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In vitro Antibacterial Activity of Naproxen and its Combination with Ciprofloxacin

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Abstract

Naproxen, a nonsteroidal anti-inflammatory drug (NSAID), is commonly used to reduce fever, and to treat pain and inflammation caused by several conditions. Previously, naproxen was evaluated for its antimicrobial potency in various studies. In our study, we aimed to demonstrate the antibacterial and synergistic activities of naproxen and ciprofloxacin against various Gram-positive and Gram-negative bacteria including, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Klebsiella pneumoniae* ATCC 700603. The results showed promising antibacterial activity against the tested Gram-positive bacteria. However, there was no effect on Gram-negative bacteria. Additionally, checkerboard assay did not reveal any additive or synergistic activity when combined with ciprofloxacin. Collectively, our study's data show naproxen's selectivity against Gram-positive bacteria. This result suggests that naproxen can further be used as a potential source of antibiotics against Gram-positive bacteria.

Keywords

Antibacterial, **c**heckerboard, ciprofloxacin, *Enterococcus faecalis*, naproxen, *Staphylococcus aureus*.

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used mainly in the treatment of pain and inflammation. Naproxen (Figure 1), 2-(6-methoxynaphthalen-2-yl) propionic acid, which is an NSAID and one of the most utilized propionic acid derivatives is used as the first-line treatment of musculoskeletal pain, acute gout, and ankylosing spondylitis. Additionally, it is commonly used to reduce fever, treat headache, muscle and tooth pains, and inflammation caused by several conditions (Stoev et al., 2021).

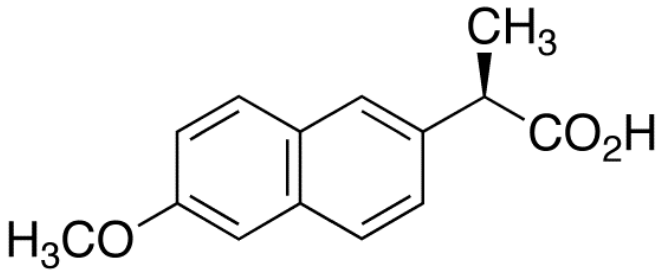

Figure 1: Structural formula of naproxen.

NSAIDs are the widest pharmacological group that has been researched for anticancer and antimicrobial activities (Hasan and Das, 2019). Especially, antibacterial activity has been of importance due to increased resistance to currently available antibiotics and a decrease in the rate of novel antibiotic discovery. Hussein and Al-Janabi (2011) demonstrated a promising antimicrobial activity of naproxen against several microorganisms.

In our study, we aim to reveal the potential antibacterial activity of naproxen, while also demonstrating its possible additive or synergistic activity with ciprofloxacin, one of the most frequently prescribed commercial antibiotics against which the resistance has been increasing worldwide.

MATERIALS AND METHODS

Extraction of naproxen sodium and its conversion to naproxen

The coating of the pills (Apranax Ford, Abdibrahim, Turkiye) was removed using methanol. The pills were then crushed into powder. Following the addition of 15 mL of water to the powder, a filtration process was carried out. At this stage, the filtrate was acidified with HCl. After filtering the precipitate by vacuum filtration, pure naproxen was obtained (Figure 2).

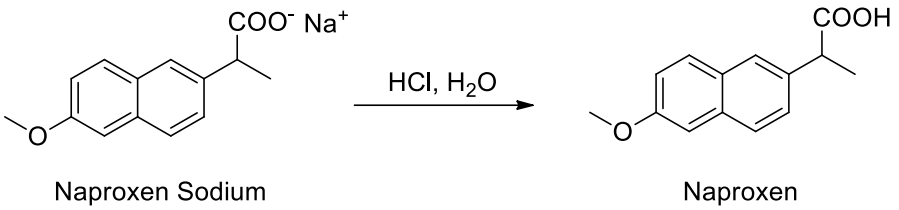

Figure 2: Synthesis scheme of naproxen.

Bacterial inoculum and the sample preparation

Enterococcus faecalis ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Klebsiella pneumoniae* ATCC 700603 were sub-cultured on Mueller-Hinton agar (MHA). The media were incubated at 37 ^{o}C overnight. Upon incubation, strains from individual colonies were inoculated into Mueller-Hinton broth (MHB), and the turbidity was adjusted to 0.5 McFarland standard for each bacterial strain.

The stock solution of the sample was prepared using pure dimethyl sulfoxide (DMSO). For detecting minimum inhibitory concentration (MIC) of naproxen by microdilution test and investigating its interaction with ciprofloxacin by checkerboard assay, the concentration of DMSO of the sample was adjusted to 3% using sterile distilled water.

Microdilution test

MIC determination

MIC was investigated by broth microdilution method (Wikler, 2006). The inocula of four quality control strains were adjusted to 1 x 10^6 cfu/mL using MHB. The final concentration of the sample ranged from 0.125 to 4 mg/mL. Ciprofloxacin was used as the positive control and the highest concentration of sample in MHB was used as the negative control for all tests.

The microplates were incubated at 37 ^{o}C for 18 hours. The MIC was regarded as the minimum concentration of the sample that inhibited the growth of bacteria. MIC was confirmed by addition of 10 µl of 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Formation of blue color after 2 hour incubation at 37 ^{o}C was regarded as bacterial growth.

Minimum bactericidal concentration (MBC) determination

To determine the MBCs of the sample against the tested bacteria, 10 µL of the sample taken from the wells of each of the concentrations that are equal to and greater than MIC were inoculated onto MHA. The media were incubated at 37 ^{o}C for 18 hours. The MBC was regarded as the minimum concentration of the sample that prevented bacterial growth on MHA.

Checkerboard assay

The interaction between naproxen and ciprofloxacin was investigated by checkerboard assay as previously proposed (Bellio *et al*, 2021). The final concentration of the sample ranged from 4 to 0.06 mg/mL, whereas that of ciprofloxacin ranged from 0.001 to 1 mg/L. Ciprofloxacin and the sample in MHB were used as controls. The microplates were incubated at 37 ^{o}C for 18h.

To determine the interaction of the naproxen and ciprofloxacin tested in combination, fractional inhibitory concentration index (FICI) calculation (FICI = A / MICA + B / MICB) was used where 'A' and 'B' are respectively the MIC of ciprofloxacin and naproxen in combination within a single well plate, and MICA and MICB are the MIC of ciprofloxacin and naproxen individually, respectively. The interaction is synergistic at < 0.5, additive between 0.5-0.9, indifference between 1-4, and antagonistic at >4.

Statistical analyses

All of the experiments were performed in triplicates and the data were examined as means ± standard error of mean (SEM). Students t-test was carried out to determine the statistical significance ($p \geq 0.05$).

RESULTS AND DISCUSSION

MIC and MBC determinations

The microdilution method was used to assess MIC of naproxen against *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *K. pneumoniae* ATCC 700603. Naproxen showed promising antibacterial activity against the Gram-positive bacteria*: S. aureus* and *E. faecalis* with 2 mg/mL and 4 mg/mL, respectively (Table 1).

Table 1: MIC values of naproxen and ciprofloxacin against the tested 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)	Naproxen	2 ± 0.33	4 ± 0.67	NC	NC
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). NC: No change.

However, naproxen did not have any antibacterial activity against the tested Gram-negative bacteria (Table 1). Additionally, no bactericidal effect was observed with naproxen against tested Gram-positive bacteria at the MIC concentrations and above. In line with our results, naproxen showed considerable antibacterial activity against various Gram-positive bacteria (Hasan and Das, 2019).

Furthermore, Mamatha *et al*. (2011) synthesized a title compound, by the reaction of naproxen and 4-methylpentan-2-one to evaluate the potential in vitro antibacterial activity against various Gram-positive and Gram-negative bacteria. The results of the title compounds revealed promising antibacterial activity towards various strains of bacteria including *S. aureus*.

Checkerboard assays

Checkerboard assay was not conducted for Gram negative bacteria because no antibacterial activity was detected. FICI was used to assess the interaction of naproxen and ciprofloxacin against the two Gram-positive bacteria: *E. faecalis* and *S. aureus*.

Table 2: FIC of naproxen in combination with ciprofloxacin against *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212.

Samples	Optimal Combination		FIC Index	
	Ciprofloxacin (mg/L)	Naproxen (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 naproxen were used against *S. aureus* and *E. faecalis*, the results revealed an indifference effect. None of the combinations showed any antagonistic activity against any of the tested bacteria as shown in Table 2.

CONCLUSION

Naproxen, an NSAID, is commonly used to reduce fever, and to treat pain and inflammation caused by several conditions. Previously, naproxen was evaluated for its antimicrobial potency in various studies (Hasan and Das, 2019; Han and Kucukguzel, 2020). In parallel to the previous studies, our results reveal promising antibacterial activity of naproxen against Gram-positive bacteria. However, no additive or synergistic activity was observed when combined with ciprofloxacin. Collectively, the data from our study show the selectivity of naproxen against Gram-positive bacteria in terms of antibacterial activity. This result suggests that naproxen can further be used as a potential source for novel antibiotics against Gram-positive bacteria by various modifications and/or combinations.

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