Pharmaceutical Research, Vol. 8, No. 9, 1991

Review

Liposomes and Nanoparticles in the Treatment of Intracellular Bacterial Infections

Patrick Couvreur,[1,3] Elias Fattal,[1] and Antoine Andremont[2]

The treatment of infections caused by obligate or facultative intracellular microorganisms is difficult because most of the available antibiotics have either poor intracellular diffusion and retention or reduced activity at the acidic pH of the lysosomes. The need for antibiotics with greater intracellular efficacy led to the development of endocytosable drug carriers, such as liposomes and nanoparticles, which mimic the entry path of the bacteria by penetrating the cells into phagosomes or lysosomes. This Review assesses the potential of liposomes and nanoparticles in the targeted antibiotic therapy of intracellular bacterial infections and diseases and the pharmaceutical advantages and limitations of these submicron delivery systems.

KEY WORDS: intracellular infections; liposomes; nanoparticles; intracellular targeting of antibiotics; lysosomes.

INTRODUCTION

Most intracellular infections are difficult to eradicate because bacteria inside phagosomes are protected from antibiotics (1). Although macrophages constitute the primary line of natural defense against infections, a variety of bacterial diseases originates from obligate or facultative intracellular parasites which are often located within macrophages. Infected cells may also constitute a "reservoir" for microorganisms, which are released from time to time, causing the recurrence of systemic infections. The need for intracellular chemotherapy has been recognized for many years (2).

Resistance of intracellular infections to chemotherapy seems to be related to the low intracellular uptake of commonly used antibiotics or to their reduced activity at the acidic pH of lysosomes (Fig. 1). Thus, antibiotics with a basic character—aminoglycosides—lead to lysosomal overloading, whereas they display a reduced activity in acidic environments (3). Conversely, acidic antibiotics—β-lactams—do not diffuse through the lysosomal membrane because of their ionic character at neutral extracellular or cytoplasmic pH (4). Finally, certain antibiotics that penetrate the cell more rapidly and to a larger extent, such as clindamycin (5), are poorly retained in cells; hence, activity is not expected to be long-lasting.

As an alternative strategy to the search for new antibiotics with inherent intracellular efficiency, one may modify the dosage form to obtain controlled release and targeting to specific sites. By associating antibiotics with colloidal particulate carriers, such as liposomes or nanoparticles, one can develop endocytosable drug formulations.

DESIGN OF PARTICULATE CARRIERS OF ANTIBIOTICS

Liposomes

Liposomes are submicronic structures closed by one or more concentric lipid bilayers surrounding an aqueous compartment. Several points must be considered for entrapping antimicrobial agents into these phospholipidic vesicles. For example, the oil–water partition coefficient of the drug will determine to what extent the drug will be entrapped. Drugs that show both hydrophilic and lipophilic properties may be able to pass through the lipid membrane and to leak out of liposomes (6). For example, we found that ampicillin entrapped into liposomes prepared by the reverse-phase evaporation method, and composed of phosphatidylcholine, cholesterol, and phosphatidylglycerol, lost their drug content rapidly even when they were kept at 4°C (7). On the other hand, although freeze-drying was found to keep intact the size distribution of ampicillin-loaded liposomes, as soon as they were resuspended in an aqueous medium, the drug was rapidly released outside of the liposomes (7). In addition, osmotic fragility (8) or phospholipid exchange (9) can disrupt the liposomal membrane in the presence of serum components, leading to a quick leakage of the entrapped drug. Recently, liposomes were developed with improved serum stability and circulation half-times (10,11). In particular, it was shown that gangliosides and sphingomyelin reduced dramatically the rate and extent of uptake of liposomes by macrophages *in vivo*. The role of cell surface carbohydrates, in

[1] Laboratoire de Pharmacie Galénique et Biopharmacie, URA CNRS 1218, University of Paris XI, Paris, France.
[2] Laboratoire d'Ecologie Microbienne, Institut Gustave-Roussy, and Laboratoire de Microbiologie, University of Paris XI, Paris, France.
[3] To whom correspondence should be addressed.

0724-8741/91/0900-1079$06.50/0

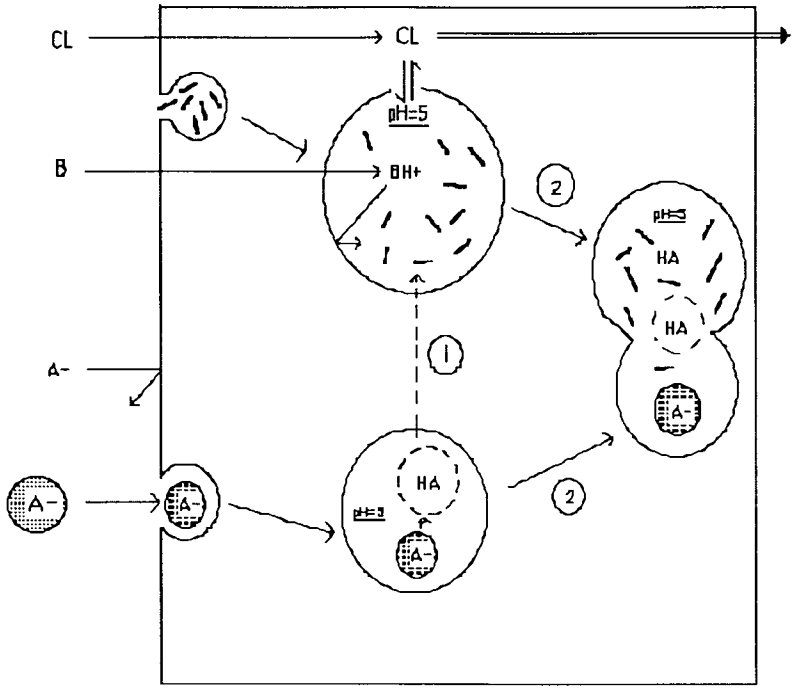

Fig. 1. Pathogeny of cellular infection and schematic intracellular traffic of antibiotics free or associated with colloïdal carriers. The cell is schematized by the rectangular box and the arrows show the possible pathway of penetration of the free or targeted antibiotic into the cell: diffusion or endocytosis. The circles represent the lysosomes (pH 5). The antibiotics are allowed to come in contact with the bacteria either after diffusion from one lysosome to another (pathway 1) or due to the fusion of two intracellular vacuoles (pathway 2). /, bacteria; ⊜, drug carrier (intact); ◌, drug carrier (degraded by lysosomal enzymes); $A^- \leftrightharpoons HA$, β-lactam; B, aminoglycoside; CL, clindamycine.

particular the surface sialic acid residues, in cellular antirecognition phenomena is widely appreciated.

Another aspect, which is rarely mentioned, is the stability of the encapsulated antibiotic itself in the aqueous compartment of the liposomes. As a general rule, one of the difficulties in the practical application of these products has been the long-term stability of the liposomes. Numerous authors (12–14) have addressed this problem, and some found that liposomes can, under certain conditions, be reduced to dry powders (15,16). However, there have been some discrepancies in results obtained by the various laboratories in which this phenomenon has been studied, with respect both to the efficacy of the sugars tested and to the degree to which the drug vesicles can be stabilized. As an illustration of this, Crowe *et al.* (16) have shown that liposomes can be preserved in the dry state in the presence of trehalose. These authors provided a physical explanation for the mechanism of the preservation: the best evidence available suggests that there is a direct interaction between the sugar and the polar head group of the phospholipid, resulting in a depression of the transition temperature of the lipid and its maintenance in a fluid state even in the absence of water (15). Other laboratories have confirmed these results with some discrepancies. For example, Madden *et al.* (17) reported that much greater quantities of the stabilizing sugar were required to stabilize the liposomes and that retention of trapped solute was significantly less efficient. The problem is that the design and evaluation of stable liposomes are a very complicated subject and comparisons are very hazardous because the authors are using different methods to prepare the liposomes, different lipids, and even different methods to record stability. In addition, a number of industrial laboratories have focused on engineering stable formulations, but few results are published (12). All these considerations show the need for a comprehensive study of the formulation and characterization of liposomes encapsulating antimicrobial agents. Given the broad versatility of liposomes in composition, charge, and size, the impact of liposome formulation on stability, pharmacokinetics, and pharmacodynamics requires further study.

Nanoparticles

Owing to their polymeric nature, nanoparticles may be more stable than liposomes in biological fluids and during storage. They can generally entrap antibiotics in a stable and reproducible way (18). However, these ultrafine particles, sized around 0.1 μm, need to be designed using polymers able to be degraded *in vivo* to avoid side effects due to intracellular polymeric overloading. Biodegradable polyalkylcyanoacrylate nanoparticles met these requirements and were extensively studied because of the ease with which they are made (19,20). They may be freeze-dried and rehydrated without modification in terms of both nanoparticle

size and drug content (18,21). However, these particles are prepared by emulsion polymerization of cyanoacrylic monomers. This reaction is classically induced by a nucleophilic agent. Attention must be paid to the fact that, in some cases, the antimicrobial agent per se may induce the anionic polymerization of the monomer. This entails the covalent linkage of the drugs to the polymer leading to their inactivation. This was observed with vidarabine, an antiviral compound, able to initiate the polymerization of the cyanoacrylic monomer through a zwitterionic pathway (22). This mechanism of interaction allows the covalent linkage of the drug with the polymer, resulting from the reaction between the methyldiene group of the monomer and the nitrogens of vidarabine in positions 3 and 7. Therefore, the antimicrobial activity of the nanoparticle drug formulation must be compared *in vitro* with the same amount of free drug.

The release rate of ampicillin from nanoparticles was found to correlate with the degradation rate of the polymer occurring through an enzymatic pathway. Thus, since ampicillin release from nanoparticles is weak in esterase-free medium, it is considerably increased in the presence of esterases (18). In serum, the liberation of ampicillin was found to follow zero-order kinetics (7).

TARGETED ANTIBACTERIAL THERAPY

Biopharmaceutical Considerations

Liposomes and nanoparticles themselves cannot escape from the circulation because of the endothelial barrier, with exception to tissues with discontinuous endothelia lining their capillaries (i.e., liver, spleen, and bone marrow). This leads to the rapid clearance of these ultrafine particulate carriers from the blood and to their capture by the cells of the reticuloendothelial system (23,24). This corresponds exactly to the same tissue distribution pattern as that of the majority of the bacteria responsible for intracellular infection. In addition, it has been shown that liposomes are taken up by circulating blood monocytes, which are known to infiltrate some infectious lesions (2). This concept of a "second carrier" such as blood monocytes is interesting since it provides possibilities for targeting to infections outside the liver and the spleen. Finally, just like for the bacteria, it is assumed that both liposomes and nanoparticles can penetrate cells by endocytosis, first forming phagosomes, which in turn fuse with lysosomes to form phagolysosomes or secondary lysosomes (25).

Tissue (liver, spleen, . . .), cellular (macrophages, Kupffer cells), and subcellular (lysosomes, phagolysosomes) localization of colloidal carriers parallels distribution of the most common bacteria responsible for intracellular infections. This fact justifies the use of these submicronic systems for an efficient delivery of antibiotics which are generally ineffective against intracellular infections due to poor penetration, poor retention, or weak activity within the intracellular milieu.

Targeting of β-Lactam Antibiotics

Numerous experiments involving particulate formulations have been performed with experimental salmonellosis, which is typical of infection by intracellular bacteria and resembles typhoid fever in humans. Liposome-associated cephalothin has been shown to be more effective than free drug in the treatment of experimental murine salmonellosis (26). Mice treated with cephalothin encapsulated into liposomes had a reduced number of salmonellosis in the liver and the spleen. It was found that liposomal encapsulation increased the uptake of cephalothin *in vitro* by infected peritoneal macrophages at 37°C. However, at 4°C, no intracellular cephalothin was detected after incubation with liposomal cephalothin (27). This observation was related to the fact that liposomes need to be endocytozed to deliver their antibiotic content intracellularly.

The effectiveness of ampicillin bound to nanoparticles of polyhexylcyanoacrylate was also tested on experimental salmonellosis in mice (28). It was observed that the linkage of ampicillin to nanoparticles dramatically increases its efficacy in treating this intracellular infection. A total dose of 0.8 mg of ampicillin bound to nanoparticles suppressed all mortality, whereas three doses of 32 mg each of the free drug were required to suppress mortality (Fig. 2). The therapeutic index of ampicillin, calculated on the basis of mouse mortality, was therefore increased by 120-fold when it was bound to nanoparticles (28). This efficacy was attributed to the combined effect of two types of targeting. First, as shown by the distribution studies, the linkage of ampicillin to nanoparticles led to the concentration of the drug in the liver and spleen; this is important, since these organs are the major foci of infection in that experimental model. Second, as discussed above, the cellular uptake of ampicillin by macrophages is probably increased more when the drug is bound to nanoparticles than when it is in the free form. The effectiveness of 0.8 mg liposome-entrapped ampicillin was less than that observed with the same dose of nanoparticle-bound ampicillin. Indeed, the mortality was reduced by roughly 50%, whereas all the mice survived with nanoparticles (29). This result was related to the lower stability of liposomes in the presence of serum, compared to nanoparticles.

On the contrary, in athymic nude mice with chronic listeriosis, a sharp decrease in the spleen and liver bacterial counts was observed after injection of liposome-entrapped ampicillin (29). This effect was greater than that observed in the spleen and liver after injection of free ampicillin and was more pronounced in the spleen than that observed after treatment with ampicillin bound to nanoparticles (30). This

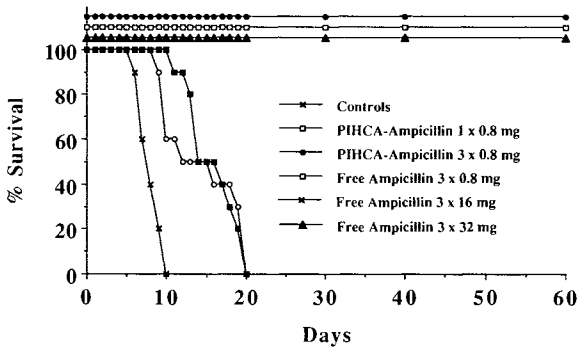

Fig. 2. Efficiency of polyalkylcyanoacrylate nanoparticles loaded with ampicillin on *Salmonella typhimurium*-infected mice compared to free ampicillin (from Ref. 29).

observation was correlated with the substantial uptake by the spleen of liposomes containing cholesterol. Nanoparticle-bound ampicillin was capable of ensuring liver sterilization after two injections of 0.8 mg nanoparticle-bound drug, but the bacterial count in the spleen was less affected by targeting of ampicillin with the aid of nanoparticles (30). This could be explained by the fact that, owing to the histological localization of the macrophages in the liver, only those which are in contact with the blood are able to concentrate nanoparticles.

The improvement in ampicillin's therapeutic index by encapsulating into liposomes was not observed by Bakker-Woudenberg *et al.* (31) in *Listeria monocytogenes*-infected nude mice. Nude mice were used in that experiment because host resistance to *L. monocytogenes,* which depends on a T cell-mediated activation of macrophages, is impaired in these mice (31). The authors speculated that lack of host defense to *L. monocytogenes* in nude mice could be compensated by intracellular delivery of ampicillin via liposomes. However, in spite of the improved therapeutic activity in normal mice, liposomal ampicillin was ineffective in nude mice with an impaired host defense system (31). It was suggested that the lack of efficacy of ampicillin was not due to the inability of the encapsulated drug to reach the intracellularly located bacteria but to the low metabolic activity of intraleucocytic bacteria in nude mice (31). Indeed, a logarithmic culture of *L. monocytogenes* is susceptible to ampicillin, whereas a stationary-phase culture is not, indicating that ampicillin is active only against multiplying bacteria.

With experimental infection caused by *L. monocytogenes* in normal mice, a considerable enhancement (90-fold) of the therapeutic activity of ampicillin resulting from liposomal encapsulation was observed (32). This effect was presumed to be due to an increased delivery of the drug to macrophages of the liver and the spleen but also to the prolonged presence of ampicillin in these organs resulting from entrapment within liposomes.

In vitro it was found that liposomal encapsulation of ampicillin resulted in an increased availability of the antibiotic for the intracellular bacteria (32). Liposomal ampicillin killed 90% of the intracellular bacteria, whereas a similar concentration of free ampicillin plus empty liposomes had no effect upon the intracellular bacteria. However, the *in vitro* growth of *L. monocytogenes* at 37°C was not influenced by intact ampicillin-containing liposomes (32). This indicates that intracellular killing of *L. monocytogenes* is the result of the cellular uptake of liposomes, followed by their degradation and the release of ampicillin intracellularly. However, it is unlikely that ampicillin-containing liposomes are localized in the same intracellular vacuoles. Therefore, one can suggest that the antibiotic may exert its antibacterial activity either following the fusion of phagolysosomes containing the liposomes with those of neighboring bacteria or, if such a fusion does not occur, after diffusion through phagosomal membrane from one to another lysosome or phagolysosome (Fig. 1).

The antibacterial activity of liposome-entrapped ampicillin against *L. monocytogenes* was also investigated *in vitro* in relation to the lipid composition of the liposomes (33). A relatively fluid type of liposomes and a less fluid type were compared. The uptake of both types of liposomes by peritoneal macrophages in monolayer culture *in vitro* was found to be similar. However, the rate of intracellular degradation appeared to be dependent on the lipid composition. A correlation was found between the relatively slow degradation of the less fluid liposomes and a delayed intracellular release of the encapsulated ampicillin, as reflected in absent or delayed intracellular killing of *L. monocytogenes in vitro.* These results were confirmed by *in vivo* observations; slow degradation of less fluid liposomes *in vivo* resulted in a reduction of the antibacterial efficiency of the drug (33). Thus, by modifying the lipid composition, it is possible to modulate the speed by which the drug will be released intracellularly and therefore its therapeutic availability.

From those results, it can be concluded that nanoparticles are clearly more efficient than liposomes in delivering β-lactams into infected liver macrophages, likely because of the better stability of nanoparticles in the blood compartment. On the other hand, the bacterial counts in the spleen seem to be less affected by nanoparticles, which are less efficient than liposomes in diffusing into the spleen's macrophage layers.

Targeting of Aminoglycosides

The influence of the type of liposome upon its antibacterial efficacy was also tested in the treatment of *Brucella melitensis* infection in mice by the use of liposome-encapsulated gentamicin (34). In this experiment, positively charged liposomes containing gentamicin were found very efficient in the elimination of *B. melitensis* residing in liver and spleen, whereas negatively charged liposomes were not as effective. It was believed that the most attractive hypothesis to explain this difference was that, depending on their charge, liposomes may interact differently with macrophages (35). So the degree of liposome–cell interaction with rat peritoneal macrophages can be improved by increasing the degree of positive surface charge.

Sunamoto (36) found that coating liposomes of sisomycin with *O*-palmitoylamylopectin increased the delivery of that drug in the lungs. In addition, these liposomes were found more efficacious in treating pigs with experimental *Legionella pneumophilia.* This result indicates that colloidal carrier-entrapped antibiotics may also be useful against infections other than those localized in the liver and the spleen.

Other aminoglycosides were reported to be capable of eliminating bacteria from infected organs when encapsulated into liposomes. Guinea pigs infected by *Brucella canis* and treated with liposome-entrapped streptomycin were free of bacteria (2 × 10 mg/kg), whereas animals treated with the free drug showed, for the same schedule of administration, only a minor reduction in the number of surviving bacteria (37). Streptomycin was also entrapped in large neutral or anionic unilamellar vesicles of egg phosphotidyl choline (38). The antibiotic in liposomal form was inactive against *Escherichia coli* in a simple tube dilution assay. When *E. coli* was located in J774.2 murine macrophages, the apparent intracellular antibacterial activity of streptomycin was increased more than 10-fold by encapsulation in neutral liposomes. This observation supports the hypothesis that liposomes need to be degraded before the antimicrobial activity appears.

The superiority of liposomal streptomycin was also evident in experimental infections with *Mycobacterium tuberculosis* and *Salmonella enteritidis* (39). On the contrary, Milward *et al.* did not observe any improvement by using liposome-entrapped streptomycin in the treatment of pigs infected with *Brucella abortus* (40).

The delivery of antibacterial drugs to phagocytic cells was also demonstrated to be feasible with amikacin (41), kanamycin, and tobramycin (37).

It is important to point out that association of aminoglycosides with liposomes is, from a theoretical point of view, somewhat illogical. Indeed, aminoglycosides are often considered to exemplify antibiotics that are spontaneously retained in cell lysosomes but with a poor antimicrobial activity at acidic pH (3). In general, higher concentrations are necessary for intracellular than for extracellular activity (42). In some cases, no intracellular activity is even recorded. For example, streptomycin was reported to display no intracellular antibacterial activity against *E. coli* or *Brucella* species, although the drug was able to penetrate intracellularly (43,44). In addition, although data in the literature do not lead easily to definite conclusions about the effect of aminoglycosides on phagocyte functions, when an effect on phagocytosis is found, it has been an inhibition of cell function (42). This could lead to a reduced cell uptake of liposomal preparations and thus a still poorer intracellular efficacy. The only rationale for encapsulating aminoglycosides into colloidal carriers (liposomes or nanoparticles) is that these compounds are most often taken up at a very slow rate. They will therefore not be very efficacious against rapidly developing bacteria. According to Tulkens (2), if the host cell has a limited life span, the intracellular bacteria, unaffected by the aminoglycoside, will be released extracellularly and will reinitiate a systemic infection. Thus, liposomes should allow a more rapid intracellular concentration of aminoglycoside.

Table I summarizes the *in vivo* studies using liposomes of different compositions with various encapsulated antibiotics.

DRUG TARGETING AND STERILIZATION OF ORGANS

In the overwhelming majority of the intracellular infections studied, the target organs were not sterilized, i.e., the infectious microorganisms were not eliminated even in the face of very high concentrations of the agent in the organ. Table I summarizes the outcome of the studies to date. This failure of sterilization may have several causes. (i) The target organisms may be in an intracellular compartment that is not reached by the carrier: indeed, the intracellular distribution of a fluorescent molecule was different depending on whether it was entrapped in liposomes or in nanoparticles (45), and further, intracellular pathogens, such as *Shigella flexneri* (46) and *Listeria monocytogenes* (47), were able to move across the intracellular bacterial compartments. (ii) It is possible that the organisms are not dividing so they may be less susceptible to antibacterial activity. This hypothesis is difficult to test, but it is widely accepted to account for the recurrence of some intracellular infections—such as tuberculosis—after years of latency. However, in experimental

Table I. Review of the Studies Using Liposomes with Encapsulated Antibiotics for the Treatment of Experimental Infections *in Vivo*[a]

Antibiotic	Composition of liposomes	Bacteria	Animal model	Efficiency compared to the free drug	Reference
Amikacin	PC/Ch/PG: 1/1/1	*Mycobacterium avium* *Mycobacterium intracellulare*	Beige mice	+	Düzgünes *et al.*, 1988 (41)
Streptomycin Oxytetracyclin	PC PC/Ch: 1/2 PC/Ch/DCP: 7/2/1	*Brucella abortus*	Pig	0	Milward *et al.*, 1984 (40)
Ampicillin	SM/Ch/PS: 4/5/1	*Listeria monocytogenes*	C57 Bl/Ka normal mice	+	Bakker-Woudenberg *et al.*, 1985 (31)
Ampicillin	SM/Ch/PS: 4/5/1	*Listeria monocytogenes*	C57 Bl/Ka nude mice	0	Bakker-Woudenberg *et al.*, 1985 (31)
Cephalotin	PC/Ch/PS: 6/3/1	*Salmonella typhimurium*	ICR mice	+	Desiderio and Campbell, 1983 (27)
Streptomycin	PC/Ch/DPPC: 5/5/0,5	*Salmonella enteridis*	Black mice	+	Tadakuma *et al.*, 1985 (39)
Sisomycin	PC/amylopectin	*Legionella pneumophilia*	Guinea pig	+	Sunamoto *et al.*, 1983 (36)
Gentamicin Kanamycin Dihydrostreptomycin Streptomycin	PC	*Brucella canis* *Brucella abortus*	Swiss mice	+	Fountain *et al.*, 1985 (37)

[a] +, increased efficiency; 0, same efficiency; PC, phosphatidylcholine; Ch, cholesterol; AP, phosphatidic acid; PG, phosphatidylglycerol; SM, sphingomyelin; PS, phosphatidylserine; DPPC, dipalmitoylphosphatidylcholine.

listeriosis, nanoparticulate ampicillin was able actually to sterilize the liver after 7 days of treatment (30). Further, we have recently observed that repeated treatments of *S. typhimurium*-infected mice with nanoparticulate ampicillin at day 30 after infection indeed sterilized the organs (unpublished results). (iii) Resistant mutants organisms may arise. This hypothesis, however, was not observed in any of the experiments performed by our group and appears rather unlikely because of the relatively low number of intracellular bacteria present when the treatment was initiated. (iv) Finally, some of the target organs can be reseeded by microbes from a privileged site in the body. This possibility is strongly suggested by results obtained with the livers of mice infected with *L. monocytogenes,* which were only transiently sterile after treatment with nanoparticulate ampicillin, whereas spleens were never sterile and may have acted as reseeding organs (30).

DRUG TARGETING AND BACTERIAL RESISTANCE

Bacterial resistance to certain antibiotics may be due to poor diffusion through the bacterial cell wall but also to the production of β-lactamases by the bacteria. Only a few papers have considered the possibility of overcoming bacterial resistance by using particulate colloidal carriers able to allow a better membrane diffusion together with drug protection.

Chowdhury *et al.* (48) were the first to show significant inhibition of some penicillin-resistant bacteria by entrapping this antibacterial agent into liposomes. In that study, *Staphylococcus aureus-, Bacillus licheniformis-,* and *Escherichia coli*-resistant strains were unaffected by penicillin, even at a concentration of 60 μg/ml, which is much higher than the minimum inhibitory concentrations on wild strains. On the contrary, growth inhibition of these resistant bacteria was obtained when penicillin was entrapped into liposomes and used at a concentration of 30 μg/ml.

In addition, positively charged liposomes were found more efficient, possibly due to an electrostatic attraction with the negative charge of the membrane surface of the bacteria, facilitating fusion of both membranes (48). Chowdhury *et al.* (48), however, did not investigate in detail the mechanism involved in overcoming bacterial resistance with the aid of liposomes. This was done by Nacucchhio *et al.* (49) using a *S. aureus*-resistant strain. The results, expressed as the percentage of bacterial growth inhibition, demonstrated that growth inhibition was highest when piperacillin was encapsulated into liposomes. Interestingly, adsorption of piperacillin at the surface of liposomes induced a significant enhancement of the antistaphylococcal activity when compared with the effect of the free drug. To confirm whether piperacillin was actually protected from hydrolysis by β-lactamase, exogenous β-lactamase was added to both free and encapsulated piperacillin (49). In that experiment, the encapsulation of piperacillin in liposomes conferred a high degree of protection against hydrolysis. It was concluded that, besides a possible facilitated transport of the drug as suggested by Chowdhury *et al.* (48), association with liposomes protects the antibiotic from hydrolysis by β-lactamases. In addition, it was proposed that adsorption of piperacillin at the surface of liposomes produced steric hindrance to the action of the β-lactamases, thus protecting the drug from hydrolysis by the enzyme.

Finally, the fact that liposomes may be useful in overcoming the cell wall barrier was confirmed by Sekeri-Pataryas *et al.* (50) with *Pseudomonas aeruginosa.* The study of the distribution of labelled penicillin in the cellular subfraction of the bacteria showed that more than half of the ^{14}C-labeled antibiotic passed the cell wall and was found associated with the cytoplasmic membranes or in the 160,000*g* supernatant. It should be of interest to compare these results with the distribution of the antibiotic when entrapped in nanoparticles where the phospholipid membrane is replaced by a synthetic biodegradable polymer.

CONCLUSION

The development of new antibacterial formulations capable of an intracellular action may improve therapy in a number of bacterial infections that are presently difficult to cure. The need for intracellular targeting of antibiotics is urgent since intracellular infections are often associated with AIDS (51).

The use of colloidal drug carriers (i.e., liposomes or nanoparticles) to deliver more efficiently antibacterial agents inside the cells is supported by numerous studies. This strategy provides one solution to the problem of the poor penetration and retention of antibiotics within the phagosomes. In addition, the use of submicron particulate carriers may serve to overcome bacterial resistance. However, the potential toxicity of particulate antibacterial preparations needs to be studied. In particular, massive drug exposure of Kupffer cells may affect reticuloendothelial function.

However, drug concentrations in the lysosomal compartment may also be accomplished by newer antibiotics such as new macrolides or fluoroquinolones. In the long run the efficacy of liposomes and nanoparticles to carry antimicrobial to intracellular targets will have to be evaluated against new drug design.

ACKNOWLEDGMENTS

This work was partly supported by the "Reseau Vectorisation" (DRED, Ministery of Education, France) and by a grant from Negma Laboratories (Buc, France).

REFERENCES

1. M. A. Horwitz. Phagocytosis of microorganism. *Rev. Infect. Dis.* 4:104–123 (1982).
2. P. Tulkens. The design of antibiotics capable of an intracellular action. In P. Buri and R. Gumma (eds.), *Aims, Potentialities and Problems in Drug Targeting,* Elsevier, Amsterdam, 1985, pp. 179–194.
3. P. Tulkens and A. Trouet. The uptake and intracellular accumulation of aminoglycosides antibiotics in lysosomes of cultured rat fibroblasts. *Biochem. Pharmacol.* 27:415–424 (1978).
4. A. Trouet and P. Tulkens. Intracellular penetration and distribution of antibiotics: The basis for an improved chemotherapy of intracellular infections. In L. Ninet, P. E. Bost, D. H. Bouanchaud, and J. Florent (eds), *The Future of Antibiotherapy and Antibiotic Research,* Academic Press, London, 1981, pp. 337–349.

5. J. D. Johnson, W. L. Hand, J. B. Francis, N. King-Thompson, and R. W. Corwin. Antibiotic uptake by alveolar macrophages. *J. Lab. Clin. Med.* 95:429–439 (1980).
6. C. E. Swenson, M. C. Popescu, and R. S. Ginsberg. Preparation and use of liposomes in the treatment of microbial infections. *Crit. Rev. Microbiol.* 15:S1–S31 (1988).
7. E. Fattal, J. Rojas, L. Roblot-Treupel, A. Andremont, and P. Couvreur. Ampicillin-loaded liposomes and nanoparticles: Comparison of drug loading, drug release and *in vitro* antimicrobial activity. *J. Microencapsul.*, in press (1991).
8. L. D. Mayer, M. B. Bally, M. J. Hope, and P. R. Cullis. Techniques for encapsulating bioactive agents into liposomes. *Chem. Phys. Lipids* 40:333–345 (1986).
9. G. Scherphoff, F. Roerdink, D. Hoekstra, J. Zborowski, and E. Wisse. Stability of liposomes in presence of blood constituants: Conequences for uptake of liposomal lipid and entrappeed compounds by rat liver cells. In G. Gregoriadis and A. C. Allison (eds.), *Liposomes in Biological Systems*, John Wiley, New York, 1980, pp. 179–209.
10. A. Gabizon and D. Papahadjopoulos. Liposomes formulations with prolonged circulation time in blood and enhanced uptake by tumors. *Proc. Natl. Acad. Sci. USA* 85:6949–6953 (1988).
11. T. M. Allen and A. Choun. Large unilamellar liposomes with low uptake into the reticuloendothelial system. *FEBS Lett.* 223:42–46 (1987).
12. S. M. Gruner, R. P. Lenk, A. S. Janoff, and M. J. Ostro. Novel multilayered lipid vesicles: Comparison of physical characteristics of multilamellar liposomes and stable plurilamellar vesicles. *Biochemistry* 24:2833–2842 (1985).
13. C. Kirly and G. Gregoriadis. Dehydratation-rehydratation vesicles: A simple method for high yield drug entrapment in liposomes. *Biotechnology* 11:979–984 (1984).
14. T. Hernandez-Caselles, J. Villalain, and J. C. Gomez-Fernandez. Stability of liposomes on long term storage. *J. Pharm. Pharmacol. Res.* 1:159–163 (1984).
15. J. H. Crowe and L. M. Crowe. Factors affecting the stability of drug liposomes. *Biochim. Biophys. Acta* 939:327–334 (1988).
16. L. M. Crowe, C. Womersley, J. H. Crowe, D. Reid, L. Appel, and A. S. Rudolph. *Biochim. Biophys. Acta* 861:131–140 (1986).
17. T. D. Madden, M. B. Bally, M. J. Hope, P. R. Cullis, H. P. Schieren, and A. S. Janoff. Protection of large unilamellar vesicles by trehalose during dehydratation: Retention of vesicle contents. *Biochim. Biophys. Acta* 817:67–74 (1985).
18. S. Henry-Michelland, M. J. Alonso, A. Andremont, P. Maincent, J. Sauzières, and P. Couvreur. Attachment of antibiotics to nanoparticles: Preparation, drug-release and antimicrobial activity *in vitro*. *Int. J. Pharm.* 35:121–127 (1987).
19. P. Couvreur, M. Roland, and P. Speiser. U.S. patent 4:489–555 (1984).
20. P. Couvreur. Polyalkylcyanoacrylates as colloidal drug carriers. *Crit. Rev. Ther. Drug Carrier Syst.* 5:1–20 (1988).
21. C. Verdun, P. Couvreur, H. Vranckx, V. Lenaerts, and M. Roland. Development of a nanoparticulate controlled-release formulation for human use. *J. Control. Release* 3:205–210 (1986).
22. V. Guise, J. Y. Drouin, J. Benoit, J. Mahuteau, P. Dumont, and P. Couvreur. Vidarabine-loaded nanoparticles: a physicochemical study. *Pharm. Res.* 7:736–741 (1990).
23. G. Poste. Liposome targeting *in vivo:* Problems and opportunites. *Biol. Cell.* 47:19–39 (1983).
24. L. Grislain, P. Couvreur, V. Lenaerts, M. Roland, D. Deprez-Decampeneere, and P. Speiser. Pharmacokinetics and distribution of a biodegradable drug-carrier. *Int. J. Pharm.* 15:335–345 (1983).
25. P. Couvreur, P. Tulkens, M. Roland, A. Trouet, and P. Speiser. Nanocapsules, a new lysosomotropic carrier. *FEBS Lett.* 84:323–326 (1977).
26. J. V. Desiderio and S. G. Campbell. Liposome-encapsulated cephalotin in the treatment of experimental murine salmonellosis. *J. Reticuloendothel. Soc.* 34:279–287 (1983).
27. J. V. Desiderio and S. G. Campbell. Intraphagocytic killing of *Salmonella typhimurium* by liposome-encapsulated cephalotin. *J. Infect. Dis.* 148:563–570 (1983).
28. E. Fattal, M. Youssef, P. Couvreur, and A. Andremont. Treatment of experimental salmonellosis in mice with ampicillin-bound nanoparticles. *Antimicrob. Agents Chemother.* 33:1540–1543 (1989).
29. E. Fattal, J. Rojas, M. Youssef, P. Couvreur, and A. Andremont. Liposome-entrapped ampicillin in the treatment of experimental murine listeriosis and salmonellosis. *Antimicrob. Agents Chemother.*, in press (1991).
30. M. Youssef, E. Fattal, M. J. Alonso, L. Roblot-Treupel, J. Sauzières, C. Tancrède, A. Omnès, P. Couvreur, and A. Andremont. Effectiveness of nanoparticle-bound ampicillin in the treatment of *Listeria monocytogenes* infection in athymic nude mice. *Antimicrob. Agents Chemother.* 32:1204–1207 (1988).
31. I. A. J. M. Bakker-Woudenberg, A. F. Lokerse, F. H. Roerdink, D. Regts, and M. F. Michel. Free versus liposome-entrapped ampicillin in treatment of infection due to *Listeria monocytogenes* in normal and athymic nude mice. *J. Infect. Dis.* 151:917–924 (1985).
32. I. A. J. M. Bakker-Woudenberg, A. F. Lokerse, J. C. V. van den Berg, and F. H. Roerdink. Liposome-encapsulated ampicillin against *Listeria monocytogenes* in vivo and in vitro. *Infection* 16(Suppl. 2):S165–S170 (1988).
33. I. A. J. M. Bakker-Woudenberg, A. F. Lokerse, and F. H. Roerdink. Antibacterial activity of liposome-entrapped ampicillin *in vitro* and *in vivo* in relation to the lipid composition. *J. Pharmacol. Exp. Ther.* 251:321–327 (1989).
34. T. Hernandez-Caselles, A. Vera, F. Crespo, J. Villalain, and J. C. Gomez-Fernandez. Treatment of *Brucella melitensis* in mice by use of liposome-encapsulated gentamicin. *Am. J. Vet. Res.* 50:1486–1488 (1989).
35. R. A. Schwendener, P. A. Lagocki, and Y. E. Rahman. The effect of charge and size on the interaction of unilamellar liposomes with macrophages. *Biochim. Biophys. Acta* 772:93–101 (1984).
36. J. Sunamoto, M. Goto, T. Tida, K. Hara, A. Saito, and A. Tomonaga. Unexpected tissue distribution of liposomes coated with amylopectin derivatives and successful use in the treatment of experimental Legionaires Disease. In G. Gregoriadis, G. Poste, J. Senior, and A. Trouet (eds.), *Receptor-Mediated Targeting of Drugs*, Plenum Press, New York, 1983, p. 359.
37. M. W. Fountain, S. J. Weiss, A. G. Fountain, A. Shen, and R. P. Lenk. Treatment of *Brucella canis* and *Brucella abortus* in vitro and in vivo by stable plurilamellar vesicle-encapsulated aminoglycosides. *J. Infect. Dis.* 152:529–535 (1985).
38. M. Stevenson, A. J. Baillie, and M. E. Richards. Enhanced activity of streptomycin and chloramphenicol against intracellular *Escherichia coli* in the J774 macrophage cell line mediated by liposome delivery. *Antimicrob. Agents Chemother.* 24:742–749 (1983).
39. T. Tadakuma, N. Ikewaki, T. Nasuda, M. Tsutsumi, S. Saito, and K. Saito. Treatment of experimental salmonellosis in mice with streptomycin entrapped into liposomes. *Antimicrob. Agents Chemother.* 28:28–32 (1985).
40. F. W. Milward, P. Nicoletti, and E. Hoffmann. Effectiveness of various therapeutic regimens for bovine brucellosis. *Am. J. Vet. Res.* 45:1825–1828 (1984).
41. N. Düzgünes, V. K. Perumal, L. Kesavalu, J. A. Goldstein, R. J. Debs, and P. R. J. Gangadharam. Enhanced effect of liposome encapsulated amikacin on *Mycobacterium avium-M. intracellulare* complex infection in Beige mice. *Antimicrob. Agents Chemother.* 32:1404–1411 (1988).
42. P. J. van den Broek. Antimicrobial drugs, microorganisms and phagocytes. *Rev. Infect. Dis.* 11:213–245 (1989).
43. W. Brumfitt, A. A. Glynn, and A. Percival. Factors influencing the phagocytosis of *Escherichia coli*. *Br. J. Exp. Pathol.* 46:215–226 (1965).
44. R. L. Magoffin and W. W. Spink. The protection of intracellular Brucella against streptomycin alone and in combination with other antibiotics. *J. Lab. Clin. Med.* 37:924–930 (1951).
45. V. Guise, P. Jaffray, J. Delattre, F. Puisieux, M. Adolphe, and P. Couvreur. Comparative cell uptake of propidium iodine associated with liposomes or nanoparticles. *Cell. Mol. Biol.* 33:397–405 (1987).

46. J. Mounier, A. Ryter, M. Coquis-Rondon, and P. J. Sansonetti. Intracellular and cell-to-cell spread of *Listeria monocytogenes* involves interaction with F-actin in the enterocytelike cell line Caco-2. *Infect. Immun.* 58:1048–1058 (1989).
47. M. L. Bernardin, J. Mounier, H. d'Hauteville, M. Coquis-Rondon, and P. J. Sansonetti. Identification of *ics* A, a plasmid locus of *Shigella flexneri* that governs bacterial intra- and intercellular spread through interaction with F-actin. *Proc. Natl. Acad. Sci. USA* 86:3867–3871 (1989).
48. M. K. R. Chowdhury, R Goswami, and P. Chakrabarti. Liposome-entrapped penicillins in growth inhibition of some penicillin-resistant bacteria. *J. Appl. Bacteriol.* 51:223–227 (1981).
49. M. C. Nacucchio, M. J. Gatto-Bellora, D. O. Sordelli, and M. D'Aquino. Enhanced liposome-mediated activity of piperacillin against staphylococci. *Antimicrob. Agents Chemother.* 27:137–139 (1985).
50. K. H. Sekeri-Pataryas, C. Vakirtzi-Lemonias, H. A. Pataryas, and J. N. Legakis. Liposomes as carriers of ^{14}C-labelled penicillin and ^{125}I-labelled albumin through the cell wall of *Pseudomonas aeruginosa*. *Int. J. Biol. Macromol.* 7:379–381 (1985).
51. C. L. Celum, R. E. Chaisson, G. W. Rutherford, J. L. Barharnt, and D. F. Echenberg. Incidence of salmonellosis in patients with AIDS. *J. Infect. Dis.* 156:998–1002 (1985).