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Kidney and Liver Pathology in Human and Experimental Leptospirosis

ANNUAL REPORT

By: Dr. Thales de Brito

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Universidade de São Paulo, Instituto de Medicina Tropical
São Paulo, S. P., Brazil

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REPORT NUMBER 1

KIDNEY AND LIVER PATHOLOGY IN HUMAN AND EXPERIMENTAL LEPTOSPIROSIS

ANNUAL REPORT

BY

T. de Brito

E. Freymüller

H.S. Santos

D.O. Penna

S. Soares de Almeida

P... Myrosa Galvão

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Universidade de São Paulo
Instituto de Medicina Tropical
Escola Politecnica - Secção de Microscopia Eletrônica
Hospital "Emílio Ribas"
São Paulo - S.P. - Brazil

1- SUMMARY - A light and electron microscopy of human and experimental leptospirosis of the guinea pig was done. The human study was carried out in kidney biopsies, but a liver biopsy study is now in progress. The experimental work, in guinea pigs, was done both in the kidney and liver and histochemical techniques were also performed.

The earliest lesion found was at the cell membrane, with partial or total disappearence of the brush border of the cells of the proximal tubuli as well as partial disappearence and distortion of the microvilli of the hepatic cells. Intercellular spaces were found to be enlarged both in the liver and kidney. Capillaries of the experimental animal showed endothelial cell tumefaction and, sometimes, disjunction of the endothelial lining, a finding also in accordance with the basic pathology of the disease. Mitochondrial pathology, seen in humans, was seen in the experimental animal only at the late phase of the disease. However, a definite increase of so called "dense bodies", whose origin was discussed, was seen in both cases. Also described, a mild but definite focal glomerular lesion, which provides anatomical basis for the proteinuria seen in the disease.

The above described pathology is in accordance with the possibility of a toxin as the main mechanism acting in leptospiral pathogenicity.

2- FOREWORD - Previous work has shown that it is possible to safety perform kidney and liver (1,3,7,9) biopsies in patients with leptospirosis, provided that a maximum of care be taken to avoid complications. New possibilities in the study of the basic lesions of the disease were opened, and techniques like the histochemical ones and those using the electron microscope could now be used.

In order to better understand the disease in humans a re evaluation of the experimental disease was in order.

In conducting the research described in this report, the investigators adhered to the Principles of Laboratory Animal Care as established by the National Society for Medical Research.

3- BODY OF REPORT - The study of the human disease has been continued and a first paper has been delivered recently (1). So far we have performed 21 kidney biopsies in leptospirotic patients without complications. The light microscopy study of these biopsies showed that the lesions were less marked but essentially similar to the ones seen in autopsy.

The electron microscopy study was done in six patients, all of them, with the exception of a 12 year-old boy, being male adults, ranging in age from 20 to 41 years. The biopsies were performed at different stages of the illness but they were always postponed until the grossly hemorrhagic phase of the disease had been superseded.

Slices 0.3-0.5 mm thick were cut with a razor blade knife from three different levels of the fragments and fixed 1.5 to 2 hours at 5ºC in 1 per cent osmium tetroxide buffered to pH 7.3 with veronal acetate buffer (PALADE). After being dehydrated in a graded series of ascending alcohols, tissues were embedded either in methacrylate or in Epon 812. Thin sections were cut on a Porter-Blum microtome equipped with glass knives. The sections were doubly staned, first in uranyl ace

tate and then in lead citrate. The preparations were examined in a Siemens Elmiskop I electron microscope with a 50 objective aperture and operating at 80 kv.

This electron microscopy study disclosed a definite glomerular lesion in human leptospirosis, characterized by focal thickening of the basal membrane and fusion of the foot process of the glomerular epithelial cell. This is in agreement with the proteinuria seen in the disease. Worth while mentioning that previous light microscopy studies by KOPPISCH and BOND (5) had also pointed out to a glomerular lesion in human leptospirosis.

However, tubular pathology was more prominent than the glomerular lesions and was characterized by a total or partial brush border loss, a finding in agreement with the poor P.A.S stain of the proximal tubules border seen in light microscopy. The tubular cells showed frequent dense bodies in their cytoplasm, about the size and shape of the mitochondria. As a matter of fact few of them had remanescent distorted cristae which was in agreement with a first interpretation, that is, that they were altered mitochondria. However, in the discussion it cannot left out the possibility that they could be lysosomes and even protein droplets inside digestive vacuoles of the cell. This idea is in accordance with the proteinuria seen in the disease.

A mitochondrial pathology was seen by us in our light microscopy study (9) and confirmed in this electron microscopy approach. There were tubular cells with a definite mitochondrial depletion. However no structural alteration of these organelles were seen, unless we interpreted some of the previously described dense bodies as altered mitochondria.

Another finding was a partial disjunction of the cellular limits between adjacent tubular cells, giving rise to an enlarged intercellular space. This cellular disjunction, among other factors, could contribute to the tubular failure through a shunt mechanism between glomerular filtrate and the kidney interstitium. However, it is necessary to mention that the junctional complexes of the epithelial cells appeared preserved. If this interpretation is correct we must postulate an increased functional permeability of those complexes which usually act as a "seal" of the intercellular space. Another interpretation is that the enlargement of the intercellular spaces is due to interstitial edema fluid which, originating from altered vessels went through the basal tubular membrane and is now dissociating the epithelial cells. Capillary pathology was poor in the human disease.

Liver biopsies are available to us, obtained through a microlaparotomy, a technique used by MONTENS (6) and which appears to be a safe procedure in human leptospirosis. However, before further studying the human liver using histochemical and electron microscopy procedures a reevaluation of the experimental disease with similar techniques was in order.

An experiment was carried out using forty-three guinea-pigs, most of them weighing an average of 421 g. Five experiments were performed. The strains of Leptospira icterohaemorrhagiae used were originally isolated from rats and cultivated from fragments of liver and kidney in Fletcher's medium for 8 to 10 days at 28ºC. Virulence was

enhanced through an initial inoculum of 0.5 ml of culture into the peritoneum of healthy guinea pigs weighing an average of 250 g. At the terminal phase of the disease these animals were killed with a blow in the head and 1:5 liver-kidney homogenates in saline were prepared. About 1 ml of the suspension was administered intraperitoneally in the animals used in the experiment. Animals were sacrificed in a initial phase of the disease, usually around 3-4days after the inoculum and in a terminal phase of the disease, usually around the 5-7 th day of illness. Two healthy guinea pigs were similarly manipulated for each experiment.

Necropsy was performed immediatelly after death and besides a light microscopy study of liver and kidney fragments a histochemical study was also carried out. Fragments of liver and kidney were cut 7 micra thick in a cryostat microtome either without or with 24 hours fixation at 5ºC in 4% formalin, pH 7.2, plus 7.5% of sacarose. The fixed fragments before cutting were transfered to a mixture of sucrose and gum acacia for 24-48 hours at 5ºC. Succinodehidrogenase activity using as substrate the Nitro B.T (ditetrazolium chloride) and the M.T.T. [3-(4,5-dimethylthiazolil-2)-2,5 diphenyl tetrazolium bromide] was studied according to techniques described by PEARSE (8) in the non fixed fragments. Also studied in the non fixed fragments but only in two experiments was the cytochrome oxidase activity using as substrate the paraaminodiphenylamine, according to technique of BURSTONE (2). In the fixed fragments alkalyne (using as substrates either Gomori's medium or sodium -naphtyl phosphate), acid phosphatase (HOLT's technique, 4) and inespecific esterase (using as substrate the sodium -naphtyl acetate) were studied. In 10 animals electron microscopy was also carried out in a way similar to that used for humans except that the inclusion was only in EPON 812.

The light microscopy study showed findings which are in agreement with previous descriptions. Histochemistry revealed that only the alkalyne phosphatase activity, demonstrated only in the kidney tubules, correlated well with the degree of the kidney lesion. At the early phase of the disease its activity was seen to disappear from groups of nephrons and, at the late phase large areas of enzyme activity depletion was observed. The P.A.S positive zone of the proximal tubules also disappeared in most of the proximal tubuli at the late phase of the disease. These findings correlate well with the electron microscopy data which showed brush border alteration similar to that seen in humans. Also seen, the cellular disjunction with the appearence of enlarged intercellular spaces and the preservation of the junctional complexes.

The respiratory enzymes, acid phosphatase and inespecific esterase did not show prominent alteration. However, these findings must be taken carefully because only a qualitative and not a quantitative study was carried out in this experiment.

Liver cells also showed a depletion and/or an alteration of the microvilli at the late phase of the disease. Biliary ductules also revealed altered microvilli. Intercellular spaces appeared enlarged and, in few cases, disappearence of junctional complexes was seen. This is particularly evident in cases where the light microscopy study showed the lack of normal trabeculation of the hepatic cells, a finding described in human autopsy examinations (5).

Regarding the hepatic and tubular cells as a whole, an increased number of so-called "dense bodies" was seen with a morphological aspect similar to the ones described in humans. Besides the previous interpretations regarding their origin, the ones located in hepatic cells could be regarded as lipofuscin granules.

Glomerular pathology was less evident than that of human cases. However, in few animals killed at the late phase of the disease a basal membrane thickening and focal foot process fusion of the epithelial cells was observed.

Mitochondrial pathology was seen in only few cases and at the late phase of the disease. It was characterized by mitochondrial depletion and swollen mitochondria with altered cristae.

Capillaries of the kidney interstitium showed swollen endothelial cells and, sometimes, areas of disjunction between cells. Kupffer's cells appeared enlarged with many "dense bodies" in their cytoplasm which could be interpreted as engulfed debris.

Leptospira were seen in this experimental study, both in the liver and kidney, made up by a central axis with spirae around it. They were located between liver cells, in the tubular and in the liver sinusoidal lumina.

Both the human and the experimental data show that the earliest lesion of leptospirosis is at the cell membrane. Although L. icterohaemorrhagiae has not been conclusively demonstrated to possess a toxin, the clinical, histological and cytological evidences suggests a toxin as the mechanism of leptospiral pathogenicity. Our work is in accordance with a circulating toxin which, in the liver of the experimental animal would produce microvilli distortion and disappearence, interfering with the normal exchanges between the cell and the blood. Both in the kidney of humans and experimental animals this so far hypothetical toxin action would be mild in the glomerulus and more definite in the tubuli, chiefly proximal tubules, where it produces the brush border pathology through an enhanced action due to their concentration power. The enlarged intercellular spaces and the capillary lesions of the experimental animal could also be explained by a similar toxin action. Only at the late phase of the disease that other organelles would deteriorate in a such way that in the more severe cases cellular necrosis supervenes.

The above findings are in accordance with a low level of serum transaminase seen in the disease. More difficult to explain is the high level of mucoproteins unless we could admit that they were mainly located at the cell membrane, being then liberated through the toxin action.

No conclusive explanation for the mechanism of the icterus in leptospirosis was found. The lesions in the biliary ductules are inespecific and found both in intra-hepatic and extra-hepatic forms of cholestasis. On the other hand, hepatic cell disjunction might provide a short cut between biliary ductules and liver sinusoidal lining. However, studies of the biopsied liver in human leptospirosis, now in progress, are showing marked icterus with similar lesions regarding the biliary ductules microvilli but without an accentuated disjunction of the liver cells. It is possible that the icterus had a genesis si -

milar to that seen in other forms of cholestasis, like the one produced by chlorpromazin.

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13 ABSTRACT A light and electron microscopy of human and experimental leptospirosis of the guinea pig was done. The human study was carried out in kidney biopsies, but a liver biopsy study is now in progress. The experimental work, in guinea pigs, was done both in the kidney and liver and histochemical techniques were also performed. The earliest lesion found was at the cell membrane, with partial or total disappearence of the brush border of the cells of the proximal tubuli as well as partial disappearence and distortion of the microvilli of the hepatic cells. Intercellular spaces were found to be enlarged both in the liver and kidney. Capillaries of the experimental animal showed endothelial cell tumefaction and, sometimes, disjunction of the endothelial lining, a finding also in accordance with the basic pathology of the disease. Mitochondrial pathology, seen in humans, was seen in the experimental animal only at the late phase of the disease. However, a definite increase of so called "dense bodies," whose origin was discussed, was seen in both cases. Also described, a mild but definite focal glomerular lesion, which provides anatomical basis for the proteinuria seen in the disease. The above described pathology is in accordance with the possibility of a toxin as the main mechanism acting in leptospiral pathogenicity.

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