Anti-Inflammatory Activity of Azithromycin as Measured by Its NF-κB Inhibitory Activity

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BACKGROUND

• Azithromycin is a broad spectrum antibiotic that is effective against Gram-positive, Gram-negative, and atypical pathogens.
• Recently it has been found to have some anti-inflammatory and immunomodulatory activities.
• NF-κB is a redox-sensitive, inducible transcription factor that is involved in the regulation of a large number of genes that control inflammatory response.
• There is some evidence that the anti-inflammatory activities of azithromycin are mediated via the NF-κB pathway (Aghai et al., 2007).
• This study was designed to investigate in detail the dose dependent effect of azithromycin in inhibiting the NF-κB activity.

PURPOSE

This study used an activated NF-κB assay to assess azithromycin’s anti-inflammatory potency, and compared that to commonly used anti-inflammatory agents, hydrocortisone and dexamethasone.

METHODS

• Cell line and Transfection. A549 human lung carcinoma cell line (CCS Cell Culture Service, Hamburg, Germany) was cultured in IMDM, containing 25mM HEPES, 4mM L-glutamine, 1mM sodium pyruvate, 2% fetal calf serum and 150U/ml pen/strep; and was stably transfected with a reporter gene construct expressing secreted alkaline phosphatase (SEAP) under control of NF-κB responsive elements.
• NF-κB Activation Assay. NF-κB activation was monitored using the PRINCESS® NINA Instant NF-κB assay kit (CCS Cell Culture Service, Hamburg, Germany).
• Azithromycin, hydrocortisone and dexamethasone at various concentrations were incubated with the A549 cells for 6 to 8 hours at 37°C with a final ethanol concentration of 2.5% in a humidified CO₂ environment.
• TNFα, at a final concentration of 7.5 ng/mL, was then added and further incubated for 20 to 24 hours for NF-κB activation.
• Bay11-7082, a specific NF-κB inhibitor at a concentration of 5 µM, was used as positive control.

RESULTS

• All three compounds dose-dependently inhibited TNF-α stimulated NF-κB activity.
• Hydrocortisone was approximately 4 orders of magnitude more potent than azithromycin.
• Dexamethasone was approximately 14 times as potent as hydrocortisone. The latter approximates the reported glucocorticoid activity ratio between dexamethasone and hydrocortisone.

Figure 1. Dose-dependent inhibition of NF-κB activation by azithromycin as determined by a reporter gene assay, R² = 0.868.

Table 1. Relative anti-inflammatory potency (RAP) of azithromycin, hydrocortisone, and dexamethasone

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ value (µM)</th>
<th>RAP (IC₅₀ compound/ IC₅₀ Dex)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone</td>
<td>0.0005</td>
<td>1</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>56</td>
<td>1/22,000</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>0.00018</td>
<td>14/1</td>
</tr>
</tbody>
</table>

DISCUSSIONS AND CONCLUSIONS

• In addition to its anti-microbial activity, azithromycin also possesses a weak anti-inflammatory activity relative to the glucocorticoids.
• With its high ocular tissue concentrations achievable by the recently marketed ophthalmic formulation, AzaSite, azithromycin could overcome its weak potency issue and might have utility for certain inflammatory ocular surface diseases.

AUTHOR DISCLOSURE INFORMATION

All authors are employees of InSite Vision, Inc.