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

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

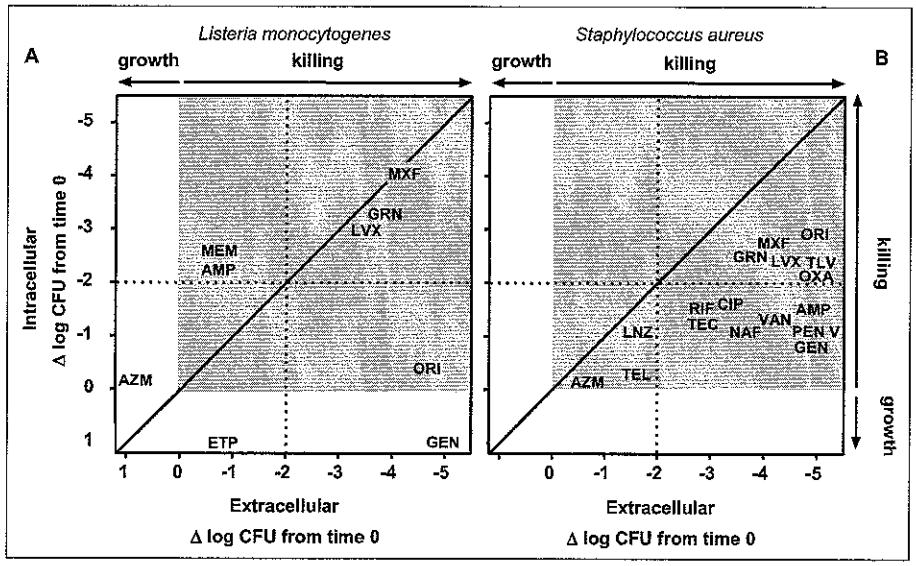
Efflux transporters expressed at the surface of eukaryotic cells are involved in the extrusion of either polar, non-

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 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 gray 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

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