Introduction

Setterstrom et al. first described the development of a biodegradable antibiotic delivery system capable of providing continuous sustained release of an antibiotic for up to 2 weeks following local application to contaminated experimental wounds in rats. Advantages of microencapsulated antibiotics over conventional systemic antibiotic therapy include the use of a single dose rather than multiple doses and maintenance of extremely high local antibiotic levels at the wound site with a minimal level of systemic antibiotic. Moreover, because the copolymer is biodegradable and does not require surgical removal after treatment, it offers advantages over other antibiotic-releasing systems. When delivered systematically with repeated doses, some antibiotics suppress lymphocyte proliferation, delayed hypersensitivity or antibiotic production whereas other antibiotics do not. The effects of slow antibiotic release from polymers, or of polymers alone, on the immune response have not yet been determined. The objective of this study was to evaluate and compare the effect of free ampicillin, microencapsulated ampicillin and antibiotic-free microspheres on cell-mediated immune responses in mice.

Materials and methods

Animals

Female Balb/c mice purchased from Charles River Laboratories (Wilmington, MA, USA) were housed at the Walter Reed Army Institute of Research Animal Facility. Food and water were given ad libitum to the animals. All investigators adhered to the national guidelines for the care of laboratory animals.

Microencapsulated ampicillin anhydrate

The microencapsulated ampicillin anhydrate (MEAA) used in these studies (composite batch D-856-038-1) was made under government contract by the Southern Research Institute (Birmingham, AL, USA). The microspheres consisted of 52:48 poly-(DL-lactide-co-glycolide), had a core load of 30.7% (by weight) ampicillin anhydrate, ranged in diameter from 45 \textmu m to 150 \textmu m and were sterilized by gamma irradiation.

Lymphocyte proliferation assay

Thirty-six mice were randomly allocated into three identically designed experiments. Each experiment
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consistent of four treatment groups each with three randomly assigned animals. Mice were injected subcutaneously (sc) twice daily for 7 days with 1 mL of one of the following: (i) 10 mg ampicillin sodium salt (Sigma Chemical Co., St Louis, MO, USA); (ii) 30.7 mg MEAA (containing the equivalent of 10 mg of ampicillin anhydride); (iii) 30.7 mg placebo microspheres (without ampicillin anhydride) or (iv) injection vehicle (2% (w/v) carboxymethylcellulose and 1% (w/v) Tween 20 in sterile H$_2$O). The selected antibiotic dose (10 mg) was equivalent to a therapeutic dose of 50 mg/kg of the average body weight (20 g) of the experimental mice. On day 8 the mice were killed by cervical dislocation and their spleens were removed and homogenized. The spleen cells from the mice in each designated group were pooled, washed twice in Hank’s buffer saline solution (HBSS) and resuspended at a concentration of 5 × 10^6 cells/mL in RPMI 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco). Cells were added in a volume of 100 μL/well to triplicate wells of 96-well flat-bottomed microtitre plates (Costar, Cambridge, MA, USA). Wells then received 100 μL of medium, 2.5 mg/L concanavalin A (Con A, Sigma) in medium, or 5.0 mg/L lipopolysaccharide (LPS, Sigma) in medium. The cells were incubated for 72 h at 37°C in 5% CO$_2$. Eighteen hours before harvest, 1.0 μCi of [3H]thymidine (6.7 mmol/Ci; New England Nuclear, Boston, MA, USA) was added to each well. Cells were harvested on glass fibre discs and placed into scintillation vials with 3 mL of Ultima Gold cocktail (Packard Instruments Co., Meriden, CT, USA). Incorporation of [3H]thymidine was measured with a Tri-Carb 2500 TR Liquid Scintillation A nalyzer (Packard).

Delayed-type hypersensitivity assay
Mice (three per group) were injected sc with ampicillin, MEAA or placebo microspheres, as described above. Animals were sensitized 24 h later with 100 μL of 3% oxazolone (Sigma) applied to the abdominal skin. On day 6, ear thickness was measured with a Vernier caliper to determine baseline thickness. Experimental and control mice were then challenged on the ear with 20 μL of 3% oxazolone. Unsensitized and untreated mice served as negative controls and sensitized but untreated animals were the positive controls. Ear thickness was measured at 24 and 48 h after challenge. Delayed-type hypersensitivity (DTH) was measured as the difference between ear thickness after challenge and baseline ear thickness.

Assay for cytokine production
A total of 24 mice were allocated randomly into two identical experiments in which three mice were assigned to one of four treatment groups. The mice were administered sc injections of the following treatments: (i) ampicillin, 25 mg/kg twice daily for 7 days (0.2 mL); (ii) MEAA, 11.4 mg/mouse suspended in 0.4 mL of injection vehicle (giving a quantity of antibiotic equivalent to that in treatment (i)); (iii) placebo microspheres, 11.4 mg/mouse suspended in 0.4 mL injection vehicle; or (iv) injection vehicle, 0.4 mL (control). MEAA, placebo microspheres and injection vehicle were administered only once. On day 8, animals were killed and their spleens harvested aseptically and homogenized. Spleen cells from each treatment group were pooled, washed twice in HBSS and resuspended at a concentration of 1 × 10^6 cells/mL in RPMI 1640 medium supplemented with 2% FBS with or without 2.5 mg/L of phytohaemagglutinin (PHA-P, Sigma) and 10 μg/L of pokeweed mitogen (Sigma). Twenty millilitres of the cell suspension were added to 25 cm$^2$ tissue culture flasks and incubated for 48 h at 37°C with 5% CO$_2$. Supernatant fluids were harvested, filtered through 0.45 μm filters and stored at −70°C until tested. Interleukin-2 (IL-2) production was quantified with the Intertest-2x Mouse IL-2 ELISA kit using a murine anti-IL-2 monoclonal antibody (Genzyme, Cambridge, MA, USA). Interleukin-4 (IL-4) production was quantified with a murine IL-4 ELISA kit using murine anti-IL-4 monoclonal antibody (Endogen, Boston, MA, USA).

Statistical analysis
Comparisons between treatment groups were made using the appropriate analysis of variance (ANOVA).

Results
Effect of antibiotic treatment on lymphoproliferation
Treatment of mice for 7 consecutive days with ampicillin, MEAA, placebo microspheres or injection vehicle (negative control) did not result in any significant difference in blastogenic response of mouse splenocytes to Con A (P > 0.10) or LPS (P > 0.25). Incorporation of [3H]thymidine (mean cpm of three experiments, ×10³) in the presence of Con A were 145.2, 141.8, 105.5 and 126.1 for animals treated with ampicillin, MEAA, placebo and injection vehicle, respectively. In the presence of LPS, incorporation of [3H]thymidine (mean cpm × 10³) were 36.7, 35.6, 36.0 and 46.1 for animals treated with ampicillin, MEAA, placebo and injection vehicle, respectively (Figure 1).

Effect of antibiotic treatment on delayed-type hypersensitivity
The optimum DTH responses were obtained 48 h after challenge. Treatment of mice with ampicillin, MEAA or placebo microspheres did not significantly alter the DTH response of mice to oxazolone as compared with the response of control animals treated with injection vehicle.
Effect of microencapsulated ampicillin on immunity

Effect of antibiotic treatment on cytokine production

There was no significant difference in IL-2 production by spleen cells from mice treated with ampicillin, MEAA, placebo microspheres and injection vehicle, respectively.

Effect of antibiotic treatment on cytokine production

There was no significant difference in IL-2 production by spleen cells from mice treated with ampicillin, MEAA, placebo microspheres and injection vehicle (control). Each bar represents the mean ± S.E.M. of three experiments.

Discussion

These results are in agreement with previous studies on the effect of antibiotics on the immune system which demonstrated no effect of β-lactam antibiotics on various immunological parameters. More recent studies also showed no significant effect of β-lactam antibiotics on cellular or humoral immune responses in mice. Similarly, a study on the effect of ampicillin and a sulbactam-ampicillin combination on the immune system of healthy subjects did not demonstrate any immunosuppressive activity by either ampicillin or the sulbactam-ampicillin combination. Di Marce et al. showed that cefepime, a new β-lactam antibiotic, has no effect on mitogen-induced lymphoproliferation, immunoglobulin synthesis or IL-2 secretion of human peripheral blood lymphocytes.

We conclude from this study that, at therapeutic doses similar to the free antibiotic, MEAA does not inhibit cell-mediated immune responses.

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References


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