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Therapeutic efficacy of liposomal gentamicin in clinically relevant rat models☆

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Abstract

Sterically stabilized liposomes are able to localize selectively at sites of infection, potentially permitting targeted drug delivery. Up to now, the majority of studies investigating therapeutic efficacy of liposomes have been conducted in animals with an intact host defense infected with high antibiotic-susceptible bacteria. In the present study, the therapeutic efficacy of gentamicin encapsulated in sterically stabilized liposomes, alone or in combination with the free drug was studied in rats with intact host defense as well as leukopenic rats. Rats were inoculated with a high gentamicin-susceptible or low-gentamicin susceptible *Klebsiella pneumoniae* in the left lung, resulting in an acute unilateral pneumonia. Survival rates demonstrate the valuable therapeutic properties of the liposome-encapsulated drug in these clinically relevant animal models.

Keywords: Sterically stabilized liposomes; Infection; Immunocompromised; Bacterial susceptiblity; Drug targeting

Clinical practice shows that, despite the availability of potent antibiotics, severe infections frequently occur and are often difficult to treat. Two major risk factors of antimicrobial treatment failure are: an impaired host defense of the patient (e.g. as a result of chemotherapy, irradiation, or the use of immunosuppressive drugs) and/or a low susceptibility of the infectious organism towards the applied antibiotic(s) (Barker, 1999; Baughman, 1999; Burgess, 1999; Reynolds, 1999). Patients with an impaired host defense are at risk because of limited control of the infection as a result of the immunodeficiency. Consequently, the bacteria can spread and multiplicate more rapidly, which increases the risk of generalization of infection resulting in sepsis, a syndrome associated with high morbidity and mortality. In the event of an infection with reduced antibiotic-susceptibility of the infectious organisms, the antibiotic levels that can be achieved at the site of infection are too low for an efficient bactericidal action. In these cases, targeted drug delivery may increase

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therapeutic efficacy by increasing drug concentrations at the site of infection.

Liposomes have attracted considerable interest as targeted drug carriers in this respect. Short-circulating, conventional, liposomes are rapidly taken up by the cells of the mononuclear phagocyte system, and their use as drug carriers seems confined to infections residing in these cells (Karlowsky and Zhanel, 1992). Long-circulating, sterically stabilized, liposomes (SSL) have been shown to localize selectively at sites of infection not restricted to the mononuclear phagocyte system. Our studies indicate that the selective target localization is a result of the locally increased capillary permeability as part of the inflammatory response. The long-circulating behavior of SSL is essential to exploit the increased capillary permeability to maximize liposome target localization (Schiffelers et al., 2000). So far, studies investigating the therapeutic efficacy of antibiotic-encapsulating SSL have mostly been conducted in animals with an intact host defense infected with high-susceptible bacteria. The objective of the present study was to assess the therapeutic efficacy of gentamicin encapsulated in SSL (LE-GEN) in clinically relevant models of serious bacterial infection.

Rats with an intact host defense or leukopenic rats were inoculated with a high or low gentamicin-susceptible strain of *Klebsiella pneumoniae* in the left lung to produce an acute unilateral pneumonia, according to Bakker-Woudenberg et al. (1982). Leukopenia was induced by i.p. injection of 60 mg/kg cyclophosphamide every 4 days starting at 5 days before bacterial inoculation, reducing leukocyte numbers 6-fold to $\sim 10^9$/l throughout the study period (8). In all models, untreated animals died between 3 and 5 days after inoculation. LE-GEN was prepared according to Bakker-Woudenberg et al. (1995). Treatment was started at 24 h after inoculation, and ten animals were used per experimental group. Survival rate was monitored for 14 days and compared using the log rank test.

In rats with an intact host defense infected with the high gentamicin-susceptible *K. pneumoniae*, a single dose of LE-GEN 5 mg/kg was superior to an equivalent dose of the free drug (GEN). The improved efficacy of LE-GEN seems to be a result of the targeted drug delivery of the liposome-encapsulated drug. Treatment for only 3 days with GEN 5 mg/kg/day resulted in complete survival, which makes the clinical applicability of LE-GEN in this setting seem limited.

In rats with an impaired host defense infected with the high susceptible *K. pneumoniae*, 5 day treatment with GEN 5 mg/kg/day twice daily significantly prolonged survival during treatment, but after termination of treatment almost all rats died. Only when rats were treated for 5 days with the maximum tolerated doses of GEN (40 mg/kg/day twice daily) complete survival could be obtained. Interestingly, addition of a single dose of LE-GEN (5 mg/kg) to the 5 day GEN-treatment (5 mg/kg/day twice daily) produced complete survival. LE-GEN alone did not even prolong survival during treatment at all doses (5 up to 20 mg/kg/day) tested. We suggest that in rats with an impaired host defense, GEN protects against the rapid spreading of the bacteria thereby preventing the development of a lethal septicemia during treatment. However, GEN has limited effect on bacteria in the left lung because of relatively low drug levels in this organ. The targeted delivery of LE-GEN to the left lung enhances the therapeutic effect in this organ. Yet, LE-GEN has limited protective effect against the septicemia because of low therapeutically active drug levels in the circulation. Therefore, GEN and LE-GEN act complementary and produce significantly higher survival rates compared to treatment with equivalent doses of GEN alone or LE-GEN alone.

In rats with an impaired host defense infected with the low gentamicin-susceptible *K. pneumoniae*, treatment with either GEN alone at the maximum tolerated dose (40 mg/kg/day) or LE-GEN alone (20 mg/kg/day) was unsuccessful. Combination of these maximum doses of LE-GEN and GEN was partly successful as it prevented death of half of the rats. By changing the lipid composition of LE-GEN, from liposomes having a more rigid lipid bilayer to liposomes with a more fluid character, complete survival could be obtained. It is suggested that the faster release of gentamicin from the fluid liposomes enhances bacterial killing compared to rigid lipo-

somes. These data show the importance of lipid composition in optimizing liposomal drug formulations. The general conclusion is that liposomal formulations of gentamicin can provide valuable therapeutic prospects in these clinically relevant rat models of serious bacterial infection.

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