Expert Opinion

Biodegradable gentamicin delivery systems for parenteral use for the treatment of intracellular bacterial infections

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Gentamicin is an aminoglycoside with a wide spectrum of antibacterial activity. However, as a highly water-soluble drug, it penetrates cells poorly. This constitutes a particularly important drawback for treating intracellular bacterial infections. This major hurdle may be solved by the use of vectors to deliver and target bioactive agents to the intracellular sites of infection. Thus, in the case of antimicrobials, drug delivery systems may help to increase their therapeutic index in intracellular locations. The development and evolution of pharmaceutical forms of gentamicin for the parenteral treatment of intracellular pathogens is reviewed in this paper.

Keywords: drug delivery systems, liposomes, microparticles, nanoparticles, treatment

Expert Opin. Drug Deliv. (2007) 4(6):677-688

1. Introduction

Aminoglycosides are extremely active antimicrobial agents, particularly against bacteraemia caused by aerobic Gram-negative Bacilli [1]. Gentamicin (GM), in particular, is an aminoglycoside with a wide spectrum of antibacterial activity [2-4]. However, as a highly water-soluble drug, it penetrates cells poorly. This is an important disadvantage for the treatment of intracellular susceptible pathogens. A series of different drug delivery system vectors have been used to deliver these agents into intracellular compartments. In order to rationally design vectors, it is important to recognize first the transport of GM by itself (**Figure 1**) [5].

Two main types of endocytosis are distinguished on the basis of the size of the endocytic vesicles formed, phagocytosis and pinocytosis. Phagocytosis refers to the internalization of large particles that must bind to specific plasma membrane receptors capable of triggering their own uptake, usually by causing the formation of F-actin-driven pseudopods that envelop the bound particle. In contrast, pinocytosis involves the ingestion of fluid and solutes via small vesicles (< 200 nm) through different possible pathways. So, when gentamicin is administered in the free form may be incorporated into cells by a pinocytosis process that is not concentrative and depends on the extracellular concentration of the drug. Internalized molecules are delivered to early endosomes (intracellular organelles that are on the major receptor-recycling pathway), and a subsequent transport to late endosomes and lysosomes occurs for degradation. On the contrary, if gentamicin is encapsulated into particle delivery systems, high intracellular concentrations may be reached through the endocytic pathway. The phagosome is the first intracellular vesicle, where particulated material is first ingested up by the cell. Afterwards, endosomes may fuse permanently with phagosomes or late endosomes may contact transiently (temporary connections) with phagosomes. In either cases, fused or hybrid vesicles mature to form

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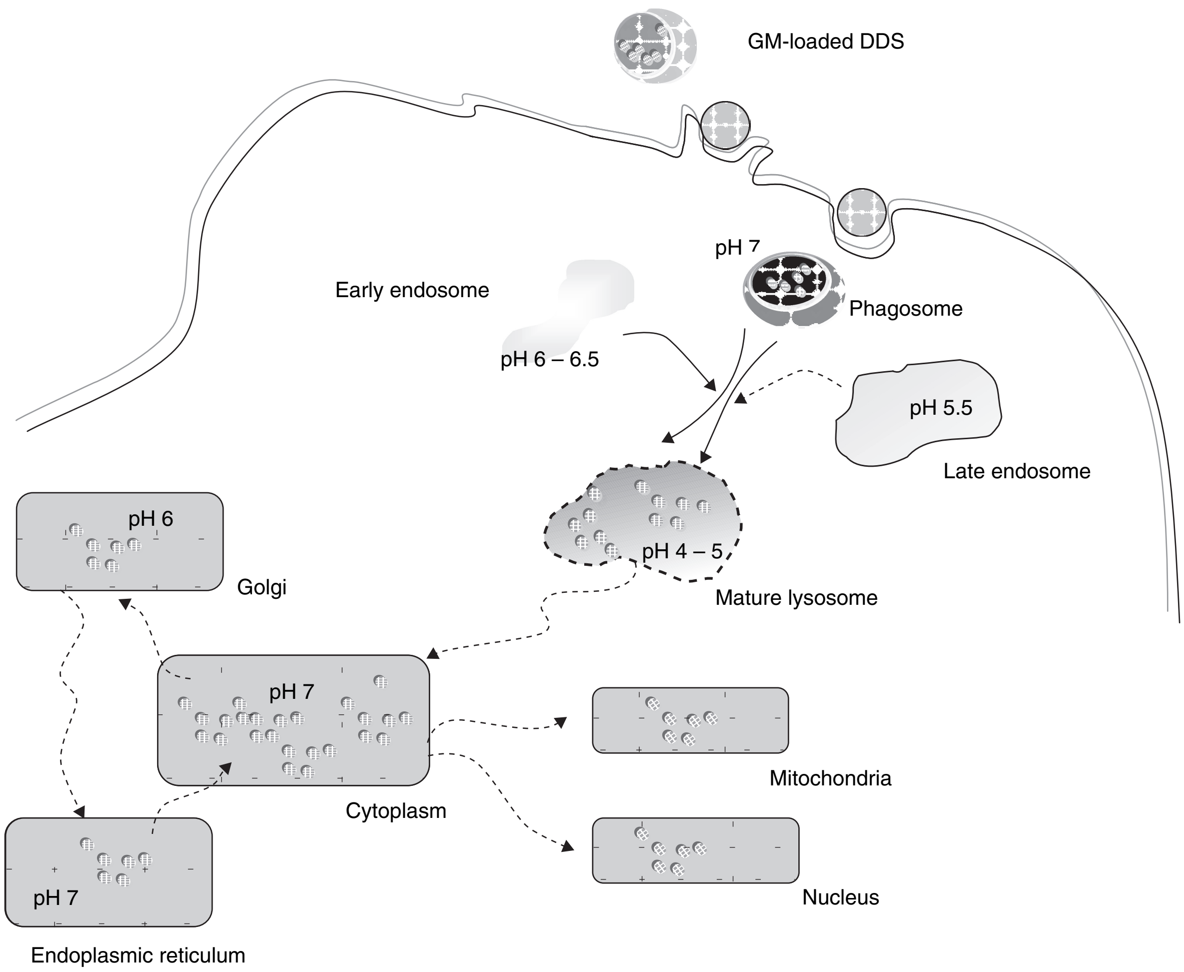

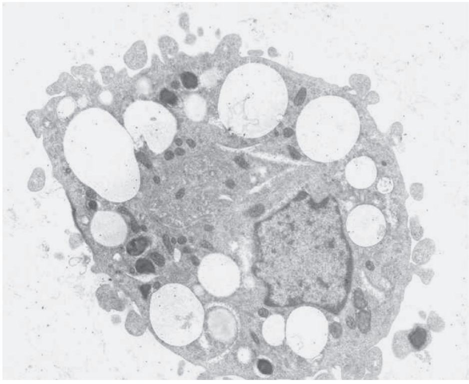

Figure 1. Internalization mechanisms of the drug delivery devices and their subsequent intracellular fate. The cellular organelles are represented as sub-compartments or boxes. The transmission electron micrograph shows gentamicin-containing poly(lactide-co-glycolide microparticles being engulfed by a THP-1 human monocyte (original magnifi cation × 20,000).
GM: Gentamicin.

lysosomes or transfer cargo to lysosomes through vesicular intermediates. Several molecules are responsible for the unique characteristics of each endocytic compartment. Thus, molecules that regulate internal pH, which is essential for the dissociation and degradation processes, vary depending on the compartment: phagosome present a pH of ~ 7; early endosomes, pH 5.9 – 6.5; late endosome, pH 5.5; mature lysosome pH 4 – 5. Acidification is an important issue considering the reduced bioactivity of the drug under such conditions. Gentamicin is polycationic at physiological pHs so it cannot easily pass through these membranous compartments. Therefore, gentamicin accumulates into phagosomes and early or late endosomes; however, lysosomal membranes contain specifi c transporters for cationic compounds. As a consequence, gentamicin may be released from lysosomes to the cytoplasm and from here may reach the Golgi and endoplasmic reticulum, where eventually contact is made with the intracellular pathogen. In effect, for optimized drug delivery, a knowledge of the internalization mechanisms of the vector and its subsequent intracellular fate is a crucial issue that should be considered (**Figure 1**).

Aminoglycosides may be incorporated into cells by a pinnocytosis fluid-phase clathrin-independent entry mechanism when high concentrations and long incubation times are used [6,7], but, nevertheless, cellular uptake occurs primarily through endocytosis after binding to acidic phospholipids and the multiligand receptor megalin [8]. The uncharged GM molecules, in their maximally lipophilic states, diffuse across lipid membrane barriers much more readily than the charged hydrophilic forms; however, once inside at the acidic pH of the endosome (pH 6) GM is protonized, resulting in a weak organic base that passes poorly through biological membranes and, as a result, concentrates in acidic compartments (i.e., lysosomes). Therefore, this phenomenon implies that most of the GM accumulates into lysosomes (lysosomotropic). Different aminoacid transporters have been described in lysosomal membranes [9,10], which could help antibiotic translocation to the cytoplasm and subsequently allow entry to the Golgi compartments. Studies conducted with fluorescent markers suggest that GM uses channels located within the endoplasmic reticulum to achieve translocation to the cytoplasm, where it associates with mitochondrial membranes and the nucleus. In addition, the Golgi complex accumulation of GM results from direct trafficking after endocytosis from the surface membrane (~ 10%) [11,12].

Another important consideration in the course of this complex traffcking is the effect of the physicochemical conditions that may be encountered intracellularly. GM is exposed to a range of pH between neutral (7 for cytoplasm and endoplasmic reticulum) and acid values (~ 6 for endosomes and Golgi, and 5 for lysosomes) [13], which may influence its antibacterial activity. At low pH, these antibiotics increase their minimal inhibitory concentration due to changes in their degree of ionization. Thus, an acidification from pH 7.5 to 6.5 would increase the minimal inhibitory concentration of GM 16-fold, and, at pH 5.0, by 64-fold [14-16]. The lack of GM activity is related to the protonation of the molecule at acidic pH [14], and as the antibiotic enters the bacteria by active transport, factors affecting this mechanism (divalent cations, hiperosmolarity, anaerobiosis and acid pH) would reduce GM antibacterial activity [17].

In summary, aminoglycosides show a limited intracellular activity compared with their strong bactericidal potential in extracellular medium. Therefore, the goal is to reach a sufficiently high concentration in the target cells by using drug delivery systems to cope with the eventual reduction in its intracellular activity. The development and evolution of pharmaceutical forms of gentamicin for the parenteral treatment of intracellular pathogens is reviewed in this paper, from liposomes to nanoparticles.

2. Liposomal formulations

Liposomes are vesicles of one or several lipidic bilayers enclosing aqueous compartments [18,19]. Depending on their size and number of lamellae, liposomes can be grouped into multilamellar or stable plurilamellar vesicles and large and small unilamellar vesicles. The main advantage of these carriers consists of their structural versatility, encompassing variable membrane fluidity, size, charge and the possibility of entrapping drugs of different sizes and solubilities. Liposomal carriers have been extensively investigated for the intracellular delivery of aminoglycosides [20-22]. The passive targeting of liposomal aminoglycosides to phagocytic cells of the mononuclear phagocytic system can improve treatment outcome for intracellular infections, compared with treatment with free aminoglycosides [21,23]. Drug entrapment into liposomes can markedly alter its pharmacokinetics (e.g., drug distribution can be shifted from the kidney to other organs, thereby reducing the nephrotoxicity associated with the use of aminoglycosides) [21]. Indeed, liposomes have been used successfully as carriers for aminoglycosides in experimental models of bacterial infections, such as those caused by *Staphylococcus aureus, Francisella tularensis, Bartonella* spp., *Legionella pneumophila, Listeria monocytogenes, Mycobacterium* spp., *Salmonella* spp., *Klebsiella pneumoniae* and *Brucella* spp. (for a review, see [24]). In these studies, the influence of the preparation method and vesicle composition on the liposome physico-chemical characteristics, (size and size distribution, lamellarity and GM sulphate encapsulation efficiency), stability in serum and interaction capacity *in vitro* with infected monocytes was demonstrated.

Overall, entrapment efficiency in liposomes is normally low for water-soluble drugs such as GM [25,26]. Liposome size distribution affects the stability, biodistribution and pharmacokinetics of liposomal formulations [25,27]. Hydrogenated phosphatidylcholine or cholesterol may be added to the composition, as they are known to modify the structure of vesicles by reducing the freedom of motion of the phospholipids' hydrocarbon chains, thus, reducing membrane fluidity, vesicle permeability and increasing liposome stability [18,28,29]. The use of charged lipids was most important when investigating the efficient interaction of the vesicles with target cells; however, some differences in the physico-chemical characteristics of the vesicles could be also attributed to their charge. GM sulphate may be dissolved in a pH 6.0 phosphate buffer to promote the protonation of the amino groups of the drug and, thereby, its interaction with the negatively charged phospholipids [30,31]. Consequently, both the electrostatic interaction between GM and lipids, and the cholesterol effect on membrane fluidity has been found to influence GM entrapment in anionic liposomes. Nonetheless, the positively charged stearylamine-containing liposomes entrapped GM more efficiently than the corresponding vesicles bearing negatively charged dipalmitoyl phosphoglycerol – an effect that cannot be explained by electrostatic drug–lipid interactions [26,32]. A possible explanation has been provided by Karlowsky and Zhanel [21], who hypothesized that positively charged drugs are readily incorporated into negatively charged bilayers favoring the formation of multilamellar vesicles and neutralizing the otherwise repulsive ionic forces between the multiple negatively charged bilayers [33]. Conversely, positively charged bilayers would not bind positively charged GM, due to the overall repulsive ionic forces between multiple bilayers; however, they are likely to separate and form relatively large uni- or oligolamellar vesicles, which, at a comparable size, must enclose a larger aqueous core volume [34], thereby entrapping aqueous drugs efficiently.

Purification is crucial for the size-related quality of vesicles. Non-purified GM-loaded liposomes tend to aggregate, probably due to the presence of non-encapsulated polycationic GM that might bind to the external surface of anionic liposomes [31,35]. Cationic liposomes have been shown to exhibit different behaviours, with small and similar vesicle size distribution before and after elution through the Sephadex column [33]. In this case, free GM might have stabilized the liposomes due to electrostatic repulsion between the vesicles and the cationic molecule.

When administered parenterally, liposomes are captured by the cells of the mononuclear phagocytic system. However, before liposomes can interact with phagocytic cells, they are generally exposed to the destabilizing interaction of certain serum components, such as high-density lipoproteins (HDLs) [36,37]. Although the exact mechanism of destabilization is not clear, it has been suggested that upon contact between vesicles and lipoproteins, lipids from the liposomes leak out and are transferred to the lipoproteins [36,38,39] leaving pores of different sizes through which the liposome content is released [40]. Therefore, the efficient treatment of intracellular infections with liposomal formulations requires good liposome stability and efficient interaction with target cells [41]. Higher molar ratios of cholesterol in the lipidic composition increases the half-life of the liposomes in the presence of HDL [40,42-44]. However, cholesterol lowered drug entrapment efficiency, compared with vesicles formed of phosphatidylcholine alone. Nevertheless, reduced entrapment has been found to be more than counterbalanced by the greater stability of the cholesterol-stabilized vesicles in the presence of HDL, resulting in higher drug content [45]. In addition, incorporation of a negative charge further improved the resistance liposomes stabilized with cholesterol to the action of the serum lipoproteins, and incorporation of a positive charge has been found not to produce such an effect [46]. It has also been reported that, the binding of serum proteins to the vesicle surface depends on the charge and lipid composition of liposomes [47].

The uptake of liposomes by monocytic-macrophagic cells takes place in two stages: unspecific opsonization of the vesicles by serum proteins (e.g., immunoglobulins, complement factors, fibronectin), followed by the phagocytosis of the opsonized liposomes [48]. During the phagocytosis stage, the cells are activated and release signaling molecules. Increasing the molar ratio of cholesterol content in the liposome reduces monocyte activation [29]. Similarly, the antimicrobial activity of GM is reported to be more moderate with high cholesterol content liposomes, which has been ascribed to the increased rigidity of the vesicles [49,50]. Moghimi and Patel [51] suggested that the incorporation of cholesterol into liposomes may change the distribution of phospholipids in the membrane, rendering the opsonization, and hence the phagocytosis, more difficult. Although cholesterol concentrations of ≥ 50% give optimal stability to the liposomes in the presence of HDL, 30% cholesterol is preferred to provide sufficient liposome stability without compromising the capture by phagocytic cells [29]. Surface charge is another major parameter influencing the interaction of liposomes with the monocytic/macrophagic cells, although the effect of this parameter is controversial. Some authors claim that negative charges favor vesicle–cell interactions [51-53], but positive charges do not exert such an effect [37,54]. Others found that positively charged liposomes interact better with cells [55-57]. Although other factors may play a role, it is suggested that positively charged liposomes interact with the negatively charged cells by electrostatic adsorption, followed by internalization by fusion or endocytosis [56]. The contradictory observations in various studies on the interaction and capture of liposomes might be attributed to differences in the method of liposome preparation, type and quality of phospholipids, or the cell line used.

A protective effect of high doses of placebo liposomes has been described [58,59]. On the contrary, several studies revealed an aggravation of intracellular *Mycobacterium* and *Listeria* infection in mice after treatment with placebo liposomes of high lipid content, whereas no effect was seen with placebo liposomes of low lipid content [23,60,61]. It has also been described that a high proportion of intact liposomes is retained in the liver when small doses are administered. However, larger doses might saturate the capacity of the liver to clear particles from the circulation, resulting in a higher amount of vesicles remaining in the circulation and available for uptake by the spleen and bone marrow. Thus, larger doses of liposomes might be able to exert an immunomodulatory effect and enhance the host's defense against bacterial infection. This explanation would be in agreement with the reported macrophage stimulatory effect of certain lipids [62]. Therefore, it may be necessary to balance carefully the advantages and potential disadvantages of lipid doses to achieve an optimal therapy for intracellular diseases such as brucellosis.

In summary, the physico-chemical characteristics, stability and interaction of liposomes with monocytes/macrophages are governed in a complex manner by their lipidic composition. Thus, detailed protocols to obtain well-defined uniform vesicles need to be established. A careful balance between the molar ratio of cholesterol to ensure low permeability to the water soluble GM, and stability in serum without compromising the interaction with the target cells seems to be essential. The surface charge exerts great influence on liposome–cell interaction. In the present authors' studies, positive charges benefited the interaction of the vesicles with monocytes/macrophages – results that correlated *in vivo* with a significant protection against *Brucella* in mice. However, stability issues, both during storage and after inoculation, and reproducibility in terms of the production of a well-defined and consistent formulation, still need attention. Conversely, biodegradable particles (micro- and nanoparticles) represent a more stable system and offer the advantage of providing controlled release of the encapsulated GM, which could minimize the need for multiple shots.

3. Microparticles and nanoparticles

Biodegradable microparticles represent an alternative system for targeting infected cells and are also useful for prolonged drug release [63-67]. For the efficient treatment of intracellular infections, a high initial dose of the antibiotic should be delivered immediately and be followed by sustained antibiotic release to reduce the number of administrations. These kinetics would improve patient compliance and minimize relapses. In fact, controlled release of the encapsulated drug and formulation stability represent the main advantages of these particulates over liposomal formulations. Particle size, entrapment efficiency, release profiles and pharmacological effects also depend greatly on the microencapsulation method [68], polymer characteristics, and hence copolymer composition and molecular weight [69,70]. Among the numerous methods for encapsulation, the double emulsion solvent evaporation (w/o/w) and spray drying have been proven to be the most suitable for water-soluble drugs [71].

GM sulphate has been encapsulated by spray drying into different poly(lactide-co-glycolide) (PLA/PLGA) types [72]. The GM sulphate loading data suggest that the encapsulation was influenced by physico-chemical interactions (H-bonding, acid-base, polar, hydrophobic and ionic interactions) between polymer, drug and solvents [73,74]. In addition, the formulation parameters of polymer type and concentration, physical state of the drug (solid or dissolved in water), and nominal loading all influence substantially encapsulation efficiency, microparticle morphology and size distribution.

Studies on the interaction between polymeric particles and cells, and on biodistribution of particles after injection in animals, have frequently used poly(styrene) and poly(acrylate) particles [75-77]. Particle uptake by phagocytic cells is largely affected by the size and surface properties of the particles [76,78,79]. The efficient uptake of PLA and PLGA microparticles by phagocytes has been reported for particle diameters in the low micrometer or nanometer range, although larger particles can be phagocytosed in smaller number [79-81]. It is noteworthy that the total mass of a few large particles may largely outweigh the mass of many small particles [76]. The mean size of the microparticles produced by spray drying has been reported to range 1.0 – 3.5 µm, depending on the PLA/PLGA type. Generally, the number of cells engaged in observable particle uptake decreased with increasing polymer hydrophilicity (PLA > PLA-H > PLGA 752 > PLGA 502 > PLGA 502H) [82]. This is consistent with the decreased particle clearance from the bloodstream observed for hydrophobic particles coated with hydrophilic polymers [79,82,83]. The most hydrophilic end-group uncapped PLGA 502H microparticles best stimulated the oxidative burst of the cells (measure of general cell activation) [82,84]. Phagocytosis may not correlate with the activation of the cells. Overall, the phagocytosis of microparticles has been shown to depend mainly on hydrophilicity of the polymer, whereas cell activation is influenced by the presence of uncapped end-groups (free -OH and COOH groups) [85,86]. Small and less hydrophilic PLA and PLGA 752 particles may be efficiently internalized, but their surface characteristics may not activate oxidative metabolism to an extent comparable with polymer surfaces containing specific functional groups, such as -COOH and -OH in end-group-uncapped polymer types [87].

The stimulation of intracellular radical oxygen intermediates might act synergistically GM in the killing of intracellular bacteria, thus increasing treatment efficiency. In a model of *Brucella* infection, the effect of GM-loaded PLGA 502H microparticles significantly decreased the intracellular bacterial levels of infected monocytes, compared with PLGA 502 microparticles, although PLGA 502H released *in vitro* only 14% of the encapsulated GM within

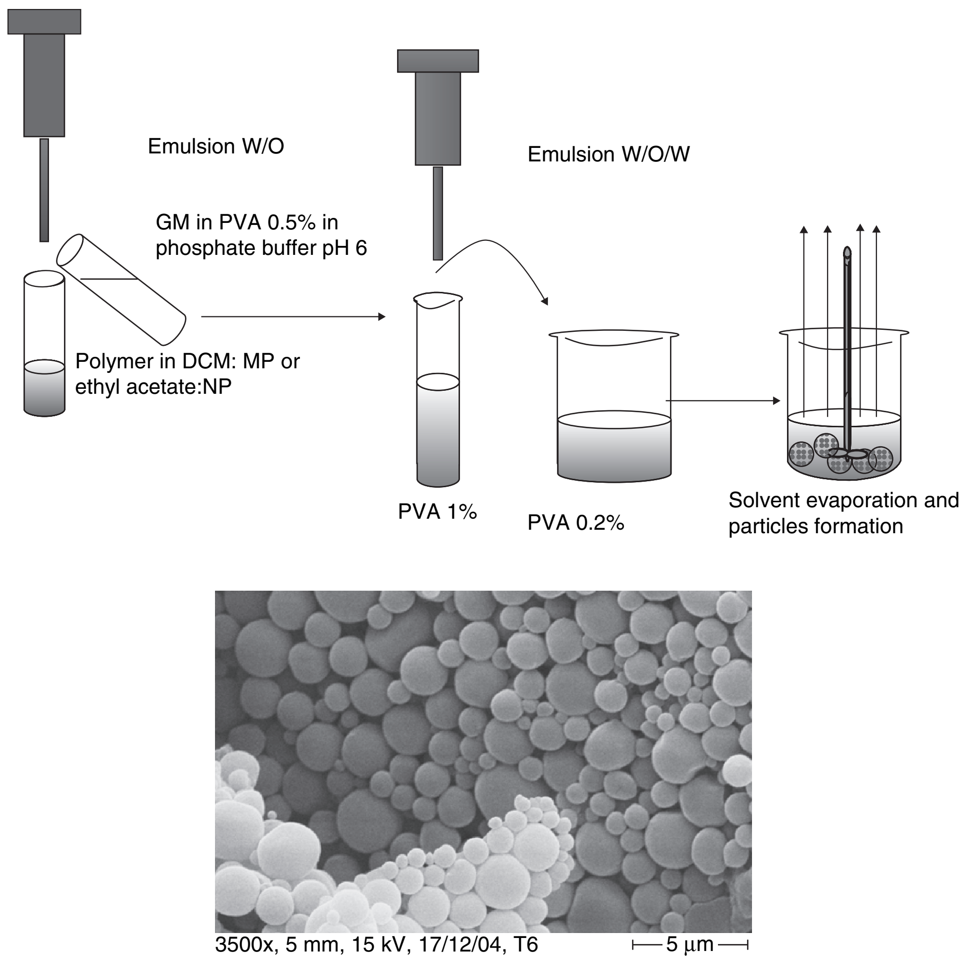

Figure 2. Micro- and nanoparticle preparation as controlled drug delivery devices for parenteral use. A schematic representation of preparing poly(lactide-co-glycolide) micro- and nanoparticles containing gentamicin, using a W/O/W solvent evaporation emulsion. The antibiotic dissolved in 0.5% PVA in phosphate buffer, pH 6.0, and 200 mg of polymer dissolved in dichloromethane for microparticle, or in ethyl acetate for nanoparticle, preparations are mixed by ultrasonication under cooling for 1 min, to form a W1/O emulsion. This inner emulsion is added to 2 ml of 1% PVA (W2) and homogenized again by ultrasonication. The resulting (W1/O)W2 emulsion is poured into 50 ml of 0.2% PVA and continuously stirred for at least 3 h at room temperature to allow solvent evaporation and particle formation. After preparation, particles were isolated by centrifugation (7000 g, 5 min for microparticles; and 12,000 g, 30 min for nanoparticles), washed three times with ultra pure water and freeze dried. The lower figure shows a scanning electron micrograph of the resulting microparticles.

GM: Gentamicin; MP: Microparticle; NP: Nanoparticle; PVA: Poly-vinylalcohol.

the initial 24 h, compared with the 50% burst release from the PLGA 502 microparticles [88]. Therefore, it may be hypothesized that either the pronounced stimulation of oxidative metabolites by the PLGA 502H particles enhanced the antibiotic activity of GM, or a higher number of PLGA 502H particles were phagocytosed, as a larger number of PLGA 502H particles, showing similar uptake rate, needed to be incubated to achieve identical drug doses with both polymer types [82].

Increased antibacterial activity of microencapsulated GM was achieved by dispersing the microparticles in a 2% (w/v) poloxamer 188 solution [88]. Although the mechanism responsible for this effect is unclear, several factors may have played a role. Surfactant adsorption onto the microparticles may have altered the surface polarity and subsequently enhanced cell adhesion and phagocytosis, which might have also been facilitated by a higher degree of particle dispersion in the presence of the surfactant. Speculatively, unspecific cell activation by the surfactant and synergistic enhancement of antibiotic activity might also have exerted some effect. Particle coating by poloxamers has been employed to suppress phagocytosis *in vitro*; the suggested responsible mechanisms were steric stabilization, increased particle hydrophilicity [89] and/or reversible interaction of the surfactant with cell membrane of phagocytic cells [90]. *In vivo*, such coatings have significantly slowed down the clearance of particles from the blood by the mononuclear phagocytic system [89,90], probably because poloxamer coating altered

Table 1. A comparison of gentamicin drug delivery systems: physicochemical and biological factors of interest.

	Size (μm)	Controlled release	Duration of release	Main advantages	Main disadvantages
Liposomes	0.25 – 5.0	No	Days	Main components are materials that are present in the body (good acceptability) Uptake by endocytosis (can fuse with the cell membrane)	Limited stability in biological fluids and during storage Low drug entrapment (low efficiency) Difficult to prepare as a monodisperse population
Nanoparticles	0.01 – 1.0	Yes	Weeks	Good stability in biological fluids and during storage Preparation suitable for scaling up Can be sterilized by filtration Surface funtionalization for targeting	Low drug entrapment
Microparticles	1 – 50	Yes	Months	Good stability in biological fluids and during storage Preparation suitable for scaling up High drug entrapment	The large size can cause embolism

the opsonization by proteins. The efficiency of poloxamers to reduce particle phagocytosis depends on the molecular weight and chain length of the propylene oxide and ethylene oxide blocks [89]. Thin coatings conferring poor steric stabilization have been obtained on highly polar surfaces [91,92] or by short particle incubation in poloxamer [83]. For the relatively polar and hydrophilic PLGA 502 and PLGA 502H microparticles, adsorption of poloxamer likely occurred through interaction with the ethylene oxide chains, resulting in a flat arrangement of adsorbed poloxamer and exposed propylene oxide chains, possibly increasing the hydrophobicity of the particle surface and promoting particle uptake. Improved dispersion characteristics should have also contributed to the increased phagocytosis, as observed by optical microscopy. Finally, mechanisms involving interaction between poloxamer and cell membrane [93,94] or cell activation cannot be disregarded. Some poloxamer types have indeed increased phagocytic activity, and altered bacterial cell wall integrity and permeability, yielding a synergistic effect with antibiotic agents [95-97].

GM-loaded microparticles, prepared by spray drying, have shown promising properties, but particles showed a high tendency to aggregate, rendering their injection difficult in mice [98]. Therefore, the present authors have recently focused on preparing and evaluating GM-loaded microparticles by a double emulsion solvent evaporation method to improve particle dispersion characteristics (**Figure 2**) [99,100]. Different co-polymers of PLGA were used, 502H and 752H being the most appropriate and suitable carriers for gentamicin encapsulation and targeting inside human macrophages and, thus, for potential brucellosis treatment. The results demonstrated that PLGA microparticles were efficiently captured by the macrophages and that the GM released from these particles was active, being able to exert its bactericidal effect inside macrophagic cells. By transmission electron microscopy and inmunocytochemistry (gold-labelled antibodies against GM), antibiotic released from the particles was observed in the cytoplasm, nucleus and other intracellular compartments (**Figure 1**) [101].

Regarding particle distribution *in vivo*, microparticles prepared using PLGA 502H or PLGA 752H were successfully delivered to the liver and spleen. Furthermore, microparticles of 502H and 752H PLGA released their content in a sustained manner. Pharmacokinetics parameters illustrated the markedly altered distribution of PLGA-loaded GM compared with the free drug, with higher concentrations of GM in the spleen and liver when it was administered loaded in microparticles. At the same time, no GM was detected in serum samples, precluding drug accumulation in the kidneys. Distribution studies showed that after 2 weeks, only 752H intact microparticles were observed in the spleen, and, in discrete quantities, in the liver. However, GM was detected up to 4 weeks later in the liver and spleen after a single dose of the microparticle formulations. This long persistence is probably due to the nature of the aminoglycoside. These drugs are highly stable and are not metabolized in the liver. Because of their polar nature, they penetrate cells very poorly, but, once inside, their intracellular retention is very high. When BALB/c mice were chronically infected with the virulent *Brucella melitensis* strain and treated with selected GM-containing formulations, both significantly reduced the splenic infection. Results also indicated that treatment with free GM was ineffective, in agreement with undetectable levels of GM in the liver and spleen [101].

4. Conclusions

The treatment of intracellular bacterial infection remains both a medical and economic challenge. Because of their strong antibacterial properties, aminoglycosides remain useful for the treatment of serious infections, but drug monitoring has to be strict to preserve antibacterial activity while avoiding toxicity as far as possible. A drug delivery system that helps to increase the therapeutic index of aminoglycosides by increasing the concentration of the drug at the site of infection and/or reducing the nephro- and ototoxicity would be of considerable interest. Liposomal and microparticle encapsulation of aminoglycosides provides relatively high GM entrapment efficiencies and efficient interaction with monocytes/macrophages. Liposomes with a membrane-like structure favor good cell interaction, and their versatility in terms of structure and composition represent their main advantages. Moreover, in the present authors' work liposomes have proved to exhibit important therapeutic activity in experimental models of brucellosis. However, stability issues, both during storage and after injection, and reproducibility in terms of production of a well-defined and consistent formulation, still need attention. Conversely, micro- and nanoparticles represent a more stable system and have the advantage of providing controlled release of the encapsulated GM, which could minimize the need for multiple injections. **Table 1** compares the main physicochemical and biological interests among the different formulations. The data reviewed here suggests that the use of drug delivery systems as an alternative therapeutic approach may open interesting avenues for the treatment of intracellular infections.

5. Expert opinion

The purpose of any delivery system for drugs is to optimize the pharmacokinetics and pharmacodinamics of a drug in order to enhance its therapeutic potential. Consequently, research on GM encapsulation for parenteral administration is moving, on one hand, from liposomes to microparticles and from here to nanoparticles. On the other hand, preparation techniques are moving from spray drying to solvent evaporation for the preparation of the polymeric vectors. The following conclusions can be drawn from our group's studies.

5.1 Liposomes

Drug entrapment varies according to lipidic composition, related to the molar ratio of cholesterol and to the amount of negatively charged phospholipid included, thus, favoring electrostatic interactions with the cationic drug. On the other hand, cationic liposomes seem to entrap GM more efficiently than comparable anionic vesicles, apparently associated to the vesicle structure. Some problems have been reported, resulting from the presence of free GM, which caused vesicle aggregation and subsequent sedimentation of the liposome. Finally, lyophilisation in the presence of trehalose has been found to alter GM-loaded liposome size distribution, inducing liposome fusion.

5.2 Microparticles and nanoparticles

Highly hydrophilic and cationic GM sulfate can be entrapped with acceptable efficiency into PLA/PLGA particles by spray drying. Formulation parameters such as polymer type and concentration, physical state of the drug and nominal loading all substantially influence encapsulation efficiency and microparticle morphology and size distribution. The microparticle characteristics of a size below 3 μm and a negative surface charge, were adequate for efficient monocyte uptake and activation. However, the efficiency *in vivo* (treatment of experimentally infected mice) is inappropriate, probably due to particle aggregation which may be responsible for deficient distribution. This main drawback is solved by using the solvent evaporation method. From our study, in terms of physico-chemical properties, adequate drug loading and capability to interact with macrophages promoting their oxidative burst, micro- and nanoparticles of 502H, and microparticles of 752H resulted in the most suitable formulations. Nevertheless, gentamicin loading should be improved before use in humans.

Declaration of interest

This work was supported by *Ministerio de Educación y Ciencia* from Spain (NAN2004-09159-C04-03).

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•• **Study about pharmacokinetics and pharmacodynamics of gentamicin administered in polymeric nano- and microapartciles; indirect nephrotoxicity study.**

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