Effects of octreotide on healing of mechanical ileus in rats

Suat Kutun, MD, Haluk Ulucanlar, MD, Alper Celik, MD, Erkan Cure, MD, Muhammed C. Kockar, MD, Abdullah Cetin, MD.

ABSTRACT

الأهداف: التحقق من آثار عقار أوكتريوتيد، النظير التركيبي لعقار السوماتوسمين، على الانسداد المعوي الميكانيكي بدون التدخل الجراحي. حيث أن كل عملية جراحية قد ينتج عنها التصاقات جديدة، يعتبر العلاج التحفظي على شكل سوائل كافية، والدعم المنحل الكهربائي وإزالة الضغط من الأمعاء ذات أهمية عالية في الوقاية من الإلتصاقات المتكررة.

الطريقة: صممت هذه الدراسة للتحقق من آثار عقار أوكتريوتيد (ساندوستاتين 1ug/ml – ساندوز)، وعقار سوماتوستاتين طويل المفعول في الانسداد المعوي الميكانيكي على نموذج الجرذان من نوع سبارجيو داولي. أجريت الدراسة في مستشفى الأورام بأنقرة في أبريل 2005م. تم تقسيم الحيوانات عشوائياً الى ثلاث مجموعات: 1) مجموعة التحكم (العدد10)، 2) المجموعة المصابة بالانسداد المعوي الميكانيكي (العدد12)، 3) المجموعة المصابة بالانسداد المعوي الميكانيكي + عقار أوكتريوتيد (العدد13). تم قياس حجم السائل داخل اللمعة كذلك تركيز الصوديوم والكلور والكالسيوم والبوتاسيوم في الدورة النظامية. والأجزاء القريبة والأمعاء الوسطى. كما قمنا أيضاً بتقييم تعداد كريات الدم البيضاء ومستويات الراسب الدموي في جميع الحيوانات.

النتائج: لم يكن هنالك فرقاً في تعداد كريات الدم البيضاء ومستويات الراسب الدموي. كانت آثار عقار أوكتريوتيد في إنقاص حجم السائل في المعوي والدعم المنحل الكهربائي ملحوظاً. ولكن، لم تكن هذه الآثار ذات فائدة في معدلات الوفيات والوذمة المعوية ($p>0.05$).

خاتمة: نحن نؤمن بضرورة القيام بالدراسات الإضافية على الحيوان والمحاولات السريرية المتعاقبة قبل استعمال عقار أوكتريوتيد كتابع سريري في المعالجة لانسداد الأمعاء الدقيقة.

Objective: To investigate the effects of octreotide on mechanical ileus without surgical intervention.

Methods: This study was organized to investigate the effects of octreotide (Sandostatine one microgram/ml-Sandoz), a long acting analogue of somatostatin, in a mechanical ileus model in Sprague-Dowley rats. The study was performed at Ankara Oncology Hospital, Ankara, Turkey in April 2005. The animals were randomly separated into 3 groups. 1) control group (n:10) 2) ileus group (n:12), and 3) ileus + octreotide group (n:13). Intraluminal liquid volume, as well as concentrations of sodium, chlorine, calcium, and phosphorous were measured in systemic circulation, proximal, and middle bowel segments. We also evaluated the leukocyte count, and hematocrit levels in all animals.

Results: There was no difference in leukocyte count and hematocrit levels. The effects of octreotide in decreasing intestinal liquid volume, and electrolytes were significant. However, these effects were not beneficial on the mortality rates, and intestinal edema ($p>0.05$).

Conclusion: We believe additional animal studies, and subsequently controlled clinical trials are necessary before using octreotide as a clinical adjunct in the treatment of small bowel obstruction.

Saudi Med J 2008; Vol. 29 (4): 539-543

From the Department of General Surgery (Kutun, Ulucanlar, Celik, Cetin), School of Medicine, Ankara Oncology Hospital, Ankara and the Department of Internal Medicine (Cure, Kockar), Division of Gastroenterology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey.

Received 25th September 2007. Accepted 23rd February 2008.

Address correspondence and reprint request to: Dr. Erkan Cure, Department of Internal Medicine, School of Medicine, Suleyman Demirel University, Isparta 32100, Turkey. Tel +90 (246) 2112651. Fax +90 (246) 2371758. E-mail: erkancure@yahoo.com

Intestinal obstruction is a frequently observed condition, often leading to serious fatal complications if underestimated, and not treated properly. No matter what the etiology is, the challenging problem in mechanical ileus is the accumulation of fluids within the small intestine resulting in excessive intestinal distension ischemia, necrosis, and fluid-electrolyte, and acid-base imbalance.[1-3] If accumulation of liquid and electrolytes in the bowel lumen continues, absorption through the intestinal wall decreases, and adverse changes in motility will take place.[1,3] The goal in the conservative

management of intestinal obstruction is to decrease intestinal distension, correct fluid, and electrolytes imbalance. In the absence of intestinal ischemia, decreasing intestinal distension by conservative methods, for example, nasogastric aspiration, and appropriate liquid-electrolyte replacement, would be beneficial.[2,4] In addition, bowel obstruction secondary to intra-abdominal adhesions may be relieved by conservative methods, and the need for surgical intervention may be avoided.[5] Pharmacological control of intestinal distension is a potentially helpful method of conservative treatment, which may decrease intestinal distension by inhibiting gastro-intestinal secretions. Somatostatin is a tetradecapeptide that decreases gastric, pancreatic, biliary, intestinal secretions, and motility.[6,7] As half-life of natural somatostatin is very short, its analogue with a long-term effect octreotide is used in the experimental settings of the present study. Based on this theory, we aimed to investigate the effects of octreotide in an animal model of mechanical bowel obstruction with special attention to electrolyte imbalance within the intestinal lumen.

Methods. The study was performed at the Gastroenterology Department of Ankara Oncology Hospital, Ankara, Turkey in April 2005. Thirty-five male Sprague-Dowley rats aged 2-3 months, with an average weight of 220 g (170-300 g) were used for this experiment. Ethical committee approval was obtained before study set up. The rats were followed up under room temperature in metal cages with a diurnal cycle of 12 hours during the day, and 12 hours at night. All the rats were fed with standard rat food for 3 days before the operation. All rats were anesthetized by intraperitoneal injection of 40 mg/kg ketamin hydrochloride, and 10 mg/ kg xylazin. Rats in the control group underwent laparotomy and closure. The remaining rats were subjected to laparotomy with midline incision. The intestinal obstruction method defined by Paran et al[8] was applied. The distal ileum was ligated with silk, 5 cm proximal to the cecum, and 10 cm proximal from this level so as to form a closed loop without affecting the intestinal blood circulation. In this way, 2 intestinal obstruction models were created, one as a simple obstruction model, and one as a closed ansa model. After the surgical procedure, the abdominal wall and skin were closed as separate layers with 3.0 Poliglactin (Dexon®, Ethicon). The study consisted of 3 groups: group 1 (n: 10, control group). The rats in this group were subjected to laparotomy only. Group 2 (n: 12, ileus group): intestinal obstruction created was applied using the method as described by Paran.[8] Group 3 (n: 13, ileus + octreotide group): in addition to bowel obstruction, synchronous administration of octreotide, an analogue of somatostatin, (Sandostatine®, Sandoz Medicine Group, Istanbul) was initiated 35 mcg/kg/day subcutaneously in 2 divided doses per day, and continued for 48 hours. All rats were followed up in separate cages after the operational processes. Oral intake was discontinued, and 10 ml/kg ringer lactate was given subcutaneous daily. After 48 hours, the anesthesia was applied again, and re-laparotomy was executed. The intestines were examined macroscopically, and the amounts of blood, luminal liquid, and the intestinal wet/dry rates were analyzed, and recorded. All rats were sacrificed by inhalation of overdose diethylether on the same day. For the rats subjected to re-laparotomy, 2 ml of blood samples was taken from the inferior vena cava. The blood samples were centrifuged for 5 minutes at 3000/min. The values of sodium (Na), calcium (Ca), chloride (Cl), and phosphorus (P) were measured in the supernatant obtained. The duodenum was clamped in order to inhibit regurgitation. A pipette was used to collect and measure the volume of intestinal fluid. The liquid samples were then centrifuged for 15 minutes at 5000 cycles. The supernatant was then segmented, and levels of intraluminal Na, Cl, Ca, and P were measured by an auto-analyzer (Merc-Mega, 800, Germany). After this process, the rates of electrolyte/liquid volume, the value of electrolytes in each segment, and the luminal electrolyte content expressing the amount of total electrolytes in weight in each segment were calculated. An intestinal section of 3 cm was weighed to observe the amount of intestinal edema due to obstruction. It was then kept below 100°C for 24 hours, after which the dried samples were weighed again, and the wet/dry rate was found.

The results of the experiment were analyzed by SPSS 9.0 (SPSS Inc, Chicago, IL). The categorical values were examined by the Chi-Square test. The non-categorical values were measured by ANOVA, and the differences between the groups were analyzed by the Tukey test. *P*-values below 0.05 were considered significant.

Results. After 48 hours all of the rats in the control group (n=10) were alive. Two of the 12 rats in the ileus group (17%), and 5 of the 13 rats in the ileus + octreotide group (38%) died. During autopsy, gastric and intestinal distension and intraperitoneal fluids were evaluated. Although no perforation occurred, a distinct change in color, edema, and strain were observed in the distal part of the proximal segment, and the intestinal wall of the closed segment. In the control group, we observed mild intra-luminal liquid volumes in all bowel segments. In the ileus group, the intra-luminal liquid volume in the intestinal lumen was distinctly high in both proximal and closed segments. Octreotide application caused a significant decrease in the intra-luminal liquid volume

of the proximal segment. In closed segment the effect of octreotide was also significant, as compared to the ileus group (p<0.001) however, was similar to those of the control group (**Table 1**). Levels of liquid accumulation in distal segments were similar in all groups. Rates of wet/dry tissue weight, as an indicator of intestinal edema, were clearly higher in the ileus group and ileus + octreotide group, particularly in the proximal segment (p<0.01), however, the difference between these 2 groups was not significant (p<0.35). Although there was no significant difference between groups in the middle (closed loop) segment (p<0.17), the wet/dry rate was higher in the middle (closed loop) segment of ileus + octreotide group, as compared to other groups (p<0.26). No significant difference was observed in terms of distal segmental edema between the groups (p<1.54). Octreotide application caused a slight increase in the wet/dry tissue weight rate, compared with both control and ileus groups, though the rates in proximal segment were higher in both ileus and ileus + octreotide groups, without significant difference (**Table 2**). Luminal liquid electrolyte concentrations in the measured segments of the ileus and ileus + octreotide groups were altered. Those of the sham operated animals were the same as serum levels. Electrolyte levels in bowel lumen showed significantly decreased levels of Na in the proximal segments in both ileus and ileus + octreotide groups, however, the difference between ileus and ileus + octreotide groups was insignificant. Calcium levels in the proximal segment were also lower in both ileus and ileus + octreotide groups compared to control group. However, octreotide treatment caused a better preservation of intra-luminal Ca which is statistically significant when compared to the ileus group (**Table 3**). While octreotide application decreased the levels of Ca (p<0.001) in both proximal and middle (closed loop) segments, Na, Cl, and P levels were decreased only in

Table 1 - The evaluation of the intraluminal liquid volumes from different segments of intestine and their statistical significance.

Segment liquids	Control group	Ileus group	Ileus + octreotide group
Proximal segment	0.36 ±0.1	7.7±1.8*†	5.11±2*
Middle segment (closed loop)	0.12± 0.07	0.79±0.3‡	0.33±0.23
Distal segment	0.11± 0.08	0.11±0.14	0.2±0.007

*p<0.000 in comparison with control group
†p<0.01 in comparison with ileus + octreotide group
‡p<0.001 in comparison with control and ileus + octreotide groups

Table 2 - The wet/dry weight ratio (edema) of the intestinal segments in all groups, and their statistical significance.

Wet/dry	Control group	Ileus group	Ileus + octreotide group
Proximal	3.55±0.42	4.82±0.64*	5.06±0.59*
Middle (closed loop)	3.78±0.8	4.2±0.5	4.42±0.69
Distal	4.12±0.73	3.8±0.55	4.06±0.46

*p<0.01 in comparison with control group

Table 3 - The consequences of the intraluminal electrolyte concentrations in proximal segment.

Proximal segment	Control group	Ileus group	Ileus + octreotide group
Sodium	141.9±35	129.2±8.3*	117.6±11.9*
Chloride	105±6.1	107±13.4	111±12.67
Calcium	2.65±0.53	1.23±0.77*	1.95±0.85
Phosphorus	22.9±1.5	24.3±23.2	25.5±30.8

*p<0.05 in comparison with control group, the evaluation for sodium and chloride has been in mEq/l, for calcium in mg/dl, and for phosphorus in mmol/dl

Table 4 - The consequences of the intraluminal electrolyte concentrations in middle segment.

Middle segment	Control group	Ileus group	Ileus + octreotide group
Sodium	141.9±35	125.3±24.5	123.4±17.9*
Chloride	105±6.1	104±14.5	107±13.3
Calcium	2.65±0.53	1.59±0.42†	2.35±0.87
Phosphorus	22.9±1.5	11.5±5.7	12.2±9.7

*p<0.05 in comparison with control group, †p<0.001 in comparison with control group, the evaluation for sodium and chloride has been in mEq/l, for calcium in mg/dl, and for phosphorus in mmol/dl

Table 5 - Comparison of leukocyte count, hemoglobin, and hematocrit values in the 3 groups.

Hematologic parameters	Control group	Ileus group	Ileus + octreotide group
Hemoglobin	14.5±1.56	14.2±0.41	13.9±0.28
Hematocrit	44.1±6.78	41.0±1.72	41.0±1.28
Leukocyte	4500±1759	5655±1363	3945±474

the closed segment (p>0.05). Serum electrolyte levels of blood samples showed that only Na values of the ileus + octreotide group were less than the control and ileus groups (p>0.05). No statistical significance was found in other measured electrolyte values (**Table 4**). The leukocyte count and hematocrit values were also not significant (p>0.05) (**Table 5**).

Discussion. In the present study, effects of octreotide were examined by forming a simple obstruction (a closed loop intestine model with double ends blocked). The intestinal obstruction, as was expected, caused a significant increase in the amount of luminal liquid in the obstruction model. It also was accompanied by edema in the intestinal wall determined by wet/dry weight rate. This content has not been adequately documented in the recent published literature. Zhang et al[9] reported that combined administration of octreotide and methylglucamine diatrizoate could accelerate resolution of small bowel obstruction by a specific therapeutic effect, and is much safer for the older patients with intestinal obstruction. Massacesi et al[10] reported that octreotide provides antisecretory and antiemetic activity in intestinal obstruction. Mangili et al[11] reported that nasogastric drainage decreased from 2000 to under 100 ml/day after the start of octreotide treatment in 8 patients with intestinal obstruction. Various factors contribute to intestinal distension and ischemia. These are, gastrointestinal secretions accumulated in the proximal part of the obstruction, decreased liquid-electrolyte absorption from the lumen, increased liquid and electrolyte secretion, and intestinal inflammation.[12,13] Although the mechanism of distension after obstruction is not very well understood, eliminating these factors may help in the treatment of this condition, and contribute to improved survival. In the present study, octreotide diminished the amount of luminal liquid and sodium, which were higher in the control group, as previously shown in similar intestinal obstruction models.[14] Despite the effect of octreotide in decreasing the luminal liquid and sodium content, it was not beneficial in decreasing the intestinal wall edema. This supports the idea that intestinal wall edema is an inflammatory process, and not solely related to luminal volume. As it has no essential anti-inflammatory effect, octreotide was not supposed to decrease bowel wall edema. Yamaner et al[15] studied the effects of octreotide on healing of intestinal anastomosis following small bowel obstruction in rats. They concluded that octreotide attenuates the ischemic changes, and electrolyte (Na and P) losses in the obstructed bowel. Similarly, Demetriades et al[16] found that Lanreotide, a long acting somatostatin analogue, decreases intestinal distension and luminal electrolyte (Na and P) losses in an experimental obstruction model. In the present study, decrement of Na in octreotide group may contribute to the increase in intestinal wall edema, and worsening the outcome of intestinal obstruction. In our study, positive or negative effects of octreotide on Cl was not seen in proximal and distal segments. In both segments the protective effect of octreotide on Ca losses was seen. We detected that octreotide does not effect P in proximal segments, and does not protect P losses in distal segment.

In our investigation, although octreotide decreased the liquid and electrolyte content in the intestinal lumen, it did not decrease the mortality rates. Conversely, in a study comparing the effects of octreotide in proximal intestinal obstruction model with distal intestinal obstruction, octreotide decreased mortality in proximal jejunum obstruction, however, it had no effect on mortality in distal intestinal obstruction.[12] This study might support the idea that intraluminal liquid accumulation can be reversed by octreotide in cases with proximal bowel obstruction. Heuser et al[17] investigated the effects of octreotide on mucosal and muscular microcirculation, both under normal and ischemic conditions, and concluded that octreotide impairs micro vascular perfusion of the jejunum both in physiologic conditions and ischemia-reperfusion (IR). They have shown that octreotide, without IR, decreased functional capillary density, and red blood cell velocity. After reperfusion, it led to perfusion heterogeneity demonstrated by villous stasis, and a decrease in the index of mucosal perfusion. They further underlined the harmful effects of octreotide on intestinal microcirculation by demonstrating a significant increase in adherent leukocytes (without IR). Interestingly, adherence was not further increased by IR, rather it was significantly decreased. We believe that the high mortality rates of octreotide arm might be explained by the impaired microcirculation due to this statement.

In another study by Li et al,[18] the role of pancreatic-derived proteases in the pathogenesis of ischemic intestinal injury was evaluated. Octreotide precondition can improve the hepatocellular energy reserve, and protect the liver from warm ischemia-reperfusion injury. They reported that the protective effect of octreotide on warm ischemia-reperfusion injury may be related to its influence on endocrine secretion. In 2 randomized, double blind, and placebo-controlled multicenter trials the prophylactic effect of perioperative inhibition of exocrine pancreatic secretion by octreotide was investigated. A significant reduction in fistula, abscess, fluid collection, sepsis, and postoperative pancreatitis were detected within patients undergoing pancreatic resection for cancer.[19] Octreotide significantly decreased

the liquid and electrolyte accumulation observed in intestinal obstruction, even in the proximal segment. However, octreotide could not inhibit the edema in the intestinal wall and mortality, which possibly is due to changes in the intestinal microcirculation. We believe additional animal studies, and subsequently controlled clinical trials are necessary before using octreotide as a clinical adjunct in the treatment of small bowel obstruction.

References

1. Henne-Bruns D, Lohnert M. Current status of diagnosis and nonoperative therapy of small bowel ileus. ***Chirurg*** 2000; 71: 503-509.
2. Madl C, Druml W. Gastrointestinal disorders of the critically ill. Systemic consequences of ileus. ***Best Pract Res Clin Gastroenterol*** 2003; 17: 445-456.
3. Plusczyk T, Bolli M, Schilling M. Ileus disease. ***Chirurg*** 2006; 77: 898-903.
4. Mattei P, Rombeau JL. Review of the pathophysiology and management of postoperative ileus. ***World J Surg*** 2006; 30: 1382-1391.
5. Mosley JG, Shoaib A. Operative versus conservative management of adhesional intestinal obstruction. ***Br J Surg*** 2000; 87: 362-373.
6. Cullen JJ, Eagon JC, Dozois EJ, Kelly KA. Treatment of acute postoperative ileus with octreotide. ***Am J Surg*** 1993; 165: 113-119.
7. Piqueras L, Martínez V. Role of somatostatin receptors on gastric acid secretion in wild-type and somatostatin receptor type 2 knockout mice. ***Naunyn Schmiedebergs Arch Pharmacol*** 2004; 370: 510-520.
8. Paran H, Wider T, Mayo A, Neufeld D, Susmalian S, Shwartz I, et al. The effect of the somatostatin analogue octreotide on experimental intestinal obstruction in rats. ***Acta Cir Bras*** 1998; 13: 207-211.
9. Zhang Y, Gao Y, Ma Q, Dang C, Wei W, De Antoni F, et al. Randomized clinical trial investigating the effects of combined administration of octreotide and methylglucamine diatrizoate in the older persons with adhesive small bowel obstruction. ***Dig Liver Dis*** 2006; 38: 188-194.
10. Massacesi C, Galeazzi G. Sustained release octreotide may have a role in the treatment of malignant bowel obstruction. ***Palliat Med*** 2006; 20: 715-716.
11. Mangili G, Franchi M, Mariani A, Zanaboni F, Rabaiotti E, Frigerio L, et al. Octreotide in the management of bowel obstruction in terminal ovarian cancer. ***Gynecol Oncol*** 1996; 61: 345-348.
12. Mulvihill SJ, Pappas N, Fonkalsrud EW, Debas HT. The effect of Somatostatin on experimental intestinal obstruction. ***Ann Surg*** 1988; 207: 169-173.
13. Nellgard P, Cassuto J. Inflammation as a major cause of fluid losses in small-bowel obstruction. ***Scand J Gastroenterol*** 1993; 28: 1035-1041.
14. Gittes GK, Nelson MT, Debas HT, Mulvihill SJ. Improvement in survival of mice with proximal small bowel obstruction treated with octreotide. ***Am J Surg*** 1992; 163: 231-233.
15. Yamaner S, Bugra D, Muslumanoglu M, Bulut T, Cubukcu O, Ademoglu E. Effects of octreotide on healing of intestinal anastomosis following small bowel obstruction in rats. ***Dis Colon Rectum*** 1995; 38: 308-312.
16. Demetriades H, Kanellos I, Mantzoros I, Kalfadis S, Galovatsea K, Zaraboukas T, et al. Effects of lanreotide on the healing of small bowel anastomoses following obstructive ileus in rats. ***Colorectal Dis*** 2002; 4: 23-27.
17. Heuser M, Popken O, Kleiman I, Post S. Detrimental effects of octreotide on intestinal microcirculation. ***J Surg Res*** 2000; 92: 186-192.
18. Li JQ, Qi HZ, He ZJ, Hu W, Si ZZ, Li YN. [Protective effect of octreotide on liver warm ischemia reperfusion injury] ***Zhong Nan Da Xue Xue Bao Yi Xue Ban*** 2006; 31: 792-796.
19. Friess H, Buchler MW. Efficacy of somatostatin and its analogues in pancreatic surgery and pancreatic disorders. ***Digestion*** 1996; 57: 97-102.

Statistics

Excerpts from the Uniform Requirements for Manuscripts Submitted to Biomedical Journals updated November 2003.
Available from www.icmje.org

Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as the use of *P* values, which fails to convey important information about effect size. References for the design of the study and statistical methods should be to standard works when possible (with pages stated). Define statistical terms, abbreviations, and most symbols. Specify the computer software used.