REDUCTION OF AMPHOTERICIN B INDUCED RENAL TUBULAR APOPTOSIS BY N-ACETYLCYSTEINE

Running Title: N-acetylcystein and amphotericin B nephrotoxicity

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Abstract

Reduction of AmB-induced renal tubular apoptosis and nephrotoxicity with N-acetylcysteine (NAC) was evaluated in a murine model. Four groups of rats were treated with AmB for 5 days and each group received either concomitant NAC 2x30, 60 or 120 mg/kg/day or sterile water for 5 days. When compared with animals received AmB only, groups that received concomittant NAC had significantly decreased apoptosis in all dosages (48.8% vs 27.4%, 23.6% and 23.5% respectively, p<0.001).
Nephrotoxicity is the major dose limiting side effect of amphotericin B deoxycholate (AmB) (14, 16, 33). AmB induced nephrotoxicity is usually reversible, however up to 15% of patients may require dialysis resulting in extended hospital stay and increased mortality (3, 33). Nephrotoxicity occurs secondary to renal vasoconstriction leading to tubular damage and a decreased glomerular filtration rate (11, 14, 15). The mechanism of the renal tubular damage has not been fully elucidated, however, a dose dependent renal tubular cell apoptosis by amphotericin B has been suggested. (31).

Several approaches have been proposed to in an attempt to decrease the incidence of AmB nephrotoxicity. These approaches include prehydration with saline, prolonging the infusion time (10, 18), infusing the drug in intralipid solution (7, 19, 21, 24, 26) or co-administration with mannitol (6), however none of these approaches proved to be effective. While lipid formulations of AmB were found to be less nephrotoxic, these formulations do not completely eliminate nephrotoxicity (9, 17, 22, 34).

Recently, Varlam et al. demonstrated that AmB causes a dose dependent apoptotic effect in rat renal tubular cells and the level of apoptosis with the ensuing nephrotoxicity (31). They also showed that AmB induced apoptosis and resulting nephrotoxicity could be reduced by concomitant use of recombinant human insulin-like growth-factor-1 (rhIGF-1), an anti-apoptotic agent.

N-acetylcystein (NAC) is an anti-apoptotic and antioxidant drug and administration prior to administration of radiocontrast agents prevented the associated nephrotoxicity (4, 5, 29). The purpose of our study was to evaluate the effect of concomitant administration of NAC on AmB induced renal tubular cell apoptosis.

Three week old male Sprague-Dawley rats weighing average 100g were maintained in individual cages. The animals had free access to a standard diet and received water ad libitum. Prior to the experiments, rats were randomized and divided into 4 groups consisting 10
animals. Each group of animals (group A, B, C and D) were treated with 10 mg/kg/day intraperitoneal (IP) AmB-deoxycholate (Bristol-Myers Squibb) for 5 days. In addition to AmB, group A was given IP sterile water; group B, C and D was treated with 2x30, 60 or 120 mg/kg/day IP respectively for five consecutive days. The doses of AmB (31) and NAC (1, 12) were obtained from the literature. Another 5 animals (group E) were treated with sterile water only. Rats were weighed daily and changes in the body weights were recorded. At the end of the study animals were sacrificed and kidneys were harvested and placed into formaldehyde solutions for histopathological evaluation. Kidneys were paraffinized and 4-micrometer tissue sections were prepared and examined for apoptosis by the terminal deoxynucleotidyl transferase mediated dUTP nick end-labeling (TUNEL assay, ApopTag® Plus Peroxidase In Situ Apoptosis Detection Kit, Chemicon, Millipore) technique.

A pathologist quantified the degree of apoptosis in a blinded fashion. For the estimation of the level of apoptosis, the apoptotic index (AI) was determined. The AI was determined by counting the TUNEL-positive renal tubular epithelial cells and unstained cells in 10 sequentially selected microscopic fields at 400x magnification (mean 500 cells in each field). The apoptotic cells were then divided by the total number of cells in each field. Differences between the groups were analyzed by Student’s t test. A p-value of <0.05 was considered to reflect statistically significant data.

Results are summarized in Table 1. Average weights of animals receiving only AmB was significantly lower than the animals received concomitant NAC (p<0.001) at the end of the study. During the study period, animals in group A were observed to become less active as the treatment progressed to day 5. In the AmB treated group, the mean AI was 48.8% and rats of negative control group revealed 2.8% AI in kidney tissue. When compared with the AmB treated group coadministration of NAC resulted in significantly decreased AI in group B.
(27.4%, p<0.001), C (23.6%, p<0.001) and D (23.5%, p<0.001) (Table 1 and Figure 1). There was no difference in the AI between the three NAC treated groups.

The use of NAC for the prevention of the radiocontrast agent induced nephrotoxicity led us to evaluate its effect in the prevention of AmB induced apoptosis and renal injury (27, 30). NAC is usually administered 600 mg orally twice daily on the day before and on the day of administration of the contrast agent. However, in some studies, the intravenous form of the drug has been used in doses as high as 50 mg/kg (13, 28). The mechanism of the contrast-induced nephropathy has not completely been elucidated but it is attributed to combination of renal ischemia and tubular epithelial cell toxicity, oxidative tissue damage and apoptosis that are very similar to AmB (2, 8, 23, 25, 32).

In our study we demonstrated that NAC reduced AmB induced renal tubular apoptosis. Varlam et al. showed that rhIGF-1 prevented renal tubular apoptosis in animals which were treated with AmB at 5 mg/kg/day (2% vs 43% respectively) and significantly reduced apoptosis in animals which had received AmB at 10 mg/kg/day (18% vs 52% respectively). Similarly, in our study, AmB treatment resulted in a mean AI of 52% and concomitant use of NAC decreased the AI to 23.5% - 27.4%. Nitescu et al. showed that treatment with 200mg/kg NAC decreased the reduction in glomerular filtration rate (GFR), and reduced plasma creatinine, hyperkalemia and systemic oxidative stress in rats subjected to renal ischemic injury (20). In another study, prophylactic NAC prevented decreases in the GFR associated with high doses of AmB (12). Although we could show that NAC significantly reduced the AmB induced tubular apoptosis, we didn’t evaluate the serum or urine electrolytes and serum creatinine levels because of the technical unavailability. However, Varlam et al. demonstrated that rhIGF-1 improved the ability of the kidney to concentrate urine, and prevent hypokalemia and dehydration.
In summary, we demonstrated that renal tubular apoptosis caused by AmB could be reduced by the concomitant use of the anti-oxidant NAC. Our findings are promising for clinical use of NAC for the reduction of the AmB induced nephrotoxicity. Further clinical studies are warranted.
Acknowledgements

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References


or intralipid 20% in neutropenic patients with pneumonia or fever of unknown origin: randomised study. BMJ 317:379-84.


Table 1. Average apoptotic indexes of animal groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Regimens</th>
<th>Apoptotic index (%)</th>
<th>Average weight (g)</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>AmB only</td>
<td>48.8</td>
<td>159</td>
</tr>
<tr>
<td>B</td>
<td>AmB + NAC 2x30 mg/kg</td>
<td>27.4*</td>
<td>205*</td>
</tr>
<tr>
<td>C</td>
<td>AmB + NAC 2x60 mg/kg</td>
<td>23.6 *</td>
<td>207*</td>
</tr>
<tr>
<td>D</td>
<td>AmB + NAC 2x120 mg/kg</td>
<td>23.5 *</td>
<td>211*</td>
</tr>
<tr>
<td>E</td>
<td>Sterile water</td>
<td>2.8</td>
<td>205</td>
</tr>
</tbody>
</table>

* P<0.05, P values were calculated by comparing group B, or C or D with group A
Figure Legend

**Figure 1.** Photomicrographs of renal tubular cells (at 400X magnification). Apoptotic cells were detected immunohistochemically by in situ end labeling of DNA strand breaks (TUNEL assay). All dark-stained cells are apoptotic (white arrows). Left panel: Tubular epithelial cells from rats treated with 10 mg/kg AmB. Right panel: Concomitant administration of AmB and 60 mg/kg/12h of NAC for 5 days with a significant decrease in apoptosis.
Figure 1. Photomicrographs of renal tubular cells