Antimicrobial chemotherapy directed by liposomes

Despite the availability of antibacterial agents for more than 50 years, patients still die from infection. Different strategies for potentiating antibiotic therapy in severe infection were recently outlined by O'Grady (1984). One method is to intensify the antibiotic treatment in terms of dosage, frequency or mode of administration. Another method is to direct the antibiotic to specific tissues and cells, by means of liposomes.

Liposomes are microscopic structures consisting of one or more concentric lipid bilayers surrounding aqueous compartments within which water-soluble drugs can be entrapped. Lipophilic agents can be incorporated into the phospholipid membranes. After intravenous injection liposomes are rapidly cleared from the blood and taken up by cells of the reticulo-endothelial system. Fixed macrophages in the liver (Kupffer cells) and the spleen, in particular, are actively involved in the uptake of the vesicles (Roerdink et al., 1977, 1981). In addition circulating monocytes have been reported to take up significant amounts of liposomes (Poste et al., 1982). In the lung, the liposome-loaded monocytes can subsequently migrate to the alveoli to become alveolar macrophages. Uptake of liposomes by macrophages occurs by endocytosis and is followed by extensive intralysosomal degradation of liposomal lipids and release of entrapped drugs (Dijkstra et al., 1984, 1985). Although it is possible to modify the tissue distribution of intravenously injected liposomes to some extent by varying the liposomal dose and size, the majority of the vesicles still end up in the reticulo-endothelial system. Directing liposomes to other cells, for example, by coating the vesicles with cell-specific ligands, is hampered by the inability of the vesicles to cross the capillary endothelium in most organs and the low endocytic capacity of many cell types (Poste, 1983; Juliano & Lopez-Berestein, 1985). On the other hand, the natural affinity of liposomes for macrophages can be exploited as a drug delivery system. For example, Poste, Bucana & Fidler (1982) successfully utilized the macrophage’s capacity to take up liposomes in order to activate the tumoricidal properties of the cells by means of liposome-entrapped immuno-modulators such as muramyl dipeptide.

Delivery of antimicrobial agents by means of liposomes has been demonstrated in some experimental models of the intracellular parasitic infections leishmaniasis and malaria in hamsters and mice with intact host defences (Alving et al., 1978; New & Chance, 1980; Pirson et al., 1980), and of mycotic infections such as Candida albicans infection both in normal mice (Lopez-Berestein et al., 1983) and neutropenic mice (Lopez-Berestein et al., 1984). These studies demonstrated an improved therapeutic index and reduced toxicity as a result of encapsulating the drug within liposomes. The delivery of antibacterial drugs to phagocytic cells by liposomes has been demonstrated in vitro. For Staphylococcus aureus, Escherichia coli and Salmonella typhimurium it was shown that intraphagocytic killing by antibiotics was enhanced by encapsulating the drugs within liposomes (Bonventre & Gregoriadis, 1978; Fountain, Dees & Schultz, 1981; Desiderio & Campbell, 1983; Stevenson, Baille & Richards, 1983). In experimental infection caused by Listeria monocytogenes in mice we observed a considerable enhancement (80-fold) in the therapeutic activity of ampicillin resulting from liposomal encapsulation (Bakker-Woudenberg et al., 1985). The mechanism by which liposomes improved the therapeutic index of ampicillin in this infection appeared to be an increased delivery of the antibiotic to the site of infection, the liver and spleen. Substantial amounts of liposomal radioactive ampicillin could be recovered from isolated Kupffer cells, the target cell of L. monocytogenes after intravenous inoculation. In addition, in studies of the survival of L. monocytogenes within murine peritoneal macrophages in vitro it was found that liposomal encapsulation of ampicillin resulted in an increased availability of the antibiotic for the intracellular bacteria; liposomal ampicillin killed 99% of the intracellular bac-
teria, whereas a similar concentration of free ampicillin plus empty liposomes only inhibited intracellular bacterial growth.

Although substantial evidence is now available about the potential usefulness of liposomes as drug carriers further studies are needed to establish optimal conditions for systemic therapy with liposome-encapsulated antimicrobial agents. By manipulating the lipid composition and the number of lamellae of the vesicles, rates of uptake or intracellular degradation by macrophages, or both, can be altered and thus the rates at which liposome-encapsulated drugs are released intracellularly and become available to exert their therapeutic action. For example, we found that incorporation of an aminomannosyl derivative of cholesterol into the liposomal membrane greatly enhances the uptake of the vesicles by Kupffer cells (Roerdink et al., in press). Similar results have been obtained with peritoneal macrophages (Wu, Tin & Baldeschwieler, 1981). Sunamoto et al. (1984) have recently shown that uptake of liposomes by monocytes and alveolar macrophages can be increased by coating the vesicles with an amlylopectin derivative. After intravenous injection the amyllopectin modified liposomes were concentrated in the lungs. This observation was elegantly applied in the treatment of experimental Legionnaires' disease in guinea pigs by the intravenous injection of a mixture of free and liposome-entrapped sisomycin (Sunamoto et al., 1984).

The direction of antimicrobial agents to target sites by means of liposomes may be of great value in the treatment of intracellular or extracellular infections that prove refractory to conventional forms of antimicrobial therapy. A recent preliminary clinical study (Lopez-Berestein et al., 1985) indicated that liposomal amphotericin B was beneficial and non-toxic in the treatment of fungal infections in eight out of twelve neutropenic and/or immunocompromised cancer patients who had previously failed to respond to therapy with the non-encapsulated drug.

References


Leading articles


Antiviral chemotherapy against HTLV-III/LAV infections

The identification of a retrovirus (HTLV-III/LAV) as the primary cause of the acquired immunodeficiency syndrome (AIDS) (Barre-Sinoussi et al., 1983; Gallo et al., 1984) offers a target for antiviral chemotherapy. This is not an easy target, however, owing to the presence of the viral genome as a provirus and the latent state of the infection. Several important questions require answers. It must be established which infected cells release HTLV-III/LAV, how long these infected cells survive in vivo, whether these cells can be eliminated and whether further spread of virus to new cells can be prevented.

Originally, it was thought that only the T4 helper/inducer subset of T cells was infected by HTLV-III/LAV but now it is clear that other cells, such as mononuclear cells, macrophages, glial cells and EBV-infected B cells can also be infected. However, the infection of T4+ cells with HTLV-III/LAV is a crucial event in the development of AIDS. The presence of other infections such as cytomegalovirus may contribute to the development of the disease by reactivation of HTLV-III/LAV through T4+ stimulation. It has also been found that HTLV-III/LAV infected persons cannot respond to HTLV-III/LAV antigen by T4+ activation, a unique property of HTLV-III/LAV infection (Wahren et al., 1985).

The half-lives of infected cells able to release virus are not known but it seems likely that dividing T4+ cells can be destroyed by a lytic multiplication of HTLV-III/LAV. Thus a mitogenic stimulation of T4+ cells should increase the replication rate of infected cells but, unfortunately, at the same time provide more virus for infection of new cells. However, if the HTLV-III/LAV reverse transcriptase activity was blocked by a selective inhibitor, the infection of new cells might be prevented. The expression of the viral genome, already presented as a DNA provirus, is mediated by cellular RNA polymerase II and is therefore not susceptible to inhibitors of viral reverse transcriptase.

Table I lists some inhibitors of HTLV-III/LAV reverse transcriptase and viral replication in cell culture, with their effects on cellular DNA polymerase a and on the multiplication of uninfected cells. It should be emphasized that, since the assay conditions differ, the information is still incomplete and in-vitro data might not relate to in-vivo efficacy. 3'Azidothymidine is phosphorylated by cellular enzymes...