Cytotoxicity of Locally Delivered Amphotericin B

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Statement of Purpose:

Fungal infections of implants and bone are commonly treated with parenteral amphotericin B which is highly toxic with a narrow therapeutic index making local delivery desirable. Typical MICs are 0.125 – 1.0 μg/mL. Amphotericin B complexes with cell membrane sterols of host cells forming pores that leak electrolytes similar to its antifungal action. This leads to cell death causing systemic toxicity including renal, hepatic, cardiac, blood dyscrasias and death. The target serum level is limited to 0.5 to 1.0 μg/mL.

Fungal infections are biofilm related similar to bacterial implant infections, however, it is not known how antifungal susceptibility is affected by the biofilm phenotype. If 100 – 1000x MIC is required for antifungal activity against sessile fungi, similar to the antibacterial susceptibility of sessile bacteria, local concentrations of 50 – 1000 μg/mL would be required. The concern for catastrophic local toxicity even if systemic toxicity is avoided raises the question- Is locally delivered amphotericin B toxic to local tissue? Muscle, bone, connective tissue, and wound healing are all at risk.

This investigation was carried out to test the hypothesis- Amphotericin B causes osteoblast and fibroblast cell death in concentrations of 1-1000 μg/mL.

Methods:

Mouse fibroblasts (BALB/3T3 A31**,** American Type Culture Collection (ATCC), Manassas, VA) and mouse osteoblasts (MC3T3-E1, ATCC, Manassas, VA) were grown in Dulbecco's modified Eagle's media with 2 mM glutamine, 5% bovine serum and penicillin/streptomycin (Fisher Scientific, Pittsburg, PA). Wells of 10,000 cells each were exposed to 0, 1, 5, or 10 μg/ml amphotericin B deoxycholate (Xgen Pharmaceuticals, Big Flatts, NY) for 7 days and then fresh media without amphotericin for 3 days. Cell morphology was observed under light microscopy and cell proliferation was determined using florescence from alamar blue (Invitrogen, Carlsbad, CA) at 1, 4, 7 and 10 days. Additionally the osteoblasts (3000 cells/well), exposed to 10, 100 and 1000 μg/ml for 5 hours, were observed under light microscopy for cell morphology and assayed for cell viability using an MTT assay (Promega, Madison, WI) at 1, 3, and 5 hours. Fluorescence and absorbance were measured on a Fluostar Omega UV spectrophotometer (BMG Labtech, Cary, NC). Cell proliferation results are reported in fluorescence rather than cell counts, in accordance to literature provided by Invitrogen.

Results:

Osteoblasts exposed to 1 μg/mL amphotericin B had no observable change in morphology and proliferated normally with a 4.0 fold increase in fluorescence over 7 days. Unexposed controls increased fluorescence of 4.12 fold in the same time period.

Osteoblasts exposed to 5 μg/mL of amphotericin B initially showed some rounded and some normal cells and although the increase in cell number over time was visually normal, producing a dense cell layer, with similar change to controls, fluorescence increased 6.81 fold over 7 days. After removal of the amphotericin between days 7 and 10, the osteoblasts previously exposed to 5 μg/mL increased fluorescence 9.69 fold.

Osteoblasts exposed to 10 μg/mL of amphotericin B showed a mix of rounded and normal cells, with fluorescence increase less than control cells of only 1.68 fold over 7 days; ($p<0.01$, t-test). After removal of the amphotericin between days 7 and 10, the osteoblasts previously exposed to 10 μg/mL increased fluorescence 2.9 fold.

Osteoblasts exposed to 100 and 1000 μg/mL of amphotericin B had grossly abnormal morphology and were all dead on MTT assay by 1 hour of exposure.

Fibroblasts exposed to 1 μg/mL of amphotericin B increased florescence 4.09 fold over 7 days, similar to unexposed controls, which showed a 4.8 fold increase.

Fibroblasts exposed to 5 μg/mL of amphotericin B had mixed morphology and did not visually proliferate as quickly as controls with florescence increased 1.79 fold over 7 days. Recovery occurred after removal of the amphotericin B between days 7 and 10 with increased fluorescence of 5.98 fold.

Fibroblasts exposed to 10 μg/mL amphotericin B visually showed primarily rounded morphology and reduced cell numbers and 0.89 fold decrease in florescence over 7 days Recovery occurred after removal of the amphotericin B between days 7 and 10 with increased fluorescence of 1.62 fold.

Conclusions:

Amphotericin is lethal to osteoblasts and fibroblasts at concentrations of 100μg/mL and above. It is causes sublethal cytotoxicity at 5 and 10 μg/mL. This is an in vitro study of mouse cell suspensions not established confluent colonies or living human tissues in vivo that could have different thresholds to amphotericin B toxicity. Future studies should explore cellular migration, other cell lines involved in wound healing such as macrophages and white cells, human cell lines and in vivo study designs. Local delivery of amphotericin achieving a concentration of 5 to 10 μg/mL may alter soft tissue and bone healing until the concentration decreases to 1 μg/mL or less but if concentration of 100 or 1000 μg/mL are achieved cell death could occur in both bone and soft tissue requiring repopulation of the wound after the amphotericin B falls below lethal levels.