Acta Veterinaria (Beograd), Vol. 53. No. 2-3, 121-128, 2003.

UDK 619:616.36-004

LIVER ATROPHY FOLLOWING PORTACAVAL SHUNT IN NORMAL RATS: A MORPHOLOGIC AND ULTRASTRUCTURAL STUDY

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(Received 27. January, 2003)

The aim of the current study was to examine morphological and ultrastructural changes in the rat liver in an experimental model of chronic liver disease (end-to-side portacaval shunt).

The surgical procedure providing an end-to-side portacaval shunt (PCS) was performed in Wistar rats. The liver and pancreas weights were determined 8 weeks after the operation, when liver histology and ultrastructural patterns of hepatocytes were examined.

Body weights were not significantly different between the groups 8 weeks after the operation. Liver weight was significantly lower in PCS rats than in control and sham operated (SHAM) rats. The same was observed when liver weight was expressed as a percentage of body weight. Pancreas weight was significantly greater in PCS than in control and SHAM rats.

Liver histology in rats with PCS showed glycogen depletion and sinusoidal dilatation around the hepatic vein. Kupffer's cells were filled with haemosiderin. The hepatocytes surrounding the portal space exhibited degenerative and microvesicular fatty changes. Multiplication of the biliary ductules in the portal space was present. Atrophy of hepatocytes occurred in other parenchymal zones and apoptopic hepatocytes were seen more frequently in rats with PCS.

The ultrastructural characteristics of hepatocyte cell lesions in rats with PCS at the end point of our experiment included reduction and fragmentation of rough endoplasmic reticulum with destroyed or dilated cisternae and few polysomes accompanied by proliferation of smooth endoplasmic reticulum.

The present study suggests that end-to-side PCS in rats causes liver atrophy and that the morphological and ultrastructural changes in the hepatocytes partially explain the metabolic and endocrine abnormalities.

Key words: liver atrophy, portacaval shunt, rats

INTRODUCTION

PCS exposes animals to numerous severe disorders, which confirm that sudden diversion of portal blood from the healthy liver is incompatible with normal hepatic function (Lee and Fisher, 1961; Assal *et al.*, 1971; Kravetz *et al.*, 1987; Bani *et al.*, 1994; Lin *et al.*, 1996; Milošević *et al.*, 1996, Radosavljević *et al.*, 2002). End-to-side PCS in rats is an experimental model of chronic liver disease, especially that due to cirrhosis and porta-systemic shunt (Lin *et al.*, 1996). This shunt diverts the portal blood flow from the liver, i.e. hepatofugal blood flow, and leads to a significant decrease of total liver blood flow, decreased hepatic arteriolar resistance, and hypoxia, causing atrophy and functional impairment of liver cells (Radosavljević *et al.*, 1997; 1998; 2001a; 2001b; 2001c; 2002). In such conditions blood flow through the hepatic artery is increased (Milošević *et al.*, 1996), because PCS leads to decreased pressure in the liver sinusoids. The liver is supplied with more blood from the arterial circulation and a hypercirculatory hyperdynamic syndrome with increased volume of circulating blood develops as a compensatory phenomenon (Milošević *et al.*, 1996). The aim of the current study was to examine the morphological and ultrastructural changes of the liver tissue in this experimental model of chronic liver disease (end-to-side PCS) 8 weeks after the operation.

MATERIAL AND METHODS

Wistar rats, weighing 180-250 g at the beginning of the experiment, were used. The rats were divided in to three groups: 1) control untreated (C rats, n=11), 2) sham operated (SHAM rats, n=17), and 3) experimental group (PCS, n=27). End-to-side PCS shunt was performed under diethyl ether anesthesia, according to the technique described by Lee and Fisher (1961).

Liver histology: The liver sections were analyzed under a microscope after staining with hematoxylin-eosin, Masson's trichrome, PAS and Perl's stain.

Electron microscopy of the liver: Small liver pieces were immediately placed in cold 3% glutaraldehyde (BDH, England) in 0.2 M caccodylate buffer with 0.2 M sucrose (pH 7.2) and fixed overnight at 0-4^{o}C on a rotor. After that, tissue samples were post-fixed in 1% osmium tetroxide (Fluka, Germany) (in caccodylate buffer, pH 7.4) for 1 hour at room temperature. One micrometer thick sections were cut, stained with toluidine blue and examined under the light microscope. Ultrathin sections were then cut and stained with uranylacetate followed by lead citrate.

Statistical analysis: All values are expressed as mean$\pm$SD. Statistical analysis of differences between groups was made using the Mann Whitney nonparametric test and $p<0.05$ was accepted as significant.

RESULTS

Body and liver weights: Body weights were not significantly different between the groups 8 weeks after the operation. Liver weight was significantly lower in PCS rats than in SHAM and control rats ($p<0.01$). The same was observed

when liver weight was expressed as a percentage of body weight ($p<0.01$). Pancreas weight was significantly greater in PCS than in control and SHAM rats (Table 1).

Table 1. Absolute and relative weights of liver and pancreas in C, SHAM and PCS groups of rats at the end point of the experiment

GROUP	C (N=11)	SHAM (N=14)	PCS (N=27)
BODY WEIGHT (g)	234 ± 12	233 ± 11	236 ± 34
LIVER WEIGHT (g)	6.9 ± 1.3	6.7 ± 1.1	5.1 ± 1.2**
% of body weight	2.9 ± 0.5	2.8 ± 0.4	2.1 ± 0.2**
PANCREATIC WEIGHT (g)	0.77 ± 0.27	0.75 ± 0.25	1.12 ± 0.38**
% of body weight	0.33 ± 0.11	0.31 ± 0.1	0.49 ± 0.17*

** $p< 0.01$, * $p<0.05$

Liver histology and electron microscopy: Liver histology in rats with PCS showed glycogen depletion and sinusoidal dilatation around the hepatic vein (Figure 1). Kupffer's cells were filled with haemosiderin. The hepatocytes surrounding the portal space had degenerative and microvesicular fatty changes (Figure 2). Moreover, multiplication of the biliary ductules in the portal space was present (Figure 3). Atrophy of hepatocytes in other parenchymal zones was observed. Apoptopic hepatocytes were seen more frequently in rats with PCS.

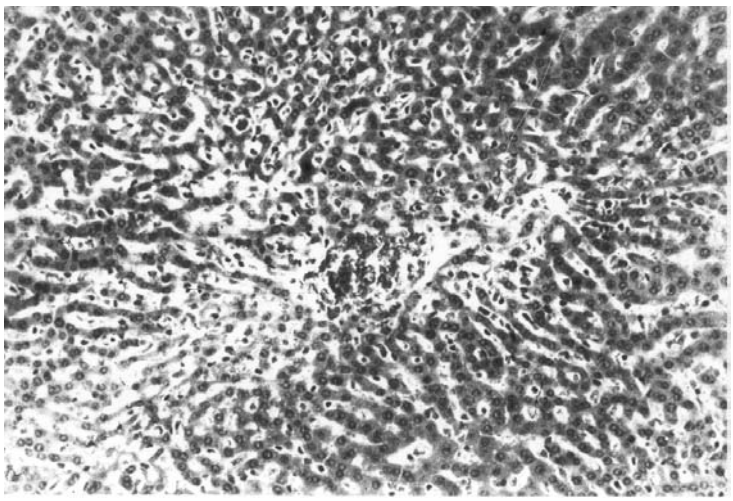

Figure 1. Sinusoidal dilatation in the liver of a rat with PCS at the end point of the experiment. Toluidine blue, x20

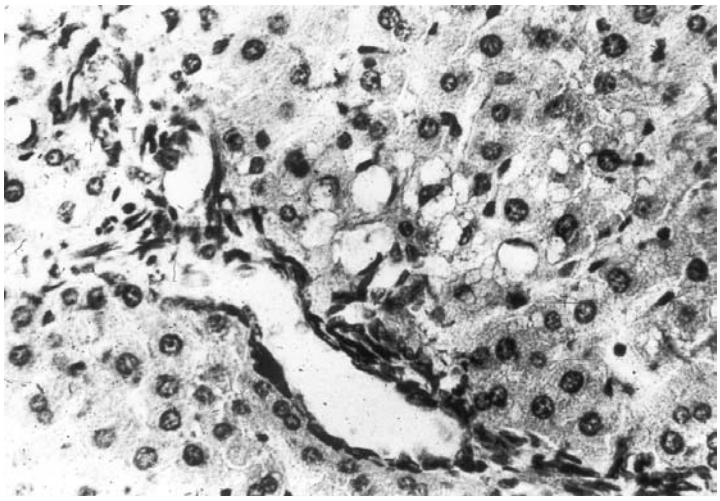

Figure 2. Microvesicular fatty changes of hepatocytes in a rat with PCS at the end point of the experiment. Toluidine blue, x40

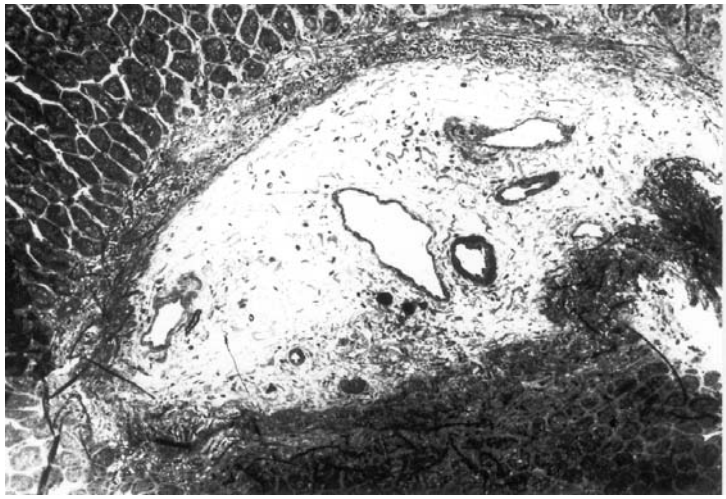

Figure 3. Multiplication of biliary ductules in the portal space in a rat with PCS at the end point of the experiment. Toluidine blue, x40

Ultrastructural characteristics of hepatocyte cell lesions of rats with PCS showed reduction and fragmentation of rough endoplasmic reticulum with destroyed and dilated cisternae and fewer polysomes accompanied by proliferation of smooth endoplasmic reticulum. An increase in the number of small lipid droplets, mitochondrial edema and a significant decrease in glycogen particle content

were also noted in PCS rats. The ultrastructural characteristics of hepatocyte cell lesions of rats with PCS in comparison to the C and SHAM rats, are summarized in Table 2.

Table 2. Summary of characteristics of hepatocyte cell lesions in rats with PCS 8 weeks after surgery

Specific alterations	GROUP TREATMENT		
	PCS (n=27)	SHAM (n=14)	C (n=11)
Mitochondrial aberrations	+	–	–
Pycnotic euchromatic nuclei	+/++	–	–
RER fragmentations	++	–	–
Poyribosome depletion	++	–	–
Glycogen depletion	+++	–	–
Lipid inclusions	++	–	–

(+) and (-) indicate presence or absence of respective feature; RER-rough endoplasmic reticulum

DISCUSSION

PCS shunts performed in normal rats caused liver atrophy (Dubuisson *et al.*, 1984; Milošević *et al.*, 1996; Šikić *et al.*, 2001) which was noted again in our study. This was manifested by a significant reduction of absolute liver weight and when expresed as a percentage of body weight. The development of liver atrophy in PCS rats is a consquence of blood flow reduction as well as deprivation of hepatotrophic substances normally present in portal blood (Starzl *et al.*, 1975). Earlier studies indicated "hepatic insulinopenia" as the most important cause of liver damage after PCS and Eck' s fistula (Starzl *et al.*, 1974; 1975). Insulin infusion in to one of the branches of the portal vein after PCS reduced atrophy, normalized ultrastructure of hepatocytes and increased the number of mitoses (Starzl *et al.*, 1974).

PCS causes pancreatic hypertrophy (Kravetz *et al.*, 1987; Nylander *et al.*, 1993) also noted in our study. It was suggested that PCS may raise the sensitivity of pancreatic CCK-A receptors through increased concentrations of intestinal factors in the circulation (Nylander *et al.*, 1993).

Our rats with PCS had less glycogen in the hepatocytes and sinusoidal dilatation around the hepatic vein, degenerative changes and microvesicular fatty changes. Atrophy of hepatocytes in other parenchymal zones was present. The unusual prominence of Kupffer's cells filled with haemosiderin was evident too. Apoptotic hepatocytes were seen in rats with PCS.

Based on the histological examinations of liver tissue obtained from rats with PCS, we have confirmed previously recorded structural changes of the liver characterized by atrophy, degeneration and regeneration of hepatocytes as well as

fatty changes and multiplication of the biliary ductules. Protein p53 and c-Ha-ras induction is closely associated with the regenerative process, whereas expression of interleukin 1-beta (IL-1beta) appears to be one of the negative growth regulators that might play an important role in atrophy of the liver (Laurent *et al.*, 2001).

Ultrastructural characteristics of hepatocyte cell lesions of rats with PCS at the end of our experiment showed reduction and fragmentation of rough endoplasmic reticulum with destroyed and dilated cisternae and fewer polysomes accompanied with smooth endoplasmic reticulum proliferation. The mitochondria were round to elongated with prominent cristae. Moreover, a significant decrease in glycogen particle content and an increase of the number of small lipid droplets were noted in PCS rats. These hepatocyte cell lesions together with pathohistological change in the liver are similar to the findings of other authors (Dubuisson *et al.*, 1984; Pasquali *et al.*, 1983; Šikić *et al.*, 2001). They cause metabolic and endocrine abnormalities i.e. disturbance of glucose homeostasis and reduction of IGF-I concentrations in serum and liver tissue, among others (Bani *et al.*, 1994; Scharf *et al.*, 1996; Radosavljević *et al.*, 1998; 2001a; 2002). Moreover, these ultrastructural changes partially explain the depression of numerous biosynthetic processes, such as production of triglycerides and cholesterol, bile acids, Krebs-Henseleit cycle of urea synthesis and the activity of microsomal enzymatic systems (Radosavljević *et al.*, 1997).

We conclude that end-to-side PCS in rats causes liver atrophy and that the morphological and ultrastructural changes in the hepatocytes partially explain the metabolic and endocrine abnormalities.

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ATROFIJA JETRE U PACOVA SA PORTO-KAVNIM ŠANTOM: MORFOLOŠKA I ULTRASTRUKTURNA STUDIJA

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SADRŽAJ

Cilj studije je bio da se ispitaju morfološke i ultrastrukurne promene tkiva jetre pacova na eksperimentalnom modelu hronične insuficijencije jetre (terminolateralni porto-kavni šant).

Na Wistar pacovima izvedena je hirurška tehnika termino-lateralnog porto-kavnog šanta (PKŠ). Osam nedelja posle operacije određivane su težine jetre i pankreasa. Takođe, ispitivane su morfološke i ultrastrukturne osobine hepatocita.

Apsolutna i relativna težina jetre je bila značajno smanjena u pacova sa PKŠ u poređenju sa kontrolnim i lažno operisanim životinjama. Težina pankreasa je bila značajno povišena u pacova sa PKŠ u poređenju sa kontrolnim i lažno operisanim pacovima. Osam nedelja postoperativno nastala je atrofija jetre u pacova sa PKŠ. Histološki nalazi jetre pokazuju redukciju glikogena i sinusoidalnu dilataciju oko hepatične vene. Kupffer-ove ćelije su ispunjene hemosiderinom. Hepatociti oko portnog prostora pokazuju degenerativne i mikrovezikularne masne promene. Takođe, u portnom prostoru je uočeno prisustvo umnoženih bilijarnih duktulusa. U drugim parenhimskim zonama razvila se atrofija hepatocita. Ultrastrukturna analiza u hepatocitima u pacova sa PKŠ na kraju eksperimenta pokazuje redukciju i fragmentaciju granuliranog endoplazmatskog retikuluma sa razorenim i dilatiranim cisternama i nekoliko polizoma, kao i proliferaciju glatkog endoplazmatskog retikuluma.

Ova studija pokazuje da termino-lateralni PKŠ u pacova uzrokuje atrofiju jetre i da morfološke i ultrastrukturne promene hepatocita delimično objašnjavaju metaboličke i endokrine abnormalnosti.