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Rifamycin Lavage in the Treatment of Experimental Intra-Abdominal Infection

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Hypothesis. **Peritoneal lavage with rifamycin reduces the number of intraperitoneal bacteria and adhesions and improves the outcome of intra-abdominal infection (IAI).**

Material and methods. **Experimental IAI was induced in Wistar rats. After 24 h, the animals underwent relaparotomy. A peritoneal fluid sample was obtained and lavage of the abdominal cavity was performed. Animals were randomly assigned to the three following groups: lavage with 0.9% sodium chloride solution (S group); lavage with rifamycin at the dose of 25 mg/kg (R25 group); and lavage with rifamycin at the dose of 12.5 mg/kg (R12.5 group). Mortality was recorded every 8 h for 7 d. All animals that died had a necropsy. Surviving rats were later sacrificed and also underwent a necropsy. At necropsy, intraperitoneal adhesions were noted and a peritoneal fluid sample was obtained for bacterial analysis.**

Results. **Peritoneal lavage with rifamycin improved survival from 50% in the S group to 91.7 and 100% in the R25 group and R12.5 group, respectively. Adhesion formation was significantly reduced in the R25 group and R12.5 group compared with the S group ($P \leq 0.01$ and $P < 0.01$, respectively). There was a greater reduction in bacterial counts in peritoneal fluid in the R25 group compared with the S group ($P = 0.003$) but there was no significant difference in the reduction of bacterial count between R25 group and R12.5 group.**

Conclusion. **These results suggest that peritoneal lavage with rifamycin improves the outcome of IAI.**

Key Words: **peritonitis; peritoneal lavage; antibiotic.**

INTRODUCTION

Intra-abdominal infection (IAI) is a crucial event in the pathogenesis of peritonitis and its local and general complications. In infectious conditions, fibrin deposits may become a nidus for abscesses and in turn become fibrous adhesions. Despite sophisticated surgical techniques, rational antibiotic therapy, and modern intensive care, IAIs remain a major threat in the pediatric and adult age group [1]. Mortality from IAI ranges from 17 to 38% [2]. Intraoperative peritoneal lavage, using physiological solutions with or without antibiotics or antiseptic solutions, have been studied extensively in both experimental and clinical settings. Rifamycin is one of the agents of choice for the management of tuberculosis. It may also play a role in infection caused by staphylococcus. Moreover, it has been shown to be active against a wide variety of microorganisms [3]. This antibiotic is widely used for local therapy and its application in the local treatment of IAI has been reported in rare studies [4, 5]. Therefore, in this study we hypothesize that peritoneal lavage with rifamycin could reduce the number of intraperitoneal bacteria and adhesions and improve the outcome of IAI.

MATERIALS AND METHODS

In accordance with the guide for the care and use of laboratory animals published by the US National Institutes of Health, 36 male Wistar rats (Institut Pasteur, Tunis, Tunisia) weighing 200 to 250 g were acclimated under controlled conditions for 1 wk before the

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experiments. Food and water were provided to the animals. Animals were randomly assigned to three groups: peritoneal lavage with 0.9% sodium chloride solution (S group, $n = 12$); peritoneal lavage with rifamycin at a dose of 25 mg/kg (R25 group, $n = 12$); and peritoneal lavage with rifamycin at a dose of 12.5 mg/kg (R12.5 group, $n = 12$).

Surgical Procedures

Bacterial peritonitis was induced by performing a cecum ligation puncture procedure (CLP) according to Wichterman *et al.* [6]. Only water was provided in the 12 h preceding the experiments. The animals were weighed and anesthetized by intramuscular injection of a combination of ketamine (50 mg/kg) and diazepam (1 mg/kg). They breathed spontaneously throughout the procedures. The abdominal skin was disinfected with 70% alcohol. All procedures were performed under sterile conditions. Routine midline celiotomy was performed with a 2 cm incision and the cecum was exposed. The cecum was ligated just distally to ileocecal valve with a 5-O polyglactin 910 (Vicryl; Ethicon, Inc., Johnson & Johnson Co., San Angelo, TX) suture to avoid intestinal obstruction and punctured twice with a 20-G needle, squeezed gently to force out a small amount of feces, and then returned to the left upper quadrant of the abdomen. The abdomen was closed in one layer with 5-O polyglactin 910 sutures. The animals received 1 mL isotonic sodium chloride solution subcutaneously for hydration. After 24 h, all animals underwent relaparotomy. Samples of peritoneal fluid were taken for microbiologic examination and the cecum was resected. The abdominal cavity was rinsed; all of the rats had initial lavage with 30 mL/kg of sterile 0.9% sodium chloride solution, followed by 15 mL/kg of one of three treatment regimens. We performed instillation of solution with rhythmic digital movements in the four quadrants of the peritoneal cavity for 2 min. After the procedure, the remaining liquid was aspirated with a syringe, followed by immediate laparorraphy. Mortality was recorded every 8 h for 7 d. All animals that died had a necropsy. Surviving rats were later sacrificed (with carbon dioxide asphyxiation) and also underwent a necropsy. At necropsy the abdomen was opened via a U-shaped incision for complete exploration. Intraperitoneal adhesions were noted and peritoneal fluid sample was obtained. Bacterial and leukocytes counts from peritoneal fluid were measured. Adhesions and the incidence of abscesses were examined in a blind manner by a study investigator according to the method of Zuhlke *et al.* [7], whereby grade 0 means no adhesions and grade IV means firm extensive adhesions that are dissectible only with sharp instruments, with organ damage almost unavoidable (Table 1). The sites of adhesions scored included the upper abdomen (liver, stomach, spleen); the omentum, and between the bowel loops and the cecum, testis, and epididymal fat body. The sum of these locations formed a total adhesion score [0–12].

Bacteriological Analysis

Peritoneal fluid samples were analyzed immediately. White blood cells were counted on Malassez cells. Samples were diluted in 10-fold steps (from 10^{-1} to 10^{-4}) in isotonic sodium chloride solutions. Quantitative bacterial culture was performed by inoculating pure and diluted samples with a measured amount via a plastic calibrated loop designed to deliver a volume of 10 μL. Blood agar medium and bromocresol purple incubated aerobically at 37°C for 24 h were used to detect aerobic bacteria. For anaerobic bacteria, blood agar medium with or without vancomycin and kanamycin added were incubated anaerobically at 37°C for 48 h. Microbial recovery was expressed as a number of colony-forming units per milliliter of peritoneal fluid.

TABLE 1

Grading of Adhesions According to Zuhlke (Grade Description)

0 No adhesions
1 Filmy adhesions: gentle, blunt dissection required to free adhesions
2 Mild adhesions: aggressive blunt dissection required to free adhesions
3 Moderate adhesions: sharp dissection required to free adhesions
4 Severe adhesions: not dissectible without damaging organs

Note. Locations scored included the following: the upper abdomen (liver, stomach, spleen); the omentum, and between the bowel loops; the cecum, testis, and epididymal fat body. The sum of these locations formed the total adhesion score (0–12).

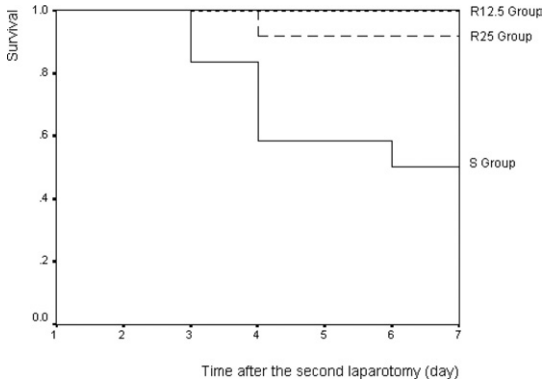

FIG. 1. Survival analysis in the three groups. Kaplan–Meier curve showing mortality rate at 7 d after surgery in the S group (lavage with saline solution), in the R25 group (lavage with rifamycin at the dose of 25 mg/mL), and in the R12.5 group (lavage with rifamycin at the dose of 12.5 mg/mL), respectively.

Statistical Analysis

All of the data were presented as mean ± SEM. Data were analyzed by using appropriate analysis of variance, or by unpaired Student's *t*-test for which P values <0.05 were considered to be significant. Kaplan–Meier analysis was used to assess differences in outcome between groups. The Mann–Whitney test was used to compare adherences.

RESULTS

Following CLP, all rats showed symptoms of peritonitis such as apathetic behavior, ocular exudates, and piloerection. The rifamycin lavage was well tolerated by rats at the two concentrations. The survival rate was 50, 91.5, and 100% in the S group, R25 group, and R12.5 group, respectively (Fig. 1). Log rank was 0.025 and 0.055, respectively, between the S group and the R25 group and the S group and the R12.5 group. It was 0.32 between the R12.5 and R25 group. The number of survival rats was 6 in the S group, 11 in the R25 group, and 12 in the R12.5 group. The cause of death in all groups was related to IAI with severe infection and generalized peritonitis at necroscopy. Culture results of the peritoneal fluid taken on the day of cecal resection revealed polymicrobial intraperitoneal infection. There was no statistical difference in the baseline level of bacterial counts (Fig. 2). Between the time of cecal resection and death or sacrifice there was a reduction in bacterial count in peritoneal fluid in the R25 group compared to the S group ($P = 0.003$) but there was no