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Original paper

SOME METABOLIC AND ENDOCRINE CONSEQUENCES IN PORTACAVAL-SHUNTED RATS

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Summary: Some metabolic and endocrine consequences of portacaval shunt were examined in rats eight weeks after the operation. Plasma levels of insulin, glucagon, somatostatin, such as pancreatic content of insulin, glucagon, and somatostatin were determined by radioimmunoassay. Numerous biochemical parameters, such as the concentration of serum ammonia, transaminase (AST and ALT), urea, bilirubin, total plasma protein and plasma albumin levels were determined by standard biochemical tests. Normal plasma levels of insulin and somatostatin, hyperglucagonaemia were observed in rats with portacaval shunt. After portacaval shunt there was a highly significant increase ($p<0.01$) in levels of blood ammonia, AST and ALT. Serum fasting glucose levels were significantly lower in portacaval shunt rats ($p<0.01$) in comparison to controls and sham operated rats. We conclude that the metabolic and endocrine consequences of portacaval shunt in rats are probably related to the functional deterioration of the liver due to diversion of the portal blood in systemic circulation.

Key words: rat, portacaval shunt, endocrine pancreas, ammonia, glucose.

Introduction

A portacaval shunt (PCS) exposes normal animals to numerous severe disorders, which confirm that sudden diversion of portal blood from the normal liver is incompatible with normal hepatic function. Complete diversion of the portal vein from the liver, hepatofugal blood flow consequential to end-to-side PCS shunt, leads to significant decrease in total liver blood flow and also to liver hypoxia and impairment of its metabolic functions (1 4).

It has been shown that end-to-side portacaval shunt in the normal rat produces a marked alteration in the metabolic, nutritive, and hormonal state (3 5). Portacaval shunting causes a variety of anatomic, metabolic and physiologic changes, such as liver atrophy (6), hyperammonaemia (7 9) and hepatic encephalopathy (9, 10).

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The end-to-side portacaval shunt performed in rats was an experimental model masking the conditions that occur in patients with liver failure, especially that due to cirrhosis, and porta-systemic shunt. Complete diversion of the portal blood flow from the liver leads to metabolic and endocrine abnormalities (4, 5).

Some endocrine consequences of PCS have been reported in the rat (5), including abnormalities in glucose homeostasis and plasma levels, pancreatic content of hormones of endocrine pancreas (11).

The purpose of the present study was to examine the metabolic and endocrine consequences of PCS in rats, eight weeks after the surgery.

Materials and Methods

Animals and experimental design

Two-month-old male Wistar rats, weighing approximately 230 g were maintained at room temperature in a 12-h dark, and 12-h light cycle. They had free access to a conventional chow diet and tap water and were kept in separate cages. The study was approved by the institutional ethics committee for animal expe-

riments. The animals were divided into three groups: control non-operated rats (C rats; n=11), rats with surgical portacaval shunt (PCS rats; n=27), and sham operated animals (SHAM rats; n=17). On day one of the experiment the rats underwent end-to-side portacaval anastomosis according to Lee and Fisher (12) as modified by Bismuth et al., (13). The sham procedure was performed in the same way, except that only venous vessels were clamped for as long as it was necessary to perform anastomosis. Surgery was performed under ether anasthesia. All animals were killed by cervical dislocation eight weeks later. Twenty-four hours before killing food was withdrawn, but not tap water.

Analytical methods

Just prior to killing blood samples were taken from the retroorbital venous plexus, and serum/plasma was stored at 20 ²C until used. All biochemical variables were measured in serum/plasma from individual rats. After killing the liver and pancreas were removed from each animal and weighed. Some of the specimens from the juxtasplenic part of the pancreas and from the left major liver lobe were extracted as follows: Tissues were weighed, placed in plastic tubes and immediately extracted for 10 min in 10 vol. of 0.5 mol/L acetic acid in a water bath at 100 ²C. After cooling, the tubes were stored at 20 ²C until analysis.

Liver function tests

Plasma glucose was measured by the glucose oxidase method using a glucose analyzer 2 (Beckman, Fullerton, CA). Total serum protein, serum albumin, bilirubin, AST and ALT were determined by routine laboratory procedures. Prothrombin time was measured using standard thromboplastin (Ortho Recombiplastin, Ortho Diagnostics, Raritan, NJ) with a Koagulab 16S (Ortho Diagnostics). Fibrinogen was determined according to Von Clauss's method. Blood ammonia concentration was determined by an enzymatic test (BioMerieux Lab., France) and blood urea concentration by Berthelot's method.

Radioimmunoassay for insulin, glucagon, somatostatin

Plasma. Basal plasma levels of insulin and glucagon were determined by radioimmunoassay (INEP-Diagnostics, Zemun and NovoBioLabs, Bagsvaerd, Denmark, respectively). Basal plasma somatostatin was measured using commercial kit (Procnix, Pharmaceuticals, Inc. Affinity Research Products Ltd).

Pancreas. Pancreatic content of insulin, glucagon and somatostatin was measured using the same RIA kits as above.

Liver histology. Multiple tissue samples from the left major liver lobe were processed for histological examination by a routine procedure. The liver sections were examined under the microscope after hematoxylineosin, Masson's trichrome, PAS and Perl's staining. In liver sections stained with Masson's trichrome, semiquantitative assessment of fibrosis was blindly performed.

Electron microscopic studies of the liver. Small liver pieces were immediately placed in cold 3% glutaraldehyde (BDH, England) in 0.2 mol/L caccodylate buffer with 0.2 mol/L sucrose (pH 7.2) and fixed overnight, at 0 4 ²C on a rotor. Then, tissue samples were post-fixed in 1% osmium tetroxide (Fluka, Germany) (in cacodylate buffer, pH 7.4) for 1 hour at room temperature. They were then embedded in Araldite (Fluka, Germany). One micrometer thick sections were cut, stained with toluidine blue and examined under the light microscope. Ultrathin sections were cut and stained with uranylacetate followed by lead citrate. The sections were examined in a JEM 1200 Joel electron microscope (Japan).

Statistical analysis

All values are expressed as mean Ò SD. Statistical analysis of data was made using the Mann Whitney nonparametric test and $p < 0.05$ was accepted as significant.

Results

Body and liver weight and serum parameters relevant for liver functions

Body weight, liver and pancreatic weights, as well as serum parameters characteristic for liver functions in each experimental group at the time of death are summarized in *Table I* and *II*.

Body weights were not significantly different between the groups after an 8-week experimental period. However, on week 8 of the experiment, both the relative and absolute weights of liver and pancreas in rats subjected to PCS were lower than in controls (C and SHAM groups).

Table I Absolute and relative weights of liver and pancreas in three groups of rats at the end point of experiment

Weights	C (N=11)	SHAM (N=14)	PCS (N=27)
Body weight (g)	234 12	237 22	236 34
Liver weight (g)	6.9 1.3	6.7 1.5	5.1 1.2**
% of body weight	2.9 0.5	3.0 0.5	2.1 0.2**
Pancreatic weight (g)	0.77 0.27	0.8 0.29	1.12 0.38**
% of body weight	0.33 0.11	0.37 0.13	0.49 0.17*

** p<0.01, * p<0.05

Table II Biochemical data in three groups of rats at the end point of experiment

Biochemical parameters	C (N=11)	SHAM (N=14)	PCS (N=27)
Basal glycaemia (mmol/L)	5.2 1.1	4.9 1.0	4.0 1.1**
Blood urea (mmol/L)	5.4 0.9	5.5 1.1	5.7 1.6
Blood ammonia (μmol/L)	59.5 9.6	53.5 7.9	183.4 38**
AST (U/L)	67 9.1	69 7.3	107 23.8**
ALT (U/L)	34 2.2	33 2.1	59 3.1**
Serum bilirubin (μmol/L)	8.03 0.17	7.69 0.17	10.08 3.42
Total plasma proteins (g/L)	70 1	69 2	67 2
Plasma albumin (g/L)	35 2	33 1	34 1
Prothrombin time (INR)	1.01 0.03	0.97 0.09	1.00 0.01
Fibrinogen (g/L)	7.0 0.9	6.9 0.6	7.3 0.3
** p<0.01			

Table III Pancreatic hormones in control rats (C), sham operated rats (SHAM) and rats with portacaval anastomosis (PCS) at the end point of experiment

Animals	Insulin		Glucagon		Somatostatin	
	Serum (mIU/L)	Pancreatic tissue content (mIU/g)	Plasma (pg/mL)	Pancreatic tissue content (pg/g)	Plasma (pmol/L)	Pancreatic tissue content (pmol/g)
C	20.6 12	14.6 6.9	196.5 44	1256 59.4	70.5 10.9	400 56
SHAM	21.3 9.7	13.2 5.0	201.5 36	1345 41.3	76.8 9.7	397 38
PCS	17.7 8.0	12.2 7.3	555 97**	2280 53.7**	73.2 8.6	385 62
** p<0.01						

After PCS there was a highly significant increase ($p < 0.01$) in levels of blood ammonia, AST and ALT, while concentrations of blood urea, bilirubin, total plasma protein, plasma albumin, prothrombin time and fibrinogen remained unchanged. Serum fasting glucose levels were significantly lower in PCS rats in comparison to controls and sham operated rats.

Basal blood levels and pancreatic contents of insulin, glucagon and somatostatin

Pancreatic hormones assays *(Table III)* showed that in rats with PCS serum insulin was slightly but not significantly reduced, plasma somatostatin was not significantly changed, and plasma glucagon was markedly increased. Also, pancreatic insulin content was significantly reduced in rats with PCS, while glucagon content was significantly increased in PCS rats. Pancreatic somatostatin content was not significantly changed.

Discussion

One of the most persistent consequences of the end-to-side PCS in rats was the reduction of body weight during the first 2 4 postoperative weeks in spite of ad libitum feeding (3). After that period, body weight gain in rats with PCS coincided with the increase in animals subjected to sham procedure as well as in controls, 8 weeks postoperatively. All metabolic changes developing in this period were PCS-specific and independent of food intake.

Portacaval shunt caused liver atrophy, manifested by a significant reduction in liver weight, and as a percentage of body weight (3, 10).

Liver histological findings in rats with PCS showed glycogen reduction in hepatocytes and sinusoidal dilatation around the hepatic vein. Moreover, Kupffer's cells filled with haemosiderin, degenerative changes, and microvesicular fatty changes in hepatic parenchyma surrounding the portal space, were visible. Atrophy of hepatocytes in other parenchymal zones was present. Apoptotic hepatocytes were visible in rats with PCS. These pathohistological changes in the liver which are similar to the findings of other authors (14, 10) cause metabolic and endocrine abnormalities, i.e. disturbance of glucose homeostasis, reduction of IGF-I concentrations in serum and liver tissue, among others.

Ultrastructural characteristics of hepatocyte cell lesions in rats with PCS at the end point 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. An increase in the number of small lipid droplets followed by

mitochondrial oedema and a significant decrease in glycogen particles content were also noted in PCS rats. These changes partially explain the depression of numerous biosynthetic processes after PCS, such as the production of triglycerides and cholesterol, bile acids, urea synthesis and activity of microsomal enzymatic systems (15, 16).

PCS causes pancreatic hypertrophy, as suggested by a significant increase of pancreas weight in PCS rats compared with C and SHAM rats, either expressed in absolute values or as a percentage of body weight. The growth-promoting effect of PCS in the pancreas suggests that PCS may raise the sensitivity of CCK-A receptors in the pancreas to CCK through increased concentrations of intestinal factors in the circulation (17, 10).

This experimental model of chronic hepatic insufficiency causes metabolic disorders, such as hyperammonaemia (3, 7, 8) which was noted in our study. Hyperammonaemia is most probably the consequence of complete diversion of portal blood rich in ammonia into the systemic circulation and a decreased liver capacity for ammonia uptake.

Hyperammonaemia has a toxic effect on the liver, inducing increased permeability of hepatocyte membranes with significantly increased concentrations of serum transaminase (AST and ALT) as obtained in our study, and a significant positive correlation between ammonia and transaminase concentrations (8). Unchanged concentrations of blood urea (18), bilirubin, total plasma protein, plasma albumin, prothrombin time and fibrinogen were due to unimpairment synthetic function of hepatocytes in PCS rats.

Endocrine consequences of PCS have been reported in rats (5), such as disorders in glucose homeostasis and in plasma concentrations of pancreatic hormones. In our rats with PCS compared with C and SHAM rats, fasting serum glucose levels were significantly reduced, basal insulin level was not significantly changed, and plasma glucagon was markedly increased. These changes were accompanied by a reduction of hepatic glycogen content (liver histology and ultrastructural changes in hepatocytes) and contribute to disorders in glucose homeostasis. Alterations in the secretion pattern of the pancreatic islets play a role in glucose homeostasis (5), but they are primarily related to the functional deterioration of the liver (diversion of the portal blood in the systemic circulation).

Pancreatic insulin content was significantly reduced and, with normoinsulinaemia, indicated that in pancreatic islets of rats with PCS there was an increased storage of insulin in B-cells and reduced synthesis of secretory proteins. Normoinsulinaemia in spite of a decrease in insulin secretion was probably a consequence of diminished extraction of insulin by the liver (5). In patients with cirrhosis hyperinsulinemia appears to be the result of hepatocellular dysfunction rather than of shunting portal blood to the systemic circulation (19). The pathogenesis of insulin resistance in liver cirrhosis includes lower plasma concentrations of IGF-I and increased plasma concentrations of GH, glucagon, catecholamines (20). Moreover, chronic hyperinsulinaemia contributes to insulin resistance in cirrhosis (21).

In the present study, plasma glucagon level and pancreatic glucagon content were increased in PCS rats compared with C and SHAM rats. Our results are similar to those in a previous study that showed hyperglucagonaemia and a normal secretion pattern of A cell. This hyperglucagonaemia was not caused by hypersecretion of A-cells, but by reduced hepatic extraction of glucagon (5, 22). Decreased hepatic catabolism resulting from progressively impaired hepatic function may play a role in the development of hyperglucagonaemia in patients with cirrhosis and portal hypertension (4). However, pancreatic hypersecretion of glucagon may also contribute to increased plasma glucagon levels in patients with cirrhosis and porto-caval anastomosis (23).

Our results indicate that in rats with PCS, basal plasma somatostatin concentrations were not changed, as well as pancreatic somatostatin content, and were associated with hyperglucagonaemia and impaired insulin release. Normal immunoraectivity and ultrastructural patterns of D-cells in rats with PCS may explain our findings (5).

We conclude, that in rats with PCS, metabolic and endocrine consequences are primarly related to the functional deterioration of the liver, which follows the diversion of the portal blood in systemic circulation. Moreower, abnormalities in carbohydrate metabolism may be related to the secretion patterns of pancreatic hormones.

NEKE METABOLIČKE I ENDOKRINE POSLEDICE PORTO-KAVNOG ŠANTA KOD PACOVA

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Kratak sadržaj: Ispitivane su neke metaboličke i endokrine posledice porto-kavnog šanta u pacova, osam nedelja od operacije. Vrednosti insulina, glukagona i somatostatina u krvi, kao i koncentracija ovih hormona u tkivnom ekstraktu pankreasa određivana je radioimunološkim metodama. Standardnim biohemijskim testovima određivana je serumska koncentracija brojnih parametara kao što su glukoza, amonijak, transaminaze, ureja, bilirubin, ukupni proteini, albumini. Vrednosti insulina i somatostatina su bile normalne, a postojala je hiperglukagonemija u pacova sa porto-kavnim šantom. Takođe, vrednosti amonijaka i transaminaza u operisanih životinja su bile značajno više ($p<0,01$), dok je koncentracija glukoze značajno snižena ($p<0,01$). Na osnovu analize dobijenih rezultata može se reći da ispitivane metaboličke i endokrine posledice porto-kavnog šatna u pacova nastaju zbog funkcionalnog oštećenja jetre usled skretanja portalne krvi u sistemsku cirkulaciju.

Ključne reči: pacov, porto-kavni šant, endokrini pankreas, amonijak, glukoza.

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