

EXPERIMENTAL ANTIBACTERIAL ACTIVITY OF SELECTIVE CYCLOOXYGENASE ANTAGONIST

Hayder M. Al-kuraishy¹, Ali I. algareeb¹ and Salah A. Al-windy²

¹Department Of Pharmacology, College Of Medicine,
Al-Mustansiriya University, P.O. Box 14132, Baghdad, Iraq.

²Salah A. Al-windy, Lecturer in department of biology
College of sciences, Baghdad University. Iraq.

ABSTRACT

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 fields of medicine and different studies showed that newer antimicrobials have revealed antimicrobial action involved in the management of diseases of non-infectious etiology. This study was done to determine in vitro antibacterial activity of selected selective cyclooxygenase-2 inhibitor. Twenty two 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 (meloxicam, celecoxib, valdicoxib and nimesulide). Inhibitors determined by measuring zone of inhibition and minimal inhibitory concentration (MIC). Results showed that MIC of celecoxib and meloxicam in $\mu\text{g/ml}$ was ranged from 5-80 $\mu\text{g/ml}$ on selected bacteria compared with negative control distilled water (D.W), valdicoxib was 80-160 $\mu\text{g/ml}$, while and nimesulide was ranged from 5-40 $\mu\text{g/ml}$. All the selected bacteria were showed sensitivity for all coxib used in this experimental study except *Pseudomonas aeruginosa* which showed resistant to meloxicam and valdicoxid, *Klebsiella pneumoniae* resist to nimesulide while *Staphylococcus aureus* was resist to valdicoxib. The smaller zone of inhibition showed by valdicoxib and celecoxib which was 3mm against *Klebsiella pneumoniae*, while the larger zone of inhibition showed by nimesulide which was 26mm against *Escherichia coli*. In conclusion selective cyclooxygenase (cox-2) inhibitor possesses antibacterial activity this is especially for nimesulide and little by valdicoxib. *Escherichia coli* are sensitive bacteria to all coxib. Consequently; coxib may be regarded as anti-inflammatory and antibacterial agent especially for urinary tract infection where *Escherichia coli* are the major causative organism.

Keywords: antibacterial; cyclooxygenase-2 inhibitor and zone of inhibition.

INTRODUCTION

The symposium of antibiotics and antibacterial chemotherapy is becoming more limited in spite of the fact that they exist in large numbers, the reason behind such a rapid turn down in the antibiotics is mainly attributed to the emergence of drug resistant bacteria, which render even some of the most broad spectrum antibiotics unsuccessful¹. Moreover, the toxic side effects produced by antibiotics reducing

their demand and remarkable antimicrobial action is present in several compounds², belonging to various pharmacological categories, such as the antihistamines³, tranquilizers⁴; the antihypertensive⁵; the antipsychotics⁶ and the anti-inflammatory agents⁷.

Such compounds, having antimicrobial properties in addition to their original pharmacological actions, have been named 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 fields of medicine¹⁰ and different studies showed that newer antimicrobials have revealed antimicrobial action involved in the management of diseases of non-infectious etiology. 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 physiology¹². 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 and COX-2 however, is expressed only in certain tissues such as the kidney, brain and pancreatic islet cells¹³ and not found in most other tissues but is induced in response to cytokines and growth factors in inflammatory conditions¹⁴. One of the serious drawbacks of NSAID is gastrointestinal irritation and ulceration, a side effect attributed to COX-1 inhibition. Therefore; COX-2 specific inhibitors have been developed primarily as anti-inflammatory agents¹⁵ and they are better tolerated than non-specific NSAID with a comparable desired 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 bacteria¹⁶. Diclofenac at concentration of 1.5 - 3.0 mg /gm bodyweight of Swiss strain of white mice could protect these animals when challenged with *Salmonella typhimurium* NCTC 74¹⁷, and demonstrated significant clearance of the pathogenic bacteria from liver and spleen^{18; 19}.

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

MATERIAL AND METHODS

This study was carried out in Department of Pharmacology, College of Medicine, Al-mustansiriya University, Baghdad – Iraq, 2012. 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 Macconky agar. Bacterial cultures were tested against selective cyclo-oxygenase inhibitors celecoxib, meloxicam, valdicoxib and nimesulide by 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 (0) control, 5 µg/ml, 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, 160 µg/ml were done. Then the Agar plates were incubated for 24 hours at 37°C.

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 that preventing the 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 µg/ml, 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml, and 160 µg/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. 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 of growth in percentage was determined based on the average diameter of colony on growth medium to their respective control²².

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

RESULTS

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

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

Bacterial type	Zone of inhibition(mm)				
	meloxicam	Celecoxib	valdicoxib	nimesulide	control
<i>Staph.aureus</i>	15	15	0	6	0
<i>Escherichia coli</i>	18	4	12	26	2
<i>Pseudomonas aeruginosa</i>	0	18	0	15	0
<i>Klebsiella pneumoniae</i>	6	3	3	0	1

Meloxicam showed inhibitory effects on all selected bacteria except of *pseudomonas aeureginosa*, celecoxib produced inhibition zone on all selected bacteria and valdicoxib produced minimal antibacterial effects on *Klebsiella pneumoniae* and no effects on *staphylococcus aureus* and *pseudomonas*

aeureginosa, nimesulide produced greater zone of inhibition 26mm and valdicoxibe as celecoxib produced lesser zone of inhibition 3mm regarding *Escherichia coli* as sensitive bacteria for all type of selective cyclooxygenase inhibitors figure (1).

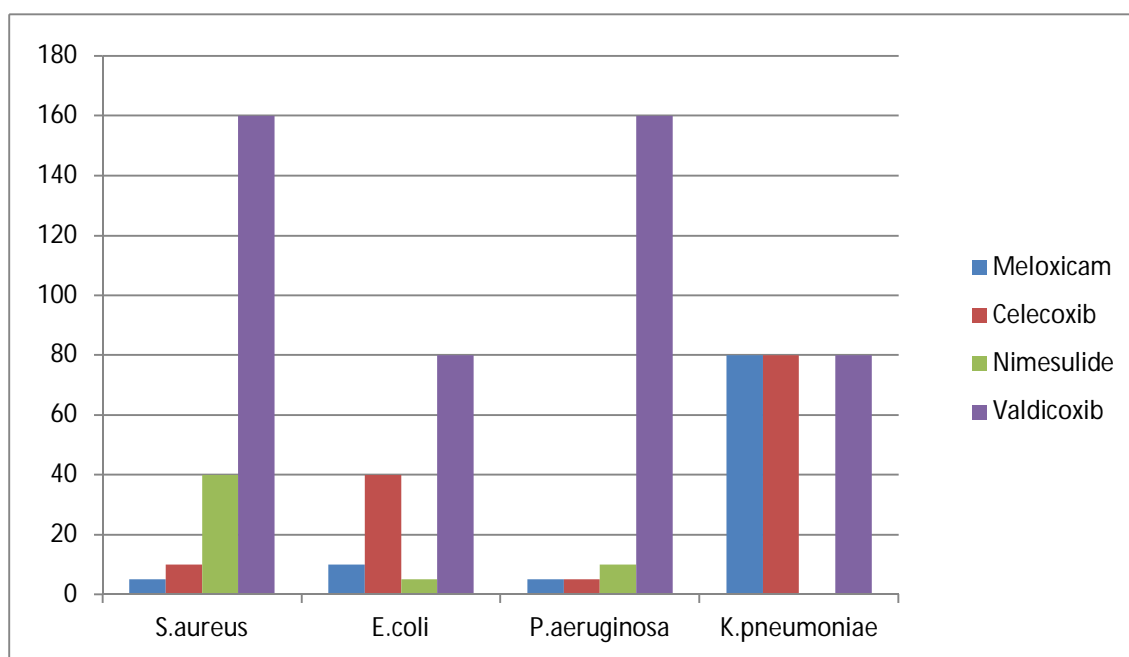


Fig. 1: Minimal inhibitory concentration (MIC) of selective cox-2 inhibitor

For 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 3×10^8 at zero time with subsequent addition of drug at sequential concentration; the CFU measured each two hours they were 4×10^6

, 3×10^5 and 2×10^4 after 2,4,6 hours correspondingly. Figure (2).

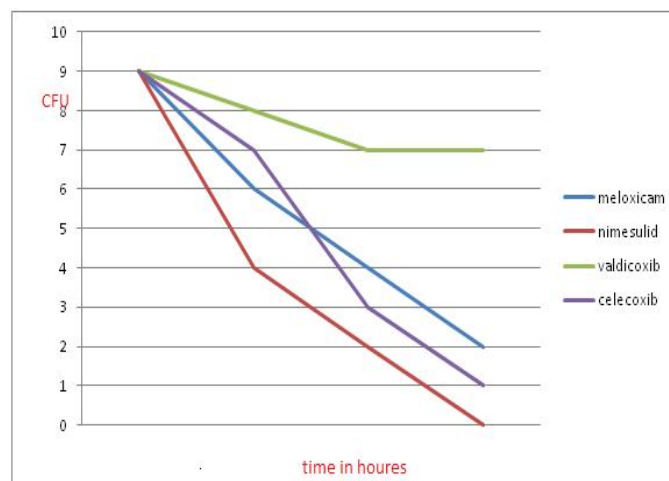


Fig. 2: kinetic and sequential effects of selective cyclo-oxygenase Inhibitors against *Escherichia coli* growth

DISCUSSION

The present study demonstrated effective antibacterial action of coxib in comparison with negative control (distilled water), nimesulide produced greater zone of inhibition against *Escherichia coli* and no effect against *Klebsiella pneumoniae* while nimesulide showed significant antibacterial effects. From sequential coxib addition, results showed 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 because bactericidal effects (progressive decreasing in bacterial colony number/per time) while bacteriostatic effects (non-progressive in bacterial colony number/per time)²³

The use of NSAID has been up evaluated not alter host response to infection²⁴⁻²⁷. 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 had maximum inhibitory effects with aspirin up to 95% inhibition, while celecoxib and ibuprofen also inhibit the bacterial biofilm but to a lesser extend²⁸ .Moreover; coxib act by blocking prosglandin 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 enhance the lipoxygenase pathway ,consequently; increasing of inflammatory leukotrienes²⁹. 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 study³⁰.

The mechanism of antibacterial activity of the coxib was not well understood but in this study coxib have dual bacteriostatic and bactericidal effects, these results supported by Annduri 2008 in a trail of experimental antimicrobial activity of diclofenac sodium, showed that diclofenac possessed significant antimicrobial properties against *salmonella typhimurium*. The antibacterial action of diclofenac was found to be via inhibition of bacterial DNA which was demonstrated using 2 μ Ci(3H) deoxythymidine uptake³¹. On contrary Steven 2009 incriminate the coxib as predisposing factor for bacterial infection due to inhibition of prostaglandin mediated granulocyte function, but coxib in 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 clearance³² unfortunately leukotrienes and prostaglandin levels were not measured in this study.

Moreover; inflammation promote bacterial growth because the inflammation lead to fluid buildup in the area of injury due to increasing in the vascular permeability leading to limited to oedema which may actually support bacterial growth and causing tissue damage that provide a good media and nutrient for bacteria³³. Therefore; coxib inhibiting bacterial growth via inhibition of inflammatory process³⁴.

Additionally cox-1 and cox-2 have critical but contrasting effects on host immune response to infection possibly mediated via altered production of prosglandin (PG) and Leukotriene(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 release³⁵.

Proposed 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 tricyclic antidepressant³⁶. As a result coxib has two benzene ring this *per se* might explain their antibacterial activity³⁷.

Furthermore; celecoxib and meloxicam are potent COX-2 inhibitors that have been shown formerly to interact with the same binding site of the COX-2 enzyme in the submicromolar range, even so, celecoxib possessed antibacterial activity against *Francisella tularensis* and that the MIC of celecoxib for *Francisella tularensis* is (32 μ g/ml) which is much higher than was reported for COX-2 (0.21 g/ml)³⁸. These findings advocate that the antimicrobial activity of celecoxib is independent of the structural features that dictate its binding to COX-2.

Accordingly; we assume that the supposed bacterial target of celecoxib for 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, 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-1³⁹. 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.

From all these previous studies we can conclude that coxib produced diversity of effects on host and microorganism regarding the antibacterial activity, hence; regarding host-microorganism relationship, coxib is regarded as harmful agent for bacteria and marginally not hazardous for host effects.

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