



Cytokines Released from Kidney Cells Exposed to Amphotericin B

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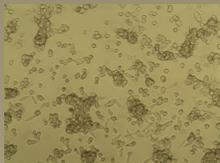
ABSTRACT

A human proximal tubule kidney cell culture (HK-2) was established as a cell model for the study of Amphotericin B induced nephrotoxicity. Optimal conditions for the culturing of HK-2 cells consisted of a keratinocyte serum-free medium supplemented with 50 µg/mL bovine pituitary extract and 5 ng/mL epidermal growth factor in a 5% CO₂, 37°C environment. HK-2 cells formed epithelial cell monolayers on collagen coated plates but remained in clustered masses without collagen. Exposure of HK-2 cells to 5 µg/mL deoxycholate-amphotericin B (DAmB) for 5 hours was done to identify inflammatory cytokines released from cells in response to the drug. Using an antibody array panel of 42 different cytokines, only IL-6 and IL-8 were constitutively released from untreated cells. Exposure to DAmB caused no increase in release of inflammatory cytokines, and no *de novo* release of additional cytokines.

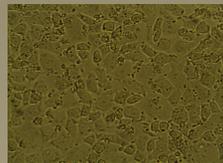
BACKGROUND

Amphotericin B (AmB) is an antifungal drug most commonly used in the treatment of systemic fungal infections (1,3). After binding to ergosterol located in fungal cell wall AmB forms transmembrane channels, disrupting cellular homeostasis and allowing leakage of cellular components, leading to eventual cell death (1,3). Though this is an effective and efficient treatment method against fungal infections, the ability of Amphotericin B to bind the cholesterol found in mammalian cells can cause adverse side effects in patients, including hemodynamic instability, oxidative damage, and immune suppression (1,3). Though AmB has a lower affinity for cholesterol than ergosterol, some mammalian cells such as renal proximal tubule cells contain sufficient quantities of cholesterol to promote binding, making nephrotoxicity the most therapeutically limiting side effect (1). The limiting of dose concentration due to adverse effects has decreased AmB use despite its effectiveness, and has led to new formulations and procedures in an attempt to alleviate some of the less desirable results (1,3). The most common alteration to AmB involves complexing it with lipid (L-AmB) or detergent (deoxycholate-AmB), forming a complex with less affinity for host immune and kidney cells, and therefore reducing unwanted side effects (3). This allows for use of higher doses and therefore, less damaging treatment of patients.

HK-2 cells were the first perpetually dividing human proximal tubule cell line to be created for laboratory use to avoid complications of working with entire organisms or organs (4). Human proximal tubule kidney cells were transduced using recombinant human papilloma virus, immortalizing them while maintaining the phenotypic traits necessary for their use as a research model for *in vivo* cells (2,4). Developed from transplantable cadaver kidneys, HK-2 cells can be assumed to be representative of the normal population (4).



•Clustering of HK-2 cells grown in 50mL filtered flasks



•Cells grown on collagen coated plates were much more confluent

HYPOTHESES

- Exposure of HK-2 cells to deoxycholate-Amphotericin B will cause an increase in the release of inflammatory cytokines as compared to the control.
- Exposure of HK-2 cells to deoxycholate-Amphotericin B will cause additional cytokines to be released as compared to the control.

METHODOLOGY

Cell Culture

1. HK-2 cells were grown in 50mL filtered angle-necked flasks maintained in a 5% CO₂, 37°C environment using keratinocyte serum-free medium. The medium contained 50 µg/mL bovine pituitary extract, 5 ng/mL epidermal growth factor, 100 µg/mL streptomycin, and 100 µg/mL penicillin as stated in the literature (2,4).
2. Cells were passed to new flasks at approximately 75% confluence (about 7 days) until the study was conducted, when they were passed to 60mm collagen coated plates and allowed to reach about 80% confluence.
3. Media was then refreshed with 3mL of fresh media and the test plate was exposed to 5 µg/mL deoxycholate-amphotericin B in media for 5 hours and the control was exposed to normal media.
4. Media was then drawn off and frozen until analysis.

Microarray

5. Cytokine release was observed using a human cytokine antibody array and chemiluminescence.
6. Analysis was conducted with Microsoft Excel spreadsheet.

RESULTS

- IL-6 and IL-8 (both inflammatory cytokines) were expressed in treated and untreated cells

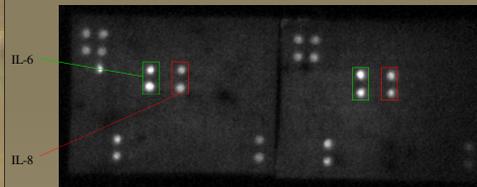


Figure 1

•Left: Microarray of cytokines isolated from untreated cells

•Right: Microarray of cytokines present after 5 hour exposure to 5 µg/mL deoxycholate-amphotericin B

Region	Total Density
IL-6 Control	4813.92
IL-6 Treated	6779.77
IL-8 Control	3262.08
IL-8 Treated	3985.67
IL-6 Treated	6209.72
IL-6 Treated	5203.41
IL-8 Treated	4150.82
IL-8 Treated	3261.36
	Fold Difference
IL-6	1.29
IL-8	0.77
IL-8	1.27
IL-8	0.82

Table 1. Pixel density analysis of treated versus untreated cells after a two minute exposure. A greater than two fold difference indicates significance.

CONCLUSIONS AND FUTURE WORK

- No significant increase in release of IL-6 or IL-8 in DAmB treated cells compared to the control
- No additional cytokines released from DAmB treated cells compared to the control
- These results warrant future research using higher concentrations of DAmB and/or longer exposure times

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