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## Pharmacodynamics of doxycycline

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Accepted 20 December 1999

Doxycycline is a long-acting/second generation tetracycline antibiotic, and currently is one of the most commonly prescribed antibiotics in the world, used to treat a wide variety of infectious agents including susceptible intracellular/zoonotic pathogens [1–6].

Because doxycycline was introduced prior to an appreciation of pharmacodynamic concepts, the optimal dosing regimen has not been determined. This in vitro study was conducted to determine the optimal dosing regimen for doxycycline based upon pharmacodynamic data.

Doxycycline was tested against selected Gram-positive pathogens, e.g. *Staphylococcus aureus* and *Streptococcus pneumoniae* and Gram-negative pathogens, e.g. *P. multocida* and *E. coli*. Time-kill studies were performed with each of these organisms at various serum concentrations representing two, four, eight, and 16 times the MIC of each test organism. The growth of the organisms was assessed by colony counts at various time points during a 24 h period to determine if doxycycline kills susceptible organisms by concentration or time-dependent kinetics. Studies were also carried to determine the PAE of doxycycline.

*Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pasteurella multocida*, and *Streptococcus pneumoniae* (ATCC 49619) were used as the test bacteria.

Inocula were prepared using Mueller–Hinton broth for *E. coli*, and Mueller Hinton broth with 1% Fildes and 20 mM NaCl for *S. aureus* and *P. multocida*, and cation-adjusted Mueller–Hinton broth (CAMH) with 5% lysed horse blood (LHB) for *S. pneumoniae*. The inocula were incubated at 37 °C with shaking until turbid (~3–4 h). The turbidity was adjusted to 0.5 McFarland standard (representing a concentration of  $\sim 10^8$  CFU/mL). A portion of the 0.5 McFarland standard was diluted 1:100 ( $\sim 10^6$  CFU/mL) with broth. One milliliter of this dilution was added to each cuvette well containing varying concentrations of doxycycline (0.02–1.6 mg/L). One cuvette well containing no antibiotic was used as a control for organism viability. The cuvettes were placed in an agitator and incubated at 37 °C for 24 h. The lowest concentration of doxycycline that inhibited visible growth for 24 h was recorded as the minimum inhibitory concentration (MIC) of the organism. The MIC for *E. coli* (ATCC 25922) was 1.5 mg/L, for *Staphylococcus aureus* (25923) 0.28 mg/L, for *P. multocida* 0.09 mg/L, and for *Streptococcus pneumoniae* (ATCC 49619) 0.16 mg/L [7,8].

The post-antibiotic effect (PAE) was determined for doxycycline for each of the organisms mentioned above by the method described by Craig and Gudmundsson [9]. An overnight growth of *E. coli*, *Staphylococcus aureus*, *P. multocida*, and *Streptococcus pneumoniae* was diluted into fresh broth, appropriate for each organism, to  $10^6$  CFU/mL and then incubated on a shaker at 37 °C for 3–4 h until logarithmic growth phase was achieved. At the end of this period, the inoculum size was determined and each tube containing the test organisms was then exposed to doxycycline at twice the MIC, four times the MIC, and 16 times the MIC for 1 h at 37 °C in a shaker. A suspension of each organism that was not exposed to antibiotics was used as control and was subjected to the same procedures described. Antibiotics were introduced at time zero of antimicrobial exposure. At the end of the exposure period the supernatant was decanted after centrifugation at 1200g for 10 min and the pellet was re-suspended in fresh broth. The same procedure was repeated and after removal all tubes were again incubated at 37 °C.

Counts of CFU/mL were performed on all cultures at time zero, before and after washing and every hour thereafter until 6 h and the counts of CFU/mL were graphed. The PAE was determined by the following equation:  $PAE = T - C$  where  $T$  is the time required for the counts of CFU/mL in the test culture to increased one  $1 \log_{10}$  above the count observed immediately after antibiotic removal and  $C$  is the time required for the count of CFU/mL in an untreated control culture to increase  $1 \log_{10}$  above the count observed immediately after completion of the same procedure used on the test culture for antibiotic removal [9,10]

The data indicate that at low concentrations of doxycycline, i.e. at 2 to 4 times the MIC, inhibition of the organisms tested occurs in a time-dependent fashion. However, at higher concentrations, i.e. 8 to 16 times the MICs of the organisms, doxycycline exhibits concentration-dependent killing (Figure 1).

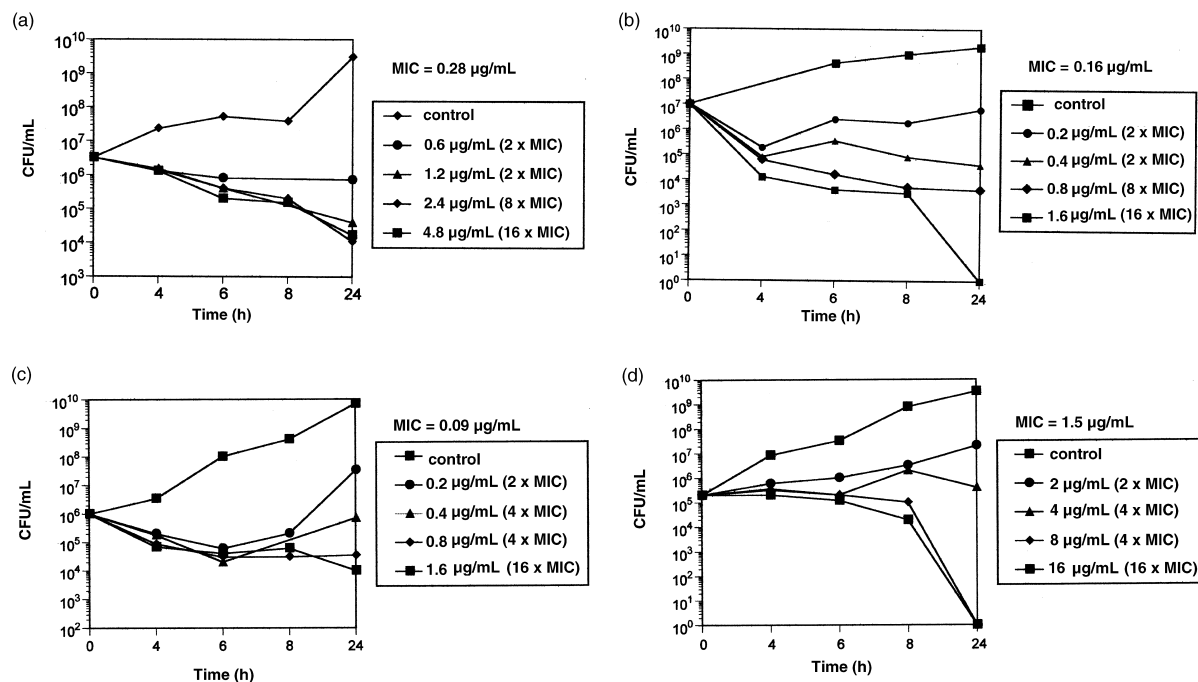
The PAE of doxycycline was demonstrated for the Gram-positive and Gram-negative organisms tested. Doxycycline's post-antibiotic effect is concentration dependent. The post-antibiotic effect for the Gram-positive and Gram-negative organisms tested is approximately equal (Figure 2).

For serious infections such as Legionnaire's disease, doxycycline therapy should be initiated with a 72 h loading regimen because of its high lipid solubility. A dose of 200 mg intravenously every 12 h is the preferred regimen to provide rapid and high serum/tissue concentration of doxycycline. If doxycycline is administered as a 100 mg intravenous dose every 12 h then 4–5 days of therapy are needed before the patient is fully saturated and a therapeutic effect can be expected. As with other antibiotics, a period of four to five serum half-lives is required before steady-state kinetics are achieved. Since the serum half-life ( $t_{1/2}$ ) of doxycycline is 22 h, it should be apparent that an initial 72 h loading regimen is required if a rapid therapeutic effect is desired in seriously ill patients [1,6]

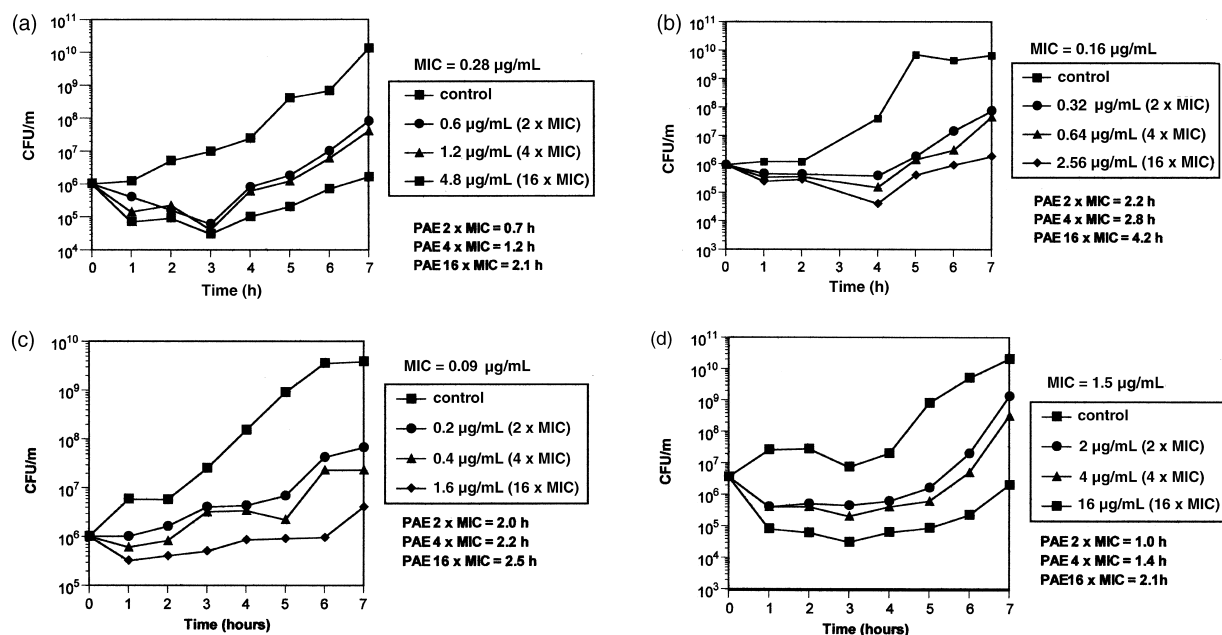
The post-antibiotic effect of doxycycline differs from other tetracyclines, but is not clinically relevant since therapeutic serum levels are present over the entire duration of the dosing period [7,12,13]. Our data suggest that doxycycline may be administered on a 12 h or 24 h dosing basis when a 400 mg (intravenous/oral) daily dose is used. Clinically, with moderate to severe infections, it is important to use a doxycycline loading regimen to rapidly achieve high blood and tissue concentrations. Non-loading dose regimens should be used for patients who are not seriously ill. In terms of pharmacoeconomics, it is most cost effective to administer doxycycline intravenously on a once-daily basis because there is a hospital charge for each intravenously administered dose. A 24 h-dosing regimen eliminates the additional administration cost of a 12 h-dosing regimen.

Doxycycline is the most commonly used tetracycline for the treatment of a wide variety of infectious diseases. Introduced decades before pharmacodynamic considerations were appreciated, the dosing of doxycycline has been based on pharmacokinetic parameters. This is the first pharmacodynamic study of doxycycline.

Doxycycline exhibits time-dependent killing at two to four times the MIC, but dose-dependent killing at eight to 16 times the MIC of the organisms tested. Optimal dose-dependent killing may be achieved using 200 mg (intravenous/oral) every 12 h or 400 mg (intravenous/oral) every 24 h. In mild to moderate infections, time-dependent killing is sufficiently effective and doxycycline may be dosed as 100 mg (intravenous/oral) every 12 h or 200 mg (intravenous/oral) every 24 h.



**Figure 1** Doxycycline kill curves: (a) *Staphylococcus aureus*; (b) *Streptococcus pneumoniae*; (c) *Pasteurella multocida*; (d) *Escherichia coli*.



**Figure 2** Doxycycline post-antibiotic effects (PAEs) (a) *Staphylococcus aureus*; (b) *Streptococcus pneumoniae*; (c) *Pasteurella maltophilia*; (d) *Escherichia coli*.

Doxycycline exerts a PAE which is dose dependent and varies between 2.1 and 4.2 h.

In conclusion, doxycycline kills by time-dependent kinetics at low serum concentrations, but optimally at high serum concentrations by concentration-dependent kinetics. Non-criti-

cally ill patients may be treated effectively using either 100 mg (intravenous/oral) every 12 h or 200 mg (intravenous/oral) every 24 h regimen. Based on our in vitro pharmacodynamic data, a high-dose doxycycline regimen, i.e. 200 mg every 12 h or 400 mg every 24 h regimen should provide for optimal con-

centration-dependent killing. The PAE of doxycycline is clinically unimportant since adequate serum concentrations are maintained for the duration of the dosing interval if given on a 12 h or 24 h basis.

## ACKNOWLEDGMENTS

The authors wish to thank Nazli Chaudhry for technical assistance.

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## High rate of resistance to nalidixic acid in *Salmonella enterica*: its role as a marker of resistance to fluoroquinolones

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Accepted 21 January 2000

Gastroenteritis caused by nontyphoidal salmonellae is a common entity, usually self-limiting and not requiring antibiotic treatment. However, in immunocompromised patients or in invasive infections rapid and efficacious treatment is required. The increase in resistance displayed by this pathogen to the antibiotics classically used in salmonellosis (ampicillin, cotrimoxazol, chloramphenicol, etc.) has caused ciprofloxacin to be considered the antibiotic of choice in the treatment of invasive salmonellosis.

Resistance to fluoroquinolones in non-typhoidal salmonellae is infrequent, with very few cases reported in the literature [1–5]. However, there are reports of clinical failures in the treatment with ciprofloxacin of strains that, although susceptible, have increased MICs for this antibiotic [6,7].

The National Committee for Clinical Laboratory Standards (NCCLS) [8] considers as susceptible to ciprofloxacin all those strains of *Salmonella enterica* which have MICs  $\leq 1$  mg/L. According to this criterion, ciprofloxacin resistance in the various species of *S. enterica* is exceptional [1–4]. However, there are authors who recommend adjusting the cutoff points to consider susceptible only those strains with MICs  $\leq 0.125$  mg/L [1,9].

Resistance to nalidixic acid in *Salmonella enterica*, although of slight clinical importance given its infrequent use in the treatment of salmonellosis, may serve as the marker of a future increase in resistance to fluoroquinolones, as has happened with *Escherichia coli* [10].

In this work we studied the resistance patterns to nalidixic