Informational and Morphometric Characterization of Dog Liver Acini in Systemic Endotoxemia

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Systemic endotoxemia, a pathological state developing as a result of endogenous intoxication caused by peritonitis, pancreonecrosis, gangrene, etc., leads to liver failure [7,9]. Although there is quite a large body of evidence on the biochemical status of the liver, the information regarding hepatic morphology is scanty and descriptive, with no account taken of the regional heterogeneity of acinus structure [3,4,12].

Here we present a morphometric characterization of dog liver acini during systemic endotoxemia with subsequent information analysis.

MATERIALS AND METHODS

Experiments were performed on liver bioptates obtained from mongrel dogs (n=12) weighing 13-18 kg. The dogs were intravenously injected with *E. coli* lipopolysaccharide (LPS) in a dose of 2 mg/kg. Liver biopsy was done prior to (control) and 1, 3, 5, and 7 h after the injection. The bioptates were fixed in 10% neutral formalin and embedded in paraffin. Microtome sections were stained with hematoxylin and eosin. The areas occupied by the nuclei and cytoplasm of hepatocytes in zones I and III of the liver acini, as well as the area and perimeter of the sinusoid vessels were measured using a Microvideomat analyzer (Opton, Germany). On the basis of these measurements the nucleus-cytoplasm ratio for hepatocytes and shape factor for sinusoid vessels were calculated. Information entropy (H), relative entropy (h), redundance (R), and organization (O) were calculated from conventional formulas [1]:

$$H=-\sum p_i \log_2 p_i;\ h=H/H_{max};$$

$$R=(1-H/H_{max})\times 100\%=(1-h)\times 100\%;$$

$$O=H_{max}-X,$$

where H_{max} is the maximum entropy of the system equal to $\log_2 3=1.585$. Equivocation (D), a parameter employed as a quantitative criterion of structural disorganization of a system, was calculated from the following formula:

$$D=R_{contr}-R_{path}=(H_{path}-H_{contr})/H_{max}.$$

RESULTS

Morphometric study of hepatic tissue in the control group revealed structural heterogeneity of acini (Table 1), which agrees with the literature data [5,11]. In zone I, the area occupied by hepatocytes is 5.6% greater compared with that in zone III (p>0.05), since periportal hepatocytes have larger nuclei (16.1%, p<0.05) and more cytoplasm (3.6%, p>0.05). The area and perimeter of the sinusoid vessels were greater in zone III: 15.5 and 21.9%,

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TABLE 1. Morphometric Characterization of Liver Acini in Endotoxemia ($M \pm m$)

Parameter	Acinus zone	Control	Duration of intoxication, h			
			1	3	5	7
Area occupied by hepatocyte nuclei (S_n), arb. units	I	14.26±0.89	12.70±0.67*	13.50±0.51*	10.51±0.43	8.10±0.38
	III	12.28±0.58	11.31±0.46*	11.57±0.46*	9.82±0.37	9.71±0.38
Area occupied by hepatocyte cytoplasm (Sc), arb. units	I	60.40±3.60	56.11±2.72*	57.05±2.91*	59.32±3.11*	56.65±2.78*
	III	58.44±3.11	54.93±2.54*	53.20±2.16	50.39±2.10	46.97±1.97
Nucleus–cytoplasm ratio (S_n/S_c)	I	0.24±0.02	0.23±0.02*	0.24±0.02*	0.18±0.02	0.14±0.01
	III	0.21±0.02	0.21±0.02*	0.22±0.02*	0.19±0.02*	0.21±0.02*
Area occupied by hepatocytes (S_h), arb. units	I	74.66±3.72	68.80±3.33*	70.55±3.27*	69.83±3.21*	64.75±3.02
	III	70.72±3.60	66.24±3.27*	64.77±3.09*	60.21±2.62	56.68±2.46
Area occupied by sinusoid vessels (S_s), arb. units	I	25.34±1.02	31.24±1.31	29.45±1.16	30.17±1.22	35.25±1.62
	III	29.28±1.23	33.82±1.36	32.23±1.38*	39.77±1.84	43.32±2.53
Perimeter of sinusoid vessels (P_s), arb. units	I	11.51±0.62	11.01±0.62*	11.02±0.64*	12.33±0.71*	9.25±0.68
	III	14.02±0.78	10.05±0.43*	11.62±0.46	13.66±0.62*	9.62±0.52
Shape factor for sinusoid vessels (P^2_s/S_s)	I	5.22±0.31	3.88±0.21	4.11±0.26	5.04±0.30*	2.42±0.18
	III	6.71±0.39	2.99±0.19	4.18±0.27	4.69±0.29	2.14±0.18

Note. All values except those indicated with the asterisk are statistically significant at $p<0.05$.

respectively ($p<0.05$), which points to a more intense interchange between hepatocytes and blood in the perinvenular zones.

The redundance coefficient for the first group, which reflects the back-up reserves and increased adaptive and compensatory abilities of a morphological system, allowed us to define the current system (dog acinus) as a probability-determinate one [2], in contrast to human acinus, which is a determinate system [6]. In this case, just as in man the perivenular (central lobular) zones have a greater degree of disorderness and unreliability of functioning.

The development of systemic endotoxemia is accompanied by progressive damage to the microcirculatory bed and liver parenchyma. A heterogeneous plethora of the portal vessels, particularly of the portal vein branches, was observed 1 h after LPS injection. Small hemorrhages were seen in some portal tracts, they became large after 3 h, and by the 7th hour had spread to the periportal zones. Heterogeneous dilation and plethora of sinusoid vessels and hepatic venules, erythrocyte aggregation, and leukocyte stases were observed after 3 h of intoxication. Hepatocyte damage was manifested as protein (granular or vacuolar) dystrophy and monocellular necroses confined predominantly to zone I after 1 h of intoxication. By the 3rd hour the necroses became focal; solitary periacinar necroses were seen 5-7 h after LPS injection.

These morphological changes were reflected in the morphometry. The area occupied by hepatocytes was reduced after 1 h of intoxication (7.8 and 6.4% in zones I and III, respectively, $p>0.05$)

TABLE 2. Information Characteristics of Liver Acini in Endotoxemia

Parameter	Acinus zone	Control	Duration of intoxication, h			
			1	3	5	7
H, bit	I	1.3420	1.3949	1.3716	1.3098	1.2883
	III	1.3434	1.3593	1.3709	1.3551	1.3627
h	I	0.847	0.880	0.865	0.826	0.813
	III	0.847	0.857	0.864	0.855	0.859
R, %	I	15.3	12.0	13.5	17.4	18.7
	III	15.3	14.3	13.6	14.5	14.1
O, bits	I	0.2430	0.1901	0.2134	0.2752	0.2967
	III	0.2416	0.2257	0.2141	0.2299	0.2223
D, %	I	0	3.3	1.8	−2.1	−3.4
	III	0	1.0	1.7	0.8	1.2

and the area occupied by sinusoid vessels was increased (23.1 and 15.4%, respectively, $p<0.05$). The perimeter and shape factor of the sinusoid vessels decreased 4.3 and 25.7% in zone I and 28.3 and 55.4% in zone III ($p<0.05$), which was reflected in the microscopic studies. These changes, together with the dystrophic damage and circulatory disorders, confirm the direct action of LPS on hepatocytes and sinusoid cells [8,10]. The area occupied by sinusoid vessels progressively increased; by the 7th hour of intoxication it was 39.1 and 48.0% ($p<0.01$, zones I and III, respectively) greater than in the control. The changes in the perimeter and shape factor had a phasic character. They increased by the 3rd-5th hour of endotoxemia, which may be regarded as a compensatory adaptive reaction aimed at increasing the interface between blood and liver in developing circulatory hypoxia. The perimeter and shape factor of the sinusoid vessels then decreased, and by the 7th hour they constituted 74.5 and 39.1% of the control values. The liver parenchyma was characterized by a decrease of the relative areas of the nuclei and cytoplasm, which was consistent with the progressing dystrophy and necrosis of the hepatocytes. These parameters gradually declined in the perivenular area, while in the periportal area they changed in a phasic manner, which reflected a heterogenous response of the acini to LPS.

The information characteristics enabled us to compare the observed changes in liver acini using integral criteria. Analysis of the data given in Table 2 clearly indicates that after LPS administration, the information and relative entropies and equivocation increase, then the equivocation and entropies decrease, while organization increases. These variations were observed in zone I and zone III. Special attention should be paid to redundance, which was minimal in zone I 1 h and in zone III 3 h after LPS administration. According to Avtandilov [1], a decrease in the redundance of morphological organization occurs in pronounced pathological processes, extreme redundance values corresponding to "critical points," when a morphological system can no longer be regulated by the organism and after which the system goes over to a new reversible or irreversible state or ceases to exist as a morphologic entity.

Thus, the administration of *E. coli* LPS to dogs results in severe morphological damage to hepatic tissues, which cause liver dysfunction and failure. Morphometry allowed us to obtain objective quantitative characteristics of the structural changes in acini. Information analysis provided an integral estimation of morphometric parameters and enabled us to compare the responses of different zones of the acinus in developing endotoxemia, and to reveal the critical periods in the development of hepatic failure, which forms prior to the appearance of its clinical signs.

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