Heat-induced superaggregation of amphotericin B attenuates its ability to induce cytokine and chemokine production in the human monocytic cell line THP-1

P. David Rogers1,2*, Katherine S. Barker1, Vanessa Herring1 and Melissa Jacob3

Departments of 1Clinical Pharmacy and 2Pharmaceutical Sciences, College of Pharmacy, University of Tennessee Health Science Center, 26 South Dunlap Street, Memphis, TN 38163; 3National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA

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The cytokine and chemokine response elicited by heat-treated amphotericin B (HT-AmB) was compared with that of untreated amphotericin B (AmB-DOC) in the human monocyte cell line THP-1. AmB-DOC produced dose-dependent increases in interleukin (IL)-1β, IL-1α, tumour necrosis factor-α, macrophage inflammatory protein (MIP)-1α and MIP-1β at 2 h. HT-AmB induced cytokine and chemokine production at a lower level than those observed with corresponding concentrations of AmB-DOC, while retaining antifungal activity. These results indicate that heat treatment of amphotericin B may prove to be a cost-effective approach to improving the therapeutic index of this antifungal agent.

Keywords: amphotericin B, monocyte, cytokine, chemokine

Introduction

Amphotericin B is a polyene antifungal antibiotic with activity against a number of pathogenic fungi.1 Treatment of the amphotericin B deoxycholate micellar formulation by heating to 70°C for 20 min produces a ‘superaggregated’ form of amphotericin B.2-5 This novel formulation has been associated with less toxicity against mammalian cells while retaining antifungal activity both in vitro and in vivo.2-4 Given the potential importance of the immunomodulatory properties of amphotericin B in both the toxicity and activity of this drug, we wished to determine whether heat-treated amphotericin B (HT-AmB) would elicit an attenuated cytokine and chemokine response in human monocytes compared with untreated amphotericin B.

Materials and methods

Reagents and cell culture

The deoxycholate salt of amphotericin B (AmB-DOC) was obtained from Bristol-Myers Squibb (New York, NY, USA). RPMI 1640 medium was obtained from Sigma (St Louis, MO, USA). Low-endotoxin fetal bovine serum was purchased from Summit Biotech (Fort Collins, CO, USA) and Gibco-BRL/Invitrogen (Carlsbad, CA, USA). The human mononuclear cell line THP-1 (ATCC TIB 202) was cultured as described previously.6

AmB-DOC was reconstituted in sterile water according to the manufacturer’s directions. Heat treatment was carried out as described previously.5 Amphotericin B concentrations >10 µg/mL have been associated with low cell viability.6 Therefore, concentrations from 0.625 to 10 µg/mL were used in this study. Cell viability was assessed by erythrosin B exclusion, as described previously.6

In vitro antifungal activity

Candida albicans ATCC 90028, Candida krusei ATCC 44507 and Candida glabrata ATCC 32312 were obtained from the American Type Culture Collection (Rockville, MD, USA). Candida tropicalis LM-64 was contained in the National Center for Natural Products Research collection (Oxford, MS, USA) and C. albicans 12-99 was a gift from Dr Spencer.

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*Corresponding author. Tel: +1-901-448-1493; Fax: +1-901-448-1741; E-mail: drogers@utmem.edu
Table 1. Activity of AmB-DOC and HT-AmB against Candida species

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC₅₀ (mg/L)</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans ATCC 90028</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AmB-DOC</td>
<td>0.09</td>
<td>0.31</td>
</tr>
<tr>
<td>HT-AmB</td>
<td>0.04</td>
<td>0.31</td>
</tr>
<tr>
<td>C. albicans 12-99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AmB-DOC</td>
<td>0.08</td>
<td>0.31</td>
</tr>
<tr>
<td>HT-AmB</td>
<td>0.06</td>
<td>0.31</td>
</tr>
<tr>
<td>C. krusei ATCC 44507</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AmB-DOC</td>
<td>0.10</td>
<td>0.31</td>
</tr>
<tr>
<td>HT-AmB</td>
<td>0.07</td>
<td>0.31</td>
</tr>
<tr>
<td>C. glabrata ATCC 32312</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AmB-DOC</td>
<td>0.04</td>
<td>0.63</td>
</tr>
<tr>
<td>HT-AmB</td>
<td>0.03</td>
<td>0.31</td>
</tr>
<tr>
<td>C. tropicalis LM-64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AmB-DOC</td>
<td>0.04</td>
<td>0.63</td>
</tr>
<tr>
<td>HT-AmB</td>
<td>0.04</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Redding (University of Texas Health Science Center, USA). Susceptibility testing was carried out using a modified version of the NCCLS methods.7

Cytokine and chemokine measurements

Tumour necrosis factor (TNF-α), interleukin (IL)-1β, IL-1α, IL-1 receptor antagonist (Ra), macrophage inflammatory protein (MIP)-1α, MIP-1β and monocyte chemoattractant protein (MCP)-1 concentrations were determined with commercial ELISA kits (R&D Systems, Minneapolis, MN, USA). After exposure to experimental conditions for 2 h supernatants were collected and stored at −70°C until assay. Duplicate measurements were made for each of two wells from three individual experiments.

Statistics and presentation of data

Data are expressed as means ± S.E.M. Data sets were compared by use of Student’s two-tailed, paired t-test. The Bonferroni correction was used for multiple comparisons. Significance was considered to be achieved when the P value was <0.05.

Results

Heat treatment of amphotericin B alters its toxicity against THP-1 cells

Viability of THP-1 cells was unchanged (virtually 100% viable) after a 2 h exposure to AmB-DOC concentrations up to 2.5 µg/mL. Higher concentrations produced dose-dependent cell death. In contrast, viability was unchanged (100%) at HT-AmB concentrations up to 10 µg/mL.

Heat treatment of amphotericin B does not alter its activity against C. albicans

No difference in antifungal activity against Candida species was observed between the two preparations. As shown in Table 1, the IC₅₀ and MIC for both preparations tested in RPMI were virtually identical.

Heat treatment of amphotericin B attenuates cytokine and chemokine production

AmB-DOC at concentrations up to 5 µg/mL produced dose-dependent increases in IL-1β, TNF-α, MIP-1α and MIP-1β. A dose-dependent response was observed for IL-1α at AmB-DOC concentrations up to 2.5 µg/mL. Loss of dose-dependent responses at higher concentrations correlated with decreased cell viability. Dose-dependent increases in IL-1β, IL-1α, TNF-α and MIP-1β were observed in response to HT-AmB at concentrations up to 10 µg/mL, whereas no clear increase in MIP-1α in response to HT-AmB was observed (Table 2). These responses were substantially less than those observed with corresponding concentrations of AmB-DOC. No clear response to either AmB-DOC or HT-AmB was observed for IL-1Ra or MCP-1 (data not shown).

Discussion

As expected, AmB-DOC elicited dose-dependent toxicity against THP-1 cells as assessed by erythrosin B exclusion. In contrast, no effect on cell viability was noted at any concentration studied for HT-AmB. This is consistent with the findings of Gaboriau et al.,2 who reported decreased haemolysis of red blood cells by HT-AmB compared with AmB-DOC. These investigators also reported reduced toxicity to the cell line HT29 as measured by lactate dehydrogenase release and the MTT assay. Petit et al.,4 reported LD₅₀ values of 5 mg/kg for AmB-DOC compared with 8.1 mg/kg for HT-AmB in mice 1 week following intravenous administration. Consistent with these findings, Kwong et al.5 demonstrated that a single intravenous dose of AmB-DOC 1 mg/kg in New Zealand white rabbits resulted in a 51.7% increase in baseline serum creatinine, whereas no change in serum creatinine was observed after administration of an equal dose of HT-AmB.
To ensure that antifungal activity of amphotericin B was not compromised by heat treatment we measured the MIC and IC\textsubscript{50} for both AmB-DOC and HT-AmB against several Candida species. Consistent with previous reports for both \textit{C. albicans}\textsuperscript{2} and \textit{Cryptococcus neoformans},\textsuperscript{4} we found no appreciable difference in these parameters between the two preparations.

Amphotericin B has been shown to induce the production of IL-1\(\beta\) and TNF-\(\alpha\) in human and murine monocytes.\textsuperscript{6,8-10} Infusion-related fever, and rigors associated with amphotericin B administration are believed to be a result of the production of these cytokines.\textsuperscript{9,10} Other cytokines and chemokines that have been shown to be induced by amphotericin B include IL-1Ra, IL-8, MIP-1\(\alpha\), MIP-1\(\beta\) and MCP-1.\textsuperscript{8} Whereas these molecules may contribute to amphotericin B-associated toxicity, they may also contribute favourably to the antifungal activity of amphotericin B through their immunomodulatory actions. Consistent with the work of Hartsel \textit{et al.},\textsuperscript{3} we found amphotericin B-induced TNF-\(\alpha\) production in THP-1 cells to be abrogated by heat treatment of amphotericin B. We also observed similar results for IL-1\(\beta\), MIP-1\(\alpha\) and MIP-1\(\beta\). Concentrations of these secreted cytokines increased in a dose-dependent fashion in response to doses of AmB-DOC up to 5 \(\mu\)g/mL. These responses diminished in conjunction with reduced cell viability. Dose-dependent responses were also observed for HT-AmB, but at higher concentrations than those observed with AmB-DOC.

We also observed a dose-dependent response to AmB-DOC for IL-1\(\alpha\), but only up to a dose of 2.5 \(\mu\)g/mL. The diminished response observed with higher AmB-DOC doses is likely to reflect the cell-associated nature of this cytokine, where expression would be expected to more closely reflect cell viability compared with secreted cytokines. This is the first demonstration of the production of IL-1\(\alpha\) in response to amphotericin B.

We did not observe a dose-dependent increase in IL-1Ra or MCP-1 in response to AmB-DOC after exposure to either amphotericin B preparation for 2 h. In previous studies, production of these cytokines was measured at 6 h (for MCP-1) and at 24 and 48 h (for IL-1Ra) after AmB-DOC exposure.\textsuperscript{5,8} In the present study, we chose to measure cytokine and chemokine concentrations at 2 h after exposure, as this reflects the period of time in which amphotericin B-associated infusion-related toxicity is observed.\textsuperscript{1,9} In light of the anti-inflammatory properties of MCP-1 and IL-1Ra, it is probable that these molecules are produced to counter the initial pro-inflammatory response elicited by amphotericin B in these cells.

In conclusion, heat treatment of AmB-DOC abrogates amphotericin B-induced toxicity as well as cytokine and chemokine production in THP-1 cells, while not compromising the antifungal activity of this agent. In light of these findings, as well as previous studies demonstrating reduced toxicity with this amphotericin B preparation, heat treatment of amphotericin B may prove to be a cost-effective approach to improving the therapeutic index of this antifungal agent.

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### References


