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The Synergistic effect of non-steroidal anti-inflammatory drugs on antibacterial activity of ceftriaxone against streptococcus pyogenes Isolated from patients with pharyngitis

Suhad Traiji Zamil[1], Raad Abdulameer Alasady[2], Rana Talib Al-Nafakh[3]
[1]*Department of Pharmacology and Toxicology, Faculty of pharmacy, University of Kufa, Najaf, Iraq, Email: suhadt.alakeli@uokufa.edu.iq*
[2]*Department of Medical Microbiology, Faculty of medicine, University of Kufa, Najaf, Iraq, Email: raada.najim@uokufa.edu.iq*
[3]*Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Kufa, Najaf, Iraq, Email: ranat.alnafakh@uokufa.edu.iq*

Abstract

Background: Streptococcus pyogenes cause major infectious problems including pharyngitis, scarlet fever, and rheumatic fever Emergence of resistant strains make such infections and relevant complications (e.g., rheumatic valvular disease and glomerulonephritis) a serious clinical challenge especially in children. Aims and objectives: In a maneuver of overcoming the resistance developed by S. pyogenes and improving the ability of antibiotics to fight such bacteria, a nonsteroidal anti-inflammatory drug NSAID is repurposed as antimicrobial against S. pyogenes, in objective to be united with the usual antibiotic mentioned by WHO principle of care. The combination was evaluated for its MIC and combination index versus S. pyogenes. Materials and Methods: From patients, suffering pharyngitis a multidrug-resistant strain of S. pyogenes was insulated. The maximum dangerous isolate was cultivated for MIC determination ceftriaxonemefenamic acid combination in comparison with each alone to establish the combination index. Results and conclusions: There were a pointed synergism concerning ceftriaxonemefenamic acid (index < 1) at P= 0.012 and Z score= 2.3 additional validation of MIC folds of dilutions are to be evaluated to achieve rational evidence. Recommendations: We suggested to additional validate the antistreptococcal effect of the combined ceftriaxonemefenamic acid on more dilutions of MIC assay and to identify the benefit of this combination in patients developed pharyngitis induced by S. pyogenes.

Keywords: Antibiotic resistance, S. pyogenes, mefenamic acid, ceftriaxone, combination index.

1. Introduction

In modern decades, due to the intense raise and global spread of bacterial resistance to a sum of generally used antibacterial agents, many studies have been focused on investigating drugs whose initial therapeutic purpose is not antimicrobial action but synergistic influence of combination two drugs [(1)] such as the non-steroidal anti-inflammatory drugs (NSAIDs) which are commonly advised agents over all the world. NSAIDs are mainly used for its role as antipyretic,

analgesic, or anti-inflammatory medications. Clinically, they are excellent pain killers in many conditions. This class encounters inflammation via inhibition of prostaglandin synthesis thereby blocking the cyclooxygenases (COX) enzyme [2]. Ceftriaxone belong a very important class of antibiotics, cephalosporin which is the β-lactam family of antibiotics [3]. Ceftriaxone interacts with transpeptidase selectively and irreversibly leading to inhibition of bacterial cell wall synthesis by catalyzing the cross-linking of the peptidoglycan polymers forming the bacterial cell wall [4]. Many bacteria are sensitive to ceftriaxone including Serratia marcescens, Citrobacter, and beta-lactamase-producing strains of Homophiles and Neisseria. It is also antibiotic of choice for treatment of bacterial meningitis caused by pneumococcal, meningococcal, Homophiles influenza, and susceptible enteric Gram-negative rods, but not Listeria monocytogenes [5]. It is about 15 million of cases with acute pharyngitis per year in the United States [6,7]. Infection with Streptococcus pyogenes (group A beta-hemolytic streptococci) is the greatest universal bacterial cause of acute pharyngitis leading to 5-15% of sore throat cases among adults [8] and 20-30% of cases among children [9]. Several studies considered the antibacterial and antifungal effect of the non- steroidal anti-inflammatory drugs. NSAIDs possessed a moderate potent effect against Gram-negative bacteria like Salmonella, E. coli, Klebsiella, Helicobacter pylori and Enterobacteria, in addition to Gram positive bacteria such as Staphylococcus, Mycobacterium, Bacillus, and Listeria monocytogenes[10].Currently, a small number of antibiotics can treat methicillin-resistant Staphylococcus aureus. One alternative approach includes adjuvants to antibiotic therapy that are NSAIDs described to show antibacterial activity [11].

2. Materials and Methods

Study objectives

In this study we aimed to follow three steps including isolation resistant S. pyogenes from individuals with pharyngitis, finding the MIC value of mefenamic acid and ceftriaxone against S. pyogenes and calculating the combination and interaction index between the ceftriaxone and mefenamic acid to establish the combination index. The study proposed an *in vitro* study for determining the MIC of numerous individual and mixed antimicrobials.

Sample collection

Patients studied were randomly chosen from cases admitted for swab culture from pharynx and antibiotic sensitivity referred to private laboratory in Kufa city who exhibited positive S. pyogenes culture. Excluded cases were those with immunocompromising. Streptococcus pyogenes was isolated from a pharyngeal swab of 10 patients (7-69 years). All these patients were suffering from S. pyogenes pharyngitis confirmed by culture. The high resistant strain of S. pyogenes was considered according to MDR criteria on agar diffusion test. The isolate was chosen to be of higher virulence of MIC assessment. Virulence factor was identified according to clinical severity, serotyping and antimicrobial resistance. Patients receiving antibiotics within 3 days prior to swab cultures, extreme ages, culture negative cases, susceptible S. pyogenes strain or least resistance for single antibiotics, pharyngitis due to other bacterial infections like staph aureus pharyngitis. The ethical form was fulfilled. The patients were indirect cases from medical care referral for pharyngeal swab culture. No one was demanded for swab culture that is not needed as a part of required care.

The Antimicrobial MIC Assay:

A stock solution of the following ingredients was prepared as following: mefenamic acid 100*g*/ml, ceftriaxone alone of 100g/ml and100g/ml of both agents were prepared. Two-fold

dilutions were used. Streptococcus pyogenes was spread homogeneously on all wells to be equipped for 2xserial dilutions of test drugs

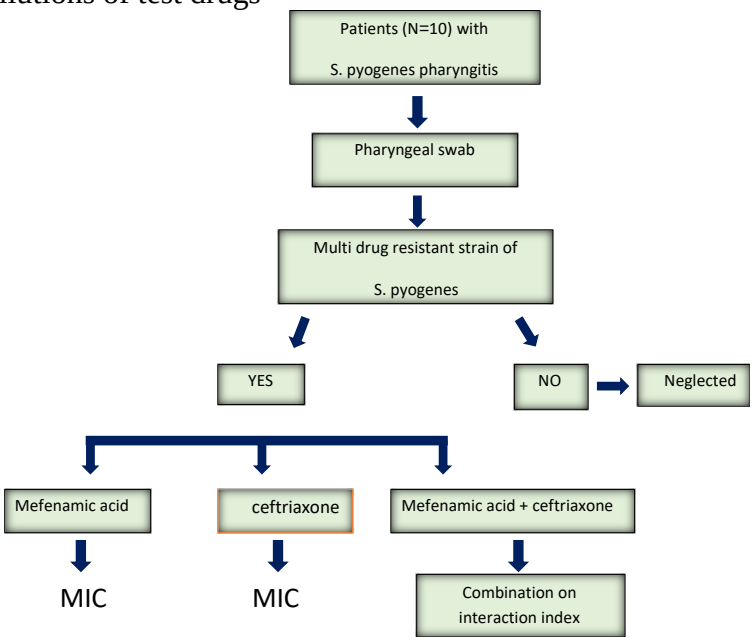

Figure (1): The main steps of NSAID, repurposing study against S. pyogenes in combination with common recommendation antibiotics

Table (1): The common materials used in MIC determinations, their vendor, Source Company and dosage form

Materials	Source contain	Source company	Dosage	Form
Mefenamic acid	alcami	USA	1 g	Powder form
ceftriaxone	Cipla	India	1 g	Powder form
Nutrient Broth medium	Hanover	Germany	50g	Powder form
NaCl	Pioneer	Jordan	1 pint	Infusion fluid
DDW	local	Iraq	1 liter	Liquid

DDW = deionized distilled water

Formulation of the stock test solution

Standard drug weight equivalence to 100 µg was used in a concentration of 100 µg /ml and serial concentrations of 50, 25, 5, 12.5, 6.25 µg /ml were used to conduct MIC assay. Count of the S. pyogenes CFU was scored as following.

Table (2): Growth scoring

Score	Growth
0	no growth
1	mild growth (10^6 CFU)
2	moderate (10^7 CFU)
3	heavy growth (>10^9 CFU)

This score was estimated as the result corresponding to each dilution

3. Results

Table (3): *In vitro* sensitivity assay using disc diffusion technique for S. pyogenes against numerous antimicrobials

Type of Antibiotic	Result
Cefepime	M
Ceftriaxone	H.S
Tobromycin	M
Doxicycline	H.S
Ciprofloxacin	R
Meropenem	H.S
Nitrofurantion	H.S
Amoxicillin	H.S
Levofloxacin	H.S
Azithromycin	H.S
Vancomycin	H.S
Trimethoprim	H.S
Amikacin	H.S

Note: R, resistant; M, moderate sensitive; H.S, highly sensitive.

A validation disc diffusion assay for S. pyogenes susceptibility was ensured in order to summarize the strain of the isolate, in this study, we purposely designated the most multidrug resistant strain to be subjected for MIC assay.

Table (4): The MIC50 values of mefenamic acid -ceftriaxone, anti S. pyogenes culture

	bacterial culture growth score			
Conc. µg / ml	Mefenamic acid	Ceftriaxone	Mefenamic Ceftriaxone	P Mann-Whitney test
100	3	0	0	0.012
50	3	0	0	
25	3	0	0	
12.5	3	0	0	
6.25	3	0	0	
0	3	3	3	

The size of sample was less than 10, thus, the estimation required additional serial dilutions. Moreover, mefenamic acid and ceftriaxone showed a significant synergistic effect even though mefenamic acid showed resistance in MIC assay. The MIC of growth score of each individual drug was compared to the combined drugs to establish type of interaction. It was significantly different (P value0.012 and Z-score 2.50). The score of culture growth was labeled as 0 = no growth, 1 = mild growth (10^6 CFU), 2 = moderate (10^7 CFU), 3 = heavy growth (>10^9 CFU).

The anti-streptococcus interaction index

Table (5): The interaction index of the united ceftriaxonemefenamic acid anti S. pyogenes

Combination	Interaction index (combination index)
Mefenamic acid-ceftriaxone	< 1

The score of the final dilution was chosen to generate the combination index. The result for mefenamic acid-ceftriaxone was below1, which show a synergetic effect given that MIC of mefenamic acid is more than that of mefenamic acid-ceftriaxone combinations.

4. Discussion

It has been increasingly difficult to ignore the number of serious resistant bacteria to antibiotics over the past numerous decades. S. pyogenes found innumerous isolates collected from throat swab samples were resistant to usual betalactam antibiotics. Additionally, several other antimicrobials, like amino glycosides and sulfonamides, have failed to eradicate several isolates of S. pyogenes [12,13]. S. pyogenes exhibited resistance to ciprofloxacin, where as this strain of S. pyogenes explained high sensitivity to amoxicillin in disc diffusion experiment. However, ceftriaxone failed to kill this strain even at a concentration of 100 µg /ml in MIC assay. On the other hand, life-threatening complications like valvular disease may be developed upon infection with S. pyogenes in children [13,14].Increased resistance in slow-growing cells is occurred. In some cases, for example in the PhoPQ system, the ultimate causation is change in lipid A that reduces negative charge and which results in electrostatic repulsion of cationic peptides to the cell wall[15]. Antipyretics are commonly given in bacterial infection, which are commonly associated with fever, treated with antibiotics. Thus, it is essential to understand the combination between these two classes of drugs. Antipyretics primarily act by inhibiting prostaglandin synthesis [16,18]. Combining mefenamic acid with the extended spectrum antibiotic, ceftriaxone is a credible rationale for this medical care in a challenge to overcome resistance, so even though synergism was substantial between mefenamic acid and ceftriaxone, mefenamic acid alone showed similar inhibitory effect. Thus, further bacterial count may reveal the difference in MIC between mefenamic acid and the combined medications. Additionally, more serial dilutions are required in future studies to identify the precise MIC of the tested agent from NSAIDs. Animal studies are suggested to support the potential antimicrobial effect of antipyretics.

5. Conclusions

Generally, mefenamic acid presented antibacterial activity against strains of streptococcus pyogenes. Although NSAIDs individually are less potent than common antibiotics, they show synergism when given with ceftriaxone and could potentially be used as adjuvants in preventing pharyngitis. We proposed to perform further studies to confirm the antistreptococcal effect of using both ceftriaxone and mefenamic acid with more dilutions of MIC assay and to investigate data of this combination in those patients with S. pyogenes pharyngitis.

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