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## Liver Injury By Experimental Portal Bacteremia: Histogenetic Recovery Study In The Rat

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# Liver Injury By Experimental Portal Bacteremia: Histogenetic Recovery Study In The Rat

## Abstract

To study the histogenetic recovery of hepatic lesions due to portal bacteremia, a complication of some clinical conditions, an experimental animal model had developed. Portal bacteremia was performed in 8-week rats and the morphological recovery of liver was histologically checked 1 to 6 days after bacteria inoculation. The major injuries, such as acute inflammatory exudate of the portobiliary spaces, piecemeal necrosis of muralium, micro-abscesses and areas of hepatocyte necrosis of the liver parenchyma, and thrombosis in the centrolobular vein were recorded 1 day after inoculation. Minimal signs of vacuolar degeneration, steatosis, necrosis areas, vessel congestion and focal hemosiderosis together with a small hepatocyte proliferative activity was instead appreciable with longer time. The results seem to suggest a role of vascular structures and Kupffer cells in the morphological repair. This experimental model could serve to understand better similar clinical hepatology conditions, such as portal bacteremia.

## Introduction

Portal bacteremia can be encountered in many clinical conditions, as primary or secondary peritonitis, bacterial translocation from the intestinal lumen, etc. The study of hepatic histological lesions after portal bacteremia and their repair mechanism has been the aim of our research. For this purpose, we have developed an experimental model in the rat, based on direct infusion of living bacteria into the portal vein [1-3]. Histology analyses will be performed to study the morphological changes and the histogenetic involvement of hepatic sinusoidal littoral cells after bacteria inoculation, whereas immunocytology analyses will be performed to study the vascular/connective response and hepatocyte regeneration.

## Methods

Experiments were performed in compliance with the Italian Laws on animal experimentation. The Ethical Committee of the University of Modena and Reggio Emilia approved the animal research protocol as requested by the Italian Law. Adult male Sprague Dawley rats (n = 30, Charles River Italia, Italy) with an average body weight of 225g (range 200–250g, 8 weeks of age) were used. The rats were housed in stainless-steel cages supplied with a self-wash system, air conditioning and lighting in agreement with Italian guidelines on housing of laboratory animals. They were given at a daily "good laboratory practice" diet (4RF21, Charles River Italia) and water ad libitum, and checked with regard to health status and body weight. Surgery was performed under general anesthesia induced by an intramuscular injection of a mixture of 30 mg/kg body weight of ketamine (Ketavet 100, Intervet prod. S.r.l., Italy), 0.75 mg/kg of acepromazine (Prequillan, Fatro, Italy), and 4 mg/kg of xylazine (Rompun, Bayer, Germany). The surgical procedure consisted in a midline upper laparotomy, retraction of the duodenal loop to the left, and exposition of the hepatic pedicle. The vena porta was then easily dissected and taped with a fine thread. Twenty rats (treatment groups) underwent 25-gauge fine needle injection of 1 ml of Escherichia Coli diluted culture (Escherichia Coli strains of clinical derivation, identified with the "Vitek two" automatic system, then diluted in saline sterile solution (NaCl 0,85%) at a concentration of 0,5 grade of the standard McFarland, equivalent to  $1,5 \times 10^8$  C.F.U./ml). Sham rats underwent injection of 1 ml of sterile saline. Spontaneous sealing of the injection point was always obtained after a few minutes of simple compression. Laparotomic incision was closed in two layers. Control rats were sacrificed without any treatment. No antibiotic treatment was performed. Rats (5 animals per group) were scheduled as: group A – control rats, no treatments, sacrifice like group B rats; group B – sham rats, saline inoculation, sacrifice after 1 day; group C – bacteria inoculation, sacrifice after 1 day; group D – bacteria inoculation, sacrifice after 2 days; group E – bacteria inoculation, sacrifice after 4

daysgroup F – bacteria inoculation, sacrifice after 6 daysRats were sacrificed by ether euthanasia; liver was excised and fixed in 10% buffered (pH 7.2) formaldehyde solution. Four large fragments of each liver were paraffin embedded and cut to obtain 7 micron-thick sections. Slides were stained with hematoxylin-eosin for cellular morphology, Masson's trichrome for connective tissue, Gomori's method for reticular fibers, Perls' Prussian blue for hemosiderin or immunocytochemically treated for Mib1/Ki67 (cell cycle proliferation), desmin (connective tissue) and actin (wall vessel).

## Results

No histological evidence of morphologic changes or different immunocytochemical expressions were recorded in livers of both control (group A) and sham (group B) rats. Normal architecture of reticular fibers (Gomori's method), no hemosiderin (Perls' Prussian blue) or evidence of increased fibrotic tissue (Masson's trichrome stain and desmin immunodetection) were found in livers of both control (group A) and sham (group B) rats. In rats sacrificed 1 day from inoculation (group C) the portal triad displayed acute inflammatory exudate with infiltration of granulocytes, histiocytes, plasma cells, and lymphocytes. Inflammatory cells (sometimes) invaded the peripheral plate of hepatocytes (muralium of hepatic laminae) with subsequent their piecemeal necrosis. Thus, the liver parenchyma displayed several micro-abscesses (Illustration 1A) and areas of hepatocyte necrosis (Illustration 1B and 1C) of various sizes. Paralleling inside the lobule, hepatocytic suffering was represented by areas of cellular ballooning, vacuolar degeneration (Illustration 2), localized steatosis, hemorrhage and spotty necrosis. These necrotic areas were located in the central and middle part of the hepatic lobule, seldom extended towards the periphery (Rappaport zone 1). One day after bacterial inoculation the immunocytochemical markers (Mib1/Ki67, desmin and actin) were not expressed inside lobules. In rats sacrificed 2 days from inoculation (group D) the liver parenchyma showed restricted necrosis areas, steatosis (Illustration 3A) inside the hepatic lobule (zone 1 of Rappaport), while some centrolobular vein displayed thrombosis (Illustration 3B). The wall of the portal vein inside portobiliary spaces appeared thickened with desquamated lining endothelium. Mib1/Ki67, desmin and actin were faint expressed. In rats sacrificed 4 days from inoculation (group E) the hepatic lobule

displayed only minimal signs of vacuolar degeneration and necrosis. Moderate stasis in the central lobular vein, hemorrhage, interstitial edema and dilatation of the sinusoids in the Rappaport zone 3 were detected. In rats sacrificed 6 days from inoculation (group F) Steatosis, necrosis areas, vessel congestion and focal hemosiderosis were poorly appreciable. Hepatocytes showed fairly small proliferative activity (low Mib1/Ki67 expression) and desmin was not cytoimmunologically appreciable. Activation of Kupffer cells, inside lobules (Illustration 4A), and foci of microvascular neogenesis (actin immunodetection), near the centrolobular vein (Illustration 4B), were observed.

## Discussion

The more relevant histological findings emerging after bacterial inoculation of liver characterized three different pathological expressions affecting: A) hallmark of acute inflammation; B) lesions of the hepatocytes; C) vascular alterations. The early signs of acute necrotic suppurative inflammation located inside the zone 1 of Rappaport represent the initial injury of bacteria against the hepatic lobule. The hepatocyte nonspecific lesion and the proliferative low expression observed can be ascribed to secondary metabolic and vascular outcomes, corresponding to the typical hepatic dyshomeostasis of septic conditions. The feeble desmin and actin activities seem to indicate an absence of chronic fibrotic reaction and confirm the trend of complete recovery of an acute inflammatory process. The vascular abnormalities recorded in sinusoids and centrolobular vein can be explained as secondary to the acute inflammatory reaction. Sinusoids, plugged by adherent granulocytes, activated Kupffer cells, macrophages and other inflammatory cells, showed their endothelium damaged by hypoxia and relative hypoperfusion. Moreover, sinusoidal and Kupffer cells will be activated to produce pro-inflammatory mediators, which produce not only microbial killing but will also cause functional damages to microcirculation, with subsequent lesions to hepatocytes around the centrolobular vein [4-5]. Our observations demonstrate the important and direct responsibility of whole bacteria in liver damage, as it happens in sepsis. The histological demonstration of these lesions gives direct evidence of liver action as forceful filter and barrier against portal bacteremia [6]. The secondary cytological and microcirculatory lesions correspond to those that can be found in hepatic dysfunction of

septic conditions [7-11]. Injections of single bacterial components, such as lipopolysaccharides [6, 12], produce results similar to that of our study.

## Conclusion(s)

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The results of our experimental model, matching features of similar clinical conditions, particularly bacteremia, seem to confirm this animal model as appropriate for the histogenesis hepatic recovery studies.

## Authors Contribution(s)

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A. Manenti, A.R. Botticelli contributed to the research design. A. Manenti, L. Trenti contributed to the surgeries. L. Reggiani Bonetti, D. Zaffe, A.R. Botticelli contributed to the histopathological analysis. A. Manenti, D. Zaffe, A.R. Botticelli contributed in drafting the paper. A. Manenti, D. Zaffe, A.R. Botticelli worked on the critical revision of the paper.

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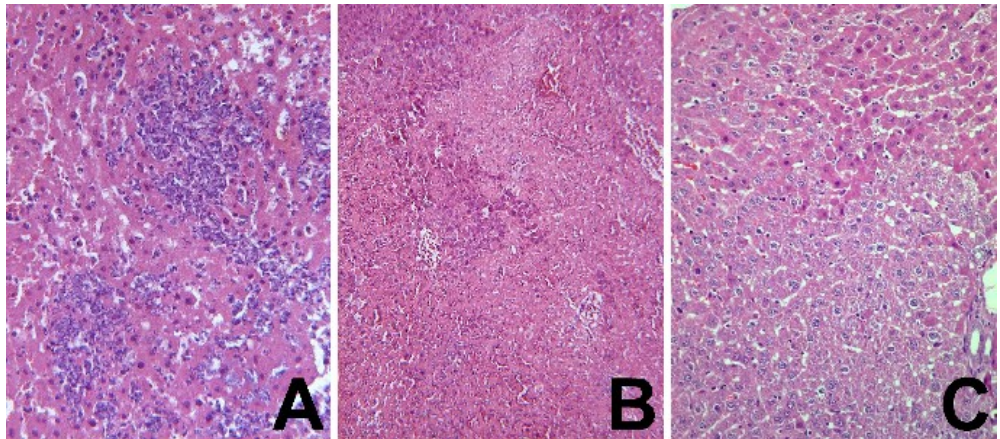
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## Illustrations

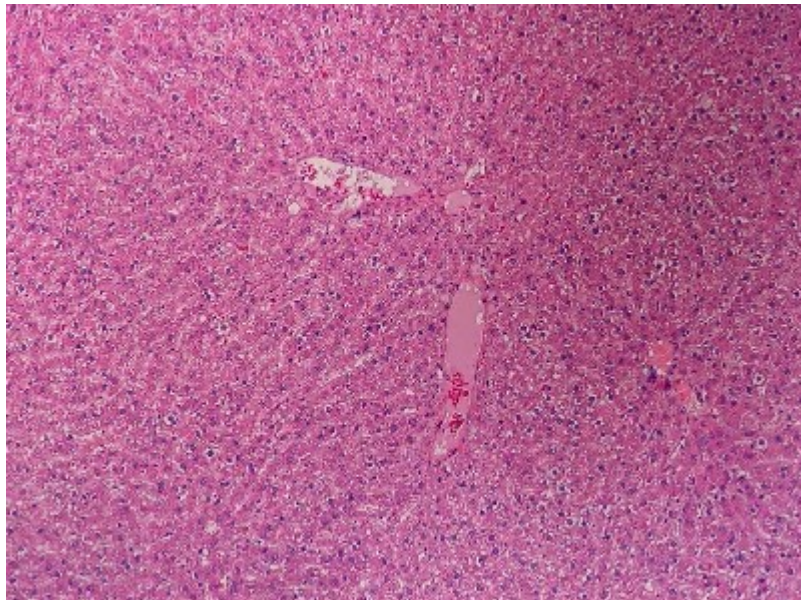
### Illustration 1

Micro-abscesses (A), necrosis (B), focal granular degenerations (C) in group C rats.



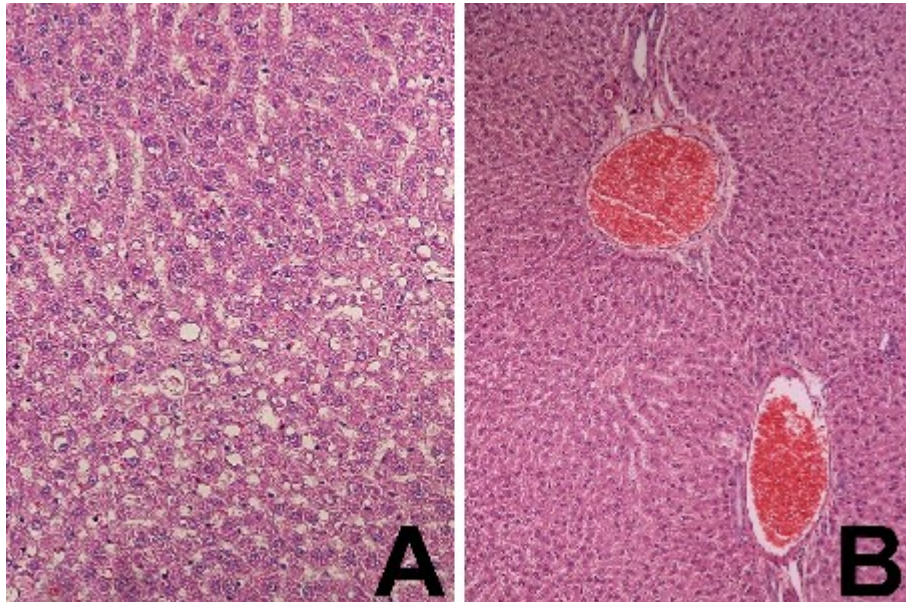
### Illustration 2

Vascular stasis and microvacuolar degeneration of hepatocytes were also detectable in group C rats.



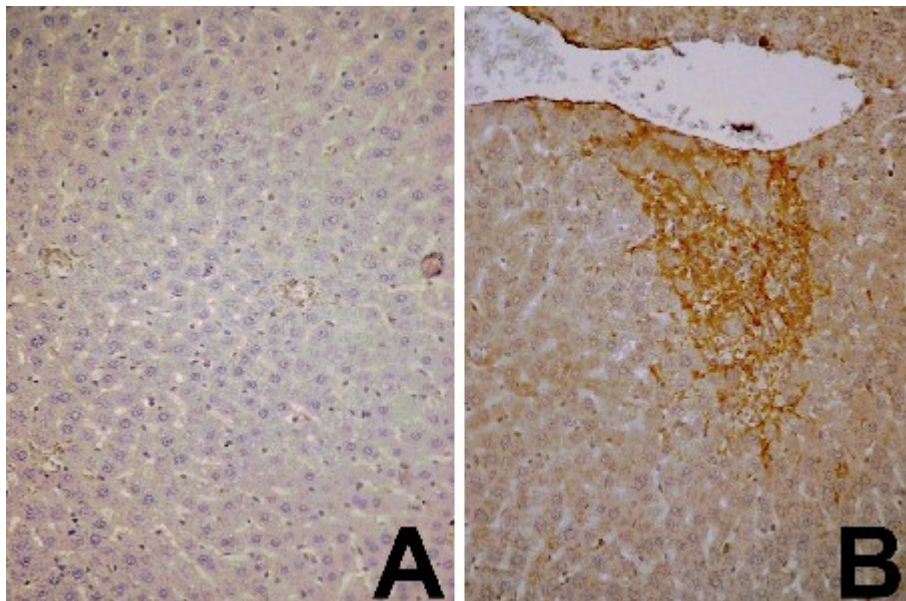
### Illustration 3

Steatosis, displayed by hepatocytes (A), and vascular thrombosis (B) were detected in group D rats.



### Illustration 4

Kupffer cells inside Disse spaces (A) and new vessels (B) were detectable in group F rats.



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