Effects of melatonin on bacterial translocation in an experimental short bowel syndrome

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The most important reasons for morbidity and mortality in short bowel syndrome (SBS) are septic complications. There is no specific treatment to prevent bacterial translocation (BT) which causes these complications. Melatonin is proven to have positive effects on the gastrointestinal system. The aim of this study was to evaluate the effects of exogenously administered melatonin on BT in SBS. Fifty Sprague-Dawley rats were divided into six groups. In control groups, the rats underwent laparotomy (Group I, Control + saline, n = 5); (Group II, Control + Melatonin, n = 5, 20 mg/kg, IM). In Sham groups, the rats underwent ileal transection (Group III, Sham + Saline, n = 8); (Group IV, Sham + Melatonin, n = 8). In SBS groups, the rats underwent 75% small bowel resection (Group V, SBS + Saline, n = 12); (Group VI, SBS + Melatonin, n = 12). Histopathological and microbiological studies were performed at the 3rd postoperative day. No difference was detected in sites of BT and colony numbers between the various groups. BT was more common in SBS groups than sham groups. However, melatonin administered rats were observed to have decreased the BT rates as compared to their control groups. The number of Kupffer cells in the liver decreased in the ongoing surgery groups while melatonin administration, on the other hand, increased Kupffer cells. Sinusoids were observed to expand due to the surgical operation, while melatonin administration increased parenchymal areas in mesenteric lymph nodes (MLN). In SBS + Melatonin group, there were increased villus heights and total mucosal thickness than those of the control and sham groups. This study demonstrated that melatonin can ensure protection against BT in SBS as it contributes to the maintenance of immune defensive mechanisms and increases intestinal adaptive response.

Key words: Bacterial translocation, short bowel syndrome, experimental, melatonin.

INTRODUCTION

Short bowel syndrome (SBS) is a condition in which the remaining bowel is not enough to absorb fluids, nutrients and electrolytes necessary for the survival of the body. In SBS, complications occur according to the extent of intestinal failure and particularly to parenteral nutrition (Terra et al., 2000). The most important reasons for mortality and morbidity in SBS are septic complications (Coran et al., 1999). Recurrent sepsis attacks may be caused by central line infection or may be of intestinal origin. Microorganisms coming from intestines increase the frequency of infections occurring in SBS patients who are in need of parenteral nutrition (Alverdy et al., 1988).
Central line infections can be minimized with the experience and care of the nutrition team (Duran, 2005). However, there is no specific treatment to prevent bacterial translocation (BT) which causes these complications.

Main parameters to be blamed for enteric bacteria invasion in SBS are bacterial overgrowth and impairment of anatomic-immune barriers (Wiest and Rath, 2003). There are some opposing views on the effect of impaired intestinal permeability on BT in SBS in animal models (O’Brien et al., 2001; O’Brien et al., 2002; Dibaise et al., 2006). One of the reasons why bacteria and their products reach mesenteric lymph nodes and then peripheral organs like blood, liver, spleen can be the decrease of total gut associated lymphoid tissue (GALT) mass as a result of resection and weakening of immune defense mechanisms (Wiest and Rath, 2003; Dibaise et al., 2006).

Melatonin, a derivative of the essential amino acid tryptophan, secreted from pineal gland, is an effective hormone in the neuro-immune-endocrine system (Reiter, 2003; Bubenik, 2002). In experimental studies involving administration of exogenous melatonin, it is demonstrated that it regulates gastrointestinal motility, microcirculation at the intestinal epithelium depending on the dose (Drago et al., 2002) and increases adaptive response in short bowel syndrome (Ozturk et al., 2006). The effect of melatonin on BT is investigated in major liver resection, intestinal ischemic reperfusion damage and experimental colitis model, and it is shown that it decreases bacterial translocation by increasing epithelial regeneration and stimulating the immune system (Cetinkaya et al., 2002; Sileri et al., 2004; Akcan et al., 2008).

The purpose of this study was to evaluate the effects of exogenously administered melatonin on BT after short bowel syndrome.

MATERIALS AND METHODS

Animals

The experimental protocol was in accordance with ‘Helsinki Declaration’ and guide for the care and use of laboratory animals of locally ethical committee. Fifty male Sprague-Dawley rats (Selçuk University, Experimental Research Center, Konya, Turkey) weighing 200 to 250 g were used in this study. All animals were kept under constant environmental conditions. The animals were given free access to water and standard pelleted rat food 3 h prior to operation.

Experimental design

The animals were divided randomly into six experimental groups. The rats in control groups (Group I and II) underwent only laparotomy and was given saline (Group I, Control + Saline, n = 5) or melatonin (Group II, Control + Melatonin, n = 5) for three days. The rats in sham groups, (Group III and IV) underwent bowel transection and was given saline (Group III, Sham + Saline, n = 8) or melatonin (Group IV, Sham + Melatonin, n = 8) for three days. The rats in SBS groups (Group V and VI) underwent 75% small bowel resection and was given saline (Group V, SBS + Saline, n = 12) or melatonin (Group VI, SBS + Melatonin, n = 12) for three days.

Surgical procedure

Animals were anesthetized with ketamine hydrochloride 50 mg/kg and xylazine hydrochloride 10 mg/kg via intramuscularly to the back part of right leg. Before the operation, operating area on the abdomen was shaved and cleaned with povidone-iodine 10%. Using sterile techniques and instruments, midline laparotomy was performed. Sham animals underwent transection of the ileum 10 cm proximal to the ileocecal junction. In SBS animals group, small bowels were resected from the 10 cm distal duodenoejunal junction to the 10 cm proximal of the ileocecal junction. Approximately 75% small bowel resected with this method. Intestinal continuity was restored with end to end anastomosis using a single layer and interrupted sutures with 6/0 prolene. Before abdominal closure, all of the animals received 10 ml 0.9% saline intraperitoneally to supply postoperative fluid necessary. Abdominal wall was closed two layer with 4/0 vicryl running sutures. Animals of melatonin treatment groups II, IV, VI (Control + Melatonin and Sham + Melatonin and SBS + Melatonin) received melatonin (N-Acetyl-5-Methoxytryptamine, Sigma, USA) (20 mg/kg IM daily), while animals in groups I, III, V (Control + Saline and Sham + Saline and SBS + Saline) received same volume of saline solution for three times up to sacrifice. Melatonin or saline solution administration was applied after abdomen was closed. The animals were allowed ad libitum water and chow on postoperative first day.

Sacrificing and bacterial translocation

After 24 h of third administration of melatonin or saline, anesthesia was performed with ketamine hydrochloride 50 mg/kg and xylazine hydrochloride 10 mg/kg via intramuscularly. Under sterile circumstance, another vertical incision was performed where first incision 0.5 cm left laterally because of preventing of contamination. Peritoneal swabs and biopsies of liver, spleen, mesenteric lymph nodes and ileum luminal content were obtained for aerobic cultures. Blood samples were obtained from portal vein and Inferior Vena Cava and than injected into aerobic culture vials Brain Heart Infusion Broth for incubations during seven days and then passages were performed on Blood Agar and Eosin-Methylene-Blue (EMB) mediums. One gram samples were obtained from the mesenteric lymph nodes, liver left lobe and inferior pole of spleen and homogenized aseptically in one ml ordinary buyyon and placed onto sterile Petri dishes Blood agar and EMB. All cultures incubated aerobic condition. Peritoneal swabs and tissue cultures were examined at the end of 24 to 48 h while blood cultures were examined end of the 15th day. A blinded microbiologist interpreted the cultures. Colonization was expressed as the number of colony-forming unit per gram of tissue homogenate (CFU/g). BT was diagnosed when a microorganism was detected simultaneously in the distal ileum and in collected organ.

Histopathological examination

Tissue samples were collected and fixed in 10% formalin solution; and were embedded paraffin waxes using standard techniques. Sections were cut (5 µm each) and each slide was stained with H and E for histopathological study. A blinded histopathology evaluated the slides. Kupffer cells and sinusoidal endothelial cells
Table 1. Frequency of bacterial translocation (% of animals exhibiting bacterial translocation).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>MLN</th>
<th>Liver</th>
<th>Spleen</th>
<th>Vena Porta</th>
<th>Vena Cava</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2(33.3%)</td>
</tr>
<tr>
<td>IV</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1(20%)</td>
<td>0</td>
<td>1(14.2%)</td>
</tr>
<tr>
<td>V</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>*4(40%)</td>
<td>*1(10%)</td>
<td>4(40%)</td>
</tr>
<tr>
<td>VI</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>*2(20%)</td>
<td>*1(10%)</td>
<td>2(20%)</td>
</tr>
</tbody>
</table>

*positive culture on two different sites at the same rate $\chi^2 = 1.825$, $p = 0.610 > 0.05$.

RESULTS

One rat from the SBS + Melatonin group and 2 rats from the SBS + Saline group, who expired in the early postoperative period, were excluded from the study. 2 rats in the Sham + Saline group, 1 rat in the SBS + Melatonin group and 1 rat in the Sham + Melatonin group were accepted as contaminated because of bacterial growth on peritoneal swab and excluded from the study.

Bacteria growing on the ileal lumen, according to their frequency, were 65 Escherichia coli, 20 Proteus, 9 Enterococcus, 43 klebsiella and 2% Candida. No difference was detected in the type of growing microorganisms and CFU between the groups ($p > 0.05$).

Histopathological evaluation

The mean and standard deviation (SD) values of Kupffer cell counts of the groups are shown in Figure 1. There was difference in Kupffer cell count of all the groups ($p < 0.05$). Nevertheless Kupffer cell count decreased in comparison with the control groups ($p < 0.05$), melatonin administration, on the other hand, increased Kupffer cell count in all the 3 groups (Group II, IV, VI). No difference was identified between SBS and sham operation and between Control + Saline and SBS + Melatonin groups as regards the cell count ($p > 0.05$).

The ratio of sinusoidal to parenchymal area in mesenteric lymph nodes was observed increasing.
Sham and SBS groups. There was significant difference in the SBS and Sham groups when compared to the control group (p < 0.05); nevertheless, there was no difference between the 2 groups themselves (p > 0.05). As for the melatonin administered groups, a clear difference was detected compared to their equivalent control groups (Control + Saline vs. Control + Melatonin and Sham + Saline vs. Sham + Melatonin and SBS + Saline vs. SBS + Melatonin) (p < 0.05) (Figure 2). Sinusoids were observed to expand due to the surgical operation (Figure 3A), while melatonin administration clearly increased parenchymal areas (Figure 3B).

Villus height and crypt depth were assessed under the light of the information given in the literature and their ratios to each other and total mucosal thicknesses were calculated (Sukhotnik et al., 2002; Schwartz and Kuenzler, 2001; Scolapio et al., 2001). The villi of rats with SBS were observed to be longer than those in the transection and control groups. A prominent difference was identified between the SBS + Saline and SBS + Melatonin groups. Villus height obviously extended with the administration of melatonin (Figures 4A and B). There was no difference between the groups in terms of crypt depth (p > 0.05). Similarly, no significant difference was detected in Villus height / crypt depth ratio (p > 0.05). In terms of total mucosal thicknesses, however, the mucosal thickness distinctively increased in the SBS groups when compared to the other ones. Significant difference was observed between the SBS + Saline and SBS + Melatonin groups (p < 0.05); melatonin administered group had increased mucosa thickness. Mean and SD values of villus height, crypt depth, villus height or crypt depth, mucosal thickness of the groups are given in Table 2.

According to the analysis carried out using the multiple comparisons test between culture results and histopathological data, there was a correlation only with the mesenteric lymph nodes. When the groups were analyzed according to the growth status on their mesenteric lymph nodes, it was observed that the sinusoidal ratio increased in the lymph nodes where growth took place. Sinusoidal areas of the lymph nodes with and without growth in their cultures are shown Figure 5.

**DISCUSSION**

In SBS the balance between the maintenance or sufficiency of defense mechanisms of the host and bacterial load can be the determining factor of BT. Decreasing bowel movements in patients requiring parenteral nutrition, decreasing adaptive response due to lack of enteral nutrition, overgrowth of bacteria in the lumen and weakening of defensive mechanisms to prevent the transfer of microorganisms from the lumen are the steps of increasing BT in SBS (Welters et al., 2001). Although melatonin is a hormone secreted from the pineal gland, it is found in a higher rate in many tissues, especially in the digestive system, than in blood (Bubenik, 2002; Çetinkaya et al., 2002). Melatonin is proven to have positive effects on the gastrointestinal system in various experimental models. These effects can be summarized as follows; antioxidant and microcirculation regulator (Bubenik, 2002), mucosa protector (Sjöblom and Flemstrom, 2003) immune system modulation and gastrointestinal motility regulator, increasing adaptive response in SBS (Sileri et al., 2004). With its anti-inflammatory and free radical scavenger effect, it decreases tissue damage in stress, ischemia and bacterial infection and it has been demonstrated that it limits the emergence of proinflammatory cytokines...
Figure 2. Sinusoidal / Paranchymal area in mesenteric lymph nodes.

Figure 3A. Sinusoidal area in mesenteric lymph nodes. The arrow shows sinusoidal area in mesenteric lymph node (× 40 magnification).

(Sener et al., 2003; Cho et al., 1989) and provides integrity in wound healing and in acute phase response (Sener et al., 2003).

In this study, the purpose of which was to analyze the effect of melatonin on BT in SBS, aerobic bacteria cultures were used. Although the anaerobic bacteria outnumber the aerobes in bowel flora, the aerobic culture was preferred since aerobic bacteria can perform translocation more easily and they play the leading role in most of the infective complications (Welters et al., 2001; Wells et al., 1987; Schimple et al., 1999; Samel et al., 2002). While the sensitivity of classical bacteria cultures is less according to the PCR techniques (Aldazabal et al., 2008), it was still chosen because of its low cost and widespread routine use (Welters et al., 2001). As the BT starts with the surgical operation and it reaches systemic
Figure 3B. Sinusoidal area in mesenteric lymph nodes treated with melatonin. Melatonin administration clearly decreased sinusoidal areas in mesenteric lymph nodes (×40 magnification).

Figure 4A. villus height in SBS group. Villus height extended in SBS (×100 magnification).

Figure 4B. villus height in SBS group treated with melatonin. villus height obviously extended with the administration of melatonin in SBS group (×100 magnification).
Table 2. Intestinal mucosal parameters.

<table>
<thead>
<tr>
<th>Groups (µm)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villus height</td>
<td>219.4 ± 12.0</td>
<td>245.8 ± 27.9</td>
<td>260.5 ± 67.2</td>
<td>296.0 ± 26.7</td>
<td>307.0 ± 62.3</td>
<td>348.9 ± 42.9</td>
</tr>
<tr>
<td>Crypt depth</td>
<td>142.4 ± 21.4</td>
<td>174.4 ± 21.5</td>
<td>156.1 ± 33.0</td>
<td>175.6 ± 28.3</td>
<td>177.7 ± 28.0</td>
<td>188.8 ± 36.0</td>
</tr>
<tr>
<td>Villus/Crypt</td>
<td>1.6 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.8 ± 0.5</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>Mucosal thickness</td>
<td>357.8 ± 32.0</td>
<td>420.2 ± 42.2</td>
<td>416.6 ± 95.8</td>
<td>471.6 ± 43.2</td>
<td>484.7 ± 64.2</td>
<td>530.7 ± 57.8</td>
</tr>
</tbody>
</table>

Note values are expressed as mean ±SD. *p < 0.05 Group I and II vs. Group V and VI, †p < 0.05 Group III vs. Group V and VI, §p < 0.05 Group IV vs. Group VI, #p < 0.05 Group V vs. Group VI.

Figure 5. Sinusoidal area in mesenteric lymph nodes with and without growth in their cultures.

tissues such as liver, spleen, kidneys and lungs on the postoperative 3rd day (Stenback et al., 2002), BT was assessed on the 3rd postoperative day in this study. No statistically significant difference was identified between the groups in terms of BT. However, the rats going through transection had higher BT rates compared to the control group; just as, rats with SBS had higher BT rates than the transection group. As for melatonin administered groups, on the other hand, BT rate was observed to decrease by half when compared to their equivalent control groups (Sham + Melatonin vs Sham + Saline, SBS + Melatonin vs SBS + Saline). In this study no BT was detected in MLN. Another studies on 80% small bowel resection with fed orally rats showed that 87 to 93 positive cultures in MLN postoperative 10th day (Asensio et al., 2003; Eizaguirre et al., 2002). On the other hand, in rats with SBS (75% small bowel resection and fed orally) reported 68 to 100% BT in MLN, 50 to 60% in portal blood and liver, and 25 to 40% in peripheral blood on the postoperative 15th day (Sukhotnik et al., 2008; Mogilner et al., 2007). In addition, Tian et al. (2009) observed 60% BT in MLN in rats with short bowel syndrome on the 21st day. The reason for difference between the results of the abovementioned studies and this one might be differences of the evaluation time. O’Brein et al. (2001) reported that after resection adaptation parameters was strongest and intestinal permeability was reduced on postoperative day 3. Aldazabal et al. (2008) reported that in rats with 80% small bowel resection BT compared with conventional cultures and techniques of PCR postoperative day 10, was detected 73 to 87% in MLN respectively and they suggested that PCR technique is a more sensitive method for detection of BT (Aldazabal et al., 2008). In the early postoperative period, SBS is not a situation of acute and severely disrupts anatomic-immune barriers as in ischemia/reperfusion injury. Another possible explanation may be that conventional techniques are not more sensitive than PCR for detection of BT in postoperative 3rd day in SBS because of highest adaptation on this term.

The first defense line in the translocation of microorganisms is the MLN; as a result of increasing flow towards the lymph nodes, inflammatory changes start
and sinusoids dilate (Skoog and Tani, 2003). It is demonstrated that melatonin increases the quantity and volume in lymphoid tissues (Bubenik, 2002; Sener et al., 2003; Drazen et al., 2002) and inhibits the apoptosis of immune cells in these tissues (Reiter, 2003). Bubenik (2002) demonstrated that the quantity and volume of Peyer’s patches, which are motor immune tissues of the digestive system, increased in melatonin administered rats and reported positive results in experimental colitis model because of its immunostimulant effects. In this study, the sinusoidal areas in mesenteric lymph nodes were observed to expand because of the surgical operation in rats undergoing bowel transaction and SBS, and parenchymal areas were observed to increase in melatonin administered groups. This data supports the positive effects of melatonin on the immune system.

Microorganisms passing mesenteric lymph nodes face liver, spleen, lung, reticuloendothelial system (RES) cells. Kupffer cells in the liver and sinusoids are responsible for 80% of RES activity (Kuiper et al., 1994). In cases where the Kupffer cells fail in preventing the systemic expansion of translocated bacteria, BT rates increase (Welters et al., 2001; Stenback et al., 2001; Stenback et al., 2002; Sileri et al., 2002). In this study, it was demonstrated that the surgical operation decreased the number of Kupffer cells and SBS did not differ from transaction in terms of the Kupffer cell count, however the number of Kupffer cells increased in all melatonin administered groups. According to the studies carried out; intestinal cell proliferation, villus, crypt and total mucosa thickness increase as an adaptive response to intestinal loss (Francesco et al., 1994; Yang et al., 2003). These changes are crucial in the defense of the organism against microorganisms as well as in adaptation. It is already known that subepithelial edema in ileum sections, blunting and shortening of the villi facilitate bacterial translocation (Sileri et al., 2002). In an experimental intestinal ischemia and reperfusion model, Sileri et al. (2004) demonstrated improvement in mucosal damage parameters such as edema, ulceration and necrosis in melatonin administered rats and a decrease in BT. They argued that it could be related to the decrease of bacterial overgrowth in the ileum with melatonin and the regulating function of melatonin on gastrointestinal motility. However, it is also shown that bacterial translocation does not decrease with treatments increasing gastrointestinal motility, such as lactulose, in SBS and that adaptive response deteriorated as a result of shorter contact period with nutrients (Mogliner et al., 2007). Therefore, it can be argued that melatonin is useful in SBS since it has positive effects on maintenance of immune mechanism, not just on motility regulation or protecting mucosal integrity.

Ileum sections were histologically analyzed in order to comment on mucosal integrity. As we evaluated our results, the groups with SBS had higher villus height and total mucosal thickness as compared to the control and sham groups. Administration of melatonin to the SBS group resulted in statistically significant increase in villus height and mucosal thickness. This result is consistent with the data reported by Yang et al. (2003) who demonstrated that the villus to crypt ratio did not change despite the increase in villus height and total mucosal thickness in ileum remaining after massive small intestine resection. This result is also consistent with the study conducted by Öztürk et al., (2006) in which they performed 90% small bowel resection and reported increase in villus height, mucosal thickness, and mitosis in crypt cells in melatonin administered rats and, as a result, in which they demonstrated that melatonin improved adaptive response in SBS.

This study demonstrates that melatonin can ensure protection against BT in SBS as it contributes to the maintenance of immune defensive mechanisms and increases intestinal adaptive response.

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