Optimization of drug-free nanostructured lipid carriers (NLC) for *Helicobacter pylori* eradication

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Helicobacter pylori (Hp) is a gastric pathogen that colonizes approximately half of the world´s population (ca. 4 billion people). Hp is responsible for the development of several gastric disorders, including gastric cancer [1]. The main treatments against Hp infection consist in the administration of antibiotics and proton-pump inhibitors. However, these treatments are failing, due to the increase of antibiotic resistance [2]. Drug-free nanostructured lipid carriers (NLC) have been studied for Hp eradication, showing a bactericidal effect against Hp both *in vitro* and *in vivo* [3,4,5]. However, eradication in mice was only achieved in 50% of the animals [5]. The main aim of this work is to optimize NLC efficiency by fine-tuning its physicochemical characteristics, in order to achieve full Hp clearance.

For NLC preparation, three surfactants were tested: Tween®60, Tween®80 and Cetyltrimethylammonium bromide (CTAB). NLC were prepared by hot homogenization followed by ultrasonication. For optimization purposes different sonication parameters were tested. The NLC were characterized in terms of size distribution and surface charge by dynamic light scattering (DLS) and electrophoretic light scattering (ELS), respectively. Nanoparticle concentration was measured by nanoparticle tracking analysis (NTA). Additionally, in NLC with Tween®60 and Tween®80 the effect of dialysis was evaluated. Dialyzed and non-dialyzed NLC were tested *in vitro* against Hp J99 strain.

All NLC were optimized in terms of size by altering the sonication parameters. The nanoparticles with Tween®60 and Tween®80 varied in a range of 200-430nm and 190-230nm, respectively. In terms of charge both NLC were anionic with a surface charge between -25 to -30mV. Cationic NLC with CTAB and a mix of Tween®60/CTAB had sizes from 107 to 200 nm and a surface charge around 25 and 76 mV. All NLC stocks had a final concentration in the range of 10^{14} particles/ml. NLC with Tween®60 and Tween®80 were tested against Hp J99. After 24h of incubation, both NLC formulations had similar outcomes, achieving a bactericidal effect. Non-dialyzed and dialyzed NLC showed also the same bactericidal activity. Moreover, when testing NLC with slightly different sizes smaller NLC showed less effect.

The NLC were successfully optimized in terms of size and charge. The formulations with Tween®60 and Tween®80 were effective against Hp, and the dialysis didn't affect the NLC bactericidal activity. Preliminary results indicate that size can play a role in the NLC bactericidal activity. These results support the therapeutic potential of these nanoparticles.

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