MINIREVIEW

Liposomes as Carriers of Antimicrobial Agents

GABRIEL LOPEZ-BERESTEIN

Immunobiology and Drug Carriers Section, Department of Clinical Immunology and Biological Therapy, The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston, Houston, Texas 77030

Liposomes are microscopic vesicles consisting of multiple concentric lipid bilayers formed when an aqueous solution is added to a dried lipid film. Since their initial description by Bangham et al. (5), several types of liposomes have been characterized (41). Multilamellar liposomes or multilamellar vesicles have been extensively used as carriers of antineoplastic and antimicrobial drugs. These multilamellar vesicles have two parts: an extensive lipid compartment that embodies the multiple lipid layers and an aqueous compartment that consists of the spaces between the layers.

As with most drug carriers, liposomes have been extensively used in an attempt to improve the therapeutic index of known active drugs, such as Adriamycin (14). In those studies, the major reduction observed in the target-organ toxicity of the drugs was probably related to a modification in drug distribution. Liposomes are preferentially taken up by organs rich in cells from the reticuloendothelial system. In mice (30) and humans (25), liposomes have been shown to localize in the liver, spleen, lung, and bone marrow.

The biologic and pharmacologic attributes of liposomes have been exploited by several investigators for the delivery of antimicrobial agents (Table 1). Liposome type, size, lipid composition, membrane fluidity, stability and charge, and ease of preparation are factors that must be considered when designing liposomal carriers. Multilamellar vesicles with their large lipid compartment offer an advantage for the incorporation of hydrophobic drugs; in contrast, reverse evaporation vesicles are a better choice for the incorporation of hydrophilic compounds. Usually, naturally occurring, biodegradable, and nontoxic phospholipids, such as lecithin, phosphatidyl serine, and phosphatidyl glycerol, are selected. The fluidity and charge of the liposomal membranes can be altered to facilitate specific objectives, such as in the case of macrophage targeting, in which negatively charged liposomes with more fluid membranes are preferentially phagocytosed. The incorporation of sterols (ergosterol and cholesterol) increases the rigidity and stability of liposomes. However, in the case of amphotericin B (AmpB) the use of sterol-containing liposomes results in decreased antifungal activity. This latter observation is probably related to tight drug-sterol binding which impedes the exchange of the drug with fungal membranes.

AmpB

AmpB, a heptaene antibiotic, is a highly effective but toxic antifungal drug whose activity is related to its capacity to interact with lipids in biologic membranes (3, 17). The high hydrophobicity of AmpB makes it a good candidate for liposomal incorporation. Liposome-incorporated AmpB (L-AmpB) has been studied in a variety of experimental infections. Early work by New et al. (36) demonstrated that L-AmpB was less toxic and more active against experimen-
follow-up periods greater than 1 year. In none of the nine patients cured who received additional chemotherapy for their leukemia (of these patients, two had bone marrow transplantation) was there evidence of recurrence of the fungal infection.

**Pharmacologic considerations.** The mechanisms involved in the enhanced therapeutic index observed with L-AmpB are not well understood, but tissue localization may play a role. We observed that when AmpB was given in liposomes, higher concentrations were found in the liver, spleen, lung, and kidney tissue of mice infected with *Candida albicans* (30). However, tissue localization alone may not be the explanation, since AmpB concentrations exceeding 160 μg/g of tissue (liver and spleen) were noted in patients with hepatic candidiasis failing to respond to AmpB (9).

This observation raises the possibility that L-AmpB may be delivered to the infected sites via either capillary leakage or secondary transport by phagocytes. In vitro, it has been shown that L-AmpB fungicidal activity was maintained, although its toxicity to erythrocytes was abrogated (33). However, the presence of serum reduces the toxicity of AmpB; therefore, the conditions present in the biologic microenvironment were not accurately reflected in these experiments. We also observed that the suppressive effect of AmpB on macrophages (32) was also nullified by AmpB incorporation in liposomes (34). Thus, a preponderant exchange of AmpB from sterol-free liposomes to ergosterol-containing fungal membranes may also be a factor.

**ANTIMONIAL DRUGS**

An excellent example of the use of liposomes to carry antimicrobial agents was provided by the studies of Alving et al. (1, 2) in the treatment of experimental leishmaniasis. Leishmanias are intracellular parasites of phagocytic cells of the reticuloendothelial system, offering a natural target for liposomal delivery. These investigators showed that liposome-incorporated antimicrobial agents and 8-aminoquinolines were far superior to the free drugs against this parasite.

**CEPHALOTHIN**

Liposome-incorporated cephalothin has been shown to be effective in the treatment of other facultative intracellular bacteria. Desiderio and Campbell (11, 12) showed that liposome-encapsulated cephalothin was more effective than the free drug in the treatment of experimental murine salmonellosis. Mice treated with the liposomal form of the drug had a reduced number of salmonellae in the liver and spleen.

**PRIMAQUINE**

Liposome encapsulation of primaquine phosphate has been demonstrated to reduce the acute toxicity related to the drug while allowing it to maintain its efficacy against murine malaria (39, 40). Although drug targetting was not the major objective in these experiments, its therapeutic index was enhanced. Primaquine phosphate is toxic mainly to the lungs, heart, kidneys, and brain; by incorporation in liposomes, its distribution is radically modified.

**IMMUNOMODULATORS**

Macrophages are a major component of the defenses of a host against microbial diseases. Several synthetic and biologic products can render macrophages activated to destroy microorganisms and neoplastic cells. Liposome-incorporated macrophage activators have been shown to be more effective than free agents in the activation of macrophages for antitumor activity (29). A similar rationale was used to treat herpes (18–20), hepatitis (22), and fungal (13) infections.

**PROSPECTS**

Liposomes are but one of the many new and promising drug-carrier systems being developed. Their successful use

---

**TABLE 1. Liposomes as drug carriers for antimicrobial agents**

<table>
<thead>
<tr>
<th>Drug(s)</th>
<th>Disease</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AmpB</td>
<td>Leishmaniasis</td>
<td>37, 38</td>
</tr>
<tr>
<td></td>
<td>Histoplasmosis</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>Cryptococcosis</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Candidiasis</td>
<td>24, 26, 27</td>
</tr>
<tr>
<td>Antimonials</td>
<td>Leishmaniasis</td>
<td>1, 2, 7, 8, 36, 37, 43</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>Salmonellosis</td>
<td>11, 12</td>
</tr>
<tr>
<td>Primaquine phosphate</td>
<td>Malaria</td>
<td>39, 40</td>
</tr>
<tr>
<td>Immunomodulators</td>
<td>Muramyl dipeptide</td>
<td>Herpes: 20, Candidiasis: 13</td>
</tr>
<tr>
<td></td>
<td>Interferon</td>
<td>Hepatitis: 22</td>
</tr>
<tr>
<td>Other antibiotics</td>
<td></td>
<td>4, 6, 21, 35</td>
</tr>
</tbody>
</table>

**TABLE 2. Summary of clinical trials with L-AmpB in treatment of systemic fungal infections**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Cured</th>
<th>Partial response</th>
<th>No response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candidiasis</td>
<td>7</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Aspergilosis</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**FIG. 1. Toxicity and antifungal activity of free AmpB versus L-AmpB in mice.** All drugs were administered intravenously in 0.2 ml of 0.9% NaCl solution. (A) Toxicity. Symbols: ◊, free AmpB; ○, L-AmpB. (B) Antifungal activity. The drugs were injected 2 days after inoculation of yeasts. Symbols: ◊, untreated infected control; ○, empty liposomes; △, free AmpB at 0.8 mg/kg; □, free AmpB at 0.8 mg/kg daily for 5 days; ■, L-AmpB at 4 mg/kg. Modified from reference 28.
in the treatment of several experimental diseases demonstrates that, with the selection of the right drug, the right carrier, and the right disease target, a solid rationale for the clinical development of liposomes as drug carriers can be established. Understanding of liposome behavior in the body and of the physicochemical mechanisms involved in the interactions of liposome, drug, and cellular targets is essential for their future application. Technology has recently been developed for large-scale production of liposome-incorporated drugs, so their promise will soon be verified in extensive clinical trials.

ACKNOWLEDGMENT

G.L.B. is a Scholar of the Leukemia Society of America.

LITERATURE CITED


34. Mehta, R., G. Lopez-Berestein, R. Hopfer, K. Mills, and R. L.


