Use of Fractional Analysis for Evaluation of Liver Structure and Function in Rats *In Vivo*

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 147, No. 2, pp. 237-240, February, 2009
Original article submitted June 4, 2008

Fractional and morphometrical analysis of images obtained by life-time photo- and videorecording of microhemocirculatory changes in the rats in health, experimental cirrhosis, and variants of its treatment was carried out. Differences in the fractional dimensions of the studied organs were significant and correlated with morphological values. This suggests fractional analysis for the diagnosis and prediction of hepatic tissue status *in vivo*.

Key Words: *liver; cirrhosis; microcirculation; fractional analysis*

Good prospects of using fractional analysis (FA) of organ and tissue structure and function for the diagnosis and prediction of disease development causes no doubts [6,11,12]. This method is used for evaluation of tumor process, angiogenesis, and collagen distribution in tissues [3,6,7,12,14]. Fractional dimension D (one of the values used most often) is a quantitative characteristic of a complex object with the self-likeness characteristics in a certain range of values, for which the metrical values of Euclidean geometry are difficult to determine and are little informative. Fractional dimension D is a more correct characteristic of a biological object's shape than the factors traditionally used in morphometry [3,6,7,15].

Previous studies of liver status showed a sufficiently high level of diagnostic accuracy and good reproducibility of FA in liver fibrosis [7-10,13,15]. Fractional analysis is the only quantitative method differentiating between different models of fibrosis; it is sufficiently sensitive for detecting drug-induced changes in liver fibrosis [15].

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We found no reports on the use of FA for the diagnosis of organ status *in vivo*. Virtually all the studies reported were carried out on histological preparations.

We studied the possibility of using FA for *in vivo* evaluation of reparative processes in the liver involved in cirrhosis.

MATERIALS AND METHODS

The study was carried out on 29 male rats (250-300 g) with normal liver and cirrhosis. Cirrhosis was induced by CCl_4; this condition and variants of its treatment served as experimental models for our study. Hepatotropic toxin was injected subcutaneously in a dose of 0.2 ml 40% oily solution of CCl_4/100 g twice weekly during 2 months. Animals with experimental cirrhosis of the liver were divided into 4 groups. Group 1 animals served as the reference for all steps of the study. In group 2 cryodestruction of 8-10% of liver volume was carried out on day 5 after the last injection of CCl_4. Cryotreatment of the liver was carried out with an autonomic nitrogen cryoinstrument with 2 mm applicator and temperature of -120°C on its surface. Group 3 animals received a mixture of extracts from cryopreserved fragments of newborn piglet

0007-4888/09/1472-0273

liver and adult swine spleen (ELS) with 100 μg/ml concentration of peptides; the mixture (1 ml) was injected intraperitoneally daily during 3 days. Group 4 animals received combined treatment (cryodestruction of a fragment of the liver and ELS during 3 days). The extracts were prepared as described previously [4].

The priority characteristic of cirrhosis is modification of the vascular architecture [5]; the system of blood vessels is the most typical fraction-like formation in the body [3,11,12], and hence, any changes in the microhemocirculatory system are detected by FA. Microhemocirculatory changes in the liver were registered by life-time microscopy, realized by the Lumam K-1 contact microscope. Biomicroscopic images of the liver were recorded with a Sony DSC-T5 digital photocamera and Panasonic VC 45 BSHRX-12 and CAM-690C telecameras. Fractional analysis of the resultant images was carried out using FRAM software [1]. Fractional dimension D was calculated as the angular coefficient of the straight line, approximating the relationship between the number of pixels of a preset coloring of the biological object and the area of the frame into which they got [1]. Calculations of the traditional morphometrical characteristics of the object (diameter and length of hepatic capillaries, area of vascular network per visual field) were also carried out in the FRAM medium.

Statistical analysis was carried out by ANOVA and Student's *t* test in the SPSS medium.

Experiments were carried out in accordance with the European Convention for Protection of Vertebrates Used with Experimental and Other Scientific Purposes (Strasbourg, 1985). The animals were sacrificed by intraperitoneal injection of sodium thiopental.

RESULTS

Microscopically hepatic capillaries presented as a system of sinusoids formed as a result of division of afferent arterial and portal vessels. Afferent capillaries are terminal branches of the portal venules (septal and interlobular, according to morphological terminology). Because of anastomoses between arterioles and portal venules, the afferent terminal capillaries deliver mixed arteriovenous blood.

The bloodflow in venular capillaries of intact animals is rapid, continuous, homogenous; the parietal plasmatic layer is intact. The blood content of functioning sinusoids is normal, their diameter is 9.28±2.18 μ. The mean diameter of the terminal hepatic venules is 18.75±2.51 μ. The area of vascular network in a visual field is 52.5±1.6%.

The microhemocirculatory system of the liver of rats after 2-month treatment by tetrachloromethane corresponded to the picture of manifest cirrhosis of the liver (Fig. 1). A drastic reduction in the number of functioning sinusoids and emergence of the twisting phenomenon were seen. The terminal portal venules were thinned, shortened, the diameter of hepatic venules being appreciably increased. The percent area of the vascular network in a visual field was 27.35±2.92, the diameter of sinusoids 9.33±1.39 μ. The bloodflow was perverted. Many anastomosed vessels were seen. Microhemocirculatory disorders manifested also by intravascular aggregation of erythrocytes, bloodflow deceleration, and exclusion of an appreciable part (up to 25% per visual field) of sinusoids from the bloodflow.

Cryodestruction of a portion of the liver can be used for stimulation of regeneratory processes [2]. Extracts of cryopreserved fragments of xeno-organs also stimulate the regeneratory processes in a damaged organ. The effect of liver extract is tissue-

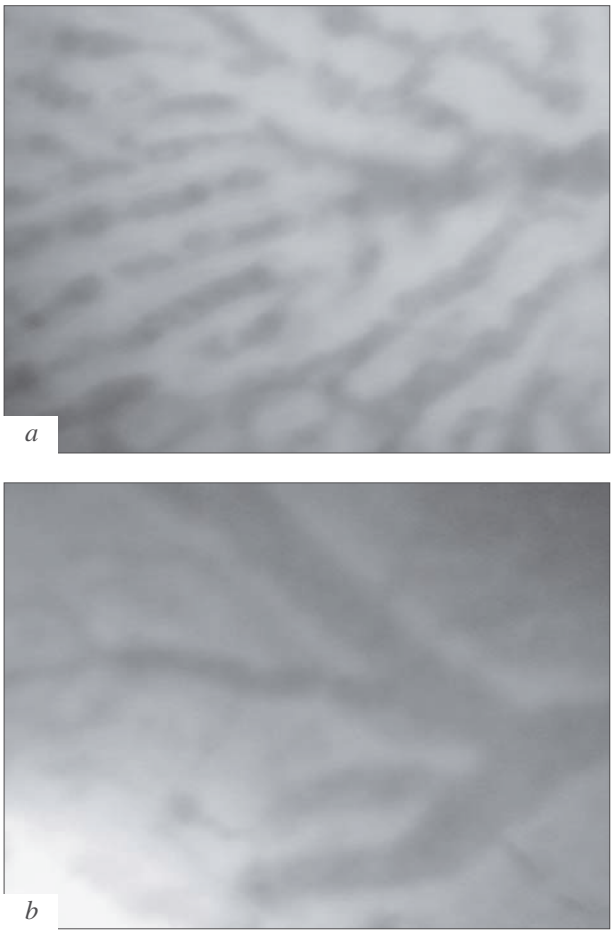

Fig. 1. Sinusoids and terminal hepatic venule in an intact animal (*a*) and in cirrhotic liver (*b*) 10 min after intravenous injection of 2% uranine solution (×175).

TABLE 1. Parameters of the Rat Liver on Day 14 after Experimental Treatment

Parameter	Group				
	baseline	1	2	3	4
Mean diameter of a sinusoid (d), μ	9.28±2.18	9.33±1.39	8.35±1.73	8.91±2.01	7.67±0.84
Percent area of capillaries per visual field (S)	0.53±0.02	0.27±0.03	0.45±0.03	0.52±0.02	0.54±0.06
Fractional dimension (D)	1.290±0.047	1.210±0.017	1.340±0.055	1.340±0.057	1.320±0.051

specific, of splenic extract tissue-nonspecific. After 3 days of injections of a mixture of extracts the liver of group 3 rats macroscopically did not differ from the liver of control animals. However biomicroscopy of the capillary network showed vascularization of the organ, manifesting by an increase in the number of functioning sinusoids, though the diameter of the vessels did not change. The percent area of vascular network was 27.3±2.9% in the control (group 1) and 35.2±3.2% in group 3.

Previous studies showed that the most pronounced changes in the biomicroscopic picture of the liver were observed on day 14 after treatments [2].

On day 14 after cryodestruction the liver in group 2 animals was more elastic than in the control. The omentum in some animals was tightly "soldered" to the site of cryoexposure. A small amount of ascitic fluid was detected in the abdominal cavity. The microcirculatory bed was characterized by increased area of vessels, the bloodflow was rapid, jet-wise, without erythrocyte aggregation.

In group 4 (cryodestruction of a fragment of the liver and injection of extracts) the twisting of the sinusoids, characteristic of cirrhosis, disappeared; the number of functioning capillaries increased.

The morphological parameters of the hepatic capillary network of rats on day 14 after experimental treatment are presented in Table 1.

The number of functioning capillaries in group 2 animals was significantly higher than in group 1. The diameters of sinusoids and numbers of capillaries were less than in groups 3 and 4. Bile tubules started to form a proper network, though sites free from vessels with straight bile tubules were seen. In groups 3 and 4 there were virtually no avascular sites and the biliary tubules formed a network close to the normal.

According to our estimation, the normal fractional dimension D is equal to 1.29, which corresponds to the intricate fraction-like architecture of hepatic capillaries, providing the maximum contact with hepatocytes. These data correspond to the previous results [8,13]. The development of cirrhosis, emergence of avascular zones as a result of necrotic degeneration of liver sites, a drastic alteration of capillary architecture (dilatation of portal veins, thinning of sinusoids and decrease in their number) resulted in a drop of the D fractional dimension to 1.21 ($p<0.001$). On the other hand, the D fractional dimension increased in all three groups of animals receiving various treatments aimed at stimulation of the regeneratory processes, reaching 1.32-1.34 in all groups; this was even higher than normally, though this difference was negligible. Presumably, a certain increase in the disorganization of the system at this stage is explained by triggering of the regeneratory processes in the liver and restoration of its microangioarchitecture. Differences in the D fractional dimension of the liver of rats receiving stimulation of the regeneratory processes and of controls (with untreated experimental cirrhosis of the liver) were significant ($p<0.001$).

Hence, the parameters calculated by FA correlated with the results of biomicroscopic study of the liver morphology and function and can be used in diagnostic studies.

The authors are grateful to S. E. Gal'chenko, Doct. Biol. Sci., for extracts of parenchymatous organs.

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