Cellular pharmacodynamics and pharmacokinetics of antibiotics: Current views and perspectives

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The treatment of intracellular infections requires the use of antibiotics presenting appropriate cellular pharmacokinetic and pharmacodynamic properties. These properties, however, cannot be predicted on the simple basis of cellular drug accumulation and minimum inhibitory concentration in broth. In most cases, intracellular activity is actually lower than extracellular activity, despite the fact that all antibiotics reach intracellular concentrations that are at least equal to, and more often higher than the extracellular concentrations. This discrepancy may result from impairment of the expression of antibiotic activity or a change in bacterial responsiveness inside the cells. It therefore appears important to evaluate the intracellular activity of antibiotics in appropriate models.

Keywords Antibiotics, cellular accumulation, cellular pharmacodynamics, cellular pharmacokinetics, intracellular infection

Abbreviations

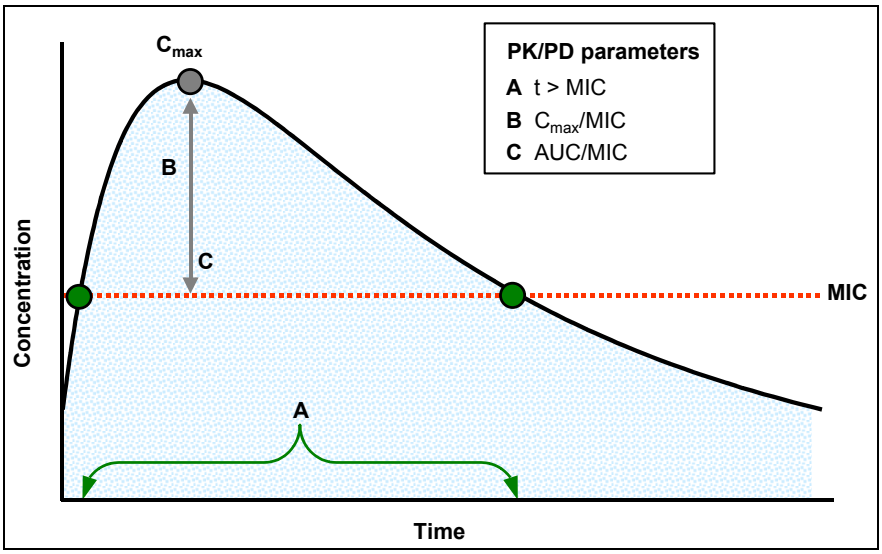
AUC	Area under the concentration-time curve
C_{max}	Peak plasma concentration
MBC	Minimal bactericidal concentration
MIC	Minimal inhibitory concentration
MRP	Multiple drug-resistance protein
PK/PD	Pharmacokinetics/pharmacodynamics

Introduction

Over the last few years, much concern has been raised regarding the optimization of antibiotic use, owing to the worrying increase of bacterial resistance and to the scarcity of new antibiotic classes under development [1]. In this context, progress in the field of anti-infective pharmacology has led to the emergence of a new discipline, referred to as pharmacokinetics/pharmacodynamics (PK/PD) of antibiotics, which is defined as the 'discipline that strives to understand the relationships between drug concentrations and effects, both desirable (eg, bacterial killing) and undesirable (eg, side effects)' [2]. Over the past 15 years, three key PK/PD parameters have been elaborated (Figure 1; for reviews, see references [3] to [6] or [7••]), which examine how antibiotic concentrations reached in body fluids over time (as predicted from the pharmacokinetic profile of the drug) compare with potentially effective antibiotic concentrations (as deduced from the minimal inhibitory concentration (MIC) or minimal bactericidal concentration (MBC) of antibiotics *in vitro*). The first parameter, time at which concentration is > MIC (t > MIC), links bactericidal effects to time and is critically dependent on the half-life of the drug, dosage and frequency of administration over a given time period. The second parameter, peak plasma concentration (C_{max})/MIC, relates bactericidal effects to concentration, and is primarily dependent on the unit dose and the volume of distribution of the drug. The third parameter, area under the concentration-time curve (AUC)/MIC, combines both types of effects, since it corresponds to the total amount of drug to which bacteria are exposed over the time period, and is directly related to the total dose given during that period and inversely proportional to the drug clearance. These parameters appear to be critical in predicting antibiotic activity and, therefore, in establishing dosages on a rational basis [8,9]. The application of these parameters, however, has so far been limited to extracellular infections in well-vascularized tissues, because they are all based on serum antibiotic levels.

The situation is, therefore, likely to be more complex when attempting to predict active antibiotic concentrations for infections developing in less accessible compartments, as is the case for intracellular infections. Some bacteria have adapted themselves to survive, and even multiply, within eukaryotic cells [10••,11]. Table 1 lists the most common pathogens responsible for intracellular infections. Besides well-known obligate or facultative intracellular organisms, several extremely common bacteria are now recognized as being able to survive intracellularly under certain circumstances. Such infections are considered as 'opportunistic', because no specific mechanism of adaptation to intracellular survival has been highlighted so far, and this survival is not an essential determinant in the life cycle of the bacteria. In the intracellular environment these bacteria become protected from humoral defenses, and probably also from antibiotic action. This may, therefore, contribute to the chronic or recurrent nature of infections in which intracellular foci are present [12,13], as classically observed for *Mycobacterium* or *Chlamydia* (for reviews, see references [14] and [15]), and also more recently demonstrated for *Staphylococcus aureus* [16-19], streptococci [20,21••], *Helicobacter pylori* [22] and *Escherichia coli* [23,24]. Thus, the selection of antibiotics endowed with intracellular activity or, preferably, with mixed extracellular and intracellular activity, appears critical in the management of such infections. For a discussion on the definition of cellular PK/PD parameters that are predictive of intracellular activity, see reference [25]. As well as considering the influence of drug concentration or the time

Figure 1. Illustration of the main PK/PD parameters that correlate with efficacy against extracellular infections.



A Time (t) during which the concentration remains above the minimum inhibitory concentration (MIC) of the antibiotic against the pathogen, **B** ratio between the peak plasma concentration (C_{max}) of the antibiotic reached in the serum and the MIC, **C** ratio between the 24-h area under the concentration-time curve (AUC) and the MIC.

Table 1. The main human pathogenic bacteria capable of intracellular survival.

Type of intracellular life	Bacterial species	Subcellular localization	Associated pathologies
Obligate	*Chlamydia pneumoniae*	Inclusions	Pneumonia
	Chlamydia trachomatis	Inclusions	Trachoma, sexually transmitted diseases
	Coxiella burnetii	(Phago)lysosomes	Q Fever, pneumonia, encephalitis, endocarditis
	Mycoplasma pneumoniae	Cytosol	Pneumonia
	Rickettsia spp	Cytosol	Fever, cat scratch, etc
Facultative	*Brucella*	Phagosomes	Brucellosis
	Francisella tularensis	Phagosomes	Tularemia
	Legionella pneumophila	Endoplasmic reticulum, lysosomes	Pneumonia
	Listeria monocytogenes	Cytosol	Meningitis, abortion
	Mycobacterium tuberculosis	Phagosomes	Tuberculosis
	Salmonella spp	Phagosomes	Digestive infections
	Shigella flexneri	Cytosol	Digestive infections
Opportunistic	*Bacillus anthracis*		Anthrax
	Borrelia burgdorferi		Lyme disease
	Campylobacter jejuni		Digestive infections
	Escherichia coli		Urinary and digestive infections
	Helicobacter pylori		Peptic ulcer
	Staphylococcus aureus	Phagolysosomes, cytosol	Skin and soft tissues infections, osteomyelitis, endocarditis, pneumonia, etc
	Streptococcus pneumoniae		Upper and lower respiratory tract infections
	Streptococcus pyogenes		Pharyngitis
	Yersinia pestis		Plague, digestive infections

of exposure on the chemotherapeutic effect at the site of infection, other parameters must be examined that will specifically modulate responses in the intracellular environment [10••]. This will result in a modulation of the MIC and MBC values within the cells, a factor which is almost never taken into account in the context of pharmacodynamics [26,27] and which may lead to inappropriate therapeutic choices and a risk of persistent infection [28,29•,30].

The objectives of this review are to present and discuss the current knowledge of the PK/PD parameters governing the intracellular activity of antibiotics, and to propose strategies for optimizing this activity.

Cellular pharmacokinetics of antibiotics

While general pharmacokinetics relate to the absorption, distribution, metabolism and elimination of drugs in the body, cellular pharmacokinetics are centered on evaluation of the penetration, distribution, degradation and efflux of drugs in individual cells [21••,31,32]. These two fields are closely related because the cellular disposition of a drug (eg, its capacity to cross biological membranes, response to enzymatic modification or transport through epithelial cells) governs its general fate (absorption, distribution and elimination) in the body. Studying the pharmacokinetics of antibiotics in eukaryotic cells is therefore of prime importance because it defines the access of the drug to the site of infection.

Mechanisms of antibiotic uptake, distribution and efflux in eukaryotic cells

To gain access to extracellular targets or to the cellular medium within the body, drugs often use non-specific routes of entry [31], such as diffusion or endocytosis, depending on their physicochemical properties. Some drugs can also take advantage of the presence of transporters that recognize them because they share some structural similarities with endogenous molecules or nutriments.

Accumulation and distribution

Diffusion

Diffusion is the most common way for molecules of a sufficiently small size (usually molecular weight < 700 Da) and with good lipid solubility (for a review on these general concepts, see reference [33]) to cross cell membranes. Among the factors that dramatically affect membrane permeation, the ionization status of the drug appears to be of prime importance, with charged species being characterized by low lipid solubility and almost no ability to cross membranes in the absence of a specific transport mechanism. The actual rate of diffusion of a drug will thus vary according to the environmental pH, with weak bases diffusing faster at basic pH than at acidic pH and weak acids exhibiting the opposite behavior. As a result, weak bases tend to accumulate in membrane-bound acidic compartments, whereas weak acids are excluded from these sites (for a discussion of these general concepts see reference [34], and for an application to subcellular compartments see reference [35]).

β-Lactam antibiotics are thought to cross the cell membrane by passive diffusion to gain access to the cellular medium. The equilibrium concentration of these antibiotics becomes equal on either side of the membrane, resulting in an accumulation factor of approximately 1 [36-38]. Being weak acids, however, β-lactams are largely excluded from lysosomes and related acidic vacuoles. Quinolones likely also enter most cells by simple diffusion, but are more concentrated inside the cells than outside at equilibrium, for reasons which are still unclear [39,40•,41,42]. Macrolides are among the antibiotics with the highest capacity for accumulation in eukaryotic cells [43]. Because of their weak basic character, cell-associated macrolides are largely trapped in their positively charged, less diffusible form in lysosomes, with dicationic molecules (eg, azithromycin, erythromycylamine and telithromycin) reaching higher levels of accumulation than monocationic molecules (eg, erythromycin, roxithromycin, clarithromycin and cethromycin) [44-47,48•].

Endocytosis

Endocytosis is a non-specific mechanism that drives poorly diffusible molecules (ie, molecules that are too voluminous or too polar) to the lysosomal compartment. Adsorption at the cell surface, or specific interaction with surface receptors, can greatly accelerate the rate and efficacy of the uptake process (for a review, see reference [49]).

Aminoglycosides are the best-characterized example of antibiotics that enter cells (kidney and ear) via a double process of adsorptive and receptor-mediated endocytosis. These highly polar molecules are polyaminated and bind to the negatively charged phospholipids of the membrane and the endocytic receptor megalin. Megalin is a protein that acts as a receptor for polyaminated compounds, and is particularly abundant in renal proximal tubules, as well as in the hair cells of the inner ear (for a review, see reference [50]). Glycopeptides, which are voluminous molecules, also enter cells via this endocytic route, and their level of accumulation in the lysosomes varies considerably depending on the type of glycopeptide. Amphiphilic glycopeptides, such as teicoplanin, dalbavancin, telavancin or oritavancin, reach much higher levels of accumulation in cells than more hydrophilic molecules such as vancomycin [51-53]. This effect is particularly evident in the case of oritavancin, the intracellular concentration of which is several hundred times higher than the extracellular concentration, which is suspected to be the result of a high level of adsorption of the molecule at the cell surface.

Inward transport

Inward transport of drugs is observed for molecules that have sufficient similarity to endogenous substrates of transporters. Active inward transport of antibiotics has been demonstrated at the surface of epithelia. This method of intracellular accumulation contributes to the intestinal absorption or re-absorption by renal tubular cells, and therefore governs the pharmacokinetics profile of antibiotics. The intestinal absorption of β-lactams (peptidomimetic drugs bearing a free acid function) is mediated by transporters of small peptides (eg, PEPT1 [54,55]) or of monocarboxylate compounds (eg, MCT1 [56]), while tubular re-absorption of β-lactams occurs via peptide transporter PEPT2 [54,55] and organic ion transporters such as OCTN2 [57]. It is worth noting that there is a huge variation in the level of recognition of different β-lactams by these transporters [55], which may explain the considerable variation in the oral bioavailability or rate of elimination of these antibiotics. Active transport is also suspected to take place in non-polarized, phagocytic cells. For example, it has been suggested that transporters of purines contribute to the accumulation of quinolones (bicyclic aromatic nuclei) in monocytes [58].

Efflux

Efflux transporters expressed at the surface of eukaryotic cells are involved in the extrusion of either polar, non-

diffusible metabolites produced within the cells, or of diffusible molecules capable of freely invading the cells. These transporters usually exhibit a broad substrate specificity by being able to recognize molecules mainly on the basis of their amphiphilicity and of the presence of ionizable functions [59]. Among these transporters, multidrug transporters of the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily (including the multidrug-resistance protein P-glycoprotein (P-gp) and multiple drug-resistance proteins (MRPs)) are ubiquitous, while organic cation or anion transporters are mainly found at the surface of epithelia [60]. When expressed in non-polarized cells, multidrug transporters reduce the cellular accumulation of drugs and, hence, affect the pharmacological activity of the drugs toward intracellular targets. For antibiotics, this transport has been demonstrated to have deleterious consequences on intracellular activity [61]. When localized at the surface of biological barriers, such as the intestine, the blood-brain barrier, the liver and the kidneys, efflux transporters contribute to reduced absorption of drugs, poor penetration of the central nervous system, or accelerated elimination by hepatic or renal routes, making serum drug concentrations suboptimal [60,62].

P-gp is thought to be involved in the transport of β-lactams, macrolides, quinolones, tetracyclines, streptogramins and trimethoprim, and MRPs are thought to transport β-lactams, macrolides, quinolones and rifamycins, at the level of epithelial cells bordering biological barriers or of phagocytic or transfected cells (see reference [60] and the references cited therein). In addition, different types of organic anion transporters contribute to the renal tubular re-absorption of β-lactams and prevent their access to the central nervous system [63,64].

Accumulation levels and subcellular distribution of the main antibiotic classes in eukaryotic cells

Table 2 summarizes the current knowledge of pharmacokinetics of antibiotics in eukaryotic cells. Macrolides and semisynthetic glycopeptides accumulate at higher levels in cells than other antibiotics, but are mainly concentrated within lysosomal vacuoles [44,45,51]. Quinolones accumulate at moderate levels and are found in the cytosolic fraction, possibly due to their high diffusibility [41]. Lincosamides and rifamycins are also concentrated within eukaryotic cells, but their localization is unknown [65,66]. Cellular concentrations of all of these antibiotic classes will be higher than serum concentrations, suggesting the potential treatment of intracellular infections located in the compartment where the drug is concentrated. Accordingly, macrolides, rifamycins and quinolones are classically considered as drugs of choice for treating intracellular infections [43,67-71]. β-Lactams penetrate, but do not accumulate within the cells, with cellular concentrations being close to extracellular concentrations, and are therefore generally considered to be of no interest for treating intracellular infections [72-74]. However, because serum levels are often quite high for this antibiotic class (peak levels > 50 mg/l), cellular concentrations might be expected to be higher than the MIC of intracellular pathogens under the conditions of their clinical use. Appropriate doses (ie, high concentration) and prolonged time of exposure (as suggested by the time-dependent activity of β-lactams in extracellular models of infection) may therefore compensate for the lack of accumulation, and confer intracellular activity to β-lactams, as was recently demonstrated in *in vitro* models [75•,76••]. Aminoglycosides accumulate slowly within cells, such that they reach active concentrations only upon prolonged exposure. They are therefore used in the management of chronic intracellular infections, such as tuberculosis [77].

Cellular pharmacodynamics of antibiotics

Based on pharmacokinetic considerations alone, it might be tempting to conclude that the intracellular activity of antibiotics can be predicted from their accumulation level. In terms of translating data obtained from cellular models into *in vivo* situations, however, a more accurate view would be obtained by considering cellular concentrations rather than accumulation factors, because this parameter also takes into account the fact that serum concentrations of antibiotics can vary considerably between classes. Some cellular concentration values are provided in Table 2, although some may be overestimated for antibiotics that are highly protein-bound, because it is essentially only the free fraction that can enter cells. It is also worth noting that local concentrations in specific compartments may be higher for antibiotics that are not distributed uniformly throughout cells.

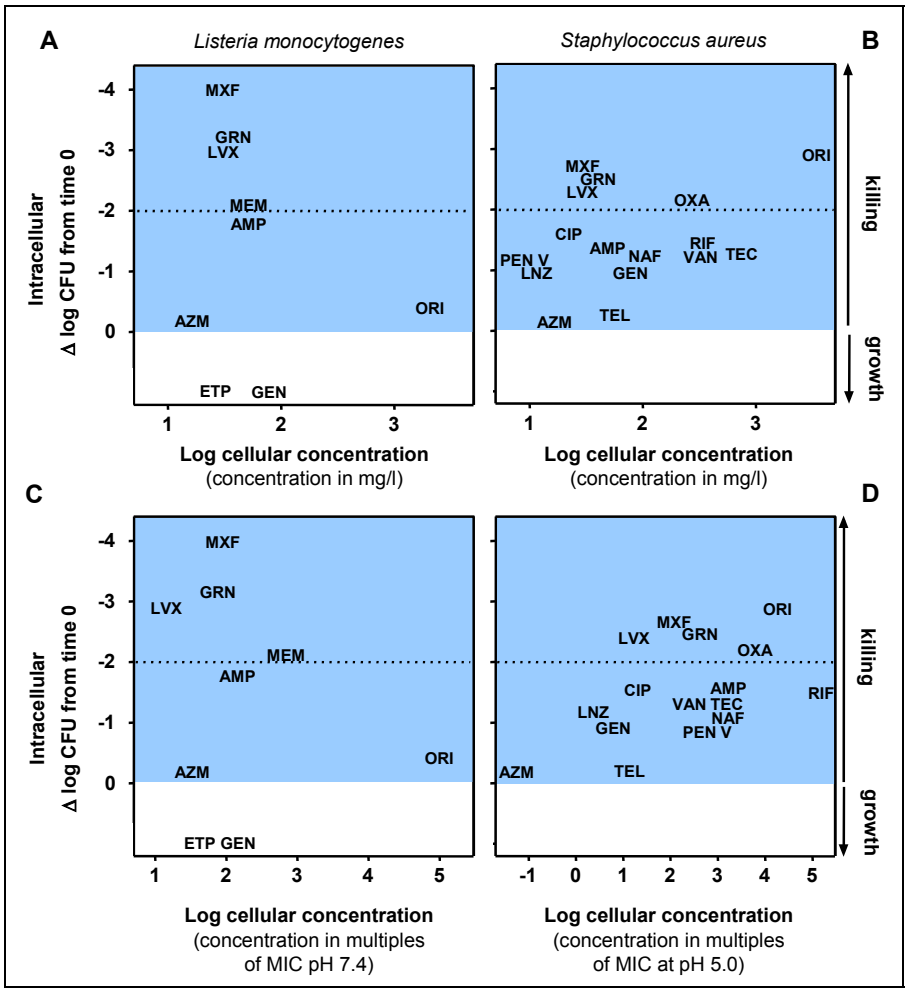
Although the cell concentrations of all antibiotics appear to be well above the MICs of susceptible organisms, studies systematically comparing the extracellular and intracellular activity of antibiotics from different classes have led to two unanticipated observations. First, there is no simple correlation between the cellular concentrations of antibiotics and intracellular activity [10••,76••,78••,79,80]. This concept is illustrated in Figure 2 (panels A and B), in which the intracellular activity of a series of antibiotics against cytosolic (*Listeria monocytogenes*) and phagolysosomal (*S aureus*) bacteria is examined and plotted as a function of log cellular concentration in THP1 macrophages, as determined in cells exposed to an extracellular concentration corresponding to the human C_{max} of each drug for 24 h. Against *L monocytogenes*, most of the antibiotics tested reached a cellular concentration of 10 to 100 mg/l, but displayed effects ranging from inactivity (bacterial growth) to a reduction in bacterial counts (-4 log). Oritavancin, which accumulated to a larger extent, was almost inactive against intracellular *L monocytogenes*, but this can be explained based on its lysosomal localization (this explanation can also be applied for the inactivity of gentamicin). Based on this explanation, it is not surprising that oritavancin is active against intracellular *S aureus*; however, it is not more bactericidal than other drugs having lower cellular concentration, such as quinolones. Second, although cellular concentrations are generally higher than extracellular concentrations, antibiotic activity can be lower intracellularly than extracellularly, at least against a phagolysosomal bacterium such as *S aureus*. The extracellular and intracellular activity of the same antibiotics as in Figure 2, are correlated in Figure 3. Against *L monocytogenes*, however, the

Table 2. Cellular pharmacokinetics of the main antibiotic classes within eukaryotic cells

Pharmacochemical class	Antibiotic	Accumulation level at equilibrium (C_C/C_E)[a]	Cellular concentration at equilibrium (mg/l)[b]	Time to equilibrium	Accelerated efflux due to active transport[c]	Predominant subcellular localization
β-Lactams	All	< 1	~ 20 to 50	Fast	P-gp, MRP, anion/cation transporters	Cytosol
Macrolides	Erythromycin	4 to 10	~ 40 to 150	Moderate (a few hours)	P-gp	2/3 Lysosomes 1/3 Cytosol
	Clarithromycin Roxithromycin Telithromycin	10 to 50	~ 20 to 400			
	Azithromycin	40 to 300	~ 16 to 120			
Fluoroquinolones	Ciprofloxacin Levofloxacin Grepafloxacin	4 to 10	~ 16 to 40	Fast (< 1 h) to very fast (< 5 min)	MRP, P-gp, anion/ cation transporters	Cytosol
	Moxifloxacin Garenoxacin Gemifloxacin	10 to 20	~ 40 to 80		Marginal or unknown	
Aminoglycosides	All	2 to 4 (after several days)	~ 40 to 80	Slow (several days)	Improbable	Lysosomes
Lincosamides	Clindamycin	5 to 20	~ 50 to 200	Fast	Unknown	Unknown
	Lincomycin	1 to 4	~ 15 to 60		None	
Tetracyclines	Probably all	1 to 4	~ 2 to 12	Unknown	P-gp	Unknown
Ansamycins (rifamycins)	Rifampin	2 to 10	~ 36 to 180	Unknown	MRP	Unknown
	Rifapentine	60 to 80	~ 1200 to 1600	Unknown		
Glycopeptides	Vancomycin	8 (after 24 h)	~ 400	Slow (several hours)	Improbable	Lysosomes (in kidney)
	Teicoplanin	60	~ 6000			Unknown
	Oritavancin	150 to 300 (after 24 h)	~ 3750 to 7500			Lysosomes
	Telavancin	50 (after 24 h)	~ 4500			Lysosomes
Oxazolidinones	Linezolid	~ 1	~ 20	Unknown	Unknown	Unknown

[a]C_C/C_E represents the accumulation factor (ie, the ratio between the cellular concentration (C_C) and the extracellular concentration (C_E)) in cultured macrophages. [b]Calculated from the accumulation ratio in cultured macrophages, using the average human C_{max} for the antibiotic under consideration as the C_E value. [c]P-glycoproteins (P-gps) and multiple drug-resistance proteins (MRPs) belong to the ABC superfamily of transporters energized by ATP hydrolysis, which are expressed in epithelial and phagocytic cells. Cation, anion and peptide transporters belong to different transporter families, but are all energized by ion gradients and are expressed essentially in epithelial cells. Table 2 is based on data from references [10••], [32] and [60].

Figure 2. Relationship between the intracellular activity of antibiotics and their cellular concentration.



The graphs show the intracellular activity of a series of antibiotics against *Listeria monocytogenes* (left) and *Staphylococcus aureus* (right) in a model of THP1 human macrophages. Activity is expressed as the change in bacterial counts following 24 h of exposure (or 5 h of exposure for oritavancin in the *L monocytogenes* model) to each of the selected antibiotics at an extracellular concentration corresponding to its human C_{max}. The cellular concentrations were all measured under the same conditions, and are expressed in terms of log of the mg/l values (panels **A** and **B**) or log of multiples of the minimum inhibitory concentration (MIC) as determined in broth at the pH of the corresponding infected compartment (panels **C** and **D**). The blue zones correspond to bacterial killing, while the dotted lines show the limit of bactericidal effect (-2 log according to the recommendations of the Clinical and Laboratory Standards Institute). The limit of detection was -4.2 log, and all values below this limit were set at -5 log. The graphs are based on data from references [75•], [76••] and [107].
AMP Ampicillin, **AZM** azithromycin, **CFU** colony forming units, **CIP** ciprofloxacin, **ETP** ertapenem, **GEN** gentamicin, **GRN** garenoxacin, **LNZ** linezolid, **LVX** levofloxacin, **MEM** meropenem, **MXF** moxifloxacin, **NAF** nafcillin, **ORI** oritavancin, **OXA** oxacillin, **PEN V** penicillin V, **RIF** rifampin, **TEC** teicoplanin, **TEL** telithromycin, **VAN** vancomycin.

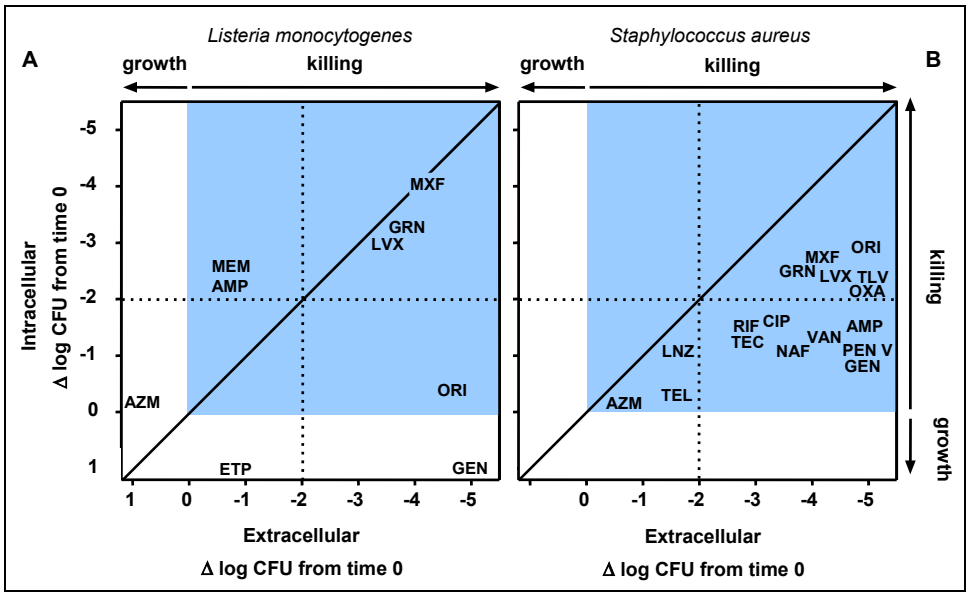
intracellular activity of antibiotics is lower, similar (eg, for quinolones), or even higher (eg, for some β-lactams) than extracellular activity. Importantly, macrolides, which are among the antibiotics accumulating in cells at a higher level, are poorly active intracellularly (see reference [69]), probably due to their intrinsic bacteriostatic nature.

The general low intracellular activity of some antibiotics could result from: (i) poor bioavailability of the accumulated antibiotic, making pharmacokinetic predictions incorrect; or (ii) a shift of MICs toward higher values in the intracellular milieu, underlining the importance of pharmacodynamic considerations. Such changes in MICs could be due to either an impaired expression of antibacterial activity within the intracellular environment, or an altered bacterial responsiveness within eukaryotic cells.

Cellular bioavailability of antibiotics

In extracellular models, activity is best predicted from the free serum concentration of antibiotics, which represents the fraction diffusing through tissues and reaching bacterial targets [81]. The absence of a correlation between the total amount of antibiotic associated with cells and the intracellular activity may suggest that part of the accumulated drug is not bioavailable because of binding to cellular constituents. The interaction of antibiotics with cellular proteins has not been documented in the literature to date, but is highly probable. What is known, however, is

Figure 3. Correlation between the intracellular and the extracellular activity of antibiotics.



The graph shows the correlation between the intracellular and extracellular activity of a series of antibiotics against *Listeria monocytogenes* (panel **A**) and *Staphylococcus aureus* (panel **B**), in a model of THP1 human macrophages. Activity is expressed as the change in bacterial count following 24 h of exposure (or 5 h of exposure for oritavancin in the *L monocytogenes* model) to each of the selected antibiotics at an extracellular concentration corresponding to its human C_{max}, both extracellularly (x-axis) and in infected macrophages (y-axis). The blue zones correspond to bacterial killing, while the dotted lines point to the limit of bactericidal effect (-2 log according to the recommendations of the Clinical and Laboratory Standards Institute). The limit of detection was -4.2 log, and all values below this limit were set at -5 log. The diagonal line delineates the experimental points expected for drugs displaying equal extracellular and intracellular activities, with points above this line corresponding to bactericidal activities that are higher intracellularly than extracellularly, and below the line to activities that are higher extracellularly than intracellularly. The graphs are based on data from references [75•], [76••] and [107].
AMP ampicillin, **AZM** azithromycin, **CFU** colony forming units, **CIP** ciprofloxacin, **ETP** ertapenem, **GEN** gentamicin, **GRN** garenoxacin, **LNZ** linezolid, **LVX** levofloxacin, **MEM** meropenem, **MXF** moxifloxacin, **NAF** nafcillin, **ORI** oritavancin, **OXA** oxacillin, **PEN V** penicillin V, **RIF** rifampin, **TEC** teicoplanin, **TEL** telithromycin, **VAN** vancomycin.

that some antibiotic classes such as aminoglycosides and macrolides, and also oritavancin, tightly bind to the lipid constituents of membranes, causing even lipid deposition within the lysosomes [46,82,83].

Intracellular expression of antibiotic activity

Environmental effects on antibiotic expression of activity can partly be taken into account by plotting activity as a function of the cellular concentration, expressed in multiples of the MIC, as determined at neutral pH for the cytosolic *L monocytogenes*, but at acidic pH for the phagolysosomal *S aureus*. Figures 2C and 2D show that, in acidic milieu, this correction negatively affects the cellular concentration of macrolides, gentamicin and, to a lesser extent, quinolones, but enhances the cellular concentration of rifampin, and marginally that of β-lactams, while not altering the cellular concentration of glycopeptides and linezolid. This correction does not, however, improve the correlation between cellular concentration and intracellular activity, suggesting that the influence of the cellular environment extends beyond pH effects.

Among other factors specific to the intracellular milieu of phagocytes, cell defense mechanisms can either cooperate with or antagonize antibiotic action. For example, inhibiting oxidative burst in macrophages reduces the intracellular activity of quinolones against *L monocytogenes*, suggesting that oxidant species reinforce the efficacy of this class of antibiotic [84]. In contrast, global impairment of cell defense mechanisms does not prevent the unanticipated intracellular bactericidal effect of β-lactams against *L monocytogenes* [85], suggesting that bacteria have increased susceptibility to these antibiotics within the cells.

Intracellular bacterial responsiveness to antibiotics

Bacteria growing inside eukaryotic cells may undergo drastic changes in their metabolism to adapt to the new and sometimes hostile environment of cells compared with the extracellular environment. Such changes have been well characterized for obligate and facultative bacteria, which need to produce additional proteins to escape from phagosomes and move in the cytosol (as observed for *Listeria* or *Shigella* [86,87]), or to prevent the fusion of phagosomes with lysosomes to enable the infection of phagosomes (as observed for *Legionella* or *Chlamydia* [88]). Recent studies examining, in a global fashion, genetic expression or protein profiles of intracellular bacteria or bacteria exposed to a mild acidic environment have demonstrated multiple metabolic modifications [89-91]. It is probable that some of these changes may influence antibiotic action, as suggested above, which might explain the increased sensitivity of intracellular *Listeria* to β-lactams. Also, the growth rate of some bacteria is generally reduced inside the cells [92-94], highlighting their need to adapt to a hostile environment. This delay in growth can contribute to

impaired antibiotic activity, since many classes of antibiotics act upon bacteria in the active stage of multiplication, as demonstrated by the growth-cycle-dependent efficacy of antibiotics against *Chlamydia* [95]. Moreover, local pH can affect not only antibiotic action, but also bacterial response to antibiotics. A surprising example of this is methicillin-resistant *S aureus*, which becomes sensitive to β-lactams intracellularly [96], probably because of a favorable effect of acidity [97]. Finally, mechanisms of resistance can affect bacterial responses to antibiotics, although it is not known how the intracellular environment may influence the expression of inducible mechanisms. Among such mechanisms, efflux pump overexpression is widespread and contributes to antibiotic resistance, both in Gram-positive and Gram-negative bacteria [98], including in pathogens capable of surviving inside eukaryotic cells. The expression of efflux pumps is essentially based on the role they play in bacterial virulence or survival within the host, as demonstrated for Gram-negative bacteria [99,100]; whether or not efflux pumps express *in vivo* is, however, still under debate. In this context, studies aimed at determining whether bacterial efflux pumps are expressed within eukaryotic cells and whether they reduce the concentration, and consequently the activity, of antibiotics in this compartment, would be welcome in the future.

Strategies to optimize intracellular activity of antibiotics

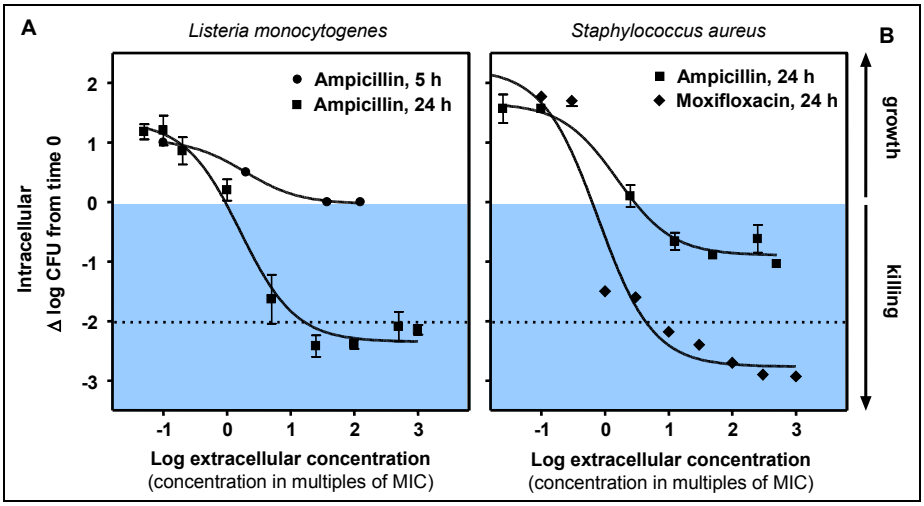
Optimizing cellular pharmacokinetics

Although there is no correlation between accumulation *per se* and activity, the intracellular activity of a given antibiotic can be optimized by maximizing its cellular concentration and the time of exposure. This effect is exemplified in Figure 4 (panel A), in which the influence of the time and concentration on the activity of a β-lactam against *L monocytogenes* is shown. The activity develops in a sigmoidal manner with concentration, and a marked bactericidal effect is obtained only with high concentrations of, and prolonged exposure to, antibiotics. Strategies aimed at optimizing drug content inside the cells over time are therefore liable to improve intracellular activity.

In the case of β-lactams, which do not accumulate to a large extent in eukaryotic cells, appropriate chemical modifications may alter their cellular pharmacokinetic profile and favor their uptake by eukaryotic cells. For example, grafting a weak basic function to and masking the acidic character of penicillin makes the molecule prone to accumulate within the lysosomes [37], whereas masking the acidic character of ampicillin in a cleavable prodrug ester markedly increases the cellular concentration of free ampicillin as well as its activity against intracellular *L monocytogenes* [38]. In the case of aminoglycosides, which slowly accumulate in cells, using an appropriate formulation such as antibiotic-loaded microspheres, improves intracellular activity by increasing the phagocytic rate of the drug [101,102]. This strategy is also efficient for increasing the efficacy of rifampin toward *Mycobacterium tuberculosis*, by allowing the slow release of the drug inside infected cells [103].

On the other hand, for antibiotics extruded out of cells by active efflux mechanisms, inhibition of the corresponding transporters increases the cellular drug content and, as a consequence, the intracellular activity. This is demonstrated by the increase in activity of quinolones against *L monocytogenes* in the presence of gemfibrozil, or the increase in activity of macrolides against both *L monocytogenes* and *S aureus* when cells are exposed to verapamil [104,105].

Figure 4. Influence of time and of concentration on the intracellular activity of antibiotics.



A Shows the influence of time and concentration on the intracellular activity of ampicillin against *Listeria monocytogenes* in infected THP1 macrophages exposed for 5 or 24 h to increasing concentrations of the drug, expressed as the log of multiples of its minimum inhibitory concentration (MIC). **B** Provides a comparison of the dose-effect relationship of the activity of ampicillin and moxifloxacin against *Staphylococcus aureus* in infected THP1 macrophages exposed for over 24 h to increasing multiples of their MIC. The blue zones correspond to bacterial killing, while the dotted lines show the limit of bactericidal effect (-2 log according to the recommendations of the Clinical and Laboratory Standards Institute). The graphs are based on data from references [75•], [76••] and [78••].

Taking into account cellular pharmacodynamics

Currently, we have only a partial view of factors influencing antibiotic activity or bacterial responsiveness to antibiotics inside cells. Antibiotic selection should be based on MIC data, as determined at the pH of the compartment in which infection develops [76••,80,96]; however, it has been observed that many parameters other than pH affect the intracellular activity of antibiotics, such that the final choice of a drug should also be based on studies of pertinent *in vitro* cellular models, in which pharmacokinetic parameters are optimized. This is exemplified in Figure 4 (panel B), in which the dose-effect relationship of two antibiotics with similar MICs are compared against intracellular *S aureus* over 24 h and at neutral pH. The pharmacological responses appear to differ in maximal effect and EC_{50} values, suggesting the importance of examining the bacterial response to a drug within the physiological environment.

Developing appropriate models

Models need to be developed that closely mimic the clinical conditions of antibiotic use in terms of concentration and antibiotic exposure, and that integrate these pharmacokinetic parameters with pharmacodynamic considerations. Currently, most *in vitro* models use constant static concentrations of antibiotics, but modulate either the time of exposure or the extracellular concentration [26,69,75•,76••,78••,94,106-109]. These current models may be appropriate to study the impact of antibiotic combinations on pharmacokinetics (eg, competition for transport [110]) and pharmacodynamics (eg, synergy or antagonism [111-113]). In addition, modeling of the variation in antibiotic concentrations over time using dynamic *in vitro* models (Figure 1) could help to reproduce more accurately the actual exposure of infected cells to antibiotics. Currently, however, *in vitro* models often use facultative intracellular pathogens, which are grown in broth [114,115••,116]. A more ideal situation would be to develop dynamic models with bacteria growing inside eukaryotic cells [117••]. Recent efforts have also been directed toward developing methodologies that allow for the sensitive and rapid detection of intracellular bacteria [118••,119••] or for the routine evaluation of intracellular efficacy of antibiotics [120,121]. These types of studies should be included in the early development of new antibiotics, especially if their spectrum of activity includes bacteria capable of intracellular survival (eg, see references [51], [69] and [122] to [125]).

In vivo models have been developed for several opportunistic or facultative bacteria [126-129], and these are essential for the appraisal of therapeutic schemes established based on *in vitro* data, to correctly address drug bioavailability issues and assess cooperation with host defenses. In this respect, direct measurement of intracellular concentrations of antibiotics *in vivo* through non-invasive approaches [130•] will allow significant progress to be made in correlating activity with actual drug concentration at the infected site. *In vivo* models can confirm unanticipated intracellular activity observed *in vitro*, for example, in the case of a new derivative of ethambutol that proved to be as active *in vivo* as *in vitro* in infected cells against *M tuberculosis*, and demonstrated a high tissue concentration, despite low oral bioavailability [131], or for quinolones, which proved to be efficient *in vivo* against *L monocytogenes* [132], in accordance with their *in vitro* behavior in infected macrophages [78••]. *In vivo* models can also help to provide a greater understanding of the pharmacokinetic issues responsible for lack of efficacy, such as the inappropriate dosing of the β-lactam mecillinam (which generates concentrations that remain above the MIC for only 6 to 7 h) associated with intracellular survival of *E coli* [29•], or the poor bioavailability of aminoglycoside-loaded microspheres, which originally showed promise *in vitro* against *Brucella abortus*, but which proved extremely disappointing *in vivo* [133].

Conclusion

The cellular accumulation of antibiotics has long been considered to be predictive of activity against intracellular infections. This concept needs to be revisited, based on recent observations that expression of activity of antibiotics and bacterial responsiveness may be considerably modified in the intracellular environment. Activity should therefore be tested in appropriate models of intracellular infections that take into account pharmacokinetic considerations (eg, time of exposure and concentrations achievable *in vivo*), and which can be used to investigate the parameters modulating pharmacodynamic behavior.

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