Animal Models for PK/PD Studies

William A. Craig, M.D.

University of Wisconsin

USA

Animal Versus In-Vitro Models

- Infection of specific body sites
- Interaction of multiple host factors
- Growth environment less favorable than broth
- Faster drug elimination than in humans, but can still simulate human pharmacokinetics by inducing renal impairment or computer-controlled drug administration

Use of Animal Models in PK/PD Evaluation of Anti-Infective Agents

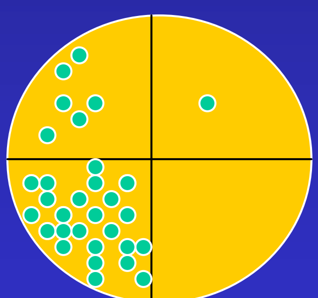
- Describing the time-course of antimicrobial activity at sites of infection

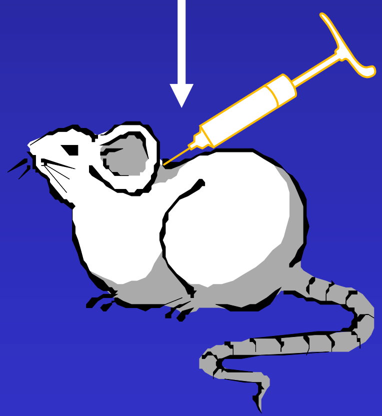
  - pattern of killing (concentration or time-dependent)
  - presence or absence of persistent effects

Neutropenic Mouse Thigh-Infection Model

1. Neutropenia induced by 2 injections of cyclophosphamide on days -4 and -1

2. Bacteria injected into thighs on day 0 (10^{6-7})

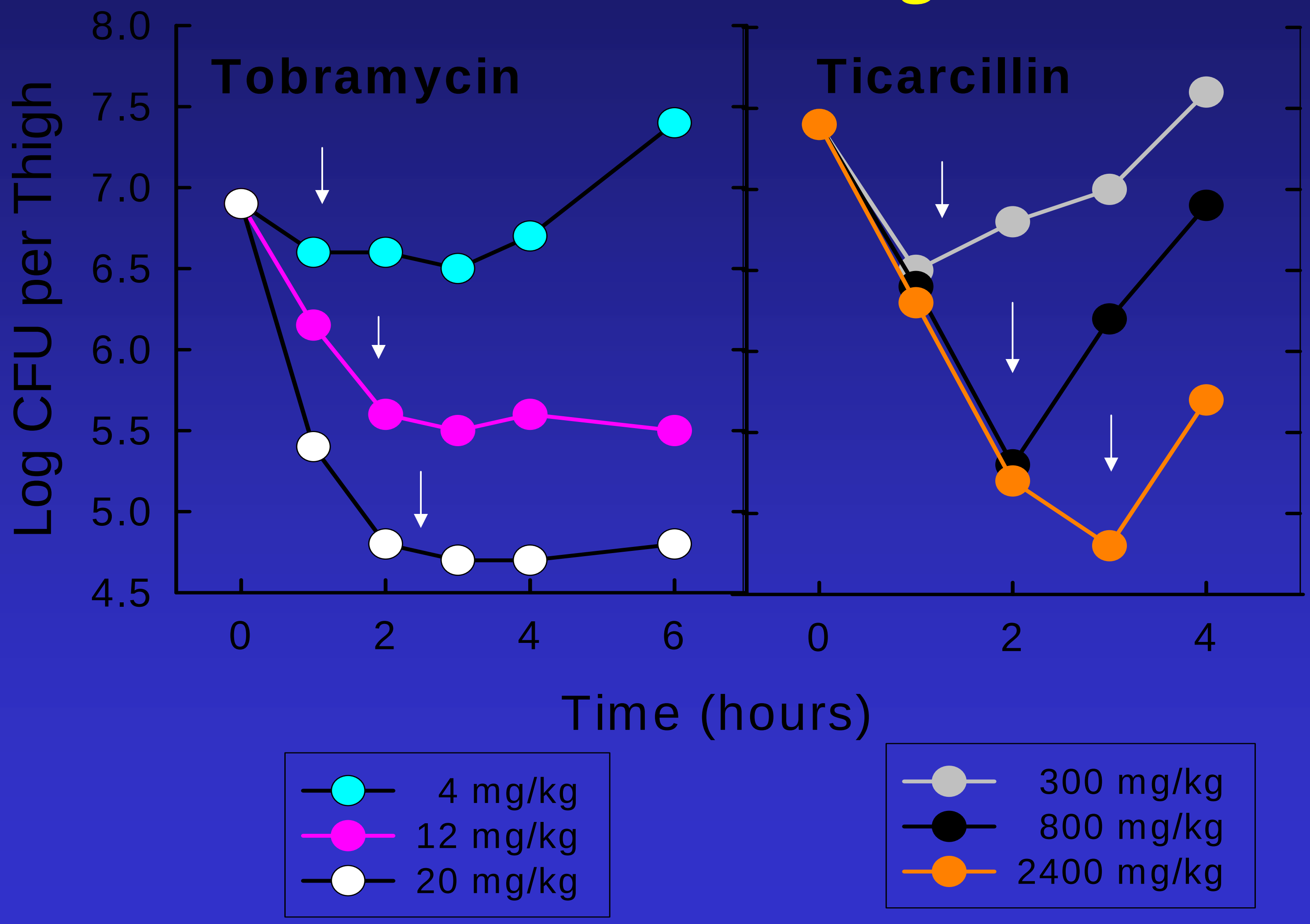




4. Thighs removed, homogenized, serially diluted and plated for CFU determinations

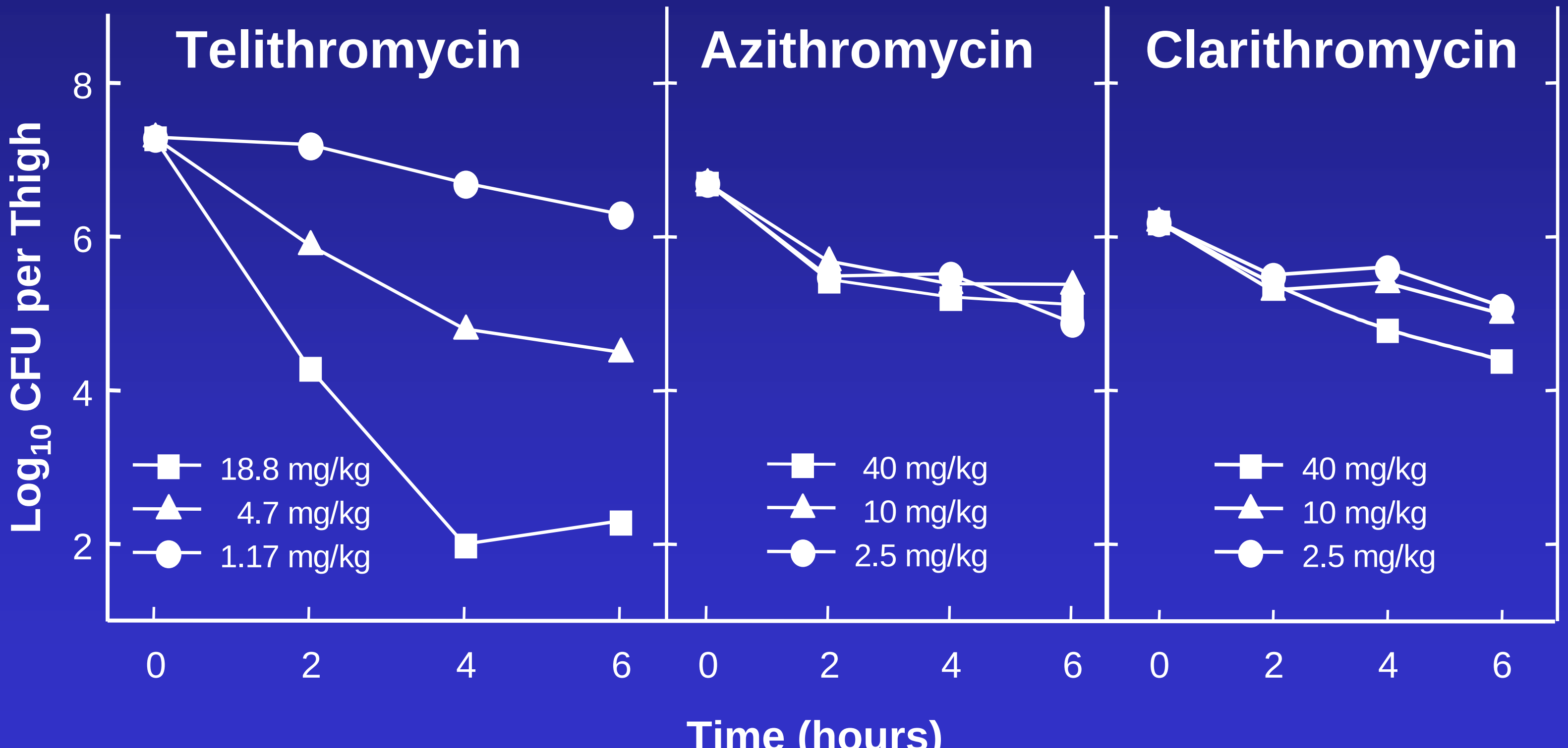
3. Treatment (usually given SQ) started 2 hr after infection and continued for 1-5 days

Time Course of Antibacterial Activity of Tobramycin and Ticarcillin Against *Pseudomonas aeruginosa*



Craig and Gudmundsson 1996

Effect of Increasing Concentrations on Killing of Pneumococci in Thighs of Neutropenic Mice



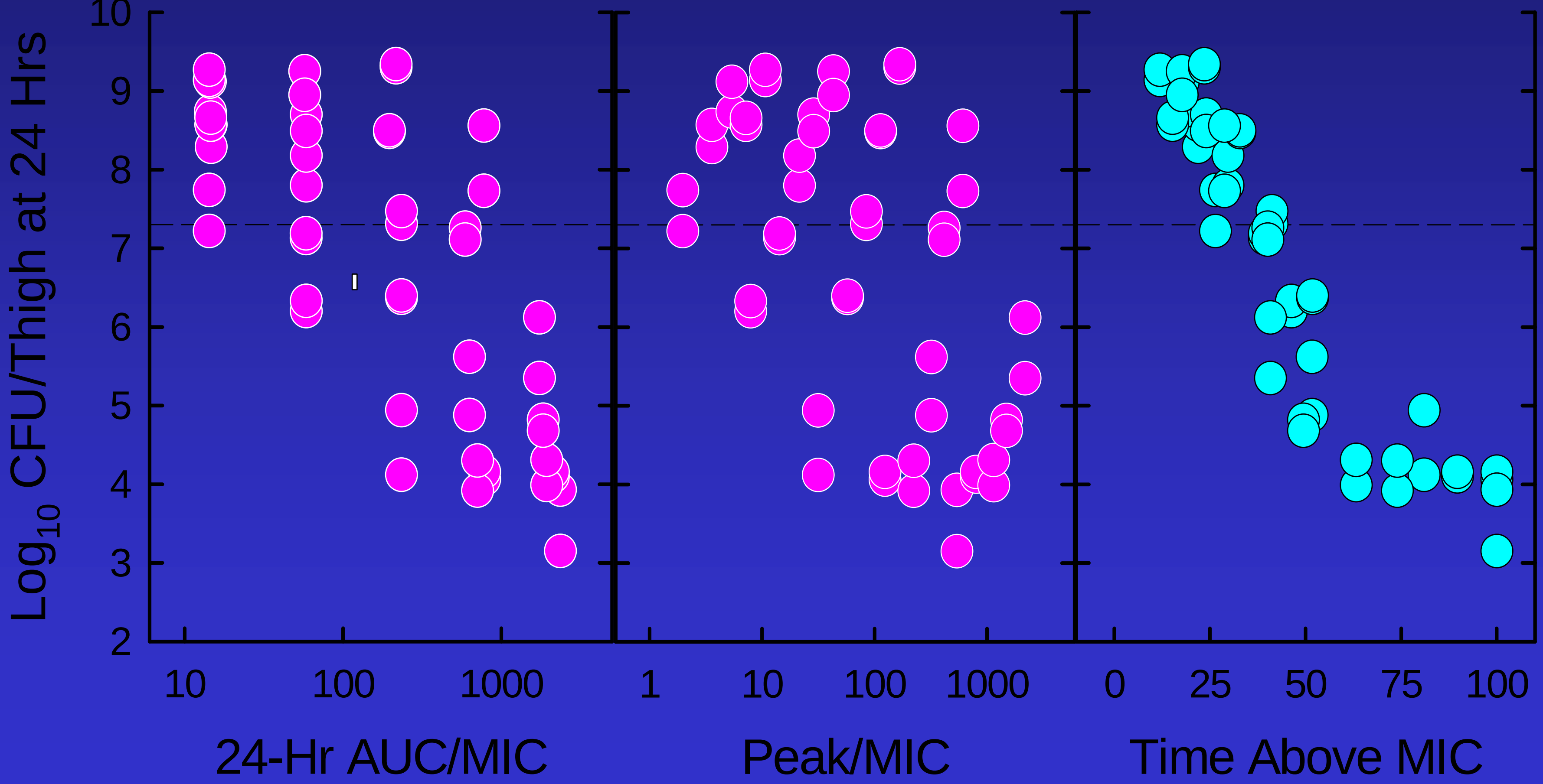
CFU = colony-forming unit.

Craig WA, et al. 40th ICAAC Toronto, Ontario, September 17-20, 2000.

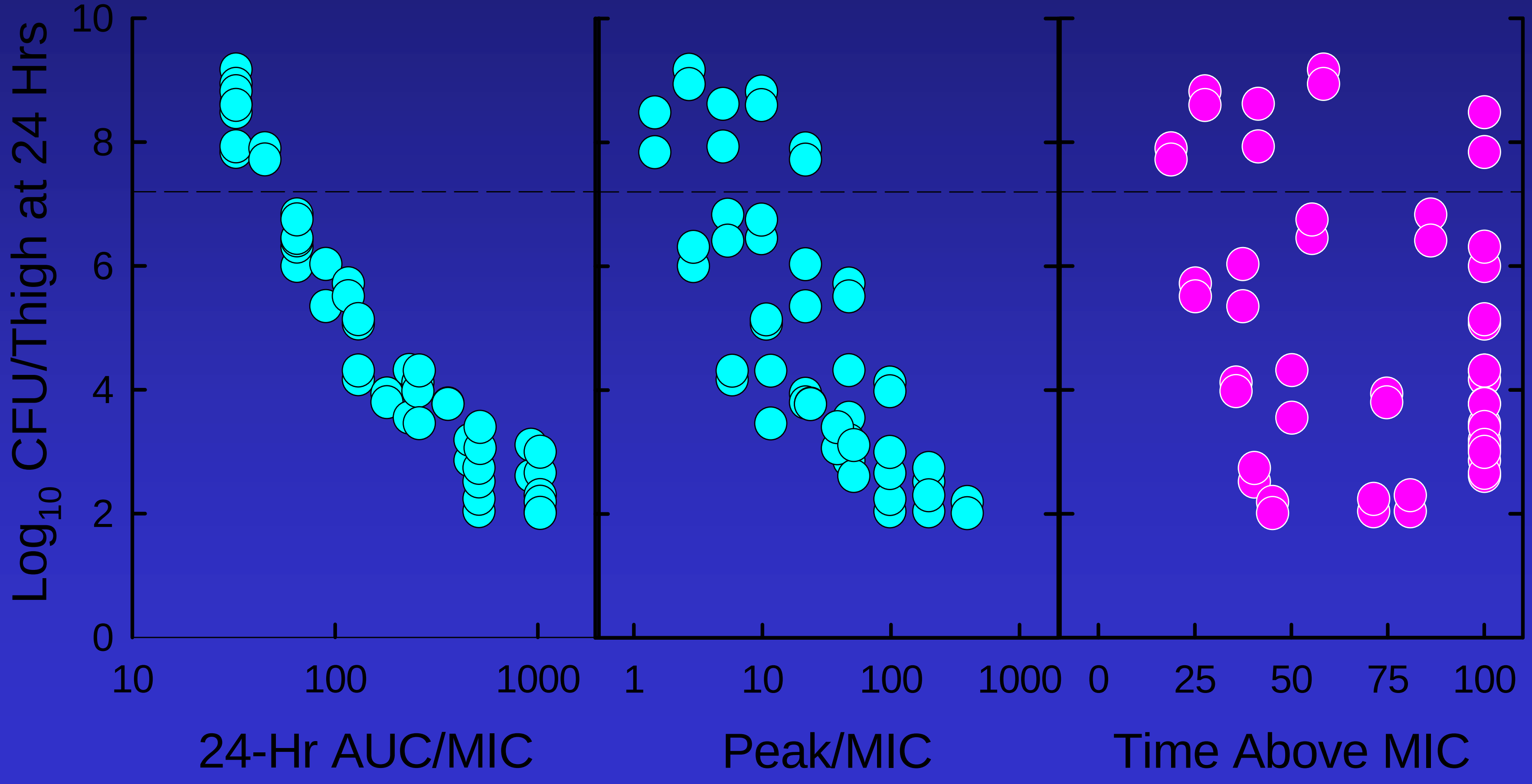
Use of Animal and In Vitro Models in PK/PD Evaluation of Anti-Infective Agents

- Describing the time-course of antimicrobial activity at sites of infection
- Identifying PK/PD indices correlating with efficacy (Peak/MIC, AUC/MIC, Time>MIC)
  - dose-fractionation studies to reduce inter-dependence among the various indices

Relationship Between PK/PD Indices and Efficacy for Ceftazidime against *Klebsiella pneumoniae* in a Murine Pneumonia Model



Correlation of PK/PD Indices with Efficacy of Levofloxacin against *Streptococcus pneumoniae* in Thighs of Neutropenic Mice



Use of Animal and In Vitro Models in PK/PD Evaluation of Anti-Infective Agents

- Describing the time-course of antimicrobial activity at sites of infection
- Identifying PK/PD indices correlating with efficacy (Peak/MIC, AUC/MIC, Time>MIC)
- Determining magnitudes of the PK/PD indice required for efficacy and identifying factors that affect the magnitude
  - cfu changes (short durations of therapy) vs survival (longer courses)
  - multiple factors can alter magnitudes
  - predicting efficacy in humans

PK/PD Magnitude Variables

- Animal
- Antibiotic Class
- Protein binding
- Organism/strain
- Presence of resistance mechanism(s)
- Immune status (normal vs neutropenic)
- Infection site
- Location of organisms (intracellular vs extracellular)
- Kinetics and shape of concentration-time curve
- Duration of therapy
- Time survival is determined

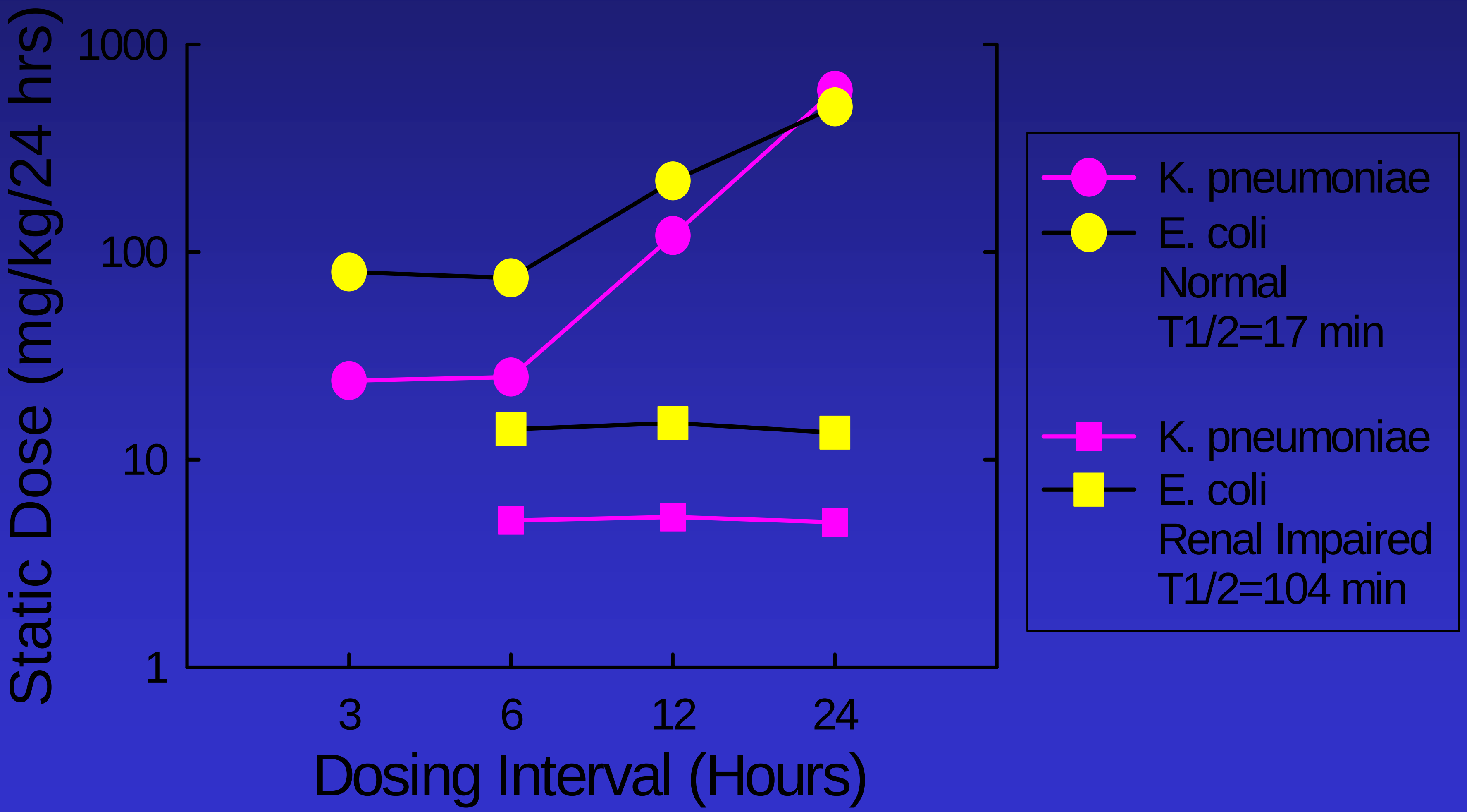
Half-Lives in Mice and Humans

	Half-life in Minutes	
Drug	Mice	Humans
Penicillin G	5	30
Imipenem	8	60
Cefazolin	15	108
Amikacin	17	150
Ciprofloxacin	32	240
Erythromycin	35	180
Minocycline	120	1080

Efficacy of Once-Daily Dosing of Amikacin against *K. pneumoniae* (MIC=0.5 mg/L) in Neutropenic Mice with Murine and Human Pharmacokinetics

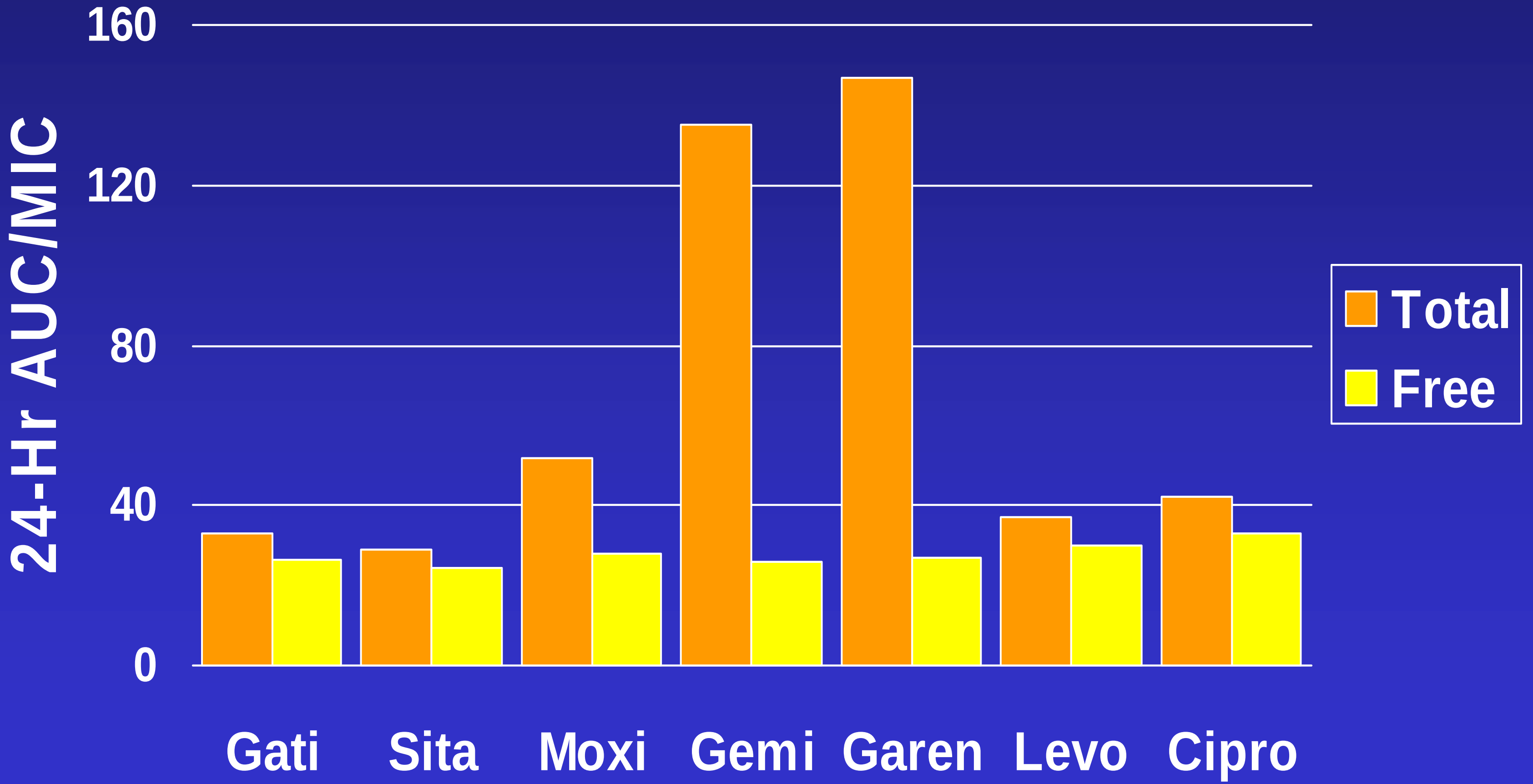
	Murine	Human
Dose (mg/kg)	15	15
Peak (mg/L)	16	46
T1/2 (min)	17	104
AUC	14	128
T>MIC (hr)	1.9	11.7
PAE (hr)	4.1	12.3
Efficacy	NO	YES

Impact of Dosing Interval on Static Dose for Amikacin against *K. pneumoniae* and *E. coli* in Mice with Normal and Impaired Renal Function



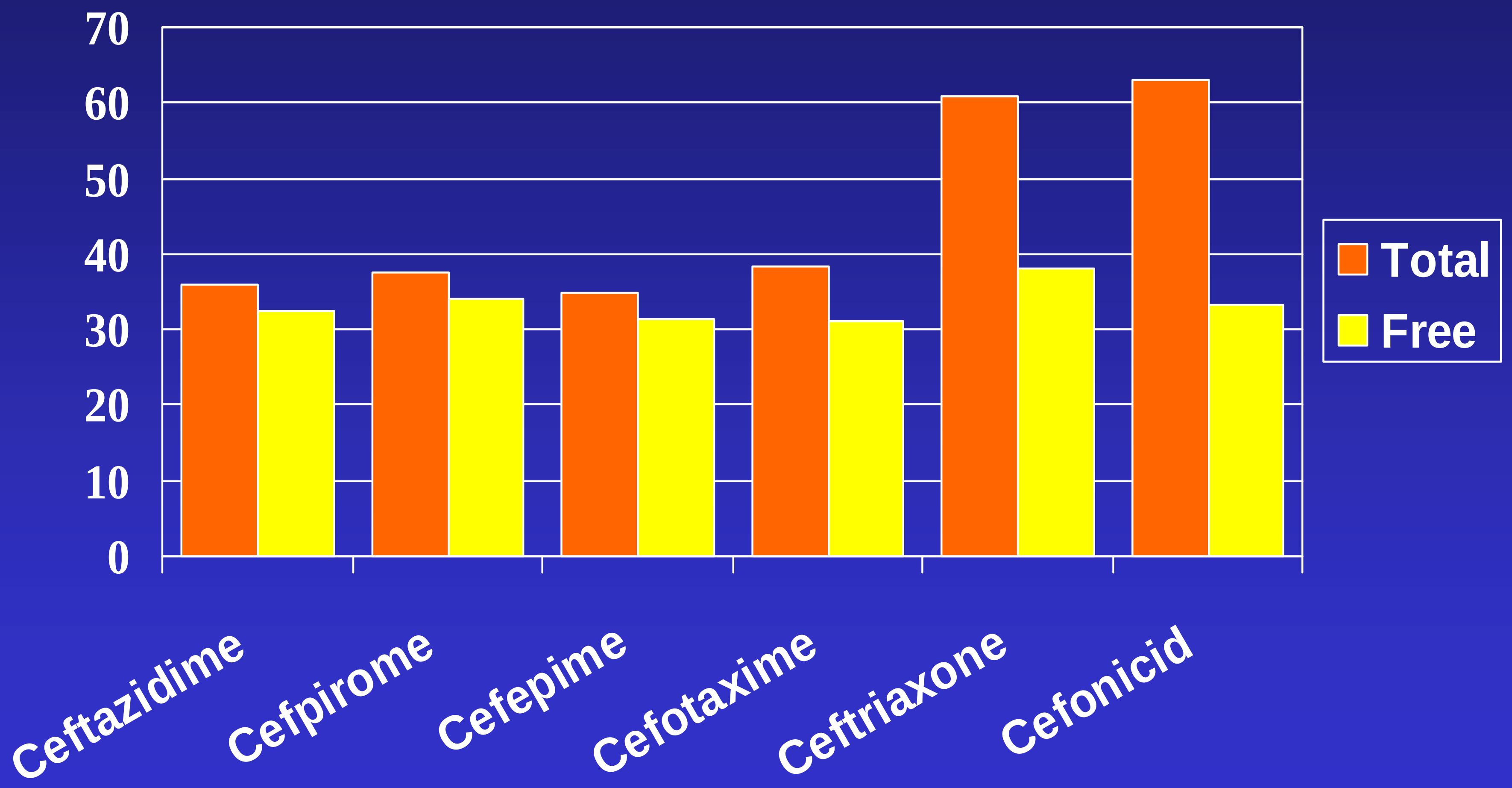
Reddington and Craig, JAC 1995

24-Hr AUC/MIC with Total and Free Drug for the Static Dose of Different Fluoroquinolones with *S. pneumoniae* ATCC 10813



Andes & Craig 40th and 41st ICAAC, 2000 and 2001

Time Above MIC for Total and Free Drug for the Static Dose of Different Cephalosporins with *Klebsiella pneumoniae* ATCC 43816



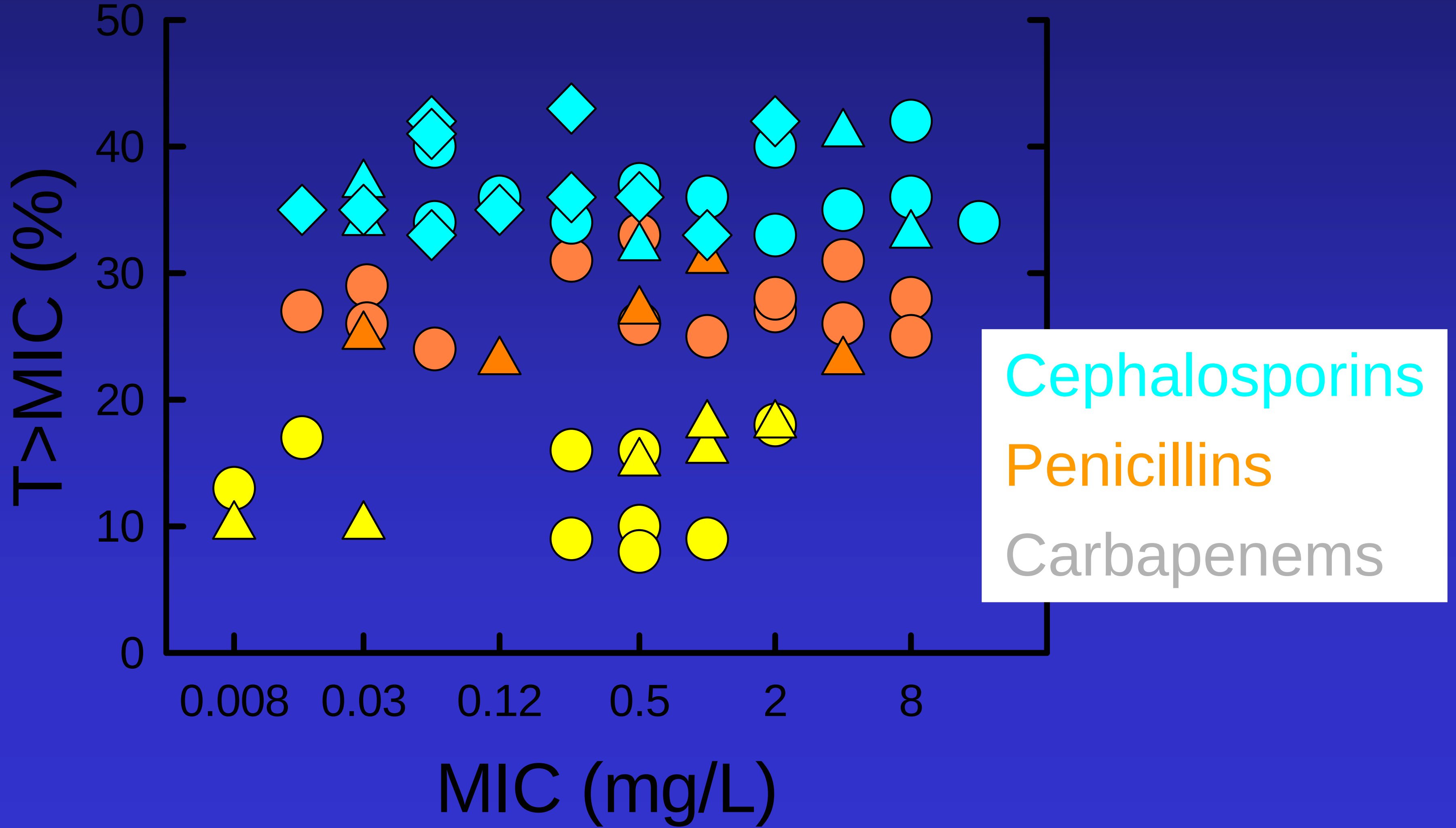
Craig Infect Dis Clin N Amer 2003

Time Above MIC Required for a Static Effect with 4 Cephalosporins

	Time Above MIC (% of Dosing Interval)		
Drug	GNB	S. pneumoniae	S.aureus
Ceftazidime	36 (27-42)	39 (35-42)	22 (19-24)
Cefpirome	35 (29-40)	37 (33-39)	22 (20-25)
Cefotaxime	38 (36-40)	38 (36-40)	24 (20-28)
Ceftriaxone	38 (34-42)	39 (37-41)	24 (21-27)

Craig Diagn Microbiol Infect Dis 22:89, 1995

T>MIC for Cephalosporins, Penicillins and Carbapenems with Susceptible and Non-Susceptible Strains of *S. pneumoniae*

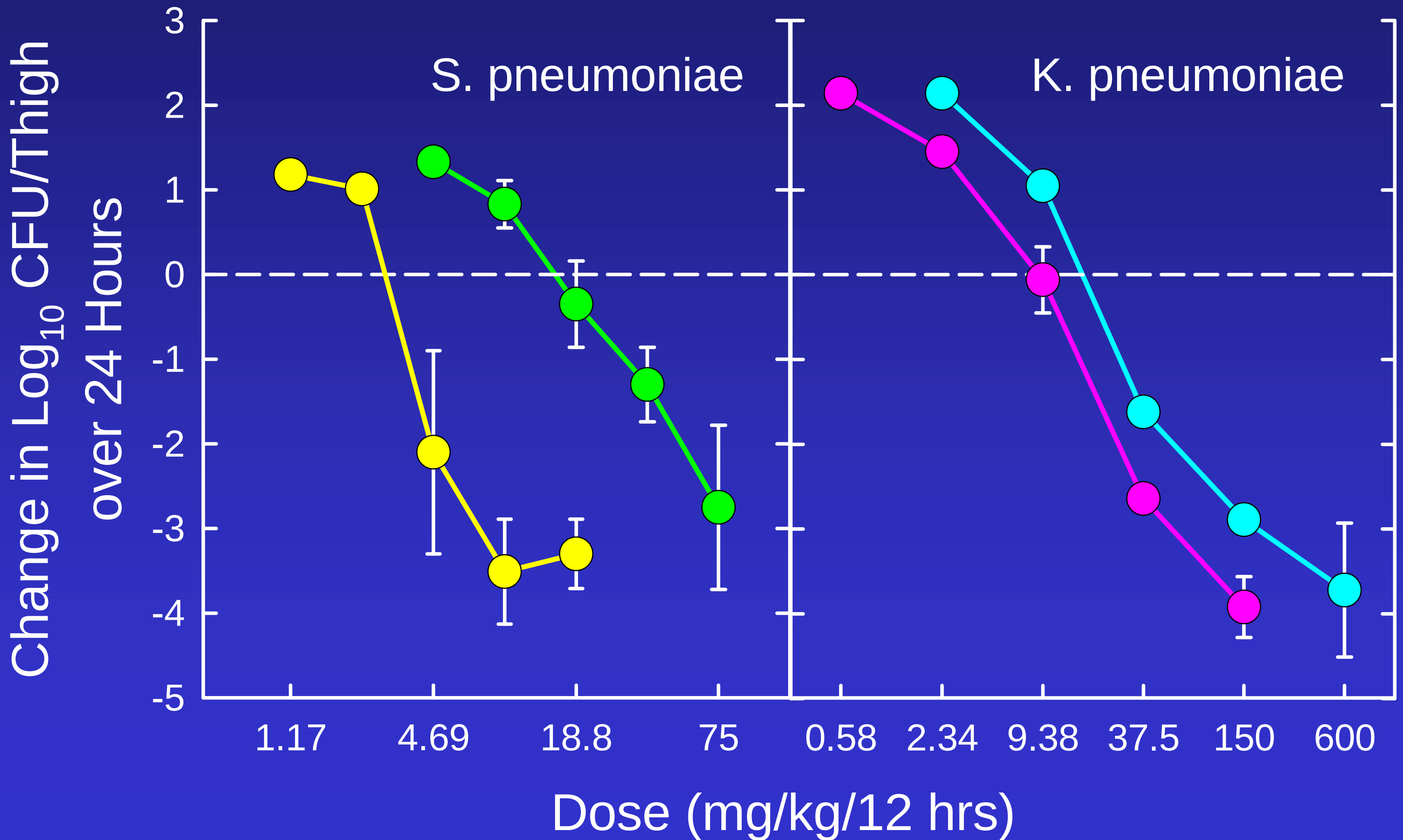


Magnitude of PK/PD Indice for Free Drug Required for Static Dose of Gemifloxacin Against *S. pneumoniae* in Thighs of Neutropenic Mice

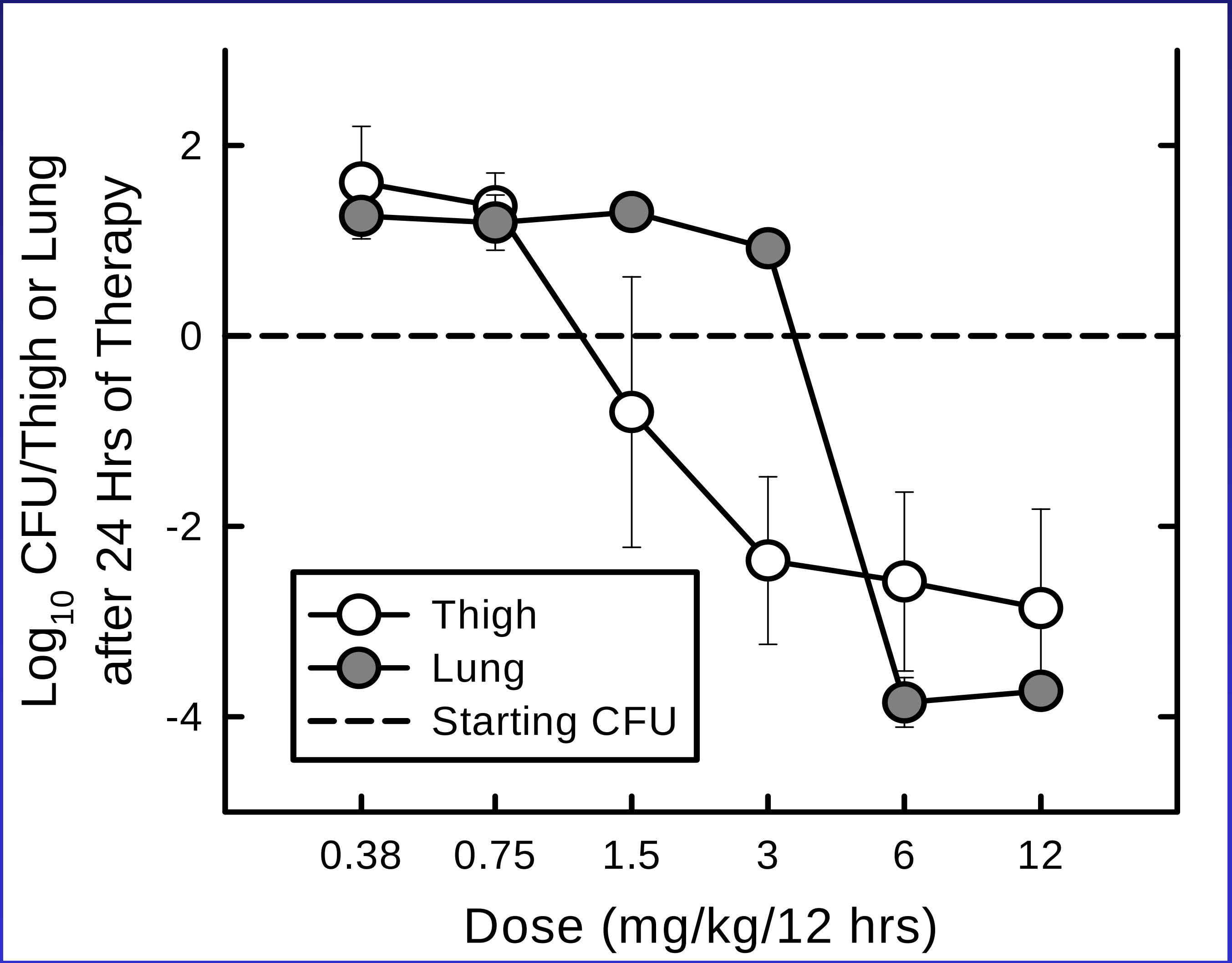
Drug	MIC	Mean AUC/MIC
Susceptible	0.015	28.3
Gyrase, PAR C or E	0.03-0.5	31.3
Efflux	0.12-0.25	5.8

Craig & Andes 2005 ECCMID

In Vivo Activity of Moxifloxacin against *S. pneumoniae* and *K. pneumoniae* in Thighs of Normal and Neutropenic Mice



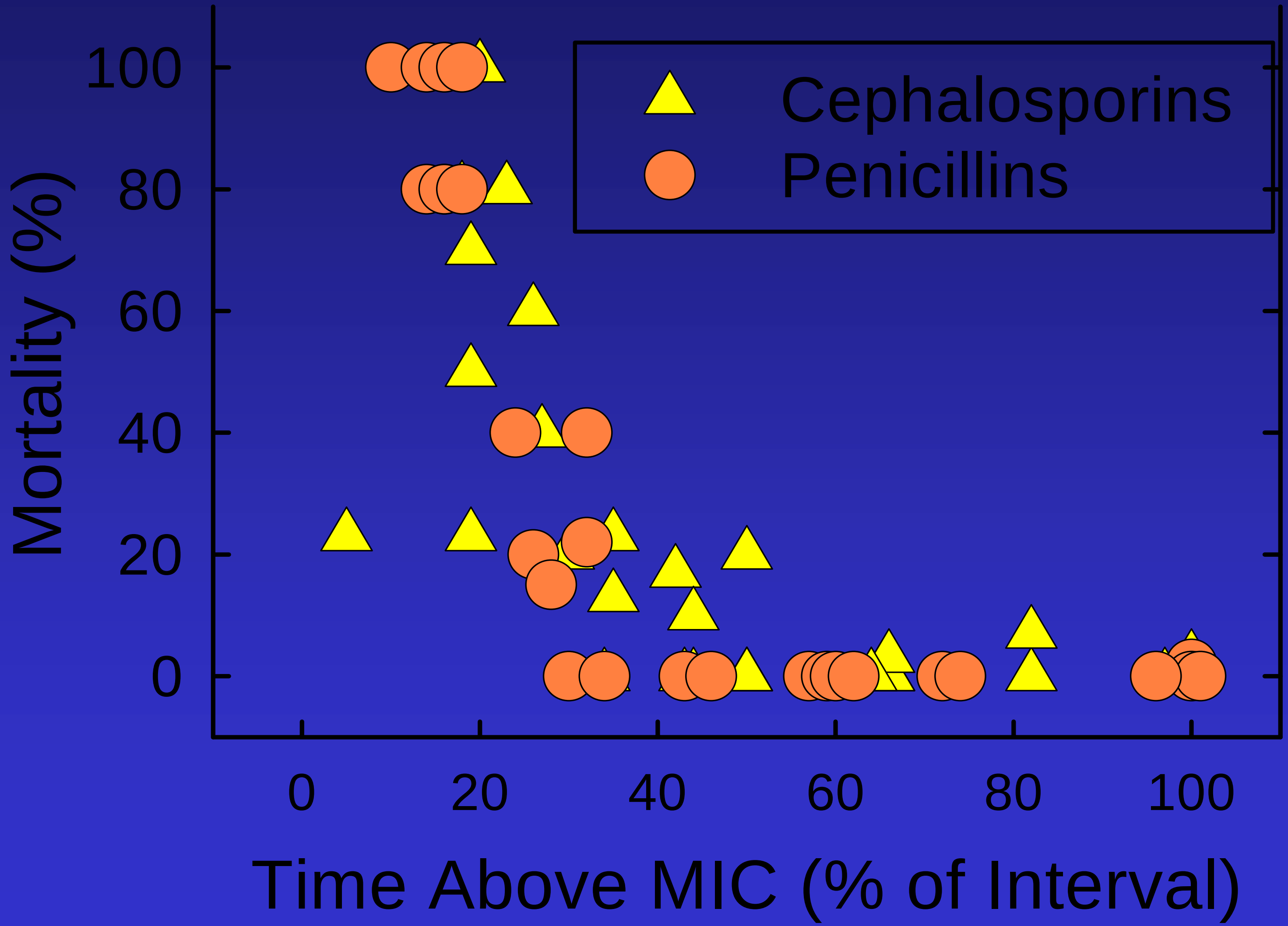
Activity of Vancomycin against *S. pneumoniae* in Lungs and Thighs of Neutropenic Mice



Survival/Mortality Studies

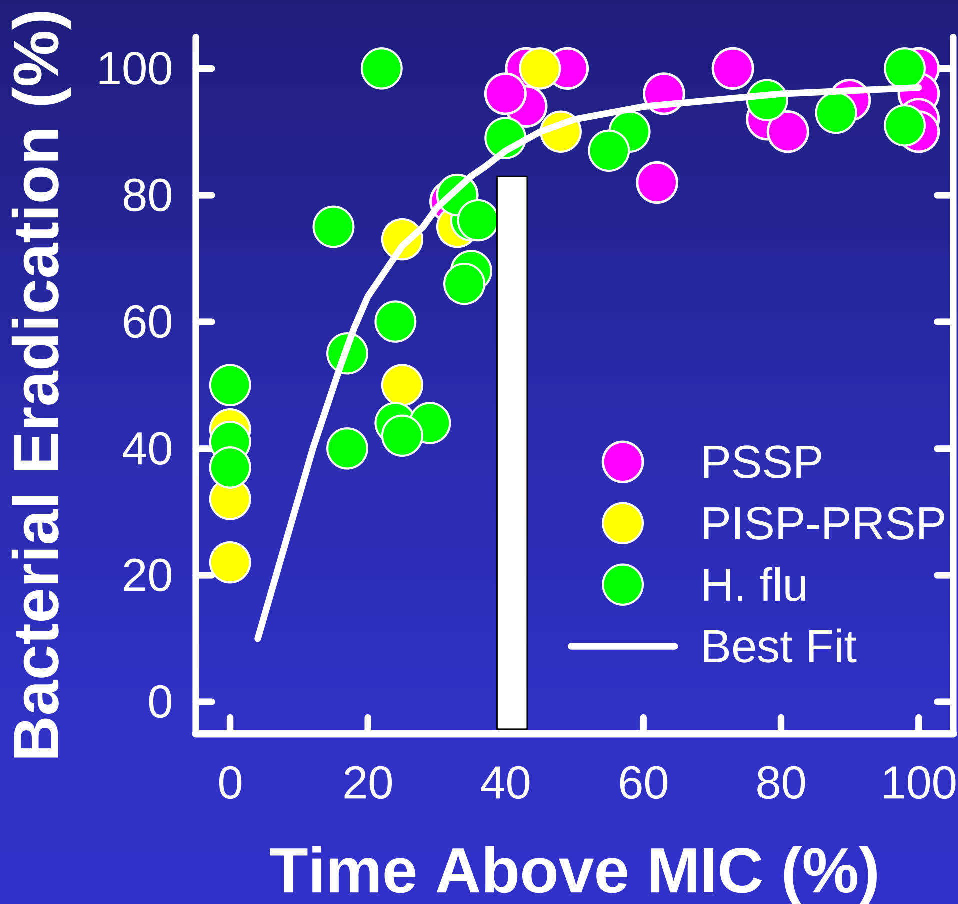
- Mortality in control animals 80-100% by end of therapy
- Animals treated for at least 48 hrs
- Mortality assessed within 24 hrs of end of therapy
- Pharmacokinetics included so PK/PD parameters could be estimated

T>MIC for ß-Lactams Versus Mortality in Animal Models: Literature Review



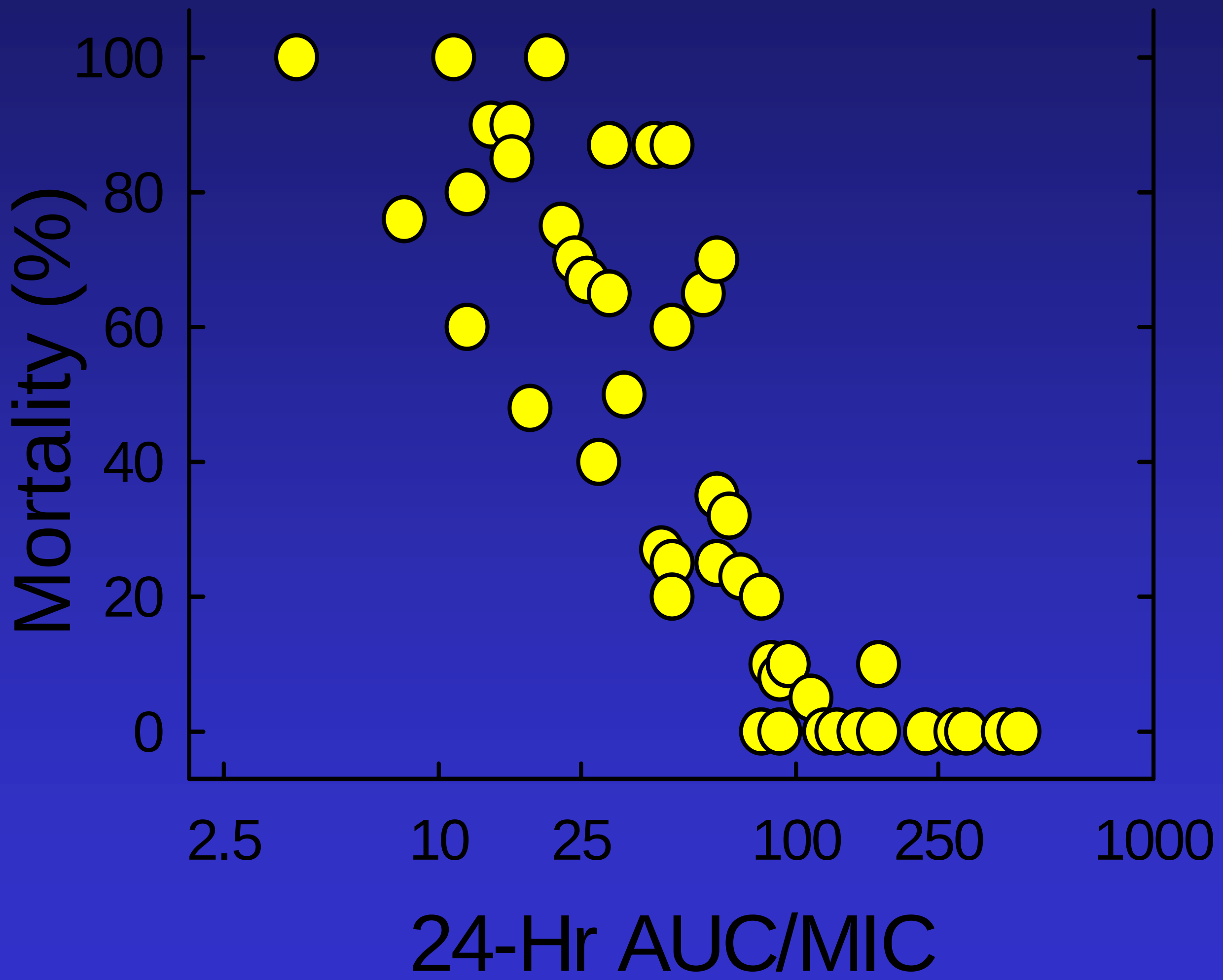
Craig Antimicrobial Pharmacodynamics in Theory and Practive 2002

Relationship of T>MIC with Bacterial Eradication in Acute Otitis Media



Craig Infect Dis Clin N Amer 2003

Relationships Between Mortality and 24-Hr AUC/MIC for Fluoroquinolones in Animal Models

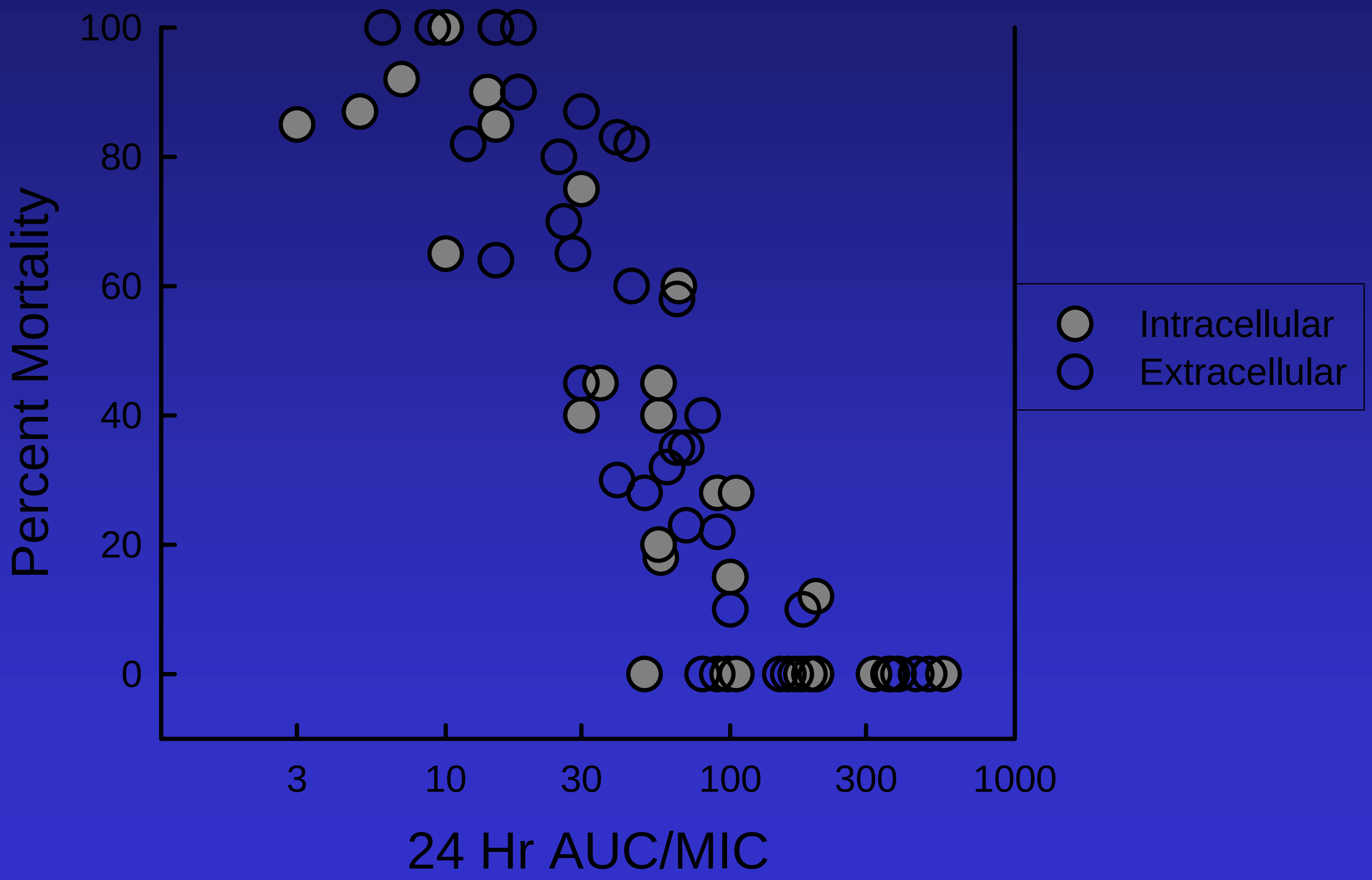


Andes, Craig Int J Antimicrob Agents, 2002

Intracellular Concentrations of Fluoroquinolones vs Intracellular Pathogens

- Tissue homogenate concentrations from multiple tissues are 2 to 8-fold higher than plasma levels
- A variety of efficacy studies using survival as the end point have been conducted with fluoroquinolnes against both extracellular and intracellular pathogens.
- The primary intracellular pathogens studied were M. tuberculosis, C. psittaci, L. pneumophila, and L. moncytogenes.

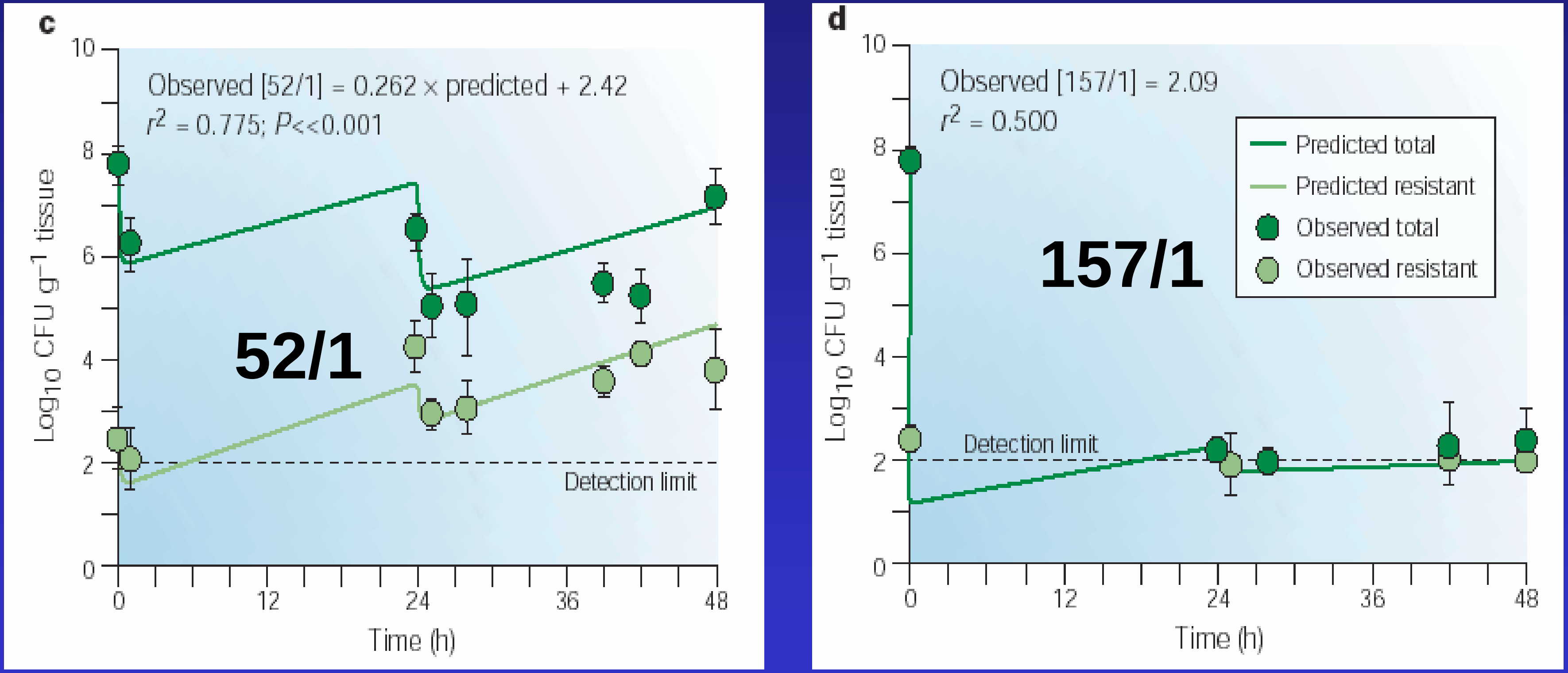
Relationship Between 24 Hr AUC/MIC and Mortality for Intracellular and Extracellular Pathogens in Various Infection Models for Fluoroquinolones



Use of Animal and In Vitro Models in PK/PD Evaluation of Anti-Infective Agents

- Describing the time-course of antimicrobial activity at sites of infection
- Identifying PK/PD indices correlating with efficacy (Peak/MIC, AUC/MIC, Time>MIC)
- Determining magnitudes of the PK/PD indice required for efficacy and identifying factors that affect the magnitude
- Identifying magnitudes of the PK/PD indice promoting and suppressing the emergence of resistance

Relationship of AUC/MIC to Enhancement and Suppression of Resistant P. aeruginosa in a Murine Thigh Infection



J Clin Invest 2003;112:275-285 &
Nature Rev Microbiology 2004;2:289-300

Conclusions

- Animal and in vitro models have been very useful for determining the PK/PD target for efficacy (PK/PD indice and appropriate magnitude required for bacteriologic cure and survival
- Additional studies are identifying the PK/PD targets that enhance and suppress the emergence of resistance
- Despite the variety of techniques and models, there is marked consistency in the PK/PD data from these in these animal model studies