In vitro activities of cloxyquin (5-chloroquinolin-8-ol) against

*Mycobacterium tuberculosis*

Poonpilas Hongmanee¹*, Kamolchanok Rukseree²,³, Benjamas Buabut¹, Boontiwa Somsri¹,
and Prasit Palittapongarnpim²,³

¹Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, ²Department of
Microbiology, Faculty of Science, Mahidol University, Bangkok, and ³National Science and
Technology Development Agency, Prathumthani, Thailand

* Corresponding author. Poonpilas Hongmanee
Mailing address: Division of Microbiology, Department of Pathology, Faculty of Medicine,
Ramathibodi Hospital, Mahidol University, Rama VI Road, Bangkok 10400, Thailand.
Phone: (662) 02 2011389
Fax: (662) 02 3547266
E-mail: Poonpilas@hotmail.com

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ABSTRACT

The in vitro activities of cloxyquin (5-chloroquinolin-8-ol) against 9 standard strains and 150 clinical isolates of Mycobacterium tuberculosis were studied. The MICs ranged from 0.062 to 0.25 µg/ml. The MIC$_{50}$ and MIC$_{90}$ were 0.125 and 0.25 µg/ml, respectively. These indicate that cloxyquin exhibited good antituberculosis activity, even for multidrug-resistant isolates.

TEXT

Current first-line drugs for treatment of tuberculosis (TB) consists of only 5 agents i.e. isoniazid (INH), rifampin (RIF), ethambutol (EMB), pyrazinamide (PZA), and streptomycin (STR). Resistance to the first-line drugs, especially RIF and INH, usually causes treatment failure and necessitate the use of the second-line drugs with a prolonged period of therapy. Even with that, treatment frequently fails. New antituberculous agents, especially the ones with novel mechanisms of action are urgently required.

Bi-halogenated 8-hydroxyquinolines (quinolin-8-ols) are a group of known drugs with antiamebic activities and were widely used to treat intestinal infection. The commonly used ones include broxyquinoline, clioquinol, chlorquinaldol, and iodoquinol (4, 6). They also exhibit antibacterial and antifungal activities (1, 14).

Herewith, we report the antituberculosis activities of a monohalogenated 8-hydroxyquinoline, cloxyquin (5-chloroquinolin-8-ol) against 150 clinical M. tuberculosis isolates, including multidrug-resistant strains. Cloxyquin (Fig. 1) was known to possess activities against bacteria, fungi and protozoa (3, 10, 11, 12) but the antimycobacterial activity has never been documented.
A total of 159 strains of *M. tuberculosis* including 9 reference strains: H37Rv ATCC 27294, H37Ra ATCC 25177, H37Rv-PAS-R ATCC 35821 (p-aminosalicylic acid-resistant), H37Rv-CS-R ATCC 35826 (cycloserine-resistant), H37Rv-KM-R ATCC 35827 (kanamycin-resistant), H37Rv-PZA-R ATCC 35828 (pyrazinamide-resistant), H37Rv-TAC-R ATCC 35829 (thiacetazone-resistant), H37Rv-ETA-R ATCC 35830 (ethionamide-resistant), and H37Rv-EMB-R ATCC 35837 (ethambutol-resistant) and 150 isolates from pulmonary and extrapulmonary patients in Ramathibodi Hospital, Bangkok, Thailand, including 100 sensitive strains, 20 drug-resistant strains (7, 3, 3, and 12 isolates resistant to INH, RIF, EMB, and STR, respectively), and 30 multidrug-resistant (MDR) strains (7 isolates resistant to INH and RIF, 3 isolates additionally resistant to EMB, 13 isolates additionally resistant to STR, and 7 isolates additionally resistant to EMB and STR), were investigated. The MICs of cloxyquin (Sigma Chemical Co., St. Louis, Mo.) were determined duplicated by microplate Alamar blue assay (MABA) (8), which has been showed to well correlation (>90%) with the BACTEC and the proportional methods (2, 8, 16, 19). Briefly, cloxyquin was prepared in dimethyl sulfoxide (DMSO, Sigma) and subsequently twofold diluted in 100 µl of Middlebrook 7H9GC in clear flat-bottom, 96-well microplates. Mycobacterial suspension was prepared in 0.04% Tween 80, and diluted with sterile distilled water to a turbidity of the McFarland No. 1. The suspension was then diluted 1:50 with 7H9GC and 100 µl was added to the wells, the highest final concentration of DMSO was 0.156% (vol/vol). The plates were incubated at 37°C for 7 days, 12.5 µl of 20% Tween 80 and 20 µl of Alamar Blue (SeroTec Ltd., Oxford, UK) were added to all wells. Growth of the organisms were determined after reincubation at 37°C for 16-24 h by visual determination of a color change from blue to pink. The MIC was defined as the lowest concentration which prevented the color change. RIF and INH (Sigma) were included as controls.
The MICs of 8-hydroxyquinoline, cloxyquin, cloquinol, chlorquinaldol and broxyquinoline against *M. tuberculosis* H37Ra were 0.125, 0.125, 6.25, 0.38 and 6.25 µg/ml, respectively. This suggested that 8-hydroxyquinoline and its derivatives are fairly active against *M. tuberculosis*. To elucidate more on their potentials, MICs of cloxyquin were further studied. The MICs of cloxyquin for the 9 reference strains ranged from 0.125 to 0.25 µg/ml. Similarly the MICs of cloxyquin for 150 clinical isolates, ranged from 0.062 to 0.25 µg/ml. The MIC$_{50}$ and MIC$_{90}$ were 0.125 and 0.25 µg/ml, respectively (Table 1). There were no statistically significant differences of MICs between drug-sensitive, drug-resistant and MDR strains. Nor were there any observable differences in MICs of strains with different antibiotic resistance patterns. The MICs of RIF and INH against *M. tuberculosis* H37Rv were 0.031 and 0.062 µg/ml, respectively.

The fact that cloxyquin is equally active across various mono- and multidrug-resistant clinical isolates suggested that its mechanism of action is not shared by previously known antituberculous drugs. The antimicrobial action of bihalogenated 8-hydroxyquinolines is likely to relate to their chelating activities. It is proposed that the iron chelation deprives the microbes from the essential nutrient. However, the mechanisms may be actually more complex. For example, bihalogenated 8-hydroxyquinolines was found to inhibit the RNA dependent DNA polymerase of respiratory syncytial virus by chelation of copper (17), and inhibit RNA synthesis by chelation of Mn$^{2+}$, Mg$^{2+}$ and Zn$^{2+}$ (9). Moreover, the antibacterial action may be the property of the metal complexes but not the free compounds (13, 17). It had previously been proposed that iodinated 8-hydroxyquinolines worked through the release of free iodine in the intestinal lumen, but some bihalogenated 8-hydroxyquinolines have antimicrobial activities even without containing any iodine. It was proposed later that the iodine residue may play a role in delaying the absorption of the drugs and makes the drugs stay longer in the intestinal lumen (6). Precise mechanisms of action of halogenated 8-
hydroxyquinolines remain to be investigated. There have been a few studies of the antituberculosis activity of quinolines. For example, clioquinol had good activity in guinea pigs, not in mice (14, 18). N-sulfonic acid derivative of 5-hydroxyamino-8-hydroxyquinoline and 8-butoxyquinoline also had good antituberculous activity in guinea pigs. The MICs of 5-nitro-8-hydroxyquinoline and 8-hydroxyquinoline against *M. bovis* BCG were found to be 1.9 and 0.3 µg/ml, respectively. Moreover, both showed moderate cidal activity in the *in vitro* model of dormant *M. bovis* BCG (15). The antituberculous effect of cloxyquin has never been reported. There is no clear information regarding the safety of cloxyquin either. However, clioquinol was reported as a possible cause of subacute myelooptic neuropathy (SMON), an uncommon neurological syndrome that occurred primarily in Japan (4, 7). The cause of the syndrome is, however, far from established, as environmental factors, such as B12 deficiency, are also likely to be important. Nevertheless, recently the interest in clioquinol has been increased due to their favorable effects on Alzheimer’s disease (5, 7). In conclusion, the excellent *in vitro* activity (even for MDRTB) of cloxyquin against *M. tuberculosis* deserves further investigation.

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TABLE 1. The MICs of cloxyquin for clinical isolates of *M. tuberculosis*

<table>
<thead>
<tr>
<th>M. <em>tuberculosis</em> strains (n)</th>
<th>MIC (µg/ml)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.062</td>
<td>0.125</td>
</tr>
<tr>
<td>Drug-sensitive (100)</td>
<td>15 (15%)</td>
<td>74 (74%)</td>
</tr>
<tr>
<td>Drug-resistant (20)</td>
<td>2 (10%)</td>
<td>13 (65%)</td>
</tr>
<tr>
<td>MDR (30)</td>
<td>5 (16.7%)</td>
<td>24 (80%)</td>
</tr>
<tr>
<td>Total (150)</td>
<td>22 (14.7%)</td>
<td>111 (74%)</td>
</tr>
</tbody>
</table>

MIC<sub>50</sub> are 0.125 µg/ml for all groups.

FIG. 1. Chemical structure of cloxyquin