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RECENT TREATMENT OF MUCORMYCOSIS – A REVIEW

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1. INTRODUCTION

Mucormycosis is triggered by hyaline moulds, which are recognized as Mucorales in the *Mucoromycotina*, a subphylum of lower fungi. Black Fungus is another name for it. Mucorels is made up of 11 genera and 27 species, all of which have been connected to human disorders. *Rhizopus arrhizus* is the most prevalent cause of Mucormycosis, followed by *Apophysomyces*, *Lichtheimia*, *Rhizomucor*, Mucor, and *Cunninghamella species*. The illness produced by these fungi was described as zygomycosis, Mucormycosis, a well-known name in clinical medicine, is an acceptable term to use when referring to the disease caused by fungus.[1,2,3]

1.1 History

Paltauf, a German pathologist, reported the very first case of Mucormycosis in 1885 and termed it Mycosis Mucorina. During the 1980s Mucormycosis have become more common in the late 1980s and early 1990s.individuals with poor immune systems.[5]

A study was conducted based on the prevalence rate.7.4 percent amplification was reported in France per annum.[6]

1.2 Microscopic examination and culture

The organisms can befound on decaying vegetation and in the soil and are common in nature These mushrooms reproduce quickly and produce a high number of spores that can travel thr ough the air. Mucormycosis is an uncommon human infection, demonstrating the human immune system's effectiveness. This is further supported by the finding that almost all human infections due to the agents of Mucormycosis occur in the presence of some underlying compromising condition. The genera most commonly found in human infections are Rhizopus, Mucor, and Rhizomucor; Cunninghamella, Absidia (now reclassified as

Lichtheimia), Saksenaea, and Apophysomyces are genera that are less commonly implicated in infection.[7]

Mucorales hyphae are unique, allowing for presumptive identification of clinical specimens. The hyphae are large (5 to 15 µ in diameter), irregularly branching, and lack septations. *Ascomycetous* moulds, such as *Aspergillus*, contain hyphae that are narrower (2to5 µ diameter), have regular branching, and numerous septations. The lack of regular septations may contribute to the fragile nature of the hyphae and the difficulty of growing the agents of mucormycosis from clinical specimens. Grinding clinical specimens can cause excessive damage to the hyphae. Thus, finely mincing tissues is preferred for culturing tissue samples that may contain molds.

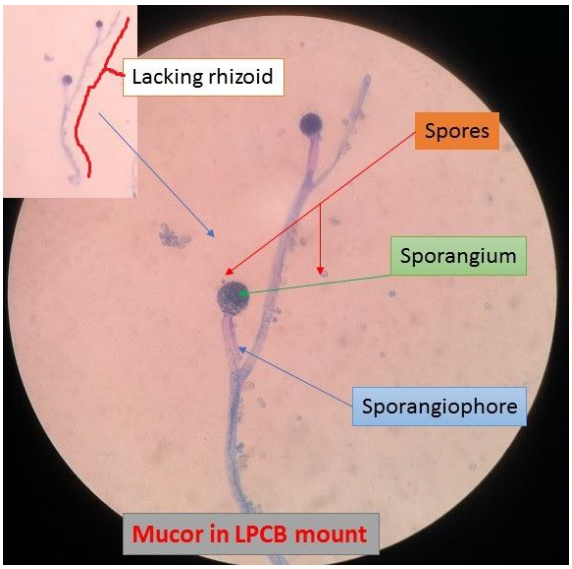


Fig no.1: laboratory diagnosis of mucorels.

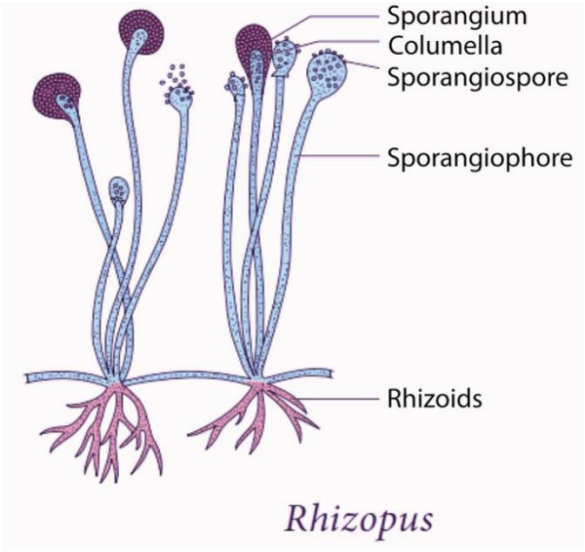


Fig no.2: Rhizopus.

2. SPECIES IDENTIFICATION AND ANTIFUNGAL SUSCEPTIBILITY TESTING

Identification of species is important for gaining a better epidemiological understanding of mucormycosis and could be useful in epidemic investigations. On culture, Mucorales fungi can easily be distinguished from *Aspergillus* fungi. Morphological characteristics are important. When evaluated by people who are experts in fungal identification, they can provide a high level of accuracy.[8] The identification of morphological species, on the other hand, is challenging. It's possible that it's linked to speciation failures.[9]

The ID32C kit has been successfully used for the identification of *Lichtheimiacorymbifera* and R. pusillus, and API 50CH[10] has been successfully used for the identification of

Lichtheimiacorymbifera and R. pusillus. Mucor genus. M. circinelloides and M. rouxii were not successful.

Neither test will be able to identify them. *L. ramosa* is detected using ID32C and positive melezitose assimilation.[11] MALDI-TOF mass spectrometry (matrix-assisted laser desorption/ionisation time-of-flight) is a promising technology, however it is not yet widely used. All Mucorales have been validated.

Fig no. 3: API 50 CH kit.

Another dependable strategy is through use of molecular-based assays with an emphasis on the transcribed internal spacer region. *M. circinelloides* has a high minimum inhibitory concentration (MIC) for posaconazole, as well as Rhizopus and Cunninghamella for amphotericin B.

3. MOLECULAR ASSAY

Conventional polymerase chain reaction (PCR), restriction fragment length polymorphism examination (RFLP), DNA sequencing of defined gene regions, and melt curve analysis of PCR products are all examples of molecular based tests. All of the mentioned assays can be used to detect or identify Mucorales signals. The great majority of molecular experiments focus on the internal transcribed spacer or the 18S rRNA genes. Various research using formalin-fixed, paraffin-embedded, or fresh tissue samples have yielded varying result. The examinations carried out have varying degrees of sensitivity (70–100%) and specificity (not estimated to be 100%), with the superior disadvantages being the small number of patients examined. Because the efficacy of these inhouse tests has not been properly investigated and clinically evaluated, they cannot be recommended as a standalone, single strategy in clinical

routine diagnostics. The use of molecular markers in blood and serum to identify illnesses has shown remarkable therapeutic results in recent years. Molecular-based identification from serum resulted in earlier diagnosis and overall verified culture-proven occurrences when compared to culture. Molecular-based diagnostic tests can already be recommended as beneficial add-on tools to support conventional diagnostic methods.[12-17]

4. ETIOPATHOGENESIS

Mucorales attack deep tissues by means of ingestion or inhalation of spores, and percutaneous injection of spores. The initial line of defense in a healthy host is capable of killing spores via oxidative metabolites and cationic peptides as soon as the spores reach lung or skin tissues. Uncontrolled diabetes mellitus, particularly ketoacidosis, steroid use, extremes of age, neutropenia; occur in patients with hematological malignancy, AIDS, renal insufficiency, organ or stem cell transplantation, iron overload, skin trauma, broad-spectrum antibiotics, intravenous drug abuse, prophylactic voriconazole for aspergillosis, and malnutrition are all risk factors.

In diabetic patients, mucormycosis occurs as a destructive and potentially critical condition due to augmented availability of micronutrients and diminished defense mechanism of the body.[18,19] Various hypotheses include.

(i) Low serum inhibitory activity against Rhizopus species,

(ii) Improved availability of iron for the pathogen at decreased PH level and

(iii)Pulmonary macrophages of persons with diabetes mellitus show diminished facility to inhibit germination of Rhizopus species.

Ketone reductase in Rhizopus allows the organism to increase the glucose and acidic environment. In DM particularly with ketoacidosis Mucorales attack deep tissues by means of ingestion or inhalation of spores, and percutaneous injection of spores. As soon as the spores penetrate into lung or cutaneous tissues, the first line of defense in the healthy host is capable of destroying the spores via oxidative metabolites and cationic peptides. Risk factors include uncontrolled diabetes mellitus, especially ketoacidosis, steroid use, extremes of age, neutropenia; especially with hematological malignancy, AIDS, renal insufficiency, organ or stem cell transplantation, iron overload, skin trauma, broad-spectrum antibiotics, intravenous drug abuse, prophylactic voriconazole for aspergillosis and malnutrition.[20,21,22]

5. DIAGNOSTIC METHOD

Diagnosis of mucormycosis includes cautious evaluation of clinical manifestations, magnetic resonance imaging modalities, utilization of computed tomography (CT) in the early stages, specialist assessment of cytological and histological provision, finest application of clinical microbiological technique and execution of molecular detection. Detection of host factors contributes extensively to the estimation of a patient's possibility for invasive mucormycosis. PAS stains, direct examination, calcofluor, histopathological examination, Gomori methenamine silver stain, culture, molecular methods and fluorescent in situ hybridization are the various laboratory techniques for detecting mucor. A key challenge in the detection of mucormycosis includes its indefinable clinical presentation and repeated distribution, and consequently a need for a sensitive nonculture-based investigative method is necessary.[23]

6. CLINICAL PRESENTATION AND MANIFESTATION

There are two types of Mucormycosis infection in human

1. Superficial and visceral,
2. Are localized and disseminated.

External ear, finger, nails, and skin are examples of the superficial form.

Pulmonary, gastric, and rhino cerebral kinds are examples of visceral forms. These spores might enter the body through the cutaneous or respiratory routes. (For example, spores spread when contaminated food is consumed or tainted needles are used).[24]

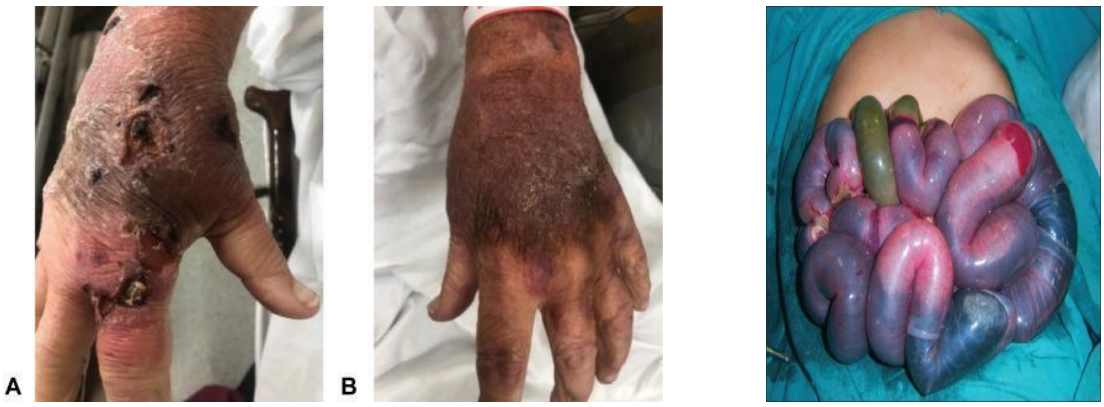


Fig no. 4: Cutaneous mucormycosis. **Fig no. 5: Gastrointestinal mucormycosis**

7. PRINCIPLE OF TREATMENT OF MUCORMYCOSIS

The treatment of mucormycosis is based on a combination of therapies that take place at the same time or at different times and with varying severity. The basic principles of

mucormycosis treatment include risk stratification for disease severity and aggressive efforts for early, clinical and laboratory diagnosis; timely initiation of an effective antifungal therapy (monotherapy or combination therapy) along with aggressive surgical debridement of necrotic lesions; and reverse of immunosuppression (discontinuation of chemotherapy and increase in immunoglobulin levels).Early detection and treatment may avoid progressive tissue invasion, as well as lessen the need for significant surgery and consequent deformity, and enhance survival.[25] In a trial of 70 patients with hematological malignancies and mucormycosis, delaying antifungal medication for 6 days after diagnosis resulted in a 2-fold increase in mortality rate (82.9 percent vs. 48.6 percent).[26] Because untreated mucormycosis is always fatal, no therapy is not an option.

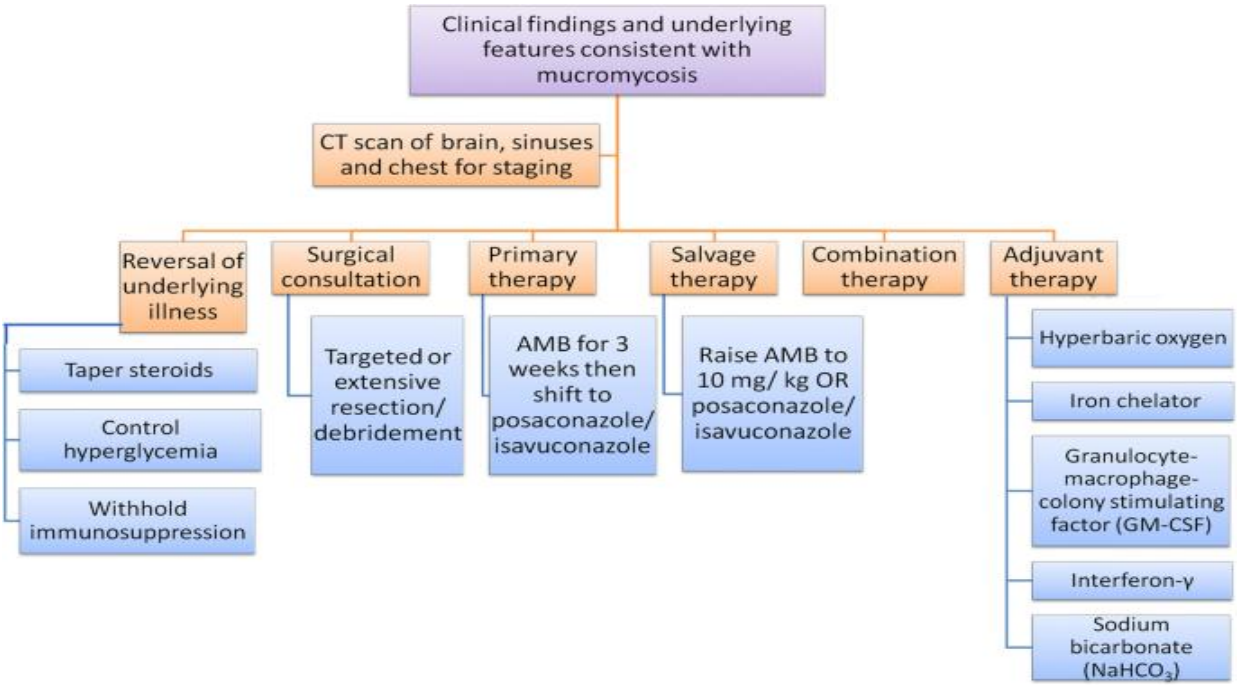


Fig. 6: Underlying consistent with mucormycosis.

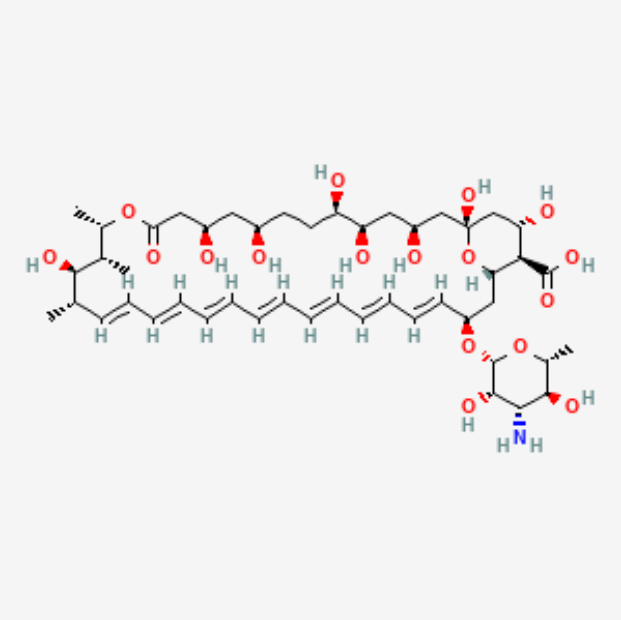
8. RECENT AND NEW ANTIFUNGAL IN MUCORMYCOSIS.

Drugs used in treatment.

Sr. No.	Class of Drug	Drug
1.	Amphotericin	Liposomal Lyophilized Lipid Deoxychelate Sonication
2.	Isavuconazole	
3.	Posaconazole	

8.1 Amphotericin B

8.1.1 Chemical structure: $C_{47}H_{73}NO_{17}$



8.1.2 IUPAC

(1*R*,3*S*,5*R*,6*R*,9*R*,11*R*,15*S*,16*R*,17*R*,18*S*,19*E*,21*E*,23*E*,25*E*,27*E*,29*E*,31*E*,33*R*,35*S*,36*R*,37*S*)-33-[(2*R*,3*S*,4*S*,5*S*,6*R*)-4-amino-3,5-dihydroxy-6-methyloxan-2-yl]oxy-1,3,5,6,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabicyclo[33.3.1]nonatriaconta-19,21,23,25,27,29,31-heptaene-36-carboxylic acid

8.1.3. Mechanism of Action

Most fungi's cell membranes include ergosterol, which amphotericin B binds to. It stimulates the development of ion channels after interaction with ergosterol, which leads to the loss of protons and monovalent cations, resulting in depolarization and concentration-dependent cell death. Moreover, amphotericin B causes oxidative damage to cells by forming free radicals, which results in increased membrane permeability. Amphotericin B also has a stimulatory impact on phagocytic cells, which aids in the clearance of fungal infections. Amphotericin B has a half-life ranging from 24 to 15 days.[31,32]

8.1.4. Dose

The daily dose varies depending on the kind of infection, the organ implicated, and the host (immunocompetent versus immunocompromised), and ranges from 0.7 to 1 mg/kg each day, administered over 2 to 4 hours as tolerated**.** Amphotericin B is amphoteric (can act as both an acid and a base) and virtually water-insoluble. It is not absorbable via oral or intramuscular

administration. It also has a prolonged post-antifungal effect of up to 12 hours**.** Premedication with a combination of acetaminophen/ibuprofen with diphenhydramine and/or hydrocortisone 30 to 60 minutes before dosing should be considered if the patient has any of the following symptoms: fever, hypertension, chills, or nausea. At doses larger than 1 mg/kg, the risk of nephrotoxicity increases, and there is no evidence to support doses higher than 1.5 mg/kg per day. Nephrotoxicity can be reduced by treating the patient with 1 litre of normal saline.[33]

8.1.5. Indication

The antifungal amphotericin B deoxycholate belongs to the polyene class. It's also known by its traditional name, amphotericin B, and it's been used to treat invasive fungal infections for over 50 years. It was discovered as a natural product of an Actinomycete sp. found in soil.[27,28] Newer lipid formulations that are less nephrotoxic as compared with conventional amphotericin B are available.[29] These include.

1. Amphotericin B, which is more commonly administered in a liposomal formulation and exhibits increased tolerability and a reduced toxicity profile
2. An amphotericin B lipid complex in which amphotericin B is tightly packed in a ribbon-like structure
3. Amphotericin B cholesteryl sulfate complex

   These lipid formulations allow for a greater daily dose, better transport to organs inside the reticular endothelium system such the lungs, liver, and spleen, and are less nephrotoxic when compared to standard amphotericin B.[30] Amphotericin B is typically reserved for selected invasive fungal infections due to the advancement of newer antifungals such as azoles (e.g., voriconazole) and Echinocandins (e.g., caspofungin).

Common indications in yeast are listed below.

- Invasive candidiasis (FDA approved). It is effective against the majority of the *Candida* species, including *Candida albicans*, *Candida krusei*, *Candida tropicalis*, and *Candida parapsilosis*.
- In neonatal candidiasis, conventional amphotericin B is less toxic than in adults and well-tolerated.
- Opportunistic fungal infections in immunocompromised children, including HIV.
- Life-threatening fungal infections in both normal and immunocompromised hosts.
- Empiric treatment in a persistently febrile neutropenic host.
- Cerebral cryptococcosis along with flucytosine for induction therapy.

- Mucormycosis and other molds, including *Fusarium spp.* and penicilliosis.
- Severe cases of sporotrichosis.
- Coccidioidomycosis and paracoccidioidomycosis, especially in severe disease.
- Histoplasmosis, for disseminated disease.
- Blastomycosis, for severe disease.

8.1.6. Adverse Effects

About 80% of the patients will develop either infusion-related or renal toxicity. Amphotericin B also interacts with cholesterol in human cell membranes, which is responsible for its toxicity. The most common side effects of amphotericin B include.

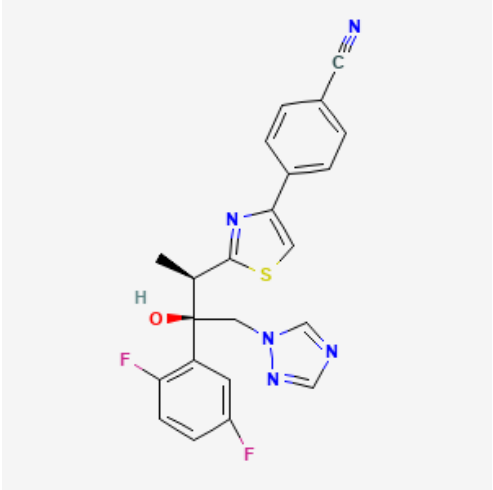
1. Loss of potassium
2. Loss of magnesium
3. Anaphylaxis
4. Fevers
5. Nephrotoxicity: Renal toxicity correlates with conventional amphotericin B use and can lead to renal failure and requirement for dialysis. But the azotemia often stabilizes with therapy and renal damage is reversible after discontinuation of amphotericin B. Avoiding concomitant use of other nephrotoxic agents, and appropriate hydration with normal saline may significantly decrease the likelihood and severity of azotemia associated with amphotericin B.
6. Other potential uncommon side effect includes demyelinating encephalopathy in patients with bone marrow transplant with total body irradiation or who are receiving cyclosporine.
7. The long-term administration is associated with normochromic, normocytic anemia due to low erythropoietin concentrations.
8. Aspergillosis, for salvage therapy in cases not responding to voriconazole
9. Visceral and cutaneous leishmaniasis (a protozoan infection)

8.1.7. Brand name

1. AMBISOME*
2. AMPHONEX*
3. ABOPHE inj*

8.2. Isavuconazole

8.2.1 Chemical structure: $C_{22}H_{17}F_2N_5OS$



8.2.2 IUPAC: 4-[2-[(2*R*,3*R*)-3-(2,5-difluorophenyl)-3-hydroxy-4-(1,2,4-triazol-1-yl)butan-2-yl]-1,3-thiazol-4-yl]benzonitrile.

8.2.3 Mode of action

ISZ comes in oral and IV forms, and it has a number of advantages, including linear pharmacokinetics, few interactions with cytochrome P450 isoenzymes, fewer drug–drug interactions, a shorter QT, no nephrotoxic cyclodextrin in the IV formulation (unlike posaconazole IV form), no need for dose adjustments in kidney or liver failure, and excellent oral bioavailability.[2]Despite having greater minimal inhibitory concentrations (MIC) than posaconazole[15], ISZ has been demonstrated to be just as effective as AmB in reducing fungal load and improving survival in a neutropenic mouse model of mucormycosis.[16] VITAL was a phase 3 single-arm, open-label, non-comparative trial that investigated ISZ. ISZ's safety and efficacy in the treatment of mucormycosis were evaluated in this study.[17]

ISZ increases Mucorales pathogenicity in a Drosophila model of mucormycosis.[19,20] ISZ prophylaxis was found to be less effective against IFD than VCZ or PSZ in comprehensive research involving 147 patients. Mucormycosis was seen in two patients who received ISZ as a prophylactic.[21] Despite the fact that ISZ appears to be less hepatotoxic than other mold-active azoles and has a better tolerance profile than L-AmB[22], the ECMM only recommends it as a second-line treatment· ISZ has been shown to permeate the blood128 brain barrier in animal models.[24] ISZ concentrations in the necrotic center of a brain abscess have been shown to be low, but concentrations in inflammatory brain tissue surrounding the abscess have been found to be adequate, equaling expected plasma concentrations. A recent retrospective study has shown that ISZ is effective in Mucorales CNS infections.[26]

8.2.4 Dose

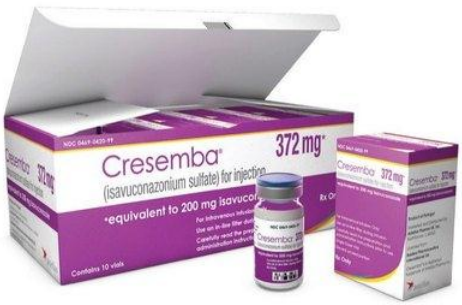
Available in oral and IV formulation of 200mg vail once daily. 200 mg/day of isavuconazole capsule daily.

8.2.5 Adverse effect

- Nausea, vomiting, diarrhoea are most common side effect along with hypokalamia, headaches.
- Hepatotoxicity
- Rashes, headache
- Elevation of liver enzyme

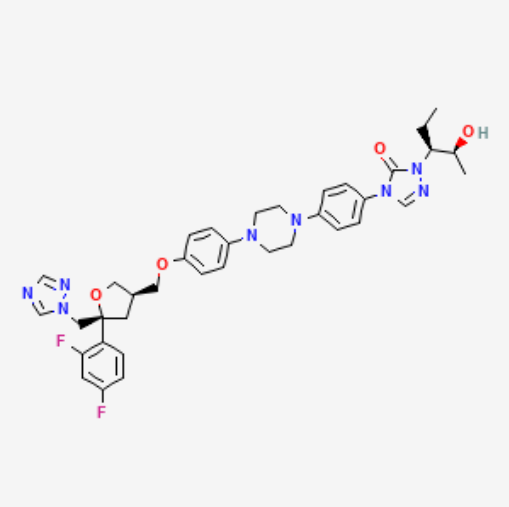
8.2.6 Brand name

CRESEMBA



8.3 Posoconazole

8.3.1 Chemical structure



8.3.2 IUPAC

4-{4-[4-(4-{[(5R)-5-(2,4-difluorophenyl)-5-(1H-1,2,4-triazol-1-ylmethyl)oxolan-3-yl]methoxy}phenyl)piperazin-1-yl]phenyl}-1-[(2S,3S)-2-hydroxypentan-3-yl]-4,5-dihydro-1H-1,2,4-triazol-5-one.

8.3.3. Mode of action

PSZ IV and Delayed Release tablets were recently created, resulting in improved bioavailability and drug exposure compared to the earlier oral solution. Greater PSZ efficiency has been linked to increased drug exposure. Furthermore, when compared to oral suspension, DR pills have less variability in absorption and are not impacted by food. Suspension DR pills and IV versions are moderately suggested due to increased blood levels. The IV version, on the other hand, is soluble in cyclodextrin and may cause renal problems. New PSZ formulations were examined in a matched paired analysis of patients treated for invasive mucormycosis.

8.3.4. Dose

800 mg/day in divided dose either 400 mg twicea day or 200 mg four times a day. the duration of the treatment depend upon the response andrisk of zygomycosis and immunosuppression.

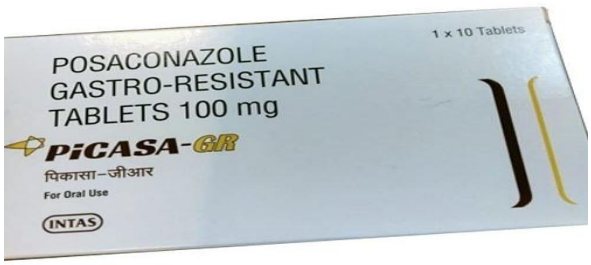
8.3.5 Brand name

POSHOPE

POSATRAL

PICASA

NOXAFIL



9. Combination Antifungal Therapy for Mucormycosis

- Echinocand
- Iron chelation therapy
- Posaconazole combination therapy

9.1 Echinocandins

R. oryzae expresses the target enzyme for echinocandins, and in DKA mice infected with *R. oryzae*, combination caspofungin plus ABLC therapy markedly improved survival, compared with monotherapy or placebo. Combination therapy with LAmB plus either micafungin or anidulafungin also improved outcome in neutropenic and DKA mice with disseminated mucormycosis. Enhanced exposure of β-glucan on the fungal surface, which results in immune stimulation, may be one of the mechanisms by which echinocandins improve outcomes in mucormycosis.[43,44,45]

9.2 Iron chelation therapy

Deferoxamine iron chelation therapy predisposes to mucormycosis, because deferoxamine actually enhances delivery of iron to Mucorales. Indeed, animals infected with R. oryzae that are treated with iron or deferoxamine have markedly worse survival than do animals treated with placebo. However, other iron chelators cannot be used by Mucorales to acquire iron . In 2005, a new orally available iron chelator, deferasirox, was approved by the US Food and

Drug Administration for the treatment of iron overload among patients with transfusion-dependent anemia.[46-49]

9.3 Posaconazole combination therapy

Two recent preclinical studies evaluated the efficacy of Posaconazole combination therapy for murine mucormycosis. In the first study, Posaconazole with AmB enhanced the survival of neutropenic mice infected with R. oryzae only when compared to a subtherapeutic dosage (0.3 mg/kg/day) of AmB monotherapy. In contrast, combination therapy was of no advantage, compared with a standard dosage of AmB monotherapy (0.8 mg/kg/d). Similarly, we recently reported that combination Posaconazole plus LAmB did not improve survival, compared with LAmB monotherapy, in either neutropenic or DKA mice with mucormycosis. No clinical studies have evaluated combination Posaconazole-polyene therapy for mucormycosis.[50,51]

CONCLUSION

To conclusion, mucormycosis is an invasive and complex fungal infection which is life threatening. This occurs in patient with Diabetes malitus, immunocompromised patient and recently found in SARS-COV-2 infected patient in large number. New radiographic, molecular, and antigenic tools are required to improve early detection and therapeutic monitoring. New antifungal agents and combinations of existing agents should be further explored in the laboratory and in clinical trials. Mortality rates are also alarming in invasive mucormycosis Current strategies mainly focus on.

1) Early and promt diagnosis of disease with the help of histopathology laboratories and microculture assay.

2) Early administration of Liposomal amphotericin B as well as Aggressive surgical intervention i.e., debridement due to lack of combination antifungal therapies that's why they are not currently recommended. And few newer antifungals are showing promising result but human's trials are awaited.

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