J Infect Chemother 21 (2015) 312–315

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Note

A novel rat model of incisional surgical site infection model developed using absorbable multifilament thread inoculated with *Escherichia coli*

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ARTICLE INFO

Article history:
Received 12 May 2014
Received in revised form
24 September 2014
Accepted 14 October 2014
Available online 18 November 2014

Keywords:
Incisional surgical site infection
Wound infection model
Aquacel Ag

ABSTRACT

The development of an effective rat model of incisional surgical site infection (SSI) has so far proven difficult. In this study, we created a novel incisional SSI model and validated it in terms of both macroscopic and microscopic aspects including its response to treatment using antimicrobial wound-dressing, Aquacel Ag®. Wounds were created on the dorsum of rats. 3-0 Vicryl® threads inoculated with *Escherichia coli* were inserted in the wound beds in the infection group ($n = 6$). The wounds were closed for two days to induce infection and then opened and covered with polypropylene sheets during the study. Aquacel Ag was placed under the polypropylene sheet in the infected wounds of the Aquacel Ag group rats ($n = 6$). The wounds in the control group ($n = 6$) contained sterile Vicryl thread that had not been inoculated with *E. coli*. The macroscopic appearance, wound area, bacterial counts, and histology of each group were evaluated. The infection group demonstrated significantly lower wound healing ($p < 0.001$), greater bacterial counts (median [interquartile range] ratings, 2.15×10^7 [0.51×10^7 –53.40×10^7] vs 2.07×10^4 [0.60×10^4–4.45×10^4] CFU/g, respectively; $p < 0.01$), and severer histological inflammation ($p < 0.001$) than the control group. The Aquacel Ag group was only able to show significantly better wound healing than the infection group ($p < 0.001$). The new incisional SSI model exhibited all clinical manifestations of incisional SSI. It could be utilized to assess the effectiveness of newly developed treatments for incisional SSI.

Surgical site infections (SSI) lead to substantial morbidity, mortality, prolonged hospital stays, hospital readmissions, and subsequent procedures resulting in high healthcare costs [1]. Among all SSIs, incisional SSI account for more than 60% of all SSI, making improvements in incisional SSI treatment paramount [1]. Animal wound infection models are important to assess the effect of antimicrobial dressings and ointments used in the treatment of incisional SSI. However, establishing a wound infection model that can sustain persistent infection and chronic ulcer formation in rodents have proven difficult [2,3]; full-thickness wounds in rodents heal naturally [4], and in some cases, the inflammatory response can lead to accelerated wound healing [5]. In addition, the characteristics of previously reported models do not resemble those of incisional SSI [2,3,6–9]. Clinical conditions have demonstrated that persistent infection due to subcutaneous sutures exacerbates incisional SSI. Therefore, we hypothesized that an incisional SSI model could be created using rats with full-thickness wounds in which the wound bed had been sutured with thread inoculated with bacteria.

Aquacel Ag® (ConvaTec, Skillman, NJ) is a hydrofiber wound-dressing material consisting of soft non-woven sodium carboxymethylcellulose fibers integrated with ionic silver. This dressing has been shown to confer broad-spectrum antimicrobial activity in various infectious settings [10]. In this proof-of-concept study, we created a new incisional SSI model using rats; in order to validate the efficacy of this model in assessing incisional SSI treatments, we evaluated the characteristics of the formed SSI and determined whether the healing process was accelerated using Aquacel Ag.

Pathogen-free male Sprague–Dawley rats (CLEA Japan, Tokyo, Japan) were used in this study in accordance with the guidelines of

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http://dx.doi.org/10.1016/j.jiac.2014.10.012
1341-321X/

National Institutes of Health. The study was approved by the Laboratory Animal Care and Use Committee, Keio University School of Medicine. All rats were provided with drinking water containing acetaminophen (0.25 mg/mL, Maruishi Pharmaceutical, Osaka, Japan) as an analgesic. Perioperative prophylactic antimicrobials were not administered in this experiment.

For the statistical analysis of the bacterial counts and the histological grading, results were expressed as median [interquartile range] ratings. Intragroup comparisons of bacterial counts and histological grading were conducted using the Kruskal–Wallis test followed by the post hoc Bonferroni correction. Comparison of wound area was conducted using two-way ANOVA followed by the post hoc Bonferroni correction. All statistical analyses were performed using SPSS 20.0 software (SPSS, Chicago, IL). Based on the Bonferroni correction, $p < 0.017$ was considered to be statistically significant in this study.

BL-21AI strain of *Escherichia coli* (Life Technologies Co., Carlsbad, CA) was used to generate a standardized bacterial load for the wound infection model. Bacterial suspensions were grown overnight at 37 °C in Luria–Bertani (LB) medium. Cultures were then centrifuged (3000× *g*) for 15 min at 4 °C and resuspended in 1 mL of 0.9% NaCl. McFarland standards were measured using a Densichek instrument (Sysmex bioMerieux Co. Ltd., Tokyo), and cell suspensions were diluted to 1.5×10^8 colony-forming units (CFU)/mL using 0.9% NaCl, which is the equivalent of McFarland standard No. 0.5 [11]. This solution was then used to inoculate 3-0 coated polyglactin910 (Vicryl®, Ethicon, Somerville, NJ) by incubating the suture in the *E. coli* suspension for 2 h at 4 °C [12].

For the wound creation, rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (30 mg/kg, Dainippon Pharmaceutical, Osaka). The dorsal trunk hair was removed using clippers, and the skin was sterilized using povidone iodine. A full-thickness rectangular wound (20 × 10 mm) was created in the shaved skin. Subsequently, the rats were randomly assigned to either the infection group, control group, or Aquacel Ag group ($n = 6$ in each group). Three stitches of 3-0 Vicryl incubated with *E. coli* were inserted in the wound beds of the infection group and the Aquacel Ag group, whereas three stitches of sterile 3-0 Vicryl were inserted in the control group. The wounds were briefly closed for two days using 3-0 nylon sutures (Ethicon) to establish infection. Two days after the initial operation (Day 0), the wounds were opened and then covered with a polypropylene sheet (Lihit Lab. Inc., Osaka) to ensure the maintenance of an enclosed and moist environment. In the Aquacel Ag group, Aquacel Ag was placed under the polypropylene sheet to allow adherence to the wound.

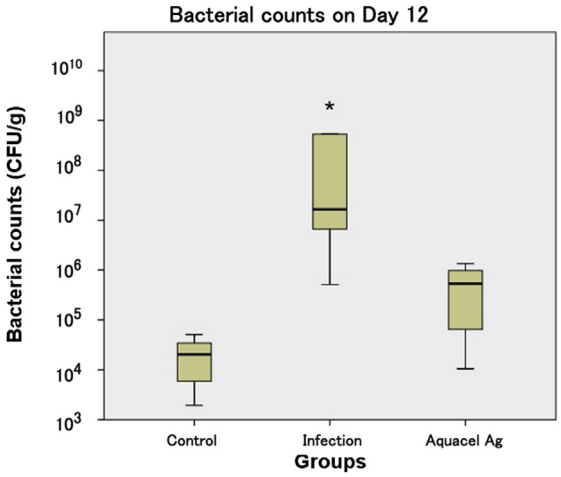

Fig. 2. Bacterial counts in samples collected from the wounds on Day 12. Data are shown using boxplots (box and whisker plots), $n = 6$. The median bacterial count is indicated by the horizontal bar. The vertical bars indicate the range, and the horizontal boundaries of the boxes represent the interquartile range. $^*p < 0.01$ compared to the control group.

The polypropylene sheets were exchanged every two days until Day 12, to record macroscopic appearance of the wound and to measure the wound area. The Aquacel Ag was exchanged along with the polypropylene sheet every two days. Wound area was measured using a slide caliper every two days from Day 0–12, and healing was expressed as the percentage of the wound that remained unhealed compared to Day 0 {(wound area of Day X/wound area of Day 0) × 100}. As shown in Fig. 1(a), stitch abscess formation was detected in all rats between Day 4 and Day 6, and increased wound exudates were observed in the infection group. In the control group, neither stitch abscess formation nor increase of wound exudates was observed. Stitch abscesses persisted in the infection group throughout the study period, and wound healing was significantly delayed compared to the control group ($p < 0.001$, Fig. 1(a and b)). The Aquacel Ag group also formed stitch abscesses between Day 4 and Day 6, however, based on macroscopic appearance, the size of the abscesses seemed smaller, and the level

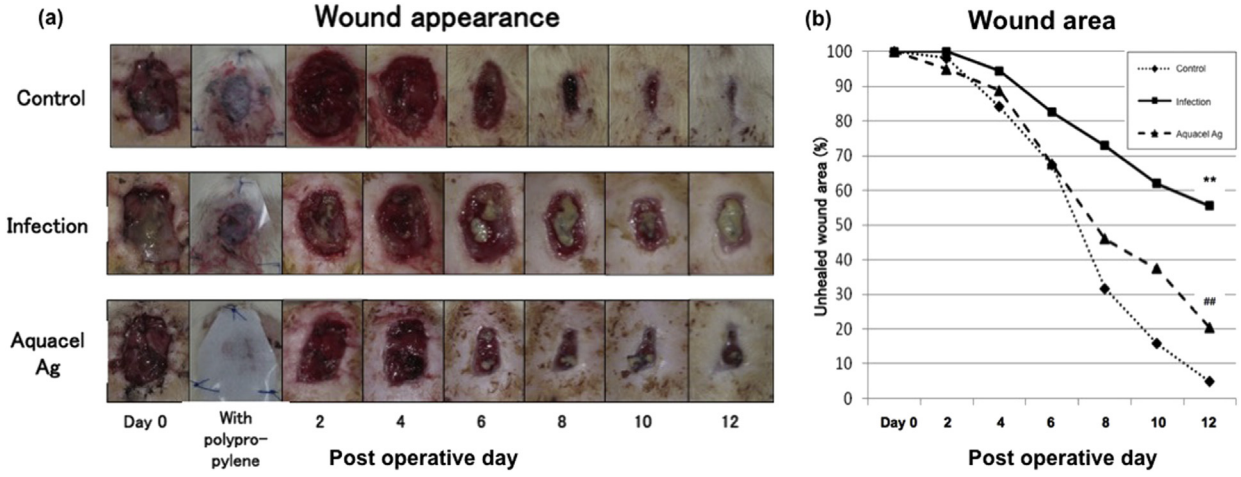

Fig. 1. (a) Chronological changes in wound appearance from Day 0 to Day 12. Pictures between Day 0 and Day 2 illustrate how the wounds were covered with the polypropylene sheet. (b) Chronological changes of wound healing expressed as the percentage of the wound that remained unhealed compared to Day 0 {(wound area of Day X/wound area of Day 0) × 100}. Each plot represents the mean, $n = 6$. $^{**}p < 0.001$ compared to the control group. $\#\#p < 0.001$ compared to the infection group.