MS; 1 – MS + metformin; 2 – MS + losartan; 3 – MS + metformin + losartan, $n = 8$)

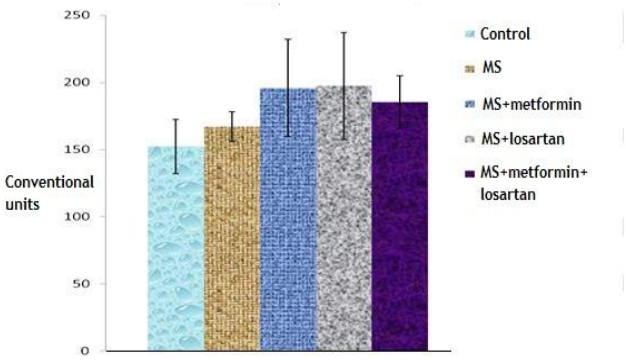

Figure 6: Average rate of *CYP3A2* mRNA expression in the liver of rats with MS and metformin and losartan administration (Mr – DNA marker; C – control; MS – model of MS; 1 – MS + metformin; 2 – MS+losartan; 3 – MS + metformin + losartan, $n = 8$, signal intensity of β-actin was taken as 100%); * – $p < 0.05$ in comparison with control, # – $p < 0.05$ in comparison with MS

Table 1: Activities of *p*-nitrophenol-hydroxylase, erythromycin-N-demethylase, and diclofenac hydroxylase in liver microsomal fraction of rats with MS and metformin and losartan administration (M ± SEM, $n = 8$)

Groups	Activity of *p*-nitrophenol-hydroxylase, nmoles×min^{-1}×mg of protein^{-1}	Activity of diclofenac hydroxylase, nmoles×min^{-1}×mg of protein^{-1}	Activity of erythromycin-N-demethylase, nmoles×min^{-1}×mg of protein^{-1}
Control	0.45 ± 0.018	403.6 ± 14.8	0.22 ± 0.02
MS	0.62 ± 0.055*	140.8 ± 14.3*	0.32 ± 0.03*
MS + metformin	0.78 ± 0.037*	1357.0 ± 46.2$^{*,\#}$	0.34 ± 0.07
MS + losartan	0.44 ± 0.040	576.7 ± 93.5$^{\#}$	0.20 ± 0.02
MS + metformin + losartan	0.58 ± 0.035	214.0 ± 21.6$^{*,\#}$	0.29 ± 0.02

Notes. * – $p < 0.05$ in comparison with control, # – $p < 0.05$ in comparison with MS.

conditions of metformin and losartan separate administration, metformin caused an increase of diclofenac-hydroxylase activity significantly higher than the levels in both control and MS groups; while losartan led to normalization of diclofenac-hydroxylase activity rate. Joint administration of these preparations led to a decrease in the activity of diclofenac-hydroxylase which was obviously related to the competitive inhibition of this enzyme active center by both of its substrates – metformin and losartan.

In rats with MS, the increase in the activity of CYP3A2 marker enzyme – erythromycin-N-demethylase – was 46% compared to the control. Separate and joint administration of metformin and losartan didn't cause significant changes in erythro-

mycin-N-demethylase activity. This is consistent with the results of the CYP3A2 gene expression study given above.

A study of indices characterizing prooxidant/antioxidant status with MS and administration of metformin and/or losartan demonstrated that glutathione-S-reductase and transferase activities remained at the level of the control group (Table 2).

In the liver of rats with MS, depletion of the reduced glutathione pool was observed: its content decreased by 1.36 times compared to the control. Metformin administration allowed normalizing this parameter, while the use of losartan or losartan in combination with metformin did not give any positive outcomes.

Simultaneously the activity of lipid peroxidation processes (LPO) was also intensified in liver microsomes of animals with MS (Table 3), which was confirmed by the increase in TBC-reactants formation rates.

Metformin administration led to the lipid peroxidation processes normalization, while both separate administration of losartan or its combination with metformin did not have effects.

Discussion

Biotransformation of the medicines as an important component of the process of their interaction with organism's physiological systems could significantly affect both the manifestation of therapeutic activity and toxicity [28]. As a result of biotransformation, we get not only pharmacologically important biologically active substances, but also biologically inactive metabolites or even toxic reactive intermediates [29, 30]. One should remember that enzymes involved in the biotransformation processes have multiple isoforms, significant individual variability of their activity, numerous stimulators and inhibitors of exogenous and endogenous origin, and various mechanisms of their genes expression regulation [31]. All this greatly complicates the metabolism of drugs and the implementation of their effects in the organism. This situation is even more aggravated when it comes to the simultaneous intake of several drugs.

CYP450 enzymes play an important role in the metabolism of numerous medicines [30, 31]. When simultaneously administered into the organism therapeutic agents can induce or inhibit the metabolism of other medicines, as well as compete for metabolism with the substrates of the corresponding CYP450 isoforms. The situation is complicated by the fact that not only different CYP450 isoforms are involved simultaneously, but the majority of drugs is metabolized by several routes, which also involved several CYP450 isoforms [28, 32]. For example, it was demonstrated that biotransforma-

Table 2: Contents of reduced glutathione, and glutathione transferase and reductase activities in livers of rats with MS and metformin and losartan administration (M ± SEM, $n = 8$)

Groups	Activity of glutathione reductase, nmoles×min^{-1}×mg of protein^{-1}	Activity of glutathione transferase, μmoles×min^{-1}×mg of protein^{-1}	Contents of glutathione, μmoles×g of tissue^{-1}
Control	115.0 ± 5.5	1.25 ± 0.093	2.58 ± 0.27
MS	114.0 ± 5.0	1.12 ± 0.06	1.48 ± 0.18*
MS + metformin	120.3 ± 3.9	1.07 ± 0.062	2.26 ± 0.33
MS + losartan	110.8 ± 4.2	1.04 ± 0.059	1.80 ± 0.18*
MS + metformin + losartan	121.2 ± 4.2	1.05 ± 0.059	1.53 ± 0.18*

Notes. * – $p < 0.05$ in comparison with control.

Table 3: NADPH-dependent LPO in rat liver microsomal fractions with MS and metformin and losartan administration (M ± SEM, $n = 8$)

Groups	NADPH-dependent LPO, nmoles×min^{-1}×mg of protein^{-1}
Control	0.105 ± 0.02
MS	0.167 ± 0.02*
MS + metformin	0.107 ± 0.01
MS + losartan	0.164 ± 0.015*
MS + metformin + losartan	0.163 ± 0.011*

Notes. * – $p < 0.05$ in comparison with control.

tion of both of metformin and losartan in rat liver involved CYP2C and CYP3A isoforms [33, 34].

Accurate CYP450 isoforms profile information allows establishing the potential interaction of drugs, including competition for specific isoforms, the individual variability associated with high polymorphism of these isoforms, and different isoenzymes possible induction.

In addition, the subjects of pharmacotherapy themselves – pathological processes – can also significantly change the rate of biotransformation processes through the induction or inhibition of CYP450 enzymes [35]. Unfortunately, the possibilities of mutual influences of the pathological processes and various medicines during their therapy, as a rule, are not taken into account by researchers and clinicians.

Given the above, we have identified expression profiles of three hepatic CYP450 isozymes in with MS and metformin and losartan administration.

Our studies did not reveal statistically significant changes in *CYP2E1* gene expression in rats both with MS and metformin administration, while losartan and its combination with metformin led to significant increase of this isoform nRNA compared to both control and MS. The addition of metformin to losartan not only did not weaken the stimulation of expression, but even somewhat enhanced it.

Subsequent studies of cytochrome *CYP2C23* gene expression levels confirmed this feature of metformin and losartan combined effects. Metformin (administered separately) against the background of MS did not lead to statistically significant changes in the *CYP2C23* gene expression level, while losartan (administered separately) induced its increase. Combined administration of metformin with losartan against the background of MS caused an increase in *CYP2C23* gene expression statistically significant in comparison with the control and the group with MS.

Changes in the nature of the effect on cytochromes CYP450 of medicines, depending on their combinations with other preparations in the background of diabetes or obesity, were also noted by other researchers [36, 37].

Our results on activities of *p*-nitrophenol-hydroxylase, erythromycin-N-demethylase, and diclofenac hydroxylase in liver microsomal fraction of rats with MS and metformin and losartan administration to some extent agree with the results of other authors noting multidirectional changes in their activities at diabetes, obesity, and different medicines administration [36–40].

In contrast to the effects on the expression of cytochrome *CYP2E1* gene, the combined administration of metformin and losartan did not cause statistically significant deviations in the level of its marker enzyme activity compared with the control and the MS group.

In animals with MS and metformin and losartan administration we observed comparatively weak changes in *CYP2C23* expression following by statistically significant distrubances in corresponding marker enzyme activity (diclofenac hydroxylase). Such differences might be due to the simultaneous operation of several mechanisms.

Metformin influences small heterodimer partner protein (SHP protein) synthesis, vitamin D (VDR), constitutive androstane (CAR), and *growth factor* (GR) nuclear receptors, coactivated with steroid receptor coactivator 1 (SRC1) and its ability to cause direct disruption of activated pregnane X receptor (PXR) interaction with SRC1 independently of PXR ligand binding pocket, as it was previously demonstrated for CYP3A regulation [41].

The metabolism of losartan in the liver of rats involves simultaneously CYP2C and CYP3A isoforms [34, 42, 43]. Some other post-translational mechanisms also could not be excluded [44]. Co-administration of metformin and losartan resulted in significant inhibition of diclofenac hydroxylase activity in comparison with the levels of groups with metformin and losartan (administered separately), which might be the result of their competition as substrates for the enzyme's active or binding sites.

The absence of significant changes in cytochrome *CYP3A2* gene expression upon administration of metformin and losartan with MS in our experiments was accompanied by the absence of significant changes in its marker enzyme (erythromycin-N-demethylase) activity.

Changes in CYP450 system caused by MS and metformin and losartan administration accompanied by lower productivity of antioxidant defense. Our previous investigations of MS age-dependent effects on rat's organism have shown a profound decrease in reduced glutathione contents [35]. Metformin administration (separate) allowed a normalizing reduced glutathione contents. Its antioxidant effects were demonstrated by several authors both *in vitro* and *in vivo,* on animal models of diabetes and in patients with insulin resistance [45, 46].

Losartan administration (separate) didn't cause any normalizing effects on glutathione level. The combined administration of metformin with losartan led to a complete leveling of metformin's normalizing effect on the reduced glutathione contents.

Thus, as in the case of the effects on the expression of cytochromes *CYP2E1*, *CYP2C23*, and *CYP3A2* genes, the total outcome of combined metformin and losartan administration on reduced glutathione contents was determined primarily by losartan.

Lower productivity of antioxidant defense creates conditions for oxidative stress. We have previously demonstrated that MS stimulated lipid peroxidation processes [35]. The nature of metformin, losartan and their combination influences on lipid peroxidation was completely similar to their respective effects on the glutathione system. Here as well as in the previous case total effect of combined metformin and losartan administration on reduced glutathione contents was determined primarily by losartan.

Our data are in good agreement with the results of other researchers [47]. They demonstrated that maternal MS (caused by high fructose exposure) induced tissue oxidative stress in their offspring organisms. The consequence of such induction in rostral ventrolateral medulla (RVLM) could be an increase in sympathetic vasomotor activity and blood pressure in organism, which are attributed to the increase in 5′-adenosine monophosphate-activated protein kinase (AMPK)-regulated angiotensin type 1 receptor (AT_1R) expression and impairment of sirtuin1 (SIRT1)-associated mitochondrial biogenesis [48].

At the molecular level, the elevated reactive oxygen species (ROS) level in RVLM was attributed by an increase in $gp91^{phox}$ subunit of the NADPH oxidase and downregulation of the antioxidant superoxide dismutase 2, suppression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and mitochondrial transcription factor A expression, together with a decrease in mitochondrial DNA copy number, events known to underpin sympathoexcitation in animal models of hypertension [48]. It is likely that activation of AT_1R plays a pivotal role in priming tissue oxidative stress in RVLM as MS long-term effects in the next generation.

Losartan as AT_1R antagonist could realize its biological effect via the AMP-activated protein kinase AMPK/SIRT1 signaling process regulation, wherein metformin may exert its beneficial effect on tissue oxidative stress directly via activation of PGC-1α [47].

Summarizing all results of our experiments it could be concluded that the combined administration of metformin and losartan affects the levels of *CYP2E1*, *CYP2C23*, and *CYP3A2* genes expression, diclofenac hydroxylase activity, reduced glutathione contents, and the activity of lipid peroxidation processes. The noted changes were not the simple summation of the effects of metformin and losartan administered separately, but in most cases were determined only by losartan. Perhaps this is due to the fact that the metformin molecule is small, chemically stable, does not bind to plasma proteins, and does not undergo intensive metabolism in the body [49], while the relatively massive losartan molecule has active hydroxyl group, chlorine atom, extended biphenyl group with a tetrazole (which is being used in place of the carboxylic acid as a bioisostere) and is metabolized in the organism (primarily by cytochrome P450 isoenzymes CYP2C9 and CYP3A4) to the 5-carboxylic acid metabolite, designated as EXP3174 [50]. Both losartan and EXP3174 are more than 98% bound to plasma proteins [50]. Thus, with the simultaneous intake of metformin and losartan into the body, the latter interacts much longer and much more actively with the molecular structures of the cell, realizing its biological activity both directly and through its even more active metabolite.

An indirect effect of losartan on the realization of the biological activity of metformin itself cannot be excluded, since it has been shown that in addition to its antihypertensive action, losartan is known to reduce blood sugar level, and therefore, its concomitant administration with antidiabetic agents can result both in hyperglycemic or hypoglycemic states [51].

Conclusions

Thus, our experiments showed that the combined administration of metformin and losartan affects the levels of *CYP2E1*, *CYP2C23*, and *CYP3A2* genes expression, diclofenac hydroxylase activity, reduced glutathione contents, and the activity of lipid peroxidation processes. The noted changes were not the simple summation of the effects of metformin and losartan administered separately, but in most cases were determined only by losartan. Obtained results indicate the need for caution in the simultaneous prescription of metformin with losartan.

Interests Disclosure

Larysa Bondarenko is the member of the Editorial Council of *Innovative Biosystems and Bioengineering* and was not involved in the editorial evaluation or decision to accept this article for publication. The other authors have no conflicts of interest to declare.

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ВПЛИВ МЕТФОРМІНУ ТА ЛОЗАРТАНУ НА ФУНКЦІОНУВАННЯ ЦИТОХРОМІВ *CYP3A*, *CYP2C* І *CYP2E1* ПЕЧІНКИ ЩУРІВ ЗА УМОВ МЕТАБОЛІЧНОГО СИНДРОМУ

Проблематика. Вивчення можливих метаболічних і біологічних взаємодій лікарських засобів на доклінічних моделях є необхідною умовою для вдосконалення розробки більшості комбінацій лікарських засобів.
Мета. Вивчити спільний вплив метформіну та лозартану на експресію мРНК *CYP3A*, *CYP2C* та *CYP2E1* у печінці, їх маркерні ферменти, антиоксидантну систему печінки та перекисне окиснення ліпідів дорослих щурів із метаболічним синдромом.
Методика реалізації. Білих щурів-самців лінії Вістар розділили на 5 груп (по 8 тварин у кожній групі): 1 – контроль (інтактні щури), 2 – метаболічний синдром (МС) (щури з МС), 3 – МС + метформін (щури з МС і введенням метформіну (266,0 мг/кг маси тіла, перорально, 60 днів)), 4 – МС + лозартан (щури з МС і введенням лозартану (4,43 мг/кг маси тіла, перорально, 60 днів)), 5 – МС + метформін + лозартан (щури з МС та введенням метформіну і лозартану). Метаболічний синдром індукували повною заміною питної води 20 %-вим розчином фруктози (200 г/л). Дози метформіну та лозартану розраховували на основі коефіцієнта видової чутливості. Через 60 днів моделювання МС досліджували експресію мРНК *CYP3A*, *CYP2C* та *CYP2E1* у печінці щурів, визначали активність їх маркерних ферментів, а також параметри перекисного окиснення ліпідів.
Результати. Показано, що комбіноване застосування метформіну та лозартану впливає на рівень експресії генів *CYP2E1*, *CYP2C23* і *CYP3A2*, активність диклофенак-гідроксилази, викликає зниження вмісту глутатіону й активність процесів перекисного окиснення ліпідів.
Висновки. Наші експерименти показали, що зазначені зміни не були простою сумою ефектів метформіну та лозартану, які вводилися окремо, а в більшості випадків визначалися лише лозартаном. Отримані результати свідчать про необхідність обережності при одночасному призначенні метформіну з лозартаном.

Ключові слова: метаболічний синдром; метформін; лозартан; CYP450.