THE EFFECTS OF LONG-TERM PARENTERAL NUTRITION ON DOG PANCREAS: AN ELECTRON MICROSCOPIC STUDY
Uzun Süreli Parenteral Beslenmenin Köpek Pankreası Üzerine Etkileri: Elektron mikroskobik Çalışma

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Abstract

Purpose: *The aim of this study was to examine the effects of total parenteral nutrition (TPN) on dog pancreas.*

Material and Methods: *Twenty-one dogs were used in this study. The dogs were divided into two groups (10 animals for control and 11 animals for experimental groups). The dogs in the experimental group were anaesthetised and the femoral vein cannulated. After a catheter was exteriorised via a subcutaneous tunnel, parenteral infusion was administered 16 hours daily for 30 days. At the end of TPN administration, the changes in the pancreatic ultrastructure and blood glucose values were evaluated and compared with the control group.*

Results: *There was no significant difference in the mean blood glucose level (BGL) between experimental and control groups (101.2 ± 36.3 mg% and 91.2 ± 14.3 mg%; p>0.05, respectively). Also there were no distinguishable ultrastructural differences in either exocrine or endocrine pancreases of both groups.*

Conclusion: *These results indicates that long term parenteral nutrition has no effects on either exocrine and endocrine pancreas.*

Key Words: *Dogs; Pancreas; Parenteral nutrition*

Özet

Amaç: *Bu çalışmanın amacı köpek pankreası üzerine total parenteral beslenmenin etkilerini araştırmaktır.*

Gereç ve Yöntem: *Çalışmada 21 köpek kullanıldı. Köpekler kontrol (10 hayvan) ve deney (11 hayvan) gruplarına bölündü. Deney grubu köpekler uyutulduktan sonra femoral venleri kanüle edildi. Katater deri altı bir tünelle dışarı çıkarıldıktan sonra 30 gün boyunca günlük 16 saat parenteral infüzyon uygulandı. Total parenteral beslenmenin uygulamasının sonunda pankreatik ultrastrüktürdeki değişiklikler ve kan glukozu düzeyleri değerlendirildi ve kontrol grubu ile karşılaştırıldı.*

Bulgular: *Deney grubundaki ortalama kan glukoz düzeyi kontrol grubundan yüksekti (deney grubu 101.2±36.3 mg%, kontrol grubu 91.2±14.3 mg%; p>0.05), fakat bu istatistiksel olarak önemli değildi. Ayrıca, kontrol ve deney gruplarının ekzokrin ve endokrin pankreaslarında ultrastrüktürel farklılıklar yoktu.*

Sonuç: *Bu sonuçlar total parenteral beslenmenin ekzokrin ve endokrin pankreas üzerine bir etkiye sahip olmadığını göstermektedir.*

Anahtar Kelimeler: *Köpekler; Pankreas; Parenteral beslenme*

Total parenteral nutrition (TPN), also defined as parenteral hyperalimentation, provides daily necessities such as calorie, electrolytes, vitamins and fluid through the veins of patients who suffer from gastrointestinal disease or malnutrition (1,2). Dutrick et al. (3) showed that normal growth and development of dogs can be realised with TPN. Later, TPN has successfully been applied to postoperative patients who suffered malnutrition (4) and insufficiency of kidney (5). Although this procedure has been applied for a number of years, a variety of complications, especially failure of liver (2), gallbladder (6) and kidney (5), have been identified.

The stimulation of synthesis and secretion of pancreatic enzymes depends on gastrointestinal hormones (GH). This stimulation disappears without oral feeding. Hence, it is expected that pancreatic secretion stops during TPN. For this reason, the aim of this study was to determine whether long-term total parenteral nutrition would result in histological changes in pancreatic ultrastructure of dogs.

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MATERIALS AND METHODS

This study was performed on 21 dogs of both sexes (approximate weight 26.6 kg). Before the experiment, rapid inoculation and antiparasitic treatment were applied and the animals were acclimated for 3 months. The dogs were divided into two groups as the control (n: 10) and the experimental (n: 11) group and then labelled.

In the experimental group, under general anaesthesia with Ketamine (15-20 mg/gr), the femoral vein was dissected by 4-5 cm transversal incision at inguinal region. After ligating distal part of the vein, 30 cm part of 1.8 mm polyethylene catheter was introduced into the lumen of v. cava inferior and tied in place. The remaining part of the catheter was taken out of skin from the back and fixed on the neck. The necks were plastered and the dogs were settled to hammock to protect the catheter during the experimental period.

TPN supplied with hypertonic glucose, essential amino acids, electrolitary solutions and vitamins were applied to the experimental group 16 hours daily for 30 consecutive days without oral feeding. In this way, fluid (50-60 ml/kg), protein (1.5-2 gr/ kg), calorie (30 cal/kg) were given daily. The parenteral solution included 0.9% NaCl, ringer lactate + 5% dextrose, 35% dextrose and amino sterile L-400 (mixture of mixprotein, electrolyte, sorbital and vitamin). Vitamin B and C were also added to parenteral fluids and vitamin K injected intramuscularly every ten days.

Blood samples were obtained from both groups on days 0, 11, 21 and 31 to determine the BGL, and then compared using Student's *t* test.

On the 31st day, under anaesthesia with Ketamine, samples of pancreatic tissue of both groups were removed. For histological studies, the samples were fixed by immersion in 2 % gluteraldehyde in phosphate buffer and then postfixed with 1% osmium tetroxide in the same buffer. Subsequently, they were dehydrated in a graded ethanol series, and embedded in Araldite. Ultrathin sections were prepared using a LKB-5 ultra microtome and sections stained with uranyl acetate and lead citrate, and examined using a JEOL 100-C electron microscope.

RESULTS

The result showed that there were no significant differences in BGL between the control (80.5±4.9 mg%) and experimental (83.3±8.7 mg%) groups on day 0. The BGL values were also nearly the same on the 11th day (control group; 82.5±12.04 mg% and experimental group; 79.4±14.04 mg%) and on the 21st day (control group; 77.3±5.33 mg% and experimental group; 85.3±19.73 mg%). On the 31st day, BGL of experimental group was slightly higher than the control group (101.2±36.3 mg% and 91.2±14.3 mg%, respectively), but it was not statistically significant ($p>0.05$) (Figure 1).

At the ultrastructural level, acinar cells of exocrine pancreas of the control group were seen to be filled with zymogen granules. These zymogen granules varied in size, although larger ones were more abundant. They were round and electron dense. Their nuclei were irregular and small. The cisternae of rough endoplasmic reticulum were also abundant (Fig. 2).

The pancreatic islets of control animals presented the well-known normal general morphological features. The beta cells showed typical secretory granules consisting of electron dense core with crystalloid in shape. The plate-like granules did not fill the rounded membranous vesicles. The granules of alpha cells were larger and more electron dense than those of beta cells. They were round and homogenous, and they filled the membranous vesicles (Fig. 3).

The acinar cells of exocrine pancreas of the experimental group on day 30 had many zymogen granules similar to the control animals. The morphological features of organelles and inclusions in these cells were similar to the control group and they were not effected by TPN (Fig. 4). Alpha and beta cells of Langerhans islets of experimental group, with ultrastructural features, were also similar to the control group (Fig. 5).

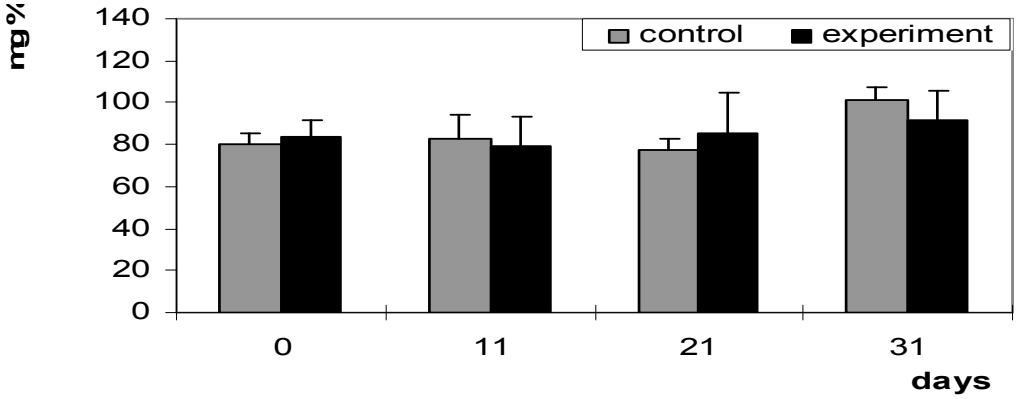

Figure 1. *Mean blood glucose levels of control and experimental group. There was no significant difference between the groups (P>0.05).*

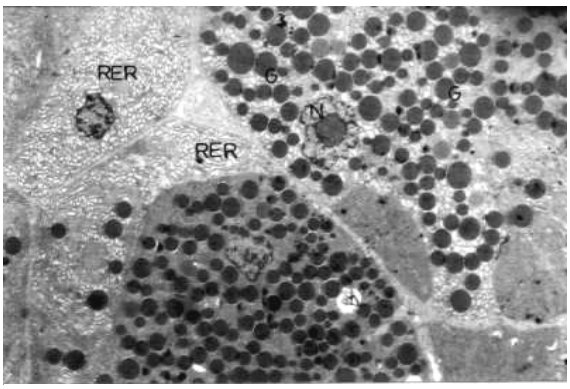

Figure 2. *Exocrine pancreas of control group. Acinar cells of pancreas including zymogen granules (G), lamellae of rough endoplasmic reticulum (RER) and nucleus (N), (X2000).*

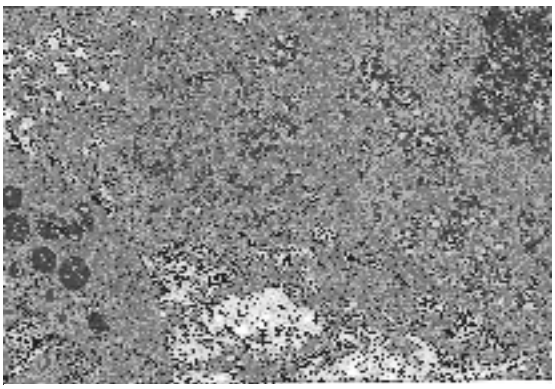

Figure 3. *Electron microscopy of endocrine pancreas of control group. Alpha (A) and beta (B) cells are containing typical secretory granules, (X4500).*

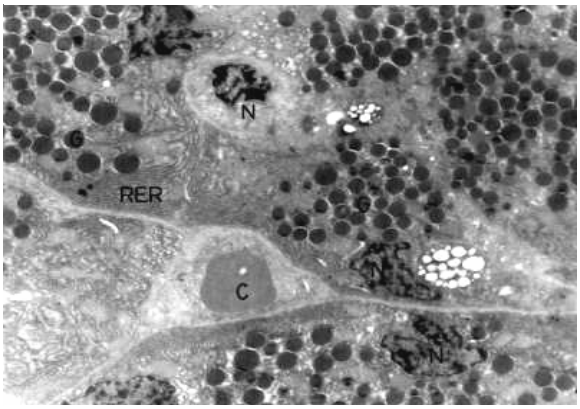

Figure 4. *There are no changes in exocrine pancreas of experimental group after TPN. Nucleus (N), rough endoplasmic reticulum (RER), zymogen granules (G) and capillary (C), (X2500).*

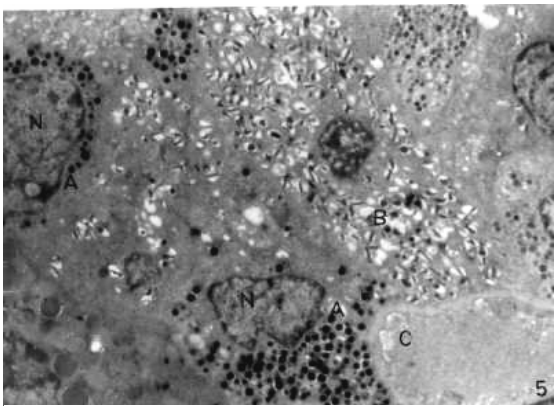

Figure 5. *Islet of Langerhans of experimental group. Alpha (A) and beta (B) cells are normal after TPN. Nucleus (N), capillary (C), (X4500).*

DISCUSSION

Because of widely used applications in clinical sciences, many experimental and clinical studies have been performed on TPN. Some of these studies are on parenteral fluids, others related to complications of TPN. A variety of complications of long-term TPN are well documented. These complications arise as a result of placement or maintenance of the intravenous catheter required for the infusion or from excessive or insufficient provision of one or more nutrients (7). During long-term infusion, TPN results in abnormal liver function (1,2), gallbladder disease (6), atrophy of digestive system, liver cell damage, gallstone formation, and increase of sepsis of gut origin (8).

Because of lack of gastrointestinal stimulation by oral feeding, reduction of GH secretion and interruption of enterohepatic circulation of bile may be found (8). The activity of the pancreas, particularly that of the exocrine pancreas, depends on GH, and the pancreatic atrophy might occur during TPN.

There are fewer studies on effects of TPN on pancreas. Most of them are clinical and biochemical studies, and histological studies are insufficient. In the present study, we have examined the changes of BGL without taking into consideration GH, insulin and glucagon levels, and the ultrastructural effects of long-term TPN on the pancreas.

BGL of experimental group was less elevated than that of control group, but it was within normal ranges. In previous studies, it has been suggested that hyperglycaemia and hyperuremia, i.e. intolerance against glucose, appeared after long-term TPN (5,9,10). The cause of this contradiction between the results of our study and the studies mentioned above may be the application of parenteral transfusion for 16 h. instead of 24 h. daily. If transfusion had been applicated for 24 h., BGL of experimental group in our study might have resembled the results of the studies above.

Furumoto (11) reported that concentration of serum gastrin was decreased but cell dynamics did not change after 3 weeks of TPN application. Traverso et al. (12) also suggested that TPN did not change the ultrastructure of the pancreas in a period of 3 days. However, this period could be too short to expect possible morphological effects of TPN on the pancreas. In the present study, despite long term parenteral nutrition without oral feeding, no morphological changes in the exocrine pancreas were found. The presence of zymogen granules indicated synthesis of pancreatic enzymes in both groups. The result of this present study was similar to those of the studies above. The present findings from endocrine pancreas led us to believe that TPN does not affect glucagon and insulin synthesis of alpha or beta cells. The presence of secreting granules in alpha and beta cells is evidence of synthesis, which might explain why BGL is in the normal range. This result was similar to the findings of Furumoto (11) who found that TPN did not change the levels of enteroglucagon and pancreatic glucagon in dogs.

In conclusion, these results suggested that long-term parenteral nutrition has no effect on either exocrine or endocrine pancreas, at least in dogs, and this could have contributions on the clinical applications of TPN.

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