EVALUATION OF ANTIMICROBIAL ACTIVITY OF SELECTIVE COX-2 INHIBITOR

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ABSTRACT
The present study was undertaken to evaluate the antimicrobial activity of Etoricoxib. At various concentrations, ranging from 20-100 µg/ml, Etoricoxib exhibits zone of inhibition of 6.5, 7, 8 and 13 respectively against P. aeruginosa while, it showed zone of inhibition of 10, 15, 18 against S. aureus and 8, 15, 21 against E. coli at the concentration range of 25-100 µg/ml. It showed more activity against S. aureus, followed by E. coli, Pseudomonas, and least activity against C. albicans and S. typhi. The MIC of the etoricoxib were ranged between 100 to 200 µg/ml. The MIC values of the three test pathogens S. aureus, E. coli, Pseudomonas were found to have MIC of 100 µg/ml, 150 µg/ml and 200 µg/ml respectively. The results indicate that Etoricoxib exhibit the antimicrobial activity compared to Doxorubicin and Ketoconazole.

Keywords: Etoricoxib, Ketoconazole, P. aeruginosa, S. aureus, E. coli

INTRODUCTION

Staphylococcus aureus is a spherical bacterium, frequently part of the skin flora (as a commensal) found in the nose frequently & in the throat less commonly. About 20% of the populations are long-term carriers of Staphylococcus aureus. Staphylococcus aureus can cause a range of illnesses from minor skin infections, such as pimples, impetigo (may also be caused by Streptococcus pyogenes), furuncles, cellulitis, folliculitis, carbuncles, scalded skin syndrome (very severe) and abscesses to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia and sepsis. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections, often causing postsurgical wound.

Staphylococcal toxins that act on cell membranes include alpha-toxin, beta-toxin, delta-toxin, and several bicomponent toxins. The bicomponent toxin Panton-Valentine leukocidin (PVL) is associated with severe necrotizing pneumonia in children. The genes encoding the components of PVL are encoded on a bacteriophage found in community-associated MRSA strains. The treatment of choice for Staphylococcus aureus infection is penicillin; but in most countries, penicillin-resistance is extremely common and first-line therapy is most commonly a penicillinase-resistant penicillin (for example, oxacillin or flucloxacillin). Combination therapy with gentamicin may be used to treat serious infections like endocarditis but its use is controversial because of the high risk of damage to the kidneys. The duration of treatment depends on the site of infection and on severity.

Pseudomonas aeruginosa is a Gram-negative, aerobic, rod-shaped bacterium with unipolar motility. An opportunistic human pathogen, P. aeruginosa is also an opportunistic pathogen of plants. P. aeruginosa is the type species of the genus Pseudomonas.

Pseudomonas is a genus of gama proteobacteria, belonging to larger family of Pseudomonads. Now this species is increasingly recognized as an emerging opportunistic pathogen of clinical relevance. Several epidemiological studies indicate that anti-biotic resistance is increasing in clinical isolates.
\( P. \text{ aeruginosa} \) is typically responsible for 12% hospital urinary tract infection, 16% nasocomial infection, 8% surgical wound infection, 10% blood stream infection. Immune-compromised patients such as patients with bone marrow depression, cystic fibrosis, cancer Aids etc. are more prone to pseudomonas infection. Healthy people do not normally carry pseudomonas infection

Nonpathogenic \( \text{Escherichia coli} \) strain Nissle 1917 also known as Mutaflor is used as a probiotic agent in medicine, mainly for the treatment of various gastroenterological diseases including inflammatory bowel disease. The Etoricoxib has not earlier been reported for its antimicrobial activity against \( \text{Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi and Candida albicans} \). The objective of the present investigation is to evaluate the antimicrobial activity of the drug Etoricoxib.

**EXPERIMENTAL**

**Materials**
\( \text{Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Candida albicans} \) purchased from the microbial Type culture collection, Chandigarh, India. All the media were purchased by Hi-media. Etoricoxib, Doxorubicin and Ketoconazole were obtained from Ranbaxy, Cadila and Glenmark respectively.

**Culture Medium & Inoculums**
The stock cultures of micro-organisms used in the assay were maintained on plate count agar slants at 4deg. for bacteria & on SDA slants for fungi. The fresh culture of micro-organisms were prepared by inoculation of each bacteria into 10ml. of nutrient broth & fungi into Muller Hilton agar broth. Incubation was performed at 37\(^\circ\)C for 24 hr. On the next day Muller Hilton agar for bacteria and SDA for fungi was prepared and cooled to 45\(^\circ\)C. 1 ml. of bacterial suspension from fresh 24hr. broth culture was then subjected for serial dilution up to 10-4 to 10-5 for \( \text{Pseudomonas} \) and 10-2 to 10-3 dilution for \( \text{S. aureus} \). All dilutions were made with distilled water. Similar procedures were adopted for fungi. The times of dilutions to be carried out for each microbial cell suspension to get the effective no. of colonies on the plate should first be optimized. For \( \text{C. albicans} \), the dilution of microbial sample was made upto 10-2 to 10-3. A sterile swab was then dipped into the suitably diluted suspension of micro-organisims and swabbed on the solidified nutrient medium.

**Antimicrobial activity assay**
The antimicrobial study was conducted for the determination of following parameters. Zone of Inhibition, MIC (minimum inhibitory concentration), MKT (minimum time kill assay), MBC (minimum bactericidal concentration).

Different concentration of Etoricoxib were tested for anti-microbial activity by disc diffusion method. Nutrient agar medium was inoculated with different micro-organism and once the media was solidified, it was punched with a 6 mm diameter well. The wells were then filled with different concentration of Etoricoxib and the blanks with distilled \( \text{H}_2\text{O} \) (concentration of Etoricoxib was 20\( \mu \text{g/ml} \) to 200\( \mu \text{g/ml} \)). Agar plates containing bacteria and Etoricoxib were incubated at 37\(^\circ\)C for 24 h. Antimicrobial activity was evaluated by measuring the inhibition zone. Inhibition zones were recorded as the diameter of growth free zone, including the diameter of the well, in millimeters of the incubation period. The tested drug was classified as active when the diameter of the inhibition zone was equal to or larger than 6 mm.

Simultaneously standard antibiotic Doxorubicin for \( \text{S. aureus, P. aeruginosa, E. coli} \) were used for comparison at a conc. 1\( \mu \text{g/ml} \) each. The dilution medium for the positive control was sterile distilled \( \text{H}_2\text{O} \). The sample was tested in triplicate.

Similar procedure was adopted for fungi, except SDA was used as a selective media in place of MHA (mullar Hilton agar) for the effective growth of fungi. Ketoconazole, anti-fungal cream was used as the standard antibiotic for the comparison of zone of inhibition with different conc. Etoricoxib.

At the end of incubation period the zone of inhibition for the Etoricoxib was measured for each bacteria & fungi and the results were tabulated. (Table –1)
Determination of minimum inhibitory concentration (MIC)  
MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube (bacteriostatic concentration). In this method, the broth dilution technique was utilized where Etoricoxib was prepared to the highest concentration of 50µg/ml (stock concentration) in sterile water and serially diluted to a working concentration ranging from 10µg/ml to 100µg/ml using nutrient broth and later inoculated with 1ml suspension of the test organisms. After 18 hours of incubation at 37ºC, the test tubes were observed for turbidity. The MIC of each sample was determined by measuring the optical density in the spectrophotometer (620 nm), comparing the sample readout with the non inoculated nutrient broth. The least concentration where no turbidity was observed was determined and noted as the minimum inhibitory concentration (MIC) value.

RESULTS AND DISCUSSION

Antimicrobial effect of Etoricoxib

From the preliminary screening studies by disc diffusion method, it was observed that the test pathogens were susceptible to the etoricoxib. However a difference in the zone sizes were observed with different pathogens (Table-1). Etoricoxib showed more activity against S.aureus, followed by E.coli, Pseudomonas, and least activity against C. albicans and S. typhi. The MIC of the Etoricoxib were ranged between 100 to 200 µg/ml. Though a variance was observed in the zones of inhibition and the MIC values, the three test pathogens S. aureus, E. coli, Pseudomonas were having MIC 100 µg/ml, 150 µg/ml and 200 µg/ml respectively (Table-2). All the test pathogens were sensitive to the antibiotic (Doxorubicin 1mg/kg) tested.

Several drugs that were not originally developed for the treatment of bacterial infections have been demonstrated to possess antimicrobial activities in vitro. For example, Eelecoxib, a broadly used anti-inflammatory agent, exhibits off-target activity against F. tularensis in vitro.  
Here, we have demonstrated that Etoricoxib, a COX-2 inhibitor with an anti-inflammatory agent, exhibits antimicrobial effect against the microorganism present in the intestinal microflora, i.e, E. coli along with the other microorganism like S. aureus and P. auregenosa. 

It was proved that Celecoxib and Rofecoxib are potent COX-2 inhibitors that have been shown previously to interact with the same binding pocket of the COX-2 enzyme with IC\textsubscript{50} in the submicromolar range. Nonetheless, our data show that Etoricoxib possessed activity against E. coli, S. aureus, P. auregenosa and the MIC of Etoricoxib for E. coli (150µg/ml). Thus, we postulate that the putative bacterial target of Etoricoxib in-vivo for E. coli in the microbial flora of intestine may be effective. Although further experiments must be performed to validate the roles of these bacterial proteins in Etoricoxib-induced growth inhibition of E. coli spp., these preliminary findings suggest that such an approach to identifying bacterial drug targets is feasible and will facilitate the development of more potent and specific, Etoricoxib derived antibacterial agents.

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REFERENCES

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Table-1: Determination of zone of inhibition by Well Diffusion Method

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Dilution</th>
<th>Conc. Of Etoricoxib(µg/ml)</th>
<th>Volume of Test drug(µl)</th>
<th>Zone of Inhibition (mm)</th>
<th>Conc. Of Antibiotic (Doxorubicin, Ketoconazole) (µg/ml)</th>
<th>Volume of antibiotic(µl)</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>4×10²</td>
<td>100</td>
<td>30</td>
<td>13</td>
<td>2</td>
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<td>15</td>
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<td></td>
<td>50</td>
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<td>12</td>
<td>10</td>
<td>9</td>
<td>8</td>
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<td>8</td>
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<td></td>
<td></td>
<td>20</td>
<td>6.5</td>
<td>6</td>
<td>7</td>
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<td>18</td>
<td>10</td>
<td>15</td>
<td>5</td>
<td>9</td>
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<td>10</td>
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<td><em>E. coli</em></td>
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<td>10</td>
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<td>8</td>
<td>5</td>
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<tr>
<td><em>S. typhi</em></td>
<td>2×10³</td>
<td>200</td>
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<td>—</td>
<td>15</td>
<td>5</td>
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<td>—</td>
<td>—</td>
<td>5</td>
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<tr>
<td><em>Candida albicans</em></td>
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<td>50</td>
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<td>20</td>
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</tbody>
</table>

Table-2: Comparison of MIC of Etoricoxib and Doxorubicin

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>MIC of Etoricoxib (µg/ml)</th>
<th>MIC of Doxorubicin (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>≤ 200</td>
<td>≤ 5</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>≤ 100</td>
<td>≤ 4</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>≤ 150</td>
<td>≤ 5</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>—</td>
<td>≤ 7</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>—</td>
<td>≤ 4</td>
</tr>
</tbody>
</table>
E. coli: (Comparison of Zone of Inhibition)

P. auregenosa (Zone of Inhibition)

S. aureus: (Comparison of Zone of Inhibition)

Fig.-1
**Fig. 2**

*P. auregenosa* (4×10^5) Blank

*S. aureus* (5×10^5) Blank

*E. coli* (16×10^5) Blank

**Fig. 3**

*Comparison of Zone of inhibition Etoricoxib Vs Doxorubicin for S. aureus*

<table>
<thead>
<tr>
<th>Concentration of drug</th>
<th>Zone of Inhibition</th>
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</thead>
<tbody>
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<td>1</td>
<td>100</td>
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<tr>
<td>2</td>
<td>50</td>
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<td>3</td>
<td>25</td>
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<td>15</td>
</tr>
</tbody>
</table>

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Comparision of Zone of Inhibition Etoricoxib Vs Doxorubicin for 
*p. aeruginosa*

Concentration of Drug — Zone of Inhibition

Fig.-4

Comparision of Zone of inhibition between 
Etoricoxib Vs Doxorubicin for *E. coli*

Concentration of drug — Zone of Inhibition

Fig.-5

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