Inhibition of Bacterial Multidrug Resistance by Celecoxib, a Cyclooxygenase-2 Inhibitor

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Multidrug resistance (MDR) is a major problem in the treatment of infectious diseases and cancer. Accumulating evidence suggests that the cyclooxygenase-2 (COX-2)-specific inhibitor celecoxib would not only inhibit COX-2 but also help in the reversal of drug resistance in cancers by inhibiting the MDR1 efflux pump. Here, we demonstrate that celecoxib increases the sensitivity of bacteria to the antibiotics ampicillin, kanamycin, chloramphenicol, and ciprofloxacin by accumulating the drugs inside the cell, thus reversing MDR in bacteria.

Multidrug resistance (MDR) to various antibiotics is exhibited by different bacteria, including staphylococci, enterococci, gonococci, streptococci, salmonellae, mycobacteria, and others. A bacterial pathogen well known for its drug resistance is Staphylococcus aureus (16), which causes a very high global mortality rate among the humans infected. Infection caused by S. aureus, particularly MRSA (methicillin-resistant S. aureus) strains, has been declared problematic in both community and clinical settings. Recently, the emergence of “panresistant” strains of Pseudomonas aeruginosa and Acinetobacter baumannii has posed a serious threat to humankind (8).

There are several mechanisms by which bacteria develop resistance. Mutation in the target genes, altered target sites, changes in the cell wall’s permeability to antibiotics, enzymatic degradation of antibiotics, transfer of resistance (R) plasmids from one bacterium to another, increased active efflux of antibiotics, etc. are some of the mechanisms (8). The active efflux of antibiotics contributes significantly to acquired resistance in bacteria due to their broad-spectrum substrate recognition, expression, and cooperation with other resistance mechanisms (15). In prokaryotes, there are five different families of efflux pumps involved in antibiotic efflux, the (i) major facilitator, (ii) multidrug and toxic efflux, (iii) resistance-nodulation-division, (iv) small multidrug resistance, and (v) ABC (ATP-binding cassette) transporter families.

In human cancers, MDR due to ABC transporters is known to play a major role in resistance to a variety of anticancer agents. The conversion of arachidonic acid to prostaglandins and other eicosanoids is catalyzed by the key enzyme cyclooxygenase (COX). COX has two distinct isoforms, COX-1 and COX-2, which differ in genetic coding (7). Although the two isoforms have similar amino acid sequences and catalytic activities, they were demonstrated to have different functions. COX-1 is constitutive and cytoprotective, while COX-2 is an inducible enzyme in inflamed tissues. Recently, a causal link between COX-2 and MDR1 gene expression, implicated in cancer chemoresistance, has been demonstrated. It has been well documented that COX-2 regulates MDR1 expression in cancers, and the use of celecoxib, a COX-2-specific inhibitor, reversed drug resistance (1, 3, 4, 10, 12).

Bacterial MDR1 (for example, LmRA from L. lactis) is homologous to human MDR1, and the two forms also have overlapping substrate specificities. However, the presence of a COX-2-like gene in bacteria has not been reported and therefore we hypothesize that celecoxib might act through some unknown protein and regulate MDR1 in bacteria. To evaluate this hypothesis, the present study was undertaken. This study included three bacterial strains, S. aureus ATCC 29213, MRSA ATCC 335913, and Mycobacterium smegmatis ATCC MC²155, and four different antibiotics, namely, ampicillin (AMP), kanamycin (KAN), ciprofloxacin (CIP), and chloramphenicol (CHL). The results clearly demonstrated that celecoxib alone is not bactericidal but in combination at lower concentrations helped in increasing the sensitivity of the bacteria to the antibiotics. These effects of celecoxib are probably due to the blockage of MDR transporters that are involved in pumping antibiotics out of bacterial cells.

**Results and discussion.** Resistance to many classes of antibiotics to treat bacterial infections is a major problem worldwide. Efflux pumps have been implicated in the development of multidrug resistance in both Gram-positive and Gram-negative bacteria. Inhibitors of efflux pumps are promising therapeutic agents, as they would help antibiotics to render their

### TABLE 1. MIC determinations

<table>
<thead>
<tr>
<th>Strain</th>
<th>AMP (µg/ml)</th>
<th>KAN (µg/ml)</th>
<th>CHL (µg/ml)</th>
<th>CIP (µg/ml)</th>
<th>Celecoxib (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> ATCC 29213</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>0.25</td>
<td>&gt;100</td>
</tr>
<tr>
<td>MRSA ATCC 335913</td>
<td>200</td>
<td>100</td>
<td>100</td>
<td>0.25</td>
<td>&gt;100</td>
</tr>
<tr>
<td><em>M. smegmatis</em> ATCC</td>
<td>250</td>
<td>0.5</td>
<td>10</td>
<td>0.5</td>
<td>&gt;100</td>
</tr>
<tr>
<td>MC²155</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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effect on bacteria. Combination therapy using an efflux pump inhibitor and an antibiotic has been shown to increase the intracellular concentration of antibiotics, thereby reversing resistance. In humans, it has been well documented that celecoxib inhibits MDR1 via COX-2 inhibition. In this scenario, the present study aimed to investigate the effect of an indirect MDR1 regulator, celecoxib, a cyclooxygenase-2 inhibitor, on the growth and multidrug resistance of *S. aureus*, MRSA, and *M. smegmatis*.

To evaluate the hypothesis, first celecoxib’s effect on the growth of the bacterial strains ATCC 29213, ATCC 335913, and ATCC MC²155 was studied where the bacteria were grown in the presence or absence of different concentrations (10 nM to 100 μM) of celecoxib for 24 or 48 h. The results demonstrated that celecoxib alone was not bactericidal and/or bacteriostatic at concentrations of up to 100 μM to any bacterial strain in this study. Similarly, the MIC of each antibiotic for each individual strain of bacteria was also determined (Table 1) and the two different concentrations at which a minimum or no bactericidal effect of the antibiotic was seen were considered for further experiments. The combined effects of celecoxib and different antibiotics were studied using a broth checkerboard method as described earlier (6). The FIC (fractional inhibitory concentration) index, which demonstrated synergism between celecoxib and the antibiotics used in this study (Table 2), was calculated as described previously (13). However, in combination, celecoxib at lower concentrations (6.25 and 12.5 μM) increased the sensitivity of the bacteria to the antibiotics AMP, KAN, CHL, and CIP significantly (Fig. 1) and the P values were calculated by two-way analysis of variance (Bonferroni posttests) using GraphPad Prism (Table 3).

To further confirm the inhibitory activity of celecoxib on the MDR efflux pump, an ethidium bromide (EtBr) efflux assay was carried out as described previously (2) using *S. aureus*, MRSA, and *M. smegmatis* in the presence of four different antibiotics (AMP, KAN, CHL, and CIP) and two different concentrations of celecoxib (6.25 and 12.5 μM). The increase in the fluorescence intensity of EtBr due its accumulation was used as a measure of MDR efflux pump inhibition. Results of an EtBr efflux assay using a flow cytometer clearly indicated

![FIG. 1. Bar graphs showing the effects of celecoxib in combination with antibiotics (AMP, KAN, CHL, and CIP) on S. aureus (A), MRSA (B), and M. smegmatis (C).](http://aac.asm.org/)

<table>
<thead>
<tr>
<th>Strain</th>
<th>FIC index</th>
<th></th>
<th>AMP</th>
<th>KAN</th>
<th>CHL</th>
<th>CIP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.28</td>
<td>0.53</td>
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</tr>
<tr>
<td>S. aureus ATCC 29213</td>
<td>0.3125</td>
<td>0.3125</td>
<td>0.3125</td>
<td>0.3125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRSA ATCC 335913</td>
<td>0.0665</td>
<td>0.3125</td>
<td>0.5625</td>
<td>0.1875</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. smegmatis ATCC  MC²155</td>
<td>0.0665</td>
<td>0.3125</td>
<td>0.5625</td>
<td>0.1875</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
that celecoxib increased the sensitivity of bacteria to antibiotics by blocking the MDR efflux pump and increasing drug accumulation, as shown by an increase in EtBr fluorescence in the cells (Fig. 2). There was a significant dose-dependent shift in the intensity of the red fluorescence of EtBr inside the cells in the presence of celecoxib. These results are in agreement with the EtBr accumulation assay results obtained in various studies (9, 14).

![FIG. 2. Flow cytometry histograms showing EtBr accumulation in S. aureus (A), MRSA (B), and M. smegmatis (C) treated with antibiotics and celecoxib alone and in combination. Each panel (A, B, or C) has four histograms corresponding to treatments with four different antibiotics (AMP, KAN, CHL, and CIP).](http://aac.asm.org/)
The synergistic effect of celecoxib and antibiotics was not observed at higher concentrations of celecoxib, and this is a subject for future studies.

In conclusion, the present study clearly demonstrated the potent effects of celecoxib in the reversal of multidrug resistance in MRSA and the increased sensitivity of M. smegmatis and S. aureus to antibiotics, suggesting celecoxib to be a novel and potential therapeutic intervention for treating infections with drug-resistant bacteria.

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REFERENCES