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Susceptibility of Methicillin-Resistant *Staphylococcus aureus* to the Selenium-Containing Compound 2-Phenyl-1,2-Benzoisoselenazol-3(2*H*)-One (PZ51)

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The growth of *Staphylococcus aureus* 209P was inhibited by 0.20 μg of 2-phenyl-1,2-benzoisoselenazol-3(2*H*)-one (PZ51) per ml, while strains of the family *Enterobacteriaceae* were more resistant to the drug. The MIC for 90% of methicillin-resistant *S. aureus* strains was 1.56 μg/ml, and the drug was bactericidal. The selenium in PZ51 was essential, since its sulfur analog (PZ25) lost the antibacterial activity.

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first detected in 1961 (1). Its incidence has continued to increase, especially in the 1980s (2, 8, 9). Since MRSA resists not only methicillin, but also cephems, aminoglycosides, tetracyclines, macrolides, and new quinolones (2, 3, 6, 8, 9), its increase and prevalence pose serious clinical and epidemiological problems all over the world. Therefore, it is necessary to find a new type of drug with anti-MRSA activity.

Selenium is an essential trace element for mammals, since it is contained in glutathione (GSH) peroxidase, which catalyzes the reduction of a variety of hydroperoxides and undergoes a catalytic redox cycle between the selenol and selenenic and/or seleninic acids (11). The seleno-organic compound 2-phenyl-1,2-benzoisoselenazol-3(2*H*)-one (PZ51; Fig. 1), exhibits GSH peroxidase-like activity in vitro, in contrast to its sulfur analog, PZ25 (5, 7, 10). In addition, PZ51 had an antioxidant activity in microsomal lipid peroxidation even in the absence of GSH (5), offering a novel type of anti-inflammatory agent with low toxicity (10). We studied the antibacterial spectrum of PZ51 and found that some gram-positive cocci were more susceptible to PZ51 than some gram-negative species. We then examined the susceptibility of clinically isolated MRSA strains to PZ51.

PZ51 and PZ25 were solubilized in dimethyl sulfoxide to 10 mg/ml, and heart infusion agar plates containing twofold serially decreasing concentrations of the drug (100 to 0.1 μg/ml) were made. Each bacterial strain was cultured overnight in NZY broth (5 g of NaCl, 2 g of $MgSO_4 \cdot 7H_2O$, 5 g of yeast extract, and 10 g of casein hydrolysate per liter [pH 7.5]), and the broth was diluted 100-fold in saline G (4). Ten microliters of the diluent (approximately 10^5 cells) was spotted by a microdispenser on the heart infusion agar plate and was cultured overnight at 37°C. The spectrum of PZ51 against some gram-negative and -positive standard strains is shown in Table 1. The growth of strains of the family *Enterobacteriaceae* was inhibited by 12.5 to 50 μg of PZ51 per ml. Other gram-negative aerobic strains exhibited somewhat similar susceptibility to the drug, but one *Flavobacterium* strain did not. On the other hand, the growth of two staphylococcal strains was inhibited by a lower concentration of PZ51 (0.20 μg/ml). *Streptococcus pyogenes* and *Streptococcus mitis*, however, were much more resistant to the drug. MICs for clinically isolated streptococci (*Streptococcus agalactiae*, *Streptococcus salivarius*, *Streptococcus pneumoniae*, and *Enterococcus faecium*, one isolate each) ranged from 1.56 to 6.25 μg/ml on Mueller-Hinton agar as well as on heart infusion agar (data not shown).

Seventy-five MRSA strains, which grew on agar plates containing 3.13 μg of methicillin per ml, were isolated in hospitals in Tokyo in 1987. They were highly resistant not

TABLE 1. Antibacterial spectrum of PZ51

Organism	MIC (μg/ml)
Escherichia coli NIHJ	12.5
Shigella flexneri 2A and 5503	12.5
Salmonella enteritidis IID 604	50
Hafnia alvei IID 978	50
Citrobacter freundii IID 976	50
Proteus vulgaris 08601	12.5
Proteus morganii IID 602	50
Providencia rettgeri 08500	50
Proteus inconstans 08303	50
Proteus mirabilis 08103	25
Klebsiella pneumoniae, type 1	50
Klebsiella oxytoca 07600	50
Enterobacter cloacae 03400	50
Enterobacter aerogenes ATCC 8329	50
Serratia marcescens 10100	50
Yersinia enterocolitica TE591	25
Alcaligenes faecalis ATCC 19108	50
Pseudomonas aeruginosa 32104	50
Pseudomonas cepacia IID 1340	50
Pseudomonas maltophilia IID 1275	3.13
Pseudomonas putida IID 5121	50
Flavobacterium meningosepticum ATCC 13253	0.39
Acinetobacter anitratum ATCC 19606	12.5
Achromobacter xylosoxidans ATCC 27061	50
Staphylococcus aureus 209P	0.20
Staphylococcus epidermidis 56500	0.20
Streptococcus pyogenes G-36	3.13
Streptococcus mitis IID 685	25
Enterococcus faecalis ATCC 19433	0.39
Bacillus subtilis ATCC 6633	0.39

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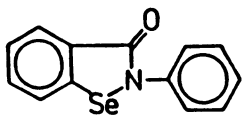
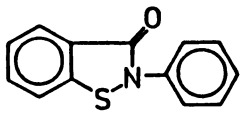


PZ 51



PZ 25

FIG. 1. Structures of PZ51 and PZ25.

only to methicillin (MIC for 90% of strains, 100 μg/ml), but also to cefazolin, erythromycin, gentamicin, tetracycline, and ofloxacin (MIC for 90% of strains, 25 to 100 μg/ml [3]). No MRSA strains, however, grew on agar containing 3.13 μg of PZ51 per ml, and more than 90 and 50% of them were susceptible to 1.56 and 0.78 μg of PZ51 per ml, respectively. The susceptibility of 7 of 75 MRSA strains to PZ25 was then tested. PZ25 had little anti-MRSA activity (MIC, 25 to 50 μg/ml). Dimethyl sulfoxide, a solvent of PZ51 and PZ25, was not at all inhibitory to the growth of MRSA at concentrations of up to 1%. Survival of an MRSA strain (MIC, 1.56 μg/ml) in NZY broth was determined in the presence of 0.5 and 1 times the MIC of PZ51 (Fig. 2). The MRSA cells were quickly killed, depending on the concentration of PZ51. Few MRSA cells survived after a 24-h exposure to 1.56 μg of PZ51 per ml. (There were no live cells in 0.3 ml of undiluted cell suspension.) There was little inoculum effect on the MIC when the MRSA strain was inoculated at 10^1 to 10^6 cells. Furthermore, susceptibility of clinically isolated coagulase-negative staphylococci (n = 33) and *Enterococcus faecalis* (n = 39) to PZ51 was determined. The MIC for 90% of the strains of either bacterium was 0.78 μg/ml.

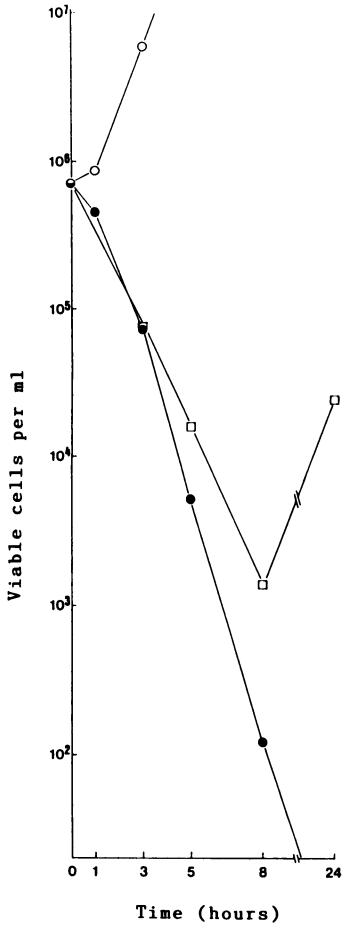


FIG. 2. Bactericidal effect of PZ51 on a MRSA strain. The number of viable cells was determined at 37°C in NZY broth in the presence of no PZ51 (○) and 0.5 (□) and 1 (●) times the MIC of PZ51. Cells in the broth were serially diluted 10-fold with saline G, and 0.1 ml of each diluent (10^0- to 10^6-fold) was plated on heart infusion agar. The MIC of PZ51 for this strain was 1.56 μg/ml when 10^6 to 10^1 cells were inoculated.

Since PZ25, a sulfur analog of PZ51 (Fig. 1), had little anti-MRSA activity, it was suggested that selenium is essential for the antibacterial activity of PZ51. PZ25 also lost its GSH peroxidase-like activity and its antioxidant activity against microsomal peroxides in the absence of GSH, while PZ51 retained both activities (5, 7, 10). Thus, the antioxidant activities of PZ51 are apparently correlated with its antibacterial activity. Studies using structural analogs of PZ51 other than PZ25 could clarify the above correlation. Also, studies on the bactericidal activities of other compounds with antioxidant activity could be helpful. The fact that PZ51 was not very effective on gram-negative strains or on streptococcal species (Table 1) might offer a clue to the antibacterial mechanism of the drug. There is now a strong demand for the development of a drug with anti-MRSA activity, and studies on the antimicrobial mechanism of PZ51 could be useful for that development.

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