ABSTRACT

Background: The emergence of small drug resistant MRSA strains with reduced vancomycin susceptibility has intensified the search for alternative therapies against these difficult-to-treat pathogens.

Methods: MIC values for WCK 771 A, Levofloxacin, Minocycline, Tobra, Cipro, Neomycin, Lincomycin, and Oxacillin were determined against 175 clinical isolates comprising of MRSA (99), MSSA (23), MRSA (73) and MSSA (20) by NCCLS recommended agar dilution method. Bactericidal kinetics up to 24 h (50% killing of 10^6 CFU/ml) was evaluated against MRSA 032, 034, 097 and 5203 strains at 3 x 10^-8 mg/ml in Mueller Hinton Broth (MHB) employing Moxi and Tobra as comparators. Bactericidal activity of WCK 771 A at 100x higher inoculum (10^8 CFU/ml) against MRSA 032 and 097 was evaluated in MHB and compared with Moxi (both at 3 x 10^-8 mg/ml) up to 8 h exposure.

Results: At 0.1, 0.16 mg/ml WCK 771 A and other comparators inhibited (at 24 h) following percentage of strains:

- WCK 771 A: 3.12 mg/ml was active against 4 MRSA strains, however, Moxi and Tobra were bacteriocidal (≤ 0.5 mg/ml). With high inoculum MRSA 032 and 097 cultures, WCK 771 A exhibited idiobiotic effect by lowering CFU by 3 kg, whereas Moxi failed to show effect under similar condition.

Conclusion: The excellent in vitro potency of WCK 771 A coupled with its potential to inhibit MRSA targeting its development for the therapy of difficult-to-treat infections caused by quinolone resistant MRSA.

INTRODUCTION

Current anti-MRSA agents are either bacteriostatic (tobramycin) or slow killers (vancomycin). In vitro exposure for 3 to 4 h of staphylococcal bacteria with improved idiosyncrasies may provide superior eradication rates in clinical settings. Generally, the clinical potencies of antibiotics are assessed at a cell count of 10^6 CFU/ml as recommended by NCCLS. However, in a typical clinical scenario, cell count in patients at the site of infection can reach as high as 10^10 cells/ml. Such high load of organisms may severely compromise the clinical potency of antibiotic agents. From this angle the potency of WCK 771 A was evaluated in comparison with other potent anti-staphylococcal agents and its clinical potential was assessed at normal inoculum as well as at 100x higher cell density in comparison with moxifloxacin.

MATERIALS & METHODS

Bacterial strains: The staphylococcal strains used in the study were obtained from various hospitals from India and USA.

Antibacterial agents: The antibacterial agents were either in-house synthesised at Wockhardt Research Center, or isolated from their respective pharmaceutical preparation.

MIC determination:
- At 0.8 mg/ml, WCK 771 A inhibited 130 strains out of 175 strains tested (Table 1). At this concentration, Sita and Clinia showed better potency by inhibiting 161 and 156 strains respectively. However, at 1.56 mg/ml, WCK 771 A was the most potent agent showing inhibition of 171 strains followed by Clinia > Sita > Vanco (168 strains), Vanco (168 strains), Moxi > Tobra (151 strains). Levofloxacin (91 strains). Imp > (85 strains), Clinia > Tobra (74 strains), and Sita (56 strains).

The high anti-staphylococcal potency of WCK 771 A appears to be due to its ability to overcome bound macrolide efflux (as shown in the poster F-537) and also due to its ability to recognize mutated FQ targets viz, DNA gyrase and topoisomerase IV. This is evident from the significant differences in percentage of strains inhibited by other FQs such as Levofloxacin on the one hand and WCK 771 A, Sita and Clinia on the other hand.

RESULTS & DISCUSSION

The bactericidal activity of WCK 771 A against MRSA 032, 034, 097 and 5203 strains was studied in cell adjusted Mueller Hinton broth at initial inoculum of 10^8 CFU/ml. Plates were inoculated in shaking condition at 35°C and aliquots were drawn at different intervals of time to determine CFU. A graph of Log CFU/ml against time in hours was plotted (Figs. 1-4).

High cell density cidal potential:
- Moxi kinetics of WCK 771 A and moxifloxacin at 5 mg/ml was evaluated against MRSA 032 and 097 using 100 times higher cell density (10^10 CFU/ml) than what is recommended by NCCLS (i.e., 10^8 CFU/ml). WCK 771 A were monitored for both MRSA strains up to 8 h (Figs. 5, 6).

Table 1: MIC of WCK 771 A and Other Antibiotics for Clinical Isolates of Staphylococcus

<table>
<thead>
<tr>
<th>Agents</th>
<th>≤0.05</th>
<th>≤0.2</th>
<th>≤0.4</th>
<th>≤0.8</th>
<th>≤1.56</th>
</tr>
</thead>
<tbody>
<tr>
<td>WCK 771 A</td>
<td>71</td>
<td>90</td>
<td>99</td>
<td>130</td>
<td>171</td>
</tr>
<tr>
<td>Levof</td>
<td>0</td>
<td>41</td>
<td>69</td>
<td>89</td>
<td>91</td>
</tr>
<tr>
<td>Moxi</td>
<td>10</td>
<td>50</td>
<td>74</td>
<td>74</td>
<td>74</td>
</tr>
<tr>
<td>Clinia</td>
<td>55</td>
<td>71</td>
<td>81</td>
<td>98</td>
<td>115</td>
</tr>
<tr>
<td>Tobra</td>
<td>52</td>
<td>77</td>
<td>82</td>
<td>95</td>
<td>115</td>
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<tr>
<td>Sila</td>
<td>67</td>
<td>88</td>
<td>95</td>
<td>156</td>
<td>168</td>
</tr>
<tr>
<td>Vanco</td>
<td>72</td>
<td>91</td>
<td>102</td>
<td>161</td>
<td>168</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>18</td>
<td>56</td>
</tr>
<tr>
<td>Imp</td>
<td>37</td>
<td>76</td>
<td>79</td>
<td>82</td>
<td>85</td>
</tr>
</tbody>
</table>

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Kil kinetics:
- Kil kinetics study of WCK 771 A against 4 MRSA strains employing Moxi and Tobra as comparators, at a concentration of 3.12 mg/ml. WCK 771 A was most cidal bringing down CFU by 3 kg, Moxi and Tobra, however, by 24 h were either static or even failed to suppress the growth beyond 0 h.

CONCLUSIONS

WCK 771 A is a potent anti-MRSA agent comparable to rifaxoxacin and cinoxacin.

WCK 771 A cidal features are superior to trovafloxacin and moxifloxacin.

WCK 771 A retains cidal effect on high density MRSA cultures.

REFERENCES


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