Research Article

INCREASED SERUM NITRITE AND NITRATE LEVELS IN AMPHOTERICIN-B ADMINISTERED ALBINO RATS

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ABSTRACT

Amphotericin B (Amp-B) usefulness is associated with a number of toxic cellular effects. We investigated the in-vivo effects of Amp-B on the nitrite (NO\textsubscript{2}) and nitrate (NO\textsubscript{3}) levels in the serum of rats administered with 0.5 or 1.5 mg/kg/wt of Amp-B over 4 or 10 weeks (i.v, weekly doses). The rat serum NO\textsubscript{2}/NO\textsubscript{3} levels appeared to be enhanced in the Amp-B administered rats compared to the untreated control group of rats and the changes observed were found to be in a dose and time – dependent manner. Based on the results, it is reported that Amp-B by way of enhancing the production of nitric oxide (NO) may contribute for more production of NO end products like NO\textsubscript{2}/NO\textsubscript{3} and these intern may be helpful in reducing the fungal affects, since Amp-B is the drug of choice used against fungal infections in clinical trails.

Keywords: Amphotericin B, Nitric oxide, Nitrite, Nitrate.

INTRODUCTION

The therapeutic use of polyene antibiotic Amphotericin-B (Amp-B) is known to produce a number of side effects such as fever, chills, nausea, vomiting, headache, anoxia, hypoxalamia, renal dysfunction and number of toxicologic side effects in clinical trials as well as in experimental models. L-Arginine in the presence of the enzyme nitric oxide synthase (NOS) is known to produce nitric oxide (NO) and citrulline. NO diffusing into the lumen of blood vessels will be very rapidly converted to nitrate, because of the high concentration of hemoglobin in the red blood cell. NO as soon as it is produced is converted to its end products nitrite (NO\textsubscript{2}) and nitrate (NO\textsubscript{3}) \cite{1}. Present study is designed to study the in vivo effect of Amp B on rat serum NO\textsubscript{2} and NO\textsubscript{3} levels in vivo.

MATERIAL AND METHODS

Animals

![Chemical structure of Amphotericin B](image)

Fig 1: Chemical structure of Amphotericin -B

Albino rats of the weight range 150±10 gm were selected for the present study. The animals were kept under constant temperature of 21±5°C and were fed Ad libitum on the commercial diet supplied by kamadhenu Agencies, Bangalore. They were divided into six groups of seven each and were maintained in separate cages.

Chemicals

Amp-B (C\textsubscript{47}H\textsubscript{73}NO\textsubscript{57}) (Fig 1) was a product from Bristol-Myers SQmb company, USA. All other chemicals used were of technical grade supplied by SBH, or BDH, India.

DISCUSSION

Present study has demonstrated that Amp-B in doses tested in vivo has enhanced the rat serum NO\textsubscript{2}/NO\textsubscript{3} levels (table:1) and the trends obtained were in agreement with the reports of earlier authors \cite{3} where they observed elevated macrophage NO\textsubscript{2} levels in their experiments involving Amp-B. The data in table 1 & 2 also confirm that a high dose of Amp-B treated over 10 weeks (Fig: 1 & 2).

RESULTS

The data in table 1 and 2 shows the levels of the control and Amp-B treated rat serum NO\textsubscript{2}/NO\textsubscript{3} levels. In the control rat serum the NO\textsubscript{2} levels were found to be more than NO\textsubscript{3} levels and the Amp-B treated rat serum appeared to show increased levels of its serum NO\textsubscript{2}/NO\textsubscript{3} levels compared to the control over. All the changes were found to be statistically significant ones the control (P<0.01). More percent elevation of Amp-B treated rat serum NO\textsubscript{2}/NO\textsubscript{3} levels were observed for rat serum receiving 1.5 mg/kg/wt of Amp-B over 10 weeks (Fig: 1 & 2).

TREATMENT OF ANIMALS

Group I and IV rats acted as control over group II rats received 0.5 mg/kg bwt of Amp-B (i.v) in saline over four weeks (weekly doses). Group III rats received 0.5 mg/kg/wt of Amp-B over 10 weeks. Groups V rats were administered with 1.5 mg/kg/wt of Amp-B (i.v) over 4 weeks and group VI rats received 1.5 mg/kg/wt AmpB (i.v) over 10 weeks (Weekly doses) in saline.

After treatment of the animals the control and experimental group of rats were anaesthetized with Ketamine HCL (40mg/kg) and from the individual animals the blood was collected by cardiac puncture, allowed to clotting and the control and experimental samples were subjected for centrifugation at 2500 rpm/15 minutes and the serum was collected into separate cuvettes.

In the control and experimental samples the serum NO\textsubscript{2}/NO\textsubscript{3} levels were determined following the method of Guarner et al.,(1993)\cite{2}.

Statistical analysis of the data:

For each parameter, the mean of individual observations (for both control and experimental groups) were taken into considerations and statistical significances of the data was analysed through one way (ANOVA).

ACKNOWLEDGMENT

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REFERENCES

The author attempted to study the in vivo effect of Amp-B on rat serum NO$_3^-$ levels for the reasons were due to diffusion of more NO formed under Amp B stress which thereafter be converted to NO$_2^-$/NO$_3^-$ levels. For cytokines. Anticryptococcal effect of amphotericin B is mediated through macrophage production of nitric oxide. Amphotericin B activation of human genes encoding nitric oxide. A further independent effect on rat serum NO$_3^-$/NO$_2^-$ levels and the data was presented in Table 1-2. Amp-B administration appeared to enhance the levels of rat serum NO$_3^-$/NO$_2^-$ levels for this the reasons were due to diffusion of more NO formed under Amp B stress which thereafter be converted to NO$_2^-$/NO$_3^-$ levels. The data supports that NO is known to kill pathogens in the body [9], based on the present experimental data it is reported that one of the antifungal mechanisms of Amp-B may involve the production of more NO in conditions of fungal infections.

**CONCLUSION**

Further the antifungal agent Amp-B appeared to exert dose and time - dependent effect on rat serum NO$_3^-$/NO$_2^-$ levels. The data supports that the increased rat serum in Amp-B administered rats could be due to more production of NO. Amp-B interacting with NO producing path ways and there by coming the production of more NO is well documented [4-8] and in the present observed trend of result with reports of the above authors. As NO is known to kill pathogens in the body [9], based on the present experimental data it is reported that one of the antifungal mechanisms of Amp-B may involve the production of more NO in conditions of fungal infections.

**REFERENCES**


### Table 1: Impact of Amphotericin-B on rat serum Nitrite (NO$_3^-$) levels in vivo (values expressed as ng NO$_3^-$/ml of serum).

<table>
<thead>
<tr>
<th>Name of the tissue</th>
<th>Control</th>
<th>0.5mg/kg Amp-B</th>
<th>1.5 mg/kg Amp-B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks treated</td>
<td>10 weeks treated</td>
<td>4 weeks treated</td>
</tr>
<tr>
<td>Rat Serum NO$_3^-$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AV</td>
<td>62.901</td>
<td>72.600</td>
<td>87.427</td>
</tr>
<tr>
<td>SD</td>
<td>± 3.112</td>
<td>±2.590</td>
<td>±10.250</td>
</tr>
<tr>
<td>PC</td>
<td>15.42*</td>
<td>38.99*</td>
<td>36.69*</td>
</tr>
</tbody>
</table>

Each Value is the mean ± SD of 7 Samples. AV: Average, SD: Standard deviation, PC: Percent change over control: *P<0.01

### Table 2: Impact of Amphotericin-B on rats serum nitrate(NO$_3^-$) levels in vivo (Values expressed as ng of NO$_3^-$/ml of serum).

<table>
<thead>
<tr>
<th>Name of the tissue</th>
<th>Control</th>
<th>0.5mg/kg Amp-B</th>
<th>1.5 mg/kg Amp-B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 weeks treated</td>
<td>10 weeks treated</td>
<td>4 weeks treated</td>
</tr>
<tr>
<td>Rat Serum NO$_3^-$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AV</td>
<td>46.193</td>
<td>61.323</td>
<td>65.014</td>
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<tr>
<td>SD</td>
<td>± 4.029</td>
<td>±3.334</td>
<td>±3.577</td>
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<tr>
<td>PC</td>
<td>32.75*</td>
<td>40.75*</td>
<td>44.31*</td>
</tr>
</tbody>
</table>

Each Value is the mean ± SD of 7 Samples. AV: Average, SD: Standard deviation, PC: Percent change over control: *P<0.01