Antifungal activity of Taurolidine against Mucorales: An in vitro study on clinical isolates

Hadis Jafarian1, Ali Amanati2-3*, Parisa Badiee1

1Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
2Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3Department of Pediatrics, Division of Pediatric Infectious Diseases, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Article type: Original article

Article History:
Received: 30 October 2021
Revised: 25 January 2022
Accepted: 19 March 2022

* Corresponding author: Ali Amanati
Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; Department of Pediatrics, Division of Pediatric Infectious Diseases, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. Email: ali_amanati_1356@yahoo.com

How to cite this paper
Jafarian H, Amanati A, Badiee P. Antifungal activity of Taurolidine against Mucorales: An in vitro study on clinical isolates. Curr Med Mycol. 2022; 8(1): 26-31. DOI: 10.18502/cmm.8.1.9211

Introduction

Taurolidine (4-[1,1-dioxo-1,2,4-thiadiazinan-4-yl] methyl)-1,2,4-thiadiazinan 1,1-dioxide) is derived from the amino acid taurine which is made naturally within the body. Taurolidine has antifungal, antibacterial, anticoagulant, and potential antiangiogenic activities [1, 2]. Taurultam, taurinamide, and taurine are the main metabolites of taurolidine. Taurinamide molecule generates three methylol-containing fragments (methylol- taurultam, methylol-taurinamide, and taurultam) which are considered to be active derivatives with antibiotic and endotoxin properties through irreversible binding to the cell walls of organisms [3]. Methylol-containing moieties appeared to react with bacterial or fungal cell wall components to prevent the adherence of microorganisms to biological surfaces, such as epithelial cells [1, 4]. Taurolidine has antimicrobial activity against various bacteria and fungi and effectively prevents biofilm formation in central venous catheters [5-7]. To date, no antimicrobial resistance has been observed in vitro [8].

Mucormycosis is a life-threatening opportunistic fungal infection in immunocompromised hosts and certain metabolic diseases such as diabetes [9]. During the COVID-19 pandemic, rhino-orbital mucormycosis reemerged as an opportunistic infection [10-12].

Rhino-orbital, pulmonary, and cutaneous infections are the most common forms of mucormycosis [13-15]. Delayed and inappropriate antifungal therapy is one of the leading causes of morbidity and mortality (ranging from 41% to 52%) in the affected individuals [15-17]. Delayed antifungal therapy (>6 days) is associated with a significant increase (2-fold and even more) in the mortality rate of mucormycosis [18].

There are promising reports on taurolidine treatment of bacterial/fungal catheter-related bloodstream infections, including gram-positive, gram-negative, and Candida infections [5-7]; however, there...
is a lack of data on the anti-Mucorales activity of taurolidine.

This study aimed to investigate the in vitro antifungal activity of taurolidine to evaluate its effects as an antifungal agent against clinical isolates of Mucorales.

Materials and Methods

Clinical isolates

A total of ten previously collected clinical Mucorales isolates were included in this study. The isolates of clinical samples obtained from Namazi and Amir hospitals, Shiraz, Iran, were referred to the mycology department at the Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. Samples were collected from different sites, including sinus and skin, by deep tissue biopsy from clinically symptomatic immunocompromised patients. A total of seven isolates were identified through amplification of the D1/D2 rDNA region and subsequent sequencing. Data were compared to the NCBI nucleotide database and deposited in GenBank (accession number: MZ695808-11, MZ695830, MZ695831, MZ695844).

Antifungal susceptibility testing

Susceptibility testing was performed using the CLSI M38-A2 [19]. All isolates were cultured on Sabouraud dextrose agar before susceptibility testing to ensure viability. Stock spore suspensions were prepared by washing the slant’s surface with 2 mL of sterile saline containing 0.05% Tween 80. Antifungal drugs were obtained from their respective manufacturers as standard powders. Stock solutions of amphotericin B (Sigma-Aldrich, Germany), itraconazole (Sigma-Aldrich, USA), posaconazole (Sigma-Aldrich, Germany), and voriconazole (Sigma-Aldrich, USA) were dissolved in dimethyl sulfoxide (Merck, Germany), and caspofungin (Sigma-Aldrich, USA) was dissolved in sterile water. The drug concentration ranged from 0.03 to 16 µg/mL in all compounds. Serial two-fold dilutions of the various drugs were prepared in Roswell Park Memorial Institute (RPMI) 1640 medium (with L-glutamine, without bicarbonate) (Sigma-Aldrich, USA) and buffered to pH 7.0 using a 0.165 M solution of MOPS (Sigma-Aldrich, USA). Spore suspensions were diluted into RPMI to a concentration of 2×10^4 CFU/mL. MICs were determined in 96-well plates with conidial suspensions in RPMI. Inoculated plates were incubated at 35°C and read visually after 24 and 48 h. For amphotericin B, MIC was checked and recorded at 24 and 48 h to determine the concentration of the drug that elicited complete (100%) growth inhibition. Afterward, MICs were checked and recorded at 24 and 48 h to determine the growth inhibition (50%) for itraconazole, posaconazole, and voriconazole. Minimum effective concentration (MEC; the lowest concentration at which the morphological changes of fungal hyphae could be observed) was used to assess in vitro antifungal susceptibility of caspofungin to Mucorales. The ATCC 22019 strain of Candida parapsilosis was included as a control strain. To determine the Mucorales growth inhibition by taurolidine, NutriLock solution (TauroPharm, Germany) was used, which did not contain citrate or heparin. A serial dilution of taurolidine ranging from 2000 µg/ml to 3.9 µg/ml was prepared in RPMI. The clinical isolates were first cultured on Sabouraud dextrose agar at 35°C. Standard conidial suspensions were prepared at final concentrations of 2×10^6 CFU/mL in RPMI. Subsequently, the conidial suspensions were added to serial dilutions of taurolidine which was previously prepared in RPMI and incubated at 35°C. The inhibitory effect compared with the growth control well was evaluated visually and microscopically after 24 and 48 h. The MIC is defined as the lowest drug concentration at which complete inhibition of growth occurs.

Results

Antifungal MICs for the quality control isolate were within the expected range. The in vitro activity of the five antifungal agents tested against the seven strains is summarized in Table 1. There was a slight difference between MIC data collected after 24- and 48-h incubation. Overall, amphotericin B and posaconazole were the most active drugs against tested organisms. For all the strains, the MIC of voriconazole and caspofungin were estimated at ≥2 µg/ml and 16 µg/ml, respectively.

Table 1. MIC and MEC (µg/mL) at 24 h and 48 h exposure of selected antifungals against tested strains

| No. | Species              | AMP (µg/mL) | CAS (µg/mL) | VOR (µg/mL) | POS (µg/mL) | ITR (µg/mL) |
|-----|----------------------|-------------|-------------|-------------|-------------|-------------|
| 1   | *Rhizopus oryzae*    | 0.032       | 16          | 2           | 0.032       | 0.064       |
| 2   | *Rhizopus oryzae*    | 0.032       | 16          | 2           | 0.032       | 0.125       |
| 3   | *Rhizopus oryzae*    | 0.032       | 16          | 4           | 0.064       | 0.125       |
| 4   | *Saksenia vasiformis*| 0.032       | 16          | 2           | 0.032       | 0.064       |
| 5   | *Rhizopus microsporus*| 0.032     | 16          | 2           | 0.032       | 0.125       |
| 6   | *Rhizopus spp.*      | 0.032       | 16          | 2           | 0.064       | 0.125       |
| 7   | *Rhizopus microsporus*| 0.032     | 16          | 4           | 8           | 8           |
| 8   | *Rhizopus spp.*      | 0.064       | 8           | 8           | 4           | 8           |
| 9   | *Rhizopus spp.*      | 0.5         | 4           | 2           | 8           | 8           |
| 10  | *Rhizopus spp.*      | 0.5         | 8           | 4           | 2           | 8           |

AMP: amphotericin B; CAS: caspofungin; VOR: voriconazole; POS: posaconazole; ITR: itraconazole.

* Minimum Effective Concentration (MEC) is reported
Anti-Mucorales activity of Taurolidine

On visual observation, the growth of isolates was completely (100%) inhibited at a 1000 µg/mL concentration of taurolidine. On microscopic observations, morphological effects on hyphal growth were observed at 500 µg/mL concentration compared to the controls (Figure 1-4). There was not any difference between reading time end-points at 24h or 48h.

Discussion

Antifungal resistance developed rapidly due to the misuse and overuse of these agents [20]. Amphotericin B, posaconazole, and isavuconazole are available choices for treating mucormycosis [21, 22]. Liposomal formulation of amphotericin B is the first-line recommended antifungal, while intravenous isavuconazole and intravenous or delayed-release tablet posaconazole are considered alternative choices, especially in those with preexisting renal diseases [22]. The antifungal MIC distributions of azoles and amphotericin B reported here for members of the Mucorales are similar to those in previous studies [16, 23]. Based on obtained results, taurolidine efficiently inhibited the growth of Mucorales in vitro in this study comparable with other antifungals active against Mucorales. Taurolidine, a taurine derivative, is a known antibacterial adjuvant that is successfully used during surgery in cases of peritonitis to reduce the severity of inflammatory peritoneal adhesions [24], for lavage of the wounds, and difficult-to-treat cases of osteomyelitis [25].

In their study, Bosch et al. estimated a 0.30 pooled incidence rate ratio with a confidence interval of 0.19-0.46 for 918 patients in nine studies that favored taurolidine-containing lock solutions. They also reported mild and scarce adverse events [26]. In another study, Roden et al. reported that removal of central venous catheters due to infection or catheter malfunction occurred less often in the presence of taurolidine-based lock solutions [27].
In addition to the chemical reaction with bacterial cell walls to prevent adhesion of the bacteria to the biological surfaces, taurolidine was documented to have anti-inflammatory activities.

Ezzat et al. reported that the use of taurolidine for temporary hemodialysis catheters was associated with lower inflammation markers, lower incidence of catheter-related bloodstream infections, and better catheter performance [28].

Taurolidine is shown to have anti-interleukin-1 and anti-tumor necrosis factor-alpha activity in in vitro and in vivo studies [29, 30]. In addition, it is effective against catheter-related bloodstream infections, even in the presence of biofilms [31]. Several reports confirmed that taurolidine could successfully prevent central venous catheter microbial colonization and infections, including a wide range of gram-positive and gram-negative pathogens, and some fungi, such as Candida spp. [5, 7, 32, 33].

Taurolidine is a safe antimicrobial agent; however, reversible thrombocytopenia and neutropenia are associated with intravenous use [34]. Moreover, localized pain was when taurolidine was administered to pediatric cancer patients via a peripheral cannula [35]. No other significant side effects have been reported from either intravenous or intraperitoneal use [36]. Taurolidine is compatible with other medications when used concurrently [37].

In the present study, caspofungin demonstrated complete resistance against tested organisms except for one organism, which also demonstrated lower MIC against taurolidine. There are reports of using combination therapy with amphotericin B and either posaconazole or an echinocandin for the treatment of Mucormycosis [15, 16, 38].

Based on our microscopic observation, the taurolidine mechanism of action may in some ways be similar to echinocandins. Taurolidine can bind to cell wall polysaccharides [2, 39]. In our study, short, distended, and balloon-shaped hyphae were observed in 500 µg/mL concentration of taurolidine, which may indicate cell wall disruption. Gorman found that bacterial cells grown in the presence of sub-inhibitory concentrations of taurolidine lost their ability to complete cell division and appeared filamentous [4].

In previous studies, taurolidine solution resulted in the significant killing of Candida albicans [34]. Shah reported the fungicidal activity of taurolidine-citrate solution against Candida albicans at 135 µg/mL concentration after 24 h exposure [40]. Olthof and his coworkers also found that various taurolidine-containing dilutions could prevent the growth of Candida glabrata in vitro [41]. In our study, fungidical activity of taurolidine was observed at 1000 µg/mL concentration, and morphological effects were observed at 500 µg/mL concentration. The effect of taurolidine on fungal structures seems to be concentration-dependent. It was able to eradicate all Mucorales in 1000 µg/mL concentration.

Accordingly, taurolidine could be considered a highly effective wound-compatible antifungal and antibacterial agent. While the consensus on wound antisepsis (last updated in 2018) does not currently recommend taurolidine as effective wound antisepsis, our findings provide new insight regarding taurolidine activity against Mucorales, which could be helpful in the local treatment of difficult-to-treat invasive mucormycosis infection of the skin and soft tissue [42].

Moreover, in another study, topical taurolidine was used against rabbit experimental Staphylococcus aureus keratitis. The results suggested that topical taurolidine was an effective ocular chemotherapeutic agent [43].

Regarding the limitations of this study, one can refer to the lack of access to an electron microscope for a better understanding of structural changes. Probable differences in sensitivity to taurolidine between species can also be evaluated in the future. Moreover, we analyzed relatively few clinical strains; therefore, generalization of the results should be done with caution. However, our findings might help researchers in future studies.

Conclusion

This study was an updated experience of using taurolidine against Mucorales which confirmed the antifungal activity of taurolidine. Our results may have generated valuable data regarding the alternative antifungal agents for the treatment of invasive mucormycosis. However, further clinical studies should be conducted to investigate their potential clinical efficacy against Mucorales infections.

Acknowledgments

Our thanks go to Professor Alborzi Clinical Microbiology Research Center staff for their technical support and assistance.

Authors’ contribution

AA was responsible for the organization and coordination of the trial. AA, HJ, and PB were the chief investigators and were responsible for the data analysis. AA, HJ, and PB conducted the study design. All authors contributed to the writing of the final manuscript and managed/ administered the trial.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in any way.

Financial disclosure

The authors received no financial stable support/ funding for this study.

References

1. Browne MK. Pharmacological and clinical studies on Taurolin: a new concept in antimicrobial chemotherapy for surgical infection. United States: Urban & Schwarzenberg; 1985.
2. Gorman SP, McCafferty DF, Woolfson AD, Jones DS. Reduced
adherence of microorganisms to human mucosal epithelial cells following treatment with Tauroline, a novel antimicrobial agent. J Appl Bacteriol. 1987; 62(4):315-20.

3. Torres-Viera C, Thauvin-Eliopoulos C, Souli M, DeGirolami P, Farris MG, Wennersten CB, et al. Activities of tauroline in vitro and in experimental enterococcal endocarditis. Antimicrob Agents Chemother. 2000; 44(6):1720-24.

4. Gorman SP, McCafferty DW, Woolfson AD, Jones DS. Electron and light microscopic observations of bacterial cell surface effects due to tauroline treatment. Lett Appl Microbiol. 1987; 6(5):103-105.

5. Handrup MM, Moller JK, Schroder H. Central venous catheters and catheter locks in children with cancer: a prospective randomized trial of tauroline versus heparin. Pediatr Blood Cancer. 2013; 60(8):1292-8.

6. Justo JA, Bookstaver PB. Antibiotic lock therapy: review of technique and logistical challenges. Infect Drug Resist. 2014; 7:343-63.

7. Dürmichen M, Seeger K, Lode NH, Kuhl JS, Degenhardt P, Singer M, et al. Randomized controlled trial of tauroline citrate versus heparin as catheter lock solution in paediatric patients with haematological malignancies. J Hosp Infect. 2012; 80(4):304-9.

8. Mermel LA, Parenteau S. Efficacy of the Biolink Catheter Lock Solution (CLSM) for Dialysis® hemodialysis access port and catheters – An in vitro model (abstract). 4th Decennial International Conference on Nosocomial and Healthcare-Associated Infections, March 2000

9. Kwon-Chung KJ. Taxonomy of fungi causing mucormycosis and entomophthoromycosis (zygomycosis) and nomenclature of the disease: molecular mycologic perspectives. Clin Infect Dis. 2012; 51(1):8-15.

10. Mehta S, Pandey A. Rhino-orbital mucormycosis associated with COVID-19. Cureus. 2020; 12(9):1-5.

11. Sharma S, Grover M, Bhargava S, Samdani S, Kataria T. Post coronavirus disease mucormycosis: a deadly addition to the pandemic spectrum. J Laryngol Otol. 2021; 135(5):442-7.

12. Singh AK, Singh R, Joshi SR, Misra A. Mucormycosis in COVID-19: a systematic review of cases reported worldwide and in India. Diabetes Metab Syndr. 2021; 15(4):1-8.

13. Badere P, Choopanizadeh M, Khosravi A. Sequence base identification of respiratory mucormycosis. Jundishapur J Microbiol. 2018; 11(3):1-6.

14. Badere P, Jafarpour Z, Alborzi A, Haddadi P, Rasuli M, Kalani M. Orbital mucormycosis in an immunocompetent individual. Iran J Microbiol. 2012; 4(4):210-4.

15. Patel A, Khan H, Hess L, Michael JS, Savio J, Rudramurthy S, et al. A multicenter observational study on the epidemiology, risk factors, management and outcomes of mucormycosis in India. Clin Microbiol Infect. 2020; 27(7):944-9.

16. Jeong W, Keighley C, Wolfe L, Leng Lee W, Slavin MA, Chen SC, et al. Contemporary management and clinical outcomes of mucormycosis: A systematic review and meta-analysis of case reports. Int J Antimicrob Agents. 2019; 53(5):589-97.

17. Kashkouli MB, Abdolalizadeh P, Oghazian M, Haji A, Ashoori N, Ghazizadeh M, Kashkouli MB, et al. A multicentric study on the epidemiology, risk factors, management and outcomes of mucormycosis in Iran. Iran J Microbiol. 2012; 4(4):210-4.

18. Chamilos G, Lewis RE, Kontoyiannis DP. Delaying amphotericin B–based frontline therapy significantly increases mortality among patients with hematologic malignancy who have zygomycosis. Clin Infect Dis. 2008; 47(4):503-9.

19. Rex JH, Alexander BD, Andes D, Arthington-Skaggs B, Brown SD, Chaturlave V, et al. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard M38–A2. Clin Lab Stand Inst. 2008; 28(16):1-13.

20. Sieradzki J, Melnick LS, Bergan JB, Kersten D, Hargrave J, Tomasz A. Recurrent peritonitis in a patient on dialysis and prophylactic vancomycin. Lancet. 1998; 351(9106):880-1.

21. Tissot F, Agrawal S, Pagano L, Petrikos G, Groll AH, Skienda A, et al. ECIL-6 guidelines for the treatment of invasive candidiasis, aspergillosis and mucormycosis in leukemia and hematopoietic stem cell transplant patients. Haematologica. 2017; 102(3):433-44.

22. Comely OA, Alastreuey A, Azen D, Chen SCA, Dannaoui E, Hochhegger B, et al. Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. Lancet Infect Dis. 2019; 19(12):1045-21.

23. Borman AM, Fraser P, Palmer MD, Szekely A, Houldsworth M, Patterson Z, et al. MIC distributions and evaluation of fungicidal activity for amphotericin B, iraconazole, voriconazole, posaconazole and caspofungin and 20 species of pathogenic filamentous fungi determined using the CLSI broth microdilution method. J Fungi. 2017; 3(2):1-14.

24. Maciver AH, McCall M, James Shapiro AM. Intra-abdominal adhesions: cellular mechanisms and strategies for prevention. Int J Surg. 2011; 9(8):899-904.

25. Blenkhard J. The antibacterial and antifungal activity of tauroline in combination with antibiotics. Surg Res Commun. 1987; 2:149-155.

26. van den Bosch C, Jeremiaas B, van der Bruggen JT, Frakking FNJ, Loeffen YGT, van de Van CP, et al. The efficacy of tauroline containing lock solutions for the prevention of central-venous-catheter-related bloodstream infections: a systematic review and meta-analysis. J Hosp Infect. 2022; 123:143-55.

27. van Roeden S, van Oevelen M, Abrams JC, Dekker FW, Rotmans JL. The best solution down the line: an observational study on tauroline-versus citrate-based lock solutions for central venous catheters in hemodialysis patients. BMC Nephrol. 2021; 22(1):1-8.

28. Ezzat H, Elsharkawy M, Rezk K, Mohsen R, Esmara A. Effect of tauroline citrate and unfractionated heparin on inflammatory state and dialysis adequacy in hemodialysis patients. J Vasc Access. 2021:1-7.

29. Bedrosian I, Sofia RD, Wolff SM, Dinarello CA. Tauroline, an analogue of the amino acid taurine, suppresses interleukin 1 and tumor necrosis factor synthesis in human peripheral blood mononuclear cells. Cytokine. 1991; 3(6):568-75.

30. Monron JR, Ramsey PS, Donohue JH. Tauroline inhibits tumour necrosis factor (TNF) toxicity—new evidence of TNF and endotoxin synergy. Eur J Surg Oncol. 1993; 19(3):226-31.

31. Percival SL, Kite P. Intravascular catheters and biofilm control. J Vasc Access. 2007; 8(2):69-80.

32. Trible S, Brandt CF, Petersen AH, Petersen JH, Fuglsang KA, Staun M, et al. Tauroline-citrate-heparin lock reduces catheter-related bloodstream infections in intestinal failure patients dependent on home parenteral support: a randomized, placebo-controlled trial. Am J Clin Nutr. 2017; 106(3):389-48.

33. Gudiol C, Nicolae S, Royo-Cebrecos C, Aguilar-Guisado M, Montero I, Martín-Gandul C, et al. Administration of tauroline-citrate lock solution for prevention of central venous catheter infection in adult neutropenic haematological patients: a randomised placebo-blinded, placebo-controlled trial (TAURCAT). Trials. 2018; 19(1):264-73.

34. Sherritz RJ, Boger MS, Collins CA, Mason L, Raad II. Comparative in vitro efficiencies of various catheter lock solutions. Antimicrob Agents Chemother. 2006; 50(5):1865-8.

35. Simon A, Annmann RA, Wisniewsky G, Bode U, Fleischhack G, Besuden MM. Tauroline-citrate lock solution (TauroLock) significantly reduces CVAD-associated grampositive infections in pediatric cancer patients. BMC Infect Dis. 2008; 8(1):1-8.

36. Koldehoff M, Zakrzewski JL. Tauroline is effective in the treatment of central venous catheter-related bloodstream infections in cancer patients. Int J Antimicrob Agents. 2004; 23(4):491-5.

37. Johnston DA, Phillips G, Perry M, McAlpine H, Richards J, Pennington CR. Tauroline for the prevention of parenteral nutrition related infection: antimicrobial activity and long-term use. Clin Nutr. 2017; 36(3):858-64.

38. Gargouri M, Marrakkchi C, Feki W, Charfi S, Maaloul I, Lahiani D, et al. Combination of amphotericin B and caspofungin in the treatment of mucormycosis. Med Mycol Case Rep. 2019; 26:32-7.

39. Caruso F, Darnowski JW, Opazo C, Goldberg A, Kishore N, Agoston ES, Rossi M. Tauroline antiadhesive properties on interaction with E. coli; its transformation in biological
environment and interaction with bacteria cell wall. PLoS One. 2010; 5(1):1-10.

40. Shah CB, Mittelman MW, Costerton JW, Parenteau S, Pelak M, Arsenaul R, et al. Antimicrobial activity of a novel catheter lock solution. Antimicrob Agents Chemother. 2002; 46(6):1674-9.

41. Olthof ED, Nijland R, Gulich AF, Wanten GJA. Microbicidal effects of various taurolidine containing catheter lock solutions. Clin Nutr. 2015; 34(2):309-14.

42. Kramer A, Dissemond J, Kim S, Willy C, Mayer D, Papke R, et al. Consensus on Wound Antisepsis: Update 2018. Skin Pharmacol Physiol. 2018; 31(1):28-58.

43. Oguz H, Ozbilge H, Oguz E, Gurkan T. Effectiveness of topical taurolidine versus ciprofloxacin, ofloxacin, and fortified cefazolin in a rabbit *Staphylococcus aureus* keratitis model. Curr Eye Res. 2005; 30(3):155-61.