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In Vitro and In Vivo Effects of Penicillin and Clindamycin on Expression of Group A Beta-Hemolytic Streptococcal Capsule

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Encapsulation of group A beta-hemolytic streptococci (GABHS) is an important virulence factor. The changes that occur in the frequency of encapsulation of GABHS during pharyngotonsillitis, in 20 patients treated with penicillin and 20 treated with clindamycin, were investigated. The effects of subinhibitory concentrations of these agents were also evaluated in vitro. At day 4, 8 of 10 (80%) GABHS isolates recovered from children treated with penicillin were encapsulated, compared with 1 of 5 (20%) of those from children treated with clindamycin ($P < 0.05$). Two days following 10 days of therapy, GABHS was eliminated from 13 of the 20 (65%) children treated with penicillin and from all treated with clindamycin ($P < 0.05$). At that time, six of the seven GABHS isolates recovered in patients treated with penicillin were encapsulated. GABHS were not detected after 4 days of therapy in those treated with clindamycin. Incubation of GABHS isolates with one-half of the MIC of clindamycin reduced the frequency of encapsulation, compared with that after incubation with one-half of the MIC of penicillin (12.5 versus 67.5%). These data illustrate the superiority of clindamycin over penicillin in reducing the expression of a capsule by GABHS.

The virulence of group A beta-hemolytic streptococci (GABHS) has been associated with two cell wall structures: the M protein and the hyaluronic acid capsule (6, 14). The virulence was assessed by resistance to phagocytosis (6, 7, 14) and induction of mortality in mice after intraperitoneal inoculation (18). Variation in the elaboration of M protein during the natural course of respiratory infections was observed (14). Compared with characteristics during the acute stages of the infection, about half of GABHS isolates gradually lost their ability to elaborate type-specific M-protein antigen and became susceptible to the antibacterial activity of the human blood during the convalescent and carrier stages. However, variations in the presence of capsule of GABHS and the effects of antimicrobial therapy on expression of a capsule were not evaluated during the different phases of pharyngotonsillitis in children.

This study was performed to investigate the dynamics of expression of capsule by GABHS during pharyngotonsillitis in patients treated with penicillin or clindamycin. The in vitro effects of subinhibitory concentrations of these agents were also evaluated.

MATERIALS AND METHODS

Patients. Children who were seen for acute GABHS pharyngotonsillitis were considered for inclusion in the study. These children had symptoms of acute uncomplicated pharyngotonsillitis of 1 to 4 days' duration. They had sore throats, swollen and enlarged tonsils with or without exudate, tender cervical lymph glands, and temperatures above 38°C. More than 10 colonies of GABHS were isolated from their pharyngotonsillar cultures. None had received antimicrobial therapy for the past 6 weeks, and none had had an acute pharyngotonsillar inflammation in the past 3 months. Included in the analysis were the first 20 consecutive patients who received penicillin and the first 20 who received clindamycin and fulfilled the criteria described above, completed their course of therapy, and were monitored for subsequent cultures as outlined below.

No randomization of antimicrobial agents was done. Penicillin was the drug given routinely to all patients with GABHS pharyngotonsillitis. Clindamycin was administered to those with a history of allergy to penicillin. Another group of 20 children seen consecutively during the time period of the study, whose pharyngotonsillar cultures showed repeated growth of GABHS on at least three cultures taken over a period of 3 to 4 months without clinical signs of infection (carriers), served as a control group. None had received antimicrobial therapy for the past 3 months and during the whole period of follow-up. The ages of children in the two patients' groups and the control group were similar and ranged from 8.5 to 16 years (average, 11 years and 4 months), and 23 of the 40 patients and 13 of the 20 controls were males.

Collection of specimens and follow-up. The retropharynx and both tonsillar surfaces were swabbed with two sterile cotton swabs (Culturette; Marion Scientific Corp., Rockford, Ill.). One was immediately plated in sheep blood (5%) agar and chocolate plates that were incubated in 5% CO_2 at a temperature of 37°C for 48 h. The plate was inspected at 24 and 48 h. Characterization of GABHS was done by determining bacitracin sensitivity, by serologic grouping using Phadebact coagglutination (Pharmacia Diagnostics, Inc., Piscataway, N.J.), and with M and T antiserum.

The other swab was plated for anaerobic bacteria on prereduced vitamin K_1-enriched *Brucella* blood agar. The anaerobic plate was incubated in GasPak jars (Baltimore Biological Laboratories, Cockeysville, Md.) and examined at 48 and 96 h.

Anaerobes were identified by techniques described previously (16). Aerobic bacteria were identified by conventional methods (11). β-Lactamase activity was determined for all isolates by the chromogenic cephalosporin nitrocefin method (13).

Six cultures were obtained from patients on the following days: day 0 (prior to administration of antimicrobial agents) and 2, 4, 7, 12, and 21 days after initiation of therapy. Four specimens were taken from controls (days 0, 7, 12, and 21). Patients and controls had complete physical examinations at these dates, and their findings were recorded.

Antimicrobial therapy. The oral antimicrobial agents were administered for 10 days. They were penicillin-V potassium tablets (250 mg four times a day) and clindamycin capsules (150 mg four times a day). Compliance with therapy was monitored by examining the unused medicine and individual checkoff cards.

In vitro antimicrobial assays and capsular studies of GABHS. The MICs of penicillin and clindamycin for each GABHS isolate recovered prior to and after therapy were determined by the agar dilution method (11). Mueller-Hinton agar supplemented with 5% sheep blood was used, as outlined by the National Committee for Clinical Laboratory Standards (12). The final inocula contained approximately 1×10^4 to 3×10^4 CFU per spot. The MIC was defined as the lowest drug concentration that prevented visible growth or yielded fewer than six discrete colonies.

The in vitro effects of antimicrobial agents were studied in GABHS isolates recovered from children before receiving antimicrobial therapy (day 0). Fresh isolates of GABHS collected from the original plates (10^6 organisms in 1 ml) were incubated for 48 h at 37°C in 9 ml of Todd-Hewitt broth medium that included one-half of the MIC. A tube without antimicrobial agents served as a control.

The presence of a capsule was evaluated each time that the patients were examined. It was determined in all newly recovered GABHS isolates that had not been subcultured and all isolates exposed in vitro to one-half of the MIC for 48 h. This was established by a blinded observer in all cases using electron microscopy after staining with ruthenium red (9). An isolate was considered to be encapsulated if a capsule was seen in more than 75% of 500 bacterial cells.

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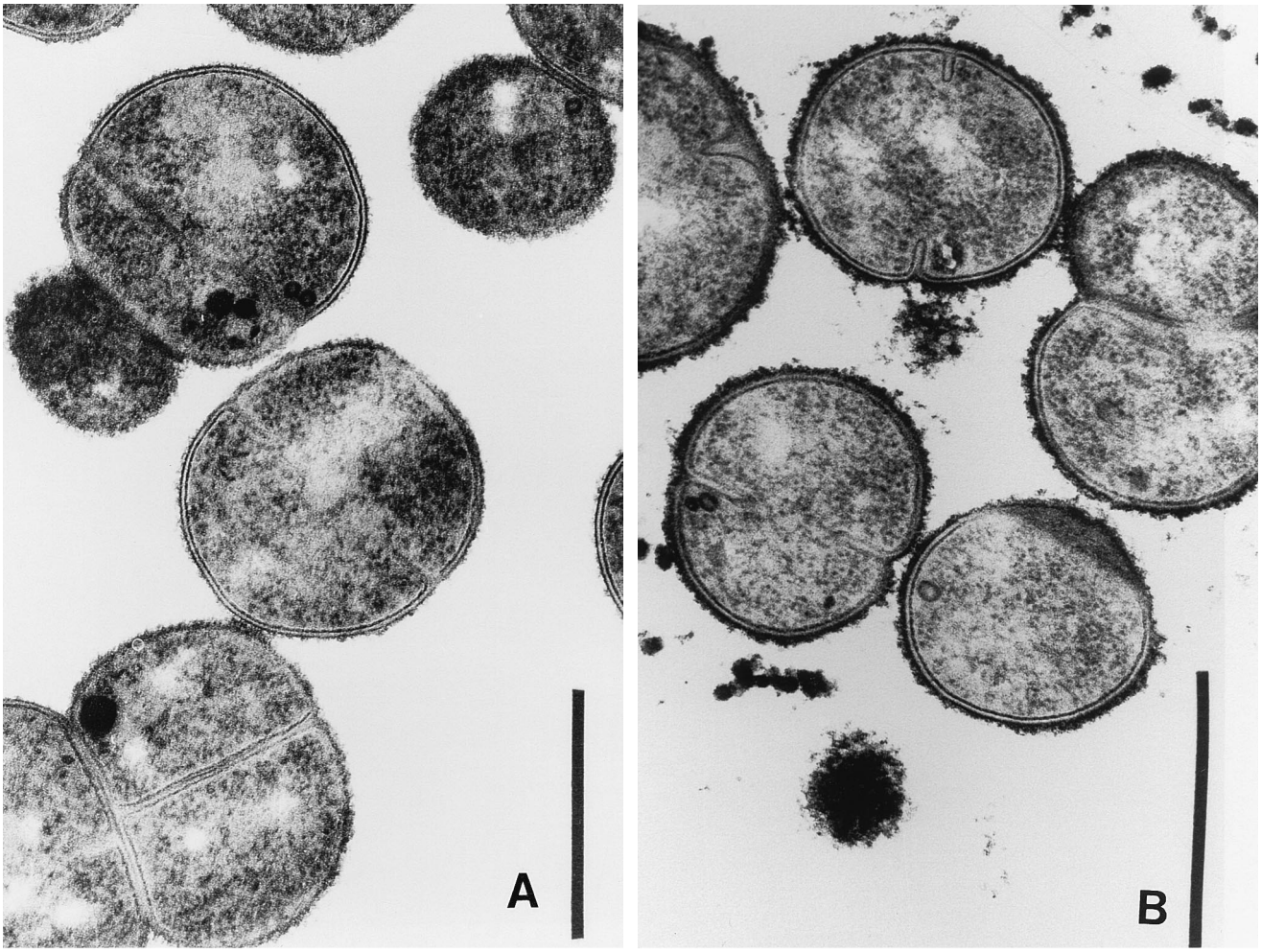

FIG. 1. Electron micrograph of a thin section of a 48-h culture of nonencapsulated (A) and encapsulated (B) GABHS stained with ruthenium red. Magnification, ×32,500. Bar = 1 μm.

Statistical analysis was done by the χ^2 test and Fisher's exact test.

RESULTS

Recovery of GABHS in patients and controls. Following therapy (at day 12) GABHS was eliminated from 13 of the 20 (65%) children treated with penicillin and from all treated with clindamycin ($P < 0.05$) (Table 1). More than 10 colonies of GABHS per plate were isolated in all these cases. At that time, six of the seven GABHS isolates from penicillin-treated children were encapsulated. Four of these children were symptomatic at that time with active pharyngotonsillitis and therefore represent clinical failure of penicillin. No GABHS were detected after day 4 from those treated with clindamycin. At day 4, 8 of 10 (80%) GABHS isolates were encapsulated in those treated with penicillin, as compared to only 1 of 5 (20%) in those treated with clindamycin ($P < 0.05$). Encapsulated GABHS were isolated in 10 to 15% of controls throughout the study period.

Serology. Serologic typing of GABHS before, during, and after therapy revealed identical isolates in all individual cases.

TABLE 1. Frequency of recovery of encapsulated (>75% of cells encapsulated) GABHS

Source of samples (*n*)	No. encapsulated/no. of samples (%) on indicated day after infection diagnosis[a]					
	0	2	4	7	12	21
Carriers (control) (20)	3/20 (15)	ND	ND	2/20 (10)	2/20 (10)	3/20 (15)
Patients with pharyngo-tonsillitis (40)						
Penicillin treated (20)	17/20 (85)	14/18 (78)	8/10 (80)	5/7 (71)	6/7 (86)[b]	5/6 (83)[b]
Clindamycin treated (20)	18/20 (90)	4/10 (40)[c]	1/5 (20)[c]	NG	NG	NG

[a] ND, not done; NG, no growth of GABHS.
[b] Four of the patients were symptomatic at day 12.
[c] $P < 0.05$ compared with penicillin-treated group.

TABLE 2. Effects of subinhibitory concentrations of penicillin and clindamycin on production of capsule by 40 strains of GABHS after 48-h incubation

Patient group[a] (no. of strains)	No. of encapsulated strains (%) after incubation with:		
	Penicillin	Clindamycin	Saline
Penicillin (20)	14 (70)	2 (10)[b]	17 (85)
Clindamycin (20)	13 (65)	3 (15)[b]	18 (90)
Total (40)	27 (67.5)	5 (12.5)[b]	35 (87.5)

[a] Strains were isolated from patients later treated with penicillin or clindamycin as indicated.
[b] $P < 0.05$ compared with penicillin incubation results.

The most common types represented were T-1, T-4, and T-12, and the most common M types were M-1, M-2, and M-4.

Antimicrobial susceptibility. Quality control strains were used to assess antimicrobial susceptibility. The MIC of penicillin for GABHS isolates ranged between 0.01 and 0.05 μg/ml, and the MIC of clindamycin ranged between 0.025 and 0.05 μg/ml. No change in MIC for any of the isolates was noted after therapy.

In vitro encapsulation of GABHS after inoculation in one-half of the MIC. Incubation with clindamycin of all original GABHS isolates recovered from patients later treated with either penicillin or clindamycin significantly reduced the prevalence of encapsulation, as compared with incubation with penicillin (12.5 versus 67.5%) (Fig. 1; Table 2).

BLPO. β-Lactamase-producing organisms (BLPO) were detected prior to therapy in three (15%) of the patients receiving clindamycin, two (10%) of those receiving penicillin, and four (20%) controls (Table 3). These BLPO were *Staphylococcus aureus*, *Haemophilus influenzae*, pigmented *Prevotella* species, and *Fusobacterium* species. Following therapy the number of children with BLPO increased to five (25%) in those treated with penicillin, was unchanged in those treated with clindamycin, and decreased to two (10%) in the controls. None of the changes were significant.

DISCUSSION

This study provides support for the role of the capsule as an important virulence factor of GABHS. The association of capsule with virulence is not unique to GABHS. It has also been described in *Streptococcus pneumoniae*, in which the capsular material has been shown to inhibit phagocytosis (19), and in *Prevotella* and *Porphyromonas* spp., in which encapsulated isolates were found more often in acutely inflamed tonsils than in normal flora (1). The recovery of capsule-forming GABHS in the majority of patients during the acute stage of the illness, as compared with their lower recovery rate in the convalescent stages of the illness as well as in carriers (control group), suggests the importance of the capsule during acute inflammation. The ability to produce a capsule may be inherited in the GABHS strains but may be expressed more often in the early stages of the infection. Similar changes in the expression of capsule during the invasive stages of illness were shown in other organisms such as *S. aureus* (8) and *Bacteroides* spp. (3), in which the presence of a capsule was correlated with increased invasiveness and virulence.

The formation of a capsule by GABHS has been shown to vary also in vitro. It is especially pronounced in the early stages of bacterial growth in vitro and diminishes as the culture gets older (6, 18). Reemergence of the capsule occurred following reinoculation in animals (6, 18).

This study illustrates the effects of antimicrobial therapy on one of the important virulence factors of GABHS. The frequency of capsular expression was significantly lower at the fourth day of therapy in GABHS isolates recovered from patients treated with clindamycin (20%), as compared with that in those treated with penicillin (80%). While clindamycin therapy completely eradicated GABHS within 7 days of therapy, GABHS was recovered from seven (35%) of the patients 2 days after completion of penicillin therapy; six of these seven isolates were still encapsulated, and four of these patients were still symptomatic. Whether the superiority of clindamycin over penicillin in eradicating GABHS is due to its ability to inhibit expression of a capsule as was shown in vitro or due to other factors has yet to be determined.

Another explanation for the increased clinical efficacy of clindamycin over penicillin in eliminating GABHS from infected tonsils may be its resistance to the enzyme β-lactamase. BLPO present in the core of recurrently inflamed tonsils were shown to protect GABHS from penicillin in vitro (5) and in vivo (4). Clindamycin's intrinsic resistance to the enzyme β-lactamase enabled it to eliminate BLPO such as *S. aureus*, pigmented *Prevotella* species, and *Fusobacterium* species. This feature explained its efficacy in the treatment of acute (15) and recurrent (2) GABHS pharyngotonsillitis and eradication of the carrier state (17). However, since there were no differences in the recovery rates of BLPO in this study of acutely inflamed tonsils, the increased activity of clindamycin over penicillin in this report cannot be explained by its enhanced activity against BLPO.

Our description of the reduction in the frequency of encapsulation of GABHS after growing the organism with one-half of the MIC of clindamycin is similar to the observed effect of clindamycin on M-protein formation by GABHS (7). Clindamycin potentiated the opsonization and phagocytosis of GABHS after the organism was grown in the presence of one-half of the MIC of clindamycin. Morphological changes were also seen in the surface of the streptococcal cells. The surface layer which contained the M protein was absent after growth within one-half of the MIC of clindamycin. Clindamycin therapy of experimental *S. aureus* osteomyelitis was found to inhibit the formation of external cell wall glycocalyx by the

TABLE 3. Frequency of recovery of BLPO

Source of samples (*n*)	No. of samples with BLPO (%) on indicated day after infection diagnosis					
	0	2	4	7	12	21
Carriers (control) (20)	4 (20)	ND[a]	ND	3 (15)	2 (10)	2 (10)
Patients with pharyngotonsillitis (40)						
Penicillin treated (20)	2 (10)	2 (10)	3 (15)	4 (20)	5 (25)	5 (25)
Clindamycin treated (20)	3 (15)	3 (15)	2 (10)	1 (5)	2 (10)	3 (15)

[a] ND, not done.

organism and enhanced phagocytosis of the organism by macrophages (10).

It is plausible that clindamycin therapy affects clinical GABHS infection in multifactorial ways by inhibiting M-protein and capsular expression of GABHS and suppressing BLPO. Reducing M-protein and capsular expression may facilitate phagocytosis, and elimination of BLPO removes a potential interfering factor that can prevent cure by penicillin. Further studies are warranted to evaluate all of these factors in acute and recurrent infections as well as the carrier state.

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