CONCENTRATIONS OF MITOCHONDRIAL AND MICROSOMAL CYTOCHROMES IN LIVER TISSUE IN EXPERIMENTAL DRUG-INDUCED FATTY DEGENERATION OF THE LIVER

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KEY WORDS: fatty degeneration of the liver; mitochondrial and microsomal cytochromes; resistance to hypoxia

Fatty degeneration of the liver is a disease with multiple etiology. The causes of this pathological state include not only diabetes, general obesity, and overindulgence in alcohol, but also various xenobiotics and drugs, including the tetracyclines.

The mechanism of action of the tetracycline antibiotics has been the subject of much research, and the principal causes of hepatotoxicity are considered to be metabolic processes such as protein synthesis [1], aerobic respiration and oxidative phosphorylation [14], hormonal regulation of fat and carbohydrate metabolism [13], and activation of lipid peroxidation [4]. However, the specificity of the triggering mechanisms leading to the development of fatty degeneration remains unknown. Research on isolated hepatocytes and mitochondria has revealed disturbances of mitochondrial oxidation of fatty acids [9] and of synthesis of mitochondrial apoproteins, which are responsible for the elimination of triglycerides from the liver [12]. A special role of the microsomal oxidation system in the pathogenesis of toxic hepatitis has been established [6].

The aim of this investigation was to assess the state of the systems of mitochondrial and microsomal oxidation in a tetracycline model of fatty degeneration of the liver. The existence of animals differing in individual sensitivity to hypoxia, which may play a role of pathogenetic factor in the development of fatty degeneration of the liver, determined the choice of rats with low and high resistance to hypoxia as the test object.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats (180-220 g), kept on the standard animal house diet. Fatty degeneration of the liver was induced with tetracycline hydrochloride (TC), which was administered in a dose of 500 mg/kg in 1% starch mucilage by the intragastric route daily for 5 days. Control animals received 1% starch mucilage alone under the same conditions [3].

The rats were divided initially on the basis of their resistance to hypobaric hypoxia, into those with low (LR) and high (HR) resistance [11]. The reaction of the animals to acute hypoxia was assessed by the method generally adopted in the writers' laboratory [7].

The liver of the animals, anesthetized with ether, was perfused with physiological saline, after which a homogenate was prepared in Hanks' solution with glucose.

Alanine aminotransferase (ALAT) activity [8], the cytochrome P-450 level [10], and the content of mitochondrial cytochromes b, c^+, c_1, and *a* [2] were determined in the liver homogenate. The total lipid content in the liver homogenate was determined with the aid of standard kits ("Lachema," Czechoslovakia).

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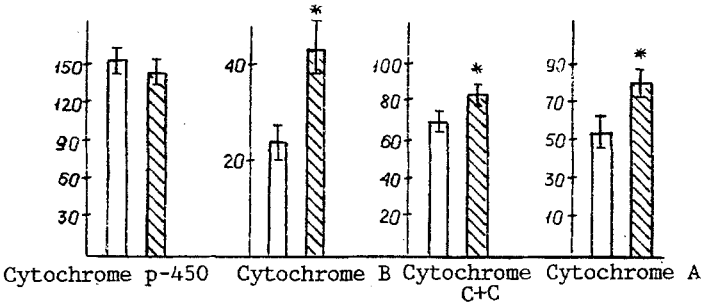

Fig. 1

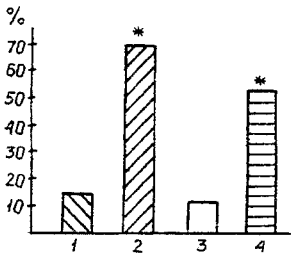

Fig. 2

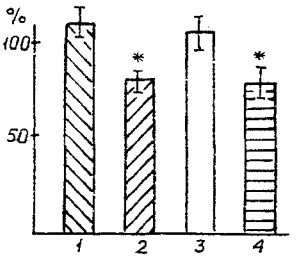

Fig. 3

Fig. 1. Content of cytochromes in liver tissue of HR and LR rats (in nmoles $\cdot g^{-1}$ dry weight). Unshaded columns – HR, obliquely shaded – LR. *$p < 0.05$.

Fig. 2. Degree of development of fatty degeneration of the liver in HR and LR rats in response to administration of tetracycline hydrochloride (in percent): 1) HR control; 2) HR experiment; 3) LR control; 4) LR experiment. *$p < 0.05$ for comparison of two groups of animals.

Fig. 3. Alanine-aminotransferase activity in liver tissue of HR and LR rats in response to administration of tetracycline hydrochloride (in moles NADH $\cdot min^{-1}g^{-1}$ dry weight). Legend as to Fig. 2.

For the morphologic control the liver tissue was stained with Oil Red. The degree of development of fatty degeneration was assessed on the basis of the volume of fat in the hepatocytes, expressed as a percentage of the total volume of the cell (100%).

EXPERIMENTAL RESULTS

Determination of the content of mitochondrial cytochromes in the liver tissue of the control HR and LR animals revealed a significant difference between them. The content of cytochromes b, c^+, c_1, and *a* in the liver of LR rats was significantly higher than in that of HR rats. With respect to the cytochrome P-450 level, there was no difference between the HR and LR rats (Fig. 1). Histologic investigations showed that under normal conditions a small quantity of lipid inclusions was found in the HR rats more often than in LR (Fig. 2). Similar results were obtained when the total lipid content of the liver tissue was determined. Initially, therefore, there were significant differences between the HR and LR rats with respect to content of mitochondrial, but not of microsomal cytochromes in the liver and the content of intracellular fat.

Administration of TC in a dose of 500 mg/kg for 5 days led to intensive accumulation of fat in the hepatocytes, its volume amounting to about 70% of the total volume of the cell in HR and about 55-60% in LR rats (Fig. 2). Thus the tetracycline model of fatty degeneration of the liver led in fact to a significant increase in the accumulation of fat in the liver, more so in the case of HR than of LR rats. Processes of cytolysis characteristic of this

TABLE 1. Level of Cytochromes in Tissues of Rats with High and Low Resistance to Hypoxia in Response to Administration of Tetracycline Hydrochloride (500 mg/kg, 5 days) (M ± m, n = 6)

Parameters	HR				LR			
	control		experiment		control		experiment	
	nmoles·g^{-1}	%	nmoles·g^{-1}	%	nmoles·g^{-1}	%	nmoles·g^{-1}	%
Cytochrome P = 450	152.1±7.5	100	109.5±13.5	72*	146.9±6.9	100	110.5±9.1	75*
Cytochrome b	22.96±3.46	100	22.63±1.78	99	43.52±5.43	100	30.48±4.71	70*
Cytochrome c+, c_1	67.65±3.43	100	62.33±3.07	92	84.01±2.75	100	70.85±2.66	84*
Cytochrome a	53.48±6.05	100	38.88±2.28	73*	79.17±7.23	100	54.44±5.59	69*

Legend. *$p < 0.05$.

pathology, expressed as a decrease in aminotransferase activity of the liver tissue of the HR and LR rats by 26-28%, were intensified under these circumstances (Fig. 3).

On the 5th day of experimental fatty degeneration of the liver a roughly equal decrease in the content of cytochrome P-450 was found in the liver of the HR and LR rats by 28 and 25% respectively, evidence that the system of microsomal oxidation is involved in this pathological process (Table 1). Our results differ from those found in [5], in which tetracycline in high doses was found to have no effect on the cytochrome P-450 content.

We also found a significant decrease in the content of cytochromes c^+, c_1, and *a* and a small (not significant) decrease in cytochrome b in the liver of the LR rats. Unlike the LR, in the liver of the HR rats a significant decrease was found only for cytochrome *a* (Table 1).

The results are evidence of disturbance of the function of the respiratory chain of the hepatocytes in tetracycline-induced fatty degeneration of the liver; the degree of disturbance, moreover, may be linked with the initial state of the mitochondrial energy-forming system. An inhibitory action of TC was found on the microsomal oxidation system, independent of the individual sensitivity of the rats to hypoxia, was discovered.

REFERENCES

1. A. Laskin, Mechanism of Action of Antibiotics [Russian translation], Moscow (1969), pp. 313-337.
2. E. N. Mokhova and I. V. Zhigacheva, Mitochondria: Accumulation of Energy and Regulation of Enzymic Processes [in Russian], Moscow (1977), pp. 139-141.
3. L. A. Porokhnyak, "Pharmacologic correction of toxic liver damage," Author's Abstract of Dissertation for the Degree of Doctor of Medical Sciences, Khar'kov (1988).
4. N. P. Skakun and I. Yu. Vysotskii, Antibiotiki, No. 3, 223 (1984).
5. N. P. Skakun and A. N. Oleinik, Vrach. Delo, No. 11, 91 (1984).
6. I. S. Chekman and A. I. Grinevich, Farmakol. Toksikol., No. 1, 119 (1984).
7. G. N. Chernobaeva and L. D. Luk'yanova, Pharmacologic Correction of Hypoxic States [in Russian], Moscow (1989), p. 160.
8. H. U. Bergmeyer, Methods in Enzymatic Analysis, 3rd Edition, Weinheim (1974).
9. E. Freneaus, G. Labre, P. Letteron, et al., Hepatology, **8**, 1056 (1988).
10. D. P. Jones et al., J. Biol. Chem., **255**, 2383 (1980).
11. T. P. Singer, E. B. Kearney, and N. S. Kenney, Adv. Enzymol., **37**, 189 (1973).
12. H. I. Swarz, Med. Chir. Dig., **5**, 323 (1976).
13. E. G. Trams, Antibiot. Med., **1**, 677 (1955).
14. H. I. Zimmerman and F. Humoller, Am. J. Physiol., **175**, 468 (1953).