The effect of octreotide on postoperative adhesion formation

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Objective: To investigate the effect of octreotide, a long-acting analogue of somatostatin, on postoperative adhesion formation, because somatostatin inhibits secretion of some growth factors that have modulatory effects on collagen synthesis.

Design: An experimental study.

Setting: Surgical Research and Biochemistry laboratories at Hacettepe University, Ankara, Turkey.

Subjects: Male Swiss albino mice.

Interventions: Both sides of a 5-cm ileal segment from Swiss albino mice were scraped 10 times, and transient ischemia was induced by clamping the segmental artery. Animals were injected subcutaneously with 1 mL/d of saline for 3 days (group 1), a single 5-mL intraperitoneal dose of saline (group 2), subcutaneously with 10 μg/kg daily of octreotide for 3 days (group 3) or a single 10 μg/kg intraperitoneal dose of octreotide (group 4). In half of the animals repeat laparotomy was performed on postoperative day 5. After adhesions were graded, the scraped ileal segments were excised for determination of hydroxyproline quantity. The same procedure was repeated on postoperative day 14 for the remaining animals.

Outcome measures: Adhesion grading, hydroxyproline levels.

Results: On postoperative day 5, the intraperitoneal octreotide group (group 4) had a significantly lower median adhesion score than groups 1 and 2. On postoperative day 14, both octreotide groups (3 and 4) had a significantly lower median adhesion grading than both saline groups (1 and 2). Hydroxyproline levels of the groups were not significantly different on either day 5 or day 14.

Conclusion: Octreotide has a beneficial effect in decreasing adhesion formation in the early postoperative period.

Objectif : Examiner l'effet de l'octréotide, un analogue à effet prolongé de la somatostatine, sur la formation d'adhérences postopératoires, en raison de la capacité de la somatostatine d'inhiber la sécrétion de certains facteurs de croissance qui peuvent avoir des effets modulatoires sur la synthèse du collagène.

Conception : Étude expérimentale.

Contexte : Laboratoires de biochimie et d'études chirurgicales à l'Université Hacettepe, Ankara, Turquie.

Sujets : Souris albinos mâles suisses.

Interventions : On a d'abord gratté dix fois les deux côtés d'un segment iléal de 5 cm provenant des souris albinos suisses puis on a provoqué une ischémie transitoire en pinçant l'artère irriguant ce segment. Les animaux ont ensuite reçu par injection hypodermique une solution salée de 1 mL/j pendant trois jours (groupe 1), une dose unique de 5 mL de solution saline par voie intrapéritonéale (groupe 2), une injection hypodermique quotidienne de 10 μg/kg d'octréotide pendant trois jours (groupe 3) ou une dose unique d'octréotide de 10 μg/kg par voie intrapéritonéale (groupe 4). La moitié des animaux ont subi une laparotomie répétée cinq jours après l'opération. Une fois les adhérences évaluées, on a retiré les segments iléaux grattés afin de déterminer leur

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teneur en hydroxyproline. Cette intervention a été répétée 14 jours après l'opération pour le reste des animaux.

MESURES DE RÉSULTATS : Évaluation des adhérences, concentrations d'hydroxyproline.

RÉSULTATS : Cinq jours après l'opération, le groupe ayant reçu l'injection intrapéritonéale d'octréotide (groupe 4) présentait un taux d'adhérences beaucoup moins élevé que la médiane par rapport aux groupes 1 et 2. Quatorze jours après l'opération, les deux groupes (3 et 4) ayant reçu de l'octréotide présentaient un taux d'adhérences largement inférieur à la médiane comparativement aux groupes 1 et 2 qui avaient reçu une solution saline. La concentration d'hydroxyproline n'a pas été significativement différente dans l'un ou l'autre des groupes aux cinquième ou quatorzième jours.

CONCLUSION : L'octréotide réussit à diminuer la formation d'adhérences au début de la période postopératoire.

Adhesion formation postoperatively is a frequently encountered, major problem that adds to the costs and workload for the medical profession. Approximately 1% of all surgical admissions, 3% of laparotomies and 28.8% of all admissions for obstruction are the result of adhesive intestinal obstruction.[1,2] Also, 15% to 20% of female infertility is caused by adhesions.[3,4] The cost of lysing pelvic adhesions alone was estimated to be more than $1 billion annually in the United States.[5]

Octreotide is an analogue of somatostatin, with the same biologic effects but a longer half-life. In clinical practice it is used for its inhibitory effects on gastrointestinal tract peptides.[6–8] It also decreases the release of some growth factors, inhibits angiogenesis and prevents cell proliferation.[9–15]

Considering these data we planned to investigate the effect of octreotide on postoperative adhesion formation. As this substance has inhibitory effects on growth factors that have modulatory effects on wound healing, we hypothesized that postoperative adhesions might be decreased with somotostatin.

MATERIAL AND METHODS

This study was performed in the laboratories of the Department of Surgical Research and Department of Biochemistry at Hacettepe University School of Medicine. Guidelines for the care and use of laboratory animals were followed.

Procedures

Male Swiss albino mice weighing between 30 and 40 g were used. After an overnight fast, all animals were anesthetized with 60 mg/kg ketamine and 4 mg/kg xylazine. Adhesion formation was induced as previously described.[16] For this purpose, the terminal ileum and cecum of all animals were mobilized and taken out on a wet gauze. Both sides of a 5-cm terminal ileal segment just proximal to the cecum were scraped 10 times until serosal petechiae appeared. Then the artery supplying the scraped ileal segment was clamped for 60 seconds to produce transient ischemia. Animals were divided into 4 groups of 30 each. They were injected as follows: group 1, 1 mL/d of saline subcutaneously for 3 days; group 2, a single intraperitoneal dose of 5 mL of saline; group 3, 10 μg/kg daily of octreotide acetate subcutaneously for 3 days; and group 4, a single intraperitoneal dose of 10 μg/kg of octreotide acetate. The first doses of saline and octreotide in groups 1 and 3 were injected after induction of anesthesia. In groups 2 and 4, intraperitoneal injections were made before the abdomen was closed.

All groups were divided into 2 subgroups: half the animals in each group had repeat laparotomy on postoperative day 5. After adhesions were blindly graded as 0 (no adhesions), 1 (spontaneously separating adhesions), 2 (adhesions separated by traction) and 3 (adhesions separated by dissection),[17] the scraped ileal segments were resected for determination of hydroxyproline levels. These procedures were repeated on postoperative day 14 for the remaining animals in each group.

Hydroxyproline determination

All tissue samples were stored at –20 °C. Protein content was determined by Lowry's method.[18] Hydroxyproline content was determined spectrophotometrically in the Department of Biochemistry according to Bergman's modified Stegman method.[19] The tissue hydroxyproline level, which was used as an indicator of adhesion severity, was calculated as micrograms of hydroxyproline per milligram of protein.

Statistical analysis

Results of intragroup hydroxyproline values were analysed using one-way analysis of variance followed by Neuman–Keuls post hoc analysis. Pairwise comparison of the results obtained on postoperative days 5 and 14 was by Student's *t*-test. The Kruskal–Wallis test, followed by the Mann–Whitney U test, was used for differences in adhesion grading as a nonparametric test. Linear regression analysis was performed for significance of correlation of adhesion grading and hydroxyproline levels. A p value less than 0.05 was considered significant.

Adhesion grading

On day 5 postoperatively, group 4 animals had a significantly lower median adhesion score than group 1 (p = 0.043) and group 2 (p = 0.023) animals (Fig. 1). On day 14, both octreotide groups (3 and 4) had significantly lower median adhesion grading than both group 1 (p = 0.007 versus

group 3 and 0.034 versus group 4) and group 2 ($p = 0.007$ versus group 3 and 0.034 versus group 4) (Fig. 2). (p values were the same for groups 1 and 2 by chance.) Differences were not significant in intragroup comparison of adhesion grading scores between days 5 and 14.

Hydroxyproline levels

There was no significant difference in hydroxyproline levels between the groups on either of the repeat laparotomy days (Figs. 3 and 4). Also there was no significant difference in pairwise comparison of hydroxyproline levels among groups on days 5 and 14.

Correlation of adhesion grading and hydroxyproline levels

Linear regression analysis revealed a significant correlation between adhesion grading and hydroxyproline levels ($r = 0.243$, $p = 0.009$).

Discussion

Adhesion formation starts with the formation of fibrin mesh followed by collagen bands at 3 to 5 days, increasing until day 14 and stabilizing thereafter.[20,21] Using this information, we selected to evaluate adhesion formation on postoperative days 5 and 14. Our results support those of a previous study which showed that octreotide decreases adhesion formation evaluated by scor-

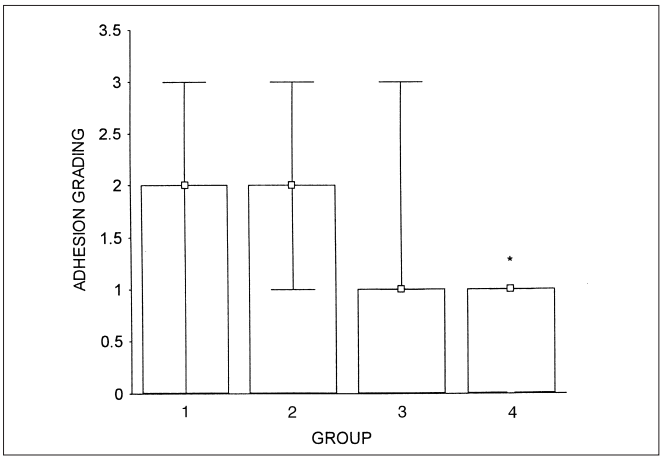

FIG. 1. Median and maximum and minimum non-outlier values of adhesion grading on postoperative day 5. Group 1 = subcutaneous injection of normal saline; group 2 = intraperitoneal injection of normal saline; group 3 = subcutaneous injection of octreotide; group 4 = intraperitoneal injection of octreotide. *Value significantly lower than groups 1 and 2.

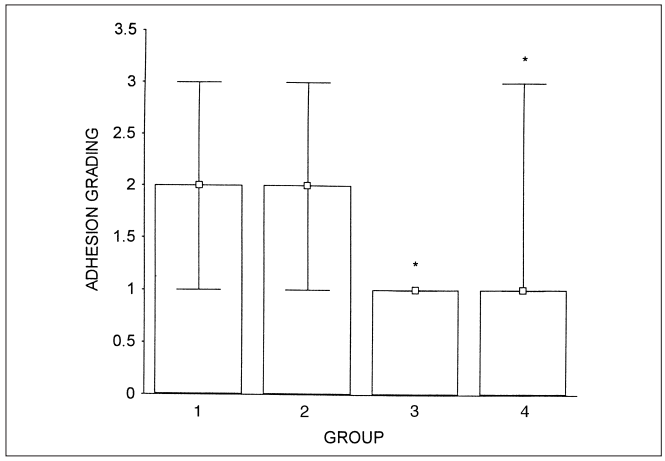

FIG. 2. Median and maximum and minimum non-outlier values of adhesion grading on postoperative day 14. Group 1 = subcutaneous injection of normal saline; group 2 = intraperitoneal injection of normal saline; group 3 = subcutaneous injection of octreotide; group 4 = intraperitoneal injection of octreotide. *Values significantly lower than groups 1 and 2.

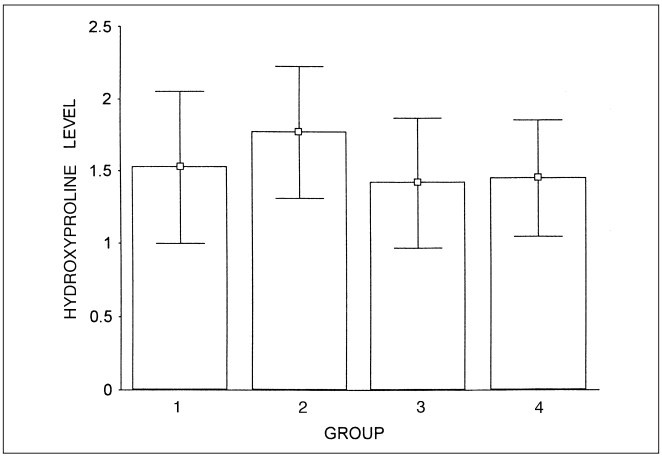

FIG. 3. Mean and standard deviations of hydroxyproline levels on postoperative day 5. Group 1 = subcutaneous injection of normal saline; group 2 = intraperitoneal injection of normal saline; group 3 = subcutaneous injection of octreotide; group 4 = intraperitoneal injection of octreotide.

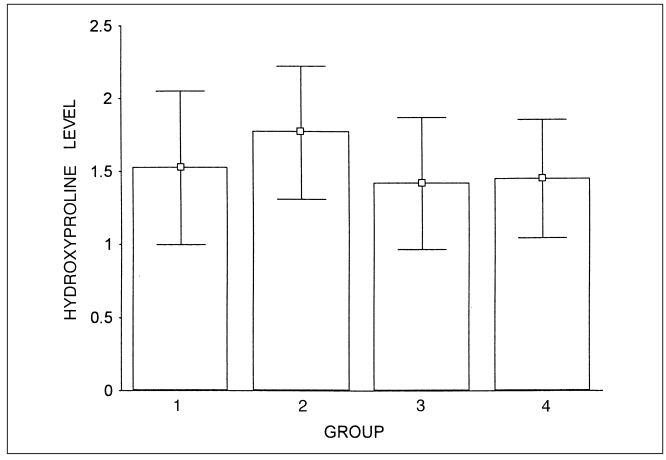

FIG. 4. Mean and standard deviations of hydroxyproline levels on postoperative day 14. Group 1 = subcutaneous injection of normal saline; group 2 = intraperitoneal injection of normal saline; group 3 = subcutaneous injection of octreotide; group 4 = intraperitoneal injection of octreotide.

ing of adhesions.[22] In that study, a single intraperitoneal dose of 120 to 150 μg/kg per rat or that intraperitoneal dose combined with an intramuscular dose of 20 μg/kg for 14 days was chosen for 2 different groups. Our results revealed that octreotide can effectively decrease adhesion formation with much smaller doses, and a single intraperitoneal dose of 10 μg/kg is enough for this purpose. Moreover, the intraperitoneal route seems to be slightly more effective for this purpose, as it led to a significantly lower adhesion grading than both control groups at day 5, whereas the subcutaneous route did not show a significant difference at that time.

There are several mechanisms to explain the effect of octreotide on decreasing postoperative adhesion formation. Growth factors have important modulatory roles on wound healing. In this process, transforming growth factor β (TGFβ),[23] epidermal growth factor (EGF),[24] fibroblast growth factor (FGF),[25] platelet-derived growth factor (PDGF)[26] and insulin-like growth factors (IGF-1)[23] are especially important.

Because wound healing and adhesion formation have similar pathways, modulation of the release of these growth factors could be expected to affect adhesion formation. The study of Williams and colleagues[27] showed that exogenous TGFβ administration increases adhesion formation. Angiopeptin, a somatostatin analogue, decreases EGF levels, IGF-1 and PDGF.[28,29] Also, smooth-muscle cell proliferation induced by IGF-1 and basic-FGF is inhibited by both octreotide[29,30] and angiopeptin.[29] Octreotide also inhibits angiogenesis.[31] In addition, somatostatin is known to have an anti-inflammatory effect.[32]

Adhesion formation follows the sequence of tissue inflammation, fibrin deposition, fibrin organization, collagen formation and maturation with the formation of adhesions.[20,21] It is possible to reduce adhesion formation with various precautions or chemicals at each of these steps. Injury, in any form, results in depression of fibrinolytic activity by release of plasminogen activator inhibitors 1 and 2 from mesothelial, endothelial and inflammatory cells.[33] Drugs that interfere with fibrinolytic activity can affect adhesion formation at the fibrin organization phase; whereas anti-inflammatory drugs such as steroids can modulate later phases of the process, interfering with collagen formation.

Data regarding the inhibitory effect of octreotide on growth factors involved in wound healing indicate that its effect might be at the collagen formation or organization phase, in other words at the later phases of wound healing. On the other hand, the fact that in our study a single intraperitoneal dose of octreotide significantly decreased adhesion grading and also proved to be the more effective route suggests that octreotide might exert its effect primarily at the early stages of adhesion formation. One possible mediator that octreotide might interfere with is plasminogen activator inhibitor.

Tagged hydroxyproline rapidly appears in the collagen of healing wounds and increases progressively.[34] Therefore its determination may be a valid indicator of adhesion severity. Indeed, in the present study, tissue hydroxyproline content showed a significant linear correlation with adhesion grading, supporting this method's reliability in the assessment of adhesions as well as other studies.[16,35,36] Although both octreotide groups had lower hydroxyproline levels than the control groups, at both 5 and 14 days, differences were not statistically significant. Supporting our results, 2 studies evaluating the effect of octreotide on anastomotic healing showed that it did not significantly alter tissue hydroxyproline levels and did not lead to increased anastomotic dehiscence or lower bursting pressures.[37,38] These data support the hypothesis that the effect of octreotide on decreased adhesion formation could be to prevent depressed fibrinolysis in the early postoperative period rather than to inhibit various growth factors and thereby, collagen synthesis.

On the other hand, a recent study revealed that octreotide adversely effects anastomotic healing and decreases hydroxyproline levels.[39] Differing from other studies, in this study octreotide was started 5 days before surgery. It is possible that octreotide might modulate different steps of wound healing, depending on the timing of administration. It seems that this issue needs further study.

Conclusions

Our results suggest that octreotide has a beneficial effect in decreasing adhesion formation in the postoperative period. Blockade of fibrinolysis or mediators involved in the early stages of adhesion formation might be the underlying mechanism for this effect.

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