Effect of Levofloxacin-Albumin Dacron Graft on Graft Infection

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ABSTRACT—The susceptibility of Dacron grafts to infection is compared with Dacron grafts applied with levofloxacin-bonded albumin (LVFX-ALB) following the inoculation of $10^7$ cells Staphylococcus aureus in rats. Staphylococcus epidermidis was inoculated in the same manner. While the control grafts were infected at the time of removal, the LVFX-ALB Dacron grafts resisted infection, thus demonstrating their effectiveness.

Keywords: Levofloxacin, Albumin, Graft infection

The highly porous Dacron graft fabricated from polyester filaments is generally considered to be one of the most suitable synthetic vascular prostheses in arterial reconstructive surgery (1). To prevent blood leakage through the pores during an operation, several sealing materials including albumin (ALB), collagen and gelatin have been employed (2). As vascular graft infection is a disastrous complication of vascular surgery, great effort must be taken to avoid this. Preoperative intravenous antibiotic prophylaxis is generally administered at the site of implantation to prevent prosthetic graft infection. However, only a small fraction of any given dose actually reaches the operated site. To reduce the systemic effects while maintaining an increase in local resistance to graft infection, it seems reasonable that an antibiotic-loaded graft prolongs the release of antibiotics from the graft at the operated site. Recent clinical and experimental studies have suggested that the development of infection-resistant vascular grafts, such as the gelatin-sealed Dacron grafts with ionically-bonded rifampicin, could prevent both early and late graft infections (3). However, it should be noted that when gelatin or collagen is used extensively, fever and allergic reactions have been reported (4). It is believed that the fever reaction was of immunological origin. To our knowledge, no report has examined the fever response to ALB-sealed grafts. To prepare ALB-sealed grafts, the graft is soaked in ALB solution and then autoclaved. As a consequence, ALB microspheres prepared by heat denaturation prevent blood leakage through the pores. Therefore, incorporation of antibacterial agents in ALB at the time of presealing grafts may be useful. The antibacterial agent chosen should not only be active against both types of staphylococci (S. aureus and S. epidermidis) and E. coli (these organisms are responsible for approximately 75% of all early and late prosthetic graft infections (5)), but also resistant to heat by autoclaving. A potential problem with using rifampicin is that resistance against it is rapidly developed (6). In addition, we observed by HPLC that rifampicin was heat-degradable. Levofloxacin (LVFX) was chosen because it shows a broad protective spectrum against Gram-positive and Gram-negative bacteria, particularly staphylococci (7), and 100% stability of LVFX solutions (1–10 mg/ml) was determined after autoclaving by HPLC. Greco et al. reported that an antibiotic drug may be necessary for only a short time, if a high initial concentration of the agent is administered, and that could prevent adherence of bacteria to the graft and reduce the inoculum (8). Therefore, this initial study is designed to load the maximum dose of LVFX into the ALB-sealed Dacron graft. In this paper, we investigated the antibacterial effects of the LVFX-ALB Dacron graft for enhancing local resistance to graft infection, by comparison with an intravenous (i.v.) injection in rat models.

Albumin (Albuminar®; 25% w/v, 50 ml/vial) was obtained from Yamanouchi Pharmaceutical Co. (Tokyo). LVFX was a gift from Daiichi Pharmaceutical Co. (Tokyo). Trypticase soy agar (TSA) was obtained from Nissui Pharmaceutical Co. (Tokyo). Potassium phosphate, monobasic and phosphoric acid were obtained from Katayama Chemical Industries (Osaka). Distilled water and methanol were of HPLC quality. All other

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Reagents were of analytical reagent quality. A double velour knitted Dacron® (Sauvage filamentous Dacron fabric; 1600 ml H2O/cm²/min/120 mmHg in porosity and 0.7 mm in thickness) was supplied from Bard Implants, Inc. (Bard Europe Division, Murray Hill, NJ, USA). Eleven-week-old male Sprague-Dawley rats (Nippon SLC, Hamamatsu) weighing 340–360 g each were used for all experiments.

The LVFX-ALB Dacron graft was prepared as follows: The amount of LVFX incorporated in the Dacron disk (1.2 cm in diameter) was predicted from the water accessible volume. The weight difference between 10 wet Dacron grafts after being soaked in water and 10 dry Dacron grafts before being soaked in water was assumed to be equivalent to the volume of aqueous phase loaded.

LVFX was dissolved in ALB solution at a concentration of 4.8 mg/ml (approximate saturated solution). Next, the Dacron graft with LVFX-ALB solution was autoclaved for 30 min at 115°C (LVFX-ALB Dacron graft; LVFX: 248 µg/graft). The in vivo release rates of LVFX from the Dacron grafts were studied. Four LVFX-ALB Dacron grafts were implanted in the subcutaneous tissue of the anterior abdominal wall of a rat, and these were removed at 0.5, 1, 3, 5, 8 and 24 hr. The removed grafts were placed into distilled water at 5°C for 3 days, and the LVFX released in the solution was determined as the remaining LVFX in the grafts. LVFX Dacron grafts were each performed in the same manner. The concentration of LVFX was assayed by HPLC under the following conditions: a reversed phase column, µ-Bondapak C18 (10 µm, 30 cm x 3.9 mm i.d.; Waters Co., Milford, MA, USA); eluent: methanol-water (7 : 3, V/V) containing 0.05 M potassium phosphate, monobasic and 0.025 M phosphoric acid; flow rate, 1.0 ml/min; and detection, UV at 330 nm. The assay was linear for the concentrations that we tested.

The susceptibility of the LVFX-ALB Dacron grafts to infection was studied. The rats were divided into four groups: 1) LVFX-ALB graft group: One LVFX-ALB Dacron graft was implanted in the subcutaneous tissue of the anterior abdominal wall of a rat; the graft was inoculated with S. aureus ATCC 25923 (0.1 ml of 10⁸ CFU/ml). 2) LVFX graft group: The LVFX graft (without ALB) was performed in the same manner. 3) Control group: The control graft (without LVFX-ALB) was performed in the same manner, and 4) i.v. graft group: LVFX was injected intravenously at 250 µg/rat right after implantation of one control graft and inoculation. S. epidermidis ATCC 14990 was each performed in the same manner. All grafts were aseptically harvested two days following the operation. Each sample was soaked in a test tube containing saline (5 ml) and sonicated for 3 min. This procedure has been proven to facilitate the quantitative removal of the attached microorganisms (9). The solutions were then serially diluted, and 0.1-ml aliquots were subcultured on TSA and incubated at 37°C for 48 hr. Fisher’s exact probability test was used to perform a statistical analysis.

Figure 1 shows the percentage of remaining LVFX in the Dacron grafts after implantation. In vivo release of LVFX from the LVFX Dacron grafts reached almost 100% 1 hr following implantation, whereas 35% of the LVFX remained in the LVFX-ALB Dacron grafts 1 hr later. From these findings, we can presume that release rates of LVFX from the LVFX-ALB Dacron graft were retarded more than that of LVFX from the LVFX.

![Graph showing remaining LVFX percentage over time](image)

**Fig. 1.** Remaining (%) of levofloxacin (LVFX) in the disks implanted in rat abdominal subcutaneous tissues at various time intervals. ○: LVFX-ALB disk, ●: LVFX disk. Values are expressed as means±S.E. (n=8).
Dacron graft because LVFX was entrapped in the ALB microspheres prepared by heat denaturation (10). Table 1 shows the results of bacterial cultures. All control grafts (12/12) and surrounding tissues were infected at the time of removal. Three of the six i.v. grafts inoculated with S. aureus became infected and two of the six i.v. grafts inoculated with S. epidermidis became infected. One of the six LVFX grafts inoculated with S. aureus (P < 0.01) and none of the six grafts with S. epidermidis became infected (P < 0.005). None of the six LVFX-ALB Dacron grafts inoculated with S. aureus (P < 0.005) nor the six LVFX-ALB Dacron grafts inoculated with S. epidermidis, were infected (P < 0.005). In the present study, the LVFX-ALB graft delivery showed higher antibacterial effects than those from an equal dose of i.v. infection. These findings lead us to presume that a lesser amount of LVFX incorporated in the graft may be useful to prevent graft infection, since there is evidence that toxicity is related to the given dose of the drug. We believe that for the clinical use of this delivery, further studies are needed to clarify the relationship between the loaded dose and activity against various clinical isolates such as staphylococci and E. coli.

**Table 1. Bacteriologic evaluation of infection in LVFX-ALB Dacron grafts**

| Inoculated strain | Number of infected grafts/Total number of grafts |
|-------------------|-----------------------------------------------|
|                   | Control | i.v. | LVFX | LVFX-ALB |
| ATCC 25923        | 6/6     | 3/6  | 1/6* | 0/6*     |
| ATCC 14990        | 6/6     | 2/6  | 0/6* | 0/6*     |

Statistical differences were analyzed by Fisher's exact probability test. *P < 0.01 vs control, **P < 0.005 vs control. Control: colonized grafts implanted for 2 days without LVFX-ALB. i.v.: colonized grafts implanted for 2 days with LVFX i.v. injection. LVFX: colonized grafts implanted for 2 days with LVFX. LVFX-ALB: colonized grafts implanted for 2 days with LVFX-ALB.

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