COMPARATIVE EVALUATION OF THE EFFECTS OF α-TOCOPHEROL, S-ADENOSYL-METHIONINE AND NIKETHAMIDE ON THE LIVER MONOOXYGENASE, GLUCURO- AND GLUTATHIONE TRANSFERASE SYSTEMS IN RATS WITH BILE-DUCT LIGATION

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Introduction

Liver diseases and injuries of various etiologies are accompanied by impairment in the functions of the organ, especially its capacity to metabolize xenobiotics [22]. In particular, the stimulation of cholestasis in rats by bile-duct ligation or administration of α-naphthiolysothiocyonate has found out the decrease of the catalytic activity of cytochromes P450, 1C2, 2C1, 2C6, 2C11, 2E1, 3A2 and other isoforms as well as the levels of the corresponding proteins [10, 3, 21, 6]. Similar changes also have been observed in human beings with cholestatic liver cirrhoses [9]. In addition, the activities of glutathione S-transferases are decreased in parallel with the drop in the concentrations of liver and erythrocyte glutathione [14, 16, 20, 21]. Enhanced drug hepatotoxicity may be a consequence of the inhibition of the above enzyme systems and the disturbance in the biliary excretion of reactive xenobiotics and their metabolites. This has convincingly been demonstrated by the paracetamol, with the principal detoxication pathway being formation of glutathione conjugates [23, 27]. The above data show the significance of a search for the substances capable to normalize the hepatic xenobiotic metabolizing function in cholestatic disturbances of the liver.

We carried out a comparative study of the effects of α-tocopherol, S-adenosylmethionine and nikethamide on the liver monooxygenase, glucuro- and glutathione transferase systems in rats with cholestasis.

Materials and methods

The experiments were carried out with 53 male rats weighing 170-200 g. The animals were subjected to bile-duct ligation under ether anesthesia. α-Tocopherol (50 mg/kg), S-adenosylmethionine (100 mg/kg) or nikethamide (50 mg/kg, intraperitoneally, once a day, 7 days) were administered at the postoperative period. The control rats (with and without bile-duct ligation) received an equal volume of a 0.85% solution of sodium chloride. The animals were decapitated in 24 hours after the last injection of the substance (8 days after the bile-duct ligation). The microsomal fraction was assayed to determine the total concentration of protein, cytochromes b5 and P450, the rates of NADPH and NADH oxidation, amidopyrine and ethyl morphine demethylation, aniline hydroxylation as well as the activity of NADPH-cytochrome P-450 and NADH-cytochrome b5 oxidoreductases, UDP-glucuronyl- and glutathione S-transferase, UDP-glucose dehydrogenase by the methods described previously [5]. The concentration of reduced glutathione in whole blood was determined according to Ellman [7], whereas that of bilirubin and its fractions was measured by the method of Jendrashik et al. [13]. The extent of the disruption of the hepatocyte plasma membrane was assessed by the activity of blood serum alanine amino transferase (ALAT) determined according to Raitmon and Frenkel [13].

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Results and Discussion

It was found out that on the 9th day after bile-duct ligation the blood serum of rats receiving 0.85% solution of sodium chloride showed an increase in ALAT activity and more than 20-fold rise in bilirubin level attributed to both non-conjugated and conjugated fractions. Under these conditions the activity of the enzyme system of microsomal oxidation and xenobiotic glucuro- and glutathione conjugation was considerably inhibited. The concentration of cytochromes P450 and b_5, the rates of NADPH and NADH oxidation, amidopyrine and ethyl morphine demethylation as well as the activity of NADPH-cytochrome P-450 and NADPH-cytochrome b_5 reductases were decreased by 17-73% as compared with sham-operated animals. As it appeared cytochro-me P-450 was partly converted into inactive form, cytochrome P420. The activity of UDP-glucuronyltransferase was inhibited in parallel with decreasing biosynthesis of its substrate, UDP-glucuronic acid (UDP-glucose dehydrogenase activity). The function of membranous CNDB-glutathione S-transferase was diminished, whereas that of the cytosolic enzyme isoforms remained unchanged. The concentration of reduced glutathione was maintained at the control level (Table).

The administration of α-tocopherol (intra-peritoneally, 50 mg/kg, 7 days) to bile-duct ligated animals was accompanied by normalization of ALAT activity. However, hyperbilirubinemia was maintained at the initially high level. The rate of NADPH oxidation as well as the activity of UDP-glucose dehydrogenase, cytosolic CDNB-glutathione S-transferase was increased in comparison with the untreated bile-duct group (Table).

S-adenosylmethionine (intraperitoneally, 100 mg/kg, 7 days) normalized the rate of NADH oxidation, the activity of NADPH-cytochrome b_5 reductase, UDP-glucose dehydrogenase, UDP-glucuronyl- and microsomal glutathione transferases. The activity of the cytosolic isoforms of the latter enzyme even exceeded the corresponding values in the control

Table. Effect of 8-day intraperitoneal administration of α-tocopherol, nikethamide (50mg/kg) and S-adenosylmethionine (100 mg/kg) on the activity of the rat monooxygenase, glucuro- and glutathione transferase system after 8 days following bile-duct ligation.

	Sham-operation	Bile-duct ligation			
		0.85% NaCl	α-tocophe-rol	S-adenosy-lmethionine	Nike-thamide
Cytochrome P450, nmol/mg	0,72±0,04 _100_	0,25±0,08 _35*_ 136	0,43±0,04 _47*_ 136	0,20±0,02 _28*_ 80	0,20±0,02 _29*_ 84
Cytochrome b_5, nmol/mg	0,52±0,02 _100_	0,38±0,02 _73*_ 100	0,33±0,02 _64*_ 87	0,40±0,03 _77*_ 105	0,42±0,04 _81*_ 111
NADPH oxidation, nmol/min/mg	4,99±0,39 _100_	2,40±0,26 _48*_ 100	2,44±0,25 _49*_ 102	2,85±0,26 _57*_ 119	4,50±0,51 _90_ 188*
NADH oxidation nmol/min/mg	3,76±0,50 _100_	2,33±0,30 _62*_ 100	4,05±0,50 _108_ 174*	2,92±0,38 _78_ 125	3,46±0,41 _92_ 149*
NADPH cyt.P450 re-ductase, μmol/min/mg	0,23±0,01 _100_	0,19±0,01 _83*_ 100	0,19±0,01 _83*_ 100	0,19±0,01 _83*_ 100	0,19±0,02 _83*_ 100
NADH cyt. b_5 reduc-tase, μmol/min/mg	4,40±0,54 _100_	3,05±0,18 _69*_ 100	3,25±0,23 _74*_ 107	3,35±0,24 _76_ 110	3,05±0,28 _69*_ 100
Amidopyrine N-demethylase, nmol/min/mg	9,67±0,54 _100_	4,32±0,39 _45*_ 100	4,29±0,72 _44*_ 99	4,36±0,60 _45*_ 101	6,87±1,13 _71*_ 159*
Ethylmor-phine N-de-methylase, nmol/min/mg	10,46±1,09 _100_	2,79±0,36 _27*_ 100	3,12±0,53 _30*_ 105	2,12±0,17 _20*_ 76	4,95±0,85 _47*_ 177*
Aniline p-hydroxylase, nmol/min/mg	0,55±0,03 _100_	0,25±0,02 _46*_ 100	0,28±0,02 _51*_ 112	0,25±0,05 _46*_ 100	0,29±0,03 _53*_ 116
UDP-glucuronyltransferase, nmol/min/mg	7,11±0,75 _100_	5,11±0,61 _72*_ 100	5,54±0,45 _78_ 108	8,31±1,25 _117_ 163*	9,29±0,56 _131*_ 182*
UDP-glucose dehydro-genase, nmol/min/mg	11,46±0,74 _100_	7,28±0,50 _64*_ 100	8,85±0,48 _77*_ 122*	10,23±0,61 _89_ 169*	9,15±0,71 _80*_ 126*
Glutathione transferase: microsomes, μmol CDNB/min/mg	0,12±0,07 _100_	0,09±0,01 _69*_ 100	0,10±0,01 _81*_ 118	0,14±0,01 _117_ 170*	0,16±0,01 _133*_ 193*
cytosol, μmol CDNB/min/mg	0,77±0,04 _100_	0,71±0,03 _88_ 100	0,83±0,03 _103_ 118*	1,19±0,11 _147*_ 168*	1,27±0,07 _158*_ 180*
Reduced glutathione, mg%	42,45±1,34 _100_	38,19±0,93 _90_ 100	38,64±1,93 _91_ 100	35,02±2,02 _83*_ 92	36,29±1,89 _85*_ 95

Note. Above the line there is the percentage of changes as compared with sham-operated rats; under the line there is the percentage of changes as compared with untreated bile-duct – ligated rats.
* - P< 0.05

animals by 47-71%, whereas the level of reduced blood glutathione was decreased (Table).

Nikethamide exerted the most pronounced enzyme-activating effect. Its intraperitoneal administration at the dose of 50 mg/kg for 7 days activated nearly all the studied liver enzyme systems. This drug elevated the rates of NADPH and NADH oxidation, amidopyrine and ethyl morphine N-demethylation, the activity of UDP-glucuronyl-, microsomal and cytosolic glutathione transferases and UDP-glucose dehydrogenase by 26-93% as compared with the untreated bile-duct ligated rats. The ALAT activity and bilirubin level remained increased, whereas the content of reduced blood glutathione was decreased (Table).

The inhibition of the activity of the enzyme systems of microsomal oxidation and xenobiotic glutathione and glucuroconjugation in cholestasis seems to be mediated by high concentration of bile acids, especially their most hydrophobic forms. The phospholipase-like, detergent and prooxidant properties of cholates and their capacity of playing a role of cytochrome P450 substrates are involved into the mechanism of such an effect. The attack of membranous phospholipids by cholate impairs synthesis and assemblage of endoplasmic reticulum membranes. As a consequence, cytochrome P450 is not built into the membrane: it is either "washed" out of it or inactivated [3, 4]. Hydrophilic derivatives of bile acids causing dissociation of the components of membranous enzyme complexes and cytochrome P450 conversion into catalytically inactive form, cytochrome P420 also have detergent properties [2, 4, 10]. The ability of endogenous cholates to be oxidized by cytochrome P450 creates conditions for their competition with other substrates, which is accompanied by a decreased metabolic rate of many xenobiotics. Besides, the hydroxylation of the steroid nucleus of bile acids is also affected, leading to accumulation of their cytotoxic mono- and dihydroxy derivatives in hepatocytes [2, 4, 11, 17, 18].

The activation of lipid peroxidation (LPO) in cholestasis and the consequent decrease of the activity of the enzyme systems of xenobiotic biotransformation are realized by several mechanisms. On the one hand it is a decrease of the metabolic rate by glutathione S-transferase and an accumulation of 4-hydroxy-2,3-nonenal, a cytotoxic LPO product, in the cholestatic liver, and on the other hand, it is a depletion of the pool of endogenous antioxidants: glutathione, ubiquinones, tocopherols [14, 16, 19, 20, 21]. In this situation the main targets for the action of reactive lipid peroxidation compounds are enzyme complexes localized in membranes [14, 20, 21]. This suggestion is confirmed by our data on preferable inhibition of membranous enzymes, which is distinctly manifested in compare with the activity of glutathione S-transferases. We can similarly regard that the disturbed permeability of the hepatocyte plasma membrane caused "washing out" of ALAT from hepatocytes and increase of its activity in blood serum.

The other reasons of decreased activity of the enzyme systems of xenobiotic oxidation and conjugation can be a generalized damage of cytochrome P450 gene transcription, a high level of estradiol (endogenous inhibitor of cytochromes P450, IIC11 and IIIA2), disturbed energy metabolism and solubilisation of lysosome hydrolases [2, 6, 10].

The obtained data show that the membrane protective action of α-tocopherol is to a great extent expressed for the hepatocyte plasma membrane (as judged by ALAT activity) and is weakly manifested for endoplasmic reticulum membranes. These data confirmed by the study of Serbinova et al. [24] who have shown inefficiency of α-tocopherol (but not the other tocol, 2,2,5,7,8-pentamethylchromane) in the damage of cytochrome P450 by lipid peroxidation products. The mechanism of such a selective α-tocopherol effect on different cell membranes is still unclear.

The capacity of S-adenosylmethionine to activate the reactions of xenobiotics conjugation with glucuronic acid and glutathione in the liver of rats with bile-duct ligation may be due to its key role in transmethylation reactions, especially in the methylation of phosphatidyl ethanolamine to form phosphatidylcholine. The latter plays an important role in the function of membrane bound enzymes of xenobiotic biotransformation [8, 12]. The methylation involving S-adenosyl-methionine of 45sRNA protects the acid from the inactivatory effect of nuclease. This process is pivotal in the mechanism of action of inducers of enzymes of xenobiotic metabolism [26]. Moreover, the enzyme protective effect of S-adenosylmethionine observed in cholestasis may be due to antioxidant properties of the substance and its capacity to increase microsomal membrane fluidity [21]. The less pronounced enzyme protective effect of S-adenosylmethionine on the liver monooxygenase system in comparison with the action of UDP-glucuronyl- and glutathione S-transferase remains to be unclear. We may suggest that S-adenosylmethionine recovered slower the activity of the components of the cytochrome P450-containing complex due to their stronger inhibition.

The ability of nikethamide to increase the function of the xenobiotic microsomal oxidation, glucuro- and

glutathione conjugation enzyme systems, which is decreased in rats with bile-duct ligation, is determined by its enzyme activating and antioxidant properties [1, 15]. The decreased by S-adenosylmethionine and nikethamide endogenous pool of reduced glutathione in the blood of rats with cholestasis seems to result from their enhanced "expenditure" in the conjugation of the substances with glutathione because of the activation of glutathione S-transferases.

Therefore, our experimental results indicate inhibition of the activity of the membrane-bound enzyme systems of xenobiotic microsomal oxidation, glucuro- and glutathione conjugation as well as impairment of the integrity of hepatocyte plasma membranes and development of pronounced hyperbilirubinemia. The comparative analysis of the efficiency of the substances with different mechanisms of action has shown that although α-tocopherol does not keep the activity of the enzyme systems of xenobiotic microsomal oxidation, glucuro- and glutathione conjugation from inhibition, it substantially prevents damaging hepatocyte plasma membranes. S-adenosylmethionine activates the enzyme systems of xenobiotic glucuro- and glutathione conjugation while unchanging the function of the monooxygenase system. In spite of the persisting low levels of cytochromes b_5 and P450, nikethamide considerably increases the catalytic activity of microsomal oxidoreductases as well as intensity of xenobiotic conjugation with glucuronic acid and glutathione.

References

1. Abakumov G.Z., Legonkova L.F., Zavodnik L.B. // Vesti AN BSSR, Ser.Biol. Navuk. - 1990, № 5. – P. 107-109.
2. Bluger A.F., Mayore A.Ya., Gorstein E.S., Zaltsmane V.K. // Pat. Fisiol. – 1978 – Vol. 6. – P. 5 – 24.
3. Bluger A.F., Mayore A.Ya., Gorstein E.S. et al. // Progr. in Hepatology, Riga. - 1981. – Vol. 9. – P. 5- 24.
4. Bluger A.F., Mayore A.Ya. // Ibid. – 1982. – Vol. 10. – P. 12 – 34.
5. Bushma M.I., Legonkova L.F., Abakumov G.Z., Zavodnik L.B. // Voprosy Pitaniya (Problems in Nutrition). – 1994, № 6. – P. 12-15.
6. Chen J., Murray M., Liddle C. // Hepatology. – 1995. – Vol. 22, № 2. – P. 580 – 587.
7. Ellman G.L. // Arch. Biochem. Biophys. – 1959. – Vol. 82. – P. 70 – 77.
8. Falani C.N., Green M.D., Swain E., Tephly T.K. // Biochem. J. – 1986. – Vol. 238, № 1. – P. 65 – 73.
9. George J., Murray M., Buth K., Farrell G.C. // Hepatology. – 1995. – Vol. 21, № 1. – P. 120 – 128.
10. Gorstein E.S., Mayore A.Ya., Zaltsmane V.K. // In.: Enzymology of Pathologic Processes, Riga. – 1980. – P. 10 – 16.
11. Gorstein E.S., Mayore A.Ya., Makarova A.Ya. // In.: Experimental Pathology of the Liver, Riga. – 1983. – P. 63 – 69.
12. Natefi Y., Djavadi-Ohanince L. // The structural basis of membrane function. N.Y., AP. – 1976. – P. 409 – 427.
13. Kolb V.G., Kamyshnikov N.S. // Handbook on Clinical Chemistry. Minsk. – 1982. – P. 111- 115, 251 – 253.
14. Krahenbuhl S., Talos C., Lauterburd B.H., Reuchen. J. // Hepatology. – 1995. – Vol. 22, № 2. – P. 607 – 612.
15. Legonkova L.F, Bushma M.I., Lukienko P.I. // Bul. Experim. Biologii i Me-ditsiny. – 1990, № 12. – P. 633 – 635.
16. Leonarduzzi G., Pavola M., Muzio G. et al. Biochem. Biophys. Res. Commun. – 1995. Vol. 214, № 2. – P. 669 – 675.
17. Little J.M., Zimniak P., Raclominska A. et al. Hepatology. – 1991. – Vol. 14, № 1. – P. 690 - 695.
18. Lyakhovich V.V., Tsyrlov I.B. Structural aspects of monooxygenase biochemistry. Novosibirsk: Nauka. – 1978. – 231p.
19. Mayore A.Ya. Functional and morphological state of hepatocytes subcellular fractions in predominant types of liver injury in the clinic and experiment. Synopsis of the doctoral thesis. Moscow. – 1988.
20. Panozzo M.P., Basso D., Balint L. et al. Clin-Exp. Pharmacol. Physiol. – 1995. – P. 266 – 271.
21. Pastor A., Collado P., Almar M, Gonzales-Gallego J. J. Hepatology. – 1996. – Vol. 24, № 3. – P. 353 – 359.
22. Posokhova E.A. Experim. i Klinich. Farmakologya. – 1996. – Vol. 59, № 4. – P. 73 – 79.
23. Salas-Coll C.A., Torrealba-de-Ron A.T., Morales-de-Martinez A. et al. G-E-N. – 1995. – Vol. 49, № 1. – P. 42 – 49.
24. Serbinova E., Tyrin V., Stoytchev S., Kagan V. Acta Physiol.-Pharmacol. Bulg. – 1985. – Vol. 11, № 3. – P. 55 – 60.
25. Shefer S., Kren B.T., Salen G. et al. Hepatology. – 1995. – Vol. 22, № 4, pt. 1. – P. 1215 – 1221.
26. Smith S.J, Liu D.K, Leonard T.B. et al. Mol. Pharmacol. – 1976. – Vol. 12. – P. 820 – 831.
27. Tsyrcunov V.M., Puzynya P.V. Novosti gepatologii i meditsiny. – 1996, № 1. – P. 20 – 44.

Summary

On the 9th day after bile-duct ligation it was shown bilirubin and its fractions content in the rat blood serum increased as well as activity of alanine aminotransferase (ALAT). The activity of the enzyme systems of microsomal oxidation and xenobiotic glucuro- and glutathione conjugation was considerably elevated. α-Tocopherol (i.p., 50 mg/kg, 7 days) normalized the activity of ALAT and the rate of NADH oxidation and enhanced the function of cytosolic glutathione S-transferase. S-adenosylmethionine activated the reactions of xenobiotic conjugation with glucuronic acid and glutathione, whereas nikethamide (i.p., 50 mg/kg, 7 days) additionally stimulated microsomal oxidation in the liver.

The detergent, phospholipase-like and prooxidant properties of cholates seem to play a leading role in the mechanisms of the increase of ALAT activity and the inhibition of the enzyme systems of xenobiotic biotransformation in the liver of bile-duct –ligated rats. The protective effects of S-adenosylmethionine and nikethamide on the xenobiotic oxidation and conjugation are likely to be due to the acceleration of transmethylation by S-adenosylmethionine, as well as to the enzyme-stimulating effect and the antioxidant properties of nikethamide.