Comparative chemotherapeutic efficacy of Balhimycin, Desgluco-balhimycin against experimental MSSA and MRSA infection in mice

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Balhimycin and desglucobalhimycin are glycopeptide antibiotics isolated from an Amycolatopsis spp during the search for novel antibacterials against MRSA from the natural product screening at the Research Centre of formerly Hoechst India Ltd. in Bombay, India. Both compounds show excellent in vitro activity against methicillin sensitive and resistant Staphylococci aureus (MSSA, MRSA). Both compounds were also found to be active against a number of MRSA strains in the animal studies. The activities were comparable to that of the reference glycopeptides vancomycin and teicoplanin used in these studies. Teicoplanin displayed better in vivo efficacy against S. epidermidis 4929H and Streptococcus pyogenes A77 than either vancomycin or desgu-ro-balhimycin in the present study. Preliminary studies on pharmacokinetic and acute toxicity were done to get some idea at the early stage of the investigation about the promise of the compounds for development.

Staphylococcal infections are widespread and are a major clinical problem in hospitalized patients mainly due to methicillin resistant strains. The situation has gradually become more serious with the emergence of vancomycin-resistant Enterococci.

An intermediate vancomycin resistant strain of Staphylococcus aureus (VRSA) was recently reported for the first time in ICAAC at Toronto in 1997. This has made it clear that vancomycin resistance is spreading to other genera as it was presumed earlier. It is therefore, of utmost importance to find out some alternate means of treatment for patients suffering from such infections.

In the course of our search for novel antibiotics against MRSA, Nadkarni et al. reported a glycopeptide, balhimycin isolated from Amycolatopsis sp. (Y-86,21022). In the present communication, chemotherapeutic efficacy of balhimycin and desgluco-balhimycin, an analogue of balhimycin, has been compared with the commercially available glycopeptide antibiotics namely, vancomycin and teicoplanin in murine septicemia model. Experimental infections were carried out using various gram positive cocci either in NMRI mice or in Swiss mice.

Materials and Methods

Antibiotics

Balhimycin (I) was isolated in the Microbiology Department of former Hoechst India Ltd. Desgluco-balhimycin (II) was obtained from the Microbiology Department of Hoechst AG, Frankfurt am Main, Germany (I 90 3325, Ch. No.V 1715). Vancomycin HCI and teicoplanin were made available from commercial sources.

In vitro sensitivity testing

The sensitivity of the strains of bacteria was determined by the agar dilution test using Mueller-Hilton agar medium (Difco) as reported earlier. Serial dilutions of the test compounds were made in agar plates and 5 x 10^3 cfu of a log phase culture of the pathogens concerned were inoculated with a Denley multipoint inoculator. The minimum inhibitory concentrations (MICs) of the antibiotics were considered as the lowest concentration that suppressed visible growth after 24 hr of incubation at 37°C. Incubation time for the methicillin-resistant Staphylococci was 48 hr at 30°C. In the case of the Streptococcus pyogenes Mueller-Hilton agar was supplemented with 10% horse blood.

Microorganisms

For the in vitro as well as in vivo studies of both balhimycin and desgluco-balhimycin one sensitive S. Aureus SG 511 (MSSA) and five methicillin resistant...
S. aureus (MRSA) namely, E705, E706, 710, E 712 and C31153 (also resistant to Cephalexin) were used. For similar studies with desgluco-balhimycin one sensitive S. aureus Giorgio and an additional MRSA E703 were also used. Besides the compound’s effect was also evaluated against Staphylococcus epidermidis 4929 H and Streptococcus pyogenes A77 in NMRI mice.

Experimental infection
For the chemotherapeutic evaluation of balhimycin and desgluco-balhimycin, both Swiss and NMRI mice, weighing 18-22g, were infected intraperitoneally with 0.3 ml suspension of the selected bacterium in 5% hog gastric mucin.

In all the experiments eight mice of either sex were used. Mice received standard food and housing with ad libitum drinking water during the course of the experiments.

Preparation of the inoculum
Challenge inoculum was prepared from freshly passaged organisms. The animals were tested at four different dilution’s of the pathogen after obtaining their respective O.D. by a spectrophotometer. The colony forming units/ml (c f u/ml) of the original culture was calculated to determine the number of c f u/ml given to each mouse. The dilution where 100% of the mice were killed was taken as LD100 O.D. of such inoculum was always found to be high in case of Staphylococcus aureus and varied between 2-4 times but there was no logarithmic difference. In NMRI mice challenge inoculum contained 4-8 times the LD100 depending upon the virulence of the infecting organism. Whereas the challenge inoculum for balhimycin and desgluco-balhimycin in Swiss mice was 1 MLD. Mortality of the infected mice occurred between 14 and 48 hr post infection depending upon the type of bacteria used.
The standard antibiotics used in the present study were vancomycin and teicoplanin. These antibiotics could protect 100% of the infected mice after the challenge inoculum was administered to them. The Median Effective Dose where 50% of the treated mice survived (ED50) was calculated for purpose of comparison with the standard drugs and the new glycopeptides under study. At least three different doses were taken for each pathogen for determination and the experiment was repeated to check the reproducibility. Details of the method of infection was earlier published by Klesel et al.5 and Chatterjee et al.6

Antibiotic treatment was given immediately after the challenge by the sub-cutaneous route. A second dose was administered 4 hr later and the third dose was 24 hr post infection. At least 8 mice were used for each dose. Similar number of mice was kept as infected untreated controls. Survival of the mice was recorded. The median effective doses (ED50 mg/kg x 3 doses) were calculated by the probit method from the number of surviving mice on day 10. The antibiotics were tested in vitro in parallel against each test strain.

**Early pharmacokinetics: Concentration of balhimycin, desgluco-balhimycin, vancomycin and teicoplanin in blood**

To study the blood level for each compound, a group of six NMRI mice and six Sprague Dawley rats, in the weight range of 18-22 g and 190-220 g respectively, were considered. Each mouse received a single injection of 40 mg/kg and each rat of 20 mg/kg by a caudal vein. 10 µl of blood was removed from a cut on the tip of the tail by a capillary tube (Wiretrol, mfr.: Drumond, Broomall, U.S.A.), wetted with sodium citrate, from each mouse or rat at various time intervals between 10 and 240 min. Until 60 min blood was collected at an interval of 10 min and thereafter, each hour unto 4 hr. The samples were frozen at -40°C for conducting bioassay.

Concentrations of the antibiotics in blood were determined microbiologically by agar diffusion method. The culture media used for the reference strains were Mueller-Hinton broth with 1.9% agar supplemented with 10% sheep’s blood for *Streptococcus pyogenes* A 77 and only Mueller-Hinton broth with 1.9% agar for *Micrococcus luteus* ATCC 9341. Details of the method was followed after Klesel et al.7

**Preliminary toxicity studies**

Acute toxicity test was conducted in Charles Foster (C F) rats having 100 g body weight. 4 male and 4 female rats were administered intravenously with the test compounds. Highest dose administered was 1000 mg/kg body weight in 1.2 ml volume of a 4% mannitol solution in 4 equal installments within 2 hr. Rats in the control group received 4% mannitol under similar conditions.

**Results**

**Antibacterial activity in vitro**

Balhimycin and desgluco-balhimycin show similar MIC values against different sensitive strains of *S. aureus* (Tables 1 and 2) whereas vancomycin HCI shows a one step higher MIC value. Teicoplanin has a MIC similar to desglucobalhimycin against most of the sensitive strains. Both balhimycin and desgluco-balhimycin show superior MIC values when compared to vancomycin or teicoplanin against most of the methicillin resistant *S. aureus* strains (MRSA) such as, E703, E705, E706, E710 and E712. Both *Staphylococcus epidermidis* 4929H and *Streptococcus pyogenes* A77 were more sensitive to teicoplanin than the other antibiotics used in the study.

<table>
<thead>
<tr>
<th>Test strain</th>
<th>Challenge dose cfu/mouse</th>
<th>Balhimycin MIC</th>
<th>Balhimycin ED50</th>
<th>Desgluco-balhimycin MIC</th>
<th>Desgluco-balhimycin ED50</th>
<th>Vancomycin MIC</th>
<th>Vancomycin ED50</th>
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</thead>
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<tr>
<td><em>S. aureus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SG 511</td>
<td></td>
<td>1.75 x 10⁶</td>
<td>0.39</td>
<td>6.4</td>
<td>0.39</td>
<td>7.1</td>
<td>0.78</td>
</tr>
<tr>
<td>E 705</td>
<td></td>
<td>3.6 x 10⁶</td>
<td>0.195</td>
<td>6.2</td>
<td>0.195</td>
<td>&lt;6.2</td>
<td>0.39</td>
</tr>
<tr>
<td>E 706</td>
<td></td>
<td>5.3 x 10⁶</td>
<td>0.78</td>
<td>7.8</td>
<td>0.78</td>
<td>9.7</td>
<td>1.56</td>
</tr>
<tr>
<td>E 710</td>
<td></td>
<td>1.0 x 10⁹</td>
<td>0.39</td>
<td>13.0</td>
<td>0.39</td>
<td>13.8</td>
<td>0.78</td>
</tr>
<tr>
<td>E 712</td>
<td></td>
<td>8.8 x 10⁸</td>
<td>0.78</td>
<td>6.2</td>
<td>0.78</td>
<td>&lt;6.2</td>
<td>0.78</td>
</tr>
<tr>
<td>C31153</td>
<td></td>
<td>2.0 x 10⁶</td>
<td>0.78</td>
<td>9.5</td>
<td>0.39</td>
<td>15.1</td>
<td>0.78</td>
</tr>
</tbody>
</table>

- Methicillin-resistant strain
- Cephalxin and Methicillin-resistant strain
Table 2—Minimum Inhibitory Concentration [MIC, mg/L] and Median Effective Dose [ED₅₀, mg/kg] Comparative activity of desgluco-balhimycin, vancomycin and teicoplanin in experimentally induced gram-positive infection in NMRI mice

<table>
<thead>
<tr>
<th>Test strain</th>
<th>Challenge dose cfu/mouse</th>
<th>Desgluco-balhimycin MIC</th>
<th>ED₅₀</th>
<th>Vancomycin MIC</th>
<th>ED₅₀</th>
<th>Teicoplanin MIC</th>
<th>ED₅₀</th>
</tr>
</thead>
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<tr>
<td>S. aureus Giorgio</td>
<td>1.0 x 10⁶</td>
<td>0.25</td>
<td>2.13</td>
<td>0.5</td>
<td>2.12</td>
<td>0.5</td>
<td>2.03</td>
</tr>
<tr>
<td>S. aureus SG 511</td>
<td>1.0 x 10⁶</td>
<td>0.25</td>
<td>3.20</td>
<td>0.5</td>
<td>2.66</td>
<td>0.25</td>
<td>2.93</td>
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<tr>
<td>S. aureus 108</td>
<td>5.0 x 10⁶</td>
<td>0.25</td>
<td>4.97</td>
<td>0.5</td>
<td>2.23</td>
<td>0.25</td>
<td>4.78</td>
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<tr>
<td>S. aureus 1806</td>
<td>1.7 x 10⁵</td>
<td>0.25</td>
<td>3.38</td>
<td>0.5</td>
<td>4.00</td>
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<td>S. aureus Kn 4</td>
<td>1.7 x 10⁵</td>
<td>0.25</td>
<td>3.28</td>
<td>0.5</td>
<td>3.04</td>
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<td>S. aureus E 703</td>
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<td>0.25</td>
<td>5.11</td>
<td>0.5</td>
<td>6.38</td>
<td>0.5</td>
<td>8.91</td>
</tr>
<tr>
<td>S. aureus E 705</td>
<td>5.3 x 10⁵</td>
<td>0.25</td>
<td>8.12</td>
<td>0.5</td>
<td>4.66</td>
<td>0.5</td>
<td>8.09</td>
</tr>
<tr>
<td>S. aureus E 710</td>
<td>5.5 x 10⁵</td>
<td>5.0</td>
<td>7.91</td>
<td>0.5</td>
<td>4.08</td>
<td>1.0</td>
<td>13.15</td>
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<tr>
<td>S. epidermidis 4929H*</td>
<td>2.3 x 10⁶</td>
<td>0.125</td>
<td>0.29</td>
<td>0.25</td>
<td>0.66</td>
<td>0.03</td>
<td>0.18</td>
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<td>S. pyogenes A 77</td>
<td>2.0 x 10⁷</td>
<td>0.125</td>
<td>0.74</td>
<td>0.25</td>
<td>0.78</td>
<td>0.008</td>
<td>0.11</td>
</tr>
</tbody>
</table>

△Ampicillin-resistant strain
●Methicillin-resistant strain
◆Unusually-sensitive to Teicoplanin

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**Graphs:**

**Graph 1:**
- Vancomycin
- Teicoplanin
- Balhimycin
- Desgluco-balhimycin

**Graph 2:**
- Time (hours) on the x-axis
- Concentration (mg/l) on the y-axis
Antibacterial activity in vivo

In Swiss mice infected with sensitive *S. aureus* SG 511, the ED\(_{50}\) of both balhimycin and desgluco-balhimycin are comparable (Table 1) with vancomycin. Similar observation was made when Swiss mice were infected with MRSA strains, except for *S. aureus* C31153 strain where desgluco-balhimycin was less active as compared to balhimycin and vancomycin.

In NMRI mice the chemotherapeutic effect of desgluco-balhimycin was studied and a comparison was made with vancomycin and teicoplanin. ED\(_{50}\) values of all the aforesaid antibiotics were very close to each other, vancomycin being marginally more active against two strains (Table 2). Against MRSA strains vancomycin displayed superior activity to either desgluco-balhimycin or teicoplanin (Table 2).

Mice infected with *Staphylococcus epidermidis* 4929H and *Streptococcus pyogenes* A77 showed better ED\(_{50}\) values with teicoplanin than either vancomycin or desgluco-balhimycin.

In the present study, it has been observed that the required size of the challenge inoculum varies in the two strains of mice. Swiss mice required more infecting organisms to get 100% mortality.

Pharmacokinetic studies in mice and rats

In mice after single intravenous injection of 40 mg/kg, achievable blood level was highest for teicoplanin (90 mg/L) after 10 min, followed by desgluco-balhimycin (40 mg/L). Both balhimycin and vancomycin displayed almost similar concentrations (~28 and 30 mg/L respectively). Teicoplanin maintained a concentration of 60mg/L up to 90 min and blood level of desgluco-balhimycin gradually decreased to 17 mg/L during the same period. Balhimycin and vancomycin had reached ~12 mg/L concentration in 60 min (Fig. 1 A).

Blood levels of the aforesaid compounds in rats after single administration at 20 mg/kg has been presented (Fig. 1 B). Teicoplanin showed peak blood level of 50 mg/L in 10 min, followed by desgluco-balhimycin at 22 mg/L, whereas balhimycin and vancomycin displayed similar concentrations, ~18 mg/L. Teicoplanin continued to show uniform blood level up to 4 hr between 30 mg and ~18 mg/L. During 20 min to 3hr both desgluco-balhimycin and balhimycin had similar blood levels which remained between 15 mg and 8mg/L and 15 mg and 6.5 mg/L respectively. The blood levels of vancomycin was ~16 to 7.5 mg/L during the same period.

Acute toxicity

No mortality was observed after treatment with 1000 mg/kg, i.v. and gain in body weight was comparable with the rats of the untreated control group. Major organs such as, liver, kidneys, spleen, heart, lung, stomach, intestine and brain did not shown any morphological abnormalities at the time of autopsy after 14 days observation.

Discussion

In *in vitro* studies balhimycin, desgluco-balhimycin and teicoplanin displayed similar activities against methicillin sensitive *Staphylococcus aureus* (MSSA) strains. They were one step better than vancomycin. Similarly both balhimycin and desgluco-balhimycin were one step superior to either teicoplanin or vancomycin against different methicillin resistant *Staphylococcus aureus* (MRSA) used in this study. Teicoplanin was found to be superior against *Staphylococcus epidermidis* 4929H and *Streptococcus pyogenes* A77.

In the animal protection studies against MRSA infections in Swiss mice all the three glycopeptides showed comparable efficacy with the exception of C31153 where vancomycin was found to be better than desgluco-balhimycin (Table 1). Against MSSA SG511 *in vivo* efficacy of all the glycopeptides tested was comparable.

In the protection test using NMRI mice, vancomycin was better against two MRSA strains but against E703 the ED\(_{50}\) values of all the three glycopeptides were comparable (Table 2). Against the methicillin sensitive strains (MSSA) teicoplanin showed better activity than either vancomycin or desgluco-balhimycin in 5 of the 7 strains tested.

The preliminary pharmacokinetic data show that both in mice and rats teicoplanin has much higher peak concentration in blood than either balhimycin, desgluco-balhimycin or vancomycin. Also teicoplanin maintains high blood levels for 90 min while other glycopeptides reached much lower blood levels.

Blood levels of balhimycin and desgluco-balhimycin were comparable to vancomycin in rats. Although teicoplanin had displayed superior bioavailability, the other glycopeptides were above their MIC levels in the present study.

Preliminary acute toxicity results indicate that rats can tolerate a high dose of intravenous administration of balhimycin without showing any adverse effects.

In conclusion balhimycin shows significant activity against gram-positive bacteria and is comparable with
the standard drugs, especially against MRSA. Desgluco-balhimycin, one of the analogues of balhimycin, displays better bioavailability than the parent compound.

Balhimycin and desglucobalhimycin are novel glycopeptides possessing the dehydrovancosamine moiety in their structure and the biological activities shown in these studies make them attractive lead compounds for semi-synthetic work.

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References