Squalenoylation of chitosan: a new platform for anti-infectives delivery?

E. Lepeltier¹, B. Loretz¹, D. Desmaele⁵, J. Zapp⁴, J. Herrmann³, P. Couvreur⁵, CM Lehr¹²

¹Drug Delivery (DDEL), Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI), Saarland University, 66123 Saarbrücken, Germany
²Biopharmaceutics and Pharmaceutical Technology, Dep. of Pharmacy, Saarland University, 66123 Saarbrücken, Germany
³Microbial Natural Products, Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI), Saarland University, 66123 Saarbrücken, Germany
⁴Institut für Pharmazeutische Biologie, Saarland University, 66123 Saarbrücken, Germany
⁵Faculté de Pharmacie, Institut Galien Paris Sud, Université Paris Sud, UMR CNRS 8612, Châtenay-Malabry Cedex, France

elise.lepeltier@helmholtz-hzi.de

ABSTRACT SUMMARY

Despite the impressive keen interest these last decades in the research field of nanomedicine, few are on the market: there is still a need to develop carriers that are biocompatible, biodegradable and easily obtained for a transposition of scale. In this work, placed in the context of infectious diseases, a new amphiphilic chitosan derivative was synthesized, the chitosan-squalene (chitosan-SQ), enabling the formation of nanoparticles in acetate buffer without difficulty by self-assembling. This new system is safe, biodegradable, preserves the anti-infective properties of the initial chitosan [1] and can be used as a carrier for hydrophilic and hydrophobic drugs.

INTRODUCTION

Among the many challenges to health, bacterial infectious diseases stand out for their ability to have a profound impact on the human species. For example, the cystic fibrosis (CF) lung is chronically inflamed and infected by Pseudomonas aeruginosa, which is a major cause of mortality in this genetic disease. Aminoglycosides, such as Tobramycin, which are growth-targeting antibiotics, are relatively effective, but due their hydrophilicity have problems to cross the Gram negative bacterial cell envelope.

The core idea of this work was to explore nanoparticulate drug carrier based on novel derivative of chitosan. This natural biodegradable polysaccharide has been reported to interact with the bacterial cell wall as well as with biofilms [1, 2], adding also anti-infective properties to its interesting spectrum of multifunctional features, including bioadhesion and penetration enhancement. Moreover, chitosan can be chemically modified in order to become an amphiphilic compound: the idea being to obtain nanoparticles by simple self-assembly. That is why the new concept of “squalenoylation” discovered by Prof. Couvreur was chosen in chemically coupling a molecule of squalene (SQ) with the chitosan: this squalene is a biomolecule, precursor of the cholesterol and has already shown spectacular self-assembling properties enabling the formation of nanoparticles with several different polar heads (siRNA, nucleosides, paclitaxel…) [3].

EXPERIMENTAL METHODS

An Ultrapure chitosan UPCL 114 (Novamatrix) with a mass average molar mass Mw=90 000 (measured by GPC) and a deacetylation percentage > 90% was used.

The synthesis of several chitosan-SQ derivatives was performed by reductive amination [4] (Scheme 1) with different percentages of modification: 2 glucosamine units for 1 SQ (2:1), 10:1, 50:1 and 100:1. The material was obtained with a mean yield of 72%.

Scheme 1: covalent coupling of the squalene on the chitosan by reductive amination.

The nanoparticles formation was studied in acetate buffer with different pH (4.0, 5.3 and 6.5), in simply adding the amphiphilic material in the solution under stirring during few hours with a final concentration of 1 mg/mL. The nanoparticles were characterized: Dynamic Light Scattering (DLS) for the size, Electrophoretic Light Scattering for the zeta potential and Scanning Electron Microscopy (SEM) for the shape.
To evaluate the biocompatibility of this new material, LDH Assay (measuring membrane leakage) and MTT Assay (measuring mitochondrial metabolism) were done on L929 (mouse fibroblast cells) and A549 (human lung adenocarcinoma epithelial cells) cell lines. Additionally the Minimum Inhibitory Concentration (MIC) was determined on two different bacterial strains: *Staphylococcus Aureus Newman* and *Escherichia Coli* (TolC-deficient). Several model drugs (fluorescein, Nile Red) possessing different hydrophilicities were loaded in the nanoparticles.

Finally, the interaction of this new chitosan material with plasmid DNA (pUC) was studied by gel electrophoresis: different N/P ratios were formulated (2, 5, 10 and 20) [5]. The transfection of pGL3 was studied on THP-1 (human monocytes) and A549 cells and quantified by Luciferase assay. The transgene expression was normalized for the protein content (BCA assay).

**RESULTS AND DISCUSSION**

Aggregation was obtained with the chitosan-SQ 2:1 whatever the buffer used, but when the amount of SQ decreases, the size of the particles also decreases and the best buffer in terms of size and biocompatibility seems to be the acetate buffer pH=5.3: with the chitosan-SQ 50:1, d=691 nm ± 13 nm (pdi=0.2) and with the chitosan-SQ 100:1, d=657 nm ± 25 nm (pdi=0.2) (*Figure 1*). The particles were stable in the fridge at least during 25 days. The zeta potential of the nanoparticles for both compounds was > 30 mV.

**Figure 1**: Representative SEM picture of Chitosan-SQ 100:1 nanoparticles.

The chitosan-SQ particles have shown no severe cytotoxicity towards the mammalian cells (*Figure 2*) and the MIC obtained on *S. aureus* and on *E. coli* was the same as the free chitosan solution: 64 µg/mL and 128 µg/mL respectively.

By gel electrophoresis, a strong interaction between the chitosan-SQ 100:1 and the pDNA was observed, the latter being totally protected from DNase degradation with N/P ratios of 10 and 20.

**CONCLUSION**

The chitosan-SQ particles have shown no severe cytotoxicity towards the mammalian cells and the same anti-bacterial activity as a chitosan solution. However, this chitosan-SQ possesses an amazing property: these innovative nanoparticles are able to encapsulate a hydrophilic or a hydrophobic antibiotic (proved with model compounds): it could be a novel, performing, non-toxic platform for the delivery of anti-infective compounds.

**REFERENCES**