ULTRASTRUCTURAL STUDIES ON LAMB LIVER WITH EXPERIMENTAL BUNOSTOMOSIS

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Received on January 28, 2020
Presented by B. Petrunov, Member of BAS, on February 26, 2020

Abstract

Ultrastructural studies were carried out on liver obtained after the appearance of clinical signs of bunostomosis from a group of experimentally infested lambs.

In the hepatocytes of the liver severe changes affecting the endoplasmic reticulum, especially the rough one, and the nucleic chromatin were determined. The formation of lipid drops into the injured hepatocytes followed the way of a fat degeneration rather than of a lipid infiltration. Both the liver parenchyma and mesenchyma compounds were affected.

This is an evidence of a direct toxic pathogenic action of the etiology agent. These data add to the knowledge of the bunostomosis pathogenesis more information about the leading role of the toxic factor.

Key words: bunostomosis, liver, hepatocytes, toxic factors

Introduction. Data of a number of authors show that organs of vital importance are often affected in helminth infections [1,2], as a result of the toxic (secondary) pathogenic effect of the parasite, while the causative agent can be absent [3-6].

In sheep host the adult Bunostomum trigonocephalum agent localizes in small intestine. In earlier studies of ours [7,8] we found out patho-histological lesions of different degrees on organs of vital importance at all stages of the infection [9,10].
These patho-morphological changes we considered a significant reason to undertake more detailed studies on their character by the method of electronic microscopy.

Material and methods. The experiments were carried out on 14 lambs of 4 months of age, divided into two groups. Group one (8 lambs) was experimentally infested with *Bunostomum trigonocephalum* [8,9] and the rest of the animals were used as a control. At appearance of clinical signs of bunostomosis (after the 45th day of invasion) all animals were euthanized. Samples were obtained immediately after slaughter.

Pieces of liver were fixed in 4% glutaraldehyde for 4 h at 0–4°C, postfixation in 1% OsO$_4$ for 1 h. The materials were embedded in Durcupan. Sections were made by Ultramicrotome LKB III and after the contrasting with uranyl acetate and lead citrate they were studied by an electronic microscope Opton 10C.

Results. Liver. Control group. The tissue was of a typical for this organ ultrastructure. Hepatocytes were of the usual measurements and form. The endoplasmic reticulum had both rough and smooth constituents. The elements of rough endoplasmic reticulum (RER) were found mostly perinuclearly, regularly arranged in several lines, and an abundance of ribosomes was evident. The Golgi zone (GZ) did not show significant deviations from the normal findings. Mitochondria were of a normal number and appearance. Hepatocyte membranes were intact. Spaces of Disse, sinusoids and sub-divisions of bile ducts, bile capillaries included, had a normal structure.

Liver. Experimental group. Haepatocytes were significantly changed, with elongated nuclei and wavy outlines of the nucleoli. The chromatin often accumulated at the nuclear periphery (Fig. 1). RER even in relatively preserved cells showed deep changes. Its quantity was reduced to a minimum and rearrange-
ment in the hepatocytes was observed. RER residues, completely disorganized, were observed around the nuclei. In other parts of the cell, where RER is usually found, we determined individual dilated cisternae of irregular form and different arrangement one towards the other as the only severely degenerated RER representatives. The smooth endoplasmic reticulum (SER) is comparatively preserved to intact in some of the hepatocytes. The mitochondria were not only polymorphous, elongated or irregularly shaped, but also their comb structure was disturbed. Various lysosomes, in the first place secondary forms like cytolysosomes, residual corpuscles, etc., were observed in increased numbers.

Interesting changes in other liver elements, outside the hepatocytes were observed too, such as enlargement of the spaces of Disse and of sinusoids, increase in microvillae in certain parts of the plasma membrane and, respectively, their disappearance in others; changes in Kupffer cells accompanied by intercellular collagen fibres. The Kupffer cells themselves were elongated and contained increased numbers of lysosomes. Figure 2 shows a part of such a cell with well elongated form, characteristic of the nuclei, too. The formation of a flagellum at one end was of a special interest. Sometimes the matrix of the organelles was erased. Transversely cut collagenic bundles and big lipid drops as well, were met. In some of the hepatocytes, the formation of fat drops was not a result of lipid infiltration but of lipid degeneration. Moreover, in the same fat drop we found fat tissue and myelinum figures too (Fig. 3).

The presence of great numbers of phagolysosomes into the Kupffer cells (Fig. 4) were a sign of a marked activity.

Various leucocytes, eosinophils, lymphocytes, mature macrophages, and plasmic cells included were seen in portal spaces, central veins and in some places into the parenchyma as well.
Discussion. By ultrastructural studies we determined that the pathogenic noxa affected the liver \([1,2]\). Its bilateral function is well known: on the one hand, the function of the parenchyma, realized by the hepatocytes, and of the mesenchima component represented by the Kupffer cells on the other \([3-5,7,8]\).

The pathogenic agent we studied was characteristic with a direct toxic effect, expressed in alterations in the hepatocytes.

The results obtained from the studies on the liver correlated with the data from our histological studies \([3,6-8]\) and proved our suggestions about the part played by the toxic factor in the bunostomosis patho-mechanism.

Analyzing all published data about the pathogenic effect of helminths \([1-5]\) and our own data as well, we tried to define the importance of the toxic effect of the metabolic products excreted by helminths and directly affecting liver hepatocytes as the only detoxifying intermediator of the organism \([1,2]\).
The changes in the lysosome apparatus of hepatocytes were most interesting \[^{10}\]. It is well known that the lysosomes react on the change in the parenchyma component of an organ and are directly related to the immunologic reaction in general \[^{10}\].

With our experimental material, the changes in the lysosomes of the hepatocytes were well correlated to those in the Kupffer cells. The lysosomal apparatus of the Kupffer cells was also activated towards heterophagocytosis \[^{1}\].

**Conclusion.** The results obtained by us proved the significant part of the toxic factor played in the bunostomosis pathogenesis in sheep.

The ultrastructural studies showed that the pathogenic agent had affected the liver. Evidences of a direct toxic effect were the changes determined in hepatocytes.

The alterations in the lysosome apparatus of the hepatocytes, expressed the undergone immunological reaction in general, supported by their correlation to the changes in the Kupffer cells.

**Acknowledgements.** The authors gratefully acknowledge the technical and consultative assistance of the specialists working at the Electronic Microscopy Laboratory of the Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences, Sofia, in carrying out this research.

**REFERENCES**


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