REPORT

In vitro evaluation of vincristine and fluconazole combination against *Candida*

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Abstract: Infections associated with cancer are a major scourge and cause of substantial morbidity and mortality in cancer patients. The aim of present study was to appraise the in vitro activity of anticancer agent vincristine and antifungal fluconazole alone and in combination against *Candida* spp. Results were interpreted in terms of fractional inhibitory concentration index (FICI). Antifungal activity of fluconazole showed marked synergism when used in combination with vincristine, with FICI ranging from 0.25-0.5 against different Candida spp. Although, the use of vincristine with fluconazole is always disputed due to its side effects including decreased peristalsis, but the present research can help to perform suitability analysis of fluconazole use in life threatening invasive candidiasis associated with cancer patients. In addition, the synergism in antifungal activity after using with vincristine also warrants further research in the direction of minimizing adverse reaction associated with combined use of fluconazole and vincristine.

Keywords: Acute lymphoblastic leukemia, vincristine, fluconazole, cancer chemotherapy, invasive candidiasis.

INTRODUCTION

Patients with hematological malignancies are at high risk of developing invasive fungal infections (Gerson *et al.,* 1984). These infections present life-threatening complications in cancer patients. Several factors are known to increase possibility of these infections including perpetuated neutropenia, cytotoxic chemotherapy and administration of broad-spectrum antibiotics and corticosteroids (Cupps and Fauci, 1982; Gerson *et al.*, 1984; Laverdiere *et al.*, 2000; Martino and Subira, 2002). Invasive fungal infections cause substantial morbidity and mortality in patients undergoing cancer chemotherapy despite advances in supportive care (Abbasi *et al.*, 1999; Viscoli *et al.*, 1999), this situation offers urgent need for diagnosis and selection of proper antifungal therapy for these infectious complications. Among these infections, *Candida* is a major organism involved in causing secondary infectious complications in cancer patients (DiNubile *et al.,* 2005). It has been reported as the fourth most common cause of hospital associated bloodstream infection in US and cause substantial mortality in hospitalized patients (Wey *et al.*, 1988; Wisplinghoff *et al.*, 2004). Moreover, *Candida* is also a major pathogen among cancer patients. It has been estimated that, candidemia was involved in about two-thirds of all deaths in infected cancer patients with either a primary or a secondary role in all cancer deaths (Viscoli *et al.,* 1999).

Management of invasive *Candida* infection involves frequent use of triazoles derivative including fluconazole, voriconazole and amphotericin B and its lipid formulations as well as echinocandins (Bohme *et al.,* 2009) but frequent association of invasive *Candida* infection with hematological malignancies offers a situation of simultaneous use of cytotoxic drug like vincristine, doxorubicin and methotraxate and enzyme asparaginase to manage cancer (Tobias and Hochhauser, 2010; Shrivastava *et al.*, 2010).

Vincristine is a mitotic inhibitor, widely used for management of pediatric oncology cases (Moore and Pinkerton, 2009). However, abovementioned complication of cancer associated invasive candidiasis requires strict monitoring, rapid and effective treatment without any drug toxicity as well as surgical interventions, if necessary. The situation of cancer associated invasive candidiasis urges for the simultaneous use of anticancer and antifungal management practices, but unexpected side effects of simultaneous drug use may reverse any putative benefits. Despite this, some combinations may boost the antifungal or anticancer management practice by providing synergistic effect on the activity. Furthermore, the use of drug combinations warrant large and expensive clinical trials in order to assess efficacy of given combination, though often limited without the availability of primary preclinical data.

The use of fluconazole is often avoided in combination with vincristine due to its effect on decrease peristalsis in intestine (Harnicar *et al.,* 2009), but life threatening invasive candidiasis requires the use of azole drugs, and

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several options for intermittent use of fluconazole and vincristine are recommended (Harnicar *et al.,* 2009).

Present study tries to analyze effect on antifungal activity of fluconazole when used in combination with vincristine against *Candida* spp. Consequently, the possible drug interaction can pave the way for developing suitable modalities for management of invasive candidiasis in cancer patients.

MATERIALS AND METHODS

Candida isolates

Seven *Candida* spp. including *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis* were used in present study. These species of *Candida* offers highest infectious complication in immunocompromised and cancer patients and thus selected for study (Pfaller and Diekema, 2007; Shokohi *et al*., 2010). Among these, *C. albicans ATCC 10231*, *C. tropicalis ATCC 66029* and *C. parapsilosis* 22019 were procured from American type culture collection (ATCC). Moreover, other *Candida* isolates were collected from King Khalid University Hospital (Riyadh, Saudi Arabia). Isolates were derived from intensive care unit patients. All isolates were grown on Sabouraud dextrose agar media and were preserved at 4^0C till the use.

Antifungal and anticancer drug

Standard powder of fluconazole was obtained from Pfizer Pharmaceutical Group, New York, N.Y., USA. Vincristine was supplied by Hospira UK Ltd. Stock solution of fluconazole was prepared in sterile distill water (5mg/1ml), while vincristine was available as vincristine sulphate (concentration 1mg/ml in distill water) from manufacturer. RPMI 1640 broth (Gibco) with L-glutamine and without sodium bicarbonate was used to perform in vitro antifungal activity of fluconazole. RPMI 1640 medium was buffered with 0.165M MOPS [3-(N-morpholino) propanesulfonic acid] to achieve final pH of media as 7.0. Fluconazole and vincristine was further diluted two fold in RPMI 1640 broth, with concentration ranging from 0.25-256µg/ml (for fluconazole) and 0.25-16 µg/ml (for vincristine).

In vitro activity of drug combinations against Candida

Minimum Inhibitory Concentration (MIC) for *Candida* isolates were determined by microdilution method as per the protocol of CLSI [M27-A3 CLSI 2008] against fluconazole (CLSI, 2008). Chequerboard test (Cuenca-Estrella, 2004; Tobudic *et al.*, 2010) was used to determine the efficacy of drug combinations against *Candida* spp. Ninety six wells micro titre plate (Corning) was added with the fluconazole concentration ranging from 0.25 to 256 µg/ml from left to right 11 wells, while the vincristine concentration of 0.25 to 16 was added to 7 wells up to down. The last well in each direction was kept as drug free control. *Candida* isolates were grown in Saboraud dextrose broth for 24 hours. The concentration of *Candida* was adjusted to $0.5\text{-}2.5\times10^3$ cells/ml in RPMI 1640 by using hemocytometer. The 100 µl of *Candida* suspension was inoculated in each well. MIC_{50} was determined by 50% reduction in absorbance (OD) compared with drug free control. OD was measured at 595nm using plate reader (multiscan EX2 LabSystem).

Effect of drug combination

Effect of drug combination was calculated on the basis of fractional inhibitory concentration index (FICI). Formula used for calculation of FICI was: FICI=Fc/Fa + Vc/Va, where Fc and Vc are MIC of fluconazole vincristine drug combination respectively (Tobudic *et al.,* 2010). Fa and Va are individual MIC of fluconazole and vincristine respectively. The interaction was defined as synergistic if the FICI was ≤0.5, indifferent, if the FICI was >0.5-≤4, & antagonistic if the >4. The ratio of Vc/Va was taken as 0 due to no effect of vincristine alone on *Candida* growth.

RESULTS

Results of experiment show decreased MIC of fluconazole except with *C. albicans ATCC 10231*, when used in combination with vincristine. The results of experiment with resultant MIC are presented in table 1. Present study found that this combination could be more deadly for invasive candidiasis, in comparison to the therapy by sole drug. However, we have found clear synergism in activity of fluconazole, while used in addition to vincristine, but the *C. albicans ATCC 10231*

Table 1: Inhibitory activity of fluconazole alone and in combination with vincristine against Candida species

Organism	MIC_{50} of fluconazole alone (Fa)	MIC_{50} of fluconazole in combination with vincristine (Fc)	Lowest concentration of vincristine required to achieve MIC_{50} (Vc)	FICI (Fractional inhibitory concentration index)
C. albicans 01	0.5 µg/ml	0.25 µg/ml	1 µg/ml	0.5
C. albicans ATCC 10231	8 µg/ml	8 µg/ml	No effect	1
C. glabrata 01	2 µg/ml	0.5 µg/ml	2 µg/ml	0.25
C. glabrata 05	4 µg/ml	2 µg/ml	0.25 µg/ml	0.5
C. glabarata 07	2 µg/ml	0.5 µg/ml	0.25 µg/ml	0.25
C. tropicalis ATCC 66029	1 µg/ml	0.5	2 µg/ml	0.5
C. parapsilosisATCC 22019	2 µg/ml	1 µg/ml	0.25 µg/ml	0.5

strain did not showed any effect of anticancer drug on fluconazole efficacy. The possible reason behind this distinct behavior may lie in the fact that, the same strain showed 2µg/ml MIC in previous study (Sabra *et al.,* 2010), but strain became refractory to this antibiotic after repeated sub-culturing (Tobudic, 2007). This fact deserve the attention, that such combination should be in vitro tested on respective patients isolate, before prescribing any further antifungal therapy in combination with other drugs.

DISCUSSION

Vincristine is an elemental component for the management of acute lymphoblastic leukemia (ALL). It is considered as principle drug for the induction and consolidation phase of disease. ALL patients frequently encounter invasive *Candida* infection, this secondary complication offer a situation of use of simultaneous management of oncologic and infectious complications in patients. Simultaneous use of drugs may have various possible interactions ranging from beneficial to harmful consequences. Certainly, the isolates from patients should be tested in vitro for interaction in the laboratory to evaluate efficacy of these combination. Such tests can direct the future drug therapy. Although, clinical data on the effect of fluconazole on vincristine therapy is available (Harnicar *et al.*, 2009), but the vice versa effect of vincristine on antifungal potential of fluconazole is lacking. We evaluated greater antifungal activity of fluconazole in combination with vincristine.

The major hurdle in the combined use of above-mentioned drug is the toxicity associated with vincristine. Vincristine is an inhibitor of microtubule formation in the mitotic spindle. Vincristine induced inhibition of microtubule causes cancer cell death but also induces toxicities including neuropathy, paresthesias, sensory deficit, and muscle weakness etc. (McCune and Lindley, 1997). In addition to this, gastrointestinal toxicities of vincristine can also manifest due to decrease peristalsis by inhibition of CYP 3A4 enzyme. CYP 3A4 enzyme is encoded by CYP3A4 gene, which is a part of a collection of cytochrome P450 gene on chromosome 7q21.1 (Hashimoto *et al.*, 1993; Inoue *et al.*, 1992) and is involved in avoiding toxicity by metabolizing vincristine (Dennison *et al.,* 2006). Fluconazole act as CYP3A4 inhibitor and increases vincristine toxicity. Despite this adverse reaction, azoles are frequently being used for prophylaxis and management of fungal infections in such patients due to its great potential in management of *Candida* infection.

The detailed in silico and laboratory tests are required to develop azole derivates, for such patients, that are weak or non-inhibitor of CYP 3A4 enzyme. Perhaps the common eukaryotic cellular organization of both fungi and cancer cells make the reason for this marked synergism in antifungal activity of fluconazole. Similar studies on other drugs can reveal some contrasting findings on use of cytotoxic anticancer drugs on fungi.

The study may be helpful in finding better combination of antifungal agents and chemotherapy regimens to manage cancer associated invasive candidiasis with less adverse reactions. Evolutionary relationships in cancer and fungal cells in terms of eukaryotic cellular organization make such test as a source for additional knowledge about management of cancer associated invasive candidiasis. Invasive *Candida* infections in cancer patients are associated with high mortality due to its delayed sufficient treatment (Garey *et al.,* 2006).

Our study indicates about positive points of using fluconazole and vincristine simultaneously, despite secondary complications associated with this combination. Furthermore, this study can be helpful for suitability analysis of these two drugs in life threatening *Candida* infection in cancer patients. Due to marked synergism of antifungal activity of fluconazole with anticancer agent vincristine, it is recommended to perform some structure activity relationships (SAR) to avoid these possible side effects of chemotherapy. Such study may invigorate about further understanding of fluconazole vincristine drug interaction with the aim of reducing its adverse complications, as this combination may be a better therapeutic regimen for cancer patients associated life threatening invasive candidiasis. Summarily it can be concluded that due to common eukaryotic nature of *Candida* and cancer cells, many other drugs are looking for a legitimate appraisal for the their use in conjunction with anticancer therapy, which is otherwise also be useful for prophylaxis and management of cancer associated invasive fungal infections.

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