EFFECT OF NASIDs ON GROWTH OF CERTAIN TYPES OF BACTERIA

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Keywords:- NSAIDs, Paracetamol, MIC

ABSTRACT

Four bacterial isolates of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis* were experimented for antimicrobial activity of four types of NASIDs (Diclofenac sodium, Meloxicam, Piroxicam, and Paracetamol) by test tube MIC and disc diffusion method. Antimicrobial activity were detected between increased NSAIDs concentrations and inhibition growth of bacterial isolates. MIC and disc diffusion methods have antimicrobial activity against bacterial isolates. These results may be an explanation of abdominal disturbances of patients those subjected to intensive course of NSAIDs.

INTRODUCTION

NSAIDs (Non Steroidal Anti Inflammatory Drugs) are amongst the most widely used of all therapies world wide, there are more than fifty different NSAIDs available, excluding Aspirin and Paracetamol, they are used for the reduction of pain, inflammation and fever, there are no significant differences in their main pharmacological actions, but there are marked differences in toxicity, and important differences in individual patient's reaction **(1)**.

Antimicrobial effects of Diclofenac had been approved by many studies. Diclofenac showed noteworthy inhibitory action [MIC]=50µg/ml on *Listeria monocytogens* with demonstrated cidal activity on this bacteria at 100µg/ml **(2)**, a total of 80 isolate of *E. coli* from UTI patients were susceptible to Diclofenac at MIC value ranging from 5-50µg/ml **(3)**, and most of 45 strains of *Mycobacterium tuberculosis* inhibited by Diclofenac Sodium at concentrations of 10-25µg/ml when tested *in vitro* **(4)**.

The antimicrobial ability of Diclofenac Sodium, Meloxicam and Paracetamol to eliminate pathogenic organisms is not limited with direct inhibitory action on those organisms, but also includes indirect effects by using the main function of such compounds as anti-inflammatory to facilitate the destruction of affected organisms, therefore Diclofenac Sodium has removal capacity of Gram negative bacteria from kidney

through effects on the function of mucosal inflammatory response represented by secretion on interleukin-6 and polymorphonuclear leukocytes (PMNL) **(5)**.

The widespread use of NSAIDs has meant that the adverse effects of these drugs have become increasingly prevalent. The two main adverse drug reaction associated with NSAIDs relate to gastrointestinal effects and renal effect of the agents. These effects are dose-dependent, and in many cases sever enough to pose the risk of ulcer perfusion, and upper gastrointestinal bleeding, and death, limiting the use of NSAIDs therapy **(6)**.

The main adverse drug reactions associated with the use of NSAIDs relate to direct and indirect irritation of the gastrointestinal tract, these drugs cause dual insult on the gastrointestinal tract, the acidic molecules directly irritate the gastric mucosa and inhibition of COX-1 reduce the levels of protective prostaglandins. Inhibition of prostaglandin synthesis in the gastrointestinal tract causes increased gastric acid secretion, diminished bicarbonate secretion, mucous secretion and tropic effects on epithelial mucosa.

Common gastrointestinal adverse drug reaction including nausea, vomiting, dyspepsia, gastric ulceration and bleeding diarrhea **(7, 8)**.

In attempting to minimize gastrointestinal adverse drug reaction, it is prudent to use the lowest effective dose for the shortest period of time, a practice which studies show is not often followed. Recent studies show that over 50% of patient taking NSAIDs have sustained damage to their small intestine **(9)**, there are also some differences in the propensity of individual agents to cause gastrointestinal adverse drug reactions. Indomethacin, Ketoprofen and Piroxicam appear to have the highest prevalence of gastrointestinal adverse drug reaction, while ibuprofen (lower doses) and Diclofenac appear to have lower rates **(7)**.

The aim of present study an attempt to clarify *in vitro* the effect of NSAIDs on some human bacterial isolates.

MATERIALS AND METHODS

Bacterial isolates

Four bacterial isolates of *Staph. aureus, E. coli*, *P. aeruginosa*, and *B. subtilis* that isolated from nasopharyngeal region, stool, burns infection, and skin respectively were used in this study, identified by conventional methodology **(10)**.

Drugs

Diclofenac sodium (Olfen®) 75mg /3ml , supplied by Mepha, the used concentration 25mg /ml, Meloxicam (Mobic®) 15mg /1.5ml supplied by Boehringer-Ingelheim, used concentration 15mg /1.5ml, Piroxicam (feldin) used concentration 20mg /ml, Paracetamol 375 mg supplied by Ibn-haian, used concentration 375 mg /5ml.

Antibacterial susceptibility

The susceptibility of four isolates were examined against four types of NASIDs by using disc diffusion method **(11)**. Nutrient agar plates were inoculated with 0.01 ml of 24 hrs. bacterial suspension of four isolates, after that discs that impregnated with NSAIDs solutes were placed on inoculated nutrient agar plates and incubated at 37◦C for 24 hrs., then the inhibition zones diameters were measured.

Minimal Inhibitory Concentrations (MICs)

MIC was determined for each drug separately by broth micro- dilution assay according to **(12),** double dilution test tubes were prepared for each drug (table 1). Nutrient broth test tubes (5 ml of each) inoculated with 0.01 ml of 24 hrs. bacterial suspension growth of four isolates, after that 1 ml from each drug dilution was added to test tubes, then incubated at 37◦C for 24 hrs. Control test tubes were carried out for each isolates without adding the NASIDs dilutions. The results were examined visually for the presence or absence of growth in each dilution, they were also read spectrophotometrically at 540 nm and optical density (OD) values were recorded to determine the percentage of bacterial culture survival.

In another step, nutrient agar plates were inoculated with 0.01 ml from MIC test tubes contents to confirm the previous results.

Table (1). NSAIDs double dilution concentrations

Concentration	Diclofenac sodium	Meloxicam	Piroxicam	Paracetamol
1	**25 mg/ml**	**1.5 mg/ml**	**20 mg/ml**	**75 mg/ml**
2	**12.5 mg/ml**	**7.5 mg/ ml**	**10 mg/ml**	**37.5 mg/ml**
3	**6.25 mg/ml**	**3.75 mg/ ml**	**5 mg/ml**	**18.75 mg/ml**
4	**3.12 mg/ml**	**1.8 mg/ ml**	**2.5 mg/ml**	**9.73 mg/ml**
5	**1.5 mg/ml**	**0.9 mg/ ml**	**1.25 mg/ml**	**4.68 mg/ml**

RESULTS AND DISCUSSION

The results of disc diffusion method of NSAIDs effect against *Staph. aureus, E. coli*, *P. aeruginosa* and *B. subtilis* were showed that Diclofenac sodium exerted more antibacterial effect against *B. subtilis* with diameter of inhibition zone about 20mm, while against *Staph. aureus* was only 15mm. Piroxicam showed only 9mm inhibition zone were recorded against *B. subtilis*. Others NSAIDs (Meloxicam, Paracetamol) were not recorded any antibacterial effect against *Staph. aureus , E. coli*, *P. aeruginosa*, and *B. subtilis* (table 2).

Table (2). Antibacterial susceptibility of NASIDS against four isolates

Bacterial isolates	***Staph. aureus***	***E. coli***	***P. aeruginosa***	***B. subtilis***
Diclofenac Sodium (25mg/ml)	**15mm***	**No effect**	**No effect**	**20mm**
Meloxicam (1.5mg/ml)	**No effect**	**No effect**	**No effect**	**No effect**
Piroxicam (20mg/ml)	**No effect**	**No effect**	**No effect**	**9mm**
Paracetamol (75mg/ml)	**No effect**	**No effect**	**No effect**	**No effect**

*** = diameter of inhibition zone , mm = millimeter**

Minimal Inhibitory Concentrations (MICs)

MIC was determined for Diclofenac sodium by broth micro-dilution assay as double dilution test tube (table 3).

Table (3). MICs of Diclofenac sodium

Diclofenac sodium	***Staph. aureus***	***E. coli,***	***P. aeruginosa***	***B. subtilis***
Control*	**0.605**	**0.322**	**0.355**	**0.321**
25 mg/ml	**0.043**	**0.062**	**0.081**	**0.081**
12.5 mg/ml	**0.135**	**0.051**	**0.088**	**0.095**
6.25 mg/ml	**0.187**	**0.08**	**0.09**	**0.057**
3.12 mg/ml	**0.104**	**0.091**	**o.106**	**0.063**
1.5 mg/ml	**0.140**	**0.095**	**0.153**	**0.109**

*** control without NSAIDs**

The MIC results were detected by spectrophotometer that showed graduated antimicrobial activity with decreasing of NASIDs concentrations against all species under the study.

MICs approach considered more accuracy than disc diffusion method, so most references rely on the obtained results from MICs, therefore the effect of NSAIDs drugs on the isolates was determined by repeating MICs of previous disc diffusion to get the exact effect of these drugs on bacterial isolates.

The results of spectrophotometer readings were recorded by comparison of the control value with graduated double dilution of NASIDs drugs in accordance to the relationship between bacterial growth and NASIDs concentrations. Some of irregular readings may be explained as a results of personal error that belong to inoculation amount that added to test tubes.

Antimicrobial activity of some NSAIDs may be a role for microbial flora shifting toward *candida* blooming than bacterial species. It may be consider intensive course intake lead to abdominal disturbances **(13)**.

Upper gastrointestinal (GI) complications are well-recognized adverse events associated with non-steroidal anti-inflammatory drug (NSAID) use **(14)**. Several studies have suggested that intraluminal bacteria play a significant role in the pathogenesis of small-bowel damage induced by NSAIDs and that enterobacterial translocation into the mucosa represents the first step that sets in motion a series of events leading to gross lesion formation **(15, 16, 17)**. Experimental and clinical investigations indicate that in the short term, antibacterial agents either reduce or abolish NSAID enteropathy **(18)**.

The activity of drug on bacteria may differ based on bacterial species or strain. The injection of indomethacin into rat gastrointestinal increased the persistence of *Enterococcus faecalis* and decreased *E. coli* growth in the same time **(7)**.

The effect of some NSAIDs compounds on immune system to antimicrobial agents needs many scientific evidences to form clear view on this activity against various microorganisms.

Further studies should be considered to evaluate antimicrobial activity as a result of NSAIDs components, acidity, or from drugs preservatives.

The present study conclude that abdominal disturbances may be as a result of intensive course of NASIDs that have antimicrobial activity that shift gastrointestinal microbial flora.

تأثير الأدوية مضادات الالتهاب غير الستيرويدية على نمو أنواع معينة من البكتريا

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الخلاصة

أختبرت الفعالية ضد الجرثومية لأربعة أنواع من الأدوية مضادات الالتهاب غير الستيرويدية (دايكلوفيناك صوديوم ، ميلوكسيكام ، بيروكسيكام والباراسيتامول)على اربعة عزلات جرثومية للمكورات العنقودية الذهبية ، الأشريكية القولونية ، الزوائف الزنجارية والعصيات البوغية الهوائية بواسطة التركيز المثبط الأدنى وطريقة الأنتشار بالأقراص . أظهرت نتائج طريقة الأنتشار بالأقراص وجود علاقة بين زيادة تركيز الادوية مضادات الالتهاب غير الستيرويدية وتثبيط النمو الجرثومي للعزلات قيد الدراسة. النتائج اعلاه ادعمت بطريقة التركيز الادنى للتثبيط بواسطة قياس نشاط الادوية مضادات الالتهاب غير الستيرويدية ضد النمو البكتيري بواسطة المطياف، حيث اظهرت نفس التاثير ضد الجرثومي تجاه العزلات الأربعة. هذه النتائج قد تعطي تفسيراً لبعض الأضطرابات المعوية الحاصلة للمرضى ممن يخضعون للعلاج المكثف من الأدوية مضادات الالتهاب غير الستيرويدية.

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