ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1985, p. 632–639
0066-4804/85/040632-08$02.00/0

Vol. 27, No. 4

Suppression of Intrinsic Resistance to Penicillins in *Staphylococcus aureus* by Polidocanol, a Dodecyl Polyethyleneoxid Ether

WOLFGANG BRUNS,* HANS KEPPELER, AND REGINA BAUCKS

Pharmakologisches Institut, Universität zu Köln, D-5000 Cologne 41, Federal Republic of Germany

Received 20 July 1984/Accepted 9 January 1985

With polidocanol, it was possible to reduce the MIC as well as the MBC of methicillin, oxacillin, penicillin G, and ampicillin against resistant staphylococci. The strongest effects were obtained with methicillin and oxacillin. All strains tested could be resensitized to these penicillins independent of the original resistance levels. Polidocanol was not inhibitory by itself for *Staphylococcus aureus*. Furthermore, it did not inhibit the activity of staphylococcal β-lactamase. This permits the conclusion that an intrinsic resistance mechanism is affected by this substance. Its action cannot be simply explained by an improved accessibility of the penicillin targets as uptake, and binding of methicillin and penicillin G in resistant cells was not changed by polidocanol. On the other hand, the lysis induced by combinations of this substance with small amounts of a penicillin was antagonized by chloramphenicol. This suggests that autolytic enzymes are involved in the polidocanol effect and possibly in the intrinsic resistance mechanism itself. Before polidocanol can trigger lysis, the penicillin must act first in some way. As could be seen with a susceptible strain, the resulting lysis did not exceed that obtained with penicillins alone. Thus, polidocanol does not exhibit an independent lytic mechanism but obviously is able to substitute penicillins in their lytic action.

Bacterial resistance to penicillins can be expressed phenotypically in different ways. The most common type of penicillin resistance is that based on the inactivation of penicillins by β-lactamases. Besides this, enzyme-independent mechanisms exist which are termed intrinsic resistance. In staphylococci, the most typical form is resistance to methicillin (and oxacillin). Although altered penicillin-binding properties were recently found in resistant staphylococci (4, 5, 12, 13), the mechanism of methicillin resistance is still unclear. In gram-negative bacteria, intrinsic resistance is due to a permeability barrier in the outer membrane (23). A special form of penicillin insensitivity is the so-called tolerance described by Sabath et al. (27) and characterized by low MICs but high MBCs of penicillins.

Many successful efforts have been made to overcome the β-lactamase-mediated type of penicillin resistance. In contrast, it was not possible until now to influence the methicillin resistance in staphylococci.

In this paper, we report on the suppression of resistance to methicillin and oxacillin in *Staphylococcus aureus* by polidocanol (PDO). Resistance to penicillin G and ampicillin was also affected but to a lower extent. The activity of β-lactamase, however, was not inhibited by PDO, indicating that its action is directed against an intrinsic resistance mechanism. Lysis induced by combinations of PDO and penicillins could be inhibited by chloramphenicol (CAP). This finding points to the participation of autolytic enzymes in the PDO effect and possibly in the resistance mechanism itself.

MATERIALS AND METHODS

Organisms. Penicillin-resistant staphylococci from different sources were employed in this study. Strains 4503, 6641, 5814 S, and S108 were resistant to penicillin G, and all others were additionally resistant to methicillin. Strain 9204 was a β-lactamase-negative variant of a methicillin-resistant wild-type strain (10). Strains 5814 R and 5814 S were isolated from a strain of mixed population (24). The strains were kindly provided by W. Cullmann, Ruhruniversität, Bochum, Federal Republic of Germany (FRG) (R 60 and 1482), G. Pulverer, Universität zu Köln, Cologne, FRG (9204, 209, 4503, and 6641), F. Rozgonyi, University Medical School, Debrecen, Hungary (5814 R and 5814 S), B. Wiedemann, Universität Bonn, Bonn, FRG (4478, 4482, and 7291), and O. Zwisler, Behringwerke AG, Marburg, FRG (S 108). The penicillin-susceptible strain used was *S. aureus* H.

Antibiotics and reagents. ^{14}C-labeled penicillin G (54 mCi/mmol), was purchased from Amersham Buchler, Brunswick, West Germany. ^{3}H-labeled penicillin G-*N*-ethyl piperidine salt (21.9 Ci/mmol) was obtained from NEN Chemicals, Dreieich, FRG. Methicillin was a gift from Beecham-Wülfing, Neuss, FRG. Oxacillin, ampicillin, and CAP were obtained from Bayer AG, Leverkusen, FRG, and penicillin G was from Hoechst AG, Frankfurt/Main, FRG. PDO, a dodecyl polyethyleneoxid ether [$C_{12}H_{25}$-(O-C_2H_4)$_{\sim 9}$-OH] was kindly provided by Desitin-Werk Carl Klinke GmbH, Hamburg, FRG.

Susceptibility tests. The MICs of the penicillins were determined by twofold serial dilution in Mueller-Hinton broth (Oxoid) as described by Ericsson and Sherris (11), with an inoculum of 5×10^5 to 1×10^6 CFU/ml. PDO, where required, was added to a final concentration of 0.1 mg/ml. After incubation at 37°C (in special cases at 30°C) for 20 h, the MIC was recorded as usual. The MBCs were determined by subculturing aliquots (0.01 ml) from each clear tube on antibiotic-free agar as described by Pearson et al. (21). The agar plates were incubated at 37°C for 24 h. The MBC was defined as the lowest penicillin concentration giving 99.9% killing.

Growth curves. The experiments were carried out in brain heart infusion broth (Oxoid). Overnight cultures were diluted 1:40 with fresh medium and shaken in a water bath at 37°C. The antibacterial agents were added at the times indicated on the figures. Growth was measured by optical density readings at 600 nm, using a spectrophotometer (Spectronic 20, Bausch & Lomb).

* Corresponding author.

TABLE 1. Effect of PDO on the MICs of penicillins against resistant *S. aureus*

Strain	MIC (μg/ml)[a]							
	MC	MC + PDO	OC	OC + PDO	PC-G	PC-G + PDO	AC	AC + PDO
5814 R	2,000	2.0	500	0.25	250	15.6	500	15.6
209	500	7.8	250	1.0	250	15.6	500	15.6
9204[b]	500	7.8	250	1.0	15.6	0.13	15.6	0.25
1482	125	3.9	125	1.0	500	7.8	1,000	7.8
4478	125	3.9	125	1.0	2,000	31.2	2,000	31.2
7291	125	3.9	62.5	0.5	2,000	125	2,000	125
R60	62.5	3.9	62.5	0.5	500	7.8	1,000	7.8
4482	31.2	2.0	31.2	0.5	2,000	62.5	2,000	62.5
5814 S	6.2	ND	1.6	ND	500	7.8	500	3.9
S108	6.2	ND	1.6	ND	1,000	3.9	2,000	7.8
6641	3.1	ND	0.8	ND	1,000	15.6	1,000	7.8
4503	3.1	ND	0.8	ND	2,000	7.8	2,000	2.0

[a] Abbreviations: MC, methicillin; OC, oxacillin; PC-G, penicillin G; AC, ampicillin; ND, not determined. The MIC of PDO alone was >100 mg/ml; the PDO concentration in the combinations with penicillins was 0.1 mg/ml.
[b] Strain 9204 does not produce β-lactamase.

Assay for β-lactamase activity. The influence of PDO on β-lactamase kinetics was determined by the acidimetric method of Rubin and Smith (26), using phenol red as pH indicator. Reaction mixtures (3 ml each) contained penicillin G (0.02 to 0.2 μmol/ml), sodium phosphate buffer (0.4 mmol/liter; pH 7.6), phenol red (0.04% [wt/vol]), PDO (0 to 10 mg/ml), and β-lactamase (1 U/ml). The change in optical density was measured at 578 nm and 37°C with an Eppendorf photometer.

The β-lactamases used were staphylococcal extracellular enzyme, isolated from strain S108 and purified as described by Richmond (22), and commercially available penicillinase from *Bacillus cereus* (Calbiochem GmbH, Giessen, FRG). One unit of β-lactamase was defined as the amount of enzyme that hydrolyzed 1 μmol of penicillin G per h at 37°C.

Binding of ^{3}H-labeled penicillin G to the penicillin-binding proteins (PBPs). Staphylococcal cells grown in brain heart infusion broth (Oxoid) were harvested in mid-logarithmic growth, washed with Tris buffer (0.05 mol/liter; pH 7.5) containing $MgSO_4$ (0.02 mol/liter), and resuspended in the same buffer. Samples of 1 ml (10 mg of dry weight per ml) were incubated with various concentrations of ^{3}H-labeled penicillin G (0.01 to 5 nmol/ml) in the presence or absence of PDO (0.1 mg/ml) for 15 min at 37°C. The reaction was terminated by adding a 1,000-fold excess of unlabeled penicillin G. The cells were washed with Tris buffer, and the cytoplasmic membranes were isolated as described previously (5). They were dissolved in sodium dodecyl sulfate (0.5% [wt/vol]), and each sample was divided into two portions. One of them was counted in a liquid scintillation counter (Intertechnique, Mainz, FRG); the other one was subjected to gel electrophoresis.

Discontinuous sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis was performed as described by Spratt et al. (29, 30), using a separating gel composed of 10% (wt/vol) acrylamide and 0.068% (wt/vol) methylene bisacrylamide. The dried gels were fluorographed on presensitized Kodak X-Omat AR film (20).

Binding of methicillin to growing cells. Staphylococci were grown in brain heart infusion broth to early logarithmic phase in a shaking water bath at 37°C. Methicillin (10 nmol/ml) and, where appropriate, PDO (0.1 mg/ml) were then added, and growth was allowed to continue for 30 min at 37°C. Then the cells were washed and the cytoplasmic membranes were prepared in the same manner as before. The membrane samples were incubated with ^{14}C-labeled penicillin G (0.3 nmol/ml) for 15 min at 37°C to saturate the remaining free binding sites. The reaction was terminated by the addition of a 1,000-fold excess of unlabeled penicillin G. After three washings with Tris buffer (0.05 mol/liter; pH 7.5),

TABLE 2. Effect of PDO on the MBCs of penicillins against resistant *S. aureus*

Strain	MBC (μg/ml)[a]							
	MC	MC + PDO	OC	OC + PDO	PC-G	PC-G + PDO	AC	AC + PDO
5814 R	4,000	7.8	4,000	2.0	2,000	31.2	4,000	31.2
209	4,000	250	2,000	125	4,000	62.5	8,000	125
9204[b]	1,000	7.8	500	2.0	31.2	0.25	62.5	0.5
1482	1,000	7.8	500	1.0	2,000	31.2	4,000	31.2
4478	500	7.8	500	3.9	4,000	125	4,000	62.5
7291	1,000	7.8	250	1.0	8,000	250	4,000	125
R 60	250	7.8	250	1.0	2,000	31.2	4,000	31.2
4482	500	3.9	250	2.0	8,000	125	8,000	500
5814 S	ND	ND	ND	ND	1,000	15.6	1,000	15.6
S108	ND	ND	ND	ND	4,000	15.6	4,000	15.6
6641	ND	ND	ND	ND	2,000	31.2	2,000	15.6
4503	ND	ND	ND	ND	2,000	15.6	4,000	7.8

[a] See Table 1, footnote *a*.
[b] See Table 1, footnote *b*.

TABLE 3. Effect of PDO on the MICs of methicillin and oxacillin against resistant *S. aureus*, determined at 30°C

Strain	MIC (<μg/ml)[a]			
	MC	MC + PDO	OC	OC + PDO
5814 R	4,000	2.0	1,000	0.5
9204[b]	2,000	3.9	1,000	0.5
4478	1,000	2.0	500	0.5
4482	1,000	3.9	500	1.0

[a] See Table 1, footnote *a*.
[b] See Table 1, footnote *b*.

the membranes were dissolved in sodium dodecyl sulfate (0.5% [wt/vol]) and counted in the liquid scintillation counter.

RESULTS

Effect of PDO on MICs and MBCs of penicillins. The 12 staphylococcal strains used in the experiments showed various MIC levels of methicillin, oxacillin, penicillin G, and ampicillin (Table 1). Eight strains expressed resistance to all four penicillins. The others were resistant only to penicillin G and ampicillin.

PDO alone was not inhibitory to these strains up to 100 mg/ml. When PDO (0.1 mg/ml) was combined with the penicillins, it resulted in a marked reduction of the MICs (Table 1). The effect was seen to different extents with the four penicillins. It was most pronounced with oxacillin. In the presence of PDO, the MICs of this penicillin were reduced to 0.25 to 1.0 μg/ml, indicating that all strains had regained normal susceptibility. Depending on the original degree of resistance, this means reduction of the MICs by factors from 62.5 up to 2,000. Similar results were obtained with methicillin. In the case of this antibiotic, the MICs could also be lowered to the susceptible range by combination with PDO. Strain 5814 R is remarkable in that its resistance to both oxacillin and methicillin was extraordinarily well influenced by PDO.

The resistance to penicillin G and ampicillin was also reduced by PDO. In general, ampicillin resistance was more suppressed than that to penicillin G. However, full susceptibility could not be attained with β-lactamase-producing strains. The highest reduction was found for strain 4503 and ampicillin, decreased by a factor of 1,000. An exceptional position was occupied by *S. aureus* 9204. This strain did not produce β-lactamase and thus exhibited a pure form of intrinsic resistance to penicillin G and ampicillin. This resistance was completely suppressed by PDO.

The influence of PDO was not restricted to the MICs of the penicillins. It referred also to their bactericidal action. This was proven by determination of MBCs (Table 2). Most of the MBCs of the four penicillins were 2 to 4 times higher than the corresponding MICs. In some cases this factor was increased up to 16.

With the methicillin-resistant strains, the influence of PDO on MBCs was more marked than that on MICs, a finding that

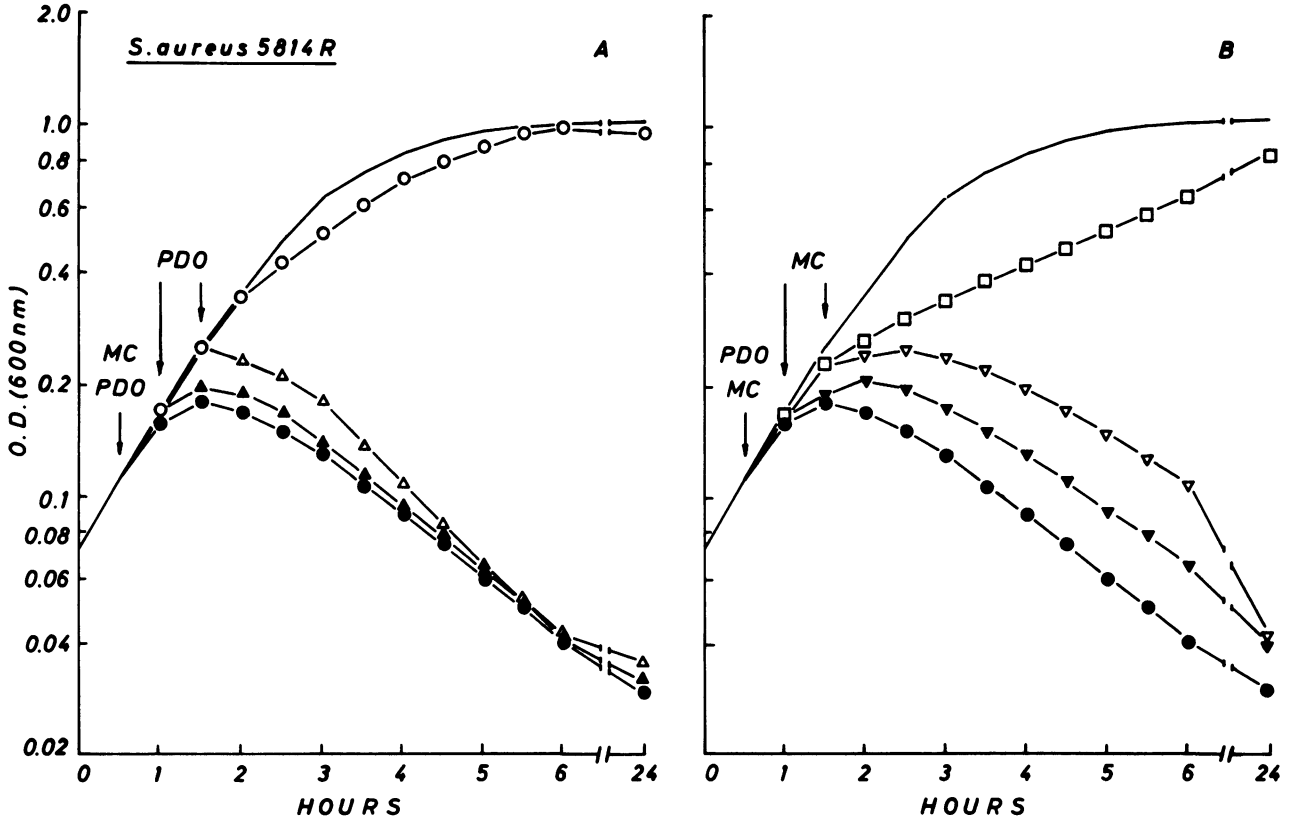

FIG. 1. Combined action of PDO (0.1 mg/ml) and methicillin (MC; 10 μg/ml) to growing cells of *S. aureus* 5814 R dependent on the order of application of the agents. MC added before PDO (A) and the opposite (B) are compared with simultaneous application. Symbols: ——, control; ○, MC added at 30 min; ●, MC plus PDO, both at 30 min; ▲, MC at 30 min plus PDO at 60 min; △, MC at 30 min plus PDO at 90 min; □, PDO at 30 min; ▼, PDO at 30 min plus MC at 60 min; ▽, PDO at 30 min plus MC at 90 min.

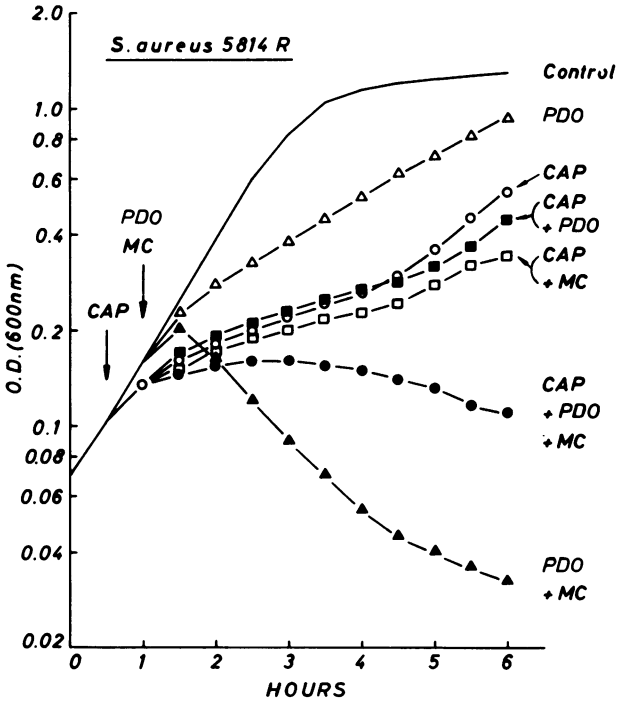

FIG. 2. Inhibition of the combined action of PDO and methicillin (MC) on growth of the MC-resistant strain 5814 R by CAP. The concentrations of the agents (added at the times indicated by arrows) were 0.1 mg/ml (PDO), 10 μg/ml (MC), and 0.1 mg/ml (CAP).

was true for each of the penicillins. This was underlined by the observation that the strains with higher MBC/MIC ratios were also well influenced. Corresponding to the situation with the MICs, the lowest MBC were obtained in the case of oxacillin and methicillin. Strain 209 did not fit this generalization as it was not susceptible to the bactericidal action of PDO-antibiotic combinations, though its MICs were well suppressed by PDO. With the methicillin-susceptible but penicillin G-resistant strains, a stronger PDO effect on MBCs than on MICs of penicillin G and ampicillin could not be obtained. It was either equal or lower.

The influence of PDO on the resistance to methicillin and oxacillin was also studied at 30°C with four strains (Table 3). At this lower temperature, the MICs were 2- to 32-fold higher than at 37°C, as is typical of methicillin-resistant staphylococci (14). In the presence of PDO, the strains became susceptible to both antibiotics also under these conditions. The MICs were reduced by factors from 256 to 2,000. Thus, the effect of PDO on the MICs is stronger at 30°C than at 37°C.

TABLE 4. Effect of PDO on MICs and MBCs of penicillins against *S. aureus* H

Compound	MIC (μg/ml)	MBC (μg/ml)
MC[a]	2.5	5.0
MC + PDO	0.62	1.25
OC[a]	0.31	1.25
OC + PDO	0.16	0.31
PC-G[a]	0.062	0.125
PC-G + PDO	0.016	0.031
AC[a]	0.125	0.25
AC + PDO	0.031	0.062
PDO	$>10^5$	$>10^5$

[a] See Table 1, footnote *a*.

Lytic action of combinations of PDO with penicillins. When PDO and penicillins were added to exponentially growing cells of resistant staphylococci, lysis occurred. The penicillin concentrations required corresponded well with the MBCs obtained in the presence of PDO (Table 2). Results of lysis experiments are demonstrated with methicillin (Fig. 1). If the two agents were applied simultaneously in the early logarithmic growth, lysis became visible after 60 min of treatment. The beginning and final point of lysis were not changed if PDO was added 30 or 60 min after methicillin. This means in the latter case that lysis started immediately after PDO had been added. When, however, PDO was given before methicillin, lysis was delayed and began always 60 min after addition of methicillin. From these findings, it can be concluded that methicillin has to act first for a certain time before PDO can trigger lysis of staphylococcal cells.

Effect of CAP on PDO-mediated cell lysis. The lytic process induced by the combinations of PDO and penicillins could be inhibited by CAP, leaving only a bacteriostatic effect. This is shown for methicillin in Fig. 2. CAP had to be added to the culture at least 30 min before application of PDO and the penicillins. The amounts of CAP required to stop lysis differed from strain to strain, but inhibitory concentrations were not necessary. Furthermore, CAP did not interact with PDO or the penicillins alone.

PDO effect in *S. aureus* H. Penicillin action on susceptible strain H was slightly influenced by PDO. In general, a fourfold reduction of both the MICs and MBCs was found in

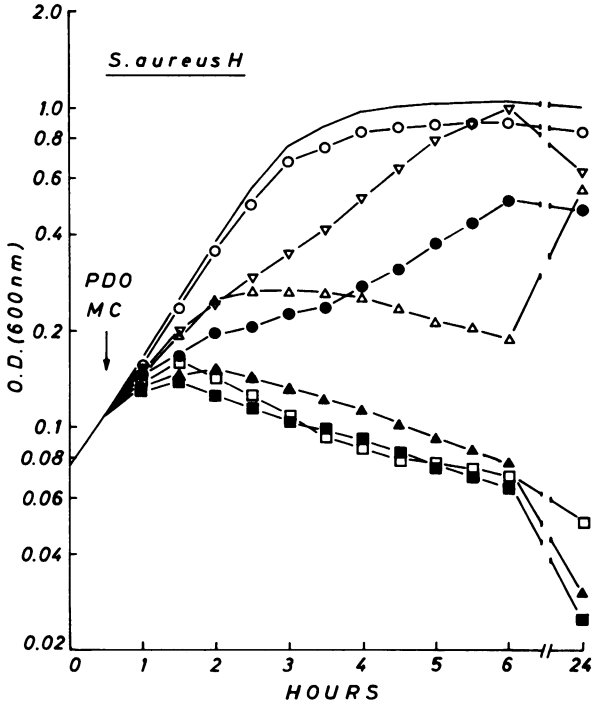

FIG. 3. Combined action of PDO (0.1 mg/ml) and methicillin (MC) to growing cells of *S. aureus* H. The agents were added simultaneously at the time indicated by an arrow. Symbols: ——, control; ▽, PDO; ○, 0.05 μg of MC per ml; △, 0.5 μg of MC per ml; □, 2.5 μg of MC per ml; ●, 0.05 μg of MC per ml + PDO; ▲, 0.5 μg of MC per ml plus PDO; ■, 2.5 μg of MC per ml plus PDO.

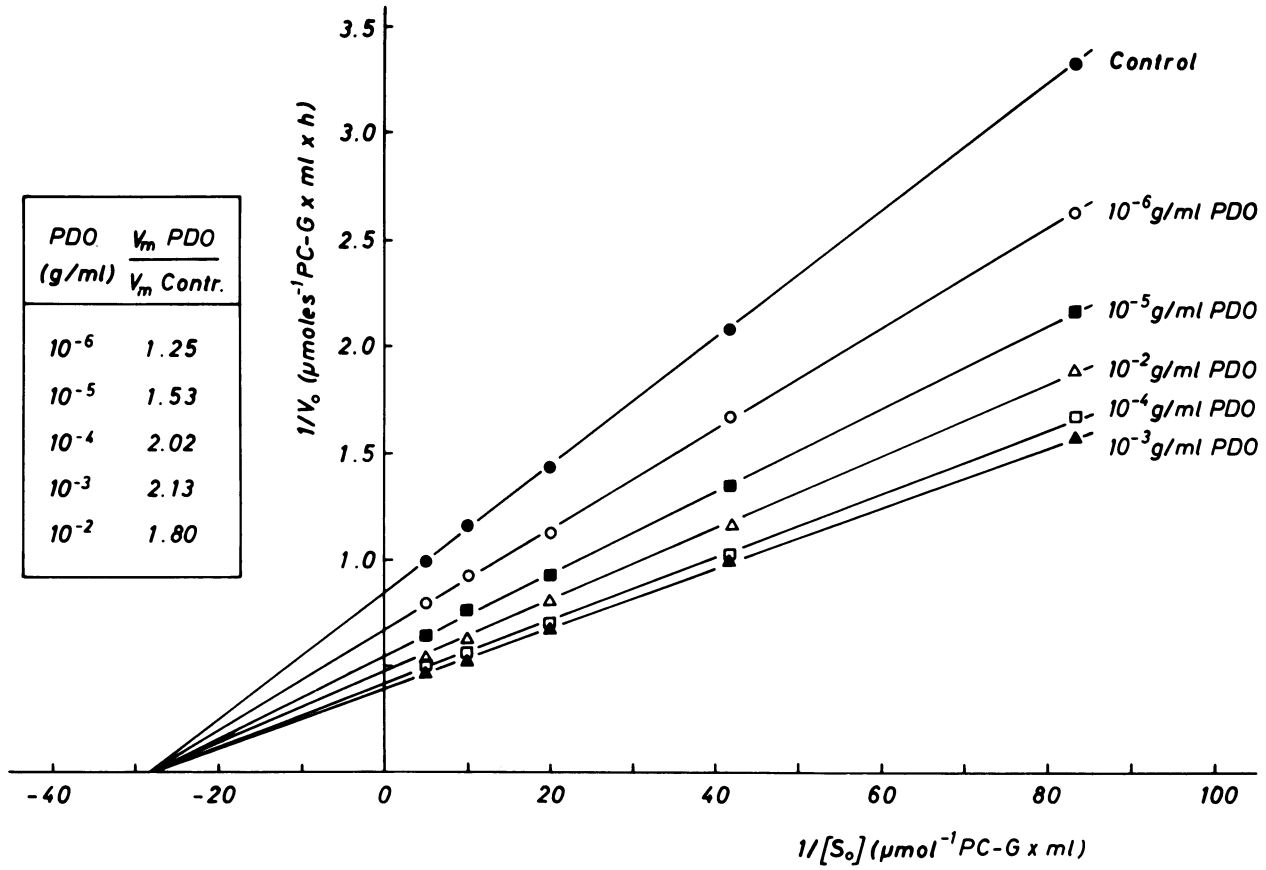

FIG. 4. Influence of PDO on the activity of staphylococcal β-lactamase (Lineweaver-Burk plot). The assay was done as described in the text. PC-G, Penicillin G.

this strain (Table 4). The MIC of oxacillin was decreased only twofold. The MIC of PDO was in the same range (>100 mg/ml) as that described for the resistant strains.

Comparable results were obtained when growth inhibition kinetics were studied. In Fig. 3 this is shown for methicillin. A 2.5-μg/ml concentration of this penicillin (1/1 MIC) caused slow permanent lysis which was in no way changed by PDO. With 0.5 μg of methicillin per ml (1/5 MIC), temporary lysis occurred ending in overnight resumption of growth. Addition of PDO produced continuous lysis, and no regrowth could be observed. However, the PDO-mediated lysis did not exceed that obtained with methicillin alone at MIC. When an inactive methicillin concentration (0.05 μg/ml = 1/50 MIC) was combined with PDO, only deceleration of growth was observed and lysis did not occur.

Influence of PDO on the activity of β-lactamase. It could not be excluded that PDO acts on the resistance to penicillin G and ampicillin in staphylococci by inhibition of β-lactamase. Therefore, the influence of PDO on the penicillin G-hydrolyzing activity of β-lactamase from *S. aureus* S108 was determined (Fig. 4). No inhibition of the enzyme could be detected. In fact, increasing stimulation of the β-lactamase activity occurred, reaching a maximum value in the presence of 1 mg of PDO per ml. At this concentration, the rate of penicillin G hydrolysis was more than twice that in the control sample.

Similar results could also be obtained with a commercially available β-lactamase from *B. cereus*. PDO again produced an increase of the enzyme activity. The maximum stimulation factor was 1.5 with a PDO concentration of 1 mg/ml (data not shown).

Binding of ^{3}H-labeled penicillin G and methicillin in the presence of PDO. The influence of PDO on binding of penicillins to their targets in the cytoplasmic membranes of resistant staphylococci was investigated with strain 9204, using ^{3}H-labeled penicillin G and methicillin. As mentioned before, this strain did not produce β-lactamase; therefore, its resistance to penicillin G is of the intrinsic type like that to methicillin.

First, the binding of ^{3}H-labeled penicillin G to the membranes was examined after incubation of whole cells suspended in buffer with various concentrations of penicillin in the presence of 0.1 mg of PDO per ml (Fig. 5). The binding curve obtained showed saturation-type kinetics, and there was no deviation from the PDO-free control.

When samples of membranes obtained from ^{3}H-labeled penicillin G-treated cells were subjected to polyacrylamide gel electrophoresis and fluorography, three PBPs were found (Fig. 6). The electrophoretic mobilities of these PBPs were identical to those known from susceptible staphylococci (19, 28). PBP 4 was not visible under these conditions because of the rapid release of penicillin G from this PBP (19). Half saturation of PBPs 1, 2, and 3 was achieved with 0.1 to 0.3 nmol of ^{3}H-labeled penicillin G per ml. These affinities were not changed by PDO.

The binding of methicillin was determined indirectly as radioactively labeled preparations of this penicillin are not available. Cells of strain 9204 were grown for 30 min in the

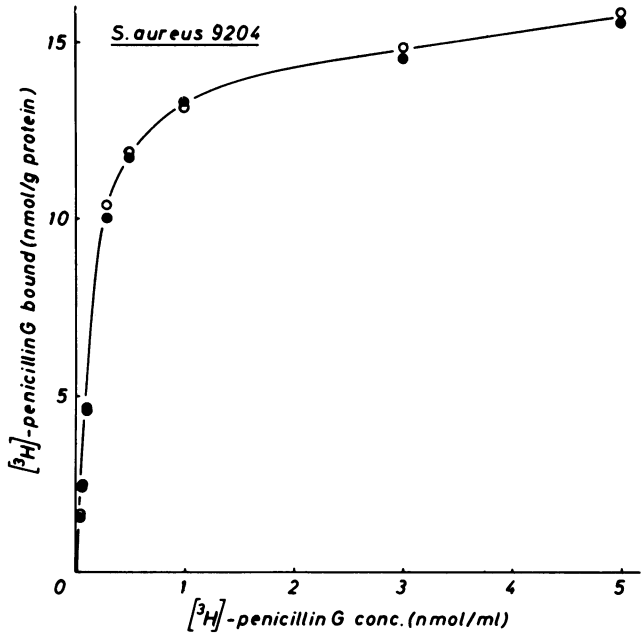

FIG. 5. Binding of ^{3}H-labeled penicillin G to the cytoplasmic membrane of *S. aureus* 9204 in the absence (○) or presence (●) of PDO (0.1 mg/ml). The experiments were performed as described in the text.

presence of methicillin alone (10 nmol/ml) or in combination with PDO (0.1 mg/ml). After isolation of the cytoplasmic membranes, the remaining free binding sites were saturated with ^{14}C-labeled penicillin G. Under these conditions, methicillin occupied ca. 60% of the penicillin targets (Table 5). These binding properties were not changed by PDO.

TABLE 5. Binding of methicillin to growing cells of *S. aureus* 9204 in the presence of PDO[a]

Methicillin concn (nmol/ml)	^{14}C-labeled PC-G bound (cpm/mg of protein)	
	Control	With PDO
0	5,500	5,465
10.0	2,213	2,299

[a] Cells were grown for 30 min with methicillin in the presence or absence of PDO (0.1 mg/ml). Membranes were prepared and then incubated with 0.3 nmol of ^{14}C-labeled penicillin G (PC-G) per ml (saturating concentration). For details, see the text.

DISCUSSION

With PDO, we were able to reduce the resistance of staphylococci to penicillins. The bactericidal action of the combinations resulted in the lysis of staphylococcal cells. PDO alone was not inhibitory but produced only a limited deceleration of cellular growth. Resistance to methicillin and oxacillin was fully suppressed in all strains. With respect to penicillin G and ampicillin, this was only possible in the β-lactamase-negative strain. In the case of β-lactamase-producing strains, variable but incomplete reduction of the resistance to these two penicillins was achieved. PDO did not inhibit the activity of staphylococcal β-lactamase. Therefore, its effect must be different from that of other surfactants which have been reported to reduce staphylococcal resistance to penicillin G but not to methicillin (32).

First, it seemed likely that PDO would stimulate uptake and binding of penicillins to the resistant staphylococci. However, this was not the case. The binding of methicillin to cytoplasmic membranes of growing cells was not changed by PDO. No alteration was found when the binding of ^{3}H-labeled penicillin G to the PBPs of resistant cells was studied in the presence of PDO. These results correspond well with findings of Hartman and Tomasz (12). They reported that in methicillin-resistant staphylococci at pH 5.2, methicillin binding was not increased, although at this pH value these staphylococci are not able to express their resistance.

The fact that cell lysis of our resistant strains was inhibited by CAP points to the participation of autolytic enzymes in the lethal effect of PDO-penicillin combinations. From *Streptococcus faecalis* it is known that nonionic detergents can interact with the autolytic enzyme system to induce cellular lysis (8). Staphylococci, however, were not lysed by PDO alone. Lysis could only be achieved if small amounts of a penicillin had acted before.

In susceptible gram-positive bacteria, it was found that autolytic enzymes are activated by the action of β-lactam antibiotics probably as a consequence of the inhibition of peptidoglycan cross-linking (1, 33). For methicillin-resistant staphylococci, however, it has been reported that lysis does not occur, though the cross-linkage of their peptidoglycan is decreased by methicillin (35). Wilkinson and Qoronfleh (34) have shown that peptidoglycan, isolated from resistant cells grown in the presence of methicillin, was sensitive to autolysin, but this was not true for the whole cells, pointing to an altered regulation of the autolytic activity in methicillin-resistant staphylococci.

Lipoteichoic acids and phospholipids (mainly cardiolipin and phosphatidyl glycerol) are known to regulate autolytic

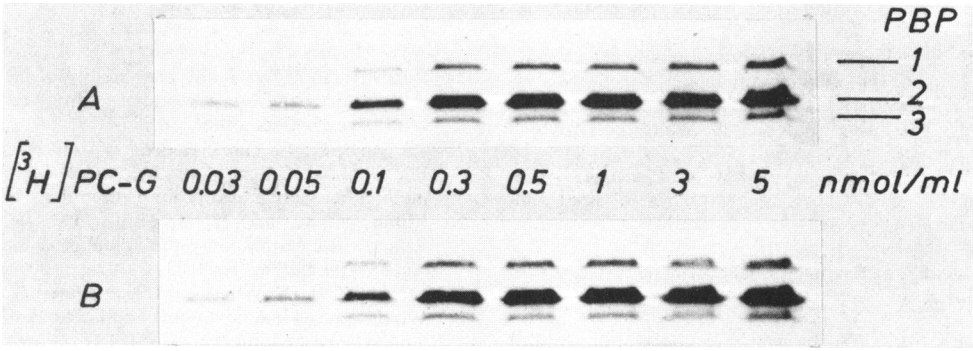

FIG. 6. Binding of ^{3}H-labeled penicillin G to the PBPs of *S. aureus* 9204 in the absence (A) or presence (B) of PDO (0.1 mg/ml).

enzymes of gram-positive bacteria (7, 15, 31). There is some evidence that these negatively charged compounds are not enzyme inhibitors in a classical sense but form micelles in which the autolytic enzymes are entrapped (2, 6). Thus, a topological barrier will be established between the enzymes and their substrate, the peptidoglycan. Tomasz and Waks originally suggested that inhibition of cell wall synthesis by penicillins triggers the bacterial autolysins by destabilizing this endogenous enzyme-inhibitor complex (33). As a consequence, lipoteichoic acids and lipids are released from the cells (3, 16, 17).

In methicillin-resistant staphylococci, this mechanism of autolysin activation appears to be blocked in some way. Earlier investigations (9, 18, 25) and newer results from our laboratory (unpublished) have revealed changes of the lipid composition in resistant staphylococci which may lead to an abnormally strong inhibition of the autolytic enzymes. Probably PDO reverses this inhibition by releasing lipoteichoic acids and lipids from the cytoplasmic membranes of the resistant cells. The fact that methicillin (or oxacillin) is additionally required for lysis points to the incapability of autolytic enzymes, activated by PDO, to attack undegraded peptidoglycan.

This is most likely due to the high rate of cross-linkage in staphylococcal peptidoglycan. That methicillin really acts as "pacemaker" in the combined action with PDO can be seen from the pretreatment experiments (Fig. 1).

In the case of resistance to penicillin G (or ampicillin), the same mode of action can be assumed. In β-lactamase producers, small amounts of active penicillin are still available that are obviously sufficient to make some relaxation of the rigid peptidoglycan structure essential for PDO to perform its lytic action. For susceptible staphylococci, there is also some evidence that sub-MICs of penicillins cooperate with PDO in the same manner (Fig. 3). With the latter experiments, it could be demonstrated that the kinetics of PDO-mediated lysis just follows that of the particular penicillin. In addition, lysis induced by inhibitory concentrations of penicillins alone was not increased. Thus, PDO does not exhibit an independent lytic mechanism but is obviously able to substitute penicillins in their lytic part of action.

ACKNOWLEDGMENTS

This work was supported by grants from the Ministerium für Wissenschaft und Forschung des Landes Nordrhein-Westfalen.

LITERATURE CITED

1. **Best, G. K., N. H. Best, and A. V. Koval.** 1974. Evidence for participation of autolysins in bactericidal action of oxacillin on *Staphylococcus aureus*. Antimicrob. Agents Chemother. **6:** 825–830.
2. **Briese, T., and R. Hakenbeck.** 1983. Interaction between choline and the *N*-acetyl-muramyl-L-alanine-amidase of *Streptococcus pneumoniae*, p. 173–178. *In* R. Hakenbeck, J. V. Höltje, and H. Labischinski (ed.), The target of penicillin. Walter de Gruyter, Berlin.
3. **Brissette, J. L., G. D. Shockman, and R. A. Pieringer.** 1982. Effects of penicillin on synthesis and excretion of lipid and lipoteichoic acid from *Streptococcus mutans* BHT. J. Bacteriol. **151:**838–844.
4. **Brown, D. F. J., and P. E. Reynolds.** 1980. Intrinsic resistance to β-lactam antibiotics in *Staphylococcus aureus*. FEBS Lett. **122:**275–278.
5. **Bruns, W., and H. Keppeler.** 1980. Mechanism of intrinsic penicillin-resistance in *Staphylococcus aureus*. Binding of penicillin G to the cytoplasmic membrane of resistant staphylococci. Arzneim. Forsch. **30:**1469–1475.
6. **Cleveland, R. F., L. Daneo-Moore, A. J. Wicken, and G. D. Shockman.** 1976. Effect of lipoteichoic acid and lipids on lysis of intact cells of *Streptococcus faecalis*. J. Bacteriol. **127:** 1582–1584.
7. **Cleveland, R. F., A. J. Wicken, L. Daneo-Moore, and G. D. Shockman.** 1976. Inhibition of wall autolysis in *Streptococcus faecalis* by lipoteichoic acid and lipids. J. Bacteriol. **126:**192–197.
8. **Cornett, J. B., and G. D. Shockman.** 1978. Cellular lysis of *Streptococcus faecalis* induced with Triton X-100. J. Bacteriol. **135:**153–160.
9. **Dunnick, J. K., and W. M. O'Leary.** 1970. Correlation of bacterial lipid composition with antibiotic resistance. J. Bacteriol. **101:**892–900.
10. **Dyke, K. G. H., M. P. Jevons, and M. T. Parker.** 1966. Penicillinase production and intrinsic resistance to penicillins in *Staphylococcus aureus*. Lancet **i:**835–838.
11. **Ericsson, H. M., and J. C. Sherris.** 1971. Antibiotic sensitivity testing. Acta Pathol. Microbiol. Scand. Sect. B **217**(Suppl.): 65–67.
12. **Hartman, B., and A. Tomasz.** 1981. Altered penicillin-binding proteins in methicillin-resistant strains of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **19:**726–735.
13. **Hayes, M. V., N. A. C. Curtis, A. W. Wyke, and J. B. Ward.** 1981. Decreased affinity of a penicillin-binding protein for β-lactam antibiotics in a clinical isolate of *Staphylococcus aureus* resistant to methicillin. FEMS Microbiol. Lett. **10:**119–122.
14. **Hewitt, J. H., A. W. Coe, and M. T. Parker.** 1969. The detection of methicillin resistance in *Staphylococcus aureus*. J. Med. Microbiol. **2:**443–456.
15. **Höltje, J. V., and A. Tomasz.** 1975. Lipoteichoic acid: a specific inhibitor of autolysin activity in Pneumococcus. Proc. Natl. Acad. Sci. U.S.A. **72:**1690–1694.
16. **Horne, D., R. Hakenbeck, and A. Tomasz.** 1977. Secretion of lipids induced by inhibition of peptidoglycan synthesis in streptococci. J. Bacteriol. **132:**704–717.
17. **Horne, D., and A. Tomasz.** 1979. Release of lipoteichoic acid from *Streptococcus sanguis*: stimulation of release during penicillin treatment. J. Bacteriol. **137:**1180–1184.
18. **Hugo, W. B., and R. J. Stretton.** 1966. The role of cellular lipid in the resistance of gram-positive bacteria to penicillins. J. Gen. Microbiol. **42:**133–138.
19. **Kozarich, J. W., and J. L. Strominger.** 1978. A membrane enzyme from *Staphylococcus aureus* which catalyzes transpeptidase, carboxypeptidase and penicillinase activities. J. Biol. Chem. **253:**1272–1278.
20. **Laskey, R. A., and A. D. Mills.** 1975. Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography. Eur. J. Biochem. **56:**335–341.
21. **Pearson, R. D., R. T. Steigbigel, H. T. Davis, and S. W. Chapman.** 1980. Method for reliable determination of minimal lethal antibiotic concentrations. Antimicrob. Agents Chemother. **18:**699–708.
22. **Richmond, M. H.** 1975. β-Lactamase from *Staphylococcus aureus*. Methods Enzymol. **43:**664–672.
23. **Richmond, M. H., and R. B. Sykes.** 1973. The β-lactamases of gram-negative bacteria and their possible physiological role. Adv. Microb. Physiol. **9:**31–89.
24. **Rozgonyi, F.** 1976. Genotypic stability of methicillin resistance in *Staphylococcus aureus* at supraoptimal temperature. Antimicrob. Agents Chemother. **10:**377–379.
25. **Rozgonyi, F., L. Vaczi, P. Sebessy-Gönczy, and I. Redai.** 1973. Phospholipid content of staphylococci sensitive and resistant to methicillin. Contrib. Microbiol. Immunol. **1:**172–179.
26. **Rubin, F. A., and D. H. Smith.** 1973. Characterization of R-factor β-lactamases by the acidimetric method. Antimicrob. Agents Chemother. **3:**68–73.
27. **Sabath, L. D., N. Wheeler, M. Laverdiere, D. Blazevic, and B. J. Wilkinson.** 1977. A new type of penicillin resistance of *Staphylococcus aureus*. Lancet **i:**443–447.
28. **Spratt, B. G.** 1980. Biochemical and genetical approaches to the mechanism of action of penicillin. Phil. Trans. R. Soc. London

Ser. B **289**:273–283.
29. **Spratt, B. G., V. Jobanputra, and U. Schwarz.** 1977. Mutants of *Escherichia coli* which lack a component of penicillin-binding protein 1 are viable. FEBS Lett. **79**:374–378.
30. **Spratt, B. G., and A. B. Pardee.** 1975. Penicillin-binding proteins and cell shape in *E. coli*. Nature (London) **254**:516–517.
31. **Suginaka, H., M. Shimatani, M. Ogawa, and S. Kotani.** 1979. Prevention of penicillin-induced lysis of *Staphylococcus aureus* by cellular lipoteichoic acid. J. Antibiot. **23**:73–77.
32. **Suling, W. J., and W. M. O'Leary.** 1975. Effect of surfactants on antibiotic resistance. Antimicrob. Agents Chemother. **8**:334–343.
33. **Tomasz, A., and S. Waks.** 1975. Mechanism of action of penicillin: triggering of the pneumococcal autolytic enzyme by inhibitors of cell wall synthesis. Proc. Natl. Acad. Sci. U.S.A. **72**:4162–4166.
34. **Wilkinson, B. J., and M. W. Qoronfleh.** 1983. Increased wall autolysis and decreased peptidoglycan cross-linking in methicillin-resistant *Staphylococcus aureus* grown in the presence of methicillin, p. 217–222. *In* R. Hakenbeck, J. V. Höltje, and H. Labischinski (ed.), The target of penicillin. Walter de Gruyter, Berlin.
35. **Wyke, A. W., J. B. Ward, and M. V. Hayes.** 1982. Synthesis of peptidoglycan in vivo in methicillin-resistant *Staphylococcus aureus*. Eur. J. Biochem. **127**:553–558.