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Experimental study on the effects of massive bowel resection on liver function and hepatocyte apoptosis

LIVER

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Original Article

ABSTRACT

Background/Aims: The effects of short-bowel syndrome on liver function and liver morphology independent of parenteral nutrition have not been thoroughly investigated. Our aim was to investigate the effects of massive bowel resection on hepatocyte apoptosis and liver function in rats.

Materials and Methods: A total of 37 female Sprague-Dawley rats were randomly assigned to five groups: Control (no procedure); Sham 1 [laparotomy (LT)/enterotomy (ET); evaluated on postoperative day (POD) 1]; Sham 2 (LT/ET; evaluated on POD7; Group 1 (80% bowel resection after LT/ET; POD1); and Group 2 (80% bowel resection; POD7). Blood samples were obtained for measuring aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase levels. For assessing hepatocyte apoptosis, liver tissue samples from the median lobe were obtained and used for a terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labeling assay.

Results: Aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase levels showed statistically significant differences among the five groups. Apoptotic hepatocyte counts there were statistically significant differences among groups for counts made in 20 consecutive high-power fields. However, liver sinusoidal cell apoptosis rates among groups showed statistically significant differences for counts made in 20 consecutive high-power fields, particularly on POD7 in rats undergoing massive bowel resection.

Conclusion: Parenteral nutrition is not the only factor involved in liver dysfunction after massive bowel resection. Massive bowel resection alone can cause liver abnormalities. Rats undergoing massive small intestinal resection show significant temporal increases in liver sinusoidal cell apoptosis rates.

Keywords: Short bowel syndrome, massive bowel resection, liver, apoptosis, liver function tests

INTRODUCTION

Short-bowel syndrome (SBS) is characterized by a state of malabsorption after extensive resection of the small intestine (1,2). There is currently no reliable data regarding the incidence and prevalence of SBS. Data derived from patients who have received home parenteral nutrition (PN) indicate an incidence of severe SBS of 1-2 cases per 100,000 people per year (3). The most common complications that result in mortality during the course of SBS are sepsis and liver failure (4,5). The causes of liver failure in SBS are PN, primary disease processes, and intestinal failure (6). However, the effects of SBS on liver function and liver morphology independent of PN have not been thoroughly investigated either in clinical or experimental studies.

Numerous diseases that involve the liver result in an increase in hepatocyte apoptosis. The most commonly involved diseases are viral hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, graft rejection, and ischemia-reperfusion injury (7).

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Table 1. Experimental groups

Groups	n	Protocol
Control	5	Normal rat LFTs and apoptosis
Sham 1	5	Effects of LT and ET on LFTs and apoptosis on POD 1
Sham 2	5	Effects of LT and ET on LFTs and apoptosis on POD 7
Group 1	9	Effects of 80% bowel resection on LFTs and apoptosis on POD 1
Group 2	13	Effects of 80% bowel resection on LFTs and apoptosis on POD 7

LFTs: liver function tests; LT: laparotomy; ET: enterotomy; POD: postoperative day

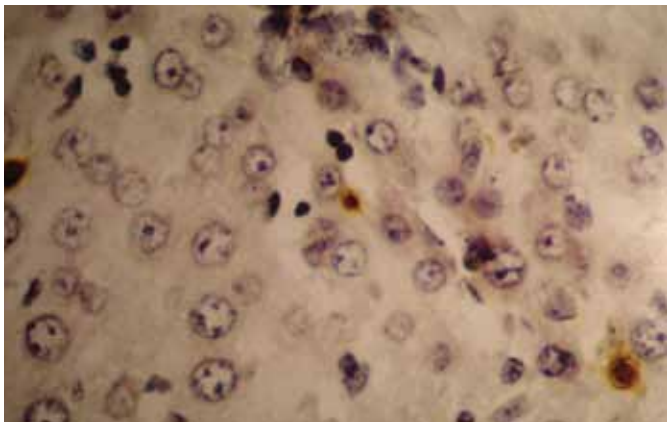

Figure 1. Liver sinusoidal cell nuclei and hepatocyte nuclei with positive reactions in a TUNEL assay (dark yellow-brown staining of the nucleus).

In this study, our aim was to investigate the effects of massive bowel resection on hepatocyte apoptosis and liver function in rats.

MATERIALS AND METHODS

Our study was approved by Ankara University Faculty of Veterinary Medicine Ethics committee (approval number: 2005/09).

A total of 37 female Sprague-Dawley rats (weighing 180-230 g) were used in our experiments. All rats were maintained at room temperature on a 12-h day/night cycle with free access to food and water. All animal procedures were performed according to approved institution protocols and under strict biological containment.

Rats were randomly assigned to five groups (Table 1) and kept in separate cages. Rats that died prior to sacrifice and that had anastomotic leakage or peritonitis during sacrifice were excluded from our analysis (n=6 in Group 1 and n=2 Group 2).

Rats were not given food or water for 3 h before the surgical procedures. Anesthesia was induced by intramuscular administration of ketamine hydrochloride at 50 mg/kg (Ketalar® 50 mg/mL, 10 mL; Ketalar; Parke-Davis, Eczacibasi, Turkey) and Xylazine HCl at 10 mg/kg (Alfazyne® 2%, 20 mg/mL, 30 mL; Alfasan Int. BV, Woerden, Holland). Under sterile conditions, the abdomen was opened with a midline incision (3-4 cm) and the small intestine and colon were exposed. Eighty percent of the small intestine was resected leaving 5-6 cm from the ligament of Treitz and the ileocecal valve. The remaining bowel ends were anastomosed with 6-0 Prolene. Warm sterile saline (10 mL) was left in the peritoneal cavity for postoperative fluid resuscitation before closing the abdomen with 4-0 silk.

For rats in the sham groups, only enterotomy and anastomosis were performed. The bowel was transected at 10 cm proximal to the ileocecal valve and the bowel was reanastomosed with 6-0 Prolene. Warm sterile saline (10 mL) was left in the peritoneal cavity for postoperative fluid resuscitation before closing the abdomen with 4-0 silk.

After administering anesthesia as described above, rats in Sham 1 and Group 1 were sacrificed on postoperative day 1 (POD1), and rats in Sham 2 and Group 2 were sacrificed POD7. The previously made midline incision was extended to the thorax and the chest was exposed. Blood (8 mL) was withdrawn from the left ventricle for aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) tests and stored at -20°C before analysis. The liver was resected en bloc and stored in bottles with 10% formalin. Paraffin blocks were prepared from liver tissues removed from the median lobes and serial sections (4-5-µm thickness) were prepared with a microtome. These tissue sections were used for a terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling (TUNEL) assay, as previously described (8).

Microscopic examinations of the tissue were performed by the same pathologist who was blinded to the experimental groups and using the same microscope (Nikon® Eclipse E200 binocular light microscope; Nikon Eclipse E200; Nikon Corporation, Tokyo, Japan). The pathologist first assessed hepatocyte apoptosis on TUNEL assayed sections for 20 consecutively chosen high-power fields (HPFs) and then 20 randomly chosen HPFs. Liver sinusoidal cell nuclei and hepatocyte nuclei that exhibited a positive reaction (dark yellow-brown staining of the nuclear) were counted (Figure 1).

Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA). Results for AST, ALT, and LDH levels as well as hepatocyte and liver sinusoidal cell apoptosis counts were expressed as means ± standard deviations. Results among groups were compared using either ANOVA or Kruskall-Wallis analysis of variance, depending on whether the data were parametric or nonparametric. Post-hoc analyses were used to identify any statistically significant differences. For all tests, a p-value of <0.05 was considered significant.

RESULTS

Liver function test results

There were statistically significant differences in AST, ALT, and LDH levels among the different groups of rats (Table 2). Signifi-

Table 2. Comparison of liver function test results among groups

	AST Mean±SD	ALT Mean±SD	LDH Mean±SD
Control	82.4±28.8	28.2±5.63	604.2±227.9
Sham 1	295.8±196.7	47±22.79	1335±592.93
Sham 2	104±18.3	31.8±1.92	1578.6±366.67
Group 1	219.8±119	56.89±27.45	768±433.75
Group 2	81±39	20.15±6.99	893.54±516.48
	X^2:22.269	X^2:19.969	F: 4.233
	p=0.000	p=0.001	p=0.007

AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; SD: standard deviation

Table 3. Comparison of apoptotic hepatocyte counts using TUNEL assays between hepatocytes and liver sinusoidal cells

	Apoptotic hepatocyte counts		Apoptotic liver sinusoidal counts	
	Consecutive HPF counts	Random HPF counts	Consecutive HPF counts	Random HPF counts
Control	2.2±1.1	3±1	20.8±5.63	21.8±5.59
Sham 1	2.6±0.89	2.4±0.89	10.8±3.35	11±2
Sham 2	1.4±0.55	1.6±0.89	15±2.83	17±1.58
Group 1	3.56±1.51	4.11±1.27	11.67±2.18	13.56±2.3
Group 2	2.92±2.18	3.46±1.66	22.54±4.35	23±4.81
	X^2: 9.368	F: 3.469	F: 16.024	F: 14.004
	p=0.053	p=0.018	p=0.000	p=0.000

HPF: high-power field

cant differences in AST levels were identified between the Control group and Sham 1, Sham 1 and Sham 2, Sham 1 and Group 2, and Group 1 and Group 2. Significant differences in ALT levels were identified between the Control group and Group 1, Sham 1 and Group 2, and Group 1 and Group 2. Significant differences in LDH levels were identified between the Control group and Sham 2, and Sham 2 and Group 1.

TUNEL assay results for hepatocyte apoptosis

There were no statistically significant differences in apoptotic hepatocyte counts among the groups of rats for consecutive counts under 20 HPFs. However, Group 1 and Sham 1 rats had higher apoptotic hepatocyte counts than rats in the other groups. For assessments of 20 randomly chosen HPFs, there was a statistically significant difference in apoptotic hepatocyte counts between Sham 2 and Group 2 (Table 3).

For liver sinusoidal cell counts under 20 consecutive HPFs, statistically significant differences were observed between Sham 1 and the Control group, the Control group and Group 1, Sham 1 and Group 2, Sham 2 and Group 2, and Group 1 and Group 2. Similarly, for 20 randomly chosen HPFs, liver sinusoidal apoptotic counts showed statistically significant differences among groups. Significant differences were observed between Sham 1 and the Control group, Control and Group 1, Sham 1 and Group 2, Sham 2 and Group 2, and Group 1 and Group 2 (Table 3).

DISCUSSION

Parenteral nutrition is considered to be the most important reason underlying the morbidity and mortality associated with SBS. Most patients with intestinal failure require PN, which results in obscure assessments of other possible causes of changes in liver function tests and liver morphology. There is currently very little information regarding the effects of SBS on liver function independent of PN.

Apoptosis is programmed cell death, and promotes normal physiological growth, differentiation, and tissue turnover. Detecting increased or decreased hepatocyte apoptosis rates in certain disease states has led to the idea of apoptosis dysregulation as an important component of pathophysiology. Almost all of the research that has been done regarding apoptosis in SBS investigated apoptosis in the remaining enterocytes and its effects on the adaptation of enterocytes. Only one study done by Toyama et al. (9) assessed changes in liver morphology and apoptosis after SBS and without PN. Our study is the first to use a TUNEL assay to identify hepatocyte apoptosis after experimentally induced SBS.

We did not find any statistically significant increase in hepatocyte apoptosis on either POD1 or POD7 after small intestinal resection compared with controls or sham-operated groups of rats. However, apoptotic hepatocyte counts were higher in groups in which 80% resection was performed. A significant decrease in apoptotic hepatocyte counts was observed at POD7 compared with POD1. We attributed this decrease to the initiation of intestinal adaptation, which subsequently decreased apoptotic signals.

Our assessment of liver sinusoidal cell apoptosis provided unique findings. There was a striking increase in liver sinusoidal cell apoptosis rates in the livers of Group 2 rats. In contrast to hepatocyte apoptosis, this response was significantly higher on POD7. This interesting finding resembled the effects of ischemia-reperfusion on the liver in previous reports (10-12). Thus, we postulate that a decrease in portal pressure after small intestinal resection creates a low-flow state that induces hypoxia in the liver, resulting in increased liver sinusoidal cell apoptosis. Our experiments are the first to show this relationship.

One intriguing finding of our study was the increased (statistically significant) liver sinusoidal cell apoptosis in the Control group compared with Sham 1 and Group 1. A similar increase, although not statistically significant, was also observed for hepatocyte apoptosis in our Control group. Because we used standard procedures and the pathologist who made these assessments was blinded to the experimental groups, we considered this to be a false-positive result. The reasons for false-

positive apoptotic signals in rat hepatocytes remain unknown. A TUNEL assay may detect single-stranded DNA breaks with free 3´-OH ends that appear during DNA repair and this process may be very active in rat hepatocytes. Even though our samples were fixed immediately, it is also possible that hepatocytes retained some post mortem DNA endonuclease activity, which could have resulted in DNA strand breaks that had TUNEL reactivity (13-15).

We did not find any correlations between the increases in AST, ALT, and LDH levels and liver sinusoidal cell apoptosis rates in contrast to hepatocyte apoptosis rates. This was expected as hepatocytes are the primary source of these enzymes. However, one study showed that increased ALT levels were correlated with necrosis rather than apoptosis after ischemia-reperfusion injury (16). We did not quantify necrosis in our study; hence, we cannot comment on this issue. Another weakness of our study is that we did not evaluate molecular markers of apoptosis. This was unfortunately due to financial constraints, although our future studies will focus on more molecular based investigations.

After experimental massive small intestinal resection in rats, there was a significant increase in liver sinusoidal cell apoptosis over time. Hepatocyte apoptosis increased initially, but decreased by postoperative day 7, although this was not statistically significant. There were no correlations between liver sinusoidal apoptosis rates and liver function test results.

Preventing hepatocyte apoptosis in SBS can prevent or decrease the morbidity and mortality of patients with intestinal failure. Parenteral nutrition is not the only factor responsible for liver dysfunction after SBS. Massive bowel resection alone can cause abnormal changes in the liver. Future studies should utilize molecular markers to detect liver apoptosis and identify pharmacological modalities to prevent this after SBS.

Ethics Committee Approval: Ethics committee approval was received for this study from Ankara University Faculty of Veterinary Medicine.

Informed Consent: N/A.

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