Abstract

Amphotericin B is an anti-fungal drug used to treat systemic fungal infections. Different formulations of Amphotericin B have been shown to activate inflammatory genes in monocytes and macrophages. A signal transduction pathway that is likely activated by Amphotericin B is the NF-κB pathway. NF-κB is a transcription factor that is activated when its inhibitor, IκB is phosphorylated which frees NF-κB to enter the nucleus. After being phosphorylated, IκB is released and degraded. These cytokines include TNF-α, IL-1, IL-6, IL-8, and GRO, among others. The exact mechanism that causes the release of these proinflammatory cytokines is not well defined, but Toll Like Receptors and its signal transduction pathways are likely involved.

Background

Amphotericin B is an antifungal drug used to treat systemic fungal infections because of its ability to form a pore complex with ergosterol in the fungal cell membrane. The side effects of this drug include fever, shaking, chills, hypotension, nausea, vomiting, and nephrotoxicity (1).

In an effort to reduce the side effects of Amphotericin B, new formulations of the drug were made. Fungizone (FZ) was the original formulation created followed by Abelcet (ABLC) and Amphotec (ABCD) with each having different delivery systems. Each of these formulations has been shown to cause inflammation in the body through the release of proinflammatory cytokines. These cytokines include TNF-α, IL-1, IL-6, IL-8, GRO, and others. The exact mechanism that causes the release of these proinflammatory cytokines has not been well defined, but Toll Like Receptors and its signal transduction pathways are likely involved.

Toll Like Receptors (TLR) are a membrane spanning receptor that recognizes foreign molecules from bacteria and viruses (Fig. 1). Amphotericin B was originally derived from bacterial cells so it is thought that the release of proinflammatory cytokines is induced through this pathway. Once a TLR binds its ligand, it activates a phosphorylation cascade that ends in transcription factors. These transcription factors enter the nucleus and activate the transcription of the proinflammatory cytokine genes.

Hypotheses

Primary Hypothesis
- Amphotericin B will activate inflammatory gene expression through NF-κB activation.

Alternate Hypothesis
- Another NF-κB pathway (P52/P100) activates inflammatory gene expression when treated with Amphotericin B

Methods

Treat Monocytes with FZ, ABLC and ABCD (5ug/mL) for 0, 5, 15, 30 and 60 minutes
Collect Protein Extracts
Western Blot
Detect on CCD Camera

Results

NF-κB is activated and free to enter the nucleus after its inhibitor, IκB is phosphorylated. After IκB is phosphorylated it is quickly degraded. Thus a decrease in the IκB/Actin B ratio overtime indicates that NF-κB has been activated (Figure 3).

Conclusions

1) The IκB/Actin B ratio did not show the expected decrease overtime which indicates that NF-κB is not activated.
2) Another transcription factor may initiate the release of proinflammatory cytokines such as P52/P100.

References


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