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Intracellular pharmacodynamics of antibiotics

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Treatment of intracellular bacterial infection remains both a medical and economic challenge. Pathogens thriving or maintaining themselves in cells, or simply taking transient refuge therein, are indeed shielded from many of the humoral and cellular means of defense. They also seem more or less protected against many antibiotics. This explains why intracellular bacteria not only are harmful for the host cells but may also constitute a reservoir for recurrence and reinfection. Because antibiotics poorly act on intracellular bacteria, selection of resistant mutants may also be fostered. All these considerations stress the importance of understanding (1) whether and to what extent antibiotics may or may not act against intracellular bacteria, (2) which are the pharmacokinetic and pharmacodynamic parameters governing their activity, and (3) how chemotherapy can be improved on that basis. This article examines these issues starting from basic knowledge about the disposition of bacteria and antibiotics in cells and moving to an integration

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of these concepts to rationalize the various but quite often contradictory experimental observations concerning intracellular activity.

Entry and fate of bacteria in cells

Antibiotics must reach and bind to their target to exert their chemotherapeutic activity. A prerequisite is that bacteria and antibiotics come into contact. Knowing where bacteria are in cells is critical. A large body of data has now been obtained in this context and is presented in a pictorial fashion in Fig. 1. The two main points to be stressed here are that the fate of bacteria is highly variable according to the pathogen considered and critically influenced

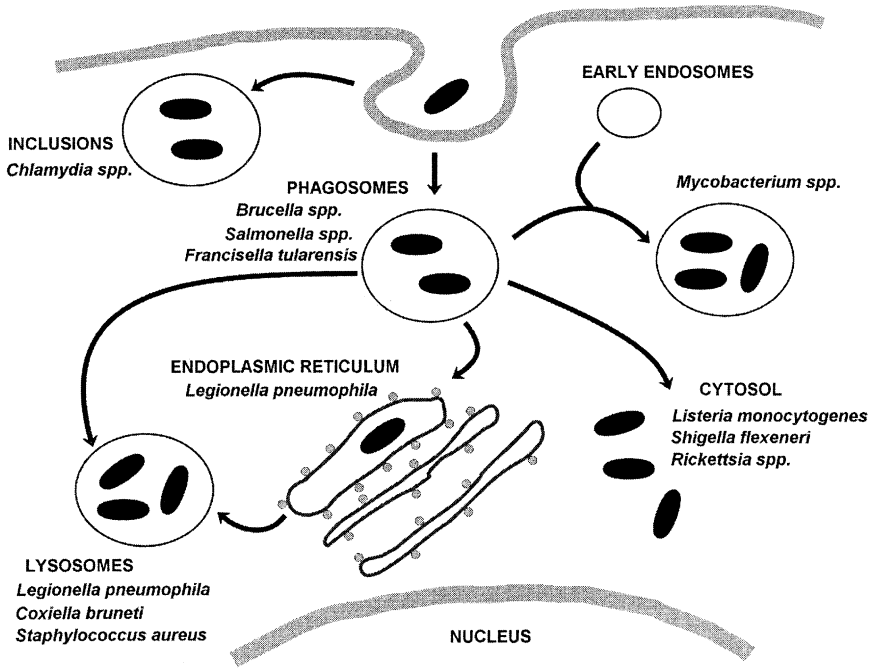

Fig. 1. Pictorial description of the various pathways followed by intracellular bacteria to evade cellular mechanisms of destruction after phagocytosis. Some bacteria (eg, *Listeria*, *Shigella*, *Rickettsiae*) escape from phagosomes early on after having been engulfed and avoid both acidification and subsequent sequestration in phagolysosomes. Others remain in phagosomes that continue to fuse with newly formed endosomes but not with lysosomes (*Mycobacteriae*); in phagosomes that are made unable to fuse with other vacuoles (*Brucellae*, *Salmonellae*, *Francisella*); or in phagosomes that are turned into specialized entities (*Chlamydiae*). In some cases (*Legionella*), phagosomes containing living bacteria may fuse with the endoplasmic reticulum in a form of abnormal autophagy. Finally, certain bacteria (*S aureus*, *Coxiella*, and to some extent *Legionella*) may simply resist the fusion of phagosomes with lysosomes and multiply within phagolysosomal vacuoles.

by its capacity to express virulence factors; and most of the pathways taken by bacteria are actually diversions from the common process of phagocytosis, the function of which is to convey particulate matters, such as bacteria, from the extracellular milieu to lysosomes and related digestive vacuoles. These diversions are geared at allowing the virulent bacteria to evade the defense mechanisms associated with phagocytosis (a pathogen is a microorganism capable of evading lysosomal destruction) [1]. Table 1 shows in a summarized fashion the present state of knowledge concerning a selected number of obligate and facultative pathogens concerning their main target cells and their prevailing subcellular localization. These various niches not only allow bacteria to be protected from the extracellular environment but they also provide distinct physicochemical conditions that affect both the bacteria and the activity of antibiotics. In their way to lysosomes, bacteria are also exposed to reactive oxygen nitrogen intermediates generated by the host NADPH oxidase [2] and nitric oxide synthase [3]. Moving to the cytosol is one way to escape those mechanisms to gain access to a neutral medium probably rich in growth-promoting factors. This may explain why some bacteria have developed quite sophisticated techniques to achieve this as quickly as possible. Those bacteria, like *Listeria monocytogenes*, multiply actively once having reached the cytosol (interferon-γ maintains *Listeria* in phagosomes and lysosomes and thereby limits severely its multiplication [4] because of continuing exposure to oxygen and nitrogen reactive species [5]). Bacteria

Table 1
Main intracellular bacteria with predominant target cells in humans and known subcellular localization of virulent forms

Organism	Type of parasite	Target cells	Subcellular localization	References
Brucella spp	Facultative	Macrophages	Phagosomes	[99]
Chlamydia spp	Obligate	Lung parenchyma cells	Inclusions	[100]
Coxiella brunetii	Obligate	Macrophages, lung parenchyma cells	Phagosomes, phagolysosomes	[101,102]
Francisella tularensis	Facultative	Macrophages	Phagosomes	[103]
Legionella pneumophila	Facultative	Macrophages	Endoplasmic reticulum, lysosomes	[102,104]
Listeria monocytogenes	Facultative	Macrophages, hepatocytes	Cytosol	[4]
Mycobacterium tuberculosis	Facultative	Macrophages	Early endosomes	[105]
Rickettsia spp	Obligate	Endothelial cells	Cytosol	[106]
Salmonella spp	Facultative	Macrophages	Phagosomes	[107]
Shigella flexeneri	Facultative	Macrophages	Cytosol	[108]
Staphylococcus aureus	Opportunist	Macrophages, PMNs	Phagolysosomes	[109–111]

that gain access to phagolysosomes have a different fate. Generally speaking, lysosomes and phagolysosomes can be considered as acidic compartments poor in nutrients (iron depletion [6], tryptophan degradation [7]). The consequence is that intracellular bacteria that sojourn in those vacuoles tend to become partially dormant. This reduces their sensitivity to many antibiotics. These bacteria also are confronted with potent lytic enzymes and specific antimicrobial agents, such as defensins [8]. Unfortunately, little is known about the cooperation (or hindrance) between these factors and antibiotics. One may suspect, however, that the reduction of bacterial metabolism induced by these agents could also decrease their sensitivity to antibiotics.

Cellular uptake and disposition of antibiotics (cellular pharmacokinetics)

Table 2 shows the key cellular pharmacokinetic properties of antibiotics that have been studied so far. As for the bacteria, one is struck by the diversity of behaviors, which is not so much of a surprise in view of the large difference in molecular structures among antibiotic classes. Common properties can, however, be delineated at the pharmacochemical class level as is reviewed here.

β-Lactams

All studies have so far reported a lack of accumulation (ie, an apparent intracellular concentration lower than the extracellular one at equilibrium) for all β-lactams whether in phagocytic [9–15] or nonphagocytic cells and tissues in general [16]. It has often been concluded that β-lactams are unable to penetrate cells, which is probably incorrect because most of the representatives of this class of drug do diffuse reasonably well through biologic membranes. All β-lactams display a free carboxylic function (or an equivalent proton-donor group), which is essential for their activity [17,18]. Modeling studies of the transmembrane distribution of weak acids show that the total concentration of such substances is always lower in acidic than in basic or neutral membrane-bounded compartments [19]. Because the cell cytosol is more acidic than the extracellular milieu, single acid β-lactams are prevented from accumulating in cells even if they can pass across membranes. Masking the free carboxyl group of a single acid β-lactam, such as penicillin G, by a basic moiety is actually all that is needed to allow substantial accumulation of the corresponding derivative [13]. The situation may be more complex for zwitterionic β-lactams, such as ampicillin, or most of the third- and fourth-generation cephalosporins, but none of them has ever been shown to accumulate in cells. One additional reason could be the presence of antibiotic efflux pumps that could actively extrude β-lactams from cells [20,21].

Table 2
Influx, accumulation levels (at equilibrium), efflux, and predominant subcellular localization of the main antibiotics (grouped by pharmacochemical classes)

Pharmacochemical class	Antibiotic	Influx[a]	Efflux[a]	Accumulation level (at equilibtrum)[b]	Predominant subcellular localization
β-Lactams	All	Fast	Variable	<1	Cytosol
Macrolides	Erythromycin	Fast	Fast	4–10	Two third lysosomes/ one third cytosol
	Clarithromycin, roxithromycin	Fast	Fast	10–20	
	Azithromycin	Fast	Slow to very slow	40–300	
	Telithromycin	Fast	Fast to slow	15–50	
Fluoroquinolones	All	Fast to very fast	Very fast	4–10	Cytosol
Aminoglycosides	All	Very slow	Very slow	2–4 (after several days)	Lysosomes
Lincosaminides	Clindamycin	Fast	Fast	5–20	Unknown
	Lincomycin	Fast	Fast	1–4	
Tetracyclines	All (?)	Fast	?	1–4	Unknown
Ansamycins (rifamycins)	Rifampin	Fast	?	2–10	Unknown
	Rifapentin	Fast	?	60–80	
Glycopeptides	Vancomycin	Slow	?	8 (after 24 h)	Lysosomes (in kidney)
	Teicoplanin	Fast	?	60	Unknown
	Oritavancin	Slow	Slow	150–300 (after 24 h)	Probably lysosomal
Oxazolidinones	Linezolid	Fast	Fast	~1	Unknown

[a] very fast: less than 3 min to equilibrium; fast: 3 to 15 min to equilibrium; slow: 15 min to 3 h to equilibrium; very slow: more than 3 h to equilibrium.

[b] C_c/C_E: accumulation factor (ratio between the cellular concentration and the extracellular concentration).

Macrolides

In sharp contrast with β-lactams, macrolides show a marked intracellular accumulation in almost all cells [5,14,15,22–27]. The extent of their accumulation, however, varies markedly among derivatives, with relatively low values for erythromycin and single-base macrolides, to extensive values for those macrolides carrying two basic functions. Overlap has been observed, however, indicating that other parameters are important. Beyond these variations, the common behavior of macrolides can be explained most easily by their character of weak bases, and applying exactly the same modeling as for the weak acids, with the result that the total drug concentration of weak bases must indeed be higher in acidic, membrane-bounded compartments. One additional factor, however, needs to be taken

into consideration. Cells contain a fairly acidic compartment, which is the lysosomal apparatus, the volume of which may not exceed 5% to 10% of the cell volume but in which the pH can be as low as 5 [28]. This creates a motive force and a potential for 100-fold accumulation of a single base drug, as compared with the extracellular milieu, to 10,000-fold for a dibasic drug [29]. Consequently, the bulk of the cell-associated macrolides is found in lysosomes and related vacuoles. Collapsing the pH gradient across the lysosomal and the pericellular membranes abolishes all accumulation [26]. Uptake and efflux of macrolides are generally rapid, with the notable exception of azithromycin, for which binding to cellular structures (mainly the phospholipids [30,31]) could play a critical role. A role of drug transporters with a link to $Ca2^{+}$ channels or a $Ca2^{+}$ channel-operated mechanism has been advocated for the uptake of macrolides but seems restricted to certain cell types. An efflux transporter modulating the accumulation of macrolides at equilibrium has been evidenced in murine macrophages but affects mainly azithromycin and erythromycin [32].

Fluoroquinolones

Fluoroquinolones have long been known to accumulate in eucaryotic cells [15,33–40]. The cellular concentrations of fluoroquinolones are generally 4- to 10-fold larger than the extracellular. This accumulation is rapid but there is no convincing explanation for its mechanism. A specific transport pathway has been tentatively identified in polymorphonuclear neutrophil leukocytes (PMN) for ciprofloxacin, together with an amino acid transporter activated by phorbol myristate acetate [41]. Uptake could also be regulated by the activation of protein kinase C and mitogen-activated protein kinase [42]. Yet, simple diffusion followed by loose binding to subcellular constituents cannot be excluded. Efflux of fluoroquinolones is faster than uptake and is probably mediated by an efflux transporter, which can be inhibited by probenecid [43] and has been provisionally identified as an multiple resistance-related protein (MRP) efflux transporter. Cell-associated fluoroquinolones have been consistently recovered in the final supernate after cell fractionation studies [35,44]. This can be interpreted in two different ways: efflux from a specific subcellular compartment is fast; or fluoroquinolones are genuinely localized in the cytosol, but probably able to diffuse in the various subcellular compartments as they do through the various organs of the body.

Aminoglycosides

Aminoglycosides have long been believed not to penetrate in eucaryotic cells. Studies in macrophages and in fibroblasts [45–47], however, have shown that cells incubated for several days in the presence of aminoglycosides accumulate these drugs to an apparent cellular-to-extracellular ratio of 2 to 4. Further studies demonstrated that intracellular aminoglycosides are almost exclusively sequestered in the lysosomes, which they access for most

cells through fluid-phase endocytosis [47–49]. This explains their slow rate of accumulation, which led many impatient observers erroneously to conclude about a lack of penetration. Cells displaying surface binding sites, such as kidney proximal tubular cells in vivo, however, accumulate aminoglycosides quite fast and extensively [50,51]. These sites have been identified as megalin (a protein binding polybasic compounds; [52–54]) on the one hand, and acidic phospholipids on the other hand [55].

Other antibiotics

Much less is known about the other antibiotics. Among the lincosaminides, clindamycin has been notorious for its large cellular accumulation [9,56], which has been ascribed to its basic character (see previous discussion for macrolides) and to the potential activity of a nucleoside transporter [57]. Surprisingly, however, its closely related congener lincomycin is only poorly accumulated by cells. The cellular pharmacokinetics of tetracyclines has not been studied in details, and apart from a few studies [58,59], there is only indirect or partial evidence of their ability to penetrate and accumulate in eucaryotic cells. The mechanisms remain obscure. PMNs incubated with chlortetracyclines have been shown to display a perinuclear fluorescent signal [60], but the data have never been further confirmed and no attempt at further studying the localization of tetracyclines by other techniques has been reported. Among ansamycins, rifampin accumulates from 2- to 10-fold according to the studies [61–63], whereas rifapentine shows a much higher accumulation (up to 60- to 80-fold [64]). The mechanism of this accumulation as well as the subcellular distribution of ansamycins remain, however, unknown. Few studies have dealt with glycopeptides. Vancomycin shows a slow uptake and modest accumulation in macrophages (up to eightfold in 24 hours [65]) and is supposed to accumulate in lysosomes (at least in proximal tubular cells of the kidney after in vivo administration [66]). Conversely, teicoplanin, a more lipophilic compound, shows a more extensive and faster accumulation (40- to 60-fold [64,67]) but its localization is not known. Oritavancin shows an exceptionally high accumulation in murine macrophages (between 150- and 300-fold after 24 hours [65]), and is probably located in lysosomes. Only one study has been published for oxazolidinones, in which linezolid was shown to reach intracellular concentrations only slightly above the extracellular one in PMNs and in McCoy cells [68]. Uptake and efflux are very fast with a maximal concentration reached within 5 minutes and 90% of the drug being released in less than 2 minutes on transfer to fresh medium.

Intracellular activity of antibiotics (cellular pharmacodynamics)

There is a massive amount of literature on the intracellular activity of antibiotics and on its relation to cellular accumulation and disposition,

dealing with a fascinating variety of different models spanning from in vitro to animal and clinical studies and a large number of drugs as can be seen from a series of key reviews over the last 15 years [69–81]. Few original studies, however, have systematically examined the relationship between drug concentration (or dosing); time of exposure (or other pertinent pharmacokinetic parameters); and chemotherapeutic response (in terms of quantitative measurement of the variation in the bacterial population). Moreover, in many experimental studies the extracellular concentrations and the timing of the experiments have often been unrealistically higher or lower than can be observed with patients. An additional difficulty that needs to be underscored is the fact that antibiotics may exert either favorable or unfavorable actions on the host cells, which modulates their activity and must be studied in detail. Quite anxiously, indeed, a recent review noted that "Overall, neutrophil-microbe interactions are complex and difficult to dissect, and carefully designed experiments using closely defined conditions are required if meaningful results are to be obtained" [82]. Clinical studies, in this context, are particularly difficult, because they tend to provide a global answer to what is actually influenced by a combination of complex extracellular and intracellular pharmacokinetic variables, together with another array of microbial and host-responses variables, and the simultaneous presence of extracellular and intracellular foci of infection. This has been evidenced clearly from studies with rifamycins [83], or more broadly speaking on antimycobacterial therapy [84]. All these factors explain why it remains so difficult to delineate the pharmacodynamic properties of antibiotics as far as intracellular activity is concerned, and why so many conflicting views have been expressed in this context. There are still lacking today in the field of intracellular infection the sound, systematic approaches that have been successfully used to determine the pharmacokinetic-pharmacodynamic parameters of antibiotics with respect to the extracellular infections.

There is nevertheless a consensus on the fact that macrolides, fluoroquinolones, tetracyclines, and ansamycins should have an activity against intracellular bacteria (and should be amenable to pharmacodynamic studies) because these drugs have been used successfully to treat a variety of both obligate and facultative intracellular organisms. A key question, however, remains whether this activity is optimal and whether it bears any relationship with the cell accumulation and subcellular disposition properties that have been summarized previously. Conversely, there is more or less also a consensus over the fact that β-lactams and aminoglycosides show no or only a poor intracellular activity. But here, one faces the realities that most of the in vitro studies supporting such a conclusion used short-term exposures only, and that β-lactams are effective in the treatment of listeriosis, and that aminoglycosides have been successfully used for decades for the treatment of tuberculosis. In both cases, a large part of the bacterial inoculum is intracellular. Many other paradoxical situations could be

discussed, but only add to the confusion if dealt in details without placing the whole issue in a broader perspective.

Actually, some of the paradoxes may become understandable if considering carefully the in vitro results presented for *L monocytogenes* in Fig. 2 and for *Staphylococcus aureus* in Fig. 3. In the first model, one sees in the left panel that a fluoroquinolone (moxifloxacin) has essentially the same activity, as function of its extracellular concentration, against intracellular and extracellular bacteria. The paradoxes here are that moxifloxacin is a concentration-dependent antibiotic (like all fluoroquinolones) and is accumulated about sevenfold in the cells where *L monocytogenes* is multiplying. Moreover, both moxifloxacin and *L monocytogenes* are in the

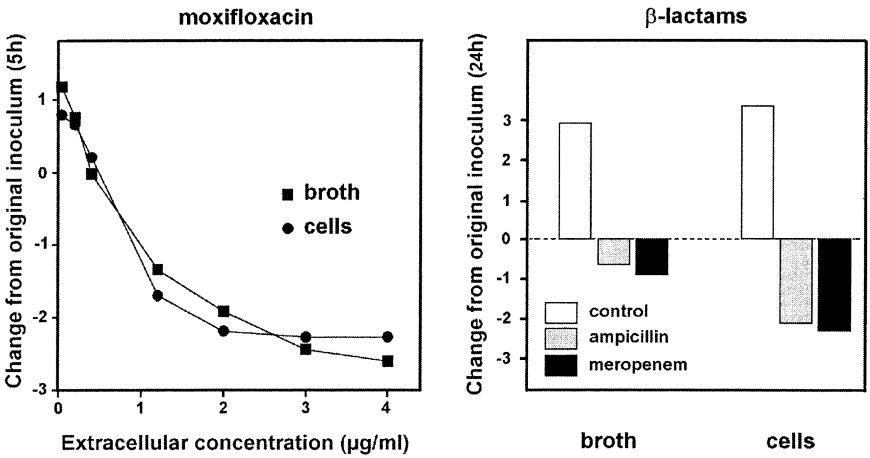

Fig. 2. Evidencing some of the paradoxes in the intracellular activity of antibiotics. The figures show the antibacterial activity of moxifloxacin (*left*) and β-lactams (ampicillin, meropenem) against extracellular (broth) and intracellular (cells [THP-1 macrophages]) *Listeria monocytogenes*. (*Left*) Influence of an increase in moxifloxacin concentration in broth or in the extracellular milieu on the change in colony-forming units in a 5-h model. The paradox here is that moxifloxacin, which is clearly a concentration-dependent antibiotic, does not act more efficaciously in cells although it accumulates about sevenfold (as determined by both fluorometric and bioassay). This suggests that a large part of the intracellular drug is prevented from acting against *Listeria*. (*From* Carryn S, Van Bambeke F, Mingeot-Leclercq MP, Tulkens PM. Comparative intracellular [THP-1 macrophage] and extracellular activities of beta-lactams, azithromycin, gentamicin, and fluoroquinolones against *Listeria monocytogenes* at clinically relevant concentrations. Antimicrob Agents Chemother 2002;46:2095–103; with permission.) (*Right*) Antibacterial activity of ampicillin and meropenem (both at 50 mg/L) against extracellular (broth) and intracellular (cells [THP-1 macrophages]) *L monocytogenes* in a 24-h model. The paradox here is that both β-lactams are more active in cells than in broth, even though they do not accumulate in cells (as determined by bioassay). This is only seen after 24 h, because only little activity is observed in the 5-h model. (*Adapted from* Carryn S, Van Bambeke F, Mingeot-Leclercq MP, Tulkens PM. Activity of {beta}-lactams (ampicillin, meropenem), gentamicin, azithromycin and moxifloxacin against intracellular *Listeria monocytogenes* in a 24 h THP-1 human macrophage model. J Antimicrob Chemother 2003;51:1051–2; with permission.)

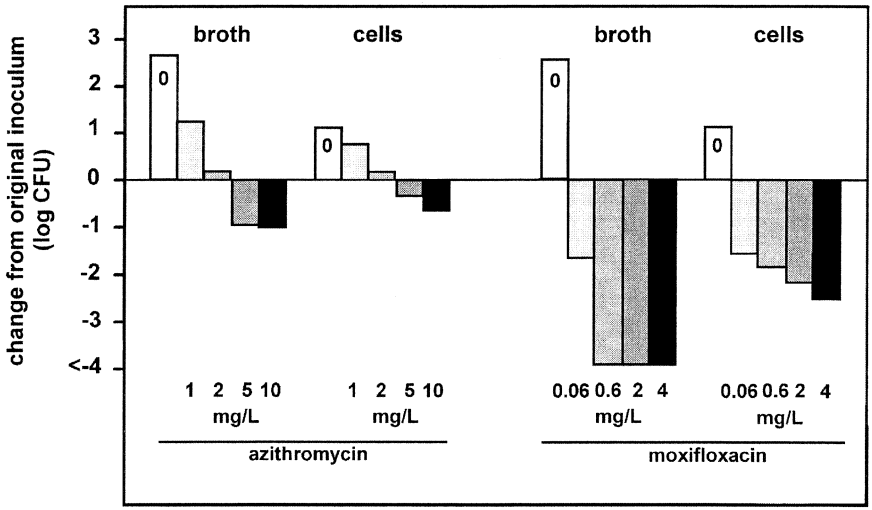

Fig. 3. Evidencing some of the paradoxes in the intracellular activity of antibiotics. The figures show the antibacterial activity of azithromycin (left) and moxifloxacin (right) at increasing concentrations against extracellular (broth) and intracellular (cells [J774 macrophages]) *S aureus* (24 h model). model; the white bars with a 0 are controls without antibiotic (broth) or with gentamicin (1 X the MIC, to prevent extracellular growth of *S aureus*; gentamicin is not added when azithromycin or moxiflxoacin are present). The paradox here is that azithromycin, which concentrates about 30-fold in cells (confirmed by bioassay) and is concentrated in phagolysosomes where *S aureus* sojourns, is less active intracellularly than extracellularly. In contrast, moxifloxacin, which is less accumulated than azithromycin and does not concentrate in phagolysosomes, shows a definite bactericidal effect against intracellular *S aureus*. Part of the paradox could be explained by the observation that the MIC and MBC of azithromycin and moxifloxacin against the strain of *S aureus* used were 0.5/8 and 0.06/0.06 at pH 7, and 512/512 and 0.25/1 mg/L at pH 5. Note also that the serum concentration of azithromycin in patients does not exceed 0.5 mg/L suggesting that the drug will be inefficacious in vivo, whereas moxifloxacin may reach a concentration of 4 mg/L, at which it shows a marked activity in this model. (*Adapted from* Seral C, Van Bambeke F, Tulkens PM. Quantitative analysis of gentamicin, azithromycin, telithromycin, ciprofloxacin, moxifloxacin, and oritavancin (LY333328) activities against intracellular *Staphylococcus aureus* in mouse J774 macrophages. Antimicrob Agents Chemother 2003;47:2283–92; with permission.)

cytosol and therefore should be in direct contact. It seems that the activity of moxifloxacin is impaired intracellularly exactly in proportion to its accumulation, which can be considered as self-defeating in this respect. The right panel of Fig. 3 shows that β-lactams are bactericidal against intracellular *Listeria* and to almost the same extent than moxifloxacin. The paradoxes here are that neither ampicillin nor meropenem accumulate in cells but their activity is nevertheless larger against the intracellular *Listeria* than against the extracellular ones; and that the β-lactams eventually appear almost as active intracellularly as moxifloxacin. There is, however an essential difference between the two models, which is that the second uses a 24-hour incubation time, whereas the first is limited to 5 hours. If the

incubation with β-lactams is limited to 5 hours, very little activity is seen [15,85]. Thus, the conclusion here is that intracellular fluoroquinolones act rapidly in a concentration-dependent manner but in a limited fashion and in a suboptimal way (interestingly enough, moxifloxacin has been found effective in animal models of listeriosis, but one lacks of clinical data). In contrast, β-lactams act slowly in a concentration-independent manner (see [15] for detailed dose-dependence studies), but become effective if prolonged contact is obtained. Opposite conclusions contradicting the clinical experience are reached if not for examining the influence of time in this setup.

Fig. 3 illustrates another paradox using the *S aureus* model and comparing azithromycin and moxifloxacin. The huge accumulation of azithromycin in cells, and its co-localization with *S aureus* in the phagolysosomes, would make many to predict a large activity. Yet, one sees that azithromycin, at an extracellular concentration of 1 mg/L, is only bacteriostatic against intracellular bacteria (no gain is obtained if moving to the clinically unrealistic extracellular concentration of 10 mg/L). This implies that eradication with azithromycin will require host factors (which are not much present in this model). Conversely, moxifloxacin, which is not concentrated in lysosomes and accumulates much less than azithromycin, is clearly bactericidal on a concentration-dependent fashion. Note, however, that as for the *L monocytogenes* model, moxifloxacin is less effective intracellularly than extracellularly. The reasons for these contrasting and apparently paradoxical behaviors are that azithromycin is, in almost all instances, a bacteriostatic antibiotic for which high local concentrations are probably useless per se (although they may ensure a prolonged exposure), whereas the local environment, and especially the acidic pH, which is highly unfavorable to the activity of azithromycin, only slightly affects that of moxifloxacin, a bactericidal drug (about fourfold increase in minimum inhibitory concentration [MIC] at pH 5 versus 7).

These and other similar observations have led the authors to propose the scheme presented in Fig. 4, which illustrates the main parameters that may critically influence the activity of antibiotics against intracellular bacteria (and explains many of the paradoxes and contradictions found in the literature). Table 3 lists the pharmacokinetic-pharmacodynamic properties that can be expected for the main classes of antibiotics in this context. Three aspects need to be underlined. First, most of the basic observations made concerning extracellular infections are observed for intracellular activity. β-Lactams are time-dependent, whereas fluroquinolones and aminoglycosides are clearly concentration-dependent. For β-lactams, this definitely justifies prolonged treatments at the maximal dose to compensate for the lack of accumulation and suggests that there is a place for continuous infusion as advocated for in systemic infections [86]. For macrolides, activity is clearly observed against phagosomal organisms (phagosomes are neutral or only slightly acidic), but to a level that has no relationship with their huge

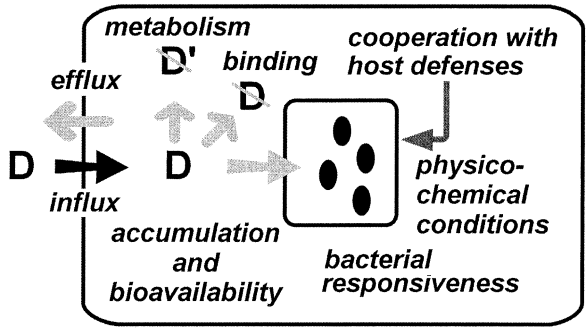

Fig. 4. Factors affecting the intracellular activity of antibiotics. The balance between influx and efflux, metabolism, and binding determines the intracellular concentration of free active drug. The latter must, however, still be able to reach its target (the box is only intended to show that such access may be prevented but does not imply that all bacteria are in membrane-bounded structures). Activity is then influenced by the state of bacterial responsiveness; the physicochemical conditions prevailing at the site of infection; and the degree of cooperation (or hindrance) with the host defenses. As a result, the final outcome may bear only a very remote correlation with the actual extracellular drug concentration and even the degree of cellular concentration. This, however, does not mean that cell penetration and cell concentration are irrelevant because an absence of penetration or an insufficient local concentration can never be associated with activity.

accumulation, probably because of their intrinsically bacteriostatic activity. The goal here probably is to reach a sufficiently high concentration to cope with the loss of activity caused by low pH or binding to cell constituents, but little gain is expected by further increasing the concentration. The point of a critical concentration should be underlined. Indeed, whereas some organisms, like *Chlamydia* and *Legionella*, are quite sensitive, this may not be the case for others, such as *S aureus*. The importance of a concentration threshold in intracellular activity has been demonstrated recently by the description of clinical failures with *Chlamydia* showing resistance to concentrations of azithromycin of 4 mg/L or higher in the BGMK cell assay system [87]. Interestingly enough, an increase in azithromycin accumulation, as obtained by inhibiting its efflux from macrophages, has been shown to decrease the extracellular concentration needed to obtain a bacteriostatic affect in the *S aureus*/J774 macrophage model depicted in Fig. 3 [44]. In contrast to both β-lactams and macrolides, concentration seems a critical determinant for fluoroquinolones and these antibiotics show typical concentration-dependent efficacy. Because fluoroquinolones also seem to kill fast, time tends to become less important, which suggests that area under the plasma-concentration curve is not a major determinant. One is struck, however, by the impairment of activity, which may make fluoroquinolones ineffective unless a sufficiently high extracellular concentration can be reached. This limitation was already underlined for *Listeria* [88,89], and may be critical for *S aureus* if considering methicillin-

Table 3
Proposed intracellular pharmacodynamic parameters for the main pharmacochemical classes of antibiotics and modulation of activity by the cellular environment

Pharmacochemical class	Proposed pharmacodynamic parameter	Modulation by the environment at the predominant localization in cell	Overall result and expected clinical implication
β-lactams	Time of exposure	Favored (after long exposure)	Activity remains low but can be larger than expected for organisms in cytosol treatments must be prolonged
Macrolides	Critical concentration (but remain essentially static; time, therefore, seems unimportant)	Markedly decreased acidic pH; binding to cellular constituents)	Act on organisms in phagolysosomes but to an extent lower than anticipated
Fluoroquinolones	Concentration (but eradication may not be obtained); act rapidly so that time may be unimportant beyond a few hours	Decreased (to an amount equal or larger than the cellular accumulation)	Activity depends on the extracellular concentration; dosing is probably essential
Aminoglycosides	Concentration (but activity develops slowly because of the low rate of uptake, so that time is important)	Severe impairment (acid pH). Note: inappropriate subcellular localization and lack of diffusion may also be critical	Activity depends on both the dose and the time of exposure; prolonged treatments are required
Glycopeptides			
Vancomycin	Time	?	Activity remains low; not recommended in clinical practice
Oritavancin	Concentration	Decreased	Effective but could be restricted to phagolysosomal organisms

The list is limited to classes for which sufficient data are available.

resistant organisms, because those tend to show elevated MIC toward fluoroquinolones. A puzzling observation for fluoroquinolones is also the lack of eradication even when concentrations are increased to several multiples of the MIC. This suggests that part of the inoculum is inaccessible or metabolically insensitive to fluoroquinolones. This has been observed not only with *L monocytogenes* [15] but also with *S aureus* [90] and *Chlamydia* spp. [91] and does not seem to be linked to selection of resistant mutants. It is tempting to speculate that this lack of eradication could be the reason for failure of fluroquinolones with *Brucella* infection in vivo [92], which is characterized by the maintenance of a residual inoculum from which reinfection is observed. For aminoglycosides, their specific pharmacokinetic properties (ie, a slow uptake) make prolonged treatments essential. Time becomes an important parameter in addition to concentration. A severe impairment of their activity is also noted, which may explain failures under conditions of inappropriate dosing or accumulation time (the low pH of phagolysosomes is probably responsible for this loss of activity because its neutralization increases activity [93]). Unfortunately, much less can be said about the other antibiotics, with the exception of oritavancin, which has been recently studied but for which more experience is needed. No or very little true pharmacodynamic data are available for ansamycins (including rifampin) or tetracyclines, even though these antibiotics were among the first to be claimed to be active in a variety of intracellular infections [94–98].

Summary

This article establishes the pharmacokinetic-pharmacodynamic parameters that are important when considering the intracellular activity of antibiotics. Generally speaking, the main classes of antibiotics seem to share globally the same properties against extracellular and intracellular organisms. The specific cellular pharmacokinetic properties may modulate those parameters so as to let other ones to become critical. Simple rules, such as equating accumulation and activity, are certainly incorrect, and other determinants need to be added to the equation. Finally, this article emphasizes the fact that much remains to be done in this area before rational therapeutic choices can be made.

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