The mechanism of amphotericin-induced distal acidification defect in rats

NARESH K. JULKA, JOSE A. L. ARRUDA AND N. A. KURTZMAN

The Sections of Nephrology, University of Illinois Abraham Lincoln School of Medicine, and the Veterans Administration West Side Hospital, Chicago, Illinois, U.S.A.

(Received 24 July 1978; accepted 29 January 1979)

Summary

1. The effect of chronic and acute administration of amphotericin B on renal function, especially urinary acidification, was studied in rats. Chronic intraperitoneal administration of amphotericin B resulted in blood concentrations similar to or higher than those seen in patients treated with the antibiotic, but failed to produce hyperchloremic acidosis. The minimum urine pH achieved during NH₄Cl-induced acidosis was significantly higher in amphotericin-treated rats than in controls; net acid excretion, however, was not significantly different from that of controls. These data were interpreted as indicating the presence of an incomplete distal acidification defect.

2. The ability to raise urine-blood (U-B)Pco₂ during alkalinization of the urine was examined in another group of amphotericin-treated rats. The data on amphotericin-treated rats could be artificially divided into two groups. One group had a normal glomerular filtration rate (GFR) and was able to raise the (U-B)Pco₂ gradient during administration of the HCO₃⁻ to the same as that seen in control rats infused with the vehicle for amphotericin, sodium deoxycholate. The second group, which comprised only 15% of the total, had a decreased glomerular filtration rate and an abnormally low (U-B)Pco₂. The normal (U-B)Pco₂ was interpreted as the result of normal H⁺ secretion during alkalinization of the urine. The low (U-B)Pco₂ in the second group was attributed to the low urinary HCO₃⁻ concentration.

3. In order to investigate the effect of large concentrations of amphotericin on distal H⁺ secretion, amphotericin was administered acutely to HCO₃⁻-loaded rats. Acute intravenous administration of amphotericin resulted in a significant decrease in (U-B)Pco₂ and urine HCO₃⁻ concentration; the decrease in urinary Pco₂ was of greater magnitude than the decrease in urine HCO₃⁻ concentration, leading to a rise in urine pH. The maximum urine pH achieved during HCO₃⁻ loading (maximal urine HCO₃⁻ was the same in both groups) was significantly higher in the animals infused acutely with amphotericin than in controls, thus indicating that amphotericin-treated animals have a distal acidification defect. These data are compatible with the hypothesis that amphotericin induces an acidification defect by increasing H⁺ back-diffusion in the form of carbonic acid or dissolved CO₂ when the urine is highly alkaline and in the form of weak acid or proton when the urine is acid.

Key words: amphotericin, distal acidification, urinary Pco₂.

Abbreviations: GFR, glomerular filtration rate; U-B, urine–blood.

Introduction

Amphotericin B is widely used for the treatment of systemic fungal infections (Butler, Bennett, Alling, Wertlake, Utz & Hill, 1964a). Administration of this antibiotic, however, is associated with nephrotoxic side-effects: in particular, development of...
renal failure, renal tubular acidosis and hyperkalaemia (Burgess & Birchall, 1972; Butler et al., 1964a; Douglas & Healy, 1969; McCurdy, Frederic & Elkinton, 1968; Patterson & Ackerman, 1971). Chronic intraperitoneal administration of amphotericin B to rats has been reported to result in nephrotoxic effects identical with those reported in man (Gouge & Andriole, 1971). Steinmetz & Lawson (1970) demonstrated that the addition of amphotericin B to the mucosal surface of the urinary bladder of the turtle results in an increased permeability of this membrane to $H^+$ and $K^+$. They suggested that the same mechanism may be operative in patients with amphotericin-induced renal tubular acidosis.

More recently, measurement of urine–blood (U–B)$P_{CO_2}$ during alkalinization of the urine has been used to assess distal acidification (Arruda, Nascimento, Kumar & Kurtzman, 1977a; Arruda, Nascimento, Mehta, Rademacher, Sehy, Westenfelder & Kurtzman, 1977b; Halperin, Goldstein, Haig, Johnson & Stinebaugh, 1974). As can be seen from the equation:

$$H^+ + HCO_3^- \Rightarrow H_2CO_3 \Rightarrow CO_2 + H_2O$$

$H^+$ secretion during alkalinization of the urine will result in a shift of equilibrium of the reaction to the right with consequent elevation of carbonic acid. Owing to the absence of carbonic anhydrase on the luminal epithelial surface of the distal nephron there is delayed dehydration of carbonic acid, which dissociates into $CO_2$ and $H_2O$ in the post-papillary region. In the lower urinary tract, the volume-to-surface relationship is unfavourable for $CO_2$ diffusion, resulting in a rise in urinary $P_{CO_2}$ (Arruda et al., 1977b). The failure to raise (U–B)$P_{CO_2}$ has been suggested to indicate either diminished $H^+$ secretion (secretory defect), by some investigators (Halperin et al., 1974), or enhanced carbonic acid back-diffusion (gradient defect) by others (Sebastian, McSherry & Morris, 1976). It is likely that both types of acidification defect will be associated with the inability to raise (U–B)$P_{CO_2}$ (Arruda et al., 1977a).

Roscoe, Goldstein, Halperin, Schloeder & Stinebaugh (1977) reported that chronically amphotericin-treated rats raise urinary $P_{CO_2}$ normally during alkalinization of the urine. In this study we examined the effects of chronic and acute amphotericin B administration to rats; we studied their ability to both raise urinary $P_{CO_2}$ and acidify the urine.

Methods

Experiments were performed on Sprague–Dawley male rats weighing 250–400 g. The rats were allowed a normal food and water intake before the day of study. They were anaesthetized with Inactin (Promonta, Hamburg, West Germany) given intraperitoneally (10 mg/100 g). Tracheostomy was performed and one carotid artery and one jugular vein were cannulated. The bladder was catheterized through an abdominal incision. Blood pressure was monitored throughout the experiment. At the start of the experiment $[^{125}I]$iothalamate, diluted in sodium chloride solution (saline), 0.75 μCi/ml, was given by an infusion pump at a rate of 0.024 ml/min throughout the course of the experiment as a marker of GFR. An equilibration period of 60 min was allowed before any collection was started. Urine samples were collected under mineral oil in preweighed glass vials and the urine volume was determined gravimetrically. Blood samples were collected from the carotid artery at the mid-portion of each clearance collection; collections were of 10–30 min duration.

Chronic amphotericin treatment was administered by injecting 5 mg of amphotericin B/kg (5.4 μmol/kg) intraperitoneally (average of five injections/week). The rats received an average of 23.0 ± 10.8 injections (range 8–50) given during a period of 10–60 days. Rats with a blood urea concentration greater than 18 mmol/l at the time of the study were discarded. The following groups of rats were studied.

**Group 1: chronic amphotericin administration + NH₄Cl**

This group was studied to determine whether animals treated chronically with amphotericin have a defect in lowering urine pH maximally in response to acidosis, as has been reported by other investigators (Gouge & Andriole, 1971; Roscoe et al., 1977). The rats used in this group were fed with 1.5% (0.28 mol/l) NH₄Cl solution for at least 3 days before the study. The following groups of animals were studied.

Group 1A (chronic amphotericin B administration): six rats chronically treated with amphotericin B were prepared for clearance studies as described above. Two to three clearance collections were obtained.

Group 1B (normal rats): six normal rats were prepared for the clearance study. Two to three collections were obtained.
Group 2: chronic amphotericin administration + NaHCO₃ loading

This group of animals was studied to determine whether animals treated chronically with amphotericin have a defect in raising urine Pco₂ in response to HCO₃⁻ loading.

Forty rats chronically treated with amphotericin B were included in this study (groups 2B and 2C). For at least 3 days preceding the study these rats were fed with 1.5% (0.28 mol/l) NH₄Cl solution in order to produce metabolic acidosis. The animals were prepared for clearance studies as described above. After baseline urine and blood collections the rats were infused with NaHCO₃ solution (0.9 mol/l) at a rate of approximately 6 ml/h in order to achieve a urine pH greater than 7.8; once this urine pH was achieved two to three urine and blood collections were obtained. An additional four rats were injected with sodium deoxycholate, the vehicle used to dissolve amphotericin B (group 2A).

Group 3: acute intravenous amphotericin infusion + NaHCO₃ loading

This group was studied in an attempt to achieve higher concentrations of amphotericin B in the urine and thus simulate conditions present in the studies in vitro, where an acidification defect induced by amphotericin is easily demonstrable (Steinmetz & Lawson, 1970; Finn, Cohen & Steinmetz, 1977). This protocol is different from those used by previous investigators, who studied only animals chronically treated with amphotericin B (Gouge & Andriole, 1971; Roscoe et al., 1977).

Group 3A: acute sodium deoxycholate administration. Nine rats were treated identically with those of group 3B, except that they were infused intravenously with sodium deoxycholate (the vehicle used to dissolve amphotericin) instead of amphotericin B. The volume infused was identical with that used in group 3. Since acute amphotericin administration to the previous group of rats resulted in a significant increase in serum K⁺, KCl was infused at a rate of 1 mmol/h simultaneously with sodium deoxycholate.

Group 3B: acute amphotericin B administration. Twenty-four rats were studied in this group. The rats were infused with NaHCO₃ solution (0.9 mol/l) as described above. When the urine pH achieved a value greater than 7.8 two to three urine and blood collections were obtained. Amphotericin B was then infused intravenously at a dose of 2 mg h⁻¹ kg⁻¹ (2.2 µmol h⁻¹ kg⁻¹) for 60 min and further collections were obtained. Fourteen rats received amphotericin B in a concentration of 2 mg/ml (2.2 µmol/ml) and the other 10 in a concentration of 0.5 mg/ml (0.54 µmol/ml).

Group 4: other studies

This group was studied to determine whether animals chronically treated with amphotericin develop metabolic acidosis and to determine blood concentrations of amphotericin.

Ten rats chronically treated with amphotericin B were exsanguinated through the abdominal aorta; amphotericin blood concentrations were determined in six rats and arterial blood pH and PaCO₂ were measured in six rats (both determinations were made in two of the rats).

GFR, plasma and urine electrolyte determinations were performed as previously described (Kurtzman, 1970). Ammonia and titratable acid were measured by the method of Cunarro & Weiner (1974). Amphotericin blood concentrations were measured by bioassay (Louria, 1958). Carbonic anhydrase activity in the urine was assayed by the method of Maren (1970).

The amphotericin B used in the study was provided by E. R. Squibb and Sons (New York), either as a commercial preparation of amphotericin B (Fungizone) or as a pure powder of amphotericin. The pure powder was prepared with sodium deoxycholate according to the same method as that used for the preparation of Fungizone.

The data were analysed by the t-test for paired and unpaired groups (Kurtzman, 1970). Statistical significance was defined as P < 0.05.

Results

Group 1: chronic amphotericin administration + NH₄Cl

The mean plasma urea concentration of all chronically amphotericin-treated rats was 10.7 ± 1.33 mmol/l. There was no difference in GFR, urine flow and blood pH between the two groups. The minimum urine pH was significantly greater in amphotericin-treated rats than in the control group. Titratable acid excretion, NH₄⁺ excretion and net acid excretion were not significantly different between the two groups.
Group 2: chronic amphotericin administration + NaHCO₃ loading

The mean baseline urine pH of all chronically amphotericin-treated rats after NH₄Cl administration in group 2 was 5.75 ± 0.03 at a blood pH of 7.26 ± 0.03. The urine pH in this group was significantly higher than that of control rats shown in Table 1 (P < 0.001). When data of all the amphotericin B-treated rats taken as one single group were compared with those of the control groups there was no significant difference between them. Analysis of the data of amphotericin-treated animals, however, showed that this group was not homogeneous. Group 2B included rats which were able to raise their (U-B)PCO₂ gradient to the same level as controls (group 2A), and group 2C rats failed to raise (U-B)PCO₂ maximally (less than 15 mmHg or 2.1 kPa) (Table 2).

In chronically amphotericin-treated rats with a decreased GFR and (U-B)PCO₂ (group 2C) urine HCO₃⁻ concentration was lower than that of control rats and lower than that of group 1A amphotericin-treated rats. The maximal urine pH achieved, however, was not different from that of the other two groups.

Group 3: acute intravenous amphotericin infusion + NaHCO₃ loading

During NaHCO₃ administration both groups of rats were able to increase urine pH, urine HCO₃⁻ concentration and (U-B)PCO₂ normally. Infusion of amphotericin B intravenously resulted in a significant decrease in GFR and fractional K⁺ excretion and an increase in plasma K⁺ concentrations (group 3B). Acute amphotericin infusion led to a significant decrease in (U-B)PCO₂; urine HCO₃⁻ concentration decreased slightly, although significantly, and urine pH rose significantly. At comparable urinary HCO₃⁻ concentrations the maximal urine pH achieved was significantly higher during amphotericin infusion than in the control group. There was no detectable carbonic anhydrase in the urine samples collected from four rats infused with amphotericin.

The control group of rats infused with sodium deoxycholate plus KCl (group 3A) did not show any significant change in GFR, urine pH, urine HCO₃⁻ concentration or fractional K⁺ excretion. (U-B)PCO₂ decreased slightly but the difference did not achieve statistical significance (Table 3).

Group 4: other studies

Blood concentrations of amphotericin were measured in six rats chronically treated with the drug; the mean value was 3.0 ± 0.45 μg/dl (0.003 ± 0.0004 μmol/ml).

Blood pH and blood Pao₂ were 7.46 ± 0.02 and 26.0 ± 3.67 mmHg (3.5 ± 0.49 kPa) respectively in six chronically amphotericin-treated rats which did not receive NH₄Cl administration.

Discussion

The results of this study demonstrate that chronically amphotericin-treated rats, when compared with control animals, have impaired ability to lower urine pH during NH₄Cl-induced acidosis; NH₄⁺ excretion and net acid excretion, however, were not different from those of controls. These data are similar to those of Gouge & Andriole (1971) and Roscoe et al. (1977).

The interest in studying urinary PCO₂ in amphotericin-treated rats arose from the fact that this drug has been shown to increase the permeability of the turtle bladder, mammalian cells and other

### Table 1. Renal acid excretion in chronically acidicotic rats treated with amphotericin B and in control rats (group 1)

<table>
<thead>
<tr>
<th>V, Urine flow. N.S., Not significant.</th>
</tr>
</thead>
<tbody>
<tr>
<td>V (ml/min)</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>Group 1A Chronic amphotericin (n = 6)</td>
</tr>
<tr>
<td>Group 1B Control (n = 6)</td>
</tr>
</tbody>
</table>
**Table 2. Effect of HCO$_3^-$ loading on rats chronically treated with amphotericin**

(U-B), Urine—blood; $T_{\text{m}}$/GFR, HCO$_3^-$ reabsorption per litre of GFR. $P$ values refer to comparisons between the two groups of amphotericin B-treated animals; the same significance was found when group 2C was compared with the sodium deoxycholate-treated group. No significant difference (N.S.) was found between group 2B and the sodium deoxycholate-treated group.

<table>
<thead>
<tr>
<th>Group</th>
<th>GFR (ml/min)</th>
<th>Plasma</th>
<th>Urine</th>
<th>(U-B) $P_{\text{CO}_2}$</th>
<th>Fractional excretion of K$^+$ (%)</th>
<th>$T_{\text{m}}$/GFR (mmol/l of GFR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pH</td>
<td>$P_{\text{a,CO}_2}$ (mmHg/kPa)</td>
<td>HCO$_3^-$ (mmol/l)</td>
<td>pH</td>
<td>$P_{\text{CO}_2}$ (mmHg/kPa)</td>
</tr>
<tr>
<td>2A: sodium deoxycholate ($n = 4$)</td>
<td>3.34 ± 0.49</td>
<td>7.67 ± 0.01</td>
<td>28.8 ± 2.37</td>
<td>32.2 ± 2.50</td>
<td>7.96 ± 0.03</td>
<td>59.0 ± 3.26</td>
</tr>
<tr>
<td>2B: amphotericin B ($n = 34$)</td>
<td>3.56 ± 0.21</td>
<td>7.68 ± 0.01</td>
<td>33.0 ± 2.00</td>
<td>39.5 ± 2.30</td>
<td>7.97 ± 0.04</td>
<td>67.0 ± 2.57</td>
</tr>
<tr>
<td></td>
<td>$P &lt; 0.01$</td>
<td>N.S.</td>
<td>$P &lt; 0.01$</td>
<td>N.S.</td>
<td>N.S.</td>
<td>$P &lt; 0.01$</td>
</tr>
<tr>
<td>2C: amphotericin B ($n = 6$)</td>
<td>2.03 ± 0.57</td>
<td>7.69 ± 0.03</td>
<td>36.0 ± 3.71</td>
<td>42.0 ± 3.48</td>
<td>7.98 ± 0.04</td>
<td>44.4 ± 2.66</td>
</tr>
</tbody>
</table>

* Significantly different when compared with the sodium deoxycholate group.
<table>
<thead>
<tr>
<th></th>
<th>GFR (ml/min)</th>
<th>V (ml/min)</th>
<th>Plasma PaCO₂ [mmHg(kPa)]</th>
<th>Plasma HCO₃⁻ (mmol/l)</th>
<th>Urine pH</th>
<th>Urine PaCO₂ [mmHg(kPa)]</th>
<th>Urine HCO₃⁻ (mmol/l)</th>
<th>(U–B) PaCO₂ [mmHg(kPa)]</th>
<th>Plasma K⁺ (mmol/l)</th>
<th>Fractional excretion of K⁺ (%)</th>
<th>Tm HCO₃⁻ (mmol/l of GFR)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 3A: sodium deoxycholate (n = 9)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2.92 ± 0.25</td>
<td>132.13 ± 22.00</td>
<td>34.0 ± 1.33</td>
<td>32.0 ± 2.31</td>
<td>7.95 ± 0.02</td>
<td>82.0 ± 4.21</td>
<td>203.9 ± 14.2</td>
<td>47.33 ± 3.91</td>
<td>2.7 ± 0.13</td>
<td>51.4 ± 4.59</td>
<td>22.9 ± 1.87</td>
</tr>
<tr>
<td></td>
<td>N.S.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>2.98 ± 0.36</td>
<td>233.26 ± 30.59</td>
<td>34.3 ± 2.29</td>
<td>33.5 ± 2.26</td>
<td>7.93 ± 0.03</td>
<td>74.5 ± 5.90</td>
<td>40.17 ± 4.58</td>
<td>5.7 ± 0.68</td>
<td>64.2 ± 5.87</td>
<td>27.5 ± 6.84</td>
<td></td>
</tr>
<tr>
<td><strong>Group 3B: amphotericin B (n = 24)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3.88 ± 0.27*</td>
<td>119.58 ± 11.87</td>
<td>35.2 ± 1.50</td>
<td>35.3 ± 1.90</td>
<td>7.96 ± 0.02</td>
<td>74.7 ± 3.00</td>
<td>197.3 ± 14.4</td>
<td>39.50 ± 2.00</td>
<td>2.8 ± 0.09</td>
<td>44.5 ± 3.38*</td>
<td>26.5 ± 1.58*</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>2.22 ± 0.33</td>
<td>113.91 ± 14.29</td>
<td>32.5 ± 1.70</td>
<td>39.5 ± 2.62</td>
<td>8.02 ± 0.03</td>
<td>57.7 ± 3.80</td>
<td>162.9 ± 12.9</td>
<td>25.30 ± 3.10</td>
<td>5.3 ± 0.70</td>
<td>35.5 ± 2.80</td>
<td>30.3 ± 2.14</td>
</tr>
<tr>
<td><strong>Comparison between sodium deoxycholate and amphotericin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C vs C</td>
<td>N.S.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E vs E</td>
<td>P &lt; 0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Measured in 20 animals.
membranes to cations (Cass, Finkelstein & Krespi, 1970; Steinmetz & Lawson, 1970). These observations suggested that increased back-diffusion of H+ from the tubular lumen of the distal nephron may represent the pathogenic mechanism in the clinical syndrome of distal renal tubular acidosis induced by amphotericin. Roscoe et al. (1977) found the ability to raise urinary Pco₂ was normal in amphotericin-treated rats. They interpreted these findings as indicating that the distal acidification defect of amphotericin administration was not associated with increased carbonic acid back-diffusion. In our study 85% of amphotericin-treated rats were able to raise (U-B)Pco₂ normally, whereas the remaining 15% had an abnormally low (U-B)Pco₂. It should be emphasized that the mean (U-B)Pco₂ of all chronically amphotericin B-treated rats was not significantly different from that of control rats. The decrease of (U-B)Pco₂ observed in 15% of the chronically amphotericin-treated animals is likely to have been the result of the decreased urinary HCO₃⁻ concentration, secondary to the concentrating defect of renal insufficiency or amphotericin (Steinmetz, Al-Awquati & Lawson, 1970; Arruda et al., 1977b).

Our data to this point are thus in complete agreement with those reported by Roscoe et al. (1977). The fact that the majority of all amphotericin-treated animals are able to raise (U-B)Pco₂ normally, while having impaired ability to lower urine pH and normal net acid excretion, has two possible interpretations. First, the acidification defect induced by amphotericin (i.e. increased H⁺ back-diffusion) could be associated with a normal ability to raise (U-B)Pco₂ because H₂CO₃ does not back-diffuse, or second, the acidification defect induced by amphotericin could be incomplete, i.e. there is an inability to lower the urine pH maximally during acidosis but H⁺ secretion is normal when the blood pH is normal or elevated (Wrong & Davies, 1959).

In order to investigate further the mechanism of amphotericin on distal acidification, the effect of acute infusion of large doses of amphotericin to HCO₃⁻-loaded rats was studied. It was reasoned that these larger doses of amphotericin might result in a concentration of amphotericin in the distal nephron similar to that used in the turtle bladder and thus cause a more severe distal acidification defect. Acute infusion of amphotericin resulted in a significant decrease in urinary Pco₂, urinary HCO₃⁻ and GFR, whereas plasma K⁺ increased significantly. The increase in serum K⁺ reflects an increased permeability of the membrane of erythrocytes and the decrease in GFR is the result of the vasoconstrictor effect of this drug (Butler, Bennett, Hill, Szwed & Cotlove, 1964b; Butler, Hill, Szwed & Knight, 1964c; Butler, Alling & Cotlove, 1965; Butler & Cotlove, 1971). The decrease in fractional K⁺ excretion probably reflects an unsteady state due to the decrease in GFR.

We have recently demonstrated that urinary Pco₂ in highly alkaline urine is critically dependent on urinary HCO₃⁻ concentration (Arruda et al., 1977b). If the decrease in urinary HCO₃⁻ concentration is the primary event, the urinary Pco₂ will decrease in the same proportion and urine pH will remain unchanged. On the other hand, if the decrease in urinary Pco₂ is the primary event, urinary HCO₃⁻ will either remain unchanged or decrease slightly, secondary to the shift in equilibrium to the right of the reaction:

\[ H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O \]

Since the decrease in urinary Pco₂ is of greater magnitude than the decrease in urine HCO₃⁻ concentration, the urine pH would rise. An increase in urine pH in highly alkaline urine associated with a fall in urinary Pco₂ has three possible interpretations: (1) the presence of carbonic anhydrase in the urine (Arruda, Roseman, Sehy, Mehta & Kurtzman, 1977c); (2) failure to secrete H⁺ (secretory defect); (3) increased carbonic acid back-diffusion (gradient defect). Since there was no carbonic anhydrase activity in the urine during acute amphotericin infusion the decrease in (U-B)Pco₂ and increase in urine pH observed must indicate the presence of a distal acidification defect. The data obtained during acute intravenous infusion of amphotericin are thus strikingly different from those obtained during chronic amphotericin administration, suggesting that the large dose of amphotericin administered intravenously disclosed an acidification defect not elicited by chronic administration of this antibiotic. The fact that, in the turtle bladder, amphotericin causes an acidification defect by increasing passive back-diffusion of H⁺ (Steinmetz et al., 1970), strongly suggests that the decrease in urinary Pco₂ observed during HCO₃⁻ loading in vivo is also the result of increased back-diffusion of H₂CO₃.

The decline in (U-B)Pco₂ however, cannot be attributed to hyperkalaemia, since we previously demonstrated that an increase in serum K⁺ does not affect (U-B)Pco₂ (Arruda et al., 1977a); furthermore, infusion of KCl to animals receiving deoxycholate did not lower urinary Pco₂.

In conclusion, these data demonstrate that
chronic amphotericin treatment is associated with impaired ability to lower urine pH during acidosis and normal ability to raise (U-B)Pco₂ in highly alkaline urine. The data suggest that chronic amphotericin treatment causes an incomplete form of distal acidification defect which is only evident during acidosis. Acute intravenous amphotericin infusion produces a more profound defect, which results in a decrease in (U-B)Pco₂ and an increase in urine pH. The fall in urinary Pco₂ is probably the result of increased carbonic acid back-diffusion. Thus the acidification defect induced by amphotericin both in vivo and in vitro appears to be the result of altered membrane permeability.

Acknowledgments

We thank Dr M. De Sa Pereria for performing the determinations of blood concentrations of amphotericin and Dr W. McDowell (from E. R. Squibb and Sons Inc.) for providing us with the amphotericin B used in this study. During the performance of these studies J.A.L.A was a Research Associate at the Veterans Administration West Side Hospital in Chicago, Illinois. This research was supported in part by the following grants: United States Public Health Service AM 20170; Veterans Administration 7083-04, 3324-01 and 052-01.

References


