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Short communication

Liposomes-in-chitosan hydrogel improves efficacy of azithromycin against methicillin-resistant *Staphylococcus aureus*

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Introduction

Azithromycin (AZT) encapsulated into different types of liposomes (AZT-liposomes) displayed pronounced *in vitro* antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) in comparison to the free drug (Rukavina et al., 2018). The present study represents a follow up to this previous work, attempting to further explore anti-MRSA potential of AZT-liposomes when incorporated into the chitosan hydrogel (CH). Namely, chitosan-based hydrogels have been proved to be suitable vehicle for the incorporation of liposomes aimed for local antimicrobial therapy (Hemmingsen et al., 2021). Intrinsic antimicrobial activity of CH is expected to further enhance antibacterial potential of AZT-liposomes for localized topical treatment of MRSA-related skin infections. Hence, several types of AZT-liposomes incorporated into CH were evaluated and compared based on their *in vitro* anti-MRSA activity.

Materials and methods

Four different types of AZT-liposomes (Table 1) were prepared by film hydration method (Rukavina et al., 2018): conventional liposomes (CL), deformable liposomes (DL), propylene glycol liposomes (PGL) and cationic liposomes (CATL), whereas extrusion through polycarbonate membranes was used to obtain liposomes with mean diameters around 200 nm.

AZT-liposomes were manually mixed into the 2.5% high molecular weight CH (30%, w/w) to obtain AZT-liposomal hydrogels: CL-in-CH, DL-in-CH, PGL-in-CH and CATL-in-CH. Antibacterial activity of AZT-liposomal hydrogels, empty CH or free AZT solution-in-CH was studied against *S. aureus* (ATCC 29213) and five different MRSA clinical isolates (MFBF 10674, MFBF 10676, MFBF 10677, MFBF 10679, and MFBF 10680). For this purpose, agar well diffusion method was applied (Čačić et al., 2023). Following incubation, confluent growth of the bacteria on the agar plates was observed, and the diameters of the bacterial growth inhibition zones were measured in millimeters. The same protocol was employed to assess anti-MRSA activity of AZT-liposomes, empty liposomes and free AZT-solution.

Table 1. Composition of different AZT-liposomes

AZT-liposomes	SPC (mg)	SDCh (mg)	DPPC (mg)	DODAB (mg)	PG (mg)	AZT (mg)
CL	100	-	-	-	-	15
DL	85	15	-	-	1	15
PGL	100	-	-	-	1	15
CATL	-	-	73	27	-	15

AZT, azithromycin; CATL, cationic liposomes; CL, conventional liposomes; DL, deformable liposomes; DPPC, dipalmitoylphosphatidyl-choline; DODAB, dimethyldioctadecylammonium bromide; PG, propylene glycol; PGL, propylene glycol liposomes; SPC, soybean phosphatidylcholine; SDCh, sodium deoxycholate. The volume of all the liposome dispersions was 5 ml.

Results and discussion

All the liposomes-in-CH formulations successfully inhibited growth of the tested MRSA strains and were significantly more effective than control (free AZT solution-in-CH). The exception was CL-in-CH, which was also more effective than control, but insignificantly on two

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out of six of the tested MRSA strains. Furthermore, for the majority of MRSA isolates CATL-in-CH and DL-in-CH demonstrated similar anti-MRSA activity. CATL-in-CH was more effective than PGL-in-CH against all tested MRSA isolates, whereas DL-in-CHG was more effective than PGL-in-CHG only against two MRSA strains (MFBF 10674 and MFBF 10676). Accordingly, CATL-in-CH was proved to exhibit the strongest anti-MRSA activity of all the tested AZT-liposomal hydrogels (Fig. 1).

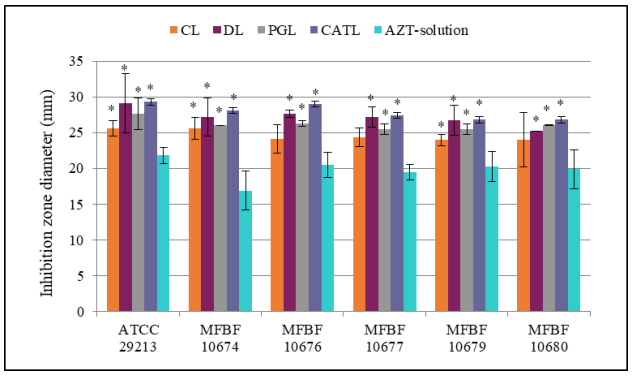

Fig. 1. *In vitro* anti-MRSA activity of the different AZT-liposomal hydrogels. Results are presented as diameter of inhibition zone (mm) including well diameter of 6 mm (mean ± SD, n=3). *Statistically significant difference (t-test, $p<0.05$) compared to free AZT solution (control).

Growth inhibition zones were not detected for the empty CH. However, when growth inhibition zones of AZT-liposomes (Fig. 2) were compared to growth inhibition zones of the corresponding AZT-liposomal hydrogels (Fig. 1), with respect to the concentration of AZT in particular formulation (AZT concentration was 3.3 times higher in AZT-liposomes in comparison to the corresponding AZT-liposomal hydrogels), it was confirmed that the incorporation into the CH remarkably enhanced anti-MRSA activity of both AZT-liposomes and AZT-solution.

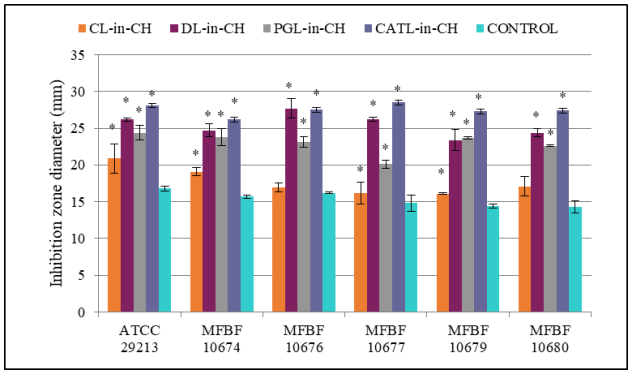

Fig. 2. *In vitro* anti-MRSA activity of AZT-liposomes (AZT conc. was 3.3-fold higher than in AZT-liposomal hydrogels). Results are presented as diameter of inhibition zone (mm) including well diameter of 6 mm (mean ± SD, n=3). *Statistically significant difference (t-test, $p<0.05$) compared to free AZT solution.

Conclusion

AZT-liposomes-in-CH exhibited anti-MRSA activity superior to AZT-solution-in-CH and AZT-liposomes (liquid dispersions). Anti-MRSA activity of AZT-liposomal hydrogels was shown to be dependent on the phospholipid composition of the entrapped liposomes, with CATL-in-CH being the most effective formulation. Although empty CH failed to inhibit MRSA growth, it was proved to enhance anti-MRSA activity of the incorporated AZT-liposomes and free AZT.

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