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Short communication

In vitro antimicrobial testing of ciprofloxacin-loaded vesicular phospholipid gels

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Introduction

Vesicular phospholipid gels (VPGs) are highly concentrated dispersions of phospholipids that can encapsulate drugs of varying lipophilicity. Since there is no concentration gradient between the inner aqueous core of the vesicles and the surrounding outer water phase, high and consistent drug load is ensured (Brandl, 2007). They have been commonly investigated for parenteral drug delivery (Brandl, 2007), but their semi-solid consistency makes them particularly appropriate for dermal application.

In this study, several different VPGs with ciprofloxacin hydrochloride (CPX) as a model hydrophilic drug were prepared. VPGs were characterized for physico-chemical properties and their antimicrobial potential was tested *in vitro* against planktonic and biofilm-forming bacteria typical for skin infections.

Materials and methods

Preparation of VPGs: VPGs were produced by hydrating (phosho)lipids (Table 1) in the drug solution (with or without addition of chitosan or propylene glycol) using a magnetic stirrer (600 rpm, 90 minutes), followed by homogenization (at different pressures) on a high-pressure homogenizer (Microfluidizer LM20, Microfluidics). The concentration of CPX in all formulations was kept constant (2%, w/w).

Physico-chemical characterization of VPGs: Mean diameters, polydispersity indexes (PDI) and zeta potentials of vesicles were determined using photon correlation spectroscopy on a Zetasizer Ultra (Malvern Panalytical Ltd, UK) (Rukavina et al., 2018). Prior to the measurements, semisolid samples of VPGs were appropriately diluted with water.

In vitro antibacterial and antibiofilm studies: Antimicrobial activities of VPGs were examined against three bacterial strains: *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6538 and methicillin-resistant *S. aureus* MFBF 10679 (MRSA).

Minimal inhibitory concentrations (MIC) for planktonic bacterial growth were determined according to the National Committee for Clinical Laboratory Standards method (NCCLS, 2000), while minimum biofilm inhibitory concentrations (MBIC) were tested by a modified method originally described by O'Toole (O'Toole, 2011).

In order to obtain a starting concentration suitable for testing, all VPGs were diluted by the appropriate growth medium for the corresponding bacterial strain. A range of concentrations was tested using the two-fold microdilution method on 96-well plates. Bacterial cultures in appropriate growth mediums were used as negative controls, whereas appropriately diluted free CPX served as a positive control. Empty (drug-free) VPGs were tested under the same conditions to examine a possible antibacterial effect exerted by the constituents of the VPGs themselves (Rukavina et al., 2018).

Results and discussion

Optimal (phospho)lipid concentrations in VPGs were found to be between 250 and 350 mg/g, while optimal system pressure applied in homogenization process was 500 bars.

As shown in Table 1, mean diameters of the vesicles within VPGs were between 120 and 350 nm, with zeta potentials around +30 mV for almost all VPGs, because

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the positively charged CPX affected the surface charge of the vesicles. PDI were in the range 0.3 – 0.8.

Table 1. Physico-chemical properties of different VPGs

VPG composition (mg/g)*	Mean diameter (nm)	PDI	Zeta potential (mV)
SPC (340)	138.7 ± 3.14	0.33 ± 0.03	+32.73 ± 0.42
SPC/CHIT (300/10)	176.2 ± 2.72	0.37 ± 0.01	+32.09 ± 0.75
SPC/P90H (165/85)	345.7 ± 10.27	0.80 ± 0.06	+18.53 ± 0.93
SPC/CHOL (283/33)	144.8 ± 2.15	0.49 ± 0.09	+27.06 ± 0.99
SPC/SLPC80 (298/53)	129.2 ± 1.96	0.45 ± 0.02	+32.12 ± 0.70
SPC/PG (350/189)	160.2 ± 15.71	0.46 ± 0.12	+29.85 ± 1.58
SPC/SLPC80/PG (224/40/52)	126.8 ± 1.42	0.43 ± 0.02	+25.16 ± 0.91

*mg of ingredient per g of formulation
CHIT, chitosan; CHOL, cholesterol; PG, propylene glycol; P90H, Phospholipon;SLPC 80, soybean monoacyl phosphatidylcholine; SPC, soybean phosphatidylcholine. The values denote the mean ± S.D. (n=3).

The results of *in vitro* antibacterial activities against the tested planktonic bacterial strains are presented in Fig.1.

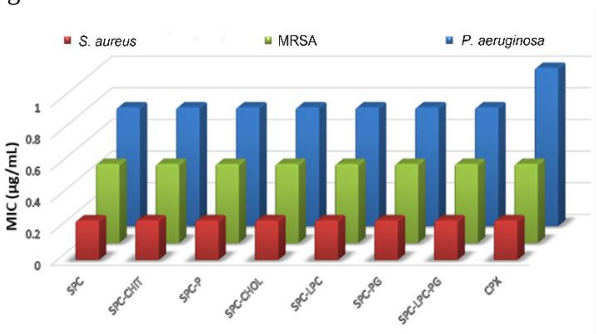

Fig. 1. *In vitro* antibacterial activities of different CPX-loaded VPGs against planktonic bacterial strains

Regardless of their composition, all VPGs were equally effective against both *S. aureus* and MRSA, although the MIC value was significantly higher for MRSA. When tested against *P. aeruginosa*, increased activity of all VPGs was achieved in comparison to free CPX (Fig. 1).

A similar trend in the efficacy of CPX-loaded VPGs was obtained at preventing *S. aureus* biofilm formation (Fig. 2). Interestingly, all CPX-loaded VPGs were more effective at preventing formation of MRSA and *P. aeruginosa* biofilms, where MBIC values for all the VPGs were two-fold lower than the value of CPX solution.

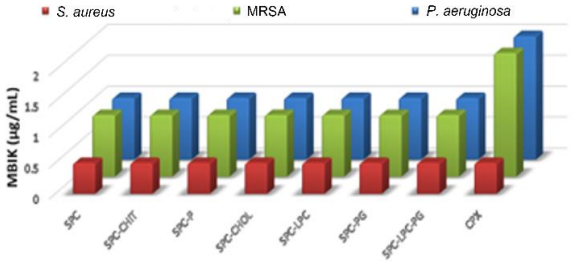

Fig. 2. *In vitro* antibacterial activity of CPX-loaded VPGs at preventing biofilm formation

Conclusion

(Phosho)lipid composition and the presence of propylene glycol or chitosan influenced the physico-chemical properties of the vesicles within VPGs but did not impact their antibacterial activity. All CPX-loaded VPGs were equally effective as free CPX against planktonic and biofilm-forming *S. aureus*, while their activity significantly increased against biofilm-forming *P. aeruginosa* and MRSA.

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