Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Antibacterial Potency of Ibuprofen and Its Interaction with Ciprofloxacin Against Gram Positive and Gram Negative Bacteria

Ertugrul Ozbil, Sultan Ogmen, Acelya Mavideniz, Mehmet Ilktac*

[1] Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, North Cyprus via Mersin 10 Turkiye.

Abstract

Ibuprofen, a nonsteroidal anti-inflammatory drug (NSAID), acts by reducing hormones for the treatment of fever, inflammation, and pain. Previously, it was only shown that the ibuprofen inhibits the effect of various bacteria that are stimulated by bacterial infections and not directly on bacterial cells. In this study, we aimed to investigate antibacterial and synergistic activities of ibuprofen against *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603. The results revealed promising antibacterial activity against tested Gram-positive bacteria, but there was no effect on Gram-negative bacteria. Furthermore, checkerboard assay did not reveal any additive or synergistic activity when ibuprofen was combined with ciprofloxacin against tested Gram-positive bacteria. Collectively, our data reveal the selective antibacterial activity of ibuprofen against Gram-positive bacteria which suggest that ibuprofen can further be investigated as a potential source for new therapeutic options.

Keywords

Antibacterial, **c**heckerboard, ciprofloxacin, *Enterecoccus faecalis*, ibuprofen, *Staphylococcus aureus*.

Article History

Submitted: 28 June 2024 Accepted: 09 July 2024 Published Online: July 2024

Article Info

***Corresponding author:** Mehmet Ilktac **email:** mehmet.ilktac@emu.edu.tr

Research Article:

Volume: 7 Issue: 1 2024 Pages: 20-24

DOI: 10.54994/emujpharmsci.1506675

INTRODUCTION

Ibuprofen (Figure 1) is (2RS)-1[4-(2-methyl propyl) phenyl] propionic acid. Ibuprofen was the first member of propionic acids that is a nonsteroidal anti-inflammatory drug (NSAID) of the 2-aryl propionic acid family. It is the most commonly used and prescribed NSAID. Ibuprofen is a non-selective inhibitor of cyclooxygenase-1 and cyclooxygenase-2 that functions by reducing hormones for the treatment of inflammation, fever, and pain (Bushra and Aslam, 2010).

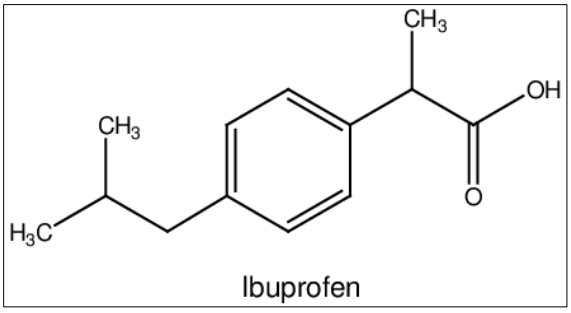

Figure 1: Structural formula of ibuprofen.

Previously, the studies that analyzed the relationship between bacteria and ibuprofen did not reveal any direct antibacterial activity but reported the anti-inflammatory action of the drug reducing the inflammation stimulated during various bacterial infections (Al-Janabi, 2010). Ibuprofen was shown to reduce the inflammation in mouse lung resulting from *Pseudomonas aeruginosa* infection but with no direct effect on the bacterium itself (Sordelli *et al.,* 1985). In this study, we investigated the antibacterial activity of ibuprofen against various pathogenic bacteria, while also demonstrating its possible additive or synergistic activity in combination with one of the most frequently prescribed antibiotics, ciprofloxacin.

MATERIALS AND METHODS

Extraction of ibuprofen from the commercial pills

The coating of the pills (Brufen, Abbott Laboratuvarlari, Turkiye) was removed by treating them with water. Then, the pills were ground into powder. Acetone was added to the powder and filtered. Acetone was removed from the filtrate by using a rotary evaporator. The residue was scratched and pure ibuprofen was obtained.

Inoculum and sample preparation

The antibacterial activity of ibuprofen was investigated against *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Klebsiella pneumoniae* ATCC

700603. The bacteria were subcultured on Mueller-Hinton Agar (MHA). The media were incubated at 37 ^{o}C under an aerobic atmosphere for 24 hours. After 24 hours, pure culture of the bacteria was obtained on MHA. 0.5 Mc Farland ($1x10^{8}$ cfu/ml) standard solutions of bacteria were prepared in Mueller-Hinton broth (MHB).

The stock solution of the sample was prepared using pure dimethyl sulfoxide (DMSO). The final concentration of DMSO was 3% for antibacterial activity tests and checkerboard assays.

Minimum inhibitory concentration (MIC) determination

Antibacterial activity of ibuprofen was investigated by the broth microdilution method (Wikler, 2006). Final inocula of the bacteria in the U-bottomed 96 well plates were 1 x 10^{6} cfu/mL and the final concentrations of the sample ranged from 0.125 to 4 mg/mL. Ciprofloxacin was utilized as the positive control and the highest concentration of the sample in MHB was negative control for all replicates. Incubation of the microplates was carried out at 37 ^{o}C for 18h. The MIC was regarded as the minimum concentration of the sample that the growth of bacteria was inhibited. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test (MTT) was used for the confirmation of the MIC.

Minimum bactericidal concentration (MBC) determination

MBC was determined as the lowest concentration of ibuprofen that killed bacteria. For this aim, 10 µL of the sample from the wells that have the concentration equal to MIC and higher was inoculated onto MHA. The media were incubated under an aerobic atmosphere at 37 ^{o}C for 18 hours.

Interaction of ibuprofen with ciprofloxacin - Checkerboard assay

Checkerboard assay was used for the determination of the interaction of ibuprofen with ciprofloxacin as previously described (Bellio *et al.*, 2021). The final concentration of the sample ranged from 4 to 0.06 mg/mL, whereas ciprofloxacin ranged from 0.001 to 1 mg/L. Ciprofloxacin and the sample each alone in MHB were used as controls. Incubation was carried out under aerobic atmosphere at 37 ^{o}C for 18h. Fractional inhibitory concentration index (FICI) was calculated using the formula:

$$FICI = A / MICA + B / MICB$$

where ‘A’ and ‘B’ are the MIC of each antimicrobial agent in combination within a single well plate; and MICA and MICB are the MIC of each drug individually. The interaction was interpreted as follows;

Synergy when FICI was < 0.5,

Additive when $0.5 \leq FICI \leq 0.9$,

Indifference when $1 \leq FICI \leq 4$

Antagonistic when FICI >4.

Statistical analyses

All of the experiments were performed in triplicates. Statistical analyses were performed by Students t-test.

RESULTS AND DISCUSSION

Antibacterial and checkerboard assays

To assess the antimicrobial activities associated with ibuprofen, the microdilution method was used to measure MICs against *E. faecalis*, *S. aureus*, *E. coli*, and *K. pneumoniae*. MIC of ibuprofen was detected as 1 and 2 mg/ml against *S. aureus* and *E. faecalis*, respectively (Table 1).

Table 1: MICs of ibuprofen against Gram-positive and Gram-negative bacteria.

Agents		Gram- positive bacteria		Gram-negative bacteria	
		S. aureus **ATCC 25923**	***E. faecalis*** **ATCC 29212**	***E. coli*** **ATCC 25922**	***K. pneumoniae*** **ATCC 700603**
Sample (mg/mL)	Ibuprofen	1 ± 0	2 ± 0	-	-
Control (mg/L)	Ciprofloxacin	0.25 ± 0	0.5 ± 0.083	0.008 ± 0	0.25 ± 0.021

Data represented as the standard error of mean (±S.E.M). ; -: Not inhibited.

On the other hand, no antimicrobial activity was detected against the tested Gram-negative bacteria (Table 1). This result may be due to the presence of an outer membrane in Gram-negative bacteria that can limit the entry of large molecules (Zgurskaya and Rybenkov, 2019). MBC of ibuprofen against *S. aureus* and *E. faecalis* was 1 and 2 mg/mL, respectively. In another study carried out, ibuprofen was found to limit the physiological activities of *E. coli* endotoxin on rabbits (Celik *et al.*, 2002) and humans (Bernard *et al*., 1997). In another study, ibuprofen showed no antibacterial effect on *Helicobacter pylori* in human body (Graham *et al.*, 1989) and on *Mycobacterium tuberculosis* in mice (Byrne *et al*., 2006). However, direct action of ibuprofen on bacterial cells has not been clearly illustrated until now.

Checkerboard assay was conducted only against *E. faecalis* and *S. aureus* because antibacterial activity was only detected against the two bacteria. The interaction was indifference for all the combinations.

Table 2: FICI of ibuprofen and ciprofloxacin combinations against *S. aureus* and *E. faecalis*.

Samples	Optimal Combination		FICI	
	Ciprofloxacin (mg/L)	**Ibuprofen (mg/mL)**	**< 0.5**	**> 0.5**
S. aureus	0.001	1		1.0 (I)
E. faecalis	0.001	2		1.0 (I)

A: Additive. I: Indifference. S: Synergy.

When different concentrations of ciprofloxacin and ibuprofen were used against *S. aureus* and *E. faecalis*, no additive or synergistic effect was observed. None of the combinations showed any antagonistic activity against any of the tested bacteria (Table 2).

CONCLUSION

Ibuprofen, an NSAID, functions by reducing hormones for the treatment of fever, inflammation, and pain. Previously, it was shown that ibuprofen has activity against bacterial infections due to its anti-inflammatory action. Although, no additive or synergistic activity was observed with ciprofloxacin, our results demonstrate promising selective antibacterial activity of ibuprofen against Gram-positive bacteria.

REFERENCES

Bellio P, Fagnani L, Nazzicone L, Celenza G (2021). New and simplified method for drug combination studies by checkerboard assay. *MethodsX* **8**: 101543.

Bernard GR, Wheeler AP, Russell JA, Schein R, Summer WR, *et al.* (1997). The effects of ibuprofen on the physiology and survival of patients with sepsis. *New Engl J Med* **336**: 912-918.

Byrne ST, Denkin SM, Zhang Y (2006). Aspirin and ibuprofen enhance pyrazinamide treatment of murine tuberculosis. *J Antimicrob Chemother* **59**: 313-316.

Celik I, Akbulut A, Kilic SS, Rahman A, Vural P, *et al.* (2002). Effects of Ibuprofen on the physiology and outcome of rabbit endotoxic shock. *BMC Infect Dis* **2**: 26-38.

Graham DY, Klein PD, Opekun AR, Smith KE, Polasani RR, *et al.* (1989). In vivo susceptibility of *Campylobacter pylori*. *Am J Gastroenterol* **84**: 233-238.

Sordelli DO, Cerquetti MC, El-Tawil G, Ramwell PW, Hooke AM, *et al.* (1985). Ibuprofen modifies the inflammatory response of the murine lung to *Pseudomonas aeruginosa*. *Eur J Respire Dis* **67**: 118-127.

Wikler MA (2006). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard, vol. 26, p M7–A7. CLSI, Wayne, Pennsylvania.

Zgurskaya HI, Rybenkov V (2019). Permeability barriers of Gram-negative pathogens. *Ann N Y Acad Sci* **1459**: 5-18.