Antibacterial Action of Discriminating Cyclooxygenase-2 Inhibitors: In Vitro Study: Consideration And Appraisal

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Antibacterial Action of Discriminating Cyclooxygenase-2 Inhibitors: In Vitro Study: Consideration And Appraisal

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Abstract

This study elected to determine in vitro antibacterial activity of selected selective cyclooxygenase-2 inhibitor (meloxicam, celecoxib, valdicoxib and nimesulide) on 22 strains of gram positive and gram negative bacteria, which were isolated from skin and urinary tract infected patient. These bacteria were being cultured on specific optimal growth media. The antibacterial activity of selective COX-2 inhibitors determined by measuring zone of inhibition and minimal inhibitory concentration (MIC).

MIC of celecoxib and meloxicam in µg/ml was ranged from 5-80µg/ml on selected bacteria compared with negative control (D.W) and for valdicoxib was 80-160µg/ml, while nimesulide was ranged from 5-40 µg/ml .All the selected bacteria were showed sensitivity for all coxib used in this experimental study except Pseudomonas aeruginosa which showed resistant to meloxicam and valdicoxib also Klebsiella pneumoniae resist to nimesulide while Staphylococcus aureus was resist to valdicoxib . The smaller zone of inhibition showed by valdicoxib which was 2mm against Escherichia coli, while the larger zone of inhibition showed by nimesulide which was 24mm against Escherichia coli.

In conclusion selective cyclooxygenas (cox-2) inhibitor possess mild to moderate antibacterial activity mainly by nimesulide and little by valdicoxib . Escherichia coli is a sensitive bacteria to all coxib, Consequently; coxib may be regarded as anti-inflammatory and antibacterial agent especially for urinary tract infection where Escherichia coli is the major causative organism.

Introduction

The symposium of antibiotics and antibacterial chemotherapy is becoming more and more limited in the percentage, in spite of the fact that they exist in large numbers. The reason behind such a rapid turn down in the market of antibiotics is largely attributed to the emergence of drug resistant bacteria, which render even some of the most broad spectrum antibiotics unsuccessful (1). Moreover, the toxic side effects produced by antibiotics reducing their demand. Different studies on search of newer antimicrobials have discovered that moderate to remarkable antimicrobial action is present in several compounds (2); belonging to various pharmacological categories, such as the antihistamines (3); tranquilizers (4); the antihypertensive (5); the antipsychotics (6) and the anti-inflammatory agents (7).

Such compounds, having antimicrobial properties in addition to their predestinated pharmacological actions, have been christened as ‘Non antibiotics’ (8,9). From the history of the development of pharmaceutical compounds it is evident that any drug may have the possibility of possessing diverse functions and thus may have useful activity in completely different spheres of medicine (10). Different studies in the search for newer antimicrobials have revealed that moderate to remarkable antimicrobial action are involved in the management of diseases of non-infectious etiology have shown some antimicrobial activity in vitro. Non-steroidal anti-inflammatory drugs produce their analgesic and anti-inflammatory pharmacological effect by inhibiting the enzyme called cyclooxygenase (COX) (11). Cyclo-oxygenase converts arachidonic acid found in cell membrane to prostacyclin, thromboxanes and various prostaglandins, each with its own effect on cell function and physiology (12). Two isoforms of COX have been identified. COX-1 is expressed constitutively in most tissues as maintenance protein and mediates physiological functions such as gastric mucosal cytoprotection and platelet aggregation. COX-2 however, is articulated only in certain tissues such as the kidney, brain and pancreatic islet cells (13). These enzyme are not expressed in most other tissues but is induced in response to cytokines and growth factors in inflammatory conditions (14). One of the serious drawbacks of NSAID is gastrointestinal irritation and ulceration, a side effect attributed to COX-1 inhibition. Therefore, COX-2specific inhibitors have been developed primarily as anti-inflammatory agents for the treatment of osteoarthritis and rheumatic pain with less induced gastrointestinal toxicity (15).In worldwide, they are better tolerated than non-specific NSAID with a comparable desired
A clinical effect; however, their toxic effect on renal function are essentially similar. Search for anti-microbial action among the non-steroidal anti-inflammatory drugs, showed that diclofenac sodium exhibited significant potential antibacterial activity against both Gram-positive and Gram-negative bacteria, while piroxicam, mefenamic acid, naproxen and oxiclofenbutazone were found to have mild to moderate antibacterial activity (16). When tested in vivo, diclofenac at concentration of 1.5-3.0 mg/gm bodyweight of Swiss strain of white mice, could protect these animals when challenged with of Salmonella typhimurium NCTC 74 (17). Diclofenac sodium further demonstrated significant clearance of the challenged pathogenic bacteria from liver and spleen (18; 19).

The aim of present study is to show the antibacterial activity of selective cox-2 inhibitors regarding celecoxibe; valdicoxibe; meloxicam and nimesulide on selected Gram-positive and Gram-negative bacteria.

**Methods**

This study was carried out in Department of Pharmacology, College of Medicine, Al-mustansiriya University, Baghdad – Iraq, 2010. It is approved by scientific jury of Department of Pharmacology, and licensed by board of medical college. A total of 22 clinical isolate were analyzed. Out of these 10 samples were of UTI and 12 from skin infection. Pus and urine samples were collected from Al-Yarmouk teaching hospital using standard protocol of sample collection. These bacteria inoculated on blood and Maconky agar. Bacterial cultures were tested against selective cyclo-oxygenase inhibitors celecoxib, meloxicam, valdicoxib and nimesulide by Replica method through agar well diffusion and tube dilution method (20,21). 10mg/ml stock solution of each drug was made in sterile distilled water. Then serial dilution of concentration(control, 5 g/ml, 10 g/ml, 20 g/ml, 40 g/ml, 80 g/ml, and 160 g/ml) was organized. Then the Agar plates were incubated for 24hours at 37.

**Tube dilution method**

Serial dilutions of the coxib were made in Muller Hinton broth which was inoculated with a standardized number of organisms and incubated for 24 hours. The lowest concentration of drug preventing of turbidity is considered to be the minimal inhibitory concentration (MIC).

**Agar well diffusion methods**

Wells in the Muller Hinton Agar plates were made by the help of 6mm borer. The culture was swabbed homogeneously across plates and the known concentration of the drug to be tested was added in the well (5 g/ml, 10 g/ml, 20 g/ml, 40 g/ml, 80 g/ml, and 160 g/ml). If the drug is effective against bacteria at a certain concentration, no colonies will grow when the concentration in the agar is greater than or equal to the effective concentration, this is the zone of inhibition. As consequence, the size of the zone of inhibition is a measure of the compound’s efficacy; the larger the clear area around the well, the more effective compound. The antibacterial activity was estimated based on size of inhibition zone formed around the well-seeded agar plates and inhibition growth in percentage was determined based on the average diameter of colony on growth medium to their respective control (22).

Drugs were obtained from private pharmaceutical company Ltd (Ajanta pharma limited, AjantaHouse, clarkopandvivil (cw) Mumbai 4000, india).

**Results**

Antibacterial property of selective cyclo-oxygenase-2 inhibitors were determined alongside different bacterial strains .The zone of inhibition of selective cyclo-oxygenase inhibitors on the selected bacterial strains are presented in table (1).

Meloxicam showed inhibitory effects on all selected bacteria except of pseudomonas aeurginosa. However celecoxib produced inhibition zone on all selected bacteria but valdicoxib produced minimal antibacterial effects on Escherichia coli and Klebsiella pneumoniae and no effects on staphylococcus aureus and pseudomonas aeurginosa .Consequently; nimesulide produced greater zone of inhibition 24mm and valdicoxibe produced lesser zone of inhibition 22mm regarding Escherichia coli as sensitive bacteria for all type of selective cyclo-oxygenase inhibitors figure (1).

Toward determining the kinetic effects of these coxib against Escherichia coli (regarding it as sensitive bacteria for all type of selective cyclo-oxygenase inhibitors);colony forming unite( CFU ) count of strain was $3 \times 10^8$ at 0(control) time with subsequent addition of drug at sequential concentrating; the CFU measured each two hours they were $4 \times 10^6$, $3 \times 10^5$ and $2 \times 10^4$ after 2,4,6 hours correspondingly. Figure (2).

**Discussion**

Non-steroidal anti-inflammatory drugs (NSAID) are the
most widely used drugs worldwide and represent a base in the therapy of acute and chronic pain. In early 1990 two isoform of cyclooxygenase(Cox) identified Cox-1 in normal tissue and Cox-2 constitutively in inflamed area (23). In current years, constitutive expression of Cox-2 in normal tissues, mainly in renal, cardiovascular, brain and gastric tissue have been proved (24). Cox-2 inhibitor drugs commonly named coxib include sulphonamide derivative (celecoxib, valdicoxib and parecoxib) and methylsulphone derivative(nimesulide and etoricoxib), later agent have antioxidant activity (25). All coxib selectively block Cox-2 with different Cox-1/Cox-2 ratio, nimesulide and celecoxib produced similar affinity for Cox-2 and less for Cox-1, while valdicoxib mainly act on Cox-2(26). The present study showed effective antibacterial action of coxib in contrast with negative control (distilled water), nimesulide produced greater zone of inhibition against Escherichia coli and no effect in opposition to Klebsiella pneumonia while valdicoxib showed little antibacterial activity but nimesulide showed significant antibacterial effects. From sequential coxib addition, results showed in this study were all coxib are bactericidal with the exception of valdicoxib which fashioned as bacteriostatic effects rather than bactericidal regarding the bacterial growth per/ml in each two hours.

The use of NSAID has been up to that time perceived as one that would not alter host response to infection(27). Previous study by Alem and Douglas(2004) in one experimental model, viability assays were accomplish on both growing and fully matured biofilm to investigate the effects of aspirin, diclofenac and other NSAID on biofilm formation, accordingly this study showed that diclofenac, aspirin and etodolac had maximum inhibitory effects with aspirin up to 95% inhibition, while celecoxib and ibuprofen also inhibit the bacterial biofilm but to a lesser significant capacity (28). Moreover; coxib act by blocking prosuglandin synthesis through inhibition of cox-2 enzyme in view of the fact that the lipoxygenase and cyclooxygenase pathway have the same precursor (arachidonic acid), inhibiting the metabolism of arachidonic acid via the cyclooxygenase pathway; would the metabolism to tend more to the lipoxygenase pathway, consequently; increasing of inflammatory leukotrienes (29). Leukotriene (LTB4) stimulate B-lymphocyte through T-lymphocyte, while, Leukotriene LTB4 and LTD4 increasing expression of IL-1, so coxib indirectly induce humoral and cellular immunity but these cytokines not measured in this study (30). The mechanism of antibacterial activity of the coxib not well understood but in this study coxib have dual bacteriostatic and bactericidal effects, these results supported by Annduri 2008 in a trial of experimental antimicrobial activity of diclofenac sodium, showed that diclofenac was found to acquire significant good antimicrobial properties against most virulent bacteria like salmonella typhimurium, the antibacterial action of diclofenac was found to be via inhibition of bacterial DNA which was demonstrated using 2µCi(3H)deoxythymidine uptake (31). On contrary Steven 2009 incriminate the coxib as predisposing factor for bacterial infection due to inhibition of prostaglandin mediated granulocyte function, but coxib in most previous showed it increase lipoxygenase pathway so elevate LTB4, LTD4 and cytokine expression so increasing in vivo bacterial clearance but toxic dose of most NSAID decrease the bacterial clearance (32). Unfortunately leukotrienes and prostaglandin levels not measured.

Moreover; inflammation promote bacterial growth because the inflammation lead to fluid buildup in the area of injury due to rising the vascular permeability leading to limited to a small area edema which may actually support bacterial growth and causing tissue damage that provided a good media and nutrient for bacteria (33). Therefore; coxib inhibiting bacterial growth via inhibition of inflammatory process (34). The therapeutic benefit of having one drug as analgesic, antipyretic, anti-inflammatory and antibacterial should be greatly explored. In addition cox-1 and cox-2 have critical but contrasting effects on host immune response to infection possibly mediated via altered production of PG and LT following infection, so deficiency of cox-1 result in enhanced inflammatory response and earlier release of pro-inflammatory cytokines, in contrast deficiency of cox-2 isoform results in reduction in inflammation and cytokine release (35).

Intended for that reason; coxib regarded as safe agent in treating bacterial infection than nonselective cox inhibitors. It was pragmatic by Anurup et al 2010 study the agents with two or more benzene ring possess strong antimicrobial activity like phenothiazine and tricycle antidepressant (36). As a result coxib has two benzene ring this per se might explain their antibacterial activity (37).

Furthermore; celecoxib and meloxicam are potent COX-2 inhibitors that have been shown formerly to interact with the same binding receptacle of the COX-2 enzyme in the submicromolar range, even so, celecoxib possessed antibacterial activity in opposition to Francisella tularensis and that the MIC of celecoxib for Francisella tularensis(32 µg/ml) is much higher than its reported for COX-2 (0.21 µg/ml) (38). These findings suggest that the antimicrobial activity of
celecoxib is independent of the structural features that dictate its binding to COX-2. Accordingly, we assume that the presumed bacterial target of celecoxib in sensitive bacteria is structurally dissimilar from the COX-2 enzyme. Moreover; coxib independent action related to inhibition of cellular enzymes and antiapoptotic effects on vital organs and induction of apoptosis in malignant cells also in addition to COX-2, celecoxib has been reported to possess inhibitory activities against other mammalian enzymes, including phosphoinositide-dependent kinase-1, carbonic anhydrase, sarcoplasmic/endoplasmic reticulum calcium ATPase, and COX-1 (39). These mammalian enzymes may serve as leads to identify the structurally similar bacterial proteins, one of which may be the hypothetical antibacterial target of celecoxib in bacteria.

References


Table (1): In vitro antibacterial activity of selective COX2 inhibitor on different bacterial strain.

<table>
<thead>
<tr>
<th>Bacterial type</th>
<th>meloxicam</th>
<th>celecoxib</th>
<th>valdicoxib</th>
<th>nimesulide</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph. aureus</td>
<td>15</td>
<td>15</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>18</td>
<td>4</td>
<td>12</td>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
Illustration 2

Figure (1): Minimal inhibitory concentration (MIC) of selective cox-2 inhibitor.
Illustration 3

Figure (2): Kinetic and sequential effects of selective cyclo-oxygenase inhibitors against Escherichia coli growth.
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