Characterisation and antimicrobial susceptibility of Staphylococcus epidermidis persister cells generated in vitro both by chemical induction and by natural isolation

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Background: Persister cells are a biofilm subpopulation, phenotypically tolerant to antibiotics, that plays a key role in chronic and relapsing infections. We developed and compared two methods to obtain in vitro S. epidermidis persister cells based on chemical induction with carbonyl cyanide m-chlorophenylhydrazone (CCCP) of planktonic bacteria and isolation from antibiotic-treated biofilms. The obtained persister cells were also tested for their susceptibility to different antimicrobials.

Materials/methods: S epidermidis ATCC 35983 and ATCC 12228 were used. For the chemical induction, different CCCP concentrations (25-200µg/ml) and incubation times (1-3h) were assayed. For the natural persister cell isolation, 24h-old biofilms formed were treated with ciprofloxacin or vancomycin (512µg/ml). The persistent phenotype was verified exposing persister cells to high concentrations (50, 100 200x MIC) of antibiotics and assessing their viability. The susceptibility of S. epidermidis persister cells to Cecropin-Melittin (CM12) peptide and to photoactivated curcumin was also tested.

Results: A higher number of persister cells was obtained after 1h treatment of S epidermidis ATCC 35983 and ATCC 12228 with 100µg/ml and 200µg/ml CCCP, respectively, with only a minor effect on cell viability. Around 1% persister cells were isolated from antibiotic-treated biofilms. CCCP-pretreated bacteria and naturally isolated persisters exhibited an antibiotic-tolerant phenotype, as no killing was observed when even the highest concentrations of antibiotics were tested. CM12 concentrations (≤2µg/ml and 8 µg/ml) exhibited a bactericidal effect (>3log CFU/ml reduction) versus CCCP-pretreated bacteria and natural persisters in both S. epidermidis strains. No difference in killing persister or normal-growing cells by CM12 was observed. Lower concentrations of photoactivated curcumin (0.5µg/ml) determined more than 3log CFU/ml reduction when tested against persister cells in both S. epidermidis strains (Figure 1).

Conclusions: Two different in vitro methods suitable to generate S. epidermidis persister cells (CCCP-induced and isolated from biofilm) were set up. Little difference in antimicrobial susceptibility were observed between the two persister models and between the tested S. epidermidis strains. CM12 and curcumin were strongly active against persisters at low concentrations, suggesting their potential role in a combination antibiotic or biocidal treatment to eradicate biofilms.

Figure 1 Activity of CM12 and photoactivated curcumin versus CCCP-induced and natural persisters