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Rat Model of Acute Tetracycline Hepatosis and Its Dynamic Predictors

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It is demonstrated that intraperitoneal administration of tetracycline hydrochloride in a dose of 125 mg/kg leads to the development of acute fatty hepatosis in rats within a 24-h period, by which time the maximum accumulation of lipids and triacylglycerides is observed in the liver. In addition, a direct dependence is established between the severity of fatty hepatosis and a decrease in the cytochrome P-450 content. The cytochrome P-450 content is a dynamic predictor of tetracycline fatty hepatosis.

Key Words: *tetracycline fatty hepatosis; triacylglycerides; cytochrome P-450*

The model of drug-induced fatty hepatosis obtained by intraperitoneal administration of tetracycline hydrochloride (TC) has found wide application in experimental studies [2,5,6]. However, published data indicate that the blood content of TC varies considerably; consequently, there are considerable individual variations of lipid accumulation in the liver due to the different absorbance of TC from the alimentary canal. The induction of fatty hepatosis by intravenous administration of TC solution has been described [8,9]. Prognostic criteria of the development of this pathology have not been established.

The aim of this study was to develop a model of acute tetracycline fatty hepatosis induced by intraperitoneal administration of the preparation and to define the prognostic criteria for assessing the severity and dynamics of the pathology. Lipid metabolism and microsomal oxidation at different times after administration of various TC doses were studied.

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MATERIALS AND METHODS

Experiments were performed on outbred male rats weighing 180-220 g maintained on the standard vivarium diet. Fatty hepatosis was induced by intraperitoneal administration of 4 ml/100 g TC (Serva). The antibiotic was dissolved in normal saline (pH 8.9) and injected in doses of 50, 125, 250, and 330 mg/kg. Biochemical studies were performed 1, 2, 3, 6, 12, 24, 48, 72, and 144 h after TC administration. Control animals received equal volumes of normal saline. The liver was perfused with normal saline under ether anesthesia, and homogenate in normal saline (1:10) was then prepared.

The degree of fatty hepatosis was assessed by the content of total lipids and triacylglycerides in the liver homogenate and blood plasma with the use of standard Lachema kits.

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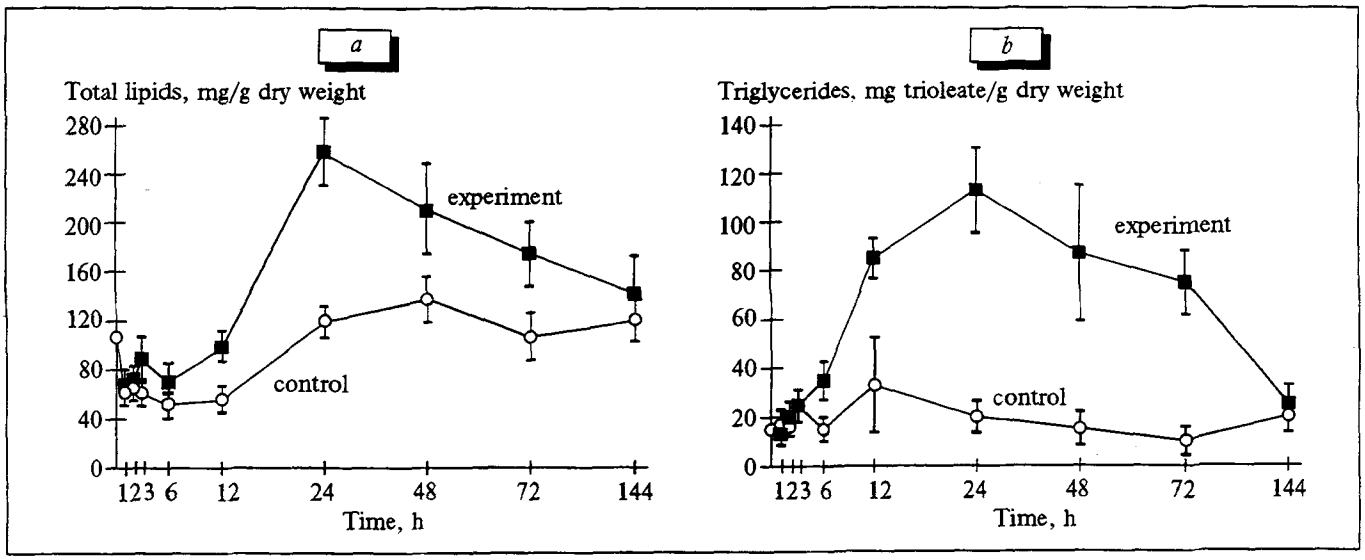

Fig. 1. Dynamics of total lipids (*a*) and triacylglycerides (*b*) in rat liver after a single administration of 125 mg/kg TC.

Hepatocyte integrity was estimated by determining the activity of alanine aminotransferase (AlAT) in the blood and liver homogenate [7].

Microsomal oxidation in the liver homogenate was estimated by the cytochrome P-450 content in the liver homogenate [10].

RESULTS

Intraperitoneal administration of TC increased the total lipid and triacylglyceride contents in rat liver homogenate 24 h after TC administration in a dose-dependent manner. Changes in the triacylglyceride content were more pronounced (Table 1).

In a dose of 50 mg/kg TC induced only a slight increase in the lipid metabolism parameters in the liver. The plasma AlAT activity increased and the cytochrome P-450 content in the liver decreased insignificantly.

At 125 mg/kg, the total lipid and triacylglyceride contents in the liver increased 47% and 5.5-fold, respectively. The plasma AlAT activity increased more than 2-fold. The cytochrome P-450 content in the liver decreased 33%.

Thus, a single intraperitoneal injection of TC in a dose of 125 mg/kg induced fatty hepatosis within a 24-h period, the triacylglyceride content in the liver being the most informative predictor of the pathology. The development of fatty hepatosis under these conditions was accompanied by the release of AlAT from hepatocytes, indicating plasma membrane disintegration, and a decrease in the cytochrome P-450 content.

At 250 mg/kg TC, the total lipid content increased 2.2-fold and the triacylglyceride content 15.8-fold. The cytochrome P-450 content in the liver decreased 46%. However, the AlAT activity was not higher but even somewhat lower than in the control. This decrease in AlAT activity indicated that the hepatocyte plasma membranes had not normalized but rather that there were marked alterations in hepatocyte structure. A similar dynamics of enzyme activity in fatty hepatosis has been described for plasma lactate dehydrogenase [4]. It was suggested [4] that an increase in the blood lactate dehydrogenase activity under the action of low doses of the xenobiotic is due to alterations in the permeability of liver cell mem-

TABLE 1. Some Biochemical Parameters of Rat Liver Homogenate and Serum 24 h after Intraperitoneal Administration of TC in Various Doses ($M \pm m$)

TC dose, mg/kg	Total lipids, mg/g dry weight	Triacylglycerides, mg trioleate/g dry weight	Activity of AlAT, nmol NADH/min×ml	Cytochrome P-450, nmol/g dry weight
–	84.25±2.55 (100)	8.84±0.37 (100)	9.01±1.08 (100)	96.27±17.79 (100)
50	85.15±3.45 (101)	12.30±1.60 (139)	13.75±1.65 (153)	72.75±4.28 (76)
125	124.10±7.20 (147)	48.25±4.25 (546)**	20.90±2.51 (232)	63.79±5.09 (67)
250	188.90±36.70 (224)	139.70±0.50 (1579)**	2.16±0.65 (24)	51.05±4.85 (54)*

Note. One asterisk indicates $p \leq 0.05$, two asterisks $p \leq 0.01$, percentage are given in parentheses.

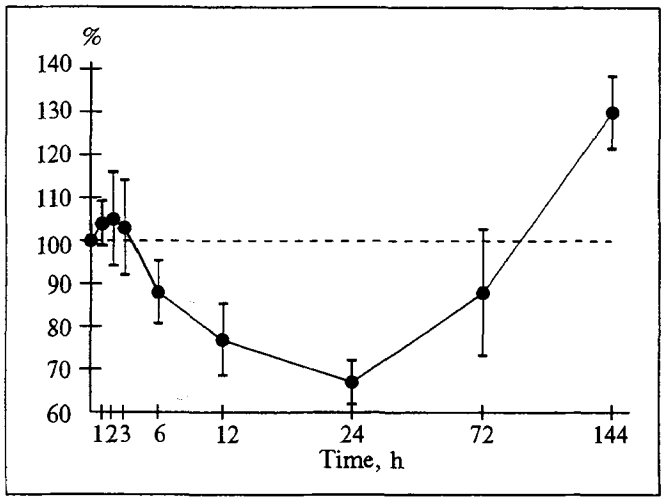

Fig. 2. Dynamics of cytochrome P-450 content in rat liver homogenate after a single administration of 125 mg/kg TC.

branes and release of the enzyme into the bloodstream, while an increase in the dose of the chemical agent causes more pronounced metabolic and structural alterations in hepatocytes, resulting in a decrease in the blood lactate dehydrogenase activity.

Thus, TC in a dose of 250 mg/kg induced severe fatty hepatosis accompanied by pronounced alterations in liver cell metabolism.

Based on the findings indicating that there is no statistically significant development of fatty hepatosis after administration of 50 mg/kg TC and that at 250 mg/kg TC provokes pronounced metabolic disorders, we decided that the use of these doses in further investigations was not warranted. The TC dose of 125 mg/kg, which induced fatty hepatosis against the background of a significant elevation of triglyceride concentration and relatively minor alterations in cell membrane permeability and cytochrome P-450 content was chosen as the working dose for the modeling of fatty hepatosis.

Investigation of the dynamics of disorders developing after a single injection of TC in a dose of 125 mg/kg showed that the maximum increase in the total lipid and triacylglyceride content occurs 24 h after TC administration (Fig. 1, *a, b*). The maximum increase in the AlAT activity in the blood and the maximum decrease in the enzyme activity in the liver (100 and 26%, respectively) were also observed after 24 h. The cytochrome P-450 content in the liver changed in a similar manner (Fig. 2). The control values for the total lipid and triacylglyceride content were restored 6 days after TC administration. This was accompanied by a gradual restoration of the cytochrome P-450 content. It is noteworthy that by the time the lipid metabolism parameters were normalized (Fig. 2), lipids were elevated compared with the control level. This indicates that in the dynamics of fatty hepatosis the cytochrome P-450 content can be regarded not only as a parameter of cytolytic processes in the liver [1], but also as an integral index of the functional status of the monoxygenase system.

Our findings provide grounds for using intraperitoneal administration of 125 mg/kg TC to obtain a model of drug-induced fatty hepatosis within a 24-h period.

In this model, not only the lipid metabolism parameters (total lipids and triacylglyceride content) but also the cytochrome P-450 content in the liver can be used as dynamic predictors of fatty hepatosis.

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