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Evaluation of the in vitro activity of levornidazole, its metabolites and comparators against clinical anaerobic bacteria

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ABSTRACT

This study evaluated the in vitro anti-anaerobic activity and spectrum of levornidazole, its metabolites and comparators against 375 clinical isolates of anaerobic bacteria, including Gram-negative bacilli (181 strains), Gram-negative cocci (11 strains), Gram-positive bacilli (139 strains) and Gram-positive cocci (44 strains), covering 34 species. Minimum inhibitory concentrations (MICs) of levornidazole, its five metabolites and three comparators against these anaerobic isolates were determined by the agar dilution method. Minimum bactericidal concentrations (MBCs) of levornidazole and metronidazole were measured against 22 strains of *Bacteroides fragilis*. Levornidazole showed good activity against *B. fragilis*, other *Bacteroides* spp., *Clostridium difficile*, *Clostridium perfringens* and *Peptostreptococcus magnus*, evidenced by MIC_{90} values of 0.5, 1, 0.25, 2 and 1 mg/L, respectively. The activity of levornidazole and the comparators was poor for *Veillonella* spp. Generally, levornidazole displayed activity similar to or slightly higher than that of metronidazole, ornidazole and dextrornidazole against anaerobic Gram-negative bacilli, Gram-positive bacilli and Gram-positive cocci, especially *B. fragilis*. Favourable anti-anaerobic activity was also seen with levornidazole metabolites M1 and M4 but not M2, M3 or M5. For the 22 clinical *B. fragilis* strains, MBC_{50} and MBC_{90} values of levornidazole were 2 mg/L and 4 mg/L, respectively. Both MBC_{50}/MIC_{50} and MBC_{90}/MIC_{90} ratios of levornidazole were 4, similar to those of metronidazole. Levornidazole is an important anti-anaerobic option in clinical settings in terms of its potent and broad-spectrum in vitro activity, bactericidal property, and the anti-anaerobic activity of its metabolites M1 and M4.

1. Introduction

Levornidazole, the levo isomer of ornidazole, is a third-generation nitroimidazole derivative newly developed after metronidazole, tinidazole and ornidazole. This novel antibiotic has shown good anti-anaerobic and antiprotozoal activity [1]. Levornidazole was approved by the China Food and Drug Administration in August 2009. The ornidazole products used in clinical settings are mostly racemic compounds composed of equal amounts of the L-isomer and D-isomer. In general, the adverse reactions of ornidazole are mainly stomach discomfort, dizziness, somnolence and other adverse reactions of the nervous system [2]. Dextrornidazole is the major component of ornidazole contributing to toxicity of the central nervous system [3]. Levornidazole is similar to or slightly better than racemic ornidazole in terms of pharmacokinetic properties [4]. Early in vitro pharmacodynamic studies also demonstrated that levornidazole has a comparable antimicrobial spectrum and more potent antimicrobial activity compared with ornidazole [5]. However, only a small sample of strains was tested in those studies without identifying each strain to specific species. Therefore, it is important to further investigate the in vitro antibacterial activity of levornidazole.

Studies have shown that five phase I metabolites are derived from ornidazole in animals and human liver. Both 1-chloro-3-(2-hydroxymethyl-5-nitro-1-imidazolyl)-2-propanol (M1) and 2-methyl-5-nitroimidazole (M2) are the oxidative products of ornidazole, whilst 3-(2-methyl-5-nitro-1-imidazolyl)-1,2-propanediol (M4) is produced by hydrolysis of the chloride in the side chain of the imidazole ring. *N*-(3-chloro-2-hydroxypropyl) acetamide (M3) and acetamide (M5) are generated by cleavage of the imidazole ring following hydrolysis of ornidazole [6]. Nanjing Sanhome Pharmaceutical Co., Ltd. (Nanjing, China) has developed the five metabolites of ornidazole (Table 1). The metabolites and metabolic pathway of levornidazole are similar to ornidazole.

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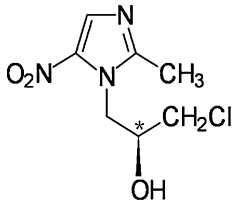
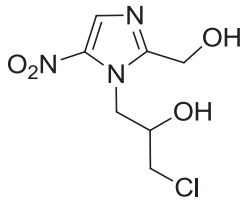
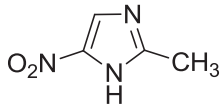
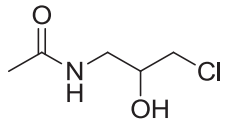
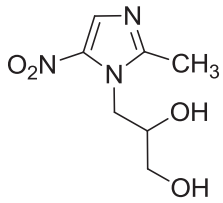
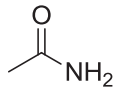
Table 1
Levornidazole and its five metabolites.

Drug	Structural formula	Molecular formula	Molecular weight (g/mol)
Levornidazole		$C_7H_{10}ClN_3O_3$	220
M1		$C_7H_{10}ClN_3O_4$	236
M2		$C_4H_5N_3O_2$	127
M3		$C_5H_{10}ClNO_2$	152
M4		$C_7H_{11}N_3O_4$	201
M5		C_2H_5NO	59

This study aimed to compare the in vitro antibacterial activities of levornidazole, metronidazole, ornidazole, dextrornidazole, and the five metabolites (M1, M2, M3, M4 and M5) of levornidazole to further characterise the antibacterial activity and spectrum of levornidazole. The results will provide useful data for future clinical use and further research of levornidazole.

2. Materials and methods

2.1. Bacterial strains

A total of 375 strains of anaerobes were collected from patients who were treated in Huashan Hospital (Shanghai, China) during the period January 2006 to February 2013. These strains were mainly isolated from blood, faeces, secretions and other specimens and included Gram-negative bacilli (181 strains), Gram-positive bacilli (139 strains), Gram-positive cocci (44 strains) and Gram-negative cocci (11 strains), covering 34 species (Table 2). Overall, 200 of the strains were isolated from faeces, including 72 strains of *Clostridium difficile*, 76 strains of *Bacteroides* spp., 45 strains of *Clostridium perfringens* and 7 strains of other *Clostridium* spp. The 72 strains of *C. difficile* and 7 strains of other *Clostridium* spp. were considered to be from infections, mainly isolated from patients with antibiotic-associated diarrhoea. The 76 strains of *Bacteroides* spp. and 45 strains of *C. perfringens* were isolated from patients with colonisation. Quality control (QC) strains included *Bacteroides fragilis* ATCC 25285, *C. difficile* ATCC 70057 and *Prevotella melaninogenica* ATCC 25845. All QC and reference strains were characterised by morphology as well as physiological and biochemical tests before they were used in this study.

2.2. Culture media

Brucella broth and *Brucella* agar were from Oxoid USA Inc. (Columbia, MD). Laked sheep blood (5% v/v) was provided by Shanghai Zhudi Germ-free Animal Blood Supplier (Shanghai, China). Haemin (5 mg/L) and vitamin K_1 (1 mg/L) were products of Sigma Chemical Co. (St. Louis, MO).

2.3. Antimicrobial agents

Levornidazole, ornidazole and dextrornidazole as well as levornidazole metabolites M1, M3, M4 and M5 were the products of Nanjing Sanhome Pharmaceutical Co. Ltd. Metronidazole and levornidazole metabolite M2 were provided by the Department of