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Lipid Nanoparticles for Reviving Antibiotics: Efficacy of a Gel of Daptomycin in a Methicillin-Resistant *Staphylococcus aureus* Rabbit Osteomyelitis Model

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ABSTRACT

Background: Daptomycin (DAP) is a bactericidal antibiotic with activity against Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, but its administration is exclusively by IV route. Lipid Nano-Capsules (LNCs) are known to vehicle medicines and could offer new therapeutic options. The efficacy of LNC-daptomycin (LNC-DAP) formulated in a gel was compared with that of other antistaphylococcal drugs in a MRSA osteomyelitis rabbit model. **Methods:** Femoral trepanation of rabbits was performed, followed by injection of 10^8 CFU *S. aureus* suspension into the knee cavity. A surgical debridement of the infected tissues was performed 3 days later and animals were randomly assigned to: no treatment (controls), LNC-DAP [one application of 50 mg (Low Dose) or 200 mg (High Dose)], linezolid (LZO, simulating a human-equivalent dose of 10mg/kg/12h), or vancomycin (VAN, constant IV infusion to reach a 20xMIC serum steady-state concentration), or daptomycin (DAP, simulating a human-equivalent dose of 6mg/kg). Surviving bacteria were counted in bone marrow (BM) and bone (BO) at day 3 and at the end of 4-days treatment (day 7). **Results**: The nanotechnology manufacturing process of daptomycin was demonstrated with an entrapment efficiency of 100%. The minimum inhibitory and bactericidal concentrations (MIC/MBC) of the free antibiotic DAP and the LNC-encapsulated DAP (LNC-DAP) against the MRSA strain were equivalent (0.5 mg/L). One topic dose of the gel formulation of LNC-DAP versus four days of IV standard antibiotics (LZO, DAP, VAN) showed significant decrease of the bacterial burdens in both BO and BM compartments as compared to IV antibiotic regimens. **Conclusions:** In this model, a gel of lipid nano-encapsulated daptomycin (LNC-DAP) showed significant *in vivo* activity after ONE topic application in comparison with 4 days of IV anti-staphylococcal drugs. The use of LNCs for local delivery of antibiotics is a promising approach to revive old antibiotics or to develop new antibacterial agents.

INTRODUCTION

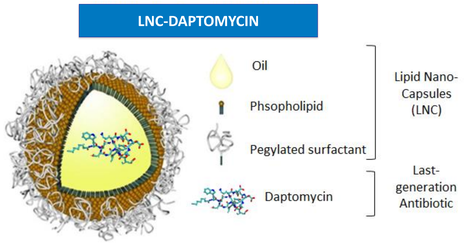
Bone and joint infections (also called osteoarticular infections, OAI) are particularly affected by the raise of multidrug-resistant bacteria. In addition, these infections are particularly difficult to treat due to local conditions (necrotic areas, bacteria sequestration in bone tissue, acidic pH, anaerobia, calcium, etc.) and biofilms production (exopolysaccharide matrix), which decrease the efficacy of antibiotics administrated through systemic delivery. Daptomycin (DAP) is a bactericidal antibiotic with activity against Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, but its administration is exclusively by IV route. Lipid Nano-Capsules (LNCs) are known to vehicle medicines and could offer new therapeutic options.

The aim of this work was to compare the efficacy of LNC-daptomycin (LNC-DAP) formulated in a gel with that of other antistaphylococcal drugs in a MRSA osteomyelitis rabbit model.

METHODS

On day 0, we used a percutaneously transarticular approach to perform a femoral trepanation of the right knee using a Jamshidi bone marrow biopsy needle (8 gauge) under general anaesthesia (ketamine, 20 mg/kg iv, and xylazine, 1 mg/kg iv). The Jamshidi needle was inserted between the two femoral condyles through the epiphysis, physis and metaphysis to reach the medullar canal. Following needle removal, the skin incision was closed. A bacterial suspension of 1 mL of *S. aureus* adjusted to 10^8 cfu/mL was injected into the knee cavity. Infection was allowed to develop for 3 days, and then a surgical debridement of the infected tissues was performed followed by an articular wash using 50 mL of 0.9% saline buffer. Samples of bone marrow and bone were removed, placed immediately on ice, weighed, homogenized in 0.5 mL of saline buffer, and then spread on agar plates using a spiral system. Treatment was started 72 h after inoculation, and antibiotics were administered for a 4 day course. At the end of the 4 day regimen, animals were euthanized, and epiphyseal bone samples and femoral bone marrow were obtained. Dilutions at 10^{-1}, 10^{-2} and 10^{-4} were performed to eliminate potential carry-over effects. Bacterial counts were determined after 48 h of incubation at 37° C. The efficacy measurement was made by comparing the bacterial load before (day 3 after infection) and after (day 7 after infection) antibacterial therapy.

Therapeutic regimens:
-Local administration of LNC-daptomycin (low (LD) and high (HD) doses).
-IV linezolid (human-equivalent dose of 600mg q12hr).
-IV vancomycin (continous infusion to reach a serum steady-state concentration of 20x the MIC (mimicking the human dose of 30 mg/kg given once daily).
-IV ceftaroline (human-equivalent dose of 600mg q12hr).
-IV daptomycin (human-equivalent dose of 6mg/kg).



RESULTS

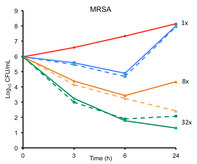


Figure 1. Time-kill curves of daptomycin and LNC-daptomycin against methicillin-resistant *S. aureus*. Daptomycin, solid lines; LNC-daptomycin, dashed lines.

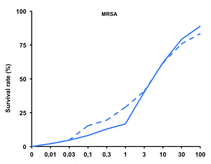
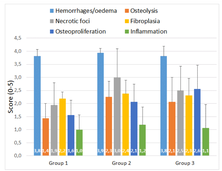


Figure 2. Mice model of MRSA-induced sepsis; determination of ED50 values. Daptomycin, solid lines; LNC-daptomycin, dashed lines.



Bone histopathologic analyses in a rat model (Group 1=control, Group 2=free LNCs, Group 3=NBT-DPT. n=8 for each group).

Figure 3. Histopathologic analysis of LNC-daptomycin in a rat experimental model.

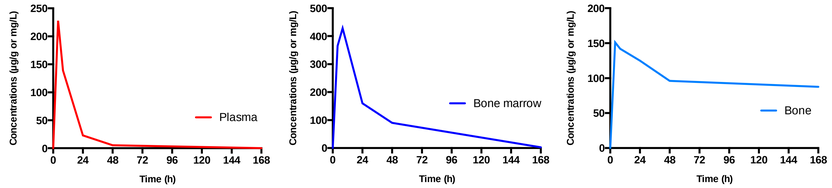


Figure 4. Pharmacokinetics of daptomycin in plasma, bone marrow and bone after a local administration of LNC-daptomycin.

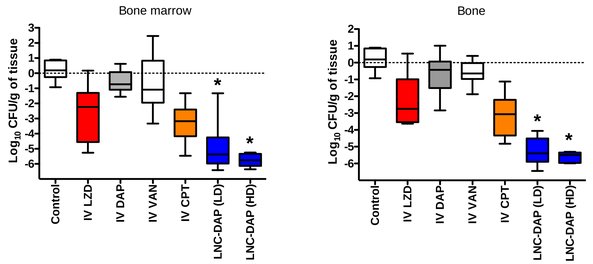


Figure 5. *In vivo* antibacterial efficacy of LNC-daptomycin (LD and HD) and comparators (linezolid, daptomycin, vancomycin, and ceftaroline) after 4 days of treatment for osteomyelitis due to methicillin-resistant *Staphylococcus aureus*.

DISCUSSION / CONCLUSIONS

- The nanotechnology manufacturing process of daptomycin was demonstrated with high entrapment efficiency (100%).
- A rabbit pharmacokinetic study highlighted a brief plasmatic and tissue distribution with persistence of high concentrations of daptomycin both in bone and bone marrow up to 4 days after one application of LNC-DAP.
- LNC-DAP implanted into rat femoral condyle defects is well tolerated in terms of weight, macroscopic observations of tissues (no lesion of the heart, the lungs, the liver, the spleen and the kidneys was observed) and bone histologic analyses (no acute toxic effect at the microscopic level, related to the presence of the gels was observed in any animal at the time of 4 days following implantation.
- A gel with lipid nano-encapsulated daptomycin (LNC-DAP) showed significant *in vivo* activity after one topic application in comparison with majors anti-staphylococcal drugs administered by IV route for 4 days in a MRSA osteomyelitis rabbit model.
- The use of LNCs for local delivery of antibiotics is a promising approach to revive old antibiotics or to develop new antibacterial agents with solubility issues for example.