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A Method for Monitoring Toxic Damage to the Liver, Based on Measurement of Total Water and Its Magnetic Relaxation Characteristics

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Acute poisoning of rats with the hepatotropic poison tetrachloromethane was accompanied by increases in the liver content of total water and its spin-lattice (T_1) and spin-spin (T_2) relaxation times and by a decrease in the T_1/T_2 ratio, with reversal of the correlation between T_1 and T_2. The antihypoxic agent sodium γ-oxybutyrate normalized water metabolism in the liver almost completely. It is concluded that total water content and T_1 are the more informative parameters for monitoring both toxic liver damage and the efficacy of its pharmacological correction.

Key Words: *liver; tetrachloromethane; water; magnetic relaxation time*

Since disturbances of water-electrolyte balance are known to play an important role in the pathogenesis of many diseases caused by toxic chemicals [6], we made an attempt to diagnose toxic liver disease and monitor the efficacy of its pharmacological correction by analyzing parameters of water metabolism in an animal model of acute tetrachloromethane (TCM) poisoning. This particular model was chosen because its pathogenetic mechanisms and physiological, biochemical, and morphological characteristics are well known [8,11,13,15] and because of evidence that there may be a water imbalance in the liver damaged by TCM [5,12].

MATERIALS AND METHODS

A total of 32 random-bred male white rats weighing 180-210 g were used. Of these, 22 were injected with

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TABLE 1. Parameters of Liver Water Metabolism in the Three Groups of Rats

Parameter	Control (intact) rats	Rats poisoned with TCM	Rats poisoned with TCM and given SGOB
Total water, %	100±2.0	104.9±2.3*	101.2±1.3**
T_1	284.6±3.7	345.4±2.6*	331.1±4.1**
T_2	38.8±0.5	52.8±0.7*	52.2±0.7
T_1/T_2	7.35±0.09	6.56±0.13*	6.34±0.1
II	-	0.898	0.735
Correlation coefficient, T_1-T_2	0.705	-0.300	0.192
b	0.070	-0.101	0.034

Note. $p<0.05$: *in comparison with intact rats; **in comparison with TCM–poisoned rats.

TCM (50% solution in oil) subcutaneously at 3.2 g/kg body weight, while the remaining 10 rats served as controls; 11 of the 22 test rats received sodium γ-oxybutyrate (SGOB) intramuscularly (20% solution) at 100 mg/kg 12 h before TCM injection and in the same dose 12 h after it. In all three groups, rats were sacrificed 24 h after TCM injection (16-18 h after the last feeding).

The percent content of total water in liver specimens (from the left lobe) was calculated by the formula: $TW=(W_w-W_d)/W_w\times100\%$, where TW is the total water content, W_w is the wet weight of the specimen, and W_d is the weight of the same specimen after being dried in an incubator at 60°C for 5 days. All samples were weighed on an analytical balance. The magnetic relaxation parameters T_1 and T_2 (spin-lattice and spin-spin relaxation times, respectively) were recorded on a Minispec PC-120 instrument (Bruker) at an operating frequency of 20 MHz (at 30±1°C). For the determination of T_1, we used two-pulse 180°-t-90° sequences on condition that $T_1>>T_2$; T_2 was determined according to Karr and Parsel. On the decay curve, 150 points were recorded in sequence (for T_2). The resultant curve was obtained by averaging 25 point superpositions. The significance of differences between the test and control results was estimated using Strelkov's statistical tables [9]. For a generalized quantification of changes in the parameters under study, an integral index (*II*) was computed by the formula:

$$II=\{(|X_t-X_c|/X_c)+(|Y_t-Y_c|/Y_c)+...\}^{1/2},$$

where X_t and Y_t and X_c and Y_c are parameters for the test and control groups, respectively. Correlation and regression analyses were carried out to establish and quantify the functional relationships between the parameters [10]. The correlation coefficients and parameters were calculated on an ACBT-40-30 computer using designated software [7].

RESULTS

Rats injected with TCM developed a typical picture of acute poisoning manifested in hypodynamia, bloody nasal discharge, and ruffling of the hair coat. As shown in Table 1, the intoxication led to increases of the total water content in the liver and of T_1 and T_2 and to a decrease in the T_1/T_2 ratio, with reversal of the correlation between T_1 and T_2 from positive (0.705 in the control) to negative (-0.3). The nature of this functional relationship also changed, as is indicated by the altered parameter b in the linear regression equation $y=a+b\times x$, where $y=T_2$ and $x=T_1$.

SGOB administration led to an almost complete normalization of liver water metabolism in the TCM-poisoned rats: the content of total water and T_1 decreased, the positive correlation between T_1-T_2 was partially restored, and the value of the integral index (which sums up deviations in all the parameters under study) fell.

Acute TCM poisoning thus markedly impairs water metabolism in the liver, this being manifested by an elevated water level in this organ and by decreased structuredness and increased mobility of the water (as is evidenced by shifts in T_1 and T_2). Data reported in the literature suggest that important factors in the pathogenesis of this toxic edema include impaired microcirculation and capillary permeability [4], hypoxia and associated metabolic acidosis [2], altered interaction of water molecules with intracellular and extracellular structures [1], lowered levels of plasma proteins, and a distorted spectrum of these proteins. A comparative analysis of these data and the results of the present study indicates that total water content and levels of T_1 protons are very informative indicators of water metabolism in the liver. It should be noted, however, that the changes in water metabolism described above for the liver of TCM-poisoned animals appear to be nonspecific, for similar changes occur in other internal organs (as well as in skeletal muscle) in a number of pathological conditions including pneumonia, burn disease, and tumor growth [3,14,16]. Hence the use of the method proposed here for evaluating the degree of toxic damage to the liver and the efficacy of its pharmacological correction may conceivably be extended to disease states other than those caused by chemicals.

The results of the present study warrant the conclusion that since TCM poisoning is accompanied by consistent changes in liver water metabolism, the parameters of water metabolism, primarily total water content and T_1, may be used to evaluate both the severity of toxic damage sustained by the liver and the efficacy of its treatment by drugs.

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