Original Research Article

THE EFFECT OF INDUCERS AND INHIBITORS OF MONOOXYGENASE ON THE ACTIVITY NITRERGIC SYSTEM IN THE MICROSOMES IN THE ISCHEMIC LIVER

Abstract

In 62 experiments on white out bred rats male weighing 180-220g. Found that inducers and inhibitors of monooxygenase 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 of the daily introduction of the inducer of drug metabolism - benzonal 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.

Keywords: ischemia, liver, monooxygenases, NO-system, benzonal, cimetidine.

INTRODUCTION

An important aspect of modern drug therapy is a personalized medicine based on research and implementations in practical health care of medicines influencing on the system of biotransformation of xenobiotics in the liver (Sivkov et al., 2010; Archakov et al. 2008). The inducers and inhibitors of drug metabolism – regulating activity of the monooxygenase system (MOS) of the liver in this problem plays the key role (Kukes et al. 2007; Villeneuve et al. 2004). In the last decade, thanks to basic research in molecular biology and medicine found that in vascular endothelium the synthesis of nitric oxide (NO^{o}) 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 NO^{o}

(Minamiyama et al., 2001; Manuhina et al., 2000). A family of isoenzymes of NO-synthase (NOS) synthesize NO from L-arginine by three major isoforms – two constitutive (neuronal (nNOS) and endothelial (eNOS) and one inducible (iNOS) (Ivashkin et al., 2000). For the production of NO with the participation of NOS along with a variety of cofactors, substrates is an important arginine, oxygen and oxidized nicotinamide dinuceotid phosphate (NADPH) (Markov, 2005; Vinogradov et al., 2005). In pathological processes accompanied by hypoxia or ischemia, the role of NO- sinus mechanism is reduced and induced activity nitrate reductase systems (Reutov, 2000). It is now established that NOS and inactive nitrate reductase system (LDCs) is found in hepatocytes, endothelium of sinusoids, the Kupffer cells/macrophages (Habib and Ali, 2011), as well as in the endothelium of the portal vein and hepatic artery (Hirst and Robson, 2011; Jaeschke et al., 2001). The presence of NOS in hepatocytes suggests a correlation with the enzymes of the 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 liver pathological process.

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

MATERIAL AND METHODS

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

The study drugs were administered after restoration of blood flow to the liver. An inducer of drug metabolism benzonal was administered

intragastrically in the form of a 1% solution in 0.5% starch gel single dose of 50mg/kg for 1, 3 and 10 days in a row (2ml). Inhibitor of drug metabolism cimetidine also was injected intraperitoneally in a 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 homogenized 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 monooxigenase 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.

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, activity of monooxygenase and oxidoreductase of nitrooxygenase systems were recorded on computerized dual beam spectrophotometer UV-2100 (Ltd, China).

The content and activity of oxidoreductase was 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).

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 was carried out using correlation analysis Pearson (r). For comparison, samples were used Student's t-test. Data were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Benzonal and cimetidine after 1 night of their introduction had no significant effect on all studied parameters characterizing the activity of NOS in the liver microsomes postchemotherapy, compared to groups, which drugs are not injected, the corresponding term monitoring (1 day.) (Table 1). 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 the 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 microsomal system in the body, and cimetidine on the

contrary an even greater extent, potentiates the effects of damage to this system. When analyzing 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, reducing the 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 increasing the activity of eNOS and restore to control values the concentrations in the ischemic liver microsomes NO administered to animals of benzonal.

As follows from literature data, iNOS and ONO_2^- and NO are components of the expression system of nitrate reductase. Its gain during ischemia/hypoxia involves an increase in the cytotoxic compounds, including NO and ONO_2^- which block the active centers of cytochrome P450 in microsomes ischemic liver [11]. Cimetidine as follows from the data obtained, reinforces these processes in microsomes of animals with ischemic liver and suppresses NOS way. However, as shown by a number of researchers during ischemia/hypoxia blockade of 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 center 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^-.

CONCLUSION

Thus, inducers and inhibitors have opposite effects on the activity of NOS in the ischemic liver microsomes. Benzonal – slow speed 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.

Table 1. 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, Δ- P<0.05 compared to hypoxia of the corresponding period

REFERENCES

1. Sivkov A.S., Paukov S.V., Ruvinov Yu.V., Kukes I.V. Individual safety of pharmacotherapy in assessing the activity of cytochrome P-450 3A4 isoenzyme (CYP3A4). Klin.med. - 2010. - №2. - P. 61-67.
2. Archakov A.I., Lisitsa A.V., Petushkova N.A., Karuzina I.I. Cytochrome P-450, drug disease and personified medicine. Part I. Clin.Med.-2008.-No. 2.-S. 4-8.
3. Kukes V.G., Sychev D.A., Shikh E.V. The study of biotransformation of drugs is a way to increase the effectiveness and safety of pharmacotherapy. Doctor. - 2007. - №1. - P. 2-5.
4. Minamiyama Y., Jmaoka S., Takemura S. Escape from tolerance of organic Nitrite by induction of cytochrome P450.Free Radical Biology et Medicine. – 2001. – Vol. 31, №11. – P. 1498-1508.
5. Manukhina E.B., Malyshev I.Yu., Archipenko Yu.V. Nitric oxide in the cardiovascular system: a role in adaptive protection. Vestn. RAMS. -2000. -№4. -C.16-21.
6. Ivashkin V.T., Drapkina O.M. Nitric oxide in the regulation of the functional activity of physiological systems. *Rus. Jour. Gastroenter., hepat., coloproctol.* -2000. -№4. -P. 16-21.
7. Markov H.M. Molecular mechanisms of vascular endothelial dysfunction. Cardiology. -2005. -12. -P. 62-72.
8. Pokrovsky V.I., Vinogradov N.A. Nitric oxide, its physiological and pathophysiological properties. Ter.arch. -2005. -№1. -P. 82-87.
9. Reutov V.P. Medico-biological aspects of cycles of nitric oxide and superoxide anion radical. Herald of RAMS. -2000. -№4. -P.35-41.
10. Yakovlev S.V., Rudakova A.V. Model pharmacoeconomic studies in antimicrobial chemotherapy: the methodology for conducting and the need to take into account additional factors. Wedge. Pharmacol. and ter. -2004. -T.13, No.2. -P.27-31.

11. Minamiyama Y., Takemura S., Imaoka S., Funae Y., Tanimoto Y., Inoue M. Irreversible Inhibition of Cytochrome P450 by Nitric Oxide. J. Pharmacol. Exp. Ther.-1997. –Vol.283(3). –P.1479-1485.

12. Lyakhovich VV, Vavilin VA, Zenkov NK, Men'shchikova EB Activated oxygen metabolites in monooxygenase reactions. Bulletin SO RAMN. - 2005. - №4. - P. 7-12.

13. Villeneuve J.P., Pichette V. Cytochrome P-450 and liver diseases. Curr. Drug Metab. -2004. –Vol.5. –P.273-282.

14. Yasui, H., Hayashi S., Sakurai H. Possible involvement of singlet oxygen species as multiple oxidants in P450 catalytic reactions. Drug Metab. Pharmacokinet. -2005. –Vol.20. –P.1-13.

15. Habib S., Ali A. Biochemistry of Nitric Oxide. Ind. J. Clin. Biochem. – 2011. – Vol. 26, №1. – P. 3-17.

16. Hirst D.G., Robson T. Nitric oxide physiology and pathology. Methods. Mol. Biol. -2011. –Vol.704. –P.1-13.

17. Jaeschke H., Gores G.J., Cederbaum A.J., Hinson J.A., Pessayre D., Lemasters J.J. Mechanisms of Hepatotoxicity. Hepatology. -2001. –Vol.15. –P. 718-724.