Hamycin treatment of candidiasis in normal and diabetic rats

Jayant N. Dhuley *

Department of Pharmacology and Toxicology, Research and Development Division, Hindustan Antibiotics Ltd., Pimpri, Pune 411 018, India

Received 30 April 1999; revised 20 August 1999; accepted 24 August 1999

Abstract

Hamycin, a heptaene antifungal antibiotic was compared with amphotericin B in the treatment of established systemic infection with *Candida albicans* in normal and diabetic rats. In normal rats, orally administered hamycin at 10 mg kg$^{-1}$ per day for 7 days reduced *Candida* colony counts in the kidneys and livers as well as amphotericin B did and was nearly as effective as amphotericin B in a 21-day treatment trial. There was no further reduction in *Candida* colony counts when normal rats were treated with hamycin at 25 mg kg$^{-1}$ twice a day for 7 days. In streptozotocin induced diabetic rats, hamycin at 20 mg kg$^{-1}$ per day for either 7 or 21 days compared favourably with amphotericin B in efficacy. Results of the present study suggest that oral hamycin may be useful in the treatment of established disseminated candidiasis in normal as well as diabetic hosts. ß 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Hamycin; diabetic rat; *Candida albicans*

1. Introduction

Disseminated candidiasis is associated with malignancies, broad spectrum antibiotics, cytotoxic chemotherapy, intra-abdominal surgery, intravascular catheters, and hyperalimentation [1,2]. Diabetes mellitus and urinary tract abnormalities predispose patients to urinary tract infections with *Candida albicans* [3]. Amphotericin B is the standard drug used to treat both disseminated and renal candidiasis, but its use may be limited by toxicity. Ketoconazole has been used for mucocutaneous *Candida* infections [4], but it is unreliable for disseminated infections. Ketoconazole can cause a decrease in serum testosterone [5], producing gynecomastia, and it can interfere with adrenal corticosteroid synthesis [6].

Hamycin is a heptaene antifungal antibiotic produced by *Streptomyces pimprina* Thirum [7]. It has potent in vitro antifungal activity against a wide range of pathogenic fungi and has therapeutic efficacy in mice infected with a variety of yeast and yeast-like and filamentous fungi such as *C. albicans*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Blastomyces dermatidis*, and *Aspergillus niger* [8]. It has been successfully used in the treatment of oral thrush [9], several other clinical forms of candidiasis as well as various superficial and deep seated mycoses [10-13]. Studies on the interaction of hamycin with yeast cells as well as other filamentous fungi...
have demonstrated that hamycin selectively binds to cell wall ergosterol of *C. albicans* and other susceptible yeasts. Therefore, it was believed that the cell wall ergosterol or other components and drug interaction may be essential for hamycin to exert its antifungal action [8]. Hamycin has been shown to increase susceptibility of *C. albicans* to phagocytosis by murine macrophages [14]. Recently aerosolised liposomal hamycin has been shown effective for the treatment of systemic *Candida* infections in mice [15].

The purposes of the present study were to compare the efficacy of oral hamycin with that of amphotericin B in the treatment of established systemic *Candida* infections in normal and diabetic rats, and to compare the efficacy of twice daily, high-dose oral hamycin with that of amphotericin B in normal rats with candidiasis.

2. Materials and methods

2.1. Chemicals

Hamycin produced in our laboratory by submerged fermentation by *S. pimprina* (Lot No. 23/97) and duly authenticated by Quality Control Laboratory of H.A. Ltd., Pimpri, Pune was used. Hamycin is a yellow amorphous powder with [α]D25 +216, UV max (80% methanol): 382 nm (ε1% 1cm 916) [16]. Hamycin solution was prepared initially in dimethyl sulfoxide (DMSO), diluted to 10 000 µg ml⁻¹ in 60% alcohol, and further diluted to a concentration of 1000 µg ml⁻¹ in phosphate buffer saline. Endotoxin contamination was less than 1 ng per g of antibiotic as measured by the Limulus ES test (Salesworth India Pvt. Ltd., Bangalore, India). Amphotericin B (Sarabhai Chemicals, Baroda, India) was diluted to a final concentration of 0.3 mg ml⁻¹ with 5% glucose injection i.p. with a pH of 4.6. Streptozotocin (Sigma Chemical Co., St. Louis, MO, USA) was prepared fresh as a 3% solution in 0.1 M citrate buffer (pH = 4.5) and was stored on ice during the injection procedures. Protamine zinc insulin (protamine, zinc, and Iltein I; U-40; Eli Lilly and Co., Indianapolis, IN, USA) was also diluted daily with sterile normal saline to a final concentration of 0.001 U ml⁻¹.

2.2. Animals and inoculum

Male albino rats of Hindustan Antibiotics (HA) strain weighing 150–200 g were used. Rats were housed eight per cage with rice husk bedding and maintained at constant temperature (24 ± 1°C), relative humidity (30–70%) on a 10 h/14 h: light/dark cycle. The HA diet and water ad libitum were provided until the start of the feeding protocol.

Mice were inoculated intraperitoneally with 0.2 ml of 10⁵ colony forming units (CFU) ml⁻¹ suspension of *C. albicans* (day 0). Verification of the concentration was done using serial Sabouraud dextrose pour plates of 10-fold dilutions of the original suspension (10⁶ CFU ml⁻¹, incubating it at 37°C for 48 h, and counting the CFU ml⁻¹). Inoculum was prepared by serially diluting the original suspension with sterile normal saline.

2.3. Induction of diabetes

In the diabetic rat trial, diabetes was induced in overnight fasted rats by intraperitoneal injection of streptozotocin (65 mg kg⁻¹) 4 days prior to the *C. albicans* inoculation. Urine glucose and ketone concentrations were monitored daily by using Combur 9 BM test (Boehringer Mannheim, Germany) reagent strips. Glucose levels in serum were determined at the time of killing by using a glucose oxidase assay. During the course of diabetic trial, the severity of the diabetes was controlled (urine glucose level 1000 mg dl⁻¹ and urine ketone level 80 mg kg⁻¹) by using regular insulin and protamine zinc insulin. Regular insulin (0.2 to 0.4 U) was given daily to each rat that appeared lethargic or moribund or had lost >15 g of weight per day. Rats with serum glucose level of >250 mg dl⁻¹ at the time of killing were used in the experiment.

2.4. Treatment regimens

Drug therapy was initiated 4 days after inoculation with *C. albicans*. Amphotericin B was given by daily intraperitoneal injection of 1.0 mg kg⁻¹ of body weight. Hamycin was given once a day by gavage with a blunt metal cannula at 10 mg kg⁻¹ per day in the non-diabetic rat trial and at 20 mg kg⁻¹ day⁻¹ in the diabetic rat trial. Control rats received...
3.3 ml of distilled water-glucose solution per kg intraperitoneally. Courses of therapy of 1 and 3 weeks were used for both normal and diabetic rats. One group of animals receiving each drug regimen was killed 28 days after they finished a 21-day course of therapy, to study relapse after treatment. In the high-dose hamycin experiment, normal rats received 25 mg kg\(^{-1}\) twice a day for 7 days.

2.5. Quantification of Candida in organs and statistical analysis

To quantitate Candida organism in organs, both kidneys and liver were removed, rinsed of any adhering blood, and homogenised in 5 ml of phosphate buffer saline, pH 7.0. Serial 200-fold dilutions of the homogenates were plated (at 0.1 ml) on petri dishes containing Sabouraud’s agar, inverted and incubated for 48 h at 37°C.

The CFU per gram of tissue for each kidney and the liver from each rat was converted to log\(_{10}\) value for statistical manipulation. The Fisher exact test was used to compare the numbers of animals in each treatment group in an experiment which had completely negative kidney and liver cultures. A \(P\) value of \(< 0.05\) was considered to be statistically significant.

2.6. In vitro susceptibility testing

In vitro susceptibility studies were done by a modification of the broth macrodilution methods described previously [17]. Amphotericin B and hamycin were tested in Antibiotic Medium 3 (Difco). Amphotericin B solution was prepared by dissolving the stabilising desoxycholate suspension in 100% dimethyl sulfoxide to yield a stock solution concentration of 5000 \(\mu\)g kg\(^{-1}\). Further two-fold serial dilutions were accomplished in Antibiotic Medium 3 to prepared final test concentrations ranging from 100 to 0.05 \(\mu\)g ml\(^{-1}\). The test concentrations were placed in 1-ml disposable tubes in 1-ml volumes and were prepared fresh and used the same day. A total of 5 mg of hamycin was dissolved in 5 ml water, resulting in a stock concentration of 5000 \(\mu\)g ml\(^{-1}\). The antimycotic was further serially diluted to achieve test concentrations ranging from 100 to 0.05 \(\mu\)g ml\(^{-1}\).

A control organism, Saccharomyces cerevisiae, and the isolate of C. albicans used for the infection studies were prepared for susceptibility testing by growing them on Sabouraud dextrose agar slants at 30°C for 48 h. A loopful of the organism to be tested was removed from the overnight slant and suspended in 5 ml of sterile saline. The saline suspension was adjusted to provide a reading of 95% transmittance when it was measured in a spectrophotometer set at 530 nm. This reading corresponded to approximately \(10^6\) CFU ml\(^{-1}\) and was verified by plate counts. The test medium was inoculated into 12 test tubes at concentrations ranging from 100 to 0.05 \(\mu\)g ml\(^{-1}\) in duplicate by using 1-ml pipettes. MICs were determined visually at the time that growth became turbid in the growth control tubes, usually at 48 h. The MIC was defined as the lowest concentration of antifungal agent which inhibited clearly visible growth.

3. Results and discussion

The model of candidiasis in rats used in the present experiment is one of a subacute systemic infection that is usually well tolerated by the animals for several weeks but is not cleared spontaneously. This model, with treatment delayed for several days, mimics human infection. This is unlike mouse lethality studies, in which therapy is initiated concomittant with injection of an otherwise lethal inoculum. In the present study hamycin in the oral form for the advantage of ease of administration was compared with that of amphotericin B. In the 7-day treatment trial, hamycin at 10 mg kg\(^{-1}\) per day, appeared to be as effective as amphotericin B. In the 21-day treatment trial at same dose, however, hamycin was as effective as amphotericin B for the liver infection but was less effective for the kidney infection. In the 21-day treatment trial designed to study relapse and cure, in which a 28-day convalescence was incorporated after the last dose, the efficacy of the two drugs was similar (Table 1). In these animals, which had well-established visceral infections, both hamycin and amphotericin B treatments produced two of five rats with microbiological cures, which was not significant with the sample size. These data suggest that in this model hamycin may be as effective as amphotericin
B and that higher total doses and longer treatment may be required for a cure with either drug.

In the diabetic rat experiments, a dose of 20 mg kg\(^{-1}\) day\(^{-1}\) was used because of the severe infection in these animals, even at the lower inoculum of \(C.\) albicans \((10^4 \text{ CFU ml}^{-1})\). At 7 days of therapy, amphotericin B and hamycin were similar in efficacy. In the diabetic rat experiment with 21 days of hamycin therapy given 20 mg kg\(^{-1}\) day\(^{-1}\), both amphotericin B and hamycin treated rats had reduced Candida titres in the liver compared with the titre in the controls. There was no difference between rats in the antifungal treatment groups in this experiment. Although both drugs also significantly reduced Candida titres in the kidneys, amphotericin B was more effective than hamycin (Table 2). Glucose concentrations measured in serum at the time of killing were similar for all groups of rats in the diabetic rat trials (amphotericin B group, 575 ± 100 mg dl\(^{-1}\) (standard deviation); hamycin group, 560 ± 120 mg dl\(^{-1}\); control group, 590 ± 110 mg dl\(^{-1}\)). The severity of diabetes plus the progressive nature of the candidiasis in this model may explain the fact that few rats had negative cultures even after 21 days of therapy with either drug.

The half-life of hamycin in rats is about 6 h. Hamycin at a dose of 25 mg kg\(^{-1}\) twice daily, had efficacy similar to that of amphotericin B in the liver, but amphotericin B was still better in the kidney. Treatment with hamycin at 50 mg kg\(^{-1}\) per day ver-

### Table 1

Hamycin (10 mg kg\(^{-1}\) day\(^{-1}\)) in candidiasis in normal rats

<table>
<thead>
<tr>
<th>Drug treatment (no. of rats)</th>
<th>Duration of treatment</th>
<th>Mean log(_{10}) CFU g(^{-1}) of tissue ± S.E.</th>
<th>Liver</th>
<th>Kidneys(^{c})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10)</td>
<td>1.55 ± 0.60</td>
<td>3.00 ± 0.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphotericin B (9)</td>
<td>7 days</td>
<td>0.40 ± 0.25*</td>
<td>0.85 ± 0.32*</td>
<td></td>
</tr>
<tr>
<td>Hamycin (9)</td>
<td>7 days</td>
<td>0.65 ± 0.30*</td>
<td>1.30 ± 0.35*</td>
<td></td>
</tr>
<tr>
<td>Control (8)</td>
<td>3.20 ± 0.25</td>
<td>5.30 ± 0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphotericin B (8)</td>
<td>21 days(^{a})</td>
<td>0.20 ± 0.25**</td>
<td>0.24 ± 0.15**</td>
<td></td>
</tr>
<tr>
<td>Hamycin (8)</td>
<td>21 days(^{a})</td>
<td>0.22 ± 0.20**</td>
<td>2.10 ± 0.55*</td>
<td></td>
</tr>
<tr>
<td>Control (5)</td>
<td>3.45 ± 0.45</td>
<td>4.55 ± 0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphotericin B (5)</td>
<td>21 days(^{a})</td>
<td>0.96 ± 0.35**</td>
<td>0.50 ± 0.36**</td>
<td></td>
</tr>
<tr>
<td>Hamycin (4)</td>
<td>21 days(^{a})</td>
<td>0.60 ± 0.25**</td>
<td>0.89 ± 0.30**</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\)Animals were killed at 4 days post-treatment.

\(^{b}\)Animals were killed at 28 days post-treatment.

\(^{c}\)Mean for right and left kidneys.

\(^{*}\)\(P < 0.05\) when compared with respective control groups.

\(^{**}\)\(P < 0.01\) when compared with respective control groups.

### Table 2

Experiment with diabetic rats treated with hamycin at 20 mg kg\(^{-1}\) day\(^{-1}\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Mean log(_{10}) CFU g(^{-1}) of tissue</th>
<th>Liver</th>
<th>Kidney(^{a})</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>3.20 ± 0.30</td>
<td>4.40 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>12</td>
<td>1.80 ± 0.25*</td>
<td>3.35 ± 0.40</td>
<td></td>
</tr>
<tr>
<td>Hamycin</td>
<td>15</td>
<td>2.10 ± 0.30*</td>
<td>3.40 ± 0.44</td>
<td></td>
</tr>
<tr>
<td>21 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>5.96 ± 0.20</td>
<td>5.55 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>15</td>
<td>1.30 ± 0.20**</td>
<td>0.92 ± 0.88**</td>
<td></td>
</tr>
<tr>
<td>Hamycin</td>
<td>14</td>
<td>2.50 ± 0.15**</td>
<td>2.80 ± 0.60**</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\)Mean for right and left kidneys.

\(^{*}\)\(P < 0.05\) when compared with respective control groups.

\(^{**}\)\(P < 0.001\) when compared with respective control groups.
sus that at 10 mg kg$^{-1}$ per day resulted in similar titres in the kidneys and livers after 7 days (Table 3). It is possible that with the isolate of *C. albicans* used in the present study, the lower dose of hamycin would be sufficient for efficacy in the rat, with higher doses conferring no advantage. Larger trials and a dose response curve in this model might demonstrate an optimal dose.

In most of our experiments, amphotericin B was superior to hamycin in the treatment of infection in the kidneys. This may be due to the severity of the *Candida* infection in the rat kidney and the propensity of this organ to involvement with *C. albicans* when inoculated hematogenously [18]. Also, hamycin may demonstrate fungistatic activity even at high doses, while amphotericin B is fungicidal.

The MICs for the *C. albicans* used were 0.22 µg ml$^{-1}$ for amphotericin B and 0.16 µg ml$^{-1}$ for hamycin. MIC of hamycin when tested in synthetic amino acid medium, fungal did not correlate with the efficacy of the drug in our model against the isolate of *C. albicans* that has been used. When complex media are used for in vitro testing of hamycin, the MIC results may be falsely high and may not correlate with clinical efficacy. This may be due to the inhibitory effect of substances contained in complex media. Furthermore, in vitro susceptibility testing of hamycin is troublesome and difficult to reproduce even when the same growth medium is used. In the present study, hamycin testing with a defined medium, the MIC was still high, emphasising the continued problem of discrepancies between the in vitro and the in vivo susceptibilities of *C. albicans* to hamycin. At present, in vivo animal models of fungal infections appear to be the best method of assessing the relative antifungal effectiveness of the drug before use in humans [19].

Hamycin has excellent activity against *C. albicans* in this animal model that is only slightly inferior to that of amphotericin B. The major advantages of the use of hamycin in humans include oral administration and the low toxicity profile found in the studies that have been done to date. A longer duration of therapy or combination therapy with other antimicrobial agents may be required to achieve mycological cure of established renal or hepatic candidiasis.

### Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean log$_{10}$ CFU g$^{-1}$ of tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Control</td>
<td>3.20 ± 0.40</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.65 ± 0.25*</td>
</tr>
<tr>
<td>Hamycin</td>
<td>0.90 ± 0.40*</td>
</tr>
<tr>
<td></td>
<td>Kidney$^a$</td>
</tr>
<tr>
<td>Control</td>
<td>5.00 ± 0.50</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.60 ± 0.40*</td>
</tr>
<tr>
<td>Hamycin</td>
<td>1.15 ± 0.44**</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E. of 10 observations.

$^a$Mean for right and left kidneys.

$^*P<0.001$ when compared with respective control groups.

$^{**}P<0.05$ when compared with respective control groups.

### References


