Free Papers D

[O72] INTRA-OSTEOBLASTIC SYNERGY OF DAPTOMYCIN WITH OXACILLIN AND CEFTAROLINE

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Aim: Intracellular persistence of S. aureus is believed to be one of the major mechanisms leading to bone and joint infection (BJI) chronicity and relapses. Despite its poor intracellular activity, daptomycin (DAP) is increasingly used in the treatment of staphylococcal BJI. The well-known in vitro synergy of daptomycin with various betalactam antibiotics consequently led us to investigate whether these combinations enhance the activity of daptomycin against the intracellular reservoir of methicillin-susceptible (MSSA) and -resistant (MRSA) ***S. aureus*** in an ex-vivo model of human osteoblast infection.

Method: Osteoblastic MG63 cells were infected for 2h with MSSA strain or its isogenic MRSA. After killing the remaining extracellular bacteria with lysostaphin, infected cells were then incubated for 24h with DAP, oxacillin (OXA) or ceftaroline (CPT) alone or in combination, at the intraosseous concentrations reached with standard human therapeutic doses. Intracellular bacteria were then quantified by plating cell lysates. Minimum inhibitory concentrations (MICs) of these molecules alone and in combination were determined using the checkerboard method at pH7, but also at pH5 to mimic intracellular conditions.

Results: Compared to untreated cells, DAP reduced significantly intracellular inoculum for MRSA only (p<10-3). OXA and CPT were active on MSSA and MRSA (p<0.05 for all). The OXA-DAP combination reduced the intracellular inoculum of MSSA and MRSA more efficiently than antibiotic alone (p<0,05). In contrast, no synergy was observed with the association DAP-CPT (Table1).

	MSSA	MRSA
DAP	+5,7%[-12,1 ; +23,6]	-27,6%[-37,0 ; -18,2]
OXA	-23,9%[-40,3 ; -7,5]	-43,2%[-52,9;-33,5]
CPT	-33,1%[-40,3 ; -25,9]	-28,9%[-44,2 ; -13,6]
DAP+OXA	-44,4%[-51,8 ; -37,0]	-57,2%[-65,7 ; -48,7]
DAP+CPT	-33,9%[-41,3 ; -26,4]	-34,2%[-45,4 ; -23,0]

Table 1: Decrease of the intracellular inoculum compared to untreated cells
In vitro, an important increase in DAP MICs was observed at acidic pH for the two strains (0.3 (pH7) to 2mg/L (pH5)). On the contrary, decreasing pH from 7 to 5 led to a drop in OXA MICs from 0.5 to 0.1mg/L for MSSA and from 128 to 0.5mg/L for MRSA.

Conclusions: Our results confirm the low activity of DAP against intra-osteoblastic ***S. aureus***, probably due to its inactivation by acidic pH condition encountered in lysosomes. On the opposite, betalactams are still active in intracellular compartment, including OXA on MRSA due to an acidic pH-related activity restauration. The OXA-DAP combination allows amplifying the intracellular effect of DAP on MSSA and MRSA. This synergy is not observed with CPT.