EXPERIMENTAL STUDY OF THE MORPHOGENESIS OF NUTMEG FIBROSIS OF THE LIVER

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Partial constriction of the inferior vena cava was carried out in dogs below the diaphragm with consequent stasis of blood in the liver. A comparative study of liver biopsy material obtained at various stages of the experiment showed that the development of fibrosis began from the central and small collecting veins as a result of proliferation of fibroblasts in the adventitia of the veins and "migration" of fuchsinophilic fibers into the adjacent parenchyma. The formation of new connective tissue took place parallel with these changes in areas of hemorrhage and necrosis in connection with proliferation of the Kupffer cells. Nutmeg fibrosis was shown to be cellular in its pathogenesis and linked with increased tropocollagen activity of the fibroblasts.

KEY WORDS: nutmeg fibrosis of the liver; constriction of the inferior vena cava; Kupffer cells.

The morphogenesis of nutmeg fibrosis of the liver is the subject of contradictory statements in the literature. Most investigatiors consider that the main role in the mechanism of its development is played by circulatory failure, retrograde stasis of blood in the liver, and acellular sclerosis at the site of dying liver cells [1, 6, 10, 12, 13, 23]. However, the view regarding acellular sclerosis is not in harmony with modern ideas of collagen formation, according to which the protein tropocollagen, from which collagen fibers are built, is formed purely by means of fibroblasts [7-9, 11, 16, 27]. Investigations using modern methods have proved the active cellular formation of collagen fibers in the liver in various pathological processes [3-5, 18, 20-22, 25, 26].

Since little work has been done on the morphogenesis of nutmeg fibrosis and since the existing contradictions cannot be resolved purely on the basis of analysis of postmortem material, it was decided to study the genesis of nutmeg fibrosis of the liver experimentally.

EXPERIMENTAL METHOD

Partial constriction of the inferior vena cava was performed above the diaphragm with consequent stasis of blood in the liver, portal hypertension, and the development of portocaval extrahepatic anastomoses and splenomegaly. Ascites appeared toward the end of the first week (the volume of fluid which collected reached 7-10 liters in some of the animals) and it persisted in most of the animals for up to 2-3 months. The liver was investigated 3 weeks and 1.5, 2.5, and 3.5 months after the beginning of the experiment. Liver biopsy was performed on 11 dogs at the above times, necessitating one or two additional laparotomy operations. Altogether 33 observations were thus made on 13 dogs. The dynamics of fibrosis were studied in two dogs for 1.5 months, in six dogs for 2.5 months, and in three dogs for 3.5 months. Material was fixed in 10% neutral formalin and embedded in paraffin wax. Sections were stained with hematoxylin–eosin, with picrofuchsin by Van Gieson's method, with fuchselin for elastin, with azocarmine by Heidenhain's method, and for fibrin by Shueninov's method; other sections were impregnated with silver by Pap's method, treated by Brachet's method and the PAS reaction, and stained with toluidine blue. Lipids were stained in the frozen sections by means of a mixture of Sudan III and Sudan IV, and ferritin was demonstrated in unstained sec-

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tions and after staining with hematoxylin–eosin by Klochkov's method [2].

EXPERIMENTAL RESULTS

After 3 weeks (10 observations) stasis was observed in the system of efferent veins of the liver. Around the central collecting veins and along the lines connecting them massive hemorrhages and necrosis were observed. The hepatocytes of the periportal zones were hypertrophied; the arteries, veins, and lymphatic capillaries of the triads were dilated. A continuous basement membrane appeared in the sinusoids. Hypertrophy of the muscular layer was observed in the efferent veins, the lumen of the large collecting veins was dilated, and that of the small veins was constricted or they were completely closed, as also were the sphincter-like veins.

The development of fibrosis began from the central and small collecting veins as a result of proliferation of fibroblasts in the adventitia of the veins (causing thickening of their wall) and "migration" of fuchsinophilic thin fibers from the wall of the veins to the adjacent parenchyma. New connective tissue was formed in the areas of hemorrhage and necrosis in the pericentral and intermediate zones parallel with these changes; the collagen fibers were arranged around the erythrocytes. Fibrosis was connected with proliferation of the Kupffer cells of the adjacent parenchyma. They became large and numerous PAS-positive pyroninophilic and metachromatic granules appeared in their cytoplasm.

After 1.5 months (11 observations) stasis of blood remained in the liver, but recent hemorrhages and necrosis were observed. The hepatocytes in the pericentral and intermediate zones were atrophied, whereas in the periportal zones they were hypertrophied. Changes in the vessels were similar to those observed after 3 weeks, but, in addition, some of the central veins were excluded from the hepatic circulation. The lumen of those veins was closed by proliferating connective tissue, and the sinusoids around them had collapsed. At the boundary with the hemorrhages and necrotic foci, in the subcapsular zones, and around the large collecting veins the sinusoids were grossly dilated.

Fibrosis of the parenchyma around the central and collecting veins increased as a result of proliferation of the fibroblasts of the adventitia. Organization of the areas of hemorrhages and necrosis by means of activated Kupffer cells took place more intensively, and the zone of fibrosis occupied a larger area.

After 2.5 months (nine investigations), despite an improvement in the clinical condition of all the animals and reduction of the ascites, venous stasis continued in the liver. The fibrosis was increased, especially along the lines connecting neighboring central and collecting veins, as the result of which "inverted" lobules appeared and the disturbances of the lymphatic circulation increased in severity, especially around the collecting veins.

After 3.5 months (three observations) decompensation of the circulation was still present in only one animal. Recent hemorrhages and necrotic foci were present chiefly in the subcapsular zones. Old hemorrhages and necrotic foci were replaced by zones of hyperplastic argyrophilic and collagen fibers. During organization of the hemorrhages, "trabeculae" of red cells were formed, bounded on both sides by a continuous basement membrane, lined with endothelium. The sinusoids between these trabeculae were usually wide and empty or they contained solitary red cells. Perierythrocytic sclerosis continued within the "red cell trabeculae," but no destructive changes were observed in the red cells. The "red cell trabeculae" are considered to indicate blocking of the hepatic veins, and they are regarded as a manifestation of intratrabecular collateral circulation [19].

After partial constriction of the inferior vena cava above the diaphragm, chronic venous stasis thus developed in the liver, with repeated hemorrhages not only around the central and collecting veins but also along the lines connecting them, following the resultant of the pressure from the neighboring veins. In these areas "straightening" of the hepatic trabeculae and atrophy and necrosis of the hepatocytes took place. As a result, "inversion" of the hepatic lobule was seen [14]. The subsequent fibrosis in these areas led to connection of neighboring central and collecting veins by fibrous septa, producing a picture of "an inverted lobular pattern" [24], characteristic of nutmeg fibrosis of the liver [13, 15, 17]. Nutmeg fibrosis of the liver is cellular in its genesis. The formation of collagen fibers close to the central and collecting veins is connected with proliferation of fibroblasts in the adventitia of the veins.

LITERATURE CITED

1. I. V. Davydovskii, in: The Pathological Anatomy of Human Diseases [in Russian], Moscow (1958), p. 303.

2. N. D. Klochkov, Arkh. Anat., No. 2, 94 (1962).
3. O. A. Kostyrev, in: Connective Tissue under Normal and Pathological Conditions [in Russian], Novosibirsk (1968), p. 183.
4. O. A. Kostyrev, Arkh. Pat., No. 10, 59 (1972).
5. O. A. Kostyrev and G. I. Borisova, Arkh. Pat., No. 1, 27 (1968).
6. V. B. Lipnitskaya, "Dynamics of morphological changes in the liver in cardiovascular failure," Candidate's Dissertation, Moscow (1968).
7. V. I. Mazurov, "A study of biosynthesis and fibrillogenesis of collagen," Author's Abstract of Doctoral Dissertation, Moscow (1972).
8. V. V. Serov, V. S. Paukov, and G. K. Mirodzhov, Arkh. Pat., No. 4, 52 (1972).
9. G. P. Sokolova, in: Connective Tissue under Normal and Pathological Conditions [in Russian], Novosibirsk (1968), p. 122.
10. E. N. Ter-Grigorova, Small Medical Encyclopedia [in Russian], No. 7, Moscow (1967), p. 519.
11. A. B. Shekhter and L. P. Istranov, Arkh. Pat., No. 7, 3 (1970).
12. M. V. Shlinchak, "Data on the pathomorphology of the liver in rheumatism and rheumatic heart disease," Author's Abstract of Candidate's Dissertation, Omsk (1959).
13. E. W. Boland and F. A. Willius, Arch. Intern. Med., 62, 723 (1938).
14. H. Elias, Am. J. Anat., 85, 379 (1949).
15. J. B. Gibson, Brit. J. Exp. Path., 40, 183 (1959).
16. H. Grossfeld, K. Meyer, G. Godman, et al., J. Biophys. Biochem. Cytol., 3, 39 (1957).
17. H. Katzin, Arch. Intern. Med., 64, 457 (1939).
18. J. Kojima, Med. J. Osaka Univ., 16, 423 (1964).
19. J. G. Leopold, T. E. Parry, and F. K. Storring, J. Path., 100, 87 (1970).
20. J. O. McGee and R. S. Patrick, Lab. Invest., 26, 429 (1972).
21. R. S. Patrick and J. S. Kennedy, J. Path. Bact., 88, 549 (1964).
22. H. Popper and F. Hutterer, Ann. New York Acad. Sci., 170, 88 (1970).
23. C. H. Rouiller, The Liver: Morphology, Biochemistry, Physiology, Vol. 2, New York (1964), p. 484.
24. S. Sherlock, Brit. Heart J., 13, 273 (1951).
25. H. Schnack, L. Stockinger, and F. Wawalka, Wien. klin. Wschr., 78, 715 (1966).
26. R. J. Stenger, Exp. Molec. Path., 4, 357 (1965).
27. T. Takeuchi and D. J. Procop, Gastroenterology, 56, 744 (1969).