Less-invasive and highly effective method for preventing methicillin-resistant *Staphylococcus aureus* graft infection by local sustained release of vancomycin

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Objective: Methicillin-resistant *Staphylococcus aureus* graft infection is one of the most serious complications of vascular surgery. Vancomycin is a potent antibiotic against methicillin-resistant *S aureus*; however, systemic administration of vancomycin is not very effective against methicillin-resistant *S aureus* graft infection. Therefore, we investigated whether a local sustained release of vancomycin prevents methicillin-resistant *S aureus* graft infection.

Methods: We have developed a poly-L-lactide-co-caprolactone sheet that enabled sustained release of vancomycin for 2 weeks. An expanded polytetrafluoroethylene vascular graft patch (1.5 mm^2) was sutured at the anterior wall of the incised murine abdominal aorta. Methicillin-resistant *S aureus* (1.0×10^3 colony-forming units) was inoculated onto the graft surface. Thereafter, the graft was treated as follows (n = 6 each): no treatment (control group), local injection of an aqueous solution of vancomycin (vancomycin solution group) and local implantation of poly-L-lactide-co-caprolactone containing vancomycin (vancomycin-PLCA group). After 7 days, the graft and blood were sampled and cultured.

Results: The methicillin-resistant *S aureus* counts in the grafts of the vancomycin-PLCA group were significantly lower than those of the other groups. Blood cultures of the vancomycin-PLCA group were all negative, whereas those of the other groups were all positive for infection. The survival rate in the vancomycin-PLCA group at 28 days was considerably higher than that in the control group (83.3% vs 16.7%).

Conclusions: A local sustained-release sheet containing vancomycin reduced methicillin-resistant *S aureus* counts in the infected vascular grafts, prevented sepsis, and drastically improved survival rates. This can be used as a highly effective and less-invasive adjunctive treatment method for preventing prosthetic methicillin-resistant *S aureus* graft infection.

Prosthetic vascular graft infection has been one of the most serious complications of vascular surgery. Although its incidence is relatively low, a high mortality rate ranging from 12% to 75% has been reported.[1-4] Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common and serious pathogens isolated from patients with vascular graft infections.[5,6] Thus, an effective strategy to prevent MRSA graft infections is urgently required.

Typically, systemic administration of antibiotics, such as vancomycin or teicoplanin, is used for treatment. However, it is often noted that this strategy is not completely effective because the antibiotic concentration in the tissues around the graft is considerably low.[7] Furthermore, repeated systemic antibiotic administration sometimes induces serious side effects, such as renal damage.

Abbreviations and Acronyms

CFU = colony-forming units
MIC = minimum inhibitory concentration
MRSA = methicillin-resistant *Staphylococcus aureus*
PLCA = poly-L-lactide-co-caprolactone

Thus, to prevent MRSA graft infection by means of a more effective strategy, we developed a local delivery system based on the local sustained release of vancomycin.[8] This system maintains sufficient antibiotic concentration in the tissues around the infected materials for a sufficient time period without requiring repeated systemic vancomycin administration. In the previous study we tested the effects of the poly-L-lactide-co-caprolactone (PLCA) sheet system in the subcutaneous graft–infected models. The animal model, however, does not completely simulate the clinical situations of vascular graft infections: thus, we developed a murine model of vascular graft infection of the abdominal aorta in the present study and investigated the effects of the vancomycin sheet in preventing vascular graft infections of the abdominal aorta.

Materials and Methods

Preparation of a PLCA Sheet Containing Vancomycin

We used PLCA[9,10] as a sustained-release carrier for vancomycin. The PLCA was purchased from BMG Co, Ltd (Kyoto, Japan). The PLCA was hydrolyzed in vivo and is absorbed over time. The PLCA sheet containing vancomycin was prepared as reported previously.[8] In brief, an aqueous phase consisting of vancomycin (20.0 mg) in 0.45 mL of double-distilled water was prepared. The aqueous phase was then added into 4.05 mL of 1,4-dioxane containing 103.5 mg of PLCA and agitated with a vortex mixer, homogenizer, and an ultrasonic generator. The solution was placed into a round glass dish (8.4 cm^2) and lyophilized to make the sheet (2-mm thickness). The sheet was cut into 4 sectors (2.1 cm^2 each, 2-mm thickness). As a result, one sector contained 5.0 mg of vancomycin. All the procedures were conducted under sterile conditions.

Preparation of MRSA

The MRSA strain SR3737 was used as the infecting organism. MRSA was supplied by Sionogi Co, Ltd (Osaka, Japan). In this study a concentration of 1.0×10^4 cells/mL was used for graft inoculation.

Animals

Male Wistar rats (body weight, 300–350 g) were purchased from Japan SLC (Shizuoka, Japan). All animals were cared for in compliance with the "Guide for the care and use of laboratory animals," Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. All of the assessments were performed by investigators who were blinded to the grouping of the study rats.

Study 1: Release Profile of Vancomycin From the PLCA Sheet and Tissue Concentration of Vancomycin Around the Sheet in the Retroperitoneal Space

Study groups. Forty-eight rats were intraperitoneally anesthetized with sodium pentobarbital (50 mg/kg). The retroperitoneal space was approached transperitoneally through a midabdominal incision. The animals were divided into 2 groups: the vancomycin–PLCA group and the vancomycin solution group (n = 24 each). In the vancomycin-PLCA group, after incision of the retroperitoneum, a vancomycin-PLCA sheet (2.1 cm^2 each, 2 mm-thickness) containing 5.0 mg of vancomycin was placed on the abdominal aorta, and the retroperitoneum was closed with suturing. In the vancomycin solution group an aqueous solution containing the same amount (5.0 mg) of vancomycin that was dissolved in 0.5 mL of physiologic saline was sprayed on the abdominal aorta, and the retroperitoneum was closed with suturing.

Tissue collection and vancomycin measurement. On days 1, 2, 4, 7, 10, and 14 after implantation, 4 rats per group were killed with an intraperitoneal administration of a lethal dose of sodium pentobarbital, and the remaining vancomycin-PLCA sheet and the tissue around the sheet were immediately harvested. Blood samples were simultaneously collected for measuring the blood vancomycin concentration. The vancomycin remaining in the harvested vancomycin-PLCA sheet was extracted into an organic solvent (dichloromethane), and the precipitant was dissolved in physiologic saline. The amount of vancomycin released was measured by using the fluorescence polarization immunoassay.

The tissue around the vancomycin-PLCA sheet was collected from a 20 × 20–mm square that included the abdominal aorta, inferior vena cava, psoas muscle, and retroperitoneum. The tissue around the vancomycin-PLCA sheet was weighed and subsequently homogenized with 5 mL of phosphate-buffered saline solution at 15,000 rpm for 15 minutes in a blender. The emulsion was then centrifuged at 5000 rpm for 15 minutes, and the amount of vancomycin in the supernatant fluid was measured by means of fluorescence polarization immunoassay. Tissue concentration was determined as the ratio of vancomycin to the volume of phosphate-buffered saline for the same weight of tissue (in micrograms per milliliter).

Study 2: Prevention of Graft Infection of the Abdominal Aorta on MRSA Inoculation

Patch-suturing model. After the aorta was crossclamped above and below the patch-suturing region, the anterior region of the aortic wall was incised 1 cm distal to the right renal artery and a 1.5 × 1.0–mm expanded polytetrafluoroethylene graft was sutured on it by using 8-0 polypropylene sutures (Figure 1). Thereafter, MRSA (1.0×10^3 colony-forming units [CFU]) was inoculated onto the graft surface. The volume of bacterial suspension was set to 0.1 mL. The retroperitoneum was closed with a 6-0 polypropylene suture. Heparin was not administered during the operation, and systemic antibiotics were not administered during the operation or in the perioperative period.

Study groups. The animals were divided into the following 3 groups (n = 6 in each cases): a control group wherein MRSA alone was inoculated onto the graft surface; a vancomycin solution group that was administered a local bolus injection of an aqueous solution of vancomycin (5.0 mg) onto the graft surface after 5
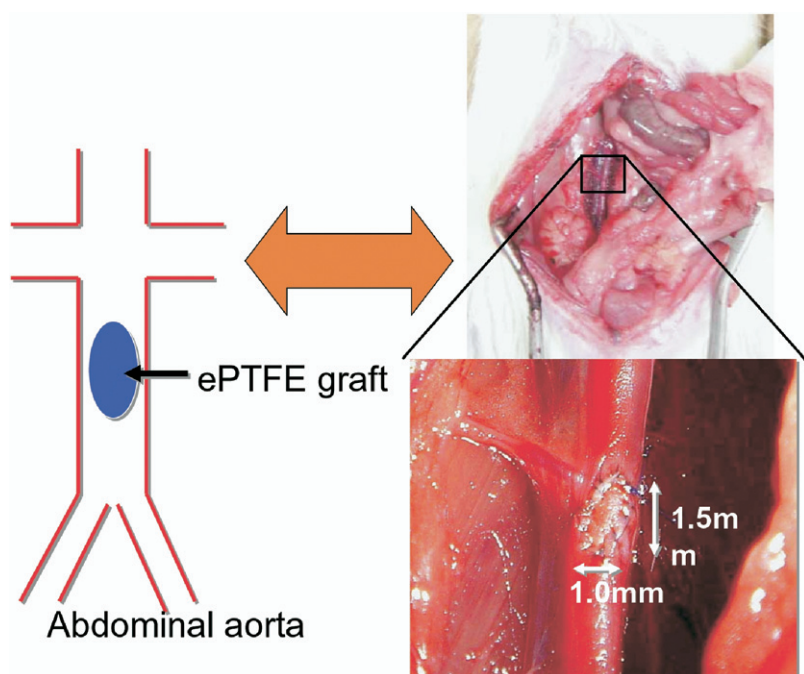
Figure 1. Graft patch-suturing model. A 1.5 × 1.0–mm expanded polytetrafluoroethylene *(e-PTFE)* graft was sutured on the incised abdominal aorta by using 8-0 polypropylene sutures.

minutes of MRSA inoculation; and a vancomycin-PLCA group wherein a vancomycin-PLCA sheet (5.0 mg) was implanted onto the graft surface after 5 minutes of MRSA inoculation.

Evaluation of bacterial colonization (CFU assay). The rats from all 3 groups were killed 7 days after the operation. Macroscopic findings of prosthetic graft infection were noted, and all grafts were carefully explanted under sterile surgical conditions. Arterial blood samples were collected for culturing. MRSA cells in the explanted grafts and in the arterial blood were counted as previously described.[8]

Study 3: Survival Study

Twelve patch-sutured models were made in the same maneuver, and the animals were divided into the following 2 groups (n = 6 in each cases): a control group wherein MRSA (1.0×10^3 CFU) alone was inoculated onto the graft surface and a vancomycin-PLCA group wherein a vancomycin-PLCA sheet (5.0 mg) was implanted onto the graft surface after 5 minutes of MRSA (1.0×10^3 CFU) inoculation. Thereafter, we observed survival for 4 weeks.

Statistical Analysis

The experimental results have been expressed as the mean ± standard deviation. For multiple comparisons among independent groups in which analysis of variance indicated significant differences, the statistical value was determined by using the Bonferroni or Dunn methods. Differences between the groups were determined by using the unpaired Student t test. Cumulative survival curves were constructed by using the Kaplan–Meier method. The log-rank test was used to compare survival curves. Statview software (Statview Corp, Cary, NC) was used for all statistical analyses.

Results

Study 1

Release profile of vancomycin from the PLCA sheet in the retroperitoneal space. The percentage of vancomycin remaining in the harvested sheet was 53.1% ± 12.5%, 29.4% ± 12.3%, 16.7% ± 8.0%, 11.9% ± 7.6%, 8.8% ± 4.5%, and 6.3% ± 4.5% on days 1, 2, 4, 7, 10, and 14, respectively. Thus the vancomycin-PLCA sheet released vancomycin for more than 2 weeks (Figure 2).

Tissue concentration of vancomycin around the sheet in the retroperitoneal space. The tissue concentrations in the vancomycin–PLCA group were 16.3 ± 8.6, 13.0 ± 10.3, 3.0 ± 1.4, 6.8 ± 3.7, 4.7 ± 3.7, and 5.8 ± 2.4 μg/mL on days 1, 2, 4, 7, 10, and 14, respectively. In contrast, the tissue concentration of vancomycin in the vancomycin solution group was 8.1 ± 6.9 μg/mL on day 1, and on day 2, this concentration decreased to 0.4 ± 0.4 μg/mL, which was less than the minimum inhibitory concentration (MIC) of vancomycin against MRSA (2.0 μg/mL). In addition, the concentration on days 4, 7, 10, and 14 was less than the detectable limit in all cases (<0.2 μg/mL). The vancomycin solution dissipated rapidly; however, the vancomycin-PLCA sheet maintained the tissue concentration higher than the MIC of vancomycin against MRSA for 2 weeks (Figure 3).

Blood vancomycin concentration. The blood vancomycin concentration at all instances was less than the detection limit (<0.2 μg/mL) in the vancomycin-PLCA group. Local implantation of vancomycin-PLCA did not increase the blood vancomycin concentration.
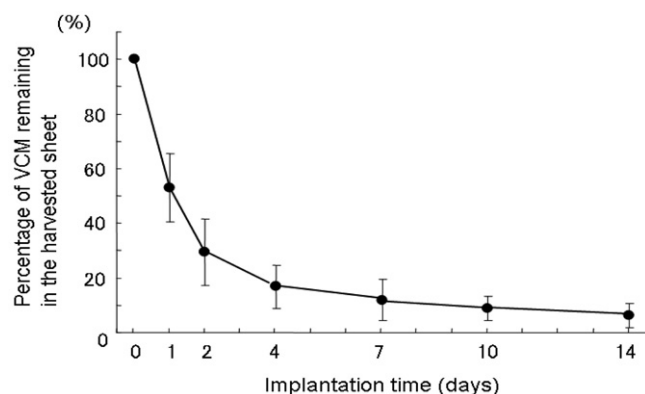
Figure 2. Release profile of vancomycin *(VCM)* from the poly-L-lactide-co-caprolactone (PLCA) sheet in the retroperitoneal space. The *x-axis* shows the time course, and the *y-axis* shows the percentage of vancomycin remaining in the harvested sheet. The vancomycin-PLCA sheet released vancomycin for more than 2 weeks in the retroperitoneal space.

Figure 3. Tissue concentration of vancomycin *(VCM)* around the sheet in the retroperitoneal space. The *x-axis* shows the time course, and the *y-axis* shows the concentration of vancomycin (in micrograms per milliliter). The vancomycin–poly-L-lactide-co-caprolactone *(PLCA)* group (*closed circle, solid line*) indicates implantation of a vancomycin-PLCA sheet. The vancomycin solution group (*open circle, dotted lines*) indicates local injection of an aqueous solution of vancomycin. The tissue concentration of vancomycin-PLCA was maintained to a concentration higher than the minimum inhibitory concentration *(MIC)* of methicillin-resistant *Staphylococcus aureus* for 2 weeks. On the other hand, the vancomycin solution concentration was maintained at greater than the MIC only for 1 day.

Study 2

Macroscopic findings of the periprosthetic graft. Seven days after the implantation of the prosthetic graft, superficial wound abscesses were not observed in all groups. The macroscopic findings of the periprosthetic graft are listed in Table 1. In the control group all rats showed periprosthetic abscesses and purulent fluid accumulation; however, the vancomycin-PLCA group showed no infection (Figure 4).

MRSA cell count in the implanted graft and arterial blood (CFU assay). In this study no colonization of other endogenous bacteria in implanted grafts was detected in all cases. Figure 5 shows the cell count of MRSA in the implanted graft and the arterial blood. The MRSA counts in the grafts of the control, vancomycin solution, and vancomycin-PLCA groups were $7.2 \pm 5.6 \times 10^7$, $1.6 \pm 2.2 \times 10^7$, and $7.9 \pm 10.5 \times 10^4$ CFU/mL, respectively. Thus the vancomycin-PLCA sheet drastically reduced the bacterial colonization in the graft by 3 orders of magnitude compared with administration of vancomycin solution. The MRSA counts in the blood of the control or vancomycin solution groups were 10^4 to 10^5 CFU/mL, whereas those of the vancomycin-PLCA group were not observed. The results suggested that the vancomycin–PLCA sheet can limit bacterial spreading from the local graft site into the systemic circulation.

Study 3

For survival data, see Figure 6. The survival curves were significantly different between the groups ($P < .05$). The survival rates at 28 days were 16.7% in the control group and 83.7% in the vancomycin-PLCA group. In the control group 2 rats di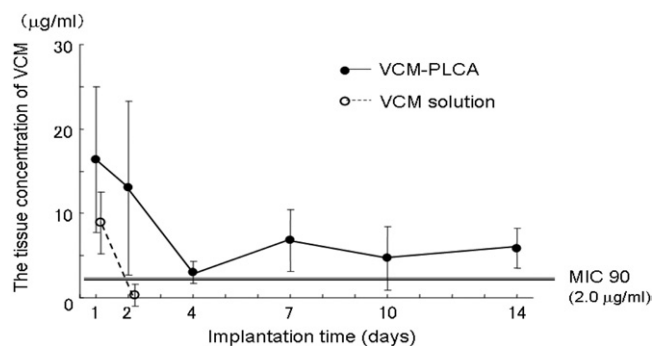ed suddenly on days 7 and 15 after the operation because of rupture of the anastomotic site, and 3 rats died on days 5, 13, and 15 after the operation because of sepsis. In the vancomycin-PLCA group only 1 rat died on day 14 after the operation of unknown reasons.

Discussion
Principal Findings

The most noteworthy finding was that the vancomycin-PLCA sheet prevented sepsis in MRSA graft infection of the abdominal aorta and drastically improved survival rates. MRSA counts in the grafts of the vancomycin-PLCA group were considerably lower than those of the other groups. The results suggest that the vancomycin-PLCA sheet has great potential in preventing MRSA graft infections.

Prosthetic Graft Infection Model of the Abdominal Aorta

To the best of our knowledge, this is the first report that demonstrates an in situ murine prosthetic vascular graft infection model. Although a simple model of subcutaneous graft infection has been widely used for prosthetic graft infection,[11-14] the model did not completely simulate the clinical situation because of the lack of exposure to the blood flow. In our previous study using the murine subcutaneous graft infection model, sepsis and death caused by MRSA infection were not observed in any of the cases,[8] although death caused by sepsis or rupture of the anasto-

Table 1. Macroscopic findings at the surgical site on day 7 after surgical intervention

	Control group	Vancomycin solution group	Vancomycin-PLCA group
Periprosthetic abscess	6/6	5/6	0/6
Nonpurulent periprosthetic fluid collection	0/6	1/6	2/6
Purulent periprosthetic fluid collection	6/6	5/6	0/6

n = 6 in all groups. In the control group all the rats showed periprosthetic abscesses and purulent fluid accumulation; however, the vancomycin-PLCA group showed no signs of infection. *PLCA*, poly-l-lactide-co-caprolactone.

motic site was observed in the present study. Therefore this life-threatening course of the model might simulate MRSA graft infection in the clinical setting.

In the present study we used less MRSA and higher doses of vancomycin than those of our previous study with a subcutaneous infection model. The reasons were as follows. In the pilot study with a retroperitoneal infection model, all rats died within 5 days when 1.0×10^4 CFU of MRSA or more was inoculated onto the graft surface. Therefore we used 1.0×10^3 CFU of MRSA in the present study. We initially used 2.38 mg of vancomycin, as used in the subcutaneous model; however, the effectiveness was insufficient. Therefore we used 5.0 mg of vancomycin. This might attribute to the difference between the subcutaneous space and retroperitoneal space that has abundant lymphatics or peritoneal fluid. It might be important to select different dosing schemes in each clinical situation.

MRSA and Graft Infection

Although recent studies have reported that MRSA is the most common and serious infectious agent isolated in vascular graft infections,[5,6] no effective strategies to prevent or treat MRSA graft infections have been established to date. There have been animal studies on graft infection by using MRSA, and most of them used the subcutaneous graft infection model. Although the use of in situ rifampicin-gelatin grafts has been used as a preventive measure against MRSA infection, it was not effective.[15] To date, the use of these grafts is common in case of rereplacement of infected prosthetic grafts; however, these results suggested that the rifampicin-gelatin grafts were inefficient against MRSA infection.[16]

Validity of the Sustained Release of Antibiotics in Preventing Graft Infection

Vancomycin was gradually released from the vancomycin-PLCA sheet for more than 2 weeks in the retroperitoneal spaces of rats, and the tissue concentration around the sheet was maintained at a concentration higher than the MIC of MRSA. On the other hand, in the solution form of the same dose, the vancomycin concentration was maintained at greater than the MIC only for 1 day. Furthermore, the serum vancomycin concentration was less than the detection limit (<0.2 μg/mL) in the vancomycin-PLCA group. These results suggest that in addition to being effective at preventing MRSA infection, a single local implantation of the vancomycin-PLCA sheet might also prevent systemic side effects. In addition, the vancomycin-PLCA sheet is pliable, easy to handle, and can be placed at any targeted site without a complicated maneuver and can therefore be used under various infectious conditions.
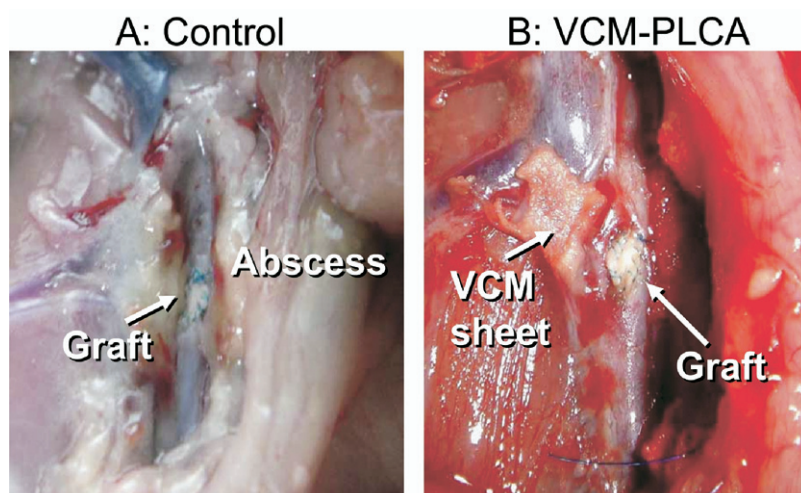

Figure 4. Macroscopic findings of the surgical site on day 7 after the operation. A, Control group: periprosthetic abscess and purulent fluid accumulation were observed. B, Vancomycin–poly-L-lactide-co-caprolactone *(VCM-PLCA)* group: no infection was observed.
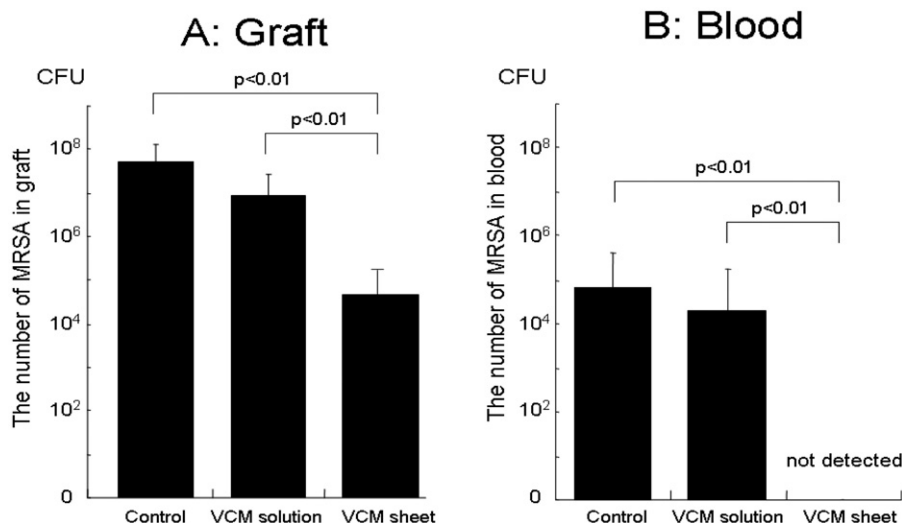
A: Graft

CFU

B: Blood

CFU

Figure 5. Cell counts of methicillin-resistant *Staphylococcus aureus (MRSA)* in the implanted graft and arterial blood. The *y-axis* shows the cell count of MRSA (logarithmic scale). *CFU*, Colony-forming units. A, The MRSA cell count in the graft. B, The MRSA cell count in the arterial blood. The MRSA counts in the graft of the vancomycin–poly-L-lactide-co-caprolactone *(VCM-PLCA)* group were considerably lower than those of the other groups. Blood cultures of the VCM-PLCA group were all negative, whereas those of the other groups were all positive for MRSA infection.

Although the macroscopic findings of the vancomycin-PLCA group did not indicate infection, MRSA cells were observed in the removed grafts on days 7 and 28 of culturing. However, the macroscopic findings showed no signs of infection; pus, dead space, and effusions were not observed. Therefore we can conclude that MRSA graft infection was

Figure 6. Cumulative survival curve of rats in the control and vancomycin–poly-L-lactide-co-caprolactone *(VCM-PLCA)* groups. The *x-axis* shows the days from the operation, and the *y-axis* shows the survival rate. VCM sheet group, *closed circle, solid line*; control group, *open circle, dotted lines*. The survival curves were significantly different between the groups (*P* < .05). The survival rates at 28 days were 16.7% in the control group and 83.7% in the VCM-PLCA group.

eventually prevented, and the possibility of recurrence can be considerably low.

Clinical Relevance

The vancomycin-PLCA sheet can be used in the prevention and adjunctive treatment of graft infection. Conventionally, prosthetic graft infection has been treated by means of the removal of the infected graft, followed by extra-anatomic bypass[17,18] or anatomic reconstruction.[16,19] If the infected graft cannot be removed, a muscle flap[20] or omentum[21] is used. However, these strategies alone cannot completely treat graft infection because of their insufficient bactericidal effects. Therefore a therapy combining the use of the vancomycin-PLCA sheet and the abovementioned procedures might improve the results of graft infection treatment.

Limitations

The current study has some limitations. First, we used rats as our experimental model, which might be more resistant to infection than human subjects. Second, the animals were only followed up for a period of 4 weeks, and therefore the long-term results are unknown. Further investigations are required, including a larger animal model and results obtained over a longer time period. Third, we did not use a control group that had daily intravenous administration of vancomycin because intravenous administration has been reported to be ineffective in graft infection. Fourth, the
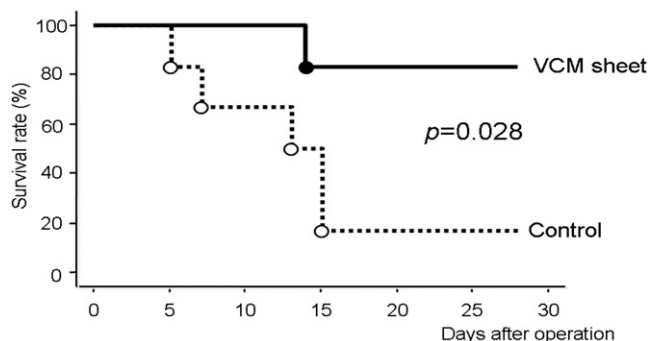
optimal release period of vancomycin was unclear. Further study with various release periods of vancomycin is necessary.

Conclusions

A local sustained-release drug (vancomycin) delivery system showed reduced MRSA counts in the infected vascular grafts, prevented sepsis, and drastically improved survival rates without increasing the blood vancomycin levels. This system can be used as a more effective and less-invasive adjuvant in the prevention of prosthetic MRSA graft infection.

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