AmBisome (AmBi) treatment stimulates increase in phagocytic activity and bead intake in RAW macrophages

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Abstract

Introduction: The RAW-264.7 (RAW) murine macrophage cell line was used to determine how the antifungal drug AmBisome (AmBi) would affect the phagocytic uptake of microscopic beads and the morphology of the cells.

Methods and Materials: RAW-264.7 cells were grown up and subcultured into 12 well, flat bottom, tissue culture plates at a concentration of 5x10⁵ cells/well and grown overnight at 37°C with 5% CO₂. Two cover slips that were placed in the wells. The next day, AmBi (50µg/ml), 50 µl/ml of Fluoresbrite Polychromatid Red microscopic beads (1.0µm microspheres), the combination of AmBi and beads or DMEM medium were added to the wells and duplicate wells were set up for each condition. Plates were incubated again at 37°C for 2h, media removed, and cells fixed with 200µl of 4% paraformaldehyde for 15 minutes at room temperature. The cells were rinsed with deionized water, and 200µl of 0.4% crystal violet was added to the wells to stain the cells. The stain remained in the wells for 10 minutes at room temperature and then removed by rinsing with deionized water. The cover slips were mounted on glass slides and sealed with nail polish.

The next day, the slides were examined with a light microscope and evaluated for cell size, shape, amount of cytoplasm, nucleus size, amount of cytoplasmic granularity, if beads were present in the cells, and if so, how many beads were present in the cell. Photographs were taken with representative fields, and 15 macrophages/experimental condition from random fields were evaluated for these characteristics.

Results: The cells incubated with beads alone had a larger size than the cells incubated with DMEM. Cells with AmBi, both with and without beads, were even larger than the cells with just beads indicating that AmBi has a marked effect on the macrophage's size. The addition of AmBi to the cells along with the beads also significantly increased the amount of beads that were taken up by the cells, suggesting that the cells incubated with AmBi had enhanced phagocytic activity. The position of the nucleus in cells treated with AmBi, both with and without beads, moved from center to side of the cell. Cells incubated with AmBi were also more granular than cells incubated with beads or DMEM alone. Cells treated with AmBi also showed an increased size and amount of cytoplasm compared to cells incubated with just DMEM. The nucleus size and the shape of the cell were generally unaffected by incubation with either the beads or the AmBi. There was also no increase in the number of cells that took up the beads whether or not the cells were incubated with AmBi.

Conclusion: AmBi increased phagocytic uptake of beads by RAW macrophages compared to RAW macrophages incubated with just the beads, and this was accompanied by an increase in cell size, size of the cytoplasm, and granularity of the cell. This increased phagocytic activity in macrophages treated with AmBi may contribute to the drug's efficacy when it is used to treat fungal infections.

Materials and Methods

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References