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Comparative imipenem treatment of *Staphylococcus aureus* endocarditis in the rat

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The efficacy of imipenem alone or in association with gentamicin against *Staphylococcus aureus* experimental endocarditis was compared to the efficacy of cloxacillin alone or in association with gentamicin. Parenteral treatment was started 24 h after intravenous bacterial challenge of rats with catheter-induced aortic valve vegetations. The cloxacillin MIC and MBC for *Staph. aureus* were 0·125 and 32 mg/l and the imipenem MIC and MBC 0·008 and 8 mg/l, respectively. *In-vitro* killing curves showed a synergistic effect between cloxacillin and gentamicin, and an additive effect between imipenem and gentamicin. Only large doses of cloxacillin (400 mg/kg tid) (producing serum levels above those obtained after intravenous injection of 2 g in man) achieved results comparable to those of imipenem 80 mg/kg tid (producing serum levels similar to those obtained after an intravenous dose of 750 mg in man) in reducing the bacterial numbers in vegetations after 3 and 5 days of treatment. There was a significantly greater reduction of bacterial numbers in vegetations after treatment with the association of cloxacillin and gentamicin than with cloxacillin alone. In contrast, the addition of gentamicin to imipenem did not improve significantly the results of treatment with imipenem alone, but imipenem alone was as good as the combination cloxacillin and gentamicin after 5 days of treatment. We conclude that imipenem is a highly bactericidal drug in this animal model, worth considering for clinical trials in the treatment of *Staph. aureus* infections.

Introduction

Imipenem is a stable modified form of thienamycin, a β-lactam antibiotic of the carbapenem class. Its spectrum of action is unusually broad, with important *in-vitro* activity against the majority of bacteria often creating therapeutic problems, such as *Bacteroides fragilis*, carbenicillin-resistant *Pseudomonas aeruginosa*, *Streptococcus faecalis*, methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* (Cherubin *et al.* 1981; Kesado, Hashizume & Ashai, 1980; Kesado *et al.*, 1982; Livingston, Elliott & Cobbs, 1981; Michael, Alford & McGee, 1981; Thompson, Fischer & Wenzel, 1982; Verbist & Verhaegen, 1981). Only a small incidence of cross-resistance to penicillins and cephalosporins has been demonstrated with imipenem. In addition to its potential application in the treatment of infections due to multi-resistant organisms, imipenem may have a place in the empirical treatment of infections which at present require the association of 2 or more antibiotics.

The high *in-vitro* activity of imipenem against *Staph. aureus* and *Staph.*

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epidermidis, including some methicillin-resistant strains (Cherubin *et al.*, 1981; Livingston *et al.*, *1981;* Thompson *et al.*, 1982) justified *in-vivo* studies of this drug in infections caused by these organisms. We selected a model of left-sided *Staph. aureus* endocarditis because it offers several advantages to test the efficacy of antibiotics. Firstly, it mimics human endocarditis, in that sterile valvular aortic vegetations (induced by the presence of a polyethylene catheter) are infected by the intravenous inoculation of bacteria, the ensuing endocarditis displaying several of the characteristics of human disease. Secondly, since in endocardial infections the polymorphonuclear leukocytes have limited access to the bacteria embedded in the valvular vegetations, the host defence mechanisms are of limited help in this type of infection (Durack, Beeson & Petersdorf, 1973; Hook & Sande, 1974). Bacteriostatic antibiotics have failed in the treatment of endocarditis (Walker *et al*, 1962), and one must rely on the bactericidal activity of antibiotics to achieve cure (Carrizosa & Kaye, 1977; Hamburger *et al.*, 1967). Left-sided *Staph. aureus* endocarditis is therefore a suitable test of the killing power of a new compound against one of the most virulent organisms. Thirdly, the treatment of *Staph. aureus* endocarditis has been extensively studied in animals, making possible the comparison of the efficacy of new drugs with that of reference antibiotics (Hamburger *et al.*, 1967; Miller, Wexler & Steigbigel, 1978; Sande & Johnson, 1975; Sande & Courtney, 1976).

The present study was undertaken to determine the *in-vivo* efficacy of imipenem in the rat model of *Staph. aureus* endocarditis, compared to that of cloxacillin. In addition, these 2 antibiotics were given either alone or in association with gentamicin in order to detect any possible synergism or antagonism.

Material and methods

Micro-organism

A strain of *Staph. aureus* isolated from a patient with bacterial endocarditis was used. Minimal inhibitory and bactericidal concentrations (MIC and MBC) of cloxacillin, gentamicin and imipenem were determined by broth dilution tests (Washington & Sutter, 1980) with an inoculum of 5×10^5 organisms from an overnight culture. To determine the MBC, 0·1 ml of an undiluted sample from each dilution of antibiotic showing no turbidity after both 24 h and 48 h of incubation, as well as 0·1 ml of 10-fold and 100-fold broth dilutions, were plated on penicillinase-containing blood agar. 10-fold and 100-fold dilution of the antibiotic-containing broth avoids carry-over of antibiotic, a phenomenon that can give falsely low MBCs. After incubation of the blood agar plates for 48 h, the number of colonies on each plate was counted, and the MBC determined as the lowest dilution of antibiotic which showed a 99·9% killing of the original inoculum.

Killing curves with various concentrations and combinations of cloxacillin, gentamicin and imipenem were performed in trypticase soya broth (Difco Laboratories, Detroit, Mich.) using an inoculum of 5×10^5 to 10^6 cfu/ml of an overnight culture of *Staph. aureus*.

Production of endocarditis

Sterile vegetations were produced in female Wistar rats (180 to 200 g) by a modification of a method already described (Héraïef, Glauser & Freedman, 1982).

Briefly, a polyethylene catheter (PP 10, Portex Ltd, Hythe, Kent, England) was inserted across the aortic valve through the right carotid artery and secured with a silk ligature. Twenty-four hours after catheterization, rats were injected in the tail vein with 0·5 ml of saline containing 10^5 cfu of *Staph. aureus*. The catheter was left in place thoughout the experiments.

Evaluation of infection

Rats were killed at different intervals, at least 8 h after the last antibiotic administration, when no antibiotic activity was detectable in blood. One ml of blood was drawn from the inferior vena cava, plated on blood agar and incubated for colony counts. Aortic vegetations, spleen and left kidney were excised, weighed, homogenized in 1 ml of saline and serially diluted and plated. Plates were counted after 24 h of incubation at 37°C. This method permitted the detection of 10^2 cfu/g of organ.

Treatment of Staph. aureus *endocarditis with various antibiotic regimens*

Treatment with antibiotics was started 24 h after injection of 10^5 cfu of *Staph. aureus* in the tail vein of the rats. In each experiment, control rats were killed at the time of starting treatment to determine the incidence and magnitude of valvular infection and of positive blood cultures. In the treatment groups, the rats were killed after 3, 5 and 10 days of treatment.

Imipenem (Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey) (without cilastatin) was reconstituted in a phosphate buffer solution pH 7.2 and injected subcutaneously at doses of 30 and 80 mg/kg tid. Cloxacillin (Beecham Research Laboratories, Brentford, England) was reconstituted with sterile water and injected subcutaneously at doses of 50 and 400 mg/kg tid. Gentamicin (Schering Corporation, Kenilworth, New Jersey) was diluted in water and injected intramuscularly at doses of 4 mg/kg tid. Control rats were injected subcutaneously with saline.

Antibiotic serum levels

Serum levels of cloxacillin, gentamicin and imipenem at various intervals after one injection were determined by the agar diffusion technique (Sabath & Anhalt, 1980).

Statistical evaluation

The Chi-square test with Yates' correction and the unpaired Student's *t*-test were used for statistical comparisons.

Results

Determination of MIC and MBC for Staph. aureus

The MIC and MBC for the strain of *Staph. aureus* tested were respectively 0·125 and 32 mg/l for cloxacillin, 0·125 and 2 mg/l for gentamicin and 0·008 and 8 mg/l for imipenem. According to Sabath *et al.* (1977), tolerance to antibiotics is defined as a MBC/MIC ratio of 32 or more. Thus, the strain of *Staph. aureus* used in these

experiments was tolerant to both cloxacillin and imipenem. We chose a tolerant strain because using our technique of MBC determination which reduces the antibiotic carry-over effect, we have found that the majority of *Staph. aureus* strains isolated from blood cultures in our hospital and tested in this way were characterized by MBC/MIC ratios of $\geqslant 32$ (for cloxacillin, this was seen in 21 out of 24 strains, and for imipenem in 21 out of 23 strains collected in 1982).

Bacterial killing of Staph. aureus *by cloxacillin and imipenem, alone and in combination with gentamicin*

The *Staph. aureus* timed kill curves determined with concentrations of antibiotics between the MIC and MBC demonstrated synergism between cloxacillin and gentamicin (Figure 1). In contrast, only an additive effect could be seen with the association of imipenem and gentamicin (Figure 1).

Serum levels of antibiotics (Figure 2)

The levels obtained in rats after subcutaneous injection of 30 and 80 mg/kg of imipenem were comparable to levels obtained in men weighing 70 kg after intravenous injection of 250 or 750 mg, respectively (Merck Sharp and Dohme, Rahway, USA, unpublished data). The levels obtained in rats after the subcutaneous injection of cloxacillin 50 mg/kg were comparable to levels obtained after intravenous injection of 0·5 g in men, whereas levels obtained after 400 mg/kg in rats were above levels obtained after 2 g in men.

Animal studies

Severity of infection at the start of treatment. Thirty-nine rats were killed 24 h after bacterial inoculation, at the time the various treatments were started. Valvular

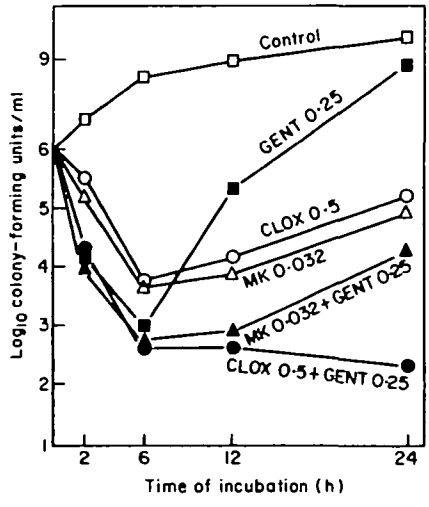

Figure 1. *Staph. aureus* timed kill curves with cloxacillin 0·5 mg/l (CLOX 0·5) and imipenem 0·032 mg/l (MK 0·032), alone and in combination with gentamicin 0·25 mg/l (GENT 0·25).

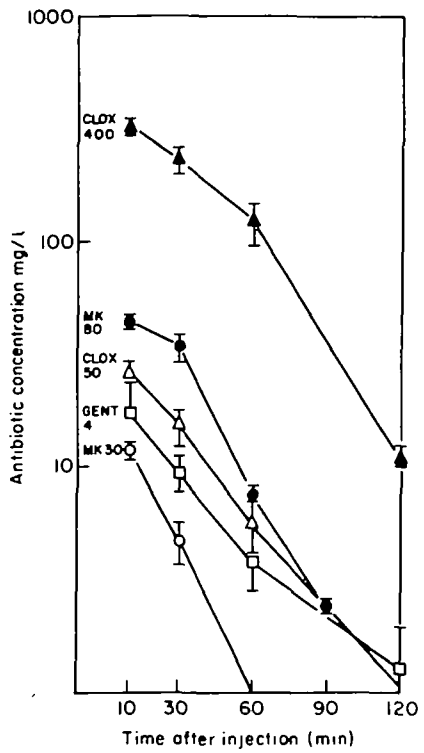

Figure 2. Rat serum levels after subcutaneous injection of cloxacillin 50 (CLOXA 50) and 400 mg/kg (CLOX 400), of imipenem 30 (MK 30) and 80 mg/kg (MK 80), and after intramuscular injection of gentamicin 4 mg/kg (GENT 4). Each point represents the mean ± S.D. of values in 3 to 5 rats.

vegetations were infected in all the animals and blood cultures were positive in 94% (37 out of 39 rats). All of the spleens and 38 of 39 left kidneys displayed severe *Staph. aureus* infection. The severity of valvular infection, expressed as colony forming units of *Staph. aureus*/g of vegetations is reported in Figure 3 (control group).

Evolution of infection in control rats. Starting 24 h after bacterial inoculation (day 0), 16 control animals were injected with normal saline tid. The cumulative mortality was 63% (10 out of 16 rats) on day 1, 88% (14 out of 16) on day 2 and 100% on day 3. All the dead animals had generalized infection as revealed by the cultures of blood, spleen, kidney and aortic vegetations.

Effect of the 3-day treatment regimen (Figure 3). In the group treated with cloxacillin 50 mg/kg tid, the mortality was significantly reduced from 100% in control rats receiving saline to 13% in rats receiving cloxacillin (2 out of 15 rats died) ($\chi^2 = 20{\cdot}45$, $P < 10^{-5}$). However, the 50 mg/kg dose of cloxacillin did not diminish the bacterial counts recovered upon cultures from the valvular vegetations when compared to bacterial numbers recovered in rats killed at the beginning of treatment. Furthermore, all rats killed after treatment with cloxacillin 50 mg/kg tid displayed *Staph. aureus* positive blood cultures, as well as positive spleen and kidney cultures.

In contrast, every other treatment regimen given for 3 days significantly reduced the valvular bacterial counts when compared to that in controls. Among the rats receiving cloxacillin or imipenem combined with gentamicin, only the combination of

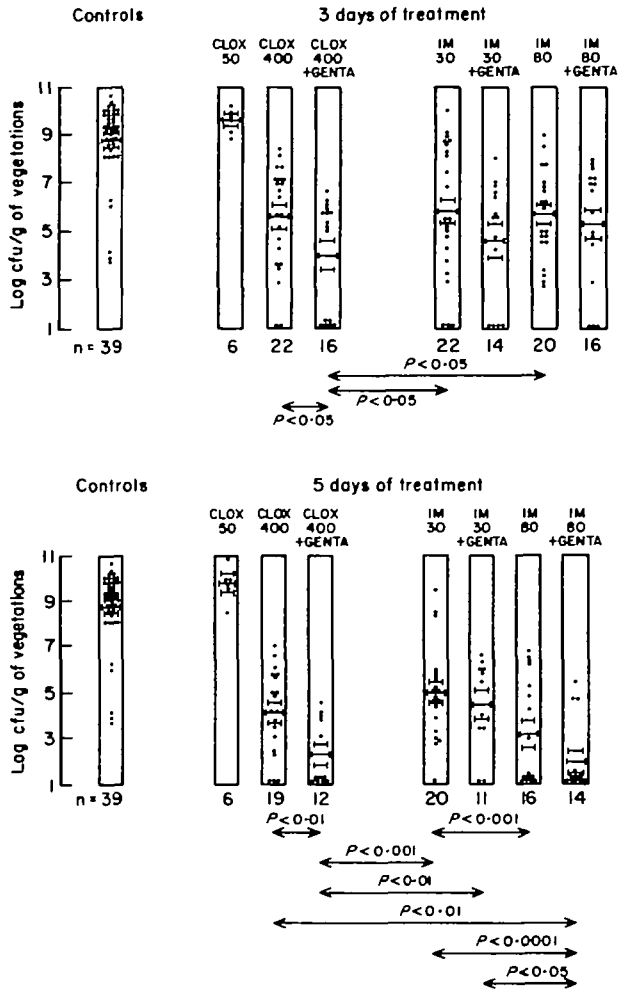

Figure 3. Effect of 3 and 5 days' treatment regimens on quantitative cultures of aortic valve vegetations. Control rats were killed 24 h after intravenous challenge with *Staph. aureus*, at the time of starting treatments. Each point represents a rat. CLOX 50 and CLOX 400: treatment with cloxacillin 50 and 400 mg/kg tid sq. Im 30 and im 80: treatment with imipenem 30 and 80 mg/kg tid sq. GENTA: treatment with gentamicin 4 mg/kg tid im. The dashes represent the mean ± S.E.M. Statistical comparisons were done using the unpaired Student's *t*-test.

gentamicin with cloxacillin 400 mg/kg tid showed a significant reduction in the bacterial numbers when compared to the effect of the β-lactam antibiotics alone.

Effect of the 5 day treatment regimen (Figure 3). As in the 3 day treatment regimen, the rats treated for 5 days with cloxacillin 50 mg/kg tid did not show a reduction in the number of cfu/g of vegetations when compared to that observed at the beginning of treatment. Blood, spleen and left kidney cultures grew *Staph. aureus* in all animals. Only the mortality was reduced (26% versus 100%) ($\chi^2 = 15, 12, P < 10^{-4}$). Therefore, this dose of cloxacillin allowed the rats to survive, but did not cause any regression of the infection.

In contrast, every other 5-day treatment regimen achieved a significant reduction in

bacterial numbers when compared to controls. Imipenem 80 mg/kg tid alone and combined with gentamicin, and cloxacillin 400 mg/kg tid combined with gentamicin, were the treatment regimens which showed the greatest reduction in valvular infection. Cloxacillin 400 mg/kg tid alone was significantly less effective than the combination of cloxacillin 400 mg/kg tid with gentamicin (an observation correlating with *in-vitro* synergism between cloxacillin and gentamicin), while the addition of gentamicin to imipenem treatment did not improve significantly the effect of imipenem alone (although a trend toward increased efficacy was observed).

Effect of the 10-day treatment regimen. The 4 treatment schedules tested (cloxacillin 400 mg/kg tid alone and in combination with gentamicin, imipenem 30 mg/kg tid alone and in combination with gentamicin) cured cardiac infection in all of the animals studied.

Discussion

In the endocarditis experiments reported here, the catheter which initiates the sterile aortic vegetations was left in place throughout the entire period of observation. Under these conditions, the evolution of *Staph. aureus* infection was particularly severe since all animals died from infection in less than 4 days in the absence of antibiotic treatment. The natural history of this model infection provided therefore a stringent test of antibiotic efficacy.

The administration of cloxacillin 50 mg/kg produced serum levels greater than the MIC for the infecting strain for approximately 90 min after injection, but a level reaching the MBC was never achieved. Using this dosage administered 3 times per day, the treatment was unable to reduce *Staph. aureus* valvular infection, nor to eradicate the micro-organism from the blood. This confirms previous observations, which established the necessity of bactericidal antibiotic to cure endocarditis (Carrizosa & Kaye, 1977; Hamburger *et al.*, 1967).

Studies using *Staph. aureus* endocarditis in rabbits have shown that penicillinase-resistant penicillins associated with an aminoglycoside bring about a more rapid eradication of bacteria than the β-lactam alone, provided the association was synergistic *in vitro*. Aminoglycosides antibiotics administered alone were ineffective (Michael *et al.*, 1981; Sande & Johnson, 1975; Sande & Courtney, 1976). These observations were confirmed in our rat model, in that the association of cloxacillin with gentamicin, synergistic *in vitro*, was shown to have increased efficacy in rats after both 3 and 5 days of treatment, when compared to the efficacy of cloxacillin alone. This advantage, however, was not apparent after 10 days of treatment, because the vegetations were sterile in both the cloxacillin alone and the cloxacillin plus gentamicin treatment groups.

In contrast to the cloxacillin treatment regimens, the addition of gentamicin to imipenem did not significantly improve the therapeutic effect of imipenem alone. This could be related to the absence of an *in-vitro* synergistic effect observed upon the addition of gentamicin to imipenem. Since a similar absence of synergism between imipenem and gentamicin has been observed with other strains of *Staph. aureus* and other bacteria (Zinner & Klastersky, 1982), one must rely on the bactericidal effect of imipenem alone for the treatment of *Staph. aureus* infections. Nevertheless imipenem alone in our experimental model provided after 5 days of treatment as great an antibacterial effect as the synergistic combination of high doses of cloxacillin plus

gentamicin. This response to infection was achieved with doses of imipenem producing serum levels in rats easily obtainable in humans, while the doses of cloxacillin necessary for similar results produced in rats serum levels higher than that usually achieved in humans.

In conclusion, in this model of experimentally-produced *Staph. aureus* endocarditis in rats, imipenem has been shown to be highly bactericidal, as bactericidal as the synergistic combination of high doses of cloxacillin plus gentamicin after 5 days of treatment. These results warrant further clinical studies to evaluate the usefulness of imipenem in *Staph. aureus* infections in man.

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