EXPERIMENTAL BIOLOGY

Changes in Liver Structure in Experimental CCl_4-Induced Hepatitis during Different Phases of Circumannual Cycle

E. F. Levitskii, E. S. Shilkina, and L. R. Mustafina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 149, No. 5, pp. 581-583, May, 2010
Original article submitted March 29, 2009

Changes in liver structure in experimental CCl_4-induced hepatitis depend on the phase of the circumannual cycle. Pronounced disorders in the liver with a model of toxic hepatitis were observed in winter and fall and minor changes were recorded in summer. In spring, the liver was characterized by higher adaptation and compensatory potential, its structural status spontaneously returned to normal on day 4 after the last injection of CCl_4.

Key Words: *biorhythms; toxic hepatitis; experiment*

The structure and function should be evaluated with consideration for the circumannual cycle phases, because the circumannual biorhythms are a hereditary fixed cyclic morphofunctional restructuring of the organism corresponding to cyclic changes in the environment [2].

We evaluated the changes in the structure of the liver with a model of CCl_4-induced hepatitis during different phases of the circumannual cycle.

MATERIALS AND METHODS

The effects of toxic CCl_4-induced hepatitis on microstructure of the liver tissue and rates of the development of destructive and compensatory adaptive processes were studied on 224 male Wistar rats (250-400 g). Experimental animals were handled in accordance with the principles of the Helsinki Declaration, Regulations of Studies on Experimental Animals (Order No. 755 of the Ministry of Health of the USSR of August 12, 1987), and Federal Law of the Russian Federation of January 1, 1997 "On Animal Protection from Cruel Handling".

The studies were carried during the same hours of the day (from 9.00 to 11.00) in order to rule out the effects of circadian fluctuations in functional parameters of the liver on experimental results. No experiments were carried out on the days with drastic weather fluctuations. Experimental animals were kept on standard rations and regimens.

The studies were carried out during different phases of the circumannual cycle in accordance with the weather and climatic conditions of Tomsk: in winter (from December 4 to February 13), summer (from May 28 to July 22), spring (from March 21 to May 15), and fall (from September 10 to November 1). The total group of animals was divided at random into two subgroups during each phase: intact animals (n=24; basal values) and animals with experimental CCl_4-induced hepatitis (n=32).

Toxic hepatitis model was modeled by 3 subcutaneous injections (at 4-day intervals) of 50% oil solution of CCl_4 (0.45 ml/100 g). The rats were decapitated on days 4, 9, 14, and 44 (for evaluation of remote results) after the last injection of CCl_4, 6 controls and 8 experimental animals per period.

Tomsk Institute of Balneology and Physiotherapy, Federal Medicobiological Agency, Russia. ***Address for correspondence:*** glualena@yandex.ru. E. S. Shilkina

0007-4888/10/1495-0647

Fragments of the liver fixed in ethanol:formalin (9:1) and embedded in paraffin by the standard method were studied. The sections (4-6 μ) were stained with hematoxylin and eosin. Micropreparations were examined and photographs were taken under a Micromed-1 microscope at ×200 and ×400.

The morphology of the liver was evaluated by the following signs: degeneration (granular, fatty), counts of binuclear cells, and portocentral necrosis (PCN). The severity of degenerative changes and binuclear cell counts were scored: 0: no sign; 1: slight manifestation of the sign (in just few hepatocytes in few fields of view); 2: moderate manifestation of the sign (in 50% of cells in one visual field in all fields of view examined); and 3: intense manifestation of the sign (in more than 90% cells in all examined fields of view).

Inflammatory changes were evaluated similarly: 0 points: no sign; 1: slight manifestation (up to 20 inflammatory infiltration cells located focally in one visual field or perivascularly); 2: moderate manifestation (small foci in several fields of view or large foci with more than 20 inflammatory infiltration cells); and 3: intense manifestation of the sign (seen in all examined fields of view perivascularly and in the stroma).

The results were processed by standard methods of mathematical statistics using nonparametric Mann–Whitney test for independent sampling [1]. Statistical significance was evaluated for the level of $p<0.05$.

RESULTS

In winter, the most pronounced structural changes in the liver parenchyma were observed on day 4 after the last injection of CCl_4 in comparison with the control (Table 1). These shifts were characterized by PCN ($p<0.001$) with manifest lymphomonocytic infiltration of necrotic foci ($p<0.05$). Medium- and large-droplet fatty degeneration of an appreciable part of hepato-

TABLE 1. Circumannual Dynamics of Liver Structure in Experimental Hepatitis (*M*±*m*, Score)

Series	Day of experiment	PCN	Fatty degeneration	Granular degeneration	Inflammatory infiltration	Binuclear cells
Intact	4	0	0	0	0	0
	9	0	0	0	0	0.25±0.07
	14	0	0	0	0	0
	44	0	0	0	0	0
Hepatitis						
spring	4	0	0	0	0	0
	9	0	0	0	0	0.50±0.29
	14	0	0	0	0	0
	44	0	0	0	0	0
summer	4	0	0	0	0	0
	9	2.50±0.29***	0	0	0	1.25±0.41*
	14	1.50±0.29*	0	0.75±0.25*	0	0
	44	0	0	0	0	0
fall	4	1.75±0.25**	2.50±0.58***	0	1.50±0.09**	0
	9	0	0	0	0	0.33±0.25
	14	0	0	0	0	0
	44	0	0	0	0	0
winter	4	1.25±0.25***	3.00±0.29***	0	2.25±0.50*	0
	9	0	0.25±0.25	2.50±0.25*	2.00±0.33*	0.50±0.29
	14	0	0	0	0	0
	44	0	0	0.38±0.29	0	0

Note. *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared to intact animals.

cytes was found in the pericentral zone ($p<0.001$). On day 9, signs of moderate granular degeneration of hepatocytes in the peripheral parts of the hepatic lobe and portocentral infiltration were seen ($p<0.05$). No appreciable changes in comparison with the intact liver were detected during later periods. The above morphological changes indicate the predominating destructive processes during the early period after the last injection of CCl_4.

In spring, a high level of spontaneous regeneration of the liver was observed, shown by the absence of significant differences in comparison with intact subgroup.

In summer, significant destructive changes in the liver parenchyma were seen on day 9 after the last injection of CCl_4. Pronounced PCN ($p<0.001$) was paralleled by a significant increase in the level of binuclear cells ($p<0.05$). The severity of necrotic changes decreased on day 14 ($p<0.05$). Slight granular degeneration was detected in the hepatocytes of the peripheral lobular zones ($p<0.05$). No appreciable differences in the liver morphology in comparison with the control were detected during the delayed period.

In fall, pronounced PCN surrounded by lymphomonocytic infiltration ($p<0.01$) were seen in the liver on day 4 after CCl_4 treatment. Small and large intracellular fatty droplets ($p<0.001$) were seen in the pericentral zone of the hepatic lobes. Later, the morphology of the liver virtually did not differ from that in intact animals.

Manifest structural disorders were observed in toxic hepatitis in winter and fall, slight ones in summer. In spring, the liver was characterized by a high adaptive and compensatory potential, because spontaneous normalization of its structure was seen on day 4 after the last injection of CCl_4.

Hence, liver structure and function and intensity of adaptive compensatory and regeneratory processes depend on the circumannual cycle phase.

REFERENCES

1. V. Borovikov, *STATISTICA: The Art of Computer-Aided Analysis of the Data. For Professionals* [in Russian], St. Petersburg (2001).
2. F. I. Komarov and S. I. Rapoport, *Chronobiology and Chronomedicine* [in Russian], Moscow (2000).