THE EFFECT OF LONG-TERM PARENTERAL NUTRITION OF DOGS ON THE HEPATOBILIARY SYSTEM

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SUMMARY: Dogs were subjected to paranteral nutrition with hypertonic glucose and aminoacids for 31 days. During the first ten days there was no significant alteration in liver function tests. The serum cholesterol began to increase on the 11th and SGPT began to increase on the 21st day. Total protein and albumin levels exhibited respective decrease. By the 31st day the rates of increase was 199.6 % for SGOT, % for SGPT, 37.3% for cholesterol, 24.4% for total lipid, 113.2% for alkaline phosphatase and 65.6% for direct bilurubine. The body weight and blood sugar showed no significant change. Marked cholestasis was detected on histopathological examination. Electron microscopic studies revealed increase in the number of the biliary canalicules and presence of inclusion bodies such as dense lipids and lysosomes within the hepatocytes.

Key Words: Parenteral Nutrition, Hepatobiliary System Liver Function tests, Cholestasis.

INTRODUCTION

In 1968, Dudrick *et al.* (9) first demonstrated that the growth, development and positive nitrogen balance can be achieved by longterm paranteral nutrition in animals and man.

In 1971 Peden *et al.* (21) noted the development of cholestasis and cirrhosis in a 1.0 kg premature infant receiving total paranteral nutrition. One year later Anderson (1) reported the occurrence of acute acalculous cholecystitis as a complication of paranteral hyperalimentation and this condition was supported by others (22-24). Various complications including impairment of liver, periportal fibrosis and rises in the incidenses of acalculous and calculous cholecystitis have been reported (2-4, 16-19).

Only the short term effects of paranteral nutrition on the hepatobiliary system have been investigated and the alterations on cell level have not been fully described.

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In this article, the biochemical, macroscopic, microscopic and ultrastructural alterations on the hepatobiliary system have been investigated with details in dogs nourished paranterally for 31 days.

MATERIALS AND METHODS

This experiment was carried out in Surgical Research Unit of Medical Faculty, Cumhuriyet University, Sivas. 21 dogs of both sexes were used. Their weight ranged between 24 and 30 kg (mean 26 kg). They were divided into two groups,

1. Control group (10 dogs),
2. Experimental group (11 dogs).

In the first day of the experiment, blood was taken for the measurements of glucose, cholesterol, total lipid, alkhaline phosphatase, SGOT, SGPT, direct and total bilirubine total protein and albumin. Later all animals were subjected to laparatomy under Kethamine (20 mg/kg) anaesthesia. Liver, gallbladder and bile ducts were examined carefully. A wedge biopsy from the liver was performed and bile cultures from gallbladder were taken.

After closure of the laparatomy incision, a transverse incision on inguinal region was made in the experimental group of dogs and a cathater was inserted into the inferior vena cava by way of femoral vein. A neck plaster was applied to protect the cathaters and dogs were put into a special hammock during the experiment.

The control group of dogs were allowed to take normal diet, but the second group of dogs were nourished paranterally for 31 days. They were given 50 ml/kg of fluid, 2gr/kg of protein and 30 cl/kg per day. Normal saline and 5% dextrose in water given for their fluid balance and their caloric and protein requirements were secured with 17.5 % dextrose in water and Aminosteryl L-400. Vitamins were added into fluids. Heparine was added into the infusates for longterm paranteral nutrition (20).

All laboratory tests were repeated in both of groups on the 11th, 21st and 31st days.

On the 31st day of the test, dogs were subjected to laparatomy again and biopsies and cultures were repeated.

Some pieces of biopsies were stained with hematoksilene eozine (HE) and examined under the light microscope, some pieces of them were sliced as no more bigger than 1 mm^3. These pieces were fixed with glutaraldehyte and 1% Osmium tetraoxide. Then they were subjected to alcoholic dehydration and blocked in araldehyte. Crosscuts in length of 300-700 A were obtained by LKB ultratome and were stained with Uranile acetate and lead citrate and later examined by JEOL C transmission electrone microscope.

Data were analyzed by X^2 analysis and p values less than 0.05 were considered significant.

RESULTS

General findings: Leg edema developed in two dogs on the second day of the experiment and regressed after seventh day. A dog from the second group died due to cathater related sepsis on the ninth day of the experiment.

Weight changes and cholesterol, glucose, total lipid, alkhaline phosphatase, SGOT, SGPT, total and direct bilirubine, total protein and albumin alterations are seen in Table 1.

Cholesterol showed a biphasic alteration and on 11th day of the experiment was found significant and later it became insignificant again.

Alkaline phosphatase values reached significant values on the 31st day. SGOT and SGPT values have gained significance on the 21st day. Direct bilirubine, total protein and albumin showed significant values on the 31st day.

Macroscopic findings: A 3 to 4 fold increase in the size of the gallbladder observed during second laparatomy in the experimental group and there was biliary mud in the gallbladder. On the contrary were gross alterations in the size of the gallbladder were not observed in the control group.

No organism grew in the blood cultures of both groups.

Microscopic findings:

A. Light Microscope : Sentrizonal necrosis, fibrosis and significant cholestasis were seen in biopsies (Figure 1) obtained during the second laparatomy in the experiment group.

Table 1

	1st Day			11th Day			21st Day			31st Day		
	CG	EG	P	CG	EG	P	CG	EG	P	CG	EG	P
W	26.7±2.4	26.5±2.9								26.7±2.4	25.6±3.1	>0.05
Gl	80.5±4.9	83.3±8.7	>0.05	82.5±2.4	79.4±14.0	>0.05	77.3±5.3	85.3±19.7	>0.05	91.2±14.4	101.2±16.3	>0.05
Chl	179.13±13.02	166.0±17.7	>0.05	179.0±11.9	223.3±44.2	>0.05	180.2±17.6	19.2±41.8	>0.05	180.4±46.2	228.0±78.1	>0.05
T.I	740±107.5	715.5±66.9	>0.05	750.4±62.4	810.3±106.4	>0.05	720.3±63.2	774.5±63.2	>0.05	710.2±65.8	890.2±204.5	<0.05
A-P	1.76±0.11	1.81±01	>0.05	1.77±0.11	2.39±1.41	>0.05	1.79±0.08	2.42±1.01	>0.05	1.89±0.41	3.86±2.87	<0.05
SGOT	26.4±1.8	25.4±7.3	>0.05	26.2±6.8	24.3±11.2	>0.05	25.3±6.5	55.3±4.2	<0.05	33.5±8.1	79.1±4.6	<0.05
SGPT	18.6±4.4	17.9±7.3	>0.05	17.8±5.4	28.0±21.4	>0.05	18.6±4.3	45.1±21.3	<0.05	18.2±6.4	46.9±2.7	<0.05
T-B	0.62±0.04	0.70±0.11	>0.05	0.66±0.06	0.63±0.09	>0.05	0.63±0.04	0.69±0.14	>0.05	0.79±0.11	0.86±0.06	>0.05
D-B	0.32±0.09	0.32±0.20	>0.05	0.35±0.07	0.33±0.09	>0.05	0.33±0.04	0.35±0.10	>0.05	0.39±0.09	0.53±0.07	<0.05
T-P	6.83±0.33	6.41±0.46	>0.05	6.38±0.39	6.14±0.89	>0.05	6.36±0.30	5.32±0.76	>0.05	6.50±0.71	5.34±1.29	<0.05
Alb	3.19±0.40	3.38±0.48	±0.05	3.34±0.53	2.94±0.64	>0.05	3.26±0.44	2.22±0.34	>0.05	3.46±0.64	2.27±0.44	<0.05

W: Weight (kg), Gl: Glucose (%mg), Chl: Cholesterol (%mg), T.I: T. Lipid (%mg), A-P: A. Phosphatase (KU), SGOT: SGOT (KU), SGPT: SGPT (KU), T-B: T. Billuribine (%mg), D-B: D. Billuribine (% mg), T-P: T. Protein (%mg), Alb: Albumine (%mg)

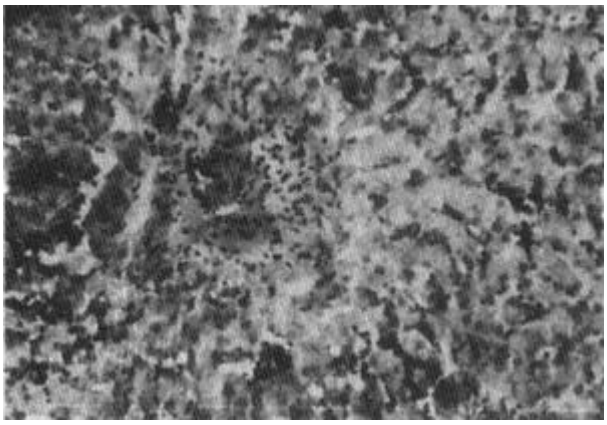

Figure 1: Sentrizonal necrosis, fibrosis and cholestasis in liver biopsies experimental group of dogs HE X 100.

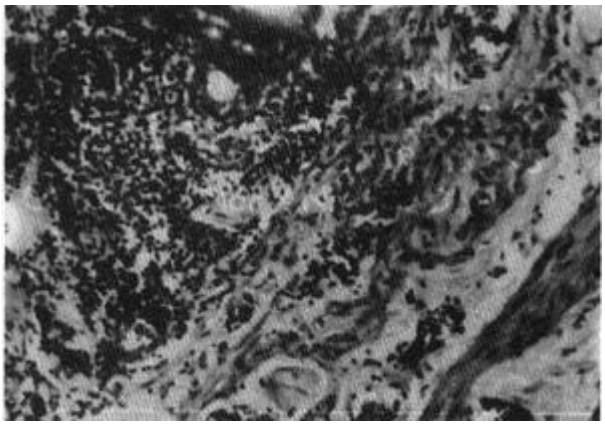

Figure 3: Mononuclear cell infiltration in the gallbladder wall in experimental group of dogs. HE X 40.

Figure 2: Cystic enlargements in gallbladder biopsies in experimental group of dogs. HE X 40.

Figure 4: Liver biopsies in the 31st day of experiment in the second group of dogs. Electromicroscopic apperance X 36500 Mc-Microvillus BC-Biliary canaliculus.

Cystic enlargements in mucosal glands were observed in gallbladder biopsies (Figure 2) and mononucleer cell infiltration were also seen in the wall of gallbladders in the experiment group (Figure 3).

These findings were not encountered in the control group.

B. Electron microscope: The most important findings in the experimental group were the significant proliferation and enlargement of blie canaliculi (Figure 4). In some of them there were also decreases of microvilli. Lipid and lysosomal inclusion bodies were frequently seen in liver cells (Figure 5).

DISCUSSION

The causes of changes of liver function tests during longterm parateral hyperalimentation have not been fully explained (8,17,26). Toxity from Tryptophan degration products (13), long-term infusion of glucose and alterations in lipid calori ratio (27) have all been blamed for these changes. Bernstein *et al.* (5) and Vilersis *et al.* (28) have stated that paranteral nutrition associated cholestatic jaundice is related to a relative nutrient excess which includes both protein and carbonhydrate but not lipid.

Linder *et al.* (17) have found a meaningful increase in SGOT levels in 68% of patients, alkaline phosphatase in 54% and serum bilirubine in 21% patients. The median time interval of peak increase for each of the three tests was between 9 and 12 days after total paranteral nutrition (TPN) was started. Grant *et al.* (13) have stated elevations in SGOT, SGPT, bilirubine and lactic dehydrogenase. On the cortrary Black *et al.* (6) have reported that SGOT, SGPT and bilirubine levels were unaffected during one week of TPN.

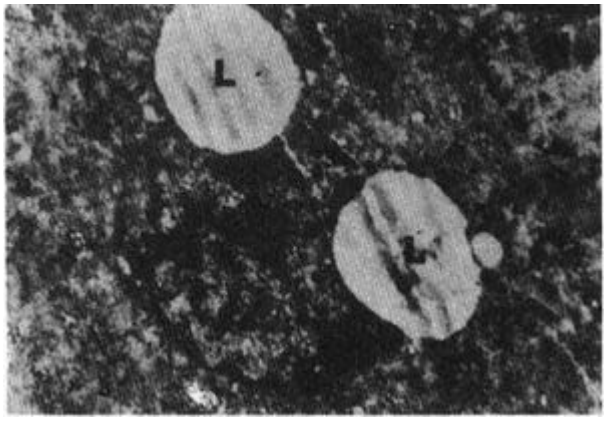

Figure 5: Liver biopsies in the 31st day of experiment in the second group of dogs. L- Lipid vacuoles N-Nucleus.

In our study we did not find any meaningful increases in SGOT, SGPT, alkaline phosphatase, total and direct bilirubine, total protein and albumin total lipid and glucose levels during the first ten days of the experiment. On the 21st SGOT and SGPT levels increased 2.2 and 2.5 fold respectively in experiment group. These increases continued until the end of the experiment, on the 31st day SGOT and SGPT levels increased 199.6 and 162.0% respectively. Increases of direct bilirubine and alkaline phosphatase reached significant values in the 31st day of the experiment. Increases of direct bilirubine and alkaline phosphatase were 65.2 and 113.2% respectively.

A significant increase in cholesterol levels was established in the 11th day. These values decreased on the 21st day and increased 31st day again. The significant elevation of total lipid was established on the 31st day. Menguy *et al.* (18) have found increases in trigliseride levels in patients receiving 25% dextrose and 4.25% aminoacid solutions. In this experiment we have used amioacide and hypertonic dextrose solutions, but lipid solutions have not been used.

If rapid changes occur in the patient's ability to tolerate glucose Loads, hyperglycemia, glucose intolerance and glycosuria appear (13,15,16,19). Grant *et al.* (13) have reported that 68% of the patients with TPN demonstrated initial hyperglycemia when blood sugar concentrations greater than 180 mg. 100 milliliters and 56% required maintenance insulin supplementation.

Many investigators have reported that the growth development and positive nitrogen balance can be achieved by long-term paranteral nutrition (7,9,10,14). Kirkpatrick *et al.* (15) have divided the patients into two groups. In the first group nutritional support was provided with dextrose, aminoacids and fat. In the second group lipid solutions had not been used. Positive nitrogen balance have been achieved in both groups.

Weight reduction had been identified in some of our experimental group of dogs and total protein and albumin also decreased on the 21st day of the experiment. These decrease have been mostly encountered in dogs whose cathaters have moved out from the lumen of the vein. These decreases have also been accounted for trauma and stress.

Trauma, sepsis and stress have been blamed for negative nitrogen balance during TPN (7,9,15). One of our dogs died due a cathater releated sepsis.

Roslyn *et al.* (25) have found gallstones (83%) and acalculous cholecystitis (6%) in their 136 patients. They have identified biliery sludge, gallstones or both in 92% of patients by abdominal ultrasonography. Similar findings have also been reported (11,22,24).

Doty *et al.* (12) have showed that in 10 dogs with TPN gallbladder volume increased 3-4 fold and identified the presence of biliary sludge in all dogs of experimental group.

Many histopathological changes occur in liver and gallbladder during TPN. Benjamin (22) has identified intracelluler and intracanlicular cholestasis, periportal fibrosis and bile duct proliferation in most of his 15 patients during TPN. Beala *et al.* (3) have found that canacilar cholestasis, direct hyperbilirubinemia cellular bile retention, and diffuse degeneration of hepatocytes. Similar findings have been reported by others (3,4,13,17).

We have found significant cholestasis on the 31st day of the experiment in the experiment group. We have also identified 65.6% increase in direct bilirubine. We have also found necrosis around the central veins and thickening of these veins with fibrosis. In gallbladder mucosa we have identified cystic enlargements and mononuclear cell infiltration.

We have also found proliferation of bile canaliculus, disappearing of microvilli and increases of lipid and lysosomal bodies in both liver and gallbladder.

In conclusion long-term TPM is associated with significant macroscopic and microscopic changes in liver and gallbladder.

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