

How Does Amphotericin B Activate Inflammatory Gene Expression in Monocytic Cells?



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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 monocytic like cells. A signal transduction pathway that is likely activated by Amphotericin B is the NF- κ B pathway. NF- κ B is a transcription factor that is activated when its inhibitor, I κ B is phosphorylated which frees NF- κ B to enter the nucleus. After being phosphorylated, I κ B is released is released into the cytoplasm where it is ubiquitinated and degraded by the proteasomes. Western blots were used to detect activation of NF- κ B in THP-1 monocytes treated with three different Amphotericin B formulations (5ug/mL) for 0, 5, 15, 30 and 60 minutes. NF- κ B activation was detected by observing a decrease in the I κ B levels overtime. The predicted decrease in I κ B levels was not detected in any of the Amphotericin B treatments which suggests that Amphotericin B may activate another pathway that results in the activation of proinflammatory genes.

Background

Amphotericin B is an antifungal drug used to treat systemic fungal infection 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(2).

Toll Like Receptors (TLR) are a membrane spanning receptor that recognize 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 active the transcription of the proinflammatory cytokine genes(3).

Hypotheses

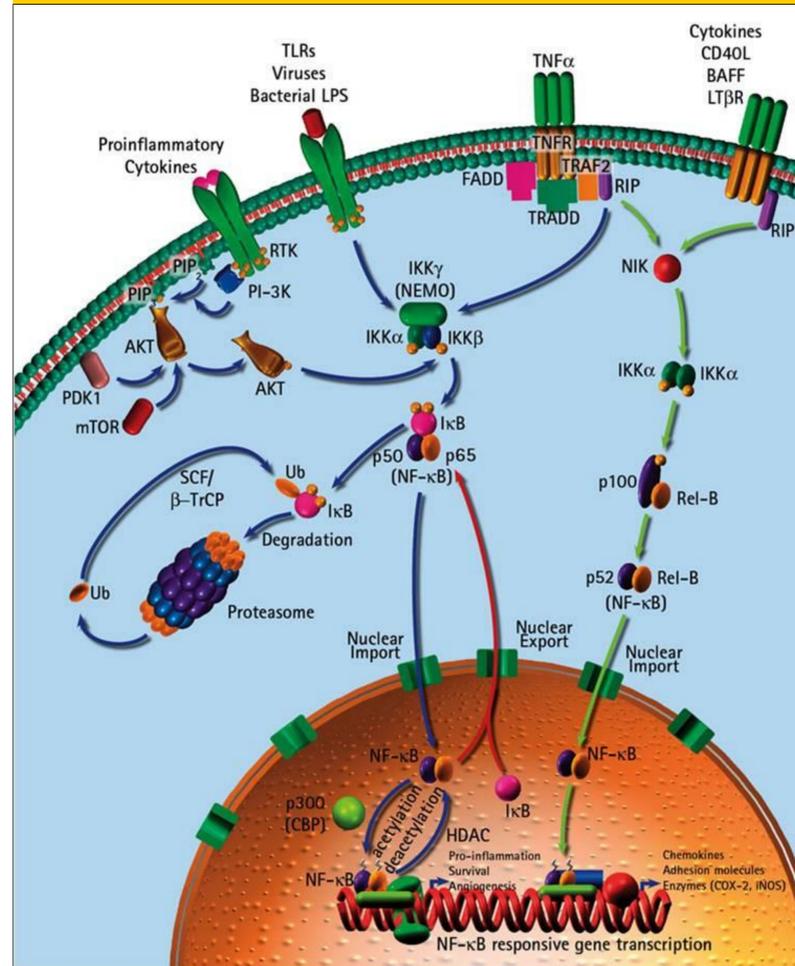
Primary Hypothesis

- Amphotericin B will activate inflammatory gene expression through NF- κ B activation.

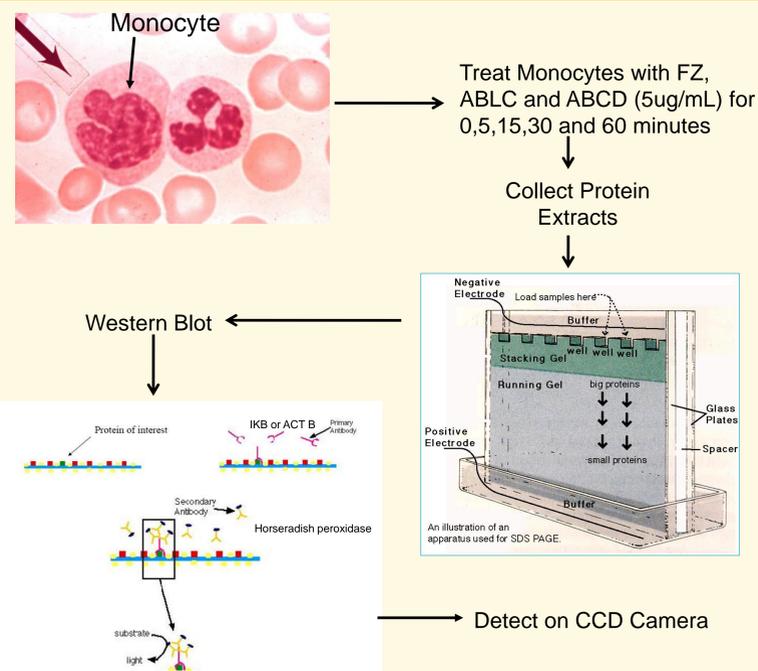
Alternate Hypothesis

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

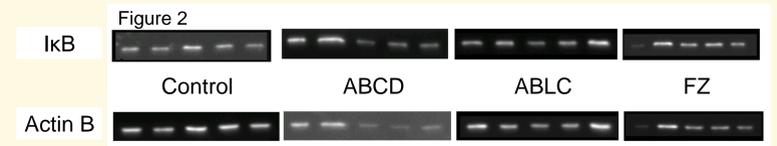
Figure 1: NF- κ B Signal Transduction



Methods

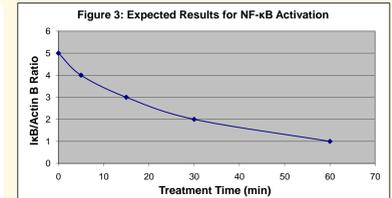


Methods

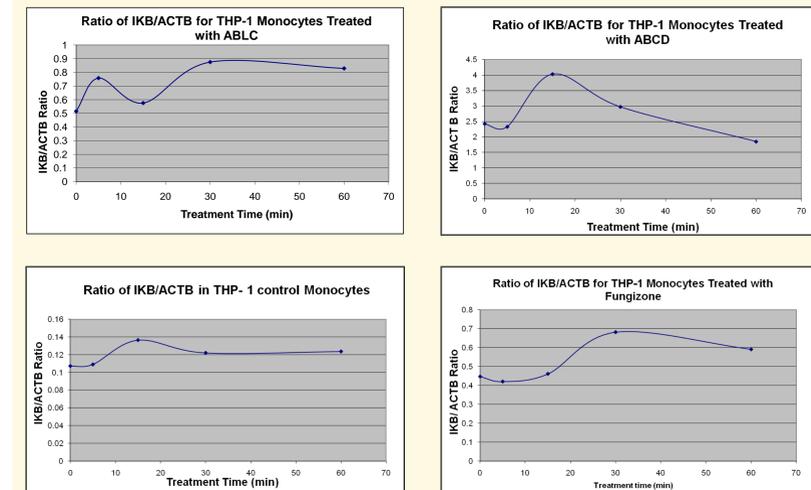


The ratio of the I κ B and Actin B bands was taken to account for loading errors and sample variations. Actin B levels has been shown to stay constant in Monocytes (Figure 2).

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



Results



Conclusions

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

References

- 1)Turtinen et al. "Antibody Array Generated Profiles of Cytokine Release from THP-1 Leukemic Monocytes Exposed to Different Amphotericin B Formulations" *Antimicro. Agents Chemother* 48.2 (2004) p396-403
- 2) Gilmore, Thomas D. "The Rel/NF- κ B signal transduction pathway: introduction" *Oncogene* 18 (1999) p 6842 - 6844
- 3) Lee, Myeong S. and Young-Joon Kim. "Signaling Pathways Downstream of Pattern-Recognition Receptors and Their Cross Talk." *Annu. Rev. Biochem.* 76 (2007) pg447-480.

Funded by a grant from the UW- Eau Claire Summer Research Experiences for Undergraduates Program