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The comparison of the intestinal adaptation effects of subcutaneous and oral insulin in a rats with short bowel syndrome

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ABSTRACT

Aim: Insulin has been reported to have positive effects on intestinal adaptation after short bowel syndrome when applicated oral or subcutaneously. The purpose of this study is to compare the intestinal adaptation effects of subcutaneous and oral routes of insulin in rats with short bowel syndrome. **Materials and Methods:** The short bowel syndrome (SBS) was performed through 70-75% of small intestinal resection and an end-to-end anastomosis. The control group rats underwent SBS only. In the second group, oral insulin (1 U/ml) was administrated twice-daily. In the last group, the insulin was administrated subcutaneously (1 U/kg) as in the control group. All rats were killed on day 15. Outcome parameters were weight of small intestine, the crypt length, villous depth, the blood levels of vascular endothelial growth factor (VEGF), and granolocyt-monocyst colony-stimulating factor (GMCSF). **Results:** Intestinal weight was significantly more in oral insulin group and subcutaneous insulin group than in the control group (72.6 ± 4.3, 78.6 ± 4.8 and 59.7 ± 4.8) ($P < 0.05$). There was no difference between the groups according to villus length, crypt depth, and villous/crypt ratio both in proximal and distal parts of the resected bowel ($P > 0.05$). VEGF values were not statistically significant between the groups (200.3 ± 41.6, 178.9 ± 30.7 and 184.3 ± 52.2) ($P > 0.05$). GMCSF was statistically higher in the control group than in other groups (3.34 ± 1.34, 1.56 ± 0.44 and 1.56 ± 0.44) ($P < 0.05$). **Conclusion:** Insulin has positive effects on intestinal adaptation in short bowel syndrome. Subcutaneous administration is slightly more effective than the oral route.

Key words: Insulin, intestinal adaptation, short bowel syndrome

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INTRODUCTION

Short bowel syndrome (SBS) is defined as massive intestinal loss with compromised bowel adaptation in congenital or acquired pathologies.[1] Patients with SBS have many complications related to prolonged total parenteral nutrition (TPN) and long-term hospitalization such as cholestasis, liver failure, multiple systemic infections, and central line complications. In SBS, intestinal adaptation is crucial for survival and weaning from TPN. Pancreaticobiliary secretions and intestinal hormones are as factors for process of intestinal adaptation.[2] Many hormones have been studied in SBS such as Growth hormone (GH), insulin-like growth factor family (IGF), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF).[3] The IGF family consist of three peptides; insulin, insulin-like growth factor I (IGF-I), and insulin-like growth factor II (IGF-II).[4] Some studies suggested that insulin has positive effects on the intestinal structure and absorptive function.[5-7] It had been used whether oral or subcutaneous route in these studies.[1,3]

We aimed to compare the intestinal adaptation effects of subcutaneous and oral administration of insulin in rats with short bowel syndrome and their differences.

MATERIALS AND METHODS

Thirty Wistar albino rats (200-250 g) were used in this study with the approval of the Animal Research and Ethic Committee of Ondokuz Mayis University. Rats were kept in room temperature and were allowed water and liquid diet *ad libitum*. All rats underwent laparotomy and 75% bowel resection with re-anastomosis.

They randomly assigned equally to three groups; first group ($n = 10$) (SBS) was the control group. They had no further intervention. The second group ($n = 10$) was the subcutaneous insulin group with SBS (SI/SBS). In

this group, insulin was applied subcutaneously at a dose of 1 U/kg, twice-daily, from day 3 to day 14. The third group (n = 10) was oral insulin group with SBS (OI/SBS). In this group, insulin was put in drinking water (1 U/ml) from day 3 to 14.

Laparotomy was performed in all rats with midline incision via intramuscular ketamin (3 mg/kg). A 75% bowel resection between 5 cm distal to ligament of Treitz and 10 cm proximal to the ileocecal valve was performed for SBS. After bowel resection, intestinal continuity was restored by end-to-end anastomosis. The rats were killed on day 15 by intramuscular ketamin injection. All rats' bowel weight was measured (g/cm/100 g), and specimens from both sides of anastomosis were obtained for histopathological and biochemical evaluation.

The intestine specimens were fixed in 10% buffered neutral formalin solution for 24 hours. Formalin-fixed tissues were embedded in paraffin and sectioned at a thickness of 4-6 μm. The sections were stained with haematoxylin-eosin and examined under light microscope. In each section, at least three villous height and crypt length parallel to each other were measured with samba analysis system morfometry module.

Each tissue was homogenized in liquid nitrogen and then sonicated for one minute 220 volt in 2 ml phosphate buffer (10 mM, pH 7,4). Homogenized tissues were stored until examination at -40°C. At the working day, frozen material was waited to melt at room temperature. After melting, homogenized tissues were centrifugated for 5 minutes at 3000 g, and separated supernatant were used for measurement.

Bender MedSystems ELISA kits (Human VEGF-C ELISA BMS297, Human GCSF BMS283 Vienna, Austria) were used for VEGF-C and GM-CSF measurement in the supernatant.

Principle of VEGF-C measurement; VEGF-C present in supernatant binds to antibodies adsorbed to the microwells. A biotin-conjugated policlonal VEGF-C antibody is added and binds to VEGF-C captured by receptors. Then, Streptavidin-HRP is added and binds to the biotin-conjugated VEGF-C. Substrate solution reactive with HRP is added to the wells. A coloured product is formed in proportion to the amount of VEGF-C present in the supernatant. The reaction is terminated by addition of acid, and absorbance is measured at 450 nm.

Principle of GM-CSF measurement; GM-CSF present in supernatant binds to antibodies adsorbede to the microwells. A HRP-conjugated monoclonal anti-GM-csf antibody is added and binds to GM-csf captured by the first antibody. Then, substrate solution reactive with HRP is added to the wells. A coloured product is formed in proportion to the amount of GM-CSF present in the supernatant. The reaction is terminated by addition of acid, and absorbance is measured at 450 nm.

The level of protein concentration in supernatant was measured by Lowry method.[8]

All results were presented as pg/mg protein.

Statistical analysis

For the statistical analysis, SPSS 13.0 was used. After normality analysis, ANOVA or Kruskal Wallis variance analysis was made. The groups were compared with t-test or Mann Whitney U tests, if the distribution was not normal.

RESULTS

The main results were shown on the Tables 1 and 2. Intestinal weight was significantly more in (SI/SBS) group and (OI/SBS) than in the control group ($P < 0.05$). Villus length and crypt depth values in both proximal and distal parts were higher in (SI/SBS) group than in other two groups, but there was no statistical difference ($P > 0.05$). There was also no difference of villus/crypt ratio between groups ($P > 0.05$). Typical histologic images of the groups are shown in Figure 1a-c.

Table 1: Measurments of villous length and crypt depth of groups

	Control	Subcutaneous	Oral
Villous length (μm) (proximal)	229 ± 28.8	301.7 ± 56.2	276.3 ± 65.9
Villous length (μm) (distal)	199.5 ± 32.6	273.9 ± 84.3	263.6 ± 56.2
Crypt depth (μm) (proximal)	172.1 ± 10.5	214 ± 25.1	141 ± 11.3
Crypt depth (μm) (distal)	168.7 ± 12.2	209 ± 11.5	194 ± 17.5

Table 2: Intestinal weight of groups, VEGF and GM-CSF values

	Control	Subcutaneous	Oral
Intestinal Weight (g/cm/100 g)	59.7 ± 4.8	78.6 ± 4.8	72.6 ± 4.3
VEGF (pg/mg protein)	184.3 ± 52.2	178.9 ± 30.7	200.3 ± 41.6
GM-CSF (pg/mg protein)	3.34 ± 1.34	1.56 ± 0.44	2.71 ± 1.04

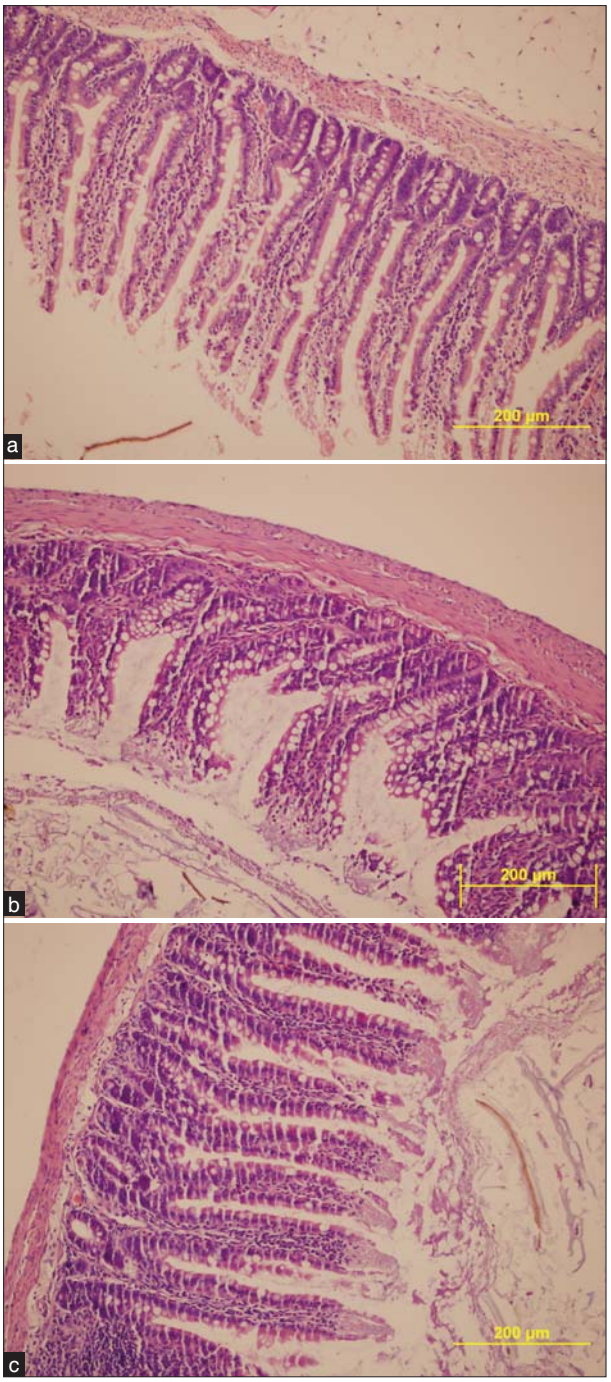


Figure 1: (a) Histologic image of Control group (Hematoxylin eosin staining, x200). (b) Histologic image of oral insulin group (Hematoxylin eosin staining, x200). (c) Histologic image of subcutaneous insulin group (Hematoxylin eosin staining, x200)

Biochemically, vascular endothelial growth factor (VEGF) value was not statistically significant between the groups ($P > 0.05$). Granulocyte-macrophage colony-stimulating factor (GMCSF) was statistically more in the control group than in the subcutaneous groups ($P < 0.05$). GMCSF was also statistically more in the oral group than in the subcutaneous group ($P < 0.05$).

DISCUSSION

Short bowel syndrome (SBS) is massive small bowel resection resulting in an inability to absorb adequate nutrients. The management goal of SBS is to provide adequate nutrition for patient and adaptation of intestine. Affected patients mostly require long-term parenteral nutrition (TPN). Following the SBS, gastrointestinal system undergoes a process of morphological and functional adaptation in terms of intestinal length, diameter and epithelial hyperplasia.[9,10] In SBS, the adaptive capacity of residual bowel determines the duration of TPN treatment and recovery rate.[11,12]

Many growth and trophic agents have been mentioned playing a role in this adaptation process.[13-15] One of the growth factors is insulin-like growth factor (IGF) family such as insulin, IGF-I and IGFII. Several studies have shown the positive role of insulin in the intestinal trophic effect.[4,5,16] In rat models, IGF-I administration improves weight gain and growth of small bowel mucosa after small bowel resection.[17] It has been also shown that IGF-I has positive effects on mucosal DNA to promote cell proliferation and crypt depth of jejunal mucosa.[18,19]

In the present study, we demonstrated that insulin has positive effect on hyperplasia of intestine in SBS. Although we could not be able to show statistical difference between groups according to villus length, crypt depth or rate of those, particularly subcutaneous insulin, is beneficial in this aspect. Our results were similar to the Sukhotnik and Ben Lulu.[4,5]

Granulocyte-macrophage colony-stimulating factor is a cytokine that promotes myeloid cell development and maturation.[20] GM-CSF is effective in treatment of inflammatory bowel disease and promote colonic mucosal restore.[21,22] In the current study, GM-CSF level was lowest in subcutaneous insulin group and was statistically significant. We suggest that GM-CSF depletion indicates the intestinal mucosal proliferation.

Vascular epidermal growth factor (VEGF) is an endogenous peptide and stimulates the intestinal mucosal restore. It may augment the adaptative process and diminish the apoptosis following the small bowel resection.[15,23] We did not demonstrate any significant differences of VEGF level between groups, but the lowest rate was in the subcutaneous insulin. This result led us to think subcutaneous insulin application has superiority on other groups in terms of intestinal adaptation effect.

Lack of further groups with insulin administration and

no SBS are the major limitations of our study.

As conclusion, administration of insulin either oral or subcutaneous has some beneficial effects in rats with SBS. Subcutaneous insulin seems to be more effective than the oral route. The findings of the current study promise improvements of SBS in clinical setting.

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