Stability of Antibiotics Bound to Polytetrafluoroethylene with Cationic Surfactants

ANTHONY P. DONETZ, RICHARD A. HARVEY, AND RALPH S. GRECO*

Departments of Surgery and Biochemistry, University of Medicine and Dentistry of New Jersey-Rutgers Medical School, New Brunswick, New Jersey 08903

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This study evaluated the effect of prolonged storage and sterilization on the noncovalent bonding of penicillin to polytetrafluoroethylene grafts. The surfactant tridodecylmethylammonium chloride (TDMAC) was unaffected by prolonged storage or sterilization, and its ability to bind penicillin remained constant for as long as 3 months. Steam or ethylene oxide sterilization markedly diminished the antibacterial activity of bound penicillin. However, the antibacterial properties of penicillin remained constant for up to 12 weeks when the grafts were stored at either 4°C or room temperature. Thus, the bonding process appears to increase the stability of the antibiotic, and the data presented suggest that vascular prostheses can be treated with a surfactant, sterilized, and stored for at least 3 months. Grafts can then be treated with an antibiotic in the operating room, washed, and implanted.

Materials and Methods

Materials. PTFE grafts were obtained from W. L. Gore and Associates, Inc. Tridodecylmethylammonium chloride (TDMAC) was obtained from Polysciences, Inc., and [14C]benzylpenicillin was obtained from the Amersham Corp. Benzylpenicillin was obtained from Sigma Chemical Co.

Binding of surfactant. One-half-centimeter segments of 6-mm internal diameter PTFE grafts were soaked in a 5% (wt/vol) solution of TDMAC in ethanol for 30 min, removed, and then allowed to air dry for a minimum of 1 h. The grafts were then vigorously washed five times in distilled water.

Binding of antibiotic. Sections of PTFE pretreated with TDMAC were immersed in a solution of 1 mg of penicillin per ml for 1 h. Graft sections were then washed in distilled water and centrifuged at 1,100 × g for 15 min in individual tubes plugged with absorbent toweling to facilitate the removal of any excess moisture or unbound penicillin. The binding of [14C]penicillin to PTFE was performed by an identical technique except that the grafts were subjected to varying concentrations of penicillin containing 1 nCi of [14C]penicillin. All graft sections were washed as above and then subjected to liquid scintillation counting in a Beckman LS 150 scintillation counter.

Bioassays. The antibiotic activity of the control and experimental grafts was evaluated in a bioassay, the methodology for which has been described in previous publications (3, 4). Briefly, grafts were embedded in a 150-mm diameter petri dish containing 65 ml of brain heart infusion agar which was seeded with 3 × 10^8 Staphylococcus aureus. The S. aureus used in these experiments was initially harvested from a wound culture, and its susceptibility to penicillin has been described elsewhere (4). The S. aureus-seeded plates containing the grafts to be studied were placed in an incubator for 18 to 24 h at 37°C. The size of the zones of inhibition of bacterial growth around the grafts was then measured. These zones of inhibition were then compared to the zones of inhibition of grafts containing a known quantity of [14C]penicillin measured by liquid scintillation counting. A standard curve was thereby achieved and utilized to convert the observed zones of inhibition in experimental grafts to micrograms of penicillin.

Penicillinase assay. The degradation of penicillin bound to the surface of PTFE grafts was investigated by using commercially available penicillinase (Sigma). Grafts were treated with TDMAC and [14C]penicillin by the techniques described above. One group of grafts received no further treatment, one group of grafts was treated with 0.05 M phosphate buffer (pH 5.9), and one group of grafts was treated with high concentrations (33 μg/ml) of penicillinase in the same buffer. Half of the grafts in each of the three groups were then counted for radioactivity, and the other half were extracted with ethanol, and the presence of penicillin and penicilloic acid was determined chemically (6) in the alcohol extract.

Evaluation of grafts. TDMAC was bound to 110 sections of PTFE. Fifteen sections were evaluated immediately after surfactant-antibiotic binding. Of these 15, 10 were subjected to steam sterilization at 15 lb (1.02 atm [ca. 101.3 kPa]) and 120°C for 15 min. Five of these grafts were steam sterilized before the binding of penicillin and five after the binding of penicillin. These grafts as well as the five grafts which had not been exposed to steam sterilization were then utilized in the bioassay.

Twenty graft sections were evaluated 1 week after treatment with TDMAC and penicillin. Of these 20, 10 were stored at room temperature and 10 were stored at 4°C. Before the bioassay, five sections from each group were subjected to steam sterilization. Twenty sections were eval-
uated by this scheme 3, 6, and 12 weeks after surfactant-

antibiotic bonding. In a separate group of bioassays, 15 TDMAC-bonded graft sections were evaluated. Ten graft sections were sterilized before the bioassay with ethylene oxide gas at 52°C for 3 h and degassed for 12 h. Five graft sections were sterilized before the addition of penicillin and five after the addition of penicillin. Five graft sections were treated only with penicillin.

RESULTS

Grafts which were incubated with [14C]penicillin in concentrations from 125 to 1,000 μg/ml were utilized to establish a correlation between the zones of inhibition expressed in the bioassay and a known quantity of freshly bound 14C-labeled antibiotic as determined by liquid scintillation counting. Thereby, a direct correlation was established between the observed zones of inhibition of bacterial growth and the concentration of antibiotic bound to the surface of the graft. This relationship is demonstrated in Fig. 1. Previous studies have shown that TDMAC has no inhibitory effect on the growth of S. aureus, and a checkerboard susceptibility study demonstrated no significant synergism between the surfactant and the antibiotic. Therefore, the antibacterial activity expressed in the bioassay is that of penicillin alone. However, the zones of inhibition measure only the amount of antibiotic which dissociates from the graft into the surrounding agar. Liquid scintillation counting, on the other hand, presumably measures the total amount of penicillin bound to the surface of the graft, and this relationship must be borne in mind when analyzing the curve shown in Fig. 1. The antibacterial activity of all experimental grafts was determined in the bioassay, and liquid scintillation counting was utilized to convert the zones of inhibition to micrograms of penicillin. The standard curve was achieved with freshly prepared grafts and, therefore, presumably measures biologically active [14C]penicillin.

The antibacterial activity of the five control grafts subjected to steam sterilization after the binding of TDMAC but before the addition of penicillin was not significantly different from the five grafts not subjected to steam sterilization. This indicates that the capacity of TDMAC to bind antibiotics is unaffected by elevated temperature and pressure. The TDMAC-bonded grafts that were exposed to ethylene oxide sterilization before the addition of penicillin showed the same zone of inhibition of growth as their control counterparts which were not sterilized. The five control grafts that were treated with penicillin before steam sterilization showed a marked decrease in the amount of biologically active penicillin on the grafts. Finally, antibiotic-bonded grafts that underwent ethylene oxide gas sterilization after the addition of penicillin also showed a marked decrease in the amount of biologically active antibiotic (Fig. 2).

The duration of graft storage had no effect on the antibacterial activity of experimental grafts when compared with freshly prepared grafts. This was true of the grafts stored at room temperature or 4°C. Steam sterilization and ethylene oxide gas sterilization caused a 90% reduction in the antibacterial activity of penicillin. On the other hand, neither form of sterilization had any effect on the surfactant TDMAC. In addition, the surfactant-treated grafts which underwent sterilization and were then incubated with antibiotic showed almost identical antibiotic bonding at any time period for up to 3 months after surfactant binding. Grafts were treated with high concentrations of penicillinase to determine how the binding properties of penicillin are affected by hydrolytic cleavage of the β-lactam ring of the antibiotic. Table 1 shows that incubation with penicillinase led to the expected cleavage of the antibiotic but that this hydrolytic reaction was not accompanied by a concomitant dissociation of the radioactive degradation product from the surface of the graft. Even in the absence of penicillinase, a substantial amount of the bound penicillin was cleaved to penicilloic acid.

DISCUSSION

Our laboratory has proposed that the biochemical bonding of anionic antibiotics to PTFE with cationic surfactants is a more efficient and effective method of infection prophylaxis than is parenteral antibiotic administration. The latter requires large doses of antibiotics to achieve very small tissue concentrations, is costly, and may be of limited use in patients with hepatic and renal insufficiency. Bonded antibiotics require the use of nominal amounts of antibiotics in the treatment process, are cost effective, and are applicable to all patients not allergic to the penicillins or cephalosporins. This study evaluated the feasibility of storing and sterilizing vascular prosthetic grafts biochemically bonded with TDMAC and penicillin. The data demonstrate no appreciable effect on antibacterial activity by the length or type of storage utilized. Thus, bonded grafts may be prepared far in advance of their clinical use and stored in a variety of ways. Steam sterilization has a deleterious effect on the antibacterial activity of bound penicillin as one would expect, since penicillin is unstable at elevated temperature. This reduction in activity precludes antibiotic bonding before sterilization procedures. On the other hand, TDMAC appears to be unaffected by steam sterilization. In addition, the effects of ethylene oxide gas sterilization are the same as those with steam sterilization. Grafts sterilized with ethylene oxide were sterilized for 3 h at 52°C, and temperature may have been as much a factor as the sterilization technique. This suggests a practical model for the clinical application of antibiotic bonding. Vascular grafts can be treated with the surfactant, sterilized, and stored for long periods of time. In the operating room, they can be treated with a specific amount of antibiotic per centimeter of graft, washed, and implanted.

Although the bound penicillin shows a remarkable resistance to chemical degradation, treatment with penicillinase results in cleavage of the amide bond of the β-lactam ring.

FIG. 1. Relationship of zones of inhibition of the growth of S. aureus in millimeters to micrograms of [14C]penicillin measured by liquid scintillation counting.
This hydrolysis, which also occurs nonenzymically in aqueous solutions, is the most commonly encountered reaction leading to the inactivation of penicillin. The data employing penicillinase emphasize that the determination of radioactivity bound to grafts cannot differentiate between intact penicillin and inactive degradation products. Since the hydrolytic cleavage of penicillin generates penicilloic acid with two negative carboxyl groups, it is not surprising that the reaction products remain largely attached to the positively charged graft. It has been widely documented in aqueous solutions that cleavage of the β-lactam ring of penicillin results in the loss of antibacterial activity. The same is true of penicillin bound to vascular grafts. That is, penicillinase treatment results in the loss of biological activity. However, our data are all based on antimicrobial activity determined in a bioassay. Thus, bound antibiotic is remarkably stable when compared with the well-known lability of the penicillins stored or kept at room temperature. This phenomenon may in part be due to the hydrophobic nature of the Gore-Tex material. PTFE, because of its nonpolar properties, does not present a favorable surface for the condensation of water. Thus, individual penicillin molecules may find themselves in an anhydrous state, being surrounded by a nonpolar environment, even when the graft is exposed to relatively high concentrations of water vapors. This contrasts with the hydroscopic nature of penicillin crystals and may explain the very low rate of hydrolytic cleavage of bound antibiotic relative to crystalline penicillin.

The studies reported herein demonstrate that penicillin and TDMAC can be bound to PTFE for prolonged periods of time and that the surfactant withstands gas or steam sterilizations. A method for the preparation of antibiotic-bonded vascular grafts is proposed which facilitates the early clinical application of this method of prosthetic infection prophylaxis.

| TABLE 1. Incubation with penicillinase leads to the expected cleavage of the antibiotic |
|---------------------------------------------|---------------------------------------------|
| Treatment               | Total 14C-ligands bound (μg/0.5 cm) | Chemical composition of bound ligands (μg/5 μl of ethanol extract) |
|                          |                                | Penicillin | Penicillinoic acid |
| Buffer                   | 996                            | 51         | 20                  |
| Penicillinase            | 722                            | 3.6        | 43                  |

LITERATURE CITED