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Antibacterial Activity of Naltrexone and Its Combination with Ciprofloxacin Against Gram Negative and Gram Positive Bacteria

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Abstract

Naltrexone, an opioid receptor antagonist, is commonly used in the treatment of alcoholism. Studies about the antibacterial activity of naltrexone are limited. In our study, we aimed to evaluate the antibacterial and synergistic activities of naltrexone against *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603. The results showed promising antibacterial activity against all tested pathogenic bacteria. Additionally, checkerboard assays revealed additive activity against *S. aureus* when combined with ciprofloxacin. Collectively, the data from our study suggest that naltrexone can further be used as a potential antibacterial source alone or in combination with other antibiotics in the treatment of bacterial infections.

Keywords

Antibacterial, checkerboard, ciprofloxacin, naltrexone.

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INTRODUCTION

Naltrexone is an opioid receptor antagonist that has been used in the treatment of alcohol and opioid dependence (Figure 1). It has been approved by Food and Drug Administration to be used in the therapy of alcohol and opioid use disorders (Lobmaier et al., 2011; Sudakin, 2016).

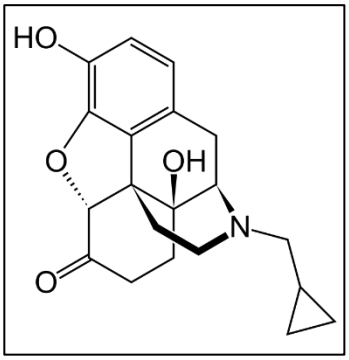

Figure 1: Structural formula of naltrexone.

Naltrexone is the most studied drug in the treatment for alcoholism (Lobmaier et al., 2011). It is commonly used for its ability to block the sedative effects of alcohols and other drugs, while also assisting in the reduction of the cravings towards addictive substances (Lobmaier et al., 2011; Sudakin, 2016). Low-dose naltrexone was reported to be a promising candidate for the off-label treatment of chronic pain due to its modulatory activity on glial cells although clinical trials are still warranted (Younger et al., 2014; Bolton MJ, et al., 2020). Another potential of naltrexone is its beneficial effect on inflammatory diseases such as Crohn's disease, multiple sclerosis, and fibromyalgia that is proposed to be via regulating the secretion of inflammatory cytokines including IL-6 and TNF-α and to provide therapeutic effects on cancers including B cell lymphoma and pancreatic cancer (Cant et al., 2017).

Due to reducing pro-inflammatory cytokine secretion, increasing the secretion of anti-inflammatory cytokines, modulating immune system via decreasing Th1 and Th17 cells, and because of immune-enhancer characteristics, naltrexone was proposed to promote the prevention and management of viral (such as COVID-19) and bacterial infections (El Shehaby et al., 2022). On the other hand, naltrexone was not studied for its direct antibacterial activity.

In this study, we aimed to demonstrate the antibacterial potency of naltrexone and its combination with ciprofloxacin, one of the most frequently prescribed commercial antibiotic against which the resistance has been increasing worldwide.

MATERIALS AND METHODS

Inoculum preparation

The antibacterial activity of naltrexone was investigated against *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Klebsiella pneumoniae* ATCC 700603. The bacteria were subcultured on Mueller Hinton Agar (MHA). The media were incubated at 37 ºC. After 24 hours, 0.5 McFarland (1.5 x 10^8 cfu/mL) standard concentration of each bacterium was prepared within Mueller Hinton broth (MHB).

Minimum inhibitory concentration (MIC) determination

Antibacterial activity of naltrexone was demonstrated by broth microdilution method (Wikler, 2006). The final inoculum of the bacteria in the 96-well plates was 1 x 10^6 cfu/mL and the final concentrations of the sample ranged from 1 to 32 mg/mL. The highest concentration of naltrexone in MHB was used as the negative control and ciprofloxacin was utilized as the positive control. Incubation was conducted for 18 hours at 37 ºC. MIC was accepted as the minimum concentration of naltrexone that prevented the growth of each strain.

Minimum bactericidal concentration (MBC) determination

MBC was accepted as the lowest concentration of naltrexone that killed bacteria. Thus, 10 µL from each well (at the the concentration of MIC and higher) was inoculated on Mueller Hinton agar (MHA). The media were incubated for 18 hours at 37 ºC.

Interaction of naltrexone with ciprofloxacin

Checkerboard assay was used for the evaluation of the interaction of naltrexone with ciprofloxacin as previously described (Bellio et al., 2021). The final concentrations of the sample ranged from 0.5 to 32 mg/mL, whereas ciprofloxacin concentration ranged from 0.002 to 1 mg/L. Incubation was carried out for 18 hours at 37 ºC.

To determine the interaction of the naltrexone and ciprofloxacin in a combination, FIC index calculation (FIC Index = A / MICA + B / MICB) was used; where ‘A’ and ‘B’ are the MICs of each agent in combination within a single well plate; and MICA and MICB are the MICs of each agent individually. The interaction is accepted to be synergistic when FIC index was < 0.5; additive when 0.5-0.9; indifference when 1-4; and antagonistic when > 4.

Statistical analyses

All of the tests were done in triplicates. Statistical analyses were conducted by Students t-test.

RESULTS AND DISCUSSION

Antibacterial activities and combination tests

In order to assess the antibacterial activities of naltrexone, the microdilution method was used to detect MIC against *E. faecalis*, *S. aureus*, *E. coli*, and *K. pneumoniae*. MICs of naltrexone against the tested bacteria are shown in Table 1.

Table 1: MICs of naltrexone against Gram negative and Gram positive 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)	Naltrexone	16 ± 0	16 ± 0	4 ± 0	2 ± 0
Control (mg/L)	Ciprofloxacin	0. 25 ± 0	1 ± 0.083	0.008 ± 0	0.25 ± 0.021

Data represented as the standard error of mean (±S.E.M).

Naltrexone showed promising antibacterial activity against all tested Gram positive and Gram negative bacteria with 16 mg/mL against *S. aureus* and *E. faecalis*, whereas 4 mg/mL against *E. coli*, and 2 mg/mL against *K. pneumoniae*. Furthermore, MBC assays revealed bactericidal effect against *S. aureus*, *E. coli*, and *K. pneumoniae* at 32 mg/mL, 16 mg/mL, and 16 mg/mL, respectively. On the other hand, no bactericidal effect was observed for *E. faecalis* even at the highest tested concentration (32 mg/mL).

Table 2: FIC index of the combinations against Gram positive and Gram negative bacteria.

Samples	Optimal Combination		FIC Index	
	Ciprofloxacin (mg/L)	**Naltrexone (mg/mL)**	**< 0.5**	**> 0.5**
S. aureus	0.125	4		0.625 (A)
E. faecalis	0.06	16		1.12 (I)
E. coli	0.002	4		1.25 (I)
K. pneumoniae	0.06	2		1.48 (I)

A: Additive. I: Indifference.

FIC index assay was used to obtain the interaction of naltrexone and ciprofloxacin against the tested bacteria. When binary combinations of naltrexone and ciprofloxacin were used against the tested bacteria, no antagonistic activity was observed as shown in Table 2. Furthermore, additive interaction was observed when 4 mg/mL of naltrexone and 0.125 mg/L ciprofloxacin was combined against *S. aureus*. The results revealed indifference effect for all other bacterial strains tested.

Naltrexone, especially low-dose naltrexone, is proposed to have antibacterial activity in addition to antifungal, antiviral, anti-helminthic, and immunomodulatory characteristics (Marangalo et al., 2024). Majority of the studies about antimicrobial effect of naltrexone focused on the immunomodulatory effects of the drug. Naltrexone was reported to be promising for management of acute endotoxic shock and viral infections such as COVID-19

because of reducing the secretion of proinflammatory cytokines such as tumor necrosis factor-α and regulating T-helper lymphocyte differentiation (El Shehaby et al., 2022; Greeneltch et al., 2004). Naltrexone was also shown to potentiate anti-HIV-1 activity of antiretroviral drugs in vitro (Gekker et al., 2001). However, studies investigating antibacterial activity of naltrexone in vitro are scarce.

CONCLUSION

Naltrexone, an opioid receptor antagonist, is commonly used in the treatment of alcoholism. The antibacterial results obtained with naltrexone revealed promising antibacterial activity against all tested Gram positive and Gram negative bacteria. Additionally, additive interaction was observed against *S. aureus* when optimal concentrations of naltrexone and ciprofloxacin was used. Collectively, our data suggest that naltrexone can be utilized as a potential source against Gram positive and Gram negative bacteria alone and/or within combinations with other antibiotics.

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