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BASIC RESEARCH STUDIES

Limitations in the use of rifampicin-gelatin grafts against virulent organisms

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Objective: **Efficacy and duration of antibacterial activity of rifampicin-gelatin grafts against virulent organisms were evaluated in an animal model.**
Materials and methods: **Rifampicin-gelatin grafts were prepared with impregnation of Gelseal (Vascutek Ltd, Scotland) graft in 1 mg/mL rifampicin solution. Rifampicin-gelatin grafts (6 cm long; n = 24) and plain Gelseal grafts as controls (n = 4) were implanted into the canine abdominal aorta with inoculation of *Staphylococcus epidermidis, Escherichia coli*, or methicillin-resistant *Staphylococcus aureus* (MRSA), and the rifampicin-gelatin grafts were retrieved after 1 to 4 weeks. Disks cut from the retrieved rifampicin-gelatin grafts were placed on agar plates streaked with one of the organisms, and the graft antibacterial activity was assessed with the width of the inhibition zone.**
Results: **In in vitro tests, initial inhibition zones (inhibition zone of 24 hours after incubation) of rifampicin-gelatin grafts against *S epidermidis*, MRSA, and *E coli* were 40.0 ± 0.3 mm, 36.0 ± 0.2 mm, and 11.8 ± 0.1 mm, respectively. In the implantation, *S epidermidis*–inoculated rifampicin-gelatin grafts had no findings of graft infection, and no colony growth was recognized on the plates streaked with the perigraft fluids. Initial inhibition zones of *S epidermidis*–inoculated rifampicin-gelatin grafts retrieved at 1 or 2 weeks were 20.1 ± 1.1 mm and 7.6 ± 1.0 mm, respectively. In *E coli*–inoculated and MRSA-inoculated rifampicin-gelatin grafts, all of the eight animals had perigraft abscess, and blood culture test results probed septicemia in five animals with patent grafts at death. Inhibition zones against *E coli* or MRSA were not formed on the plates streaked with the same organism, whereas initial inhibition zones of *E coli*–inoculated and MRSA-inoculated rifampicin-gelatin grafts on *S epidermidis*-streaked plates were 8.0 ± 0.2 mm and 18.5 ± 0.5 mm, respectively. In the MRSA group, however, recolonization of high minimal inhibitory concentration strains developed within the inhibition zones as early as 24 hours. Histologically, neither organisms nor inflammatory cells were found in *S epidermidis*–inoculated rifampicin-gelatin grafts and tissue ingrowth was recognized at 2 to 4 weeks, whereas *E coli*–inoculated and MRSA-inoculated rifampicin-gelatin grafts had aggressive neutrophil infiltration into the graft interstices, revealing establishment of uncontrollable graft infection.**
Conclusion: **These results suggested that rifampicin-gelatin grafts are clearly valid for *S epidermidis* infection, whereas no efficacy was recognized against either MRSA or *E coli* graft infection because of early development of high minimal inhibitory concentration MRSA strains or poor susceptibility. (J Vasc Surg 2002;35:779-85.)**

Vascular graft infection has a significant incidence rate of 0.77% to 3.0% in aortofemoral bypasses[1,2] or a 3% rate in thoracic aorta replacement,[3] which results in higher limb loss or mortality rates. To provide effective prophylaxis or management of established graft infection, many attempts have been made to impregnate grafts with various antibacterial agents.[4-7] Rifampicin has a broad-spectrum antibacterial activity against most aerobicgram-positive and gram-negative organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) or *Escherichia coli*,[8] and has a favorable chemical property that easily links to gelatin in a form of ionic bonding by merely soaking in a rifampicin solution.[9-11] Therefore, in current reports regarding infection-resistant grafts, the rifampicin-gelatin grafts have been exclusively tested in an experimental study[9,10,12-14] and favorable clinical results have also been reported.[15-17] Nevertheless, sufficient efficacy and duration of infection resistance to commonly causative organisms are the most important concerns for further clinical prevalence of rifampicin-gelatin grafts.[6,13,18,19] A possibility of application to established graft infections as the result of virulent organisms is an important problem to be clarified. *E coli* graft infection after primary bypass surgery occupies 12% to 30% of the causative organisms in aortoiliac or femoral

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Competition of interest: nil.
Supported by 1999 grants 11671142 from the Ministry of Education of the Japanese government.
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Published online Feb 1, 2002.
Copyright © 2002 by The Society for Vascular Surgery and The American Association for Vascular Surgery.
0741-5214/2002/$35.00 + 0 **24/1/121850**
doi:10.1067/mva.2002.121850

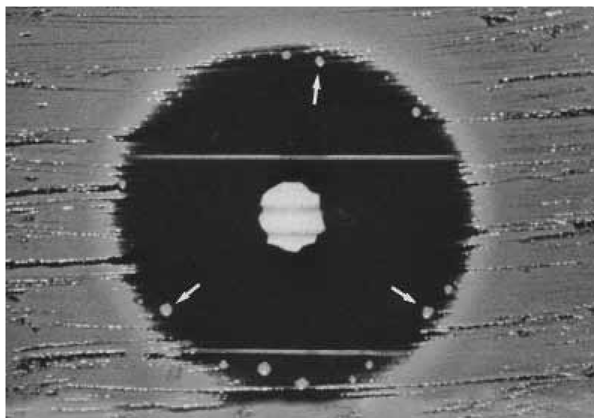

Fig 1. In vitro antibacterial susceptibility test results of rifampicin-gelatin grafts. Recolonization *(arrows)* of resistant strain of MRSA developed within inhibition zone as early as 3 days after incubation.

graft infection,[2] and the incidence rate of MRSA graft infection is high. Several investigators[20,21] have suggested that in situ replacement with rifampicin-gelatin grafts against MRSA infection is probably unwise. However, data regarding efficacy of rifampicin-gelatin grafts against these virulent organisms are limited. In this study, efficacy and duration of antibacterial activity of rifampicin-gelatin grafts against virulent organisms were examined with in vitro testing and an animal model, and the possibility of clinical application of rifampicin-gelatin grafts for management of established graft infection, and as prophylaxis against graft infection, was explored through use of a high-level inoculation model.

MATERIALS AND METHODS

Preparation of rifampicin solution and rifampicin-gelatin grafts. Because rifampicin with solvent is not available in Japan, our rifampicin solution was prepared from oral type rifampicin powder (Daiichi Pharmaceutical Ltd, Tokyo, Japan). The rifampicin solution processed in our laboratory gradually decreases antibacterial activity at room temperature because of oxidation. Therefore, the solution was prepared 24 hours before use and kept in a refrigerator. The preparation procedure was as follows: 100 mg of rifampicin powder was dissolved in 95 mL of 0.05 mol phosphate buffer saline solution (pH, 8.0), 100 mg of ascorbic acid and 490 mg of NaCl were added, and 0.1% rifampicin solution (1 mg/mL, with 270 mOsm; pH, 7.40) was then obtained and sterilized with filtration through a 0.22- m–cellomembrane filter. Before in vitro or implantation tests, a gelatin-sealed polyester graft (Gelseal graft; Vascutek Ltd, Scotland) was soaked in the rifampicin solution for 30 minutes at room temperature and excess solution was wiped off immediately before implantation. The plain Gelseal graft was implanted as a control.

Preparation of Bacteria

Bacteria used in this study were coagulase negative *Staphylococcus* (*Staphylococcus epidermidis*), MRSA, and *E coli* (American Type Culture Collection No. 25922). These rapid growing–type strains were common pathogens of postoperative graft infection, and the former two were obtained as clinical isolates from the clinical examination laboratory in the Asahikawa Medical University Hospital. A small aliquot of each isolate was streaked onto each appropriate culture plate and incubated at 35°C. After 24 hours, the colonized plate was then stored at 5°C in a refrigerator for later use. *E coli* was incubated and stored on a Trypt-soy agar plate, and the other two were stored on blood agar plates. Twenty-four hours before, an aliquot obtained from colonies on the stored plate was streaked on each specific agar plate, and the plate was incubated at room temperature to obtain sufficient colonies. In the implantation study, with the increased colonies of each organism strain, organism solutions were prepared. Briefly, some of the colonies were diluted with a physiologic saline solution (NaCl, 0.85% Medium; biomerieux sa, Marcy L'Etoile, France), and the desired concentration in compliance with the recommendation of the National Committee for Clinical Laboratory Standards (NCCLS)[22] was automatically produced with a colorimeter (ATB 1500, Vitek Systems Co, Japan). In this study, 1 10^8/mL of organism concentration was used for graft inoculation.

Antibacterial susceptibility test of rifampicin-gelatin grafts. To assess antibacterial activity of rifampicin-gelatin grafts, four round disks with a diameter of 6 mm were cut from each graft segment and placed on a Müller-Hinton agar plate, which had been inoculated with one of the preceding bacteria, and incubated at 35°C for 24 hours. Diameters of the inhibition zones formed surrounding the four disks were measured, and the antibacterial activity of the grafts was expressed with the mean diameter of the four inhibition zones. In this study, susceptibility criteria were on the basis of the NCCLS.[22] According to the criteria, clinical efficacy is divided into three levels: susceptible, intermediate, and resistant. The relations between each level and minimum inhibitary concentration (MIC) in rifampin are as follows: in *S aureus*, susceptible more than 35 mm, intermediate 26 to 34 mm, and resistant less than 25 mm; and in *E coli*, susceptible more than 11 mm, intermediate 8 to 10 mm, and resistant less than 7 mm. MICs were 0.01 to 0.5 g/mL for Staphylococci, including MRSA, and less than 12 g/mL for *E coli*.[8] In our preparation method, concentration of rifampicin was most stable at 1 mg/mL, and susceptibilities of MRSA and *E coli* to rifampicin were considered to be at significant levels when the inhibition zones were more than 35 mm and more than 11 mm or more, respectively. Because the maximal inhibition zone was established within 24 hours after incubation, the mean diameter at 24 hours was defined as the initial inhibition zone, which was compared among the three bacteria strains.

Table I. In vitro susceptibility testing of organisms to rifampicin-gelatin grafts

	Diameters of inhibition zone (mm)		
Time after incubation	S epidermidis	*MRSA*	E coli
24 hours (IIZs)	40.0 ± 0.3	36.0 ± 0.2	11.8 ± 0.1
2 days	36.5 ± 0.4	32.5 ± 0.2	9.0 ± 0.2
5 days	34.6 ± 0.4	30.4 ± 1.2	0
1 week	30.6 ± 0.4	24.2 ± 0.8	–
2 weeks	26.3 ± 0.5	15.6 ± 1.6	–
3 weeks	18.2 ± 1.0	0	–

Values are mean ± standard deviation of four discs.
IIZs, Initial inhibition zone.

Evaluation of rifampicin-gelatin graft infection resistance in an animal model. Twenty-eight randomly selected mongrel dogs of both sexes, weighing 10 to 15 kg (average, 14.4 ± 0.58 kg), were used for implantation. The study was approved by the Institutional Review Board, and animal care complied with the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (Washington: National Academy Press, 1996). All the animals were anesthetized with intravenous thiamylal sodium, intubated, and ventilated with 33% oxygen, and a median laparotomy was performed with aseptic conditions. After intravenous administration of heparin, rifampicin-gelatin grafts (6 cm long; n = 24) and plain Gelseal grafts as controls (diameter, 6 mm; n = 4) were implanted into the canine infrarenal abdominal aorta in end-to-end anastomoses with 5-0 polypropylene continuous sutures. Immediately after aortic declamping, the graft was infected with direct inoculation with 2 mL of one of the three organism solutions with a concentration of 1 10^{8}/mL, and the retroperitoneum and laparotomy then were closed in layers. No antibiotics were given during the experimental period. The implantation study was performed in compliance with the vertebrate animal biosafety-level criteria in microbiologic and biomedical laboratories.[23] The infected animals were housed in primary biosafety containment equipment, and their care complied with the guidelines of animal biosafety level 2.

Graft retrieval and examination. Of 28 animals, 20 were used for *S epidermidis* innoculation study. Of those 20, 16 were used for implantation of *S epidermidis*–inoculated rifampicin-gelatin grafts, which were retrieved at 1, 2, 3, or 4 weeks after implantation, and their healing process after organism innoculation was examined. Four were used for implantation of plain Gelseal grafts as controls, which were removed at 1 week. The remaining eight animals were used for MRSA or *E coli* innoculation study; four each were used for implantation of rifampicin-gelatin grafts inoculated with MRSA or *E coli*, which were retrieved at, or within, 1 week. At the removal, all of the animals were anesthetized, and arterial blood sampling was performed for examination of septicemia. The graft was reexplored aseptically, direct graft imprinting on each specific agar plate was used for bacterial recovery, and perigraft fluid and tissue swabs were also streaked and cultured. After intravenous administration of heparin, the animals were killed with an overdose of potassium chloride. The graft, including both anastomoses, was carefully removed and longitudinally opened for inspection of the luminal surface, and four disks were obtained from each organism-innoculated graft. At first, to examine the residual antibacterial activity of the rifampicin-gelatin grafts, the four disks were placed on a Müller-Hinton agar plate inoculated with the same bacteria and incubated at 35°C. The *E coli*–inoculated rifampicin-gelatin graft disks and the MRSA-inoculated rifampicin-gelatin graft disks also were tested on the *S epidermidis*–streaked plates. The residual portions of each graft after disk removal were rinsed once with a saline solution, and direct graft imprinting on the agar plates was performed for bacterial recovery. The agar plates were incubated overnight. When the specific colony growth was recognized, it was considered that the graft had been infected. After the direct graft imprinting, the residual portions of each graft were fixed with 10% buffered formalin for 24 hours, cross-sectioned, embedded in paraffin, and stained with hematoxylin and eosin and Elastica van Gieson.

RESULTS

In vitro susceptibility test of organisms to rifampicin-gelatin graft

For in vitro test results of rifampicin-gelatin grafts, the mean diameters of initial inhibition zones against *S epidermidis*, MRSA, and *E coli* were 40.0 ± 0.3 mm, 36.0 ± 0.2 mm, and 11.8 ± 0.1 mm, respectively (Table I). These results suggested that rifampicin-gelatin grafts used in this study were valid and useful for *S epidermidis*, whereas the efficacy against *E coli* was insufficient but susceptible. In the MRSA group, despite the sufficient values of initial inhibition zone, recolonization of MRSA strains with high level MICs developed within the inhibition zone as early as 3 days after incubation (Fig 1), which suggests that MRSA had no bacterial antibiotic susceptibility to rifampicin-gelatin grafts as well.

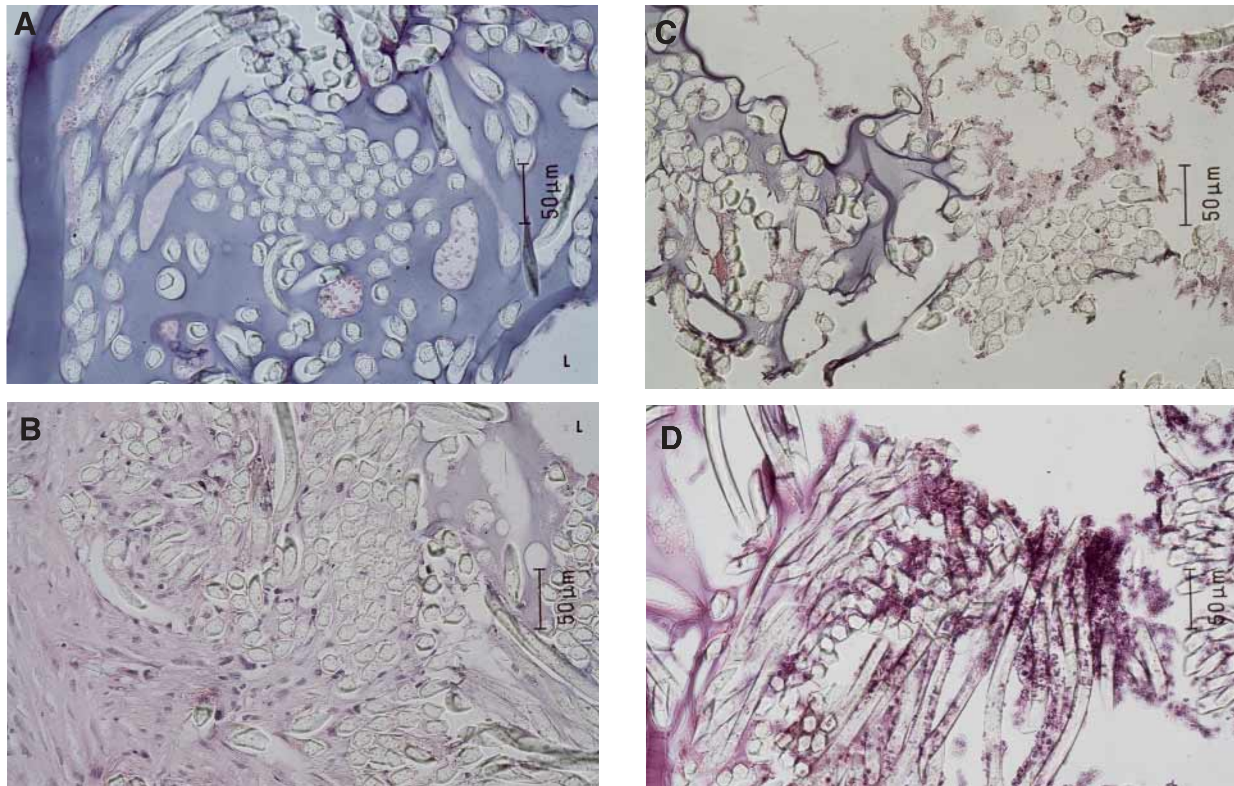

Fig 2. Photomicrographs of rifampicin-gelatin grafts after implantation. *S epidermidis*–inoculated rifampicin-gelatin grafts at day 7 show no inflammatory responses **(A)** and at day 14 show good healing **(B)**. MRSA-inoculated and *E coli*–inoculated rifampicin-gelatin grafts show aggressive neutrophil infiltration in outer capsule (**C,** MRSA) and graft interstices (**D,** *E coli*). Cross-section, hematoxylin and eosin. *L,* Luminal surface.

Implantation study

Staphylococcus epidermidis. All of the 20 grafts were patent at the time of removal, and no perigraft abscess was recognized in either the rifampicin-gelatin graft or control groups. However, in bacteriologic culture of the graft, perigraft fluid, and tissue swabs, no growth of an *S epidermidis* strain was recognized in the rifampicin-gelatin graft group, whereas many colonies were formed within 24 hours after incubation in the control group, which suggests graft infection. Mean initial inhibition zones of *S epidermidis*–inoculated rifampicin-gelatin graft disks retrieved at 1 and 2 weeks were 20.1 ± 1.1 mm and 7.6 ± 1.0 mm, respectively (Table II), which shows a significant duration of the antibacterial activity. These inhibition zones disappeared at 3 weeks, and no inhibition zones were formed in controls.

Histologically, control grafts inoculated with *S epidermidis* showed severe neutrophil infiltration in the outer tissue capsule and into the graft interstices through defects of the sealant at day 7, whereas neither organisms nor inflammatory cells were found in *S epidermidis*–inoculated rifampicin-gelatin grafts retrieved at 1 to 4 weeks (Fig 2, *A*). Sufficient gelatin sealants of *S epidermidis*–inoculated rifampicin-gelatin grafts remained after 2 weeks, but most of them had been absorbed at 3 weeks. Fibrous tissue ingrowth was recognized in *S epidermidis*–inoculated rifampicin-gelatin grafts retrieved at 2 to 4 weeks (Fig 2, *B*), proving that *S epidermidis* infection had been thoroughly prevented.

MRSA. Of four animals, one died within 7 days. Perigraft abscess was recognized in all of the four rifampicin-gelatin grafts. Three animals with patent grafts were in distress at death, and blood bacteriologic culture tests probed MRSA septicemia. In the bacteriologic culture test for the retrieved rifampicin-gelatin grafts, no colonies developed on the plates that were directly streaked with the retrieved MRSA-inoculated rifampicin-gelatin grafts, whereas many colonies were formed within 24 hours in the plates streaked with the perigraft fluid or tissue fragments. Aggressive MRSA recolonization without initial inhibition zones around MRSA-inoculated rifampicin-gelatin graft disks formed on MRSA-streaked plates. Although significant initial inhibition zones with mean diameters of 18.5 ± 0.5 mm had formed on the *S epidermidis*–streaked plates (Table II), recolonization of MRSA strains with high level MICs developed within the inhibition zone as early as 24 hours, which was earlier than that observed in rifampicin-gelatin grafts in the preimplantation test. Histologically, MRSA-inoculated rifampicin-gelatin grafts had marked perigraft abscess with aggressive

Table II. Infection resistance of organism-inoculated rifampicin-gelatin grafts on Staphylococcus epidermidis–streaked plates

	Diameters of initial inhibition zone (mm)		
Implantation periods	*S epidermidis*	*MRSA*	*E coli*
1 week	20.1 ± 1.1 (n = 4)	18.5 ± 0.5 (n = 4)	8.0 ± 0.2 (n = 4)
2 weeks	7.6 ± 1.0 (n = 4)	–	–
3 weeks	0 (n = 4)	–	–

Number of rifampicin-gelatin grafts (n = 4).

neutrophil infiltration (Fig 2, *C*), revealing establishment of graft infection.

E coli. Of four animals, two died within 7 days. Perigraft abscess was recognized in all of the four rifampicin-gelatin grafts, and blood bacteriologic culture tests probed *E coli* septicemia in two animals with patent grafts at death. In the bacteriologic culture test, no colonies developed in direct imprinting with the retrieved *E coli*–inoculated rifampicin-gelatin grafts, whereas many colonies were formed within 24 hours in the plates streaked with the perigraft fluid or tissue fragments. As with MRSA, aggressive high MIC *E coli* recolonization around *E coli*–inoculated rifampicin-gelatin graft disks formed on the plates streaked with *E coli*, without initial inhibition zone formation. However, significantly sized initial inhibition zones with a mean diameter of 8.0 ± 0.2 mm were formed on *S epidermidis*–streaked plates (Table II). These results revealed that, although the susceptibility of *E coli* to rifampicin-gelatin grafts was poor, rifampicin-gelatin grafts still retained their antibacterial activity for as long as they did against *S epidermidis.*

Histologically, *E coli*–inoculated rifampicin-gelatin grafts had marked perigraft abscess with aggressive neutrophil infiltration as well. As seen in *S epidermidis*–inoculated and MRSA-inoculated rifampicin-gelatin grafts, sufficient gelatin sealant of *E coli*–inoculated rifampicin-gelatin grafts still remained 1 week after implantation, but there was neutrophil infiltration into the graft interstices through the sealant defects (Fig 2, *D*), revealing establishment of graft infection.

DISCUSSION

Use of antibacterial vascular grafts has two objectives: prophylactic use in primary vascular reconstruction and management of mycotic aneurysms or established graft infections. In the former, unusual postoperative healing processes, such as perigraft hematoma, prolonged lymphatic discharge, or accumulation, are relevant inducing factors of graft infection. In the latter, replacement of infected grafts with extraanatomic bypasses has been preferentially used, and Yeager et al[24] reported satisfactory results for infrarenal aortic infection. Nevertheless, in situ replacement has definite surgical and anatomic advantages when compared with the extraanatomic technique, which is a longer procedure with substantial risks of morbidity and mortality. In addition, the use of autogenous material for replacement is often not practical. Hence, cryopreserved arterial homografts have been used for in situ replacement[25,26] and rifampicin-gelatin grafts[7,10-17] have been studied as an alternative material because of the potential advantages of easier procurement and handling, when compared with cryopreserved arterial homografts.

The definitive efficacy of rifampicin-gelatin grafts still remains unclear. The rifampicin retention in the graft results from the ionic bond formation between the negatively charged free carboxyl groups of the gelatin and the positively charged radicals of rifampicin.[10,16] The estimated elution half lives of protein-sealed grafts were reported to be 4 to 141 hours.[11] However, the rifampicin retention after implantation is restricted, not only by absorption of the antibiotic itself, but also by degradation of the gelatin sealant. Although Drury et al[27] reported that the gelatin sealant of Gelseal grafts was mostly absorbed within 7 to 10 days after implantation, this study confirmed that a significant amount of gelatin was retained on the graft surface for 2 weeks after implantation and sufficient antibacterial activity of the retrieved rifampicin-gelatin grafts was retained up to 2 weeks after implantation. Therefore, it was suggested that effective infection resistance is maintained for 2 weeks and that the loss of antibacterial activity is the result of the resorption of the gelatin sealant rather than of release or inactivation of rifampicin itself.

Rifampicin is not only the most effective antibiotic for mycobacterium tuberculosis but also has a wide antibacterial activity against both gram-positive and gram-negative bacterial strains. The mechanism of the action is RNA disruption with DNA-dependent RNA polymerase.[28] However, the identity of organism strains susceptible to rifampicin-gelatin graft still remains unclear. In this study, we tested *S epidermidis* as the most frequent pathogen of biofilm infection[18,19] and *E coli* as the most virulent gram-negative bacterium. In addition, the currently highlighted organism, MRSA, was also tested because there was no definitive study regarding the efficacy of rifampicin-gelatin graft on MRSA, regardless of the poor outcome of MRSA

graft infection.[20] For in vivo assessment of the efficacy of rifampicin-gelatin grafts, bacteria-challenging animal models have been used in the previous studies, where various organism concentrations for inoculation were adopted, with a range of 10^2 to 1.0 10^8 mL.[4,6,7] According to the guidelines of the NCCLS for antibiotic susceptibility testing,[29] use of Macfarand 0.5 solution, which is 1 to 2 10^8 colony forming units (CFU)/mL, is recommended. We used a direct culture method instead of a bacterial culture method with a broth, and in our preliminary study, bacterial concentration of 1 10^8 CFU/mL was obtained at McFarland 1.0. We presumed that the difference in concentration probably was the result of the use of the direct culture technique. Neverthless, 10^8 is still a high level, but the NCCLS recommended this concentration for antibacterial activity testing. In this study, the grafts were infected with inoculation with 2 mL of the organism solution with 10^8 CFU/mL, which is the concentration when graft infection was established and the organism number had reached a plateau.[28] This organism concentration was the highest when compared with those in the previous studies. However, in clinical use, it would be essential to confirm a significant efficacy of rifampicin-gelatin grafts as shown in the test results of *S epidermidis* in this study.

Rifampicin solution has two disadvantages as an antibacterial agent: the first is loss of antibacterial activity because of oxidation in room temperature. Sano and Tunakawa[9] reported that inactivation was greatly suppressed at neutrality or in lower temperatures. In this study, the loss of antibacterial activity was solved with preparation 1 day before use and storage in a refrigerator. Rifampicin-gelatin grafts were sufficiently effective against *S epidermidis* infection both in in vitro tests and animal implantation. However, *E coli* was not susceptible to the rifampicin-gelatin grafts retrieved 1 week after implantation, and no organisms were recovered from MRSA-inoculated and *E coli*–inoculated rifampicin-gelatin grafts in the direct imprinting technique. As shown in Table II, the organism-inoculated rifampicin-gelatin grafts still contained rifampicin at 1 week after implantation. Therefore, we speculated that the rifampin bonding the grafts inhibited the growth of *E coli* only on the graft surface but was in insufficient concentration to affect the surrounding tissues. There is the other possibility that *E coli* graft infection was the result of the relatively low concentration of rifampicin solution. MICs are 0.01 to 0.5   g/mL for Staphylococci, including MRSA, and less than 12   g/mL for *E coli*.[8] In our preparation method, concentration of rifampicin was most stable at 1 mg/mL, which is about 2000 to 83 times higher than the MICs. Because initial inhibition zones were more than 35 mm for MRSA and 11 mm or more for *E coli*, the susceptibilities to rifampicin of the rifampicin-gelatin grafts were considered to be significant levels, in accordance with the guidelines of the NCCLS.[29] In this study, these were 36.0 ± 0.2 mm and 11.8 ± 0.1 mm, respectively, revealing the concentration of the rifampicin solution to be sufficient. Hence, we consider that 1 mg/mL of rifampicin is sufficient in local application and the use of high concentration rifampicin may develop high MIC strains.

The other disadvantage of rifampicin-gelatin grafts is the well-known tendency to yield high MIC strains,[20,30] especially against MRSA, and topical use accelerates this tendency. Hayes et al[20] reported that in situ replacement with a rifampicin-gelatin graft is probably ineffective for established graft infection caused by MRSA. In this study, MRSA was initially sensitive to rifampicin, but high level MIC strains developed early, and MRSA-inoculated rifampicin-gelatin grafts formed severe perigraft abscesses, resulting in serious outcome. Although this implantation model did not investigate the effect of rifampicin-gelatin grafts on established graft infection, the results clearly showed that rifampicin-gelatin grafts are absolutely inapplicable against MRSA infection. In a further study, evaluation of the other alternative antibacterial agents, such as vancomycin or teicoplanin, will be necessary.

In conclusion, the in vitro susceptibility test and implantation study showed that rifampicin-gelatin grafts retained sufficient antibacterial activity for 2 weeks and may be useful both for prophylaxis and established graft infection against *S epidermidis*. However, *E coli* and MRSA showed no significant susceptibility to rifampicin-gelatin grafts. Thus, rifampicin-gelatin grafts are not applicable to management of infections associated with vascular surgery that are caused by these virulent organisms.

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Submitted Feb 7, 2001; accepted Oct 10, 2001.

Please see the related commentary by Dr A. Ross Naylor on pages 823-4.

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