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Simulated Human Serum Profiles of One Daily Dose of Ceftriaxone plus Netilmicin in Treatment of Experimental Streptococcal Endocarditis

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We performed experiments in rats aimed at determining whether a combination of ceftriaxone (CRO) and netilmicin (NET), by using once-daily administration in rats, which simulated profiles of drug in human serum, was more effective than either agent alone in the treatment of endocarditis caused by viridans group streptococci. A programmable infusion pump system enabled the production of profiles of CRO in serum that simulate those found in humans after the intravenous administration of 2 g. The subcutaneous administration of 18 mg of NET per kg of body weight produced levels in the sera of rats comparable to those after the intravenous administration of a dose of 5 mg of NET per kg in humans. Rats with catheter-induced aortic vegetations were infected intravenously with two test strains, a CRO-susceptible *Streptococcus sanguis* strain (MICs of CRO and NET, 0.064 and 8 mg/liter, respectively) and a relatively CRO-resistant *Streptococcus mitis* strain (MICs of CRO and NET, 2 and 8 mg/liter, respectively). Against both strains, the combination of CRO and NET was synergistic in vitro as determined by time-kill curves. Treatment of rats was started 48 h postinfection and lasted for 3 days. CRO alone was effective against the susceptible strain ($P < 0.001$ compared with control animals) but was not effective against the resistant organism. A significantly enhanced antibacterial activity of the CRO-NET combination in reducing the valvular bacterial counts was observed with both test strains ($P < 0.001$). The synergistic effect was obtained with a single daily injection of NET which provided detectable levels in serum for only 8 h, suggesting that in vivo synergism in the treatment of infections caused by viridans group streptococci can be obtained without 24 h of aminoglycoside coverage. These experimental data might provide a rationale for clinical trials of a once-a-day dosing regimen in the treatment of streptococcal but nonenterococcal endocarditis.

The regimen of penicillin for 4 weeks or penicillin plus two injections of an aminoglycoside per day for 2 weeks is effective for the treatment of endocarditis caused by viridans group streptococci. However, both regimens require several injections per day, and the addition of the aminoglycoside can be toxic in elderly patients or in those with impaired renal function. Two recent developments may allow for more convenient and safer treatment. First, a single daily dose of ceftriaxone (CRO) administered for 4 weeks has been found to be effective and has allowed treatment of a substantial number of patients on an outpatient basis and without a permanent venous catheter (9, 28). Second, recent experimental and clinical evidence has suggested that the administration of a total daily dose of an aminoglycoside in one daily injection is as effective as and is potentially less toxic than a regimen of two or three daily doses (1, 10, 17, 23, 29, 31, 33). Thus, it is conceivable that a combination of CRO plus one daily dose of netilmicin (NET), administered one after the other in a single intravenous (i.v.) injection, may allow shortening of the length of treatment with CRO without adding toxicity, and thus may be a very convenient treatment for endocarditis caused by viridans group streptococci. In experimental endocarditis, three doses of CRO and one dose of NET have been shown to be as effective as three doses of CRO and three doses of NET and more effective than CRO administered alone (10). Similar findings were reported with penicillin-tobramycin (25) and penicillin-gentamicin (11) combinations. However, the pharmacokinetics of antibiotics in animals are very different from those in humans. For example, because of the short half-life of ceftriaxone in rats, this drug had to be administered three times a day to achieve detectable levels in serum over 24 h. Even with this regimen, periods of subinhibitory concentrations in serum before the administration of the next dose were observed (10). The purpose of the present study was to better approach ceftriaxone administration in humans by means of a programmable pump system developed in our laboratory. This system enabled us to simulate in rats the pharmacokinetic profile of CRO in humans (8).

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MATERIALS AND METHODS

Microorganisms. Two bacterial strains were used to produce endocarditis. These strains have already been described (10).

Susceptibility studies and in vitro killing curves. The MICs of penicillin G, CRO, and NET were determined by macrodilution tests in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) by using an inoculum of an overnight culture of 10^6 CFU/ml (16). Killing curves were determined in Mueller-Hinton broth, which was incubated at 37°C for 24 h by using an inoculum of 10^7 to 10^8 CFU/ml of an overnight

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culture. The selected CRO concentrations approximate the peak (250 mg/liter) and trough (15 mg/liter) levels found in the serum of treated rats. The test concentration of NET was that found in rats at 2 h of treatment (10 mg/liter). Details of the method have already been described (10). In vitro synergy was defined as a greater than 100-fold increase in killing with the combination in comparison with that with the most active agent (19).

Pharmacokinetics. Serum antibiotic concentrations were determined by an established agar diffusion technique (24) by using *Escherichia coli* ATCC 25922 as the indicator strain for CRO and *Bacillus subtilis* ATCC 6633 (Difco) as the indicator strain for NET. The detection limit was 0.1 μg/ml in both cases. The diluent was decomplemented rat serum. The samples were taken at various times from groups of nine infected rats.

SBTs. For the two strains, serum bactericidal titers (SBTs) were determined in three rats of each treatment group by using standard methods with plates supplemented with a broad-spectrum β-lactamase (penicillin amido-beta-lactam hydrolase; Genzyme Diagnostics, Kent, England). Plates were inoculated in duplicate and were incubated either in a 10% CO_2 atmosphere or under anaerobic conditions. Samples were obtained at 1 and 24 h after the initiation of antibiotic therapy (CRO alone, NET alone, or a combination of CRO and NET).

Production of endocarditis and installation of the infusion pump device. Sterile aortic vegetations were produced in female Wistar rats (weight, 180 to 200 g) (13). Briefly, a polyethylene catheter was inserted across the aortic valve through the right carotid artery and was secured with a silk ligature. At the same time, a sterile silastic catheter (Dow Corning Corp., Midland, Mich.) was inserted through the jugular vein into the superior vena cava. The line was tunneled subcutaneously and was brought to the skin of the interscapular region. The external portion of the catheter was connected to a flow-through swivel, which permitted the animal to move in the cage (30), and then to the programmable infusion pump (Harward 44 programmable pump). The pump was set to deliver a volume of 0.2 ml of saline per h to keep the catheter open until the beginning of the antibiotic treatment. The control animals had no i.v. catheter.

Bacterial endocarditis was induced 24 h after catheterization by i.v. challenge of the animals with an inoculum of 1×10^7 to 2×10^7 CFU, which is a 10-fold higher inoculum than that necessary to induce endocarditis in 90% of the animals.

Treatment protocol. Antibiotic therapy was started 48 h after i.v. inoculation and was continued for 3 days. For animals infected with each strain, animals other than controls were randomly assigned to one of three treatment groups: (i) ceftriaxone alone, administered i.v. over 3 days by means of a programmable infusion pump which delivered drug at changing flow rates (required total dose for 24 h, 1.063 g/kg); (ii) NET alone, which was administered subcutaneously (s.c.) as a single dose of 18 mg/kg every 24 h for 3 days; and (iii) CRO administered i.v. over 3 days as described above plus NET as one daily dose administered s.c. for 3 days. The dosage of each antibiotic was chosen so as to result in levels in rat sera similar to those in human sera following the administration of 2 g of CRO and 5 mg of NET per kg, both administered i.v. as a single daily dose (22, 31). A group of untreated rats served as controls. Each experiment was repeated at least twice.

Evaluation of infection. Infection was evaluated as described previously (10). Briefly, control rats were sacrificed at the beginning of treatment. Treated rats that survived were killed 12 h after the end of CRO therapy and 36 h after the last NET dose. Aortic valves were excised, weighed, homogenized, serially diluted, and plated as described above. Colony counts were determined after 48 h of incubation at 37°C under aerobic and anaerobic conditions in order to eliminate the effects of possible residual NET in the valves. The effectiveness of therapy was determined by measuring the reduction in bacterial titers, expressed as $\log_{10}$ CFU per gram of aortic valves. The dilution technique permitted the detection of 10^2 CFU/g of aortic valves.

Statistical evaluation. The chi-square test with Yates' correction was used for proportional variables. Continuous variables were analyzed by one-way analysis of variance. The Student's t test was used to compare each group with the others. A P value of <0.05 was considered significant. For calculation of the continuous variables, a value of 10^2 CFU/g was assigned to sterile vegetations (limit of detection).

RESULTS

In vitro studies. The MICs of penicillin G and ceftriaxone are 0.032 and 0.064 μg/ml, respectively, for *Streptococcus sanguis*. *Streptococcus mitis* was considerably more resistant; MICs were 2.0 μg/ml for each agent. Both organisms were equally susceptible to NET (MIC, 8 μg/ml).

In vitro killing curves for CRO, NET, and their combination against *S. sanguis* or *S. mitis* are shown in Fig. 1A and B, respectively. A high inoculum of 10^8 CFU/ml was used in order to simulate the high bacterial densities in infected vegetations. Against *S. sanguis*, the combination of CRO at the concentration of the trough level in treated rats (15 mg/liter) plus NET at 10 mg/liter was bactericidal at 6 h, whereas CRO alone, even at the concentration of the peak level in treated rats (250 mg/liter), as well as netilmicin alone at 10 mg/liter produced no significant decrease in viable counts at this early time point. Against *S. mitis*, the combination sterilized the culture at 6 h, whereas the rate of killing by CRO and NET alone at this time was not significant.

Levels of antibiotics in serum. Using our programmable infusion pump system, we obtained levels of CRO in rat serum similar to those in human serum following an i.v. injection of 2 g (Fig. 2A). The concentration of 15.9 mg/liter in serum at 24 h represents about 200- and 8-fold the MICs for *S. sanguis* and *S. mitis*, respectively. The s.c. injection of 18 mg of NET per kg in rats resulted in a profile fairly similar to that obtained after a 30-min i.v. administration of 5 mg/kg in humans (Fig. 2B). Serum netilmicin levels were no longer detectable in rats at 8 h, whereas in humans, residual activity could still be measured at 24 h.

SBTs. For *S. sanguis*, the geometric mean SBTs at 1 and 24 h were 1:32 and 1:4 for CRO, 1:4 and 1:4 for NET, and >1:64 and 1:8 for CRO-NET, respectively. For *S. mitis*, the geometric mean SBTs at 1 and 24 h were 1:4 and >1:2 for CRO, 1:4 and >1:2 for NET, and 1:8 and >1:2 for CRO-NET, respectively. There was no difference between the results observed on the plates incubated in a 10% CO_2 atmosphere and those incubated anaerobically. Although the SBTs for rats receiving CRO-NET differed by only one tube dilution from the SBTs for those receiving CRO alone, this difference was repeatedly observed in all animals. Moreover, if the usual cutoff of 99.9% killing was increased to 99.99%, the SBTs with CRO alone were >1:2 at both 1 and 24 h for both strains compared with 1:64 and 1:8, respec-

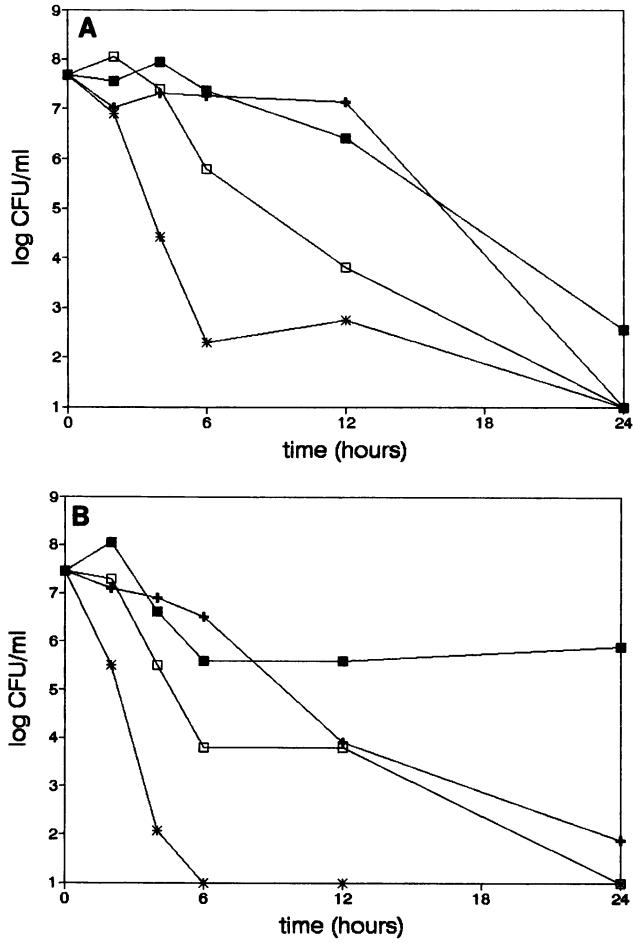

FIG. 1. In vitro killing curves for *S. sanguis* (A) and *S. mitis* (B) in the presence of CRO, NET, and their combination. (A) ■, control; +, CRO at 250 mg/liter; *, CRO at 15 mg/liter plus NET; □, NET at 10 mg/liter. (B) ■, control; +, CRO at 250 mg/liter; *, CRO at 15 mg/liter; □, NET at 10 mg/liter.

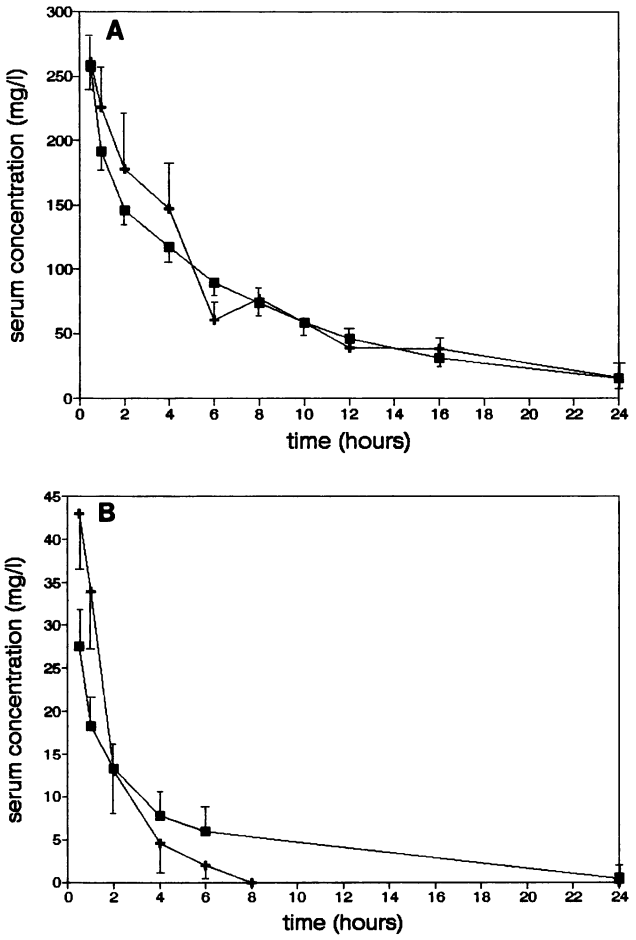

FIG. 2. Serum concentration-versus-time curves after i.v. administration of 2 g of CRO in humans (22) (A) and during simulation in rats by means of a programmable pump system after i.v. administration of 5 mg of NET per kg in humans (32) and 18 mg/kg s.c. in rats (B). Each symbol is the mean for nine animals. (A) ■, 2 g in humans; +, simulation in rats. (B) ■, 5 mg/kg administered i.v. in humans; +, simulation in rats.

tively, for *S. sanguis* and 1:8 and 1:2, respectively, for *S. mitis* with CRO-NET.

Treatment of established endocarditis. All control animals had extensive infection of the aortic valve by 48 h (Fig. 3A and B), with mean ± standard deviation (SD) bacterial counts of 9.8 ± 0.7 and 7.8 ± 1.2 $\log_{10}$ CFU/g for *S. sanguis* and *S. mitis*, respectively. For both test strains, the treatment results with NET alone did not reach significance (Fig. 3A and B). For *S. sanguis* (Fig. 3A), the regimen with CRO alone resulted in a significant diminution of the mean bacterial densities compared with those in control animals and in animals treated with NET alone (4.7 ± 1.9, 9.8 ± 1.3, and 8.3 ± 1.3 $\log_{10}$ CFU/g [mean ± SD], respectively; $P < 0.001$). A 3-day course of therapy with the combination of CRO plus one daily dose of NET (Fig. 3A) resulted in the elimination of infection in 9 of 20 animals (45%; $P < 0.006$ compared with controls) and a statistically significant reduction in the mean bacterial counts in vegetations, with values being 2 to 6 $\log_{10}$ units lower than those in the other groups ($P < 0.001$). In the case of *S. mitis* (Fig. 3B), treatment with CRO alone was ineffective, whereas the combination produced a statistically significant mean reduction in counts compared with those in animals treated with CRO alone and control animals (3.9 ± 1.9, 6.8 ± 2.2, and 7.8 ± 1.2 $\log_{10}$ CFU/g [mean ± SD], respectively; $P < 0.001$). In 3 of 10 animals (30%; P, not significant), the infection was below the detection limit by the time of sacrifice (Fig. 3B).

DISCUSSION

In the experiments described here, we explored whether CRO in combination with NET, using once-daily administration in rats, which simulated profiles of drug in human serum, was more effective than either agent alone in the treatment of experimental endocarditis caused by beta-lactam-susceptible and -resistant viridans group streptococci. In previous experiments, multiple doses of CRO and one dose of NET have been shown to be as effective as multiple doses of CRO and three divided doses of NET and more effective than CRO alone (10). However, because of the short half-life of CRO in rats, this drug had to be administered three times a day, resulting in periods of subinhibitory concentrations in serum before the administration of the next dose (10). In the experiments described here, we used a programmable pump system which delivered drug

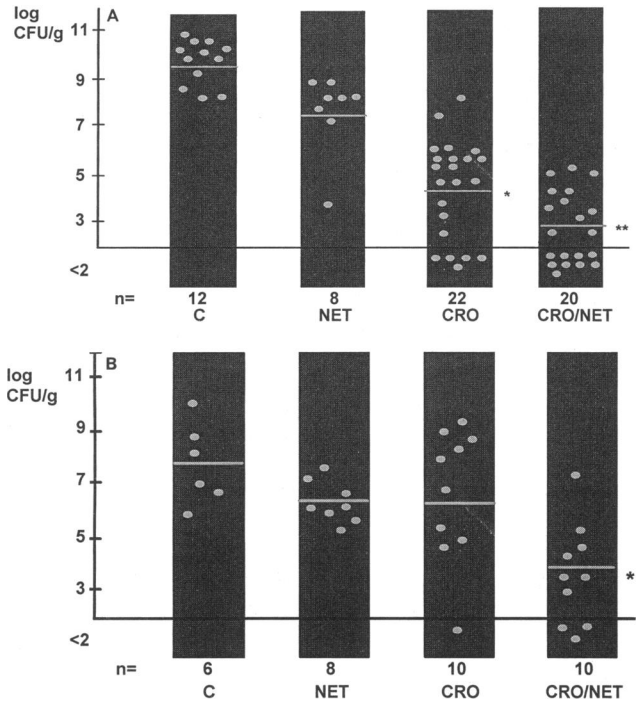

FIG. 3. Results of therapy after treating *S. sanguis*-infected (A) and *S. mitis*-infected (B) rats for 3 days with CRO i.v. (simulated levels in human serum after the administration of 2 g i.v.), NET administered s.c. once a day (18 mg/kg), or the combination. C, controls. (A) *, $P < 0.001$ compared with controls and rats treated with NET; **, $P < 0.001$ between rats treated with CRO and CRO-NET. (B) *, $P < 0.05$ compared with the other groups.

at changing flow rates during the whole treatment period, thus permitting close simulation in rats of the profile in human serum after i.v. injection of 2 g of CRO (22). Serum ceftriaxone levels stayed above the MICs for both test strains during the whole treatment period, a fact which might be important in view of the controversial presence of a postantibiotic effect of beta-lactam antibiotics with gram-positive cocci in the absence of local immunological defense mechanisms (32).

Under the experimental conditions of the present study, CRO alone showed efficacy against the ceftriaxone-susceptible *S. sanguis* strain but not against the ceftriaxone-resistant *S. mitis* strain. This confirms the results obtained in the same model but with conventional s.c. injections of the drug (10). The reason for this difference in efficacy of CRO against the two strains is not clear, since the levels of CRO in serum were well above the MICs for both strains and there was no difference between the two strains in the kinetics of killing in vitro. A nonhomogeneous diffusion of CRO in cardiac vegetations has been documented (5) and may have resulted in insufficient local levels of CRO with regard to the MIC of the CRO-resistant strain. However, when CRO was used in combination with NET, a synergistic activity was observed against both strains. In vitro, this synergistic activity was particularly obvious during the first 6 h of incubation. In animals with *S. sanguis* endocarditis, the combination of CRO plus one daily dose of NET was significantly better than CRO or NET alone in terms of the number of animals cleared of infection at the time of sacrifice and of the reduction in bacterial counts in infected vegetations. In animals with *S. mitis* endocarditis, the combination was less effective than it was against *S. sanguis*, but it still resulted in a significant reduction in the bacterial densities in vegetations compared with those in the vegetations of control rats and rats treated with either drug alone. The synergistic activity of the combination was achieved despite the low degree of susceptibility of both streptococci to aminoglycosides and despite a short period of bacterial exposure to NET, since the levels of NET in serum were detectable only during the first 8 h after injection. Thus, our data confirm and extend the results obtained in previous experiments, but in those experiments, CRO levels in human serum were not as closely simulated at they were in the present study (10). The data are also consistent with recent observations showing the improved antibacterial activity of penicillin in combination with one daily dose of an aminoglycoside in the treatment of experimental streptococcal but nonenterococcal endocarditis (11, 25). The precise mechanism of this in vivo synergism remains to be elucidated. It may be due to a genuine bactericidal synergy between the two drugs, as suggested by the in vitro killing curves presented here and by others (7). It may also be due to an important early bactericidal contribution of the aminoglycoside component (27) favored by the homogeneous penetration of aminoglycosides into vegetations, with local concentrations being rapidly similar to concentrations in plasma (4). To our knowledge, the postantibiotic effect of an aminoglycoside alone or in combination with a beta-lactam antibiotic against viridans group streptococci has never been studied. In vitro studies with *Enterococcus faecalis* have suggested that a postantibiotic effect is present with penicillin plus gentamicin (14). However, such an effect was not observed in vivo when only one daily dose of gentamicin was added to penicillin in experimental left-sided endocarditis (14). These results are in contrast to the results of the present study with viridans group streptococci, in which one daily dose of an aminoglycoside used in combination with CRO was able to produce an in vivo synergistic effect. The reason for the discrepancy might rely on differences in the mechanisms of action of aminoglycosides against enterococci or viridans group streptococci. With enterococci, it has been shown that penicillin enhances the uptake of aminoglycosides (20, 21) and that a 24-h exposure is necessary in order to obtain a synergistic effect with penicillin (6). In contrast, aminoglycoside uptake by viridans group streptococci appears to remain unchanged in the presence or absence of penicillin (20). Thus, the synergistic activity between an aminoglycoside and CRO against viridans group streptococci is probably the result of another mechanism that has yet to be determined. It appears that it does not require the constant simultaneous presence of an aminoglycoside and CRO.

It has been demonstrated that one daily dose of ceftriaxone, 2 g administered i.v. or intramuscularly for 4 weeks, is a safe and effective treatment for streptococcal endocarditis in humans, with results being comparable to those obtained by conventional penicillin therapy with or without an aminoglycoside (9). Our present data show an enhanced bactericidal activity when CRO and NET are used in combination in a way that simulates once-daily administration of both drugs. The simulation of the CRO levels in human serum allowed us to compare regimens with or without one daily dose of NET in a way which should be very similar to the situation in humans, all the more so because the levels of protein binding of both antibiotics are comparable in rats and humans (3). Thus, the results presented here provide a rationale for clinical trials of this once-a-day dosing regimen

in the treatment of streptococcal but nonenterococcal endocarditis. On the basis of the good results obtained with CRO as monotherapy (9, 28) and the demonstrated in vivo synergism with one daily dose of NET in the experiments described here, a short course of therapy (e.g., 2 weeks) could be envisioned, with the associated advantages of cost and convenience.

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