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Lack of Influence of Commonly Used Drugs on Bioassay Indicator Organisms

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Many commonly used pharmaceutical agents have been found to inhibit bacterial growth in vitro. Determinations of antimicrobial concentrations in sera of patients taking nonrecognized antibacterial agents could possibly be altered if bioassay systems are utilized for the determinations. We therefore attempted to determine the in vitro effect of commonly used drugs on bioassay indicator organisms. Fifty-one different agents (antihistamines, anticholinergics, central nervous system agents, cardiovascular agents, analgesics, steroids, muscle blockers, and other miscellaneous agents) were tested for inhibition or enhancement of the growth of *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, and *Klebsiella pneumoniae*. None of the agents tested exhibited any effect on standard in vitro bioassay organisms. Nortriptyline hydrochloride inhibited the growth of *B. subtilis* and *M. luteus* at a concentration of 500 µg/ml (zones of inhibition, 14 and 13 mm, respectively), but no inhibition was observed with concentrations of 50 µg/ml or lower.

Pharmaceutical agents not usually classified as antibacterial agents have been found to inhibit bacterial growth in vitro. Non-antibacterial agents such as promazine (4), caffeine (3), theophylline (3), heparin (10), and ascorbic acid (9) are reported to possess bacteriostatic or bactericidal properties, often at their usual serum concentrations. Other classes of agents (e.g., cancer chemotherapy drugs [11], anesthetics [8, 12], or steroid hormones [6]) or derivatives of certain classes (e.g., barbituric acids [1, 2] and benzodiazepines [7]) have demonstrated similar properties.

Since a wide variety of drugs demonstrate in vitro antibacterial activity, other agents in common clinical usage may also possess such activity. Therefore, patients taking nonrecognized antibacterial agents may have serum concentrations sufficient to influence bacterial growth in vitro. A specific concern is that determinations of antimicrobial concentrations in the sera of patients taking nonrecognized antibacterial agents may be altered if bioassay systems are utilized for the determinations.

Our interest originated when intraoperative concentrations of cephalosporins were determined in the sera of patients undergoing surgical procedures. Many such patients receive 10 or more medications in the perioperative period, and it was considered essential to determine the effect of these frequently administered agents on in vitro bioassay systems. To our knowledge, no

comprehensive information is available in the literature describing the effect of commonly used non-antibacterial agents on in vitro bioassay systems.

MATERIALS AND METHODS

Agents tested. A list of the agents tested is given in Table 1. Each of the agents was obtained from the pharmaceutical manufacturers as pure powder with no preservatives or other additives. For each agent a solution was prepared with a final concentration of 4.16 mg/ml. The agents were dissolved in distilled water or, if necessary, 95% ethanol or dilute sodium hydroxide. From each of these solutions, 0.3 ml was removed and mixed individually with 2.2 ml of serum from normal volunteers, resulting in a final drug concentration of 500 µg/ml. The solutions were mixed by vortexing and then frozen (-20°C) until analysis. For this screening of assay interference, concentrations much greater than those usually achieved in vivo were desired. Each of the solutions was then tested in the manner described below.

Bioassay. *Bacillus subtilis* ATCC 6633 spore suspensions were obtained from Difco Laboratories; *Staphylococcus aureus* ATCC 6538P, *Micrococcus luteus* ATCC 9341, and a multiply resistant strain of *Klebsiella pneumoniae* (ATCC 27799) were lyophilized preparations from the American Type Culture Collection. Assay plates were prepared the day of use with Difco antibiotic medium no. 1 (*S. aureus*), no. 5 (*B. subtilis*), or no. 11 (*M. luteus* and *K. pneumoniae*) as described previously (5). Plastic petri dishes (100 by 15 mm; Falcon Plastics) were used with agar volumes of 5 or 10 ml (*B. subtilis*) or 9 ml (*S. aureus*, *M. luteus*, and *K. pneumoniae*). Aliquots (20 µl) of the drug solutions

TABLE 1. Agents used in this study

| Type of agent | Name | Type of agent | Name |
|-------------------------------|-------------------------------|----------------------------|-------------------------------------|
| Antihistamine-anticholinergic | Atropine | Cardiovascular | Probenecid |
| | Chlorpheniramine maleate | | Theophylline |
| | Cimetidine hydrochloride | | Warfarin sodium |
| | Diphenhydramine hydrochloride | | Clonidine hydrochloride |
| | Glycopyrrolate | | Chlorothiazide |
| | Hydroxyzine hydrochloride | | Chlorthalidone |
| Central nervous system | Amitriptyline hydrochloride | | Digoxin |
| | Chlorpromazine hydrochloride | | Hydrochlorothiazide |
| | Diazepam | | Hydralazine hydrochloride |
| | Flurazepam | | Lidocaine hydrochloride |
| | Imipramine hydrochloride | | N-Acetyl procainamide hydrochloride |
| | Nortriptyline hydrochloride | Propranolol hydrochloride | |
| | Phenobarbital | Quinidine sulfate | |
| | Phenytoin sodium | Spiro lactone | |
| | Promethazine hydrochloride | Analgesic | Acetaminophen |
| Secobarbital | Aspirin | | |
| Miscellaneous | Allopurinol | | Ibuprofen |
| | Ascorbic acid | | Meperidine hydrochloride |
| | Caffeine | | Morphine sulfate |
| | Colchicine | Propoxyphene hydrochloride | |
| | Ephedrine hydrochloride | Muscle blocker | Pancuronium bromide |
| | Heparin sodium (beef) | | Succinyl choline |
| | Heparin sodium (porcine) | | Tubocurarine hydrochloride |
| | | | Steroid |
| | | Prednisolone | |
| | | Prednisone | |
| | | | Progesterone |

were pipetted onto sterile 6-mm filter paper disks (Difco) and applied to the agar surface; control disks containing the solvents and antibiotic disks (BBL Microbiology Systems) containing 10 µg of penicillin, 10 µg of ampicillin, 30 µg of cefoxitin, 30 µg of chloramphenicol, and 10 µg of gentamicin were included in each assay. The unstacked plates were incubated overnight at 35°C, and zone sizes were recorded to the nearest millimeter.

RESULTS

The majority of agents demonstrated no inhibition or enhancement of bacterial growth. Initial testing of hydrochlorothiazide and chlorothiazide resulted in zones of inhibition (for *B. subtilis* only) of 11 and 10 mm, respectively. However, similar zone sizes were seen with their sodium hydroxide solvent, and on repeat testing no inhibition was detected when concentrations of sodium hydroxide diluent were reduced. The initial sample of nortriptyline hydrochloride (diluted in distilled water) produced

zones of inhibition of 10 and 12 mm for *M. luteus* and *B. subtilis*, respectively. Subsequent testing of two additional lots of nortriptyline hydrochloride demonstrated similar inhibition. The water diluent failed to inhibit any of the microorganisms. When agar thickness was increased (a total of 10 ml of agar), the zone of inhibition for *B. subtilis* was 8 mm. Nortriptyline dilutions of 50, 5, 0.5, and 0.05 µg/ml demonstrated no inhibition of *B. subtilis* or *M. luteus*. When commercially obtained antibiotic disks were tested with the same systems, the expected large zones of bacterial growth inhibition were observed.

DISCUSSION

Pharmaceutical agents in common clinical use, although studied at greater concentrations than usually achievable in vivo, exhibited no independent effect on standard in vitro procedures used to determine in vivo antimicrobial

concentrations. However, nortriptyline hydrochloride demonstrated significant inhibition of *B. subtilis* and *M. luteus* at 500 µg/ml, but not at concentrations usually found in vivo. Since the test agents were not examined in the presence of antimicrobial agents the effect on actual antimicrobial assays cannot be stated, but it is likely that there would be minimal interference.

Despite the large number of reports of the antimicrobial activity of nonrecognized antibacterial agents the agents that we examined failed to show significant influence on bioassay indicator organisms.

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