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ORIGINAL ARTICLE

A novel antibiotic, linezolid, reduces intraperitoneal adhesion formation in the rat uterine horn model

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Abstract

Objective. To examine the effects of linezolid in prevention of adhesion formation in a rat uterine horn model. *Design.* Prospective randomized study. *Setting.* University Experimental Animal Research Laboratory Center. *Sample.* Ninety female Wistar albino rats. *Methods.* A dose response study was first conducted with 60 Wistar albino rats that were randomly assigned to six equal groups by administering vehicle (control), 5 mg/kg (Group 1), 15 mg/kg (Group 2), 50 mg/kg (Group 3), 100 mg/kg (Group 4), and 150 mg/kg (Group 5) linezolid starting three days before the adhesion inducing operation and continuing for 14 days postoperatively. Adhesion was scored clinically with a scoring system. The minimum effective dose was found to be 100 mg/kg/day. With this dose time response (starting three days before the operation and continuing for seven days), only preoperative and postoperative administration studies were conducted. *Main outcome measures.* Extent and severity of adhesions. *Results.* Total adhesion scores in the control and Groups 1 and 2 were significantly higher when compared with Groups 4 and 5, but not with Group 3. There were no significant differences in the adhesion scores between Groups 3, 4, and 5. In time response arm a total of 10 days treatment was not as effective as 17 days treatment. In postoperative and preoperative arms of the study, it was found that administration of linezolid only postoperatively or preoperatively did not affect adhesion formation significantly when compared with the control group. *Conclusion.* Linezolid was found to reduce intraperitoneal adhesion formation.

Key words: *Linezolid, adhesion formation, dose response*

Introduction

Despite intense ongoing studies, intraperitoneal adhesion formation still remains to be an inevitable consequence of surgical procedures. It has been reported that 93–100% of patients who have undergone abdominal surgery develop adhesions, some of whom might later develop bowel obstruction, infarction, fistulization, and erosion (1). It is also one of the main causes of infertility (2–4) and chronic pelvic pain (5,6), and is one of the major problems that add to the costs and workload for the medical profession. Therefore, any method by which adhesion formation could be reduced would be of great benefit.

Adhesion formation includes the release of inflammatory mediators from activated macrophages and interference with the fibrinolytic pathway (7). It has been shown that adhesion formation is a dynamic process and follows the sequence of tissue inflammation, fibrin deposition, fibrin organization, collagen formation, and maturation with the formation of adhesions (8,9). Theoretically, it is possible to reduce adhesion formation to some extent by interfering with any of these steps. Recently we have shown that modification of the inflammatory-coagulation cascade of events with an adjuvant, rosiglitazone, was effective in reducing adhesion formation (10).

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Several antibiotics are reported to be bi-functional drugs as they have an anti-inflammatory effect in addition to their microbicidal effect (11–13). Linezolid is a novel oxazolidinone antibiotic that acts against both gram-positive and selective gram-negative bacteria. It has been used for the treatment of serious, life-threatening infections associated with vancomycin-resistant *E. faecium* and complicated skin infections caused by *Streptococcus pyogenes* or methicillin-resistant *Staphylococcus aureus* (14,15). It has been shown that linezolid has the ability to modify acute-phase inflammatory response through its effects on cytokines synthesis by monocytes (16). In their study, Garcia-Roca et al. showed that linezolid suppressed the synthesis of the cytokines (IL-1β, IL-1ra, IL-6, and TNF-α) from human peripheral blood mononuclear cells significantly in a concentration-dependent manner. Theoretically, if linezolid has potent concentration-dependent suppressive effects on cytokine production and modifies acute-phase inflammatory response by disturbing the cytokine cascade, it might have a reducing effect on adhesion formation.

From this point we considered it of interest to examine the effects of linezolid in prevention of adhesion formation in a rat uterine horn model.

Materials and methods

Ninety female, non-inbred, non-pregnant, nulliparous Wistar albino rats weighing 160–200 g were used. All rats were kept under standard conditions with free access to oral intake. The study was approved by Gaziosmanpasa University Experimental Animal Research Committee. Estrous cyclicity of the rats was determined by vaginal smears and rats which were in estrus state were enrolled so that all the rats were in the same cyclic period during the operation.

The study protocol was as follows. A dose response study was first conducted with 60 rats and these 60 rats were randomly assigned into six equal groups each consisting of 10 rats before the operation. One group was assigned as the control group and the other groups were assigned as the study groups. A vehicle treatment (2 ml/day 5% dextrose) to the control group and linezolid (Zyvoxid®, Pfizer, Istanbul, Turkey) 5 mg/kg/day to Group 1, 15 mg/kg/day to Group 2, 50 mg/kg/day to Group 3, 100 mg/kg/day to Group 4, and 150 mg/kg/day to Group 5 was orally administered starting three days before the operation and continuing for 14 days postoperatively. Further studies were planned to be conducted if a significant reduction in adhesion could be achieved. With the minimum dose that significant reduction in adhesion was observed, which was found to be 100 mg/kg/day, a time response study, only preoperative and postoperative administration studies were conducted. For time response study, 100 mg/kg/day linezolid was administered to rats in Group 6 starting three days before the operation and continued for seven days after the operation. The results were compared with that of the control and Group 4. In postoperative study arm (Group 7), vehicle treatment was given for three days before the operation and 100 mg/kg/day linezolid was given for 14 days starting the day after the operation. For the preoperative arm (Group 8), 100 mg/kg/day linezolid was administered only for three days before the operation and for 14 days after the operation rats got vehicle treatment. Adhesion results in Groups 7 and 8 were compared with the control and Group 4.

The surgical technique was as follows. Following anesthesia with 75 mg/kg ketamine hydrochloride (Ketalar®, Eczacibasi, Istanbul, Turkey) and xylazine (5 mg/kg, Rompun®, Bayer, Istanbul, Turkey) intramuscularly, the abdominal skin was shaved and cleansed with povidon iodine solution. The peritoneal cavity was accessed by a 3-cm midline incision and both uterine horns were exposed, and then a 2 cm segment of the anti-mesenteric surface of the right uterine horn was traumatized in seven spots in the anti-mesenteric surface using bipolar cautery for 2 seconds with a power of 40 Watts. The peritoneum-fascia and the skin were closed separately with the use of a simple interrupted 4-0 polyglactin 910 suture. Each operation was limited to 10 minutes to control the effect of room air tissue drying. Also tissues were irrigated with warm saline during surgery to minimize variations in moisture and temperature. During operation, handling of the other tissues was minimized. About 100 mg/kg aspirin was administered orally for analgesia. All the surgeries were done by the same investigator (first author) who was blinded to the groups. The rats were randomly assigned and not sequentially operated in order to minimize bias.

In the dose response (control and Groups 1–5), postoperative (Group 7), and preoperative arms (Group 8), the rats were left for a recovery period of 14 days and were sacrificed at the end of this period. In the time response arm (Group 6), rats were sacrificed after seven days. Any adverse effects were monitored and rats were weighed daily during the treatment period. A second look laparatomy was performed to all rats at the end of the study periods. Intraperitoneal adhesions were scored according to Leach et al.'s clinical adhesion scoring system (17) by the second author, who had no prior knowledge

of which group was being evaluated. Adhesions to the uterine horn defect were scored as follows: 0 = no uterine adhesion; 1 = 1–25% involvement; 2 = 26–50%; 3 = 51–75%; and 4 = 76–100%. Adhesions were further characterized on gross examination for severity as follows: 0 = no adhesions; 1 = filmy avascular; 2 = vascular or opaque; and 3 = cohesive attachment of uterine horns to each other or other abdominal structure. The degree of adhesion formation was evaluated with the following adhesion scores: 0 = no adhesions; 1 = if the adhesion separated from tissue with gentle traction; 2 = requiring moderate traction; and 3 = requiring sharp dissection. Therefore, a total score of 10 was possible and for comparisons between groups, total scores were used.

For statistical analysis, statistical program for social sciences version 11.5 (SPSS 11.5, demo, SPSS Inc., Chicago, Illinois) was used. Variables were tested with Shapiro–Wilk test for normal distribution. It was found that the variables were not normally distributed. The data were expressed as the medians (minimum–maximum). Kruskal Wallis test was used for comparisons of the groups. A p-Value of <0.05 was assumed to be significant. When a significant result was found, Mann–Whitney U test was used in order to determine which groups were differing. A p-value of <0.0033 ($\alpha = 0.05/15$) was assumed to be significant in dose response study, <0.016 ($\alpha = 0.05/3$) for time response study and <0.008 ($\alpha = 0.05/6$) for pre and postoperative studies.

Results

One rat in the control group and two rats in Group 5 died after the operation due to complications related to surgery. The standardized surgical procedures and the administration of the protocols were well tolerated by the remaining animals. Determination of the occurrence of any side effects was done by observation. Weight of the rats was measured at the beginning and at the end of the treatment protocols and general appearance of rats, eating habits, consistency of stool, and color of urine were observed daily. The mean weights of the rats in Group 5 were lower than the other groups; however, this difference was not significant (data not shown). No other side effects related to medication were noted.

The total adhesion scores in the control and first five groups are shown in Table I. Total adhesion scores in the control and Groups 1 and 2 were significantly higher when compared with Groups 4 and 5 (all p-values were <0.0033 [$\alpha/15$] in Mann–Whitney U test), but comparison of these groups with Group 3 did not yield any significant difference (p-values >0.0033) (Table I, Figure 1). There were no statistically significant differences in the clinical total adhesion scores between Groups 3, 4, and 5 ($p = 0.043$ for Group 3 versus Group 4, $p = 0.101$ for Group 3 versus Group 5, $p = 0.274$ for Group 4 versus Group 5). However, rats in Group 5 had the lowest adhesion scores and scores were decreasing with the increase in linezolid dosage (Figures 1 and 2). About 100 mg/kg/day was found to be the minimum effective dose that reduced the adhesion formation (Figure 1).

In time response arm of the study, it was found that a total of 10 days linezolid treatment (Group 6) was not as effective as 17 days treatment (Group 4) (Figure 3). The median total adhesion score was significantly higher in Group 6 (median: 6 [0–7]) when compared with Group 4 (median: 3 [0–5]) ($p = 0.003$); however, it did not differ significantly when compared with the control ($p = 0.095$) (Figure 3).

In postoperative and preoperative arms of the study it was found that administration of linezolid only postoperatively or preoperatively did not affect adhesion formation significantly when compared with the control group (Figure 4).

Table I. Results of dose response sudy.

	Extent	Severity	Degree	Total
Control	3 (1–3)	2 (1–3)	2 (1–3)	7 (4–9)[a]
Group 1 (5 mg/kg/d)	3 (1–3)	2.5 (2–3)	2 (1–3)	7.5 (4–9)[a]
Group 2 (15 mg/kg/d)	3 (1–3)	2 (1–3)	2 (1–3)	7.5 (4–9)[a]
Group 3 (50 mg/kg/d)	1.5 (0–3)	2 (0–3)	1 (0–3)	4.5 (0–9)[b]
Group 4 (100 mg/kg/d)	1 (0–3)	1 (0–2)	1 (0–1)	3 (0–5)[c]
Group 5 (150 mg/kg/d)	0 (0–2)	0 (0–2)	0 (0–3)	0 (0–7)[c]
p*	<0.0001	<0.0001	<0.0001	<0.0001

*Kruskal Wallis test. Scores are expressed as medians (minimum–maximum).
[a,b,c]Differences between [a] and [c] are statistically significant ($p < 0.0033$, Mann–Whitney U test), between [a] and [b] or between [b] and [c] are not statistically significant ($p > 0.0033$, Mann–Whitney U test).

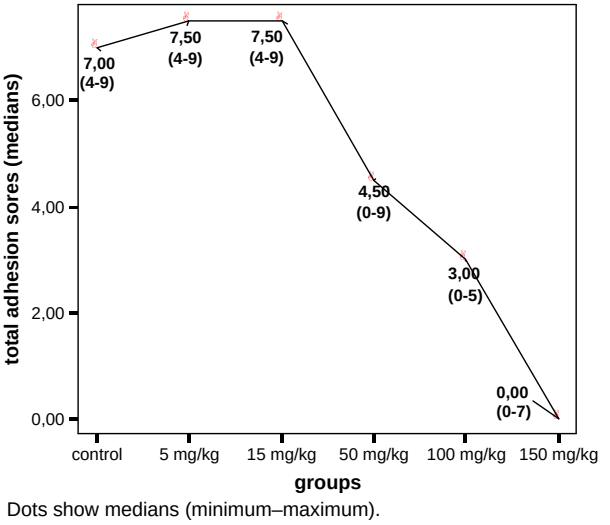

Figure 1. Dose response study. The minimum effective dose was found to be 100 mg/kg/day.

Discussion

An inflammatory reaction, which may be the result of many stimuli, is the first step in the common pathway to adhesion formation and reduction of factors that exacerbate the peritoneal response to injury without affecting actual regional inflammatory response capacity may prove a satisfactory means of ameliorating formation of adhesions (18,19). Recently, although the mechanism is unknown, linezolid has been shown to have potent concentration-dependent suppressive effects on cytokine production by LPS-stimulated monocytes in vitro, modifying the acute-phase inflammatory response by disturbing the cytokine cascade (16). From this point, the effect of this novel antibiotic on adhesion formation was assessed in the present study and it was found that linezolid has a reducing effect on adhesion formation to some extent with a minimum effective dose of 100 mg/kg in the rat model. Time response study showed that duration of administration was important and preoperative and postoperative only administrations were ineffective.

Oral, parenteral, and intraperitoneal administration of antibiotics and antiendotoxin agents has been shown to reduce postoperative adhesion formation in animals undergoing adhesion inducing operation without bacterial peritonitis (20–23). In animal models it has been shown that bacteria translocate from the gut into the peritoneal cavity around the time of laparotomy (24). It has been suggested that perioperative translocation of microbial antigens may augment the peritoneal tissue and cellular response to operative injury which is due to mechanical trauma or hypoxia at laparotomy and cause postoperative adhesion. Cahill et al. after performing adhesion inducing operation (AIO) or sham laparatomy to equal numbers of CD-1 mice that had been gavaged with fluorescein isothiocyanate-labeled lipopolysaccharide showed that AIOs resulted in significantly greater amounts of LPS translocation by peritoneal macrophages and intraperitoneal administration of bactericidal/permeability increasing protein to CD-1 mice early after AIO markedly attenuated subsequent adhesion formation (19). They suggested that peritoneal adhesion formation is exacerbated by peritoneal contamination due to translocation after laparotomy and may be attenuated by therapeutic antagonism.

Linezolid is a member of a new class of antimicrobial agents, the oxazolidinones that bind to the 50S subunit of bacterial ribosomes and block the formation of the initiation complex without inhibiting elongation or termination (25). Linezolid is a totally synthetic compound and has a good activity against virtually all important gram-positive pathogens, including methicillin-resistant *staphylococci*, penicillin-resistant *pneumococci*, macrolide-resistant *streptococci*, and vancomycin-resistant *enterococci* (26). It is rapidly and extensively absorbed after oral dosing and has an average absolute bioavailability of approximately

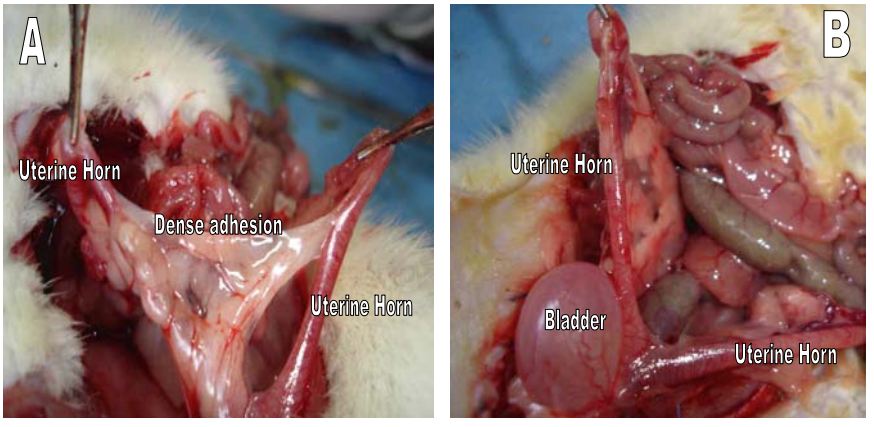

Figure 2. (A) Dense adhesion between uterine horns. (B) No adhesion.

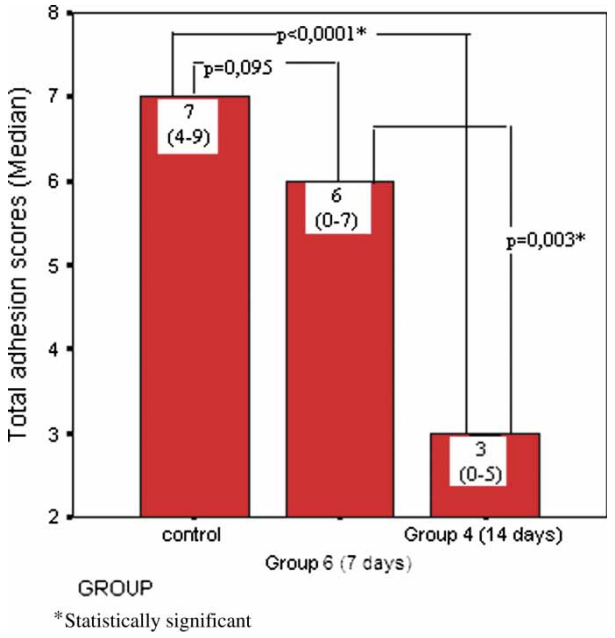

Figure 3. Time response study. The reduction in total adhesion scores in Group 6 was not significant when compared with the control; however, continuing drug administration for another week resulted in a statistically significant reduction in adhesion formation showing that duration of administration was important.

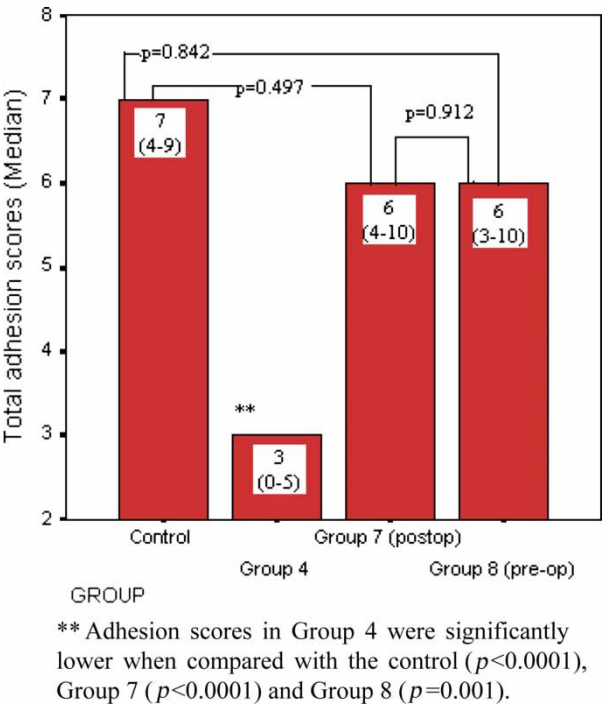

Figure 4. Comparison of only preoperative and postoperative drug administrations with the control and minimum effective dose. Only preoperative or only postoperative linezolid administrations did not reduce total adhesion scores significantly when compared with the control; however, administration of the drug throughout active phases of adhesion formation caused a significant reduction in total scores.

100% (27). The most frequently reported adverse events following linezolid administration are diarrhea, headache, nausea, and vomiting (28). One of the possible mechanism of linezolid in reduction of adhesion formation in the present study may be its bacteriostatic (and partly bacteriocidal) effect on bacteria that might have contaminated the peritoneal cavity after AIO. Another mechanism may be its immunomodulatory effect which is suppression of the synthesis of some cytokines including IL-1β, IL-1ra, IL-6, and TNF-α in a concentration-dependent manner so that the formation of adhesion cascade is inhibited to some extent in cytokine production and cell migration step (16).

Results of the time response, postoperative, and preoperative only studies in the present study did not show any statistically significant reduction in adhesion formation. Development of intraperitoneal adhesions is a dynamic process whereby surgically traumatized tissues, in apposition bind through fibrin bridges which become organized by wound repair cells (29). Highly mobile intraperitoneal structures will not permanently adhere to each other until fibroblast invasion leads to collagen deposition beginning on the third postoperative day (29). The collagen bands that begin to develop on postoperative days 3–5 continue to evolve until day 14, after which they stabilize (9). The process of postoperative adhesion begins during surgery and while the severity and extent may change over weeks and months, the incidence of an adhesion, i.e. whether it develops at all, is determined within the first five days following peritoneal trauma (30). Time response, pre and postoperative only studies indicate that duration and continuation of the treatment is important to achieve a significant reduction in adhesion formation.

There are several difficulties in assessing studies on adhesions in animal models. Firstly, the immunological properties of animals are not the same as humans and it is not appropriate to extrapolate data across species. There may also be inter-animal variability unless inbred animals are used. Secondly, there may be inter-observer variability in the scoring systems that have been used in assessment (31) and a study that demonstrates a significant change in adhesion score may not reflect a true clinical difference in the extent of adhesive disease; however, animal models are the mainstay of investigations on adhesion formation and up to today no validated scoring systems have been developed. Leach et al.'s clinical adhesion scoring system that has been used in the present study is one of the most commonly used systems (10,31,32). Another point is that linezolid is an expensive antibiotic and it may not be appropriate to solely use this agent for prevention of adhesion formation as there is

also drug resistance issue. However, the aim of the study was to assess whether the cytokine secretion inhibition effect of this novel antibiotic that had been shown before (16) could interfere with the inflammatory step of adhesion and reduce its formation.

In conclusion, the effect of a novel antibiotic, linezolid, on the formation of intraperitoneal adhesion was assessed in an animal model clinically with the aid of a scoring system. Linezolid was found to reduce intraperitoneal adhesion formation with a minimum dose of 100 mg/kg. Time response study, only preoperative or postoperative administrations showed that duration of administration was important. The possible mechanisms may be the immunomodulatory and antibacterial property of the drug. Whether this effect can be translated to humans needs further evaluation.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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