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The Effect of Inducers and Inhibitors of Monooxygenase on the Activity Nitrergic System in the Microsomes in the Ischemic Liver

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Authors' contributions

This work was carried out in collaboration between both authors. Author SS designed the study, performed the statistical analysis, wrote the first draft of the manuscript and managed the literature searches. Author KK wrote the protocol and managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Experiments were carried out on 62 white male rats and average weighing was 180-220 g. We found that inducers and inhibitors of monooxygenase showed opposite effects on the activity of NOS in the ischemic liver microsomes. Benzonal and cimetidine, after 1 night of their introduction, had no significant effect on all studied parameters. After 3 and 10 daily using drug metabolism inducer that benzonal makes a slow speed nitrate reductase system, stimulates nitroxylenes system (eNOS), and cimetidine, on the contrary – even more nitrate reductase activates the speed system, inhibits eNOS nitroxylenes. Now, in connection with the growth of liver disease and aggressive exposure to xenobiotic with induction and inhibitory action, this problem acquires a special urgency and, surely, requires further study.

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1. INTRODUCTION

An important aspect of the modern drug therapy is a personalised medicine based on research and implementations in the practical health care of medicines influencing on the system of biotransformation of xenobiotics in the liver [1,2]. The inducers and inhibitors of drug metabolism are to regulate the activity of the monooxygenase system (MOS) of the liver, and it plays the key role in this issue [3,4]. In the last decade, we appreciate basic research in molecular biology and medicine which were found that in vascular endothelium, the synthesis of nitric oxide (NOº) is the family of cytochrome P-450-like hemoproteins - NO-synthase in 5-electron oxidation of L-arginine with the formation of L-citrulline and NOº [5,6]. A family of isoenzymes of NO-synthase (NOS) synthesise NO from L-arginine by three major isoforms that two constitutive (neuronal (nNOS) and endothelial (eNOS) and one inducible (iNOS) [7]. To produce NO there are some processes are important like utilising NOS along with a variety of cofactors, substrates such as arginine, oxygen and oxidised nicotinamide dinucleotide phosphate (NADPH) [8,9]. In pathological processes accompanied by hypoxia or ischemia, the role of NO- sinus mechanism is reduced and induced activity nitrate reductase systems [10]. It is now established that NOS and inactive nitrate reductase system (LDCs) is found in hepatocytes, endothelium of sinusoids, the Kupffer cells/macrophages [11], as well as in the endothelium of the portal vein and hepatic artery [12,13]. The presence of NOS in hepatocytes suggests a correlation with the enzymes of MOS. However, in the literature, there is practically few data on the effect of inducers and inhibitors of drug metabolism on the activity of NOS in microsomes isolated from hepatocytes in the development of the pathological liver process.

In connection with the above-mentioned case, the purpose of the study was to study the activity of NOS in the liver microsomes after administration to animals in the dynamics of the postischemic period of benzonal and cimetidine.

2. MATERIALS AND METHODS

The study was carried out on 62 male rats of mixed population weighing 180-220 g, which were divided into 3 groups. First group animals after 1, 2 and 3 days ischemia/hypoxia of the liver caused by occlusion during the 180 min of the vascular pedicle of the left lateral and middle lobes.

The study drugs were administered after the restoration of blood flow to the liver. An inducer of drug metabolism benzonal was administered intragastrically in the form of 1% solution in 0.5% starch gel single dose of 50 mg/kg for 1, 3 and 10 days in a row (2 ml). Inhibitor of drug metabolism cimetidine also was injected intraperitoneally in 0.1% aqueous solution daily, once daily for 1, 3 and 10 days in a row (2^{nd} group). Control for all research groups served as data of intact animals. Each group consisted of 6-8 animals.

The animals were sacrificed by instant decapitation method under light Rausch-anesthesia. The extracted liver was perfused through the inferior vena cava by chilled (0±4ºC) 50 mM Tris *HCl* buffer, pH 7.4, containing 0.05 M *KCl* and 0.25 M saccharose. After washing the liver from the blood it was ground and homogenised in the same solution (1:3). From that fraction, which was obtained by centrifugation at VAC-602 (Germany) after 20 minutes of unscrewing, with 12 thousand g, had been beset microsomes thousand at 105 g for 60 min. All procedures were performed in the refrigerating chamber KHS-12(Russia) at 0±4ºC. In microsomes, resuspended in 100 mM Tris - HCl buffer; pH 7.4 was evaluated activity of monooxygenase system that content of cytochromes P-450, P-420, and b5 by classic method of T. Omura, R. Sato (1964), the activity of NADPH-reductase (NADPH-op.-ed.) by C. H. Williams, H. Kamin (1961), benzo(α)pyrene hydroxylase (B(a)PG) by C. H. Yang, L.P.Kicha (1978). Aniline hydroxylase (AG) by A. I. Archakov et al. (1975), N-demethylase amidopyrine (N-AP) by A. Bast, J. Nordhosck (1981), glucose-6-phosphatase (G-6-Phase) by N. S. Gnosh, N. C. Kar (1983) were assessed (Table 1).

Nitrooxygenase activity was determined by the content of stable metabolites nitrite and nitrate NO -, NO_2 - and NO_{3-} - by the method of P. P. Golikov et al.(2000), activity of endothelial NOS (eNOS) by Sumbaev V. V., Yasinska, I. M. (2000), inducible NOS (iNOS) and the concentration of peroxynitrite (ONO_{2-}) in Ravaeva M. Yu, E. N. Chuyan (2011). Content, the activity of monooxygenase and

Table 1. Dynamics of activity indicators of monooxygenase inhibitors of liver after acute ischemia/hypoxia and establishment in different dates (days) benzonal and cimetidine, M±m

Groups	P-450, nm/mg	P-420, nm/mg	b_5, nm/mg	NADFH-cyt.c-red, nm/min/mg	B(a)PG, nm/min/mg	AG, nm/min/mg	N-AP, nm/min/mg	G-6-Phase, nm/min/mg
Control group	0,97±0,031	0,036±0,001	0,63±0,026	106,9±3,95	1,69±0,078	0,88±0,023	4,85±0,151	79,8±3,06
Ischemia:								
1 day	0,30±0,018*	0,262±0,009*	0,15±0,006*	8,4±0,29*	0,65±0,022*	0,30±0,017*	1,57±0,062*	24,7±1,03
3 day	0,37±0,015*	0,191±0,008*	0,20±0,007*	13,7±0,44*	0,89±0,035*	0,37±0,015*	1,85±0,061*	39,5±1,65
10 day	0,45±0,018*	0,170±0,006*	0,25±0,008*	21,1±0,87*	0,93±0,042*	0,41±0,018*	1,91±0,067*	43,3±1,17
Ischemia+B:								
1 day	0,35±0,017*	0,231±0,010*	0,17±0,007*	8,9±0,36*	0,73±0,039	0,35±0,019*	1,62±0,048*	25,3±1,23*
3 day	0,68±0,021*$^{\Delta}$	0,107±0,013*$^{\Delta}$	0,31±0,009*$^{\Delta}$	68,1±2,48*	1,05±0,044*$^{\Delta}$	0,51±0,022*	2,24±0,079*$^{\Delta}$	45,9±1,34*$^{\Delta}$
10 day	1,55±0,059*$^{\Delta}$	0,015±0,002*$^{\Delta}$	0,77±0,032*$^{\Delta}$	120,4±6,35*	2,01±0,095*$^{\Delta}$	1,46±0,061*$^{\Delta}$	6,35±0,330*$^{\Delta}$	82,7±3,56*$^{\Delta}$
Ischemia+C:								
1 day	0,29±0,019*	0,266±0,008*	0,16±0,005*	8,5±0,33*	0,68±0,027*	0,32±0,018*	1,59±0,055*	23,9±1,16*
3 day	0,31±0,014*$^{\Delta}$	0,243±0,009*$^{\Delta}$	0,17±0,006*	13,1±0,59*	0,80±0,031*	0,34±0,015*	1,73±0,059*	37,2±1,48*
10 day	0,28±0,011*$^{\Delta}$	0,285±0,005*$^{\Delta}$	0,16±0,005*$^{\Delta}$	17,5±0,58*$^{\Delta}$	0,71±0,026*$^{\Delta}$	0,29±0,016*$^{\Delta}$	1,54±0,048*$^{\Delta}$	28,6±0,89*$^{\Delta}$

** - P<0.05 compared with control, ∆- P<0.05 compared to hypoxia of the corresponding period*

oxidoreductase of nitrooxygenase systems were recorded on computerized dual beam spectrophotometer UV-2100 (Ltd, China). The content and activity of oxidoreductase were calculated in microsomes per milligram of protein in 1 ml (mg/ml), which was determined by the method of O. N. Lowry et al. (1951).

2.1 Statistical Analysis

The obtained results were subjected to statistical analysis using the software package Excel, Statistic for Windows V.6.0. Normality of distribution of quantitative parameters was checked using the criteria Kolmogorov-Smirnov and Shapiro-Wilk test. Calculated arithmetic mean (M), standard deviation (σ), error arithmetic average (m), sample standard deviation (S). The distribution of the samples was carried out on the basis of student's criterion (t) with the computation of error probability (P). The correlations for the indicators were carried out using correlation analysis Pearson (r). For comparison, samples were used Student's t-test. Data were considered significant at $p<0.05$.

3. RESULTS AND DISCUSSION

Benzonal and cimetidine had no significant effect on all studied parameters characterising the activity of NOS in the liver microsomes after 1 night of their introduction and after chemotherapy, compared to other groups, which drugs are not injected, the corresponding term monitoring (1 day) (Table 2). In subsequent periods after 3 and 10 days benzonal significantly reduced the expression of NO, iNOS and ONO_2^- on the background of the dynamic of the studied follow-up period of increasing eNOS activity and content of microsomal protein. At the same time, after 3 and 10 of the daily administration of cimetidine in selected microsomal fractions of the liver shows a dynamic period of observation, a decrease in the activity of eNOS and increased expression of NO, iNOS and ONO_2^-, marked inhibition of microsomal protein concentration. Therefore, the introduction of animals with ischemic liver benzonal optimizes the processes of NOS in the microsomal system in the body, and cimetidine, on the contrary, an even greater extent, potentiates the effects of damage to this system. When analysing the performance of NOS is therefore with the activity of eNOS associated changes in the level of iNOS reaction rate, the content of microsomal NO and ONO_2^- in all studied groups of animals. In this regard, it is quite possible to believe that the increased NO and ONO_2^- is due to inhibition of eNOS and overexpression of iNOS. Benzonal positively influenced changes in the level of NO in microsomes, reduced an activity of iNOS and content of cytotoxic ONO_2^-. You can put that with the decreased activity of iNOS and the level of ONO_2^- was associated, although not significantly increase the activity of eNOS and restore to control values the concentrations in the ischemic liver microsomes NO administered to animals of benzonal.

According to the literature data, iNOS, ONO_2^- and NO are components of the expression system of nitrate reductase. During ischemia and/or hypoxia involves an increase in the cytotoxic compounds, including NO and ONO_2^- which block the active centres of cytochrome P450 in microsomes ischemic liver [11]. Cimetidine as followed data reinforces these processes in microsomes of animals with ischemic liver and suppresses NOS way. However, as shown by some researchers during ischemia/hypoxia blockade the active site of the isoforms of cytochrome P-450 activated oxygen metabolites, including NO and ONO_2^- have a fragile relationship [12]. In this regard, we can assume that the inducer of drug metabolism benzonal, promotes the release of the connection of active centre of cytochrome P-450 with NO and ONO_2^- ischemic liver. As a result of increased accessibility to the substrates of oxidation in particular L-arginine, which plays a major role in the regulation of functional metabolic and regenerative functions of liver [13,14]. This is evidenced by the increase of eNOS activity in microsomes when administered to animals with ischemic liver of benzonal. Therefore, benzonal as an inducer of drug metabolism when administered to animals with ischemic liver microsomes increases in NOS activity, through mechanisms of oppression nitrate reductase components, thus reducing the level in hepatocytes toxic compounds, the overexpression of NO and ONO_2^-.

3.1 Discussion

We considered that a number of basic factors like covariates in these analyzes, which can lead to an association between NO and ONO_2^-, including age, race/ethnicity, diabetes, hypertension, hyperlipidemia, cardiac vascular diseases (cardiovascular diseases), body mass index (BMI), smoking history, alcohol consumption, physical activity, prior use of

Table 2. Dynamics of indicators of activity of NO – system in the liver microsomes after playing it acute ischemia/ hypoxia and different periods (day) of benzonal and cimetidine, M±m

Group	NO, mkM/mg	eNOS, mkM/min/mg	iNOS, mkM/min/mg	ONO2-, mkM/mg	Protein мс, mg/ml
Control	5,5±0,16	17,4±0,62	0,10±0,002	0,080±0,016	36,8±1,22
Ischemia:					
1 day	8,6±0,33*	7,9±0,29*	0,35±0,017*	0,23±0,010*	29,5±1,13*
3 day	8,1±0,27*	8,5±0,35*	0,23±0,009*	0,19±0,009*	30,8±1,09*
10 day	7,6±0,28*	9,7±0,42*	0,17±0,006*	0,14±0,007*	31,2±1,18
Ischemia+B					
1 day	8,7±0,29*	8,3±0,21*	0,32±0,019*	0,22±0,011*	29,1±1,26*
3 day	6,3±0,26*$^{\Delta}$	12,5±0,43*$^{\Delta}$	0,17±0,005*$^{\Delta}$	0,16±0,006*$^{\Delta}$	31,7±1,31
10 day	5,8±0,22$^{\Delta}$	18,4±0,59$^{\Delta}$	0,11±0,004*$^{\Delta}$	0,07±0,005*$^{\Delta}$	37,5±1,42
Ischemia+C:					
1 day	8,9±0,39*	8,1±0,28*	0,36±0,019*	0,25±0,013*	28,7±1,26*
3 day	10,6±0,37*$^{\Delta}$	8,4±0,15*	0,33±0,012*$^{\Delta}$	0,21±0,011*	28,3±1,33*
10 day	13,5±0,52*$^{\Delta}$	7,2±0,18*$^{\Delta}$	0,46±0,021*$^{\Delta}$	0,35±0,014*$^{\Delta}$	32,6±1,40

** - P<0.05 compared with control, ∆- P<0.05 compared to hypoxia of the corresponding period*

hormone therapy (HT), and in the longitudinal analysis of the active HT hand for participants in clinical trials of HT. Diabetes mellitus was defined as self-administration of pills or insulin and/or serum glucose on an empty stomach> 126 mg/dl [3,9]. Hypertension was defined as a systolic blood pressure less than 140 mmHg or diastolic blood pressure >90 mmHg or took pills for hypertension [5]. Hyperlipidemia was defined as total cholesterol level> 240 mg/dl or LDL> 160 mg/dl or taking cholesterol-lowering drugs [14]. The survey history, alcohol use, previous cardiovascular diseases and previous use and duration of hormones were set in the questionnaire. Physical activity was determined using personal habits data and classified into a total metabolic equivalent (MET) per week [10,4]. This lack of effect was confirmed by Western blot, which demonstrated that the expression of these enzymes was not altered by L-NAME Discourse. Our results show that chronic treatment of rats with L-NAME is effective in hypertrophy of the walls of the arterial vessel wall (envelopes), together with perivascular fibrosis associated with the deposition of collagen fibres [15]. Also, a connection between the sinusoidal lumen and interstitial expansion (increase in cellularity, mainly of fibroblasts, and connective ability in the portal space) was achieved [16,12]. A decrease in sinusoidal calibration is associated with an imbalance in vasoactive mediator production in sinusoids when exposed to L-NAME. Acute or chronic treatment with L-NAME results that are invasive due to the lack of NO to counteract the suppression of peptides, such as angiotensin-sin and endothelin that ultimately leads to hypertension. Cells that are important regulators of the sinusoidal capillary layer are very sensitive to their predecessors (Rockey 2001), and their contraction with vasoconstrictors can reduce the sinusoidal capillary space. These cells may be an important factor in the increase in intrahepatic resistance observed in portal hypertension. By agreement with studies, Dupuis et al. (2004) reported enhanced gene expression associated with the regulation of cell proliferation, extracellular matrix remodelling, and NO/cGMP signalling in aortic tissue of rats treated with L-NAME for 15 or 30 days [11,13].

4. CONCLUSION

Therefore, inducers and inhibitors have opposite effects on the activity of NOS in the ischemic liver microsomes. Benzonal makes the slow speed of nitrate reductase system, stimulates nitroxygenase system (eNOS), and cimetidine, on the contrary, that even more nitrate reductase system activates the speed system, inhibits eNOS nitroxylenes. The difference in activity of benzonal and cimetidine explain through what mechanisms can regulate the enzyme monooxygenase, thereby positively impact on pathological processes in the liver that is critical to its hypoxic conditions. At the present time in connection with the growth of liver disease and aggressive exposure to xenobiotic with induction and inhibitory action on the person, this problem acquires a special urgency and, of course, requires further study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard was written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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