STUDY OF ANTIFUNGAL COMPOUND

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ABSTRACT: Fungal infections have become problematic in recent years. There has been an emergence of new fungal pathogens, development of antifungal resistance, and increasing prevalence. In addition, toxicity, resistance, and lack of efficacy as a single agent limit the effectiveness of current antifungal drugs. So,new drugs targeting new pathways are really needed.

Key Words : antifungal compound, mode of action , new drug , clinical data ,toxicity

I. INTRODUCTION:

Fungal infections which affect the keratinised tissue of the body like skin, hair and nail. It affect the stratum corneum of the epidermis. In worldwide Commonly called mycotic infection[1] . Opportunistic fungal infections have become problematic in recent years. There has been an emergence of new fungal pathogens, development of antifungal resistance, and increasing prevalence. In addition, toxicity, resistance, and lack of efficacy as a single agent limit the effectiveness of current antifungal drugs. High rates of morbidity and mortality continue to be associated with infections that are caused by molds and yeast[2] .The advancement in medicine and surgery has caused an alarming increase in immunocompromised patients that are susceptible to fungal infections. Many patients are at a higher risk of developing mycoses, such as those suffering from HIV infection, receiving organ transplantation and intensive cancer therapy[3]. With such a wide range of risk factors, the prophylactic use of antifungal therapies is one of the reasons of frequent resistance to antifungal drugs. This section provides information on the major fungal pathogens, the current antifungal agents, the value of mechanism for action studies, and the novel PHL5-34A are introduced.

1.1 Major fungal pathogens

Candida species are the most common pathogens associated with fungal disease, and *Aspergillus*, *Cryptococcus* sp and Zygomycetes account for many fungal infections as well [2]These fungi are very common and can be acquired from host surroundings. After antifungal treatment, the mortality rate is still very high due to the patient's immunodeficiency, late diagnosis, or fungal drug resistance [3]There are many invasive infections associated with *Candida* species. There are currently more than two hundred ascomycetous yeasts included in the genus *Candida*, and of these, only a few species of the genus are opportunistic pathogens of humans. *Candida albicans* are thought to be the most common cause of fungal infections in humans today [3]Identifying patients that are at a higher risk of developing *Candida* infections is an important

step in determining which patients should receive treatment. Some of the treatment options may include strategies that are presumptive (there are many risk factors that raise suspicion of infection) or prophylactic (preventative measure for high-risk factors). *Aspergillus fumigatus*, is an ascomycetous fungus found around the world and its spores are abundant in the environment due to their small size. *A. fumigatus* conidia are frequently present in food, tap water, at home, and in office rooms [3]. A definitive diagnosis of invasive aspergillosis requires a positive culture from a sterile site, or it requires histologic or radiologic evidence in a patient at high risk with compatible clinical findings. Unfortunately, when the fungi are positively identified in the body, it could be too late for treatment. Techniques for an early diagnosis, preventative strategies, and new treatment methods are needed to reduce the mortality in invasive aspergillosis patiets [2]

Cryptococcus neoformans is a saprophytic, basidiomycetous, dimorphic organism found worldwide. Its natural habitats are pigeon droppings and contaminated soil, and its small basidiospores can turn into yeast cells. The basidiospores or yeast cells may be inhaled by humans, then through the respiratory tract the pathogen can disseminate within the host causing pulmonary infections, and subsequently, due to the predilection of *C. neoformans* for the central nervous system, life threatening meningoencephalitis[3] It causes infections in both immunocompromised and immunocompetent patients. In addition, patients who have undergone organ transplantation and are receiving high-dose corticosteroids are at increased risk for development of cryptococcosis [3]

II. Anatomy of Skin [4-7]:

Epidermis : The epidermis is stratified squamous epithelium. The thickness of the epidermis varies between 0.4 and 1.5 mm. More than 95% of epidermal cells is constituted by keratinocytes. Other cells in epidermis are Melanocytes, Langerhans cells ,Merkel cells … Morphologically these are divided into

· Stratum basale
· Stratum spinosum
· Stratum granulosum
· Stratum lucidum
· Stratum corneum.

Stratum Basale[4] :{Stratumgerminativum }Only one cell thick, but may be two or three cells thick in glabrousskin and hyper proliferative epidermis. Basal cells - small and cuboidal (10–14 nm) with large, dark-staining nuclei and a dense cytoplasm. They containribosomes and tonofilaments, membrane-bound vacuoles which hasmelanosomes transferred from melanocytes by phagocytosis. The stratumbasale is the primary site for mitotically active cells. The cells in basal layer are Stemcells,Transient amplifying cells ,Post mitotic cells.

Stratum Spinosum[5] {prickle cell layer} : This layer contains 8 to 10 layers of cells. These cells are polyhedral with a round nucleus. The cells in the upper spinous layer are larger, more flattened and contain organelles called“lamellar granules”. Due to the presence of the spine-like appearance of the cell margins in histological sections (these spines correspond to the abundant desmosomes) this layer is called the spinous layer. keratinocytes and provide a network for stability. Mechanical couplingbetween epidermal cells is provided by the desmosomes. Physiologic

communication occurs at the gap junction.

Stratum Granulosum[6]

Due to the presence of intracellular basophilic keratohyaline granulesthis name is given to this layer. Cells are 2 to 5 layer thick. Cell containslamellated granules known as membrane coating granules

Stratum Corneum[7]

It is the outer most layer, has 20-25 layers corneocytes which are thelargest cell of epidermis which contain soft keratin and are stabilized byintermolecular disulfide bond. Cells are flat with no nucleus.

Stratum Lucidum[7]

Present over the palms and soles. This layer is electron lucent so iscalled by this name. This layer is present between the stratum granulosumand stratum corneum. These cells contain nucleus and are called as"transitional cells".

Blood vessels and lymphatics[7]:

Skin has a rich vascular network. It includes arterioles, terminal arterioles,pre-capillary sphincters, arterial and venous capillaries, post-capillary venulesand collecting venules. Cutaneous vessel network formed between the sub

cutaneous adipose tissue and the dermis arises from the deep plexus, thefascial network in the skin. From this various vessels branch out to reach theappendages and ascending arteriole arises to generate a sub papillary plexus

from which capillary loops are formed which enters the papillary dermis.Epidermis is avascular.7 Vasculature has various roles in the skinProvide nutrients and oxygenRegulate body temperature. The blood flow is regulated where opening causes dissipation of moreheat and constriction causes slowing of blood flow to the skin which in turnconserves energy.Endothelium constitutes the inner most component of the blood vessel.Arteriole contains a subendothelial layer of elastic tissue in contrast tovenules. Pericytes surround the endothelium of the capillaries, small arteriesand venules.

III. ANTIFUNGAL AGENTS CLASSES:

There are currently five classes of antifungal agents that are used orally or intravenously for the treatment of fungal infections in humans. The classes are polyenes, pyrimidine analogues, allylamines, azoles.

3.1. Mode of Action :

Azoles are the second class to target the cell membrane of fungi. The major groups of azoles are the imidazoles and the **triazoles**. These two groups have five-membered organic rings containing either two or three nitrogen molecules. Cellular and mitochondrial membranes are both affected by azoles. The azoles inhibit cytochrome P450-dependent 14α-lanosteroldemethylation, which is a critical step in the synthesis of ergosterol, an important component of fungal membranes [8]. The mode of administration is different in imidazoles and triazoles. Imidazoles are used by topical treatment while triazoles can be administered intravenously and orally. Since the azoles affect the P450 enzyme activity, their main toxicities are due to interactions with other compounds that

induce or inhibit this system [8].Since the azoles are fungistatic drugs, their widespread use has resulted in the development of drug resistance [9].

Flucytosine is a **pyrimidine**analogue since 1972. Flucytosine is the only antimetabolite available for the treatment of fungal infections[5]. Flucytosine is a fluorine analogue of cytosine that functions as an inhibitor of thymidylatesynthetase. It is only efficacious when administered in combination with amphotericin B. The major toxicity problems for flucytosine include bone marrow suppression, myocardial suppression, myocardial toxicity, and renal failure[10].

The only **allylamine** in clinical use today is terbinafine. Terbinafinediscovered as a derivative of the topical antifungal naftifine, which was the original compound of the allylamine class[5]. Terbinafine has limited activity for treatment of invasive fungal diseases and mainly effective against fungi such as dermatophytes. It can be combined with voriconazole for treatment of infections[9].

Amphotericin B is the **polyene**primarily in use therapeutically. Amphotericin B is a product of *Streptomyces nodosus*. Amphotericin B selectively and irreversibly binds fungal cell membrane sterols. The interaction of the antifungal with membrane sterols results in the formation of transmembrane pores, allowing for the leakage of ions and small molecules resulting in cellular damage or death. The major drawbacks for amphotericin B use are its significant side effects and that intravenous administration is required for treatment of invasive mycoses[8].

The Novel antifungal compound: The antifungal drugs that are being used today have several drawback including toxicity, resistance, lack of efficacy as a single agent, a limited spectrum of activity, high cost. In addition, their targets are mainly restricted to the cell membrane. Therefore, the need for new drugs targeting new pathways . Compounds that have a distinct mechanism of action (MOA) are at a higher demand because they can be used for combinatorial or chemical modifications. **PHL5-34A[11] :**PHL5-34A is a synthetic compound based on the phloeodictine class of compounds found in marine sponges such as *Pellinaeusiphonia*. These compounds have a bicyclic tetrahyropyrrolopyrimidinium core with an aliphatic side chain at C-6

A novel analog of one of these compounds, PHL5-34A was synthesized chemically at the National Center.

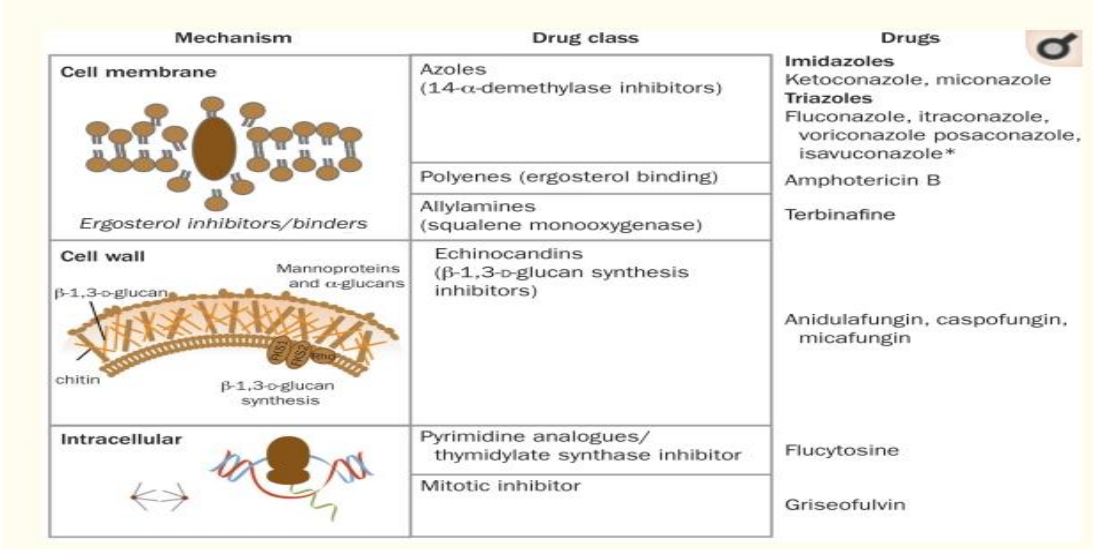

FIG. 3.1.1.Mode of action of anti-fungal drug

IV. CLINICAL TEST[12] :

Laboratory investigations play an important role
They are diagnosed by two important methods
i. Direct microscopy
ii. Culture

Direct Microscopy[12,13]:Direct microscopic examination in 10% KOH solution is consideredone of the most important procedure in medical mycology.This procedure is done on an outpatient basis to establish evidence offungal infection in skin, hair and nail. We can obtain the results within 1- 2hours. It is an easier, reliable and more useful procedure to diagnose fungalinfections. The following steps:

Scraping [13]

The presence of fungal infection of the skin, hair, nail can beestablished by doing a scraping. The scrapings are collected and examinedunder a microscope. First, the skin lesion is cleaned using alcohol. For easysampling, little distilled water can be applied. By pulling the skin above thesite with one hand and moving the scalpel edge across the lesion the sample iscollected.

KOH mount [13]

Skin scraping is collected on a black paper or directly on the slideusing a scalpel from the edge of the lesion. 10-30% KOH is usually used.After sample is collected on a glass slide from the advancing border of thelesion using a blunt scalpel, add KOH and place the cover slip. Now heat theslide gently and then examined for the presence of fungal elements. Specialtransport packs backed by black card when available can be used to transport the scrapings.

Microscopic examination [12]

In order to view the presence of fungal spores and hyphae thisprocedure is done. In this procedure first we examine under low powermagnification (x10) and then under high power magnification (x40) for betterillumination so that the morphology of the fungus can be studied. Fungalspores vary from 2-10 mm in diameter. Sometimes the lines of juncture ofnormal epidermal cells dissolve into branching network and these are easilymistaken for a fungal structure. This is called 'mosaic fungus'. Cotton fibresand synthetic fibres can also mimic fungal hyphae. For microscopic examination 10% to 40 % KOH is used.Counter stains such as Parker's blue black ink, Periodic acid Shiff orfluorescent stains such as Calcoflour can be used to visualise the fungalelements[12].

Modified Parker's ink and 1% eosin method[12]

Modified parkers stain is prepared by adding 1% eosin to Parkers inkin 2:1 proportion. Mixture is applied over the affected area and allowed todry. Then the cellophane tape is reapplied, gently pressed, removed and stuckover a glass slide and viewed. Pink appearance of background can be seenwhich is due to eosin and blue appearance of fungal elements can be seenwhich is due to ink[12].

Calcofluor white[12]

This is a colourless dye, a fluorochrome stain. When applied overscrapings from skin and mucous membrane it helps in rapid detection of thefugal elements . When viewed under ultraviolet light, fungal structures appearas brilliant apple-green or a ghostly blue-white colour.Though direct microscopy is an easy and reliable test, 5-10% of falsenegative results may occur because,Inappropriate material or an insufficient quantity was obtained foranalysis. Out dated or defective KOH used.andadequate time was spent on examining the specimen.

USES OF KOH[13]:

KOH is used in the diagnosis of, candida infection of the skin, nails , hairLaboratories select a simple glucose/peptone agar, either with 4%sugar, 1% peptone and an acid pH (Sabouraud's dextrose agar) or with 2%sugar, 1% peptone and a neutral pH (Emmon's modification). In order toreduce contamination, antibacterial antibiotics such as gentamycin (0.0025%)and/or chloramphenicol (0.005%) are added. The addition of cycloheximideat 0.04% will inhibit the growth of non-dermatophyte moulds. For moulds,the temperature of incubation should be 26–28°C and cultures should be heldfor a maximum of 3-4 weeks.7Microsporum- macroconidia are rough, thick walled, range fromfusiform to obovate in shape with 1-12 or more septa. Trichophyton- thinwalled, smooth, cylindrical, fusiform or clavate in shape with upto 12transverse septa.Epidermophyton- macroconidium is clavate, broadened androunded at its distal pole, thin walled and has up to 5 septa. In some specieslike M.audonii, T.verrucosum, T.simii, a third spore produced calledchlamydospore. In T.schoeninii and T.violaceum there may be only one kindof spore[13.14].

IV. Drug – Drug interaction[15] :

Polyene

Amphotericin B is not metabolized by hepatic CYP450 enzymes and has very few drug–drug interactions . The pertinent drug–drug interactions for amphotericin B are related to the nephrotoxicity and electrolyte similar renal side effects. One common example is the coadministration of amphotericin B with immunosuppressants, such as tacrolimus or cyclosporine[15], in transplant recipients. This combination is associated with increased risk of kidney injury and electrolyte disturbances.

Flucytosine

This **is** not a substrate or inhibitor of the CYP450 enzymes. There are very few drug–drug interactions. Because flucytosine is renally cleared, medications altering renal function may affect drug levels and the risk of toxicity[15].

Triazole

Drugs have the highest potential for seriousdrug–drug reactions. They are substrates and inhibitors of various hepatic CYP450metabolic enzymes . The possible drug–drug interactions vary by individual drug because each has a variable affinity for the isoenzymes (CYP2C19, CYP3A4, CYP2C9) [16]. As inhibitors of CYP450 enzymes, triazoles can impair metabolism of a coadministered drug, increasing the risk of toxicity. As substrates of the pathway, the concentrations ofthe triazoles can be substantially affected by concomitant use of medications thatinhibit or induce the enzymes, as has been observed for itraconazole and voriconazole.lists

commonly used classes of medications that have the potentialfor serious interactions if administered with azoles. Closely examining a patient'smedication list is recommended before starting and stopping medications given thehigh potential for drug–drug interactions. Absorption of the itraconazole oral capsulesand the posaconazole oral solution is optimized by gastric acidityso proton pump inhibitors and histamine-2 blockers should beavoided. drug–drug interactionsmay be encountered by the additive effect of additional QT prolonging agents. Isavuconazole is the only triazole that is not associated with QT prolongation[16].

V. Toxicity of Antifungal Compounds[17] :

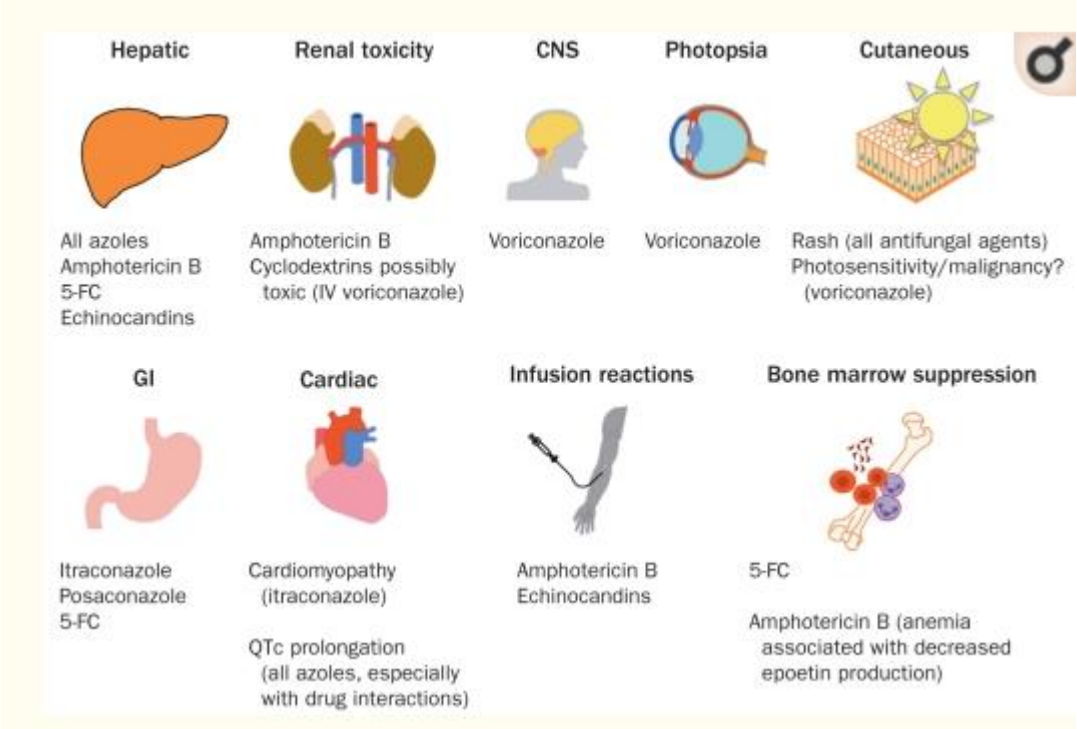

Fig.5.1. Toxicity of antifungal compounds

CNS = central nervous system; 5-FC = flucytosine; GI = gastrointestinal; IV = intravenous; QTc = corrected QT interval.

VI. FUTURE DIRECTIONS[15,16,17]

The need to develop new therapy to treat fungal infections Systematic screens of chemical compound libraries are being increasingly used in order to discover new antifungal compounds. the chemical characteristics and origin of the molecules have gained more importance in the screening phase. In combination with this strategy, genomics-era technologies promise to bring significant advances in the discovery of effective antifungal. Studies in mutant fungal cells treated with active molecules allow the identification of genes related to the mechanism of action, providing specific target assays for novel antifungal agents Developing antifungal agents that explore unique factors in fungi is a challenge, since these organisms are eukaryotes such as mammals. Essential genes for fungal survival are very interesting targets and are already being useful for the design of new drugs. In addition, agents that can block genes related to drug resistance can also be an efficient alternative .Also, studies focused on several drugs are currently being conducted aiming at the potentiation of antifungal drugs, especially fluconazole.

VII. CONCLUSIONS

Further studies are needed to determine the chemical identity of the bioactive compounds responsible for antifungal activity. a MOA study was conducted on antifungal compound, the current antifungal drugs don't inhibit cell cycle. Thus, this a potentially novel antifungal target for new drug discovery. Given the central role played by the cell cycle in cell division and growth, inhibiting the cell cycle will have a dramatic effect on cell viability. On the other hand, since the cell cycle is an important process in all eukaryotic cells, it is possible that PHL5-34A may cause an inhibitory effect on not only fungal cells but also on the human host cells.

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