O308

Abstract (oral session)

In vitro checkerboard data correlate with in vivo outcome of polyene+azole combination therapy against experimental candidiasis

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Objectives: In vivo/in vitro correlation of antifungal combination testing is necessary in order to reliably assess the efficacy of combination regimens. We therefore attempted to correlate in vitro checkerboard testing of posaconazole (POS) and amphotericin B (AMB) with the in vivo outcome of combination therapy against experimental candidiasis in a neutropenic murine model. Methods: For, the in vitro experiments, serial twofold drug dilutions were prepared in 96-well microtitration plates at concentrations of 0.008-0.5 mg/L for AMB and 0.00006-0.06 mg/L for POS alone and in combination (8x12 checkerboard) in quadruplicate. After inoculation with 2.5x103 CFU/ml of a Candida albicans clinical isolate, plates were incubated for 48h at 37oC and read spectrophotometrically at 405nm. For the in vivo experiments 4-6 weeks old CD1 female mice were rendered neutropenic before i.v. infection with 105 CFU/mouse of the C. albicans isolate. In vivo 3x3 checkerboard combination regimens of 3.6, 2 and 1 mg/kg ip of AMB with 0.9, 0.45 and 0.2 mg/kg po of POS, determined in pilot exeriments to correspond to low L (20%), intermediate I (50%) and high H (80%) fungal burden, respectively, were tested. Mice were euthanized and CFU/kidney were determined after 2 days of treatment by subculturing. The interaction was assessed with Bliss independence (BI) model, where the % of fungal burden at each in vitro and in vivo combination regimens (ECOMB) were compared with the BI no-interactive effect derived by the equation EIND=EPOSxEAMB (EPOS and EAMB are % of fungal burden of each agent alone calculated based on the drug-free control). Bliss synergy, independence and antagonism was concluded when ECOMB was significantly lower than, equal to and higher than EIND (t test $p < 0.05$). Results: In vitro experiments revealed synergy (23-27%) at 0.06-0.008 mg/L of AMB with 0.001-0.00006 mg/L of POS whereas antagonism (14-58%) was observed at higher AMB (0.125-0.03 mg/L) and POS (0.015-0.004 mg/L), concentrations. Bliss synergy was found for the LAMB+LPOS (13±4%) and LAMB+HPOS (10±4%) combination regimens whereas Bliss antagonism was found for combination of HAMB and IAMB with HPOS and IPOS (35-83%). Conclusions: In vitro concentration-dependent pharmacodynamic interactions of POS+AMB combination against C. albicans correlated in vivo dose-dependent synergistic and antagonistic interactions. Properly analyzed checkerboard data can predict in vivo outcome of antifungal combination therapy.