Effective Treatment of Acute and Chronic Murine Tuberculosis with Liposome-Encapsulated Clofazimine

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Advantages of Liposomal Delivery

• reduced toxicity
• targeted delivery
• enhanced retention
• overall improved therapeutic efficacy
Methods

• Mice infected with *Mtb* Erdman i.v.
• CLF delivered via iv route:
  – Free CLF
  – Liposomal CLF (Multilamellar liposomes, DMPC-DMPG)
  – Empty Liposomes
• Administered every 3-4 days over 2 week period (5 Rx)
  – Day 1 (acute)
  – Day 21 (subacute)
  – Day 90 (chronic)
• Day 1 post treatment: Harvest liver, spleen, lungs
L-CLF treatment in acutely infected mice

BALB/c mice ($n = 4$ per group) were infected i.v. with $10^6$ M. tuberculosis Erdman organisms and left untreated (O) or treated i.v. with L-CLF (●, 100 mg/kg; ◆, 50 mg/kg; ▼, 25 mg/kg; ●, 10 mg/kg; and ▲, 5 mg/kg), F-CLF (▲, 5 mg/kg), or empty liposomes (□, lipid content equivalent to 100-mg/kg dose) on days 1, 5, 8, 11, and 14 postinfection. The mice were sacrificed 1 day after the last drug injection (day 15), and their spleens, livers, and lungs were homogenized and plated for CFU. The results are shown as means ± standard deviations. *, $P < 0.01$. 
L-CLF treatment in mice with established infection

BALB/c mice (n = 4 per group) were infected i.v. with $10^6 M. tuberculosis$ Erdman organisms. Beginning on day 21 postinfection, the mice were left untreated (O) or treated i.v. every 3 to 4 days over a 2-week period (total, five injections) with L-CLF (■, 100 mg/kg; ●, 50 mg/kg; ▲, 5 mg/kg) F-CLF (▲, 5 mg/kg), or empty liposomes (□, equivalent to 100-mg/kg dose). The mice were sacrificed 1 day after the last drug injection (day 36), and the spleens, livers, and lungs were homogenized and plated for CFU. The results are shown as means ± standard deviations. *, $P < 0.01$. 
L-CLF treatment in chronically infected mice

BALB/c mice (n = 4 per group) were infected i.v. with $10^6$ *M. tuberculosis* Erdman organisms. Beginning on day 92 postinfection, the mice were left untreated (O) or treated i.v. every 3 to 4 days over a 2-week period (total, five injections) with L-CLF (●, 50 mg/kg; ▲, 5 mg/kg) F-CLF (△, 5 mg/kg), or empty liposomes (□, equivalent to 50-mg/kg dose). The mice were sacrificed 1 day after the last drug injection (day 107), and the spleens, livers, and lungs were homogenized and plated for CFU. The results are shown as means ± standard deviations. *, P < 0.01.
Clearance and recovery of *M. tuberculosis* in tissues of mice after treatment with L-CLF

BALB/c mice (n = 4 per group) were infected i.v. with 10^6 *M. tuberculosis* Erdman organisms. Beginning on day 97 postinfection, the mice were left untreated (○) or were treated i.v. every 3 to 4 days over a 2-week period (1st) (total, five injections) with 50 mg of L-CLF/kg (●). Groups of mice were sacrificed 1 day after the last drug injection (day 112), 2 weeks posttreatment, 1 month posttreatment, and 2 months posttreatment. Another set of mice (●) were administered a second round (2nd) of L-CLF treatments (50 mg/kg) beginning 2 weeks after the completion of the first round (day 125). Groups of mice were sacrificed 1 day after the last drug injection (day 140), 1 month posttreatment, and 2 months posttreatment. The spleens, livers, and lungs were homogenized and plated for CFU. The results are shown as means ± standard deviations. *P* < 0.01; ††, *P* < 0.001; §§, *P* < 0.0001.
Summary

• 10X higher doses delivered parenterally without toxicity
• Effective during acute, established and chronic stages of infection
• Effective in all organs tested
Drawbacks and Improvements

• iv administration
  – aerosol delivery methods
• Improved liposome formulations
• Alternate packaging/delivery systems
  – nanoparticles
  – block copolymer
  – cyclodextrin
  – microspheres
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