Imipenem-Cilastatin in the Treatment of Methicillin-Sensitive and Methicillin-Resistant Staphylococcus aureus Infections

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Imipenem-cilastatin was evaluated for efficacy and toxicity as an antistaphylococcal agent in 23 patients; 11 of these patients were infected with methicillin-resistant Staphylococcus aureus (MRSA), and 12 were infected with methicillin-susceptible S. aureus (MSSA). There were 15 soft tissue, 5 endovascular, and 3 skeletal infections and a total of nine patients with bacteremia. As determined by in vitro susceptibility testing, the MICs for 90% of the MRSA and MSSA isolates tested were 6.25 and 0.39 μg/ml, respectively. Two MRSA isolates were resistant to a concentration of >16 μg/ml. When 11 MRSA isolates and 7 MSSA isolates were incubated for 48 h the MICs for 90% of the isolates increased to >50 μg/ml for the MRSA isolates and 6.25 μg/ml for the MSSA isolates. Three S. aureus isolates emerged resistant. Ten of 11 (91%) MRSA infections and 11 of 12 (92%) MSSA infections were clinically cured. Adverse reactions occurred in 25% of the imipenem-cilastatin-treated patients. These reactions included gastrointestinal intolerance (7% of the patients), rash or pruritis (6%), eosinophilia (6%), thrombocytosis (4%), and a positive, direct Coomb test without hemolysis (3%). One of the two patients for whom therapy was discontinued because of gastrointestinal intolerance had antibiotic-associated colitis. Imipenem appears to be an effective antistaphylococcal agent against both MRSA and MSSA infections.

Because of the increasing importance of staphylococcal infections, the limited therapeutic options, and economic concerns, alternate agents to treat staphylococcal infections are needed. In Detroit, Mich., both community- and hospital-acquired methicillin-resistant Staphylococcus aureus (MRSA) infections have become widespread (6, 9, 13). As the infectious disease consultants at a large inner-city hospital, we administer care to indigent patients, including intravenous drug abusers. To the best of our knowledge, clinical isolates of vancomycin-resistant MRSA have not been reported; thus, vancomycin remains the drug of choice for MRSA infections. However, in anticipation of vancomycin resistance, alternate agents for use against MRSA infections are needed. Encouraged by previous in vitro and clinical studies (2-5, 7, 10, 11, 14, 15, 17-20; K. Shannon and I. Phillips, Abstr. 13th Int. Cong. Chemother., abstr. no. 5y53, 1983), we evaluated imipenem-cilastatin (I/C) for its efficacy, safety, and tolerance as an antistaphylococcal agent.

MATERIALS AND METHODS

Patient selection. Between June 1983 and May 1984, we enrolled 69 patients who were seen by the Infectious Disease Consultation Service at Henry Ford Hospital, Detroit, Mich. After informed consent was obtained, the patients were admitted to the study if they were hospitalized with proven or suspected bacterial infections caused by pathogens presumed or known to be susceptible to I/C. Patients were excluded for the following reasons: (i) age under 12 years; (ii) pregnancy or nursing; (iii) hypersensitivity to I/C or history of anaphylactic reactions to any β-lactam antibiotic; (iv) infection at the onset of therapy anticipated to require treatment for more than 42 days; (v) brain abscess or meningitis; (vi) clostridial infection of muscle; (vii) prosthetic valve endocarditis; (viii) antimicrobial agent use within 72 h before a patient was considered for initiation of I/C therapy; (ix) high probability of death within 48 h; (x) anuria or severe renal insufficiency; and (xi) infection with a pathogenic organism presumed to be resistant to I/C. All patients were treated intravenously with I/C at doses ranging from 1 to 4 g per day in four divided doses.

In vitro studies. All clinical specimens were identified by standard methods (8). Staphylococcus aureus isolates were tested for susceptibility to imipenem by using a disk diffusion assay and an inoculum prepared in Mueller-Hinton broth to a turbidity equivalent to 0.5 MacFarland standard. Organisms were plated onto sheep blood agar containing 10-μg imipenem disks. The plates were incubated for 18 h at 35°C. Organisms with zone sizes of ≥16 mm were interpreted as susceptible; organisms with zone sizes of 14 to 15 mm were interpreted as having intermediate susceptibility; and organisms with zone sizes of ≤13 mm were interpreted as resistant.

The MICs were determined by a microdilution broth technique (8). The MIC was defined as the lowest concentration of antibiotic which inhibited visible growth. Serial twofold dilutions containing 0.024 to 50 μg of imipenem per ml were made in Mueller-Hinton broth (pH 7.4 ± 0.2), and these preparations were inoculated with an organism at a final density of 10^5 CFU/ml. The plates were incubated at 35°C and interpreted after 18 and 48 h. Organisms with MICs of ≤8 μg/ml were defined as susceptible, and organisms with MICs of ≥16 μg/ml were defined as resistant. Methicillin resistance and susceptibility determinations were based on oxacillin MICs (>2 μg/ml, resistant; ≤2 μg/ml, susceptible).

To determine serum inhibitory concentrations (SIC) (8), serum samples were taken 1 h before administration and 1 h after administration of an intravenous I/C dose during therapy. Serial twofold serum dilutions were made on microdilution plates, by using Mueller-Hinton broth as the diluent. Individual wells were then inoculated with an organism at a final density of 10^5 CFU/ml. The plates were then incubated
at 35°C and interpreted at 18°C by using the same criteria for inhibitory concentrations as described above for MIC determinations.

Clinical studies. The patients were followed serially for clinical and bacteriologic effects by the Infectious Diseases Service. Data for hematologic parameters, serum chemistry data, and urinalysis data were obtained periodically. We defined cure as complete clinical resolution of signs and symptoms of infection and failure as no clinical response during therapy or relapse of signs and symptoms after therapy was completed. We defined bacteriologic failure as isolation of the initial organism after therapy was finished. Thereafter, we followed patients through follow-up clinic visits, telephone calls, and chart reviews at subsequent hospital admissions.

RESULTS

Of the 69 patients enrolled, 33 had Staphylococcus aureus-related infections. Single isolates were obtained from 29 of these patients before therapy or immediately after initiation of antibiotic therapy. Multiple isolates were recovered from four patients during therapy; two isolates were recovered from two patients, and three isolates were recovered from another two patients. Of the 33 patients 23 could be evaluated. Ten patients were excluded, two because of I/C resistance, one because of inadvertent oral administration of antimicrobial agents at the time of discharge, four because Staphylococcus aureus isolates were believed to be the colonizing organisms rather than the pathogenic organisms, and three because they had MSSA infections that were incurred during the last phase of the study when I/C was being conserved to increase the number of patients with MRSA infections treated. Of the 23 patients evaluated, 12 had MSSA infections, and 11 had MRSA infections. The mean age of the patients was 35.8 years; the underlying diseases included intravenous drug abuse (22 patients) and diabetes mellitus (1 patient).

In vitro susceptibility. Imipenem in vitro susceptibility testing was performed by using 39 clinical Staphylococcus aureus isolates (Table 1). The imipenem MICs for 90% of the strains tested (MIC90s) against MRSA and MSSA isolates were 6.25 and 0.39 μg/ml, respectively. Figure 1 shows the correlation between the disk diffusion data and MICs; six MRSA isolates and one MSSA isolate were resistant as determined by the diffusion method but susceptible as determined by the MIC method. In one of the four patients from whom multiple isolates were recovered during the course of therapy, the MIC increased from 0.049 to 1.56 μg/ml.

The incubation time was extended to 48 h for 7 MSSA isolates and 11 MRSA isolates (Table 1). The MIC50 of imipenem for the MSSA isolates increased to 6.25 μg/ml, while the MIC50 for MRSA isolates increased to >50 μg/ml. With this extended incubation time, three Staphylococcus aureus isolates (one MSSA and two MRSA) were resistant.

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>Taxon</th>
<th>No. of isolates</th>
<th>MIC50 (μg/ml)</th>
<th>MIC90 (μg/ml)</th>
<th>MIC range (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>MSSA</td>
<td>21</td>
<td>≤0.024</td>
<td>0.39</td>
<td>&lt;0.024-1.56</td>
</tr>
<tr>
<td></td>
<td>MRSA</td>
<td>18</td>
<td>0.049</td>
<td>6.25</td>
<td>≤0.024-50</td>
</tr>
<tr>
<td></td>
<td>MSSA</td>
<td>7</td>
<td>0.195</td>
<td>6.25</td>
<td>≤0.024-50</td>
</tr>
<tr>
<td></td>
<td>MRSA</td>
<td>11</td>
<td>0.780</td>
<td>&gt;50</td>
<td>0.097-&gt;50</td>
</tr>
</tbody>
</table>

FIG. 1. In vitro susceptibilities of Staphylococcus aureus isolates to I/C disks and MICs. Symbols: ⋄, MRSA; △, MSSA.

The MSSA isolate was obtained on the day 40 of I/C therapy from a patient who relapsed with osteomyelitis after the study was completed. One of the two MRSA isolates was obtained from a patient who could not be evaluated, while the other was obtained from a patient who was successfully treated by I/C therapy and surgical drainage of a neck abscess.

The pre- and postdose SICs of I/C were determined for 22 of the 23 patients who could be evaluated. The predose SICs ranged from <1:2 to 1:256, and the postdose SICs ranged from 1:16 to 1:2,048. Postdose SICs of ≥1:8 were readily achieved, although the SICs for MRSA isolates were one to two dilutions lower than those for MSSA isolates.

Clinical response. Among the I/C-treated patients 15 had soft tissue infections, five had endovascular infections, and three had skeletal infections; 9 patients were bacteremic. In 20 patients, the infections were caused by Staphylococcus aureus alone, and in 3 patients the infections were mixed; 10 of 11 MRSA infections (91%) and 11 of 12 MSSA infections (92%) were clinically cured. One bacteriologic failure occurred in the MRSA group (Table 2).

The MSSA failure involved a patient who was being treated for osteomyelitis of the foot and septic arthritis of the sternoclavicular joint. MSSA was detected in the blood during the first 11 days of therapy despite postdose SICs of 1:256. On day 40 of I/C therapy (2 g/day) breakthrough bacteremia occurred with a MSSA isolate, although its source could not be determined. Phage typing revealed that this MSSA isolate was the same phage type (54/85) as the original isolates. Although the MICs had increased 32-fold, in vitro resistance was not demonstrated by the usual methods. However, incubation for 48 h revealed that the MIC was 50 μg/ml. I/C therapy was extended for another 2 weeks. Eleven days after discharge, the patient was readmitted with lumbar osteomyelitis due to MSSA.

The MRSA bacteriologic failure was a woman who was treated with 2 g of I/C per day for 7 days and had a diagnosis of cellulitis and sinus tract of the right calf. This patient responded clinically. Local signs and symptoms of infection resolved completely. The SICs were 1:16. However, when she was subsequently admitted for surgical excision of the
sinus tract, MRSA was isolated. The clinical MRSA failure was in a female patient who was being treated for cellulitis of the left forearm. She was treated for 9 days with 1.5 g of I/C per day, with peak SICs of 1:256. Despite resolution of pain, erythema, and induration, she developed an abscess after discharge from our hospital, which was drained at another institution.

**Side effects.** We evaluated all 69 patients for drug tolerance. Of these patients, 17 (25%) experienced an adverse effect; a total of one adverse effect occurred for every 54 patient days of I/C therapy. The adverse effects included eosinophilia (four patients), thrombocytosis (≥800,000/mm³; three patients), a positive direct Coomb test without hemolysis (two patients), rash or pruritus and both (four patients), and nausea, vomiting, or diarrhea (five patients). Of the two patients discontinued from the study because of gastrointestinal intolerance, one had antibiotic-associated colitis. All adverse effects resolved when I/C therapy was discontinued. One patient died from non-I/C-related causes secondary to gentamicin-resistant, tobramycin-resistant *Pseudomonas aeruginosa* tracheobronchitis and respiratory failure.

**DISCUSSION**

Imipenem (N-formimidoyl thienamycin), a carbapenam antibiotic that is produced in vitro by *Streptomyces cattleya*, has a broad spectrum of antimicrobial activity against gram-negative and gram-positive bacteria, including multiply resistant species of *Pseudomonas aeruginosa*, *Serratia* spp., *Acinetobacter* spp., enterococci, *Bacteroides* fragilis, and MRSA (1, 7, 12, 17). Since imipenem is extremely resistant to β-lactamase, it appears to have little cross-resistance with penicillins and cephalosporins.

Although the mechanism of action of imipenem against gram-positive organisms is not well understood, previous in vitro susceptibility testing (Table 3) indicated that imipenem is active against both MSSA and MRSA isolates (3, 4, 7, 11, 17–20; Shannon and Phillips, Abstr. 13th Int. Cong. Chemother.). However, the MIC₉₀ for MRSA isolates may be 8 to 400 times the MIC₉₀ for MSSA isolates (3, 7, 11, 17, 18, 20). Our in vitro data also demonstrate a disparity between the MICs for MSSA and MRSA isolates, with 90% of MSSA isolates inhibited by 0.39 μg of imipenem per ml, whereas 6.25 μg/ml is required to inhibit 90% of MRSA isolates. Only 13% of the MRSA isolates (2 of 18 isolates) were resistant to imipenem. When the incubation time was extended to 48 h, 14% of our MSSA isolates (1 of 7 isolates) and 18% of our MRSA isolates (2 of 11 isolates) were resistant.

In experimental models of *Staphylococcus aureus* endocarditis in rabbits and rats, Scheld and Keeley (14) and Baumgartner and Glauser (2) demonstrated that imipenem is comparable in bactericidal activity to nafcillin and cloxacinil plus gentamicin. In a clinical trial conducted by Marier et al. (10) in which I/C was compared with cefazolin for the treatment of skin, soft tissue, lower respiratory tract, urinary tract, and bone and joint infections, all *Staphylococcus aureus* infections in the I/C group were clinically cured. Bacteriologic eradication was achieved with 93% of 30 *Staphylococcus aureus* isolates treated with I/C, compared with 96% of 29 *Staphylococcus aureus* isolates in the cefazolin group. Favorable results were obtained in other clinical trials by Eron et al. and Schreiner et al., although one drug addict with *Staphylococcus aureus* endocarditis was considered a failure when blood cultures persisted after 5 days of therapy (5, 15). While these clinical studies reported *Staphylococcus aureus* infections that were treated successfully with I/C, methicillin susceptibility was not evaluated. In our study, both MRSA and MSSA infections were treated with I/C with equal success. I/C clinically cured 91% of the MRSA infections (10 of 11 patients) and 92% of the MSSA infections (11 of 12 patients).

I/C seems to be well tolerated, although adverse reactions were observed in 25% of the 69 patients treated. However, only two patients developed serious side effects which necessitated the discontinuation of therapy; these side effects were antibiotic-associated colitis and severe nausea, vomiting, and diarrhea.

Hesitation in recommending I/C as an antistaphylococcal agent, especially against MRSA infections, has been based on in vitro work which suggested that standard MIC testing conditions are inadequate for expressing resistance to β-lactam antibiotics. Alterations in laboratory techniques, including extended incubation time, lower temperature, addition of sodium chloride, and manipulation of pH, have all been used to alter the expression of MRSA resistance to various antibiotics. Markowitz et al. (11) demonstrated a significant increase in the MIC₉₀s for MRSA isolates (from 0.4 to 50 μg/ml) when the incubation time was extended to 48 h. Witte et al. (20) concluded that there was little disparity between MICs when cultures were incubated at 30° and 35°C, although 37% of the MSSA isolates tested were tolerant to imipenem when they were incubated at 30°C, compared with 54% of the isolates incubated at 35°C. Wise et al. (19) tested nine isolates of *Staphylococcus aureus* by

### TABLE 3. In vitro activity of N-formimidoyl thienamycin against *Staphylococcus aureus* isolates after 18 to 24 h of incubation

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Reference</th>
<th>Incubation temp (°C)</th>
<th>MIC₉₀ (μg/ml) for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MSSA</td>
<td>MRSA</td>
</tr>
<tr>
<td>Witte et al.</td>
<td>20</td>
<td>30</td>
<td>0.02 (21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35</td>
<td>0.04 (21)</td>
</tr>
<tr>
<td>Thompson et al.</td>
<td>17</td>
<td>35</td>
<td>8.00 (43)</td>
</tr>
<tr>
<td>Markowitz et al.</td>
<td>11</td>
<td>35</td>
<td>0.05 (51)</td>
</tr>
<tr>
<td>Verbiest and Verhaegen</td>
<td>18</td>
<td>36</td>
<td>0.12 (26)</td>
</tr>
<tr>
<td>Cherubin et al.</td>
<td>3</td>
<td>37</td>
<td>0.01 (20)</td>
</tr>
<tr>
<td>Wise et al.</td>
<td>19</td>
<td>37</td>
<td>0.06 (25)</td>
</tr>
<tr>
<td>Enciso et al.</td>
<td>4</td>
<td>37</td>
<td>0.06 (52)</td>
</tr>
<tr>
<td>Shannon and Phillips</td>
<td>7</td>
<td>NS</td>
<td>0.12 (29)</td>
</tr>
<tr>
<td>Kahan et al.</td>
<td>7</td>
<td>NS</td>
<td>≈0.13 (1.29)</td>
</tr>
</tbody>
</table>

* The numbers in parentheses are the numbers of isolates tested.
* Five of the isolates were MRSA isolates.
* The methicillin resistance of the isolates was not specified.
* NS, Not specified.
using various culture media and pHs (pH range, 6.0 to 8.0) and found that the MICs of I/C were not greatly altered. However, the smallest discrepancy occurred at pH 7.2, and the greatest discrepancy occurred at pH 6.6 on Penassay agar.

The clinical significance of the in vitro results is not known. As our results indicate, the MICs for MRSA and MSSA isolates increased with prolonged incubation, with three isolates becoming resistant. Two of these isolates were obtained from evaluable patients; one of these patients was a clinical failure, and the other had a surgically drained abscess. These results support the hypothesis that extended incubation time is required to express imipenem resistance. Until further clinical studies are available, I/C should not be used for treatment of infections by staphylococcal isolates that demonstrate resistance after 48 h of incubation. However, we are encouraged by the overall excellent response rate and especially by the performance of imipenem against the five serious endovascular infections, three of which were due to MRSA isolates. We were able to achieve peak SICs of >1:8 in all patients, which further support the antistaphylococcal activity of imipenem.

I/C may be of greatest use against polymicrobial bone and soft tissue infections where combination therapy with more toxic agents would otherwise be used. In these instances, if Staphylococcus aureus is isolated, efficacy should be based on clinical parameters and not solely on in vitro testing since laboratory manipulation of environmental factors can alter the phenotypic expression of resistance.

Although this study was small, nonrandomized, and open, imipenem appears to be an effective agent against both MSSA and MRSA infections. Further clinical trials will be necessary to establish the efficacy of imipenem against serious Staphylococcus aureus infections and the clinical significance of altered in vitro test conditions.

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LITERATURE CITED