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Review Article

Intracellular habitat of pathogenic microorganisms: implications for antimicrobial chemotherapy

Abstract

A few pathogenic bacteria have developed various strategies to live in particular intracellular compartments. Within such specific niches the conditions for antibiotic activities may be quite different from the milieu given in the laboratory for in vitro testing of antimicrobial susceptibility according to recommended procedures such as EUCAST or CLSI. There are some examples when intracellular pathogens are not affected by an antimicrobial agent, although they are susceptible in vitro, namely Listeriae are in vitro susceptible to vancomycin but this antibiotic does not work on intracellular Listeria. On the other hand, it could happen that in the intracellular bacteria are susceptible to an agent which is ineffective under recommended in vitro test conditions, namely Listeriae are generally resistant to fosfomycin in vitro but fosfomycin is active against intracellular Listeriae. Prerequisites for effective treatment are: the microorganism is vulnerable, i.e. not refractory, during the intracellular living, the drug gets access to the site of residence (some drugs do not penetrate and some others are exported by efflux pumps) and the drug remains active under these particular conditions. Furthermore, the help of the host's own defense system is required. Therefore, antimicrobial therapy of infections with intracellular microorganisms should respect these aspects.

Keywords: intracellular bacteria, antibiotics, fosfomycin, quinolones, macrolides, transport mechanisms, efflux pumps

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Introduction

Good in vitro susceptibility of pathogens to antimicrobials is not always a reliable predictor for a therapeutic success. For example, when susceptible microorganisms reside in a biofilm or even within host cells, they may be either recalcitrant (refractory) or unaccessible.

Not only viruses but also several bacteria and protozoa (Table 1) are *obligatory intracellular* parasites. Those microorganisms may reside and multiply exclusively within host cells. They are absolutely dependent on the conditions in this particular niche; both energy and nutrients produced by the host cell are made available to the microorganisms. In addition some other bacteria as well as fungi are so-called *facultatively intracellular* pathogens (Table 1); in principle they are able to grow on non-living and even artificial media providing the required chemical substrates for their own metabolism. They adapt to the particular environmental conditions by activating some additional genes present in their genome, when they have the chance to get into a host cells. And indeed, at least in certain stages of infectious diseases with these pathogens, the bacteria and fungi are found in the intracellular environment and this fact plays a definite role in the pathogenesis. Furthermore, one has to keep in mind that a large series of bacteria and fungi (Table 1) is found *occasionally intracellularly* during an infectious process, since many bacteria are able to survive under those special conditions at least for a certain while. In fact, these categories are not always strictly separated from each other, so that this classification is rather arbitrary.

The intracellular habitat will have major consequences on the metabolic activity of pathogens [1]. Furthermore, the intracellular location of pathogens will trigger definite metabolic reactions in the host cell [2]. Moreover, the enclosed microorganisms are protected against the attack by several non-specific as well as humoral immune defense mechanisms of the host, which means that primarily cell-mediated immune reactions are required to stop the intracellular multiplication of parasites [3].

All these quite various specific features will influence the activities of antimicrobial agents on intracellular pathogens [4], [5].

Various host cells may be misused by pathogens

Several types of human or animal cells may host pathogens for more or less long periods.

a) Phagocytes: The role of phagocytes, i.e. mainly granulocytes, dentritic cells, monocytes, migrating and resident macrophages, during infection is to scavenge penetrating microorganisms from the tissues [6]. They

Table 1: Some typical examples of intracellular parasitism of microorganisms

A: obligate intracellular parasites
Viruses: all
Bacteria: Chlamydia, Rickettsia, Ehrlichia
Protozoa: Plasmodium, Toxoplasma, Leishmania, Trypanosoma
B: facultative intracellular parasites
Bacteria: Mycobacterium, Listeria, Legionella, Bartonella, Francisella, Coxiella, Yersinia, Brucella, Salmonella, Shigella, Burkholderia
Fungi: Histoplasma, Coccidioides, Cryptococcus, Microsporidia
C: occasional intracellular parasites
Bacteria: Staphylococcus, Neisseria, Borrelia, Haemophilus, Escherichia
Fungi: Candida

Table 2: Residence of some bacteria and protozoa in various intracellular compartments

a) *Phagocytic vacuole/Phagolysosome*: Leishmania,Toxoplasma gondii, Salmonella, Small colony variants of Staphylococcus aureus (some pathogens are able to inhibit the fusion of lysosomes with the phagocytic vacuole)
b) *Cytoplasm*: Listeria, Trypansosoma cruzi
c) *Nucleus*: certain bacteria such as Listeria monocytogenes are able to invade into the nucleus of host cells.

are endowed to take up particles by invaginations of the cell membrane [7] and to internalize them and to detain them in a phagocytic vacuole. By activation of several hostile components (reactive oxygen species, H^+ ions, nitric oxide, cationic antimicrobial peptides such as defensins and microbicidal enzymes) [8] most phagocytosed parasites are inactivated and destroyed rapidly.

Obviously, certain lines of monocytes which are not bactericidal effector cell, can get infected by L. monocytogenes and may allow intracellular growth; like Trojan horses they will transport the protected pathogens to other sites and even across anatomical barriers; by this way some bacteria may enter the CNS [9].

b) Epithelial cells: Escherichia coli may penetrate into uroepithelial cells and can survive in this intracellular site an antibiotic exposure; thus the intracellular residence is one reason – besides biofilm formation – for recurrence of urinary tract infections [10]. Small colony variants from Staphylococcus aureus may persist in the epithelial cells of mammary glands and hence may produce chronic infections [11].

c) Endothelial cells: The vascular endothelium is the main target of a number of infectious agents, besides several viruses especially Rickettsia and Ehrlichia are among them. These arthropod-transmitted obligate-intracellular bacteria cause serious systemic diseases that are not infrequently lethal [12].

d) Erythrocytes: Plasmodia cross the (semi)-rigid membrane of erythrocytes not by drilling a pore but the protozoa rather trigger an invagination of the host's membrane similar to the process seen in phagocytes [13]. Later on the parasites are lying intracellularly in a vacuole escaping recognition by the immune system. The intracellular pathogens are able to modify the host cells by increasing the permeability of the erythrocyte membrane due to activating channels with active transport systems [14].

e) Combination: Several bacteria are not qualified for a particular host cell but are able to infect a large array of different host cells.

The various compartments of intracellular residence

There are several possible mechanisms of entry [7], which will influence the intracellular fate and final localization of the pathogen. The intracellular site of sequestration seems to be specific for each pathogen and depends on the presence of appropriate virulence factors and alteration of particular bacterial metabolism pathways so that they are able to survive and to multiply within characteristic niches [15]. The strategies of the various pathogens are quite variable (Table 2) [16].

The conditions in the particular environments

In the standard operation procedures (SOPs) for antimicrobial in vitro testing the milieu factor plays a critical role for the test results. In general a neutral pH, physiologic salt concentrations and definite supplementations with sugars, proteins, vitamins and essential ions are given, so that the bacteria may multiply quite well under such conditions. Within host cells the milieu is quite different from those in vitro conditions and may vary in various compartments, which means that the growth and the growth rate of bacteria as well as the activity of antibiotics may be different from in vitro conditions. It is well known that fast replicating bacteria constructing plenty of new cell wall components are more susceptible to antibiotics which interfere with these processes of cell wall construction like betalactams, than those who are resting alive without multiplication [17]. During a quiescent stage the pathogens may be recalcitrant to certain antibiotics, which can be the reason for intracellular survival and endogenous exacerbation after the end of antibiotic therapy.

Table 3: Concentrations of some antibiotics within host cells

a) *high intracellular concentration* (ratio intra-/extracellular >10): macrolides, azalides, ketolides, clindamycin
b) *moderate intracellular concentration* (ratio intra-/extracellular 1–10): quinolones, rifampicin, tetracycline, chloramphenicol, fosfomycin
c) *low intracellular concentration* (ratio intra-/extracellular <1): penicillin, ampicillin, cephalosporins, imipenem, aminoglycosides

The stability of antibiotics strongly depends on many environmental factors such as ion supplementation and pH. The ion composition in the local situation may influence the ability of antibiotics to gain access to the proper target in the bacterial cell. The transport of antibiotics across the bacterial cell wall and cytoplasmic membrane or the permeability of the pores, through which the antibiotics normally penetrate, are highly sensitive to changes in the ion concentration and composition.

The implications for antimicrobial chemotherapy

Before antimicrobials can act against intracellular pathogens several prerequisites have to be fulfilled:

a) Intracellular accumulation of antimicrobials

The host cell membrane is a lipid bilayer and therefore a natural barrier for free, passive diffusion for most antibiotics. In general lipid-soluble antibiotics, such as rifampicin, chloramphenicol and trimethoprim, are rather well enabled to cross the membrane of living as well as dead host cells. Some ionized antibiotics, for example some penicillin derivatives at least in their salt configuration reacting like weak acids, may cross the barrier, however in rather small amounts only. On the other hand even active transport can take place, since there are several import systems naturally delivering nutrients, which may be misused by a few antibiotics. Furthermore, some antibiotics may gain access to the host cell via vesicle pinocytosis. By typical phagocytosis antibiotics integrated into liposomes may be taken up by phagocytes such as granulocytes, monocytes, macrophages [5], [18] but not by epithelial cells.

On the other hand, the intracellular concentration of a drug is also influenced by export systems of the host cell [5]. Several systems exist for example the so-called ABC transporters [19] or the MATE (*m*ulti-*ant*imicrobial *e*xtrusion) proteins [20] which among other host cell compounds and drugs also export antimicrobials. Some antibiotics are good substrates for those efflux pumps, which means that the antibiotics are exported by some cells before they can reach their target in the bacterium [21]. Thus, consequently the various antibiotics differ definitely in their intracellular concentrations (Table 3).

Azithromycin, for example, is notably accumulated within host cells, since about 40fold intracellular concentrations are found in granulocytes within 1 hour without disturbing their functions. These cells may serve as a vehicle and can deliver the antibiotic at the site of infection [22].

The transport of aminoglycosides via the lipid bilayer membrane into host cells is slow, so that after short-term exposition the intracellular levels do not achieve relevant concentrations; after continuous administration, however, these antibiotics are trapped and concentrated in special sites (see below).

b) Intracellular distribution of antimicrobials (Figure 1)

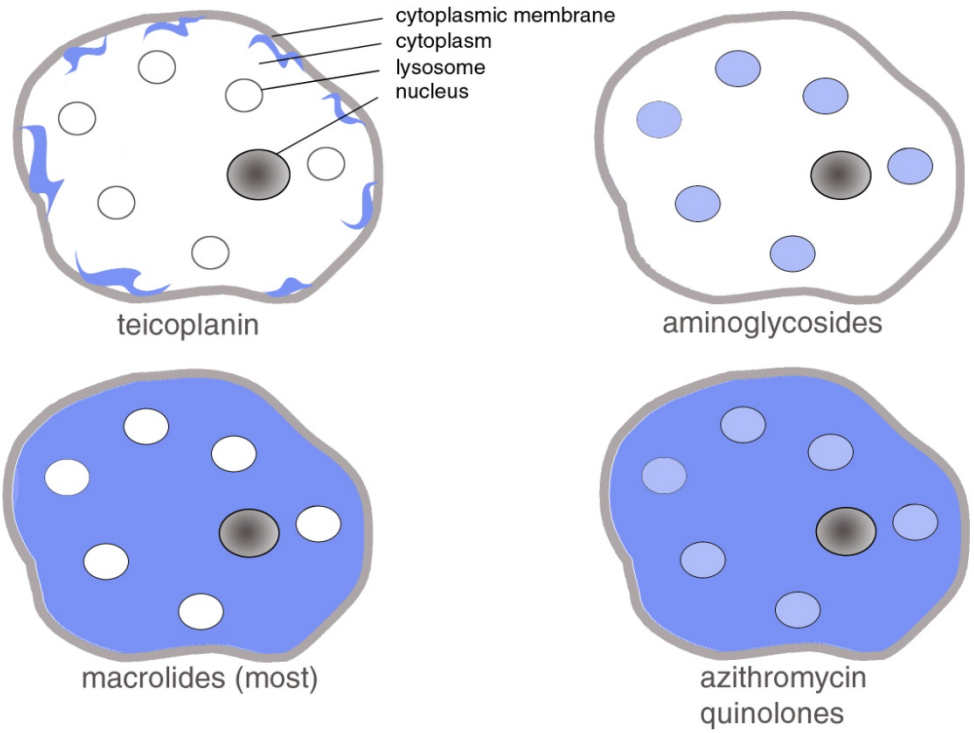
- Glycopeptides such as vancomycin and teicoplanin will stick to the lipid layer of the cellular membrane and cannot reach internalized bacteria in the inner parts of the host cell.
- By lysosomal trapping aminoglycosides are accumulated in distinct locations. In the rather acid milieu of this compartment these antibiotics will be present in a protonated form which is devoid of antibacterial activity. This depot is drained slowly over several months after end of exposition.
- Most macrolides, such as erythromycin, roxithromycin and clarithromycin are more or less equally distributed in the cytoplasm.
- Azithromycin also is found in the cytoplasm; in addition it penetrates into the lysosomal compartment, too. This also applies to quinolones.

c) Intracellular activity or inactivity of antimicrobials

A poor correlation between the intracellular accumulation of the antibiotics, for example dicloxacillin, cefuroxime, gentamicin and rifampicin, and the actual intracellular effect was found [23]. The efficiency of antibiotics against intracellular bacteria is rather dependant on several other factors such as ionic strength and pH, which will influence their chemical stability, as well as on the refractoriness of the bacteria under the given conditions. For example small colony variants of staphylococci grow slowly and therefore are recalcitrant to the activity of betalactams [17]. Furthermore, the stability of most antibiotics is largely dependent on neutral pH. The betalactams, for example, are inactivated at low pH; but the various members vary in their stability [24]; penicillin G is much more susceptible to low pH than ampicillin and amoxicillin [25]. Aminoglycosides are inactive in the lysosomal vacuoles with low pH, because in such conditions they are rendered in a protonated, i.e. inactive form [5]. In contrast, the activity of rifampicin is enhanced in an acidic environment [5].

d) Special situations

Listeriae when tested in vitro according to CLSI or EUCAST recommending test media with a high glucose content



1) Antibiotics with an lipid ancor, such as teicoplanin, get stuck to the lipid bilayer of the cell-membrane and cannot further progress
2) Other antibiotics, such as macrolides, will dissipate evenly throughout the cytoplasm avoiding some specific sites like the lysosomes
3) Some antibiotics, such as azithromycin as well as quinolones, residing in the cytoplasm can diffuse into the lysosomes and back, when the concentration in the cytoplasm decreases
4) Aminoglycosides are trapped into the lysosomes where they exclusively reside, since in this acidic environment the molecule gets protonated and this particular chemical form can hardly move from this cage (such a depot is evacuated only after long periods of time)

Figure 1: Schematic depiction of the intracellular localization of some antibiotics (according to [5])

are generally resistant to fosfomycin, because the drug cannot be transported across the bacterial wall and membrane under this situation. Once Listeriae are intracellular, they activate a transport system for the uptake of glucose only scarcely available in the host cell and this influx pump of Listeriae will – by chance – also transport fosfomycin into the bacteria. Hence, there is a paradox situation that fosfomycin is inactive in vitro but active in vivo [26]. Also intracellular Salmonellae get highly susceptible to fosfomycin [27].

e) Immunomodulatory effects of antibiotics on the host's defense cells

At least some antibiotics exert pleiotropic effects. This means that they not only attack the bacteria but by quite other mechanisms they trigger effects in host cells. The induction of cytochrome P450 enzymes in liver cells by rifampicin is a well known fact. But furthermore, some antibiotics will either enhance or block the activity of the host's defense cells, namely, granulocytes, macrophages or even lymphocytes [28], which may influence the fate of intracellular bacteria.

f) Vulnerability of intracellular bacteria

When bacteria grow rapidly, for example under favorable growth conditions as provided by in vitro testing protocols, their machineries for protein synthesis as well as cell wall construction are strongly activated. In such conditions they are obviously highly susceptible to those antibiotics which interfere with protein or cell wall synthesis. The in vivo conditions, however, are hostile or at least unfavorable, in particular when bacteria reside in the special niches within host cells, and thus will possibly allow survival only or at most slow growth, so that the bacteria are often much less vulnerable to those agents. This means that there is often no close correlation between in vitro activity of antibiotics and intracellular efficiency.

Conclusion

There is a long list of obligate, facultative or occasional intracellular pathogens (Table 1). These microorganisms have adapted by various strategies to the particular conditions within distinct compartments of different host cells. Within such sites and niches the conditions for the action of antimicrobials may be quite different from the milieu given in the laboratory according to the recommendations of either CLSI or EUCAST for in vitro testing of antimicrobial susceptibility. Hence, the practical value of these conventional test methods is limited more or less to infections with extracellular microorganisms.

Table 4: Prerequisites for good therapeutic activities of antimicrobials against intracellular pathogens

– A priori susceptibility of the pathogen to a given antibiotic (the in vitro test conditions according to EUCAST or CLSI will give results which are not unanimously relevant for intracellular pathogens)
– Vulnerability of the pathogen under the given stage of multiplication
– Penetration and eventually accumulation of drugs within the resident host cell (this may differ from cell type to cell type)
– Distribution of drugs to the right compartment (cytoplasm, phagocytic vacuole, nucleus) i.e. where the pathogen resides
– Favorable conditions for full activity of the antibiotic on the site of residence
– Support by the host's own defense and immune system against the pathogens

Therefore, the clinician should respect some prerequisites for therapy of infections with intracellular microorganisms with antimicrobials (Table 4). An effective treatment is achieved, when the microorganism is susceptible and not refractory, when the drug gets access to the site of residence and when the drug remains active under these particular conditions.

Notes

Competing interests

The author declares that he has no competing interests.

References

1. Fuchs TM, Eisenreich W, Kern T, Dandekar T. Toward a Systemic Understanding of Listeria monocytogenes Metabolism during Infection. Front Microbiol. 2012;3:23. DOI: 10.3389/fmicb.2012.00023
2. Eisenreich W, Heesemann J, Rudel T, Goebel W. Metabolic host responses to infection by intracellular bacterial pathogens. Front Cell Infect Microbiol. 2013 Jul 9;3:24. DOI: 10.3389/fcimb.2013.00024
3. Bajénoff M, Narni-Mancinelli E, Brau F, Lauvau G. Visualizing early splenic memory CD8+ T cells reactivation against intracellular bacteria in the mouse. PLoS ONE. 2010;5(7):e11524. DOI: 10.1371/journal.pone.0011524
4. Raoult D, editor. Antimicrobial agents and intracellular pathogens. Boca Raton: CRC Press; 1993.
5. Hof H. Antibiotic treatment of infections with intracellular bacteria. In: Paradise LJ, Friedman H, Bendinelli M, editors. Opportunistic intracellular bacteria and immunity. New York: Plenum Press; 1999. p. 281-93.
6. Kantari C, Pederzoli-Ribeil M, Witko-Sarsat V. The role of neutrophils and monocytes in innate immunity. Contrib Microbiol. 2008;15:118-46. DOI: 10.1159/000136335
7. Pizarro-Cerdá J, Bonazzi M, Cossart P. Clathrin-mediated endocytosis: what works for small, also works for big. Bioessays. 2010 Jun;32(6):496-504. DOI: 10.1002/bies.200900172
8. Harrison CJ. Innate immunity as a key element in host defense against methicillin resistant Staphylococcus aureus. Minerva Pediatr. 2009 Oct;61(5):503-14.
9. Drevets DA, Schawang JE, Mandava VK, Dillon MJ, Leenen PJ. Severe Listeria monocytogenes infection induces development of monocytes with distinct phenotypic and functional features. J Immunol. 2010 Aug;185(4):2432-41. DOI: 10.4049/jimmunol.1000486
10. Hunstad DA, Justice SS. Intracellular lifestyles and immune evasion strategies of uropathogenic Escherichia coli. Annu Rev Microbiol. 2010;64:203-21. DOI: 10.1146/annurev.micro.112408.134258
11. Atalla H, Gyles C, Mallard B. Persistence of a Staphylococcus aureus small colony variants (S. aureus SCV) within bovine mammary epithelial cells. Vet Microbiol. 2010 Jul;143(2-4):319-28. DOI: 10.1016/j.vetmic.2009.11.030
12. Valbuena G, Walker DH. Infection of the endothelium by members of the order Rickettsiales. Thromb Haemost. 2009 Dec;102(6):1071-9. DOI: 10.1160/TH09-03-0186
13. Chitnis CE, Blackman MJ. Host cell invasion by malaria parasites. Parasitol Today (Regul Ed). 2000 Oct;16(10):411-5. DOI: 10.1016/S0169-4758(00)01756-7
14. Hill DA, Desai SA. Malaria parasite mutants with altered erythrocyte permeability: a new drug resistance mechanism and important molecular tool. Future Microbiol. 2010 Jan;5(1):81-97. DOI: 10.2217/fmb.09.109
15. Freitag NE, Port GC, Miner MD. Listeria monocytogenes - from saprophyte to intracellular pathogen. Nat Rev Microbiol. 2009 Sep;7(9):623-8. DOI: 10.1038/nrmicro2171
16. Raoult D, Brouqui P. Intracellular location of microorganisms. In: Raoult D, editor. Antimicrobial agents and intracellular pathogens. Boca Raton: CRC Press; 1993. p. 39-61.
17. Looney WJ. Small-colony variants of Staphylococcus aureus. Br J Biomed Sci. 2000;57(4):317-22.
18. Maurin M, Raoult D. Antibiotic penetration within eukarytic cells. In: Raoult D, editor. Antimicrobial agents and intracellular pathogens. Boca Raton: CRC Press; 1993. p. 23-37.
19. Alvarez AI, Pérez M, Prieto JG, Molina AJ, Real R, Merino G. Fluoroquinolone efflux mediated by ABC transporters. J Pharm Sci. 2008 Sep;97(9):3483-93. DOI: 10.1002/jps.21233
20. Ohta KY, Imamura Y, Okudaira N, Atsumi R, Inoue K, Yuasa H. Functional characterization of multidrug and toxin extrusion protein 1 as a facilitative transporter for fluoroquinolones. J Pharmacol Exp Ther. 2009 Feb;328(2):628-34. DOI: 10.1124/jpet.108.142257
21. Nichterlein T, Kretschmar M, Schadt A, Meyer A, Wildfeuer A, Laufen H, Hof H. Reduced intracellular activity of antibiotics against Listeria monocytogenes in multidrug resistant cells. Int J Antimicrob Agents. 1998 May;10(2):119-25. DOI: 10.1016/S0924-8579(98)00030-2
22. McDonald PJ, Pruul H. Phagocyte uptake and transport of azithromycin. Eur J Clin Microbiol Infect Dis. 1991 Oct;10(10):828-33. DOI: 10.1007/BF01975835
23. Sandberg A, Hessler JH, Skov RL, Blom J, Frimodt-Møller N. Intracellular activity of antibiotics against Staphylococcus aureus in a mouse peritonitis model. Antimicrob Agents Chemother. 2009 May;53(5):1874-83. DOI: 10.1128/AAC.01605-07
24. Hou JP, Poole JW. Kinetics and mechanism of degradation of ampicillin in solution. J Pharm Sci. 1969 Apr;58(4):447-54. DOI: 10.1002/jps.2600580412

25. Jerzsele A, Nagy G. The stability of amoxicillin trihydrate and potassium clavulanate combination in aqueous solutions. Acta Vet Hung. 2009 Dec;57(4):485-93. DOI: 10.1556/AVet.57.2009.4.3

26. Scortti M, Lacharme-Lora L, Wagner M, Chico-Calero I, Losito P, Vázquez-Boland JA. Coexpression of virulence and fosfomycin susceptibility in Listeria: molecular basis of an antimicrobial in vitro-in vivo paradox. Nat Med. 2006 May;12(5):515-7. DOI: 10.1038/nm1396

27. Okada N, Nishio M, Danbara H. Intracellular activity of fosfomycin against two distinct enteropathogenic bacteria, Salmonella enterica and Listeria monocytogenes, alive inside host cells. Chemotherapy. 2003 May;49(1-2):49-55. DOI: 10.1159/000069787

28. Labro MT. Interference of antibacterial agents with phagocyte functions: immunomodulation or "immuno-fairy tales"? Clin Microbiol Rev. 2000 Oct;13(4):615-50.

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