Jpn. J. Med. Mycol.
Vol. 48, 109－113, 2007
ISSN 0916－4804

Short Report

Antifungal Potential of Disulfiram

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〔Received: 16, August 2006. Accepted: 2, February 2007〕

Abstract

Disulfiram, an alcohol antagonistic drug has been on the market since 1949 with 80% bioavailability and an established safety profile. Recently it has been reported as a P-glycoprotein efflux pump modulator. Herein we report its antifungal potential. The MIC_{50} and MIC_{90} of disulfiram for yeast isolates is 4 and 8 μg/m*l*, respectively, and the MIC range is 1-16 μg/m*l* for both fluconazole sensitive and resistant strains. Interestingly, disulfiram also showed fungicidal activity on *Aspergillus* spp. with MIC_{50} and MIC_{90} of 2 and 8 μg/m*l*, respectively.

Key words: disulfiram, antifungal, fungicidal

Introduction

Life threatening fungal infections have become increasingly prevalent among immunocompromised patients with human immunodeficiency virus, in cancer transplant recipients and in intensive care units[1-3]. Therapeutic options are often limited by the toxicity of currently available systemic antifungal agents and the emergence of resistance[4-7]. This has prompted the development of new antifungal agents, as well as rediscovery and reengineering of existing molecules and their side activities[8, 9].

Various mechanisms which contribute to development of resistance in *Candida* are over expression of/or mutations in the target enzymes and overexpression of drug efflux pumps[10, 11]. This medical problem is escalating and there is an urgent need to combat it.

One of the most frequently employed resistance strategies in both prokaryotes and eukaryotes is the trans membrane-protein-catalysed extrusion of drugs from the cell. P-glycoprotein (P-gp), an ATP driven 170 kd efflux pump, located in plasma membrane is one such example. P-gp can pump out a wide range of cytotoxic drugs, and the high level of resistance is due to its overexpression. To overcome the problem of P-gp mediated efflux, it is important to have chemicals as antichaperones to prevent P-gp maturation and transport. Similar to the P-gp efflux system, there are reports which have demonstrated the presence of an energy dependent drug efflux mechanism in *Candida albicans*[12-14]. According to Prasad *et al.*, the protein encoded by CDR1 gene is thought to encode a drug efflux protein in the ATP binding cassette (ABC) family[15].

Disulfiram (bis (dimethylthiocarbamoyl) disulphide), an alcohol antagonistic drug has been in clinical use for many years (prescription drug information (PDR)). In a recent study, it has been reported that it acts as a modulator of P-gp[16]. According to Sauna *et al.*, disulfiram inhibits ATP hydrolysis and binds to drug substrate binding sites of multiple ABC transporters, which are associated with drug resistance[16, 17]. Further, it is an attractive agent to combat

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multidrug resistance. From this study it was concluded that disulfiram can be used as an efflux pump inhibitor to overcome the resistance of azoles, mainly fluconazole resistant *Candida albicans*. Synergy studies were done during the course of which it was found that disulfiram itself has antifungal potential. Recently, Sauna *et al.* have published a review on disulfiram and its possible antifungal potential[17]. Hence, disulfiram was explored for its antifungal activity against yeast and filamentous fungi.

Materials and methods

Fungal strains

All the isolates of fungi tested were maintained in the Mycology Culture Collection of Ranbaxy Research Laboratories, Gurgaon, India. The fungal isolates were comprised of strains from ATCC and clinical isolates obtained from various medical institutions of India. ATCC strains used were *Candida parapsilosis* ATCC 22019, *C. krusei* ATCC 6258, *C. albicans* 24433, *C. albicans* 90028, *C. parapsilosis* 90018, *C.*

Table 1. WHONET analysis of antifungal activity of disulfiram

Antibiotic name	Number	MIC_{50}	MIC_{90}	Geom. Mean	MIC Range
All fungal isolates					
Fluconazole	61	32	256	32.736	0.25 - 256
Amphotericin B	61	0.25	0.5	0.235	0.03 - 1
Itraconazole	61	0.5	256	0.926	0.008 - 256
Voriconazole	61	0.5	32	0.595	0.008 - 256
Cancidas	61	0.5	32	0.823	0.03 - 32
Disulfiram	61	4	8	3.49	1 - 16
All Yeasts isolates					
Fluconazole	48	16	256	18.755	0.25 - 256
Amphotericin B	48	0.25	0.5	0.239	0.03 - 0.5
Itraconazole	48	0.5	256	1.047	0.008 - 256
Voriconazole	48	0.5	32	0.509	0.008 - 256
Cancidas	48	0.25	1	0.379	0.03 - 32
Disulfiram	48	4	8	3.513	1 - 16
All *Aspergillus* isolates					
Fluconazole	13	256	256	256	256 - 256
Amphotericin B	13	0.25	0.5	0.223	0.06 - 1
Itraconazole	13	0.5	4	0.587	0.125 - 8
Voriconazole	13	0.5	8	1.055	0.125 - 32
Cancidas	13	32	32	14.382	0.25 - 32
Disulfiram	13	2	8	3.409	1 - 16
All *Candida* isolates					
Fluconazole	45	16	256	22.454	0.25 - 256
Amphotericin B	45	0.25	0.5	0.238	0.03 - 0.5
Itraconazole	45	1	256	1.228	0.008 - 256
Voriconazole	45	0.5	32	0.578	0.008 - 256
Cancidas	45	0.25	1	0.324	0.03 - 16
Disulfiram	45	4	8	3.591	1 - 16
***Candida albicans* isolates**					
Fluconazole	18	256	256	30.791	0.25 - 256
Amphotericin B	18	0.125	0.25	0.145	0.03 - 0.5
Itraconazole	18	0.032	256	0.122	0.008 - 256
Voriconazole	18	0.032	128	0.118	0.008 - 256
Cancidas	18	0.25	1	0.411	0.03 - 16
Disulfiram	18	2	4	2.619	1 - 8
***Candida glabrata* isolates**					
Fluconazole	23	16	32	19.758	4 - 256
Amphotericin B	23	0.25	0.5	0.308	0.06 - 0.5
Itraconazole	23	4	256	8	0.5 - 256
Voriconazole	23	1	32	1.72	0.5 - 128
Cancidas	23	0.25	0.5	0.235	0.06 - 1
Disulfiram	23	4	4	4.122	2 - 16

MIC_{50}, MIC_{90} and MIC range is in $\mu g/ml$

Table 2. Antifungal activity of disulfiram (μg/ml) against non-*Candida* yeast isolates (n=3)

Organism	FCZ	ITZ	VRZ	AMB	CAN	DIS
Cr. neoformans I	2	0.06	0.06	0.25	32	1
Cr. neoformans M106	0.25	0.06	0.03	0.25	32	2
H. capsulatum	4	0.25	0.25	0.25	0.06	8

tropicalis 750, *C. glabrata* 90030, *C. albicans* 36082, *Aspergillus fumigatus* 204304, *A. flavus* 204305 and *A. niger* 16404. All the *Candida* isolates were identified using Germ tube assay and API - ATB ID 32 strips, whereas filamentous fungi were identified by making lactophenol-cotton blue slides and using slide culture techniques.

Yeasts were grown on Sabouraud's dextrose agar (SDA) overnight at 37℃ and filamentous fungi were grown on potato dextrose agar (PDA) (both from HiMedia Laboratories Pvt Ltd, Mumbai, India) for 1-2 weeks until spores appeared at 37℃.

Antifungal agents

Fluconazole and voriconazole were synthesized in house (Ranbaxy Research Laboratories, Gurgaon, India), itraconazole (M/S Lee Pharma, Hyderabad, India), cancidas (Merck & Co, INC, NJ 08889, USA), amphotericin B (Sigma) and disulfiram {"Alcobuse" (TO Pharma) and Chronol (Pravin Pharma India)} were procured commercially. Stock solutions of itraconazole (ITZ), voriconazole (VRZ), amphotericin B (AMB) and disulfiram (DIS) were made in dimethyl sulfoxide (DMSO). Fluconazole (FCZ) and cancidas (CAN) were dissolved in distilled water.

Antifungal susceptibility testing

MICs for yeasts and filamentous fungi were determined by the broth microdilution method using RPMI-1640 (Hyclone), pH 7.0 as recommended by the CLSI[18, 19]. The MIC endpoint determinations for azole antifungals and amphotericin B were as per CLSI guidelines. However, in the case of cancidas, MIC endpoint determination was 100% growth reduction in yeast and 50% growth reduction in filamentous fungi[19]. WHONET software version 5.1 was used for determining MIC_{50}, MIC_{90}, geometric mean and MIC range.

The MIC and above wells were spotted on drug free medium for each drug and each isolate and minimum fungicidal concentration (MFC) was determined. MFC is the lowest drug concentration which prevents any growth after spotting, and is presented in μg/ml.

Table 3. Minimum fungicidal activity of disulfiram (μg/ml) against yeasts and *A. fumigatus* isolates

Organism	Disulfiram	
	MIC (μg/ml)	MFC (μg/ml)
C. parapsilosis ATCC 22019	16	16
C. krusei ATCC 6258	4	4
C. albicans ATCC 36082	8	8
C. albicans YO119	4	2
C. albicans 1162	8	8
C. tropicalis ATCC 750	16	16
C. krusei ATCC 766.1	2	2
C. glabrata ATCC 90030	4	4
C. glabrata 1347	4	4
C. glabrata 1348	4	4
Cr. neoformans I	2	2
Cr. neoformans M106	1	1
H. capsulatum	32	32
A. fumigatus 1008	8	8
A. fumigatus 1019	8	8

Results

Disulfiram was screened for its antifungal potential on a spectrum of fungal isolates comprised of fluconazole sensitive and resistant strains of *C. albicans* and non-*albicans* yeasts (*C. glabrata*, *Histoplasma capsulatum*, *C. krusei*, *C. tropicalis* and *Cryptococcus neoformans*) and filamentous fungi. MIC_{50} and MIC_{90} of disulfiram for all the 61 tested fungal isolates were 4 and 8 μg/ml, respectively. However, it was found that MIC_{50} and MIC_{90} of disulfiram is better than the reported standard drugs in the case of fluconazole resistant *C. albicans* (2 and 4 μg/ml) and fluconazole resistant *C. glabrata* (4 and 4 μg/ml) (Table 1). Disulfiram was found to be active on cryptococcal strains whereas cancidas was inactive (Table 2). Further, azoles are fungistatic in nature whereas disulfiram showed fungicidal activity against the tested fungal cultures (Table 3).

Antifungal potential of disulfiram was tested against 13 *Aspergillus* isolates. Like amphotericin B, disulfiram showed fungicidal potential activity with MIC_{50} and MIC_{90} of 2 and 8 μg/ml, respectively.

Discussion

The alcohol antagonist disulfiram blocks the oxidation of alcohol at the acetaldehyde stage during alcohol metabolism. It has been on the market for more than 50 years with a well-established safety profile and 80%

bioavailability. It is well tolerated in humans and a maximum of 500 mg daily is usually administered in a single dose for one to two weeks (PDR information).

Recently, there has been a report suggesting the role of disulfiram as a P-gp efflux pump modulator[16]. According to Sauna *et al.*, disulfiram inhibits ATP hydrolysis and binds to drug substrate binding sites of multiple ABC transporters which are associated with drug resistance, and is thus potentially an attractive agent to combat multidrug resistance[16].

Azoles are fungistatic in nature. Repeated use of fluconazole leads to development of unresponsiveness. The major mechanism of resistance in azoles is due to efflux of the antifungal agent, disulfiram, an alcohol antagonistic, which is well known as a modulator of efflux pumps such as p-glycoprotein and Cdrlp of *C. albicans* should enhance the activity of the tested azoles[16]. Based on this it was thought that disulfiram might play a role in combating fluconazole resistance in *C. albicans* by inhibiting the drug efflux[16]. Hence synergy studies with fluconazole and disulfiram against resistant *C. albicans* and *C. glabrata* isolates were carried out. However, since we did not observe any synergy or enhancement in the activity, probably the mode of resistance may have been due to mutation in 14 alpha demethylase. We have not studied the cause of resistance of these isolates although it was observed that disulfiram itself had significant antifungal potential.

Various fungal pathogens were screened and, as mentioned above, disulfiram showed fungicidal activity with MIC_{50} and MIC_{90} of 4 and 8 μg/m*l*, respectively. Unlike cancidas, which is only cidal for yeasts, it was fungicidal for all the tested fungal isolates.

Disulfiram, well established as an alcohol antagonist, has additional potential as an antifungal agent, which merits further research.

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