Synergistic effect of Ceftriaxone and diclofenac against Escherichia Coli

Amir Zaman Khan[1], Shah Zaman[1], Sardar Muhammad[2], Riaz Nasim[1], Mohsina Haq[2], Muhammad Luqman[1]

ABSTRACT

Background: The emergence of resistant strains of bacteria has rendered many useful antibiotics ineffective. The researchers are forced to try combination of two or more antibiotics, or addition of a non- antibacterial to them. Ceftriaxone, a third-generation cephalosporin agent is used to treat many infectious diseases, especially those caused by members of Enterobacteriaceae family. We tested the efficacy of ceftriaxone, alone and in combination with various concentrations of diclofenac sodium, against Escherichia coli.

Objective: To study the synergistic effect of ceftriaxone and its combination with non-antibiotic drug diclofenac sodium against Escherichia coli ATCC 25922 isolate.

Material and Methods: Agar well diffusion technique was applied. 30 µl of solutions of ceftriaxone, and its combination with different concentrations of diclofenac sodium and DMSO were transferred aseptically into the wells. Agar plates were placed in incubator at 37°C for 24 hours. Mean zone of inhibition of each drug was calculated.

Results: Ceftriaxone 30µg formed a zone of inhibition of 35 mm. Its combination with 25µg and 50µg of diclofenac sodium formed zone of inhibition of 37.5mm while its combination with 100µg diclofenac sodium formed a zone of inhibition of 38.6mm and formed 39.8mm zone of inhibition when combined with 200µg diclofenac sodium.

Conclusion: Ceftriaxone activity increased when combined with different concentrations of diclofenac sodium drug showing a synergistic effect of their combination.

Key words: *ceftriaxone, Diclofenac sodium, E. coli, Synergism*

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INTRODUCTION

Antibiotics have significantly enhanced the quality of life and increased life expectancy i by combating infectious diseases. The emergence of resistant strains of various microbes has rendered the antibiotics ineffective [1]. Ceftriaxone, a third-generation cephalosporin is a broad-spectrum antibacterial drug which inhibits cell wall synthesis. It has a broad spectrum of activity against many microbes, but especially effective against E. Coli and other members of Enterobacteriaceae family) [2]. However, there are increasing reports of developing resistance against this valuable drug. World Health Organization (WHO) has recognized ceftriaxone-resistant to members of Enterobacteriaceae family as pathogens of clinical importance. Resistant microbes can lead to an increase in mortality and can cause delay in treating infectious diseases even leading to death. Reports suggest increase in the resistant strains of E.coli against third generation cephalosporins since 2004 [3].

There are many ways where the bacteria can be intrinsically resistant to cepahlosporins. One of the mechanism is that the pathogens can express certain enzymes like cephalosporinase which can deactivate and destroy the antibiotic. They can also modify the antibiotic target, Penicillin binding protein, due to which the β-lactams bind poorly and it becomes ineffective. Bacteria can express efflux pumps which exports the drug out of the cell. The microbe can prevent the drug from reaching its target by decreasing the entry or uptake of the antimicrobial agent[4].

It has also been observed in a study that there is an emerging resistance against carbapenems and colistin, which is very alarming because these drugs are our last hope. Antibiotic resistant of gram-negative bacteria is evolving into a worldwide crisis and WHO has named it as one of the top three global health threats. Therefore, urgent measures need to be taken to address the problem of increasing bacterial resistance against antimicrobials . Therefore, we must look for agents that can prolong and boost the efficacy of antibiotics against such pathogens. Treatment of Gram negative bacteria is already becoming challenging and problematic. Combining antibiotics with non-antibiotics is proving to be an innovative strategy to improve the efficacy of antibiotics fight the pathogens. Antibiotic-non antibiotic approach is successfully applied to treat many infections caused by gram-negative and gram-positive bacteria, malarial infections, tuberculosis and HIV diseases for long time quite some time [5]

1.Department of Pharmacology, Peshawar Medical College
2.Department of Microbiology, Peshawar Medical College.

Correspondence: Dr. Amir Zaman Khan
Department of Pharmacology Peshawar, Pakistan
Peshawar Medical College
Cell: 03329303201 EMail: amirzaman36@gmail.com

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So action needs to be taken to protect the already existing antibacterial agents and devise strategies to produce novel agents against resistant pathogens [6].

The synergism between antibiotic and non-antibiotic combination can emerge as a valuable tool to overcome the problem of bacterial resistance to antibiotic by increasing the efficacy of antimicrobial agents [7].

The irrational and inappropriate use of antibiotics have caused them to become resistant to pathogens [8]. To fight against this nuisance, search for new compounds is the need of the moment which can exert their own antibacterial effect or they can enhance the effect of antibiotic drugs. These compounds are known as 'non-antibiotics' as these agents are used to treat non-infectious pathological conditions but they possess their own anti-bactericidal action [9].

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID), which has shown antibacterial activity against many gram-negative and gram-positive bacteria. Its antibacterial action is reported to be due to its ability to inhibit DNA synthesis in bacteria [11]. Different antibiotic combination therapies have been introduced previously to decrease resistance such as Augmentin, co-trimoxazole and rifampicin and isoniazid. Therefore, antibiotic-non-antibiotic combinations are under trial as they can increase inhibitory effect of antibiotics and decreasing antibiotic resistance.

According to one study, gentamycin plus diclofenac combination had more effect than giving gentamycin alone [9]. In our study we searched for the combined effect of ceftriaxone with diclofenac sodium on Escherichia coli ATCC 25922.

MATERIALS AND METHODS

This experimental study was conducted in laboratory of Agriculture University of Peshawar, from January 2019- June 2019. American type culture collection (ATCC) isolate of Escherichia coli ATCC 25922 was taken. Ceftriaxone and diclofenac sodium were obtained in powdered form from Astella Pharma. The powdered drugs were dissolved in solution of dimethyle sulfoxide (DMSO). Bacteria was grown on MacConkey agar and kept in incubator for 24 hours. Bacteria grown were transferred into falcon tubes containing Mueller Hinton broth with sterilized loop. These tubes were then incubated at 37C. Mueller Hinton agar Media was used for antibiotic susceptibility test. Media used were made by Oxoid Limited, United Kingdom.

The standard inocula were spread on sterilized Mueller Hinton agar through a sterilized glass spreader to get the bacterial lawn. Wells of 4mm in diameter and 5mm depth were punched in the agar plates using a sterile borer.

The test solutions were prepared by dissolving the test drugs in 10 ml of DMSO. Powdered form of ceftriaxone alone and its combination with Diclofenac sodium were weighed and dissolved in 10ml of DMSO in separate beakers with separate stirrers to avoid any mixing of the solutions. 30µl solutions of 30µg of Ceftriaxone were prepared and 30µg Ceftriaxone in combination with 200µg, 100µg, 50µg and 25µg of diclofenac solutions were prepared and 30µl of each solution were transferred aseptically into the wells. DMSO was used as control. Agar plates were placed in around each well of the drug was measured at two planes and mean of these two were calculated. Similarly, mean zones of inhibition formed around the three wells of each drug were calculated [10].

Mean surface area of zone of inhibition was calculated by using πr^2 formula, where 'r' is the radius of zone of inhibition. Percentage change in incubator at 37°C for 24 hours. Zone of inhibition surface area with the combination against ceftriaxone alone was calculated by the formula (BA)/A×100.

(A = surface area of zone of inhibition due to ceftriaxone only and B = surface area of zone of inhibition due to the combined effect of ceftriaxone and diclofenac combination)

This experiment was performed inside a biosafety cabinet to avoid any contamination of the isolates. Statistical analysis was performed on SPSS version 21 applying student t-test on the mean of two variables. p-value less than 0.05 was taken as significant.

RESULTS

The mean zones of inhibition with increasing doses of diclofenac in combination have expanded as compared to the ceftriaxone alone zone of inhibition. This shows that Escherichia coli have high sensitivity to the action of diclofenac sodium and ceftriaxone combination as compared to ceftriaxone alone.

Ceftriaxone 30µg formed a mean zone of inhibition of 35 mm±2.29. When combined with 25µg and 50µg of diclofenac solution showed increased zone of inhibition of 37.5 mm which was statistically insignificant by applying student t-test (p-value >0.05). On the basis of surface area, ceftriaxone area of inhibition increased to 14.4% when combined with 25µg and 14.5% when combined with 50µg diclofenac sodium.

Ceftriaxone 30µg when combined with 100 µg diclofenac showed a zone of inhibition of 38.6 mm, which is significant as p-value is 0.05, showed an increase of 22% surface area of inhibition of ceftriaxone

Ceftriaxone activity increased from 35 mm to 39.8 mm when combined with 200 µg of diclofenac, which shows significance as the p-value is <0.05, an increase of 29% surface area of inhibition of ceftriaxone was noted.

DMSO, was used a solving agent and a negative control, no zone of inhibition was observed in the plates where DMSO was used alone.

Fig 1. Comparison of Escherichia coli ATCC 25922 sensitivity to ceftriaxone and its combination with different doses of diclofenac sodium.

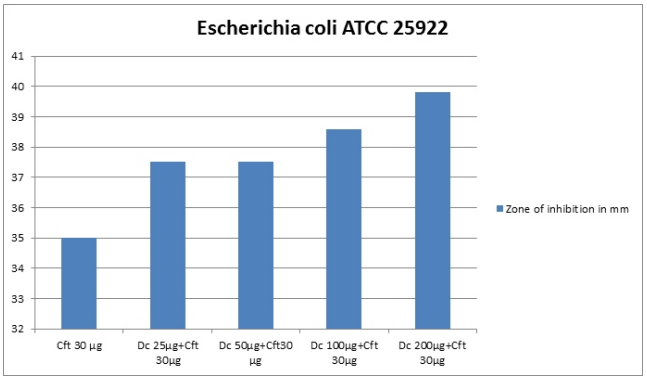

Table 1. Escherichia coli ATCC 25922 sensitivity to ceftriaxone alone and different combinations of diclofenac sodium with ceftriaxone.

Drugs	Dose	Mean zone of inhibition ± SD (mm)	p-value
Ceftriaxone	30 gµ	35±2.29	
Diclofenac Sodium + Ceftriaxone 30 gµ	25 gµ + 30 gµ	37.5±0.50	0.13
	50 gµ + 30 gµ	37.5±1.50	0.18
	100 gµ + 30 gµ	38.6±0.57	0.05
	200 gµ + 30 gµ	39.8±1.75	0.04
DMSO	99%	0	

Table 2. Escherichia coli ATCC 25922 sensitivity to ceftriaxone alone and different combinations of diclofenac sodium and ceftriaxone

Drugs	Dose	Area of inhibition (mm)	% increase of ceftriaxone on the basis of surface area
Ceftriaxone	30 gµ	964.37	
Diclofenac Sodium + Ceftriaxone 30 gµ	25 gµ + 30 gµ	1104.04	14.4
	50 gµ + 30 gµ	1105.08	14.5
	100 gµ + 30 gµ	1173.84	22
	200 gµ + 30 gµ	1247.17	29.3

DISCUSSION

The strategy of combining of antibiotic with non-antibiotic to combat resistance problems in bacteria has been successfully applied in various studies. Synergism between the antibiotics and non-antibiotics can be beneficial as many of the non-antibiotic agents have weak antibacterial effects making them unsuitable to use alone[12].

These non-antibiotic drugs can enhance the in-vitro movement of antibiotic against the bacteria. These agents increase the lethal effects and efficacy of antimicrobials when both non-antibiotic and the antibiotics are taken together, and also change the pathogenicity of bacteria by modifying their physiology [13].

Amlodipine, an anti-hypertensive agent increased the activity of an antibiotic, streptomycin, by increasing the diameter of zone of inhibition of streptomycin against standard bacterial strains on disk diffusion method [14].

Non steroidal anti-inflammatory drugs can be used to increase the efficacy of certain antibiotics and can serve as non-antibiotics. Aspirin increase the susceptibility of metronidazole, amoxicillin and clarithromycin in inhibiting growth of Helicobacter pylori by Minimum inhibitory concentration (MIC) method [15].

Diclofenac sodium also reduced the MIC of antibiotics like ciprofloxacin, amoxicillin/clavulinic acid, cefoperazone, gentamycin, tetracycline and imipenam when used against Proteus mirabilis isolated from diabetic foot ulcer patients [16].

Diclofenac sodium has been reported to increase the antibacterial activity of many antibiotics when they are co-administered. Diclofenac when given in combination with ceftriaxone in patients increased the elimination half-life of the antibiotic when compared with ceftriaxone alone treated group of patients on liquid chromatography [17].

The combined effect of diclofenac with different antibiotic agents tested on Escherichia coli showed that it reduces the MIC of the antibacterial drugs on the resistant strains of the pathogen [18].Diclofenac showed synergistic activity when used in combination with ampicillin, amoxicillin, augmentin, cephalexin, cephradine, cefotaxime, ciprofloxacin and gentamicin against Klebsiella pneumoniae ATCC 10031 and Pseudomonas aeruginosa ATCC 10145 [19].

Streptomycin 10 µg formed a zone of inhibition of 17.4 mm against Escherichia coli 74. When combined with a 100 µg of diclofenac sodium, increase was noted in the zone of inhibition of streptomycin from 17.4 mm to 18.1 mm which showed an increase of 8.20% in area of inhibition of streptomycin [20].

Streptomycin 10µg, increased the zone of inhibition from 18.6 mm to 19.4 mm when co-administered with 100 µg of diclofenac against Escherichia coli ATCC 25922, an increase 8.74% surface area of streptomycin on the basis of πr^2 [21].

In our study, increase in the activity of ceftriaxone 30 µg was noted on Escherichia coli ATCC 25922 when combined with a non-antibiotic drug, diclofenac sodium. It increased the mean zone of inhibition of ceftriaxone 30 µg from 35 mm to 37.5 mm in 25 µg, showing an increase in surface area of ceftriaxone by 14.4%.

It also showed a mean zone of inhibition increase of 37.5 mm when ceftriaxone combined with 50 µg, showing an increase in surface area of ceftriaxone by 14.5% on the basis of πr^2. The zone of inhibition was increased to 38.6 mm with 100 µg, showing an increase of 22% surface area of the antibiotic and to 39.8 mm with 200 µg diclofenac, increasing the surface area of ceftriaxone by 29.3%.

DMSO was used as a solvent for the drugs used, which were in powdered form. We also made separate wells for DMSO against Escherichia coli ATCC 25922, as negative control, where no zone of inhibition was formed by the solvent.

CONCLUSIONS

1) It is concluded that the effect of combination of 30 µg ceftriaxone is significantly effective in combining with 100 µg and 200 µg of diclofenac sodium.
2) Combined effect of antibiotics-non-antibiotics against bacteria can be used as a valuable tool to get additional anti-bacterial effect.

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DATA SHARING STATEMENT: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under

Khan AZ, Zaman S: Concept and design of study, Collection of data, statistical analysis
Muhammad S: Writing of manuscript, critical review of manuscript
Nasim R, Haq M: Analysis and interpretation of data, statistical analysis
Luqman M: Data collection, bibliography

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.