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ORIGINAL ARTICLE

In vitro anti-tuberculosis activity of azole drugs against *Mycobacterium tuberculosis* clinical isolates

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KEYWORDS
Drug resistance;
Imidazole;
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Abstract
Background: Latent tuberculosis has been associated with the persistence of dormant *Mycobacterium tuberculosis* in the organism of infected individuals, who are reservoirs of the bacilli and the source for spreading the disease in the community. New active anti-TB drugs exerting their metabolic action at different stages and on latent/dormant bacilli are urgently required to avoid endogenous reactivations and to be part of treatments of multi- and extensively-drug resistant tuberculosis (M/XDR-TB). It was previously reported that azole drugs are active against *M. tuberculosis*. For that reason, the aims of this study were to determine the *in vitro* activity of azole drugs, imidazole (clotrimazole, CLO and econazole, ECO) and nitroimidazole (metronidazole, MZ and ipronidazole, IPZ), against a collection of MDR *M. tuberculosis* clinical isolates; and to analyze their potential use in both the LTB and the active forms of M/XDR-TB treatments.
Methods: A total of 55 MDR *M. tuberculosis* isolates and H37Rv were included. MZ and IPZ activity against *M. tuberculosis* isolates were tested using anaerobic culture conditions. The activity of ECO and CLO was measured by the minimal inhibitory concentration (MIC) using a microdilution colorimetric method.
Results: MZ and IPZ showed bacteriostatic activity against *M. tuberculosis* strains. MIC_{50} and MIC_{90} to ECO was 4.0 μg/ml, while MIC_{50} to CLO was 4.0 μg/ml and MIC_{90} was 8.0 μg/ml respectively.
Conclusion: All azole compounds tested in the study showed inhibitory activity against MDR *M. tuberculosis* clinical isolates.

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PALABRAS CLAVE
Resistencia a fármacos;
Imidazoles;
Tuberculosis latente;
Nitroimidazoles

Actividad *in vitro* de azoles contra aislamientos clínicos de *Mycobacterium tuberculosis*

Resumen

Introducción: La tuberculosis (TB) latente ha sido asociada a la persistencia de *Mycobacterium tuberculosis* durmientes en el organismo de las personas infectadas, las cuales constituyen un reservorio del bacilo y una fuente de diseminación de la enfermedad en la comunidad. Urge la necesidad de contar con nuevos fármacos antituberculosos con acción sobre el bacilo en estado latente/durmiente, a fin de evitar reactivaciones endógenas y para ser incluidas en el tratamiento de la TB multirresistente y extensivamente resistente (M/XDR-TB). Se ha reportado que los azoles son activos contra *M. tuberculosis*. Por esta razón, los objetivos del presente estudio fueron determinar la actividad *in vitro* sobre aislamientos clínicos de M/XDR-TB de distintos azoles, incluyendo los imidazoles econazol (ECO) y clotrimazol (CLO) y los 5-nitroimidazoles ipronidazol (IPZ) y metronidazol (MZ), así como analizar su potencial uso contra las formas latente y activa de esta enfermedad.

Métodos: Fueron incluidos 55 aislamientos clínicos de *M. tuberculosis* MDR y la cepa de referencia H37Rv. Se evaluó la actividad del MZ y el IPZ sobre los aislamientos en condiciones de cultivo anaeróbico, mientras que la actividad del ECO y el CLO fue estimada determinando la concentración inhibitoria mínima (CIM) mediante el método colorimétrico de microdilución en placa.

Resultados: El MZ y el IPZ presentaron actividad bacteriostática frente a las cepas de *M. tuberculosis*. La CIM_{50} y CIM_{90} del ECO fue de 4 μg/ml, mientras que el CLO presentó una CIM_{50} de 4 μg/ml y una CIM_{90} de 8 μg/ml.

Conclusión: Todos los compuestos azólicos evaluados presentaron actividad inhibitoria frente a aislamientos clínicos de *M. tuberculosis*.

Introduction

Latent tuberculosis (LTB) has been associated with the persistence of dormant *Mycobacterium tuberculosis* in the organism of infected individuals, who are reservoirs of the bacilli and the source for spreading the disease in the community[10].

The Word Health Organization (WHO) estimates that one-third of the world population is infected with *M. tuberculosis* and around 10% might develop the disease during their life[31,32]. In 2013, it was estimated that almost 480 000 new multidrug-resistant tuberculosis (MDR-TB, simultaneously resistant to isoniazid, INH, and rifampicin, RIF) cases occurred worldwide. The extensively drug-resistant TB (XDR-TB) caused by MDR microorganisms with resistance also to an injectable agent (amikacin, kanamycin or capreomycin) and to a fluoroquinolone, has been reported by 100 countries and in accordance with the WHO, around 9.0% of people with MDR-TB have XDR-TB[9].

From year 2000 to 2012, Argentina reported more than 80 XDR-TB cases with 2.2% of incidence rate among new MDR-TB cases[15].

XDR-TB is considered an almost incurable disease and a limited number of active antibiotics that could be used are available. The contacts of M/XDR cases could also be infected by these resistant mycobacteria, developing and spreading the disease in the community. Therefore, new active anti-TB drugs exerting their metabolic action at different stages and on latent/dormant bacilli are urgently required to avoid endogenous reactivations and to be part of treatments for M/XDR-TB.

Azole drugs, such as imidazole, econazole (ECO) and clotrimazole (CLO) are widely used antifungal agents, which are also active against some gram positive bacteria. There are many data on their safety, side effects, pharmacokinetics and pharmacodynamics[4,32]. Furthermore, it has been previously reported that azoles are also active against *in vitro M. tuberculosis* and in the mouse model[2,17,18,24].

The distribution of Cytochrome P450 (CYP) monooxygenases in fast-growing and slow-growing mycobacteria evidence that these enzymes are highly conserved in the genus *Mycobacterium* unlike other bacteria having no CYP, such as *Escherichia coli*. Azole drugs coordinate to the heme iron of CYPs of the microorganism; therefore, they are potent CYP inhibitors. *M. tuberculosis* contains 20 different CYPs[11,12,19,23]. There is no doubt about the activity of some azoles against mycobacteria such us PA824, metronidazole (MZ), delamanid, ECO, CLO and many new molecules of chemical synthesis; however, their mechanism of action is not fully elucidated[20,24]. It has been postulated that the main mechanism of resistance to azoles is the increased drug efflux mediated by the MmpS5-MmpL5 system in *M. tuberculosis*[11,16,24].

Antimicrobial drugs that showed no significant activity against *M. tuberculosis* in aerobic culture condition may be active in the dormant state induced by a drop of the oxygen

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