Immunological Responsiveness of Guinea Pigs to Antibiotics Diffusing from Bone Cement

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The sustained release of antibiotics from bone cement provides an opportunity for the induction of hypersensitivity. We evaluated the cellular and humoral immune responsiveness of guinea pigs to antibiotics diffusing from bone cement. We were unable to detect the presence of antibodies or skin hypersensitivity to any of the antibiotics tested.

Infection is one of the most serious problems associated with prosthetic implants. Among the more recently developed techniques to control postoperative infections has been the incorporation of antibiotics into bone cement (2, 3, 5, 6). Because of their broad-spectrum activity, aminoglycosides and cephalosporins seem to be appropriate agents for this use. A potential concern for this procedure, however, is the induction of hypersensitivity.

The experiments reported here were designed to evaluate the immune responsiveness of guinea pigs to tobramycin (Nebcin), cefamandole (Mandol), and penicillin G (Eli Lilly & Co., Indianapolis, Ind.) released from bone cement.

Pellets of bone cement were prepared from Surgical Simplex P radiopaque bone cement (North Hill Plastics Division, Howmedica International, Ltd., London, England) by mixing the sterile powder and liquid monomer as described in the package literature. If antibiotic was included, the antibiotic and dry powder (1 g per 40 g of powder) were mixed by manual shaking for 5 minutes before addition of the liquid monomer. Small pellets (320 mg) were prepared by packing sterile metal cylinders (6 by 10 mm) with cement as soon as the cement was workable. Large pellets (4 by 16 mm) weighing 1 g were cut from a sheet of bone cement with a sterile cork borer. The amount of tobramycin and penicillin G in the small pellets was 5.2 mg, while in large pellets, the concentration of tobramycin was 65 mg, and that of cefamandole and penicillin G was 90 mg. Control pellets were prepared as above, but without antibiotic. The pellets were surgically implanted in the peritoneal cavity of 400-g guinea pigs.

Blood samples were collected by cardiac puncture. Urine was collected beginning 4 h after surgery and daily thereafter for 4-h intervals. Antibiotic levels in buffer, serum, and urine were determined by biological assay (1). Guinea pigs were skin tested on the abdomen by intradermal injection of 0.1 ml of saline containing either 0.1 or 1 mg of antibiotic or saline alone. Serum antibodies to antibiotics were determined by a passive hemagglutination technique (7).

In leaching experiments comparing the release of tobramycin, cefamandole, and penicillin G, release of cefamandole and tobramycin but not penicillin G was observed for a sustained period of time (14 days). Penicillin G release was observed during the first 48 h but not thereafter (Table 1). In a separate experiment (data not shown), tobramycin was detectable for 60 days, reaching maximal concentrations within the first 48 h. Sustained release of antibiotics from bone cement has been previously demonstrated (2, 5, 6).

In vivo experiments using bone cement pellets (320 mg) containing tobramycin or penicillin G we were unable to detect the presence of either antibiotic in the urine of guinea pigs. At 6 and 18 weeks, no serum antibodies to the antibiotics were found. Skin tests at 12 and 18 weeks were also negative. Because of the absence of detectable antibiotic in the urine, it appeared that a pellet with a larger amount of antibiotic should be used. Our failure to stimulate an immune response might be due to in vivo levels lower than those needed for immunogenicity.

Guinea pigs (n = 4) were implanted with large (1-g) tobramycin-containing pellets. Tobramycin was detectable in the urine of all animals 24 h after surgery and for 11 days thereafter.

The animals were bled and skin tested on day 21. Urine samples were negative for antibiotic at that time. Serum antibodies to tobramycin were not detected in either sample nor was the drug itself detected in the serum. Skin tests were also negative.
In a subsequent experiment, pellets containing tobramycin (65 mg), penicillin G (90 mg), or no antibiotic were implanted in guinea pigs. Test groups consisted of six guinea pigs. During the first 72 h, five penicillin G animals died. Urine collected from the animals with tobramycin-containing pellets beginning on day 3 contained appreciable levels of tobramycin which was detected in the urine up to day 45 after surgery (Table 2). Penicillin G was detected in the urine of the surviving animal only on days 3 and 6. Urine from control animals was negative. All animals were skin tested 6 weeks after surgery and before sacrifice (week 12). Skin tests were read at 2, 6, 24, and 48 h after injection of antigen. At 2 and 6 h, areas of erythema and induration at the sites of antibiotic and saline injections were similar. There was no evidence of an immunological or inflammatory reaction at any of the test sites at the final (48 h) period of observation. Serum from blood drawn at week 6 and before sacrifice contained no demonstrable antibodies to the antibiotics. The pellets were removed from the animals and incubated in saline with daily changes. Tobramycin, but not penicillin G, leached from the pellets for 14 days (5 to 14 µg/day).

Experiments similar to those above were performed using 1-g pellets containing cefamandole (90 mg). Cefamandole was detectable in the urine of all animals for 10 days and in one animal for 30 days. These animals gave no evidence of immunological reactivity by skin test or assays for circulating antibody.

Several factors may account for the lack of stimulation of the immune system. (i) The amount of antibiotic released may have been insufficient to stimulate the immune system. (ii) The release of large amounts of antibiotic during the first 48 h may have generated tolerance leaving the animal unresponsive. (iii) The drugs may have failed to readily conjugate with protein to form an immunogenic complex, thus reducing the possibility of stimulation of the immune system.

It is impossible to determine from these studies which factor(s) is responsible for the absence of immune stimulation in the treated animals. The fact that only very low levels of antibiotic are present after the first few days does not eliminate the risk of hypersensitivity. Industrial workers exposed to low levels of chemicals for long periods of time are known to become sensitive to them. Conditions for the induction of tolerance also exist. A monomeric immunogen, such as an antibiotic, present at high doses can bypass the macrophages and interact with the lymphocytes. The period of time that the immunogen was present at high concentrations, however, was relatively short for the induction of tolerance, particularly in mature animals (4). It is well known that for haptens such as antibiotics to be immunogenic, they must first complex with a serum protein or some other suitable large-molecular-weight carrier. Sufficient antibiotic-protein conjugates may not be formed under the conditions of release from bone cement. If the lack of immune stimulation is associated with failure to meet the proper criteria for immunogenicity, then this may explain the absence to date of reports of drug hypersensitivity in patients in whom antibiotic-containing cement has been used.

LITERATURE CITED


