Review Article

Steroid-binding receptors in fungi: implication for systemic mycoses

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(Received: 6 December 2014; Revised: 30 January 2015; Accepted: 4 February 2015)

Abstract

It has been shown that some of the mycotic infections especially systemic mycoses show increased male susceptibility and some steroids have been known to influence the immune response. Researchers found that some fungi including yeasts use "message molecules" including hormones to elicit certain responses, especially in the sexual cycle, but until recently no evidence was available to link specific hormonal evidence to this pronounced sex ratio. More evidence needed to demonstrate that a steroid (s) might in some manner influence the pathogenicity of the fungus in vivo. Therefore, the aim of this review paper is to shed some light on this subject along with effort to make mycologists more aware of this research as a stimulus for the explore of new ideas and design further research in this area of medical mycology.

Keywords: Steroid-binding receptors, Systemic mycoses

➢ How to cite this paper:
Chadeganipour M, Mohammadi R. Steroid-binding receptors in fungi: implication for systemic mycoses. Curr Med Mycol. 2015; 1(2): 46-52. DOI: 10.18869/acadpub.cmm.1.2.46

Introduction

Some of the mycoses show increased male susceptibility and some steroids have been known to influence the immune response. It has been shown that some fungi including yeasts use "message molecules" including hormones to elicit certain responses, especially in the sexual cycle [1, 2]. But until recently no firm evidence was available to link specific hormonal evidence to this pronounced sex ratio. More research and evidence are needed to demonstrate that a steroid (s) might in some manner influence the pathogenicity of the fungus in human host. Steroid binding sites with high affinity for progesterone were detected in the enriched plasma membrane fraction of the fungus *Rhizopus nigricans* [3]. The existence of a corticosteroid binding protein and an endogenous ligand (steroid) for a clinical strain of *Candida albicans* was reported [4]. Čapek, et al., reported that progesterone and its hydroxy derivatives and various sterols effect on the growth of dermatophytes [5]. It was demonstrated that all the steroid compounds employed inhibited growth of the 51 strains of dermatophytes tested in their study. These researchers hypothesized that this may represent a primitive steroid-receptor system. Since then they have also found mammalian-like receptors and steroid hormones in other fungal species which will be discussed especially in *Paracoccidiides brasiliences, Coccidioides immitis* and *Saccharomyces cerevisiae* [6-8].

Paracoccidioidomycoses

Paracoccidioidomycosis is a progressive, mostly chronic disease that is often fatal if left untreated. The lungs are the primary portal of entry and the disease usually progresses to form granulomata of the buccal and nasal mucosa. The lymph nodes are commonly involved. The etiological agent of this disease is a fungus, *Paracoccidiides brasiliences* [9]. This agent can have a long latency period (as long as 60 years) and it is difficult to tell when the infection was acquired. The disease can also be of a benign, self-limited nature as indicated by positive skin sensitivity tests in symptom- free individuals [10]. This disease is limited virtually to one continent with most cases being reported in Brazil, followed by Venezuela, and Columbia. The available data indicates that this disease has a rather distinct distribution with respect to age and sex. The

Paracoccidioidomycosis is rare in children and most common in males between 40-5- years. It is more common in males than females. The most common explanations include: 1) that children have less contact with *P. brasiliensis* than adults, 2) men have greater occupational exposure to fungus than women, 3) *P. brasiliensis* has a long latency period (seen mainly in adults). These explanations may be partially true but since the natural reservoir of *P. brasiliensis* has not been discovered, these reasons are somewhat speculative. If the fungus is present in the soil (1-2 reports exist) and woman from the endemic area work alongside males in the fields, occupation and soil exposure may not be important factors in the distribution of the disease. Skin sensitivity surveys indicated that there was equal exposure to *P. brasiliensis* and sensitivity in the population increases with age. There are no sex-based differences in prepubertal children who contract the disease [11]. For the above reasons it seems that hormonal factors may play a critical role in the distribution and pathogenesis of this disease. Muchmore, et al., was the first team of workers to report that female sex hormones (estradiol and progesterone) and synthetic estrogen (stilbestrol) inhibited the growth of both forms of *P. brasiliensis* [12]. The concentrations of hormones used in this study were at much higher concentrations than the normal. Physiological levels of this study might not be valid to compare with later hormonal studies that use hormone concentrations within the normal physiological range. One study by San-Blas, 1982 mentions a growth Inhibitor produced by the yeast phase of *P.brasiliensis* in the later part of the growth phase. This metabolite was inhibitory to *H. capsulatum* and *B. dermatitidis* but not for *S. cerevisiae.* They suggest the metabolite might be of a phenolic structure but never fully characterized this [13]. In retrospect, if self-inhibitors are produced they might be more closely related to steroids and warrant further study. Based on the studies just cited and contrary to the findings of Powell, et al., the possibility that hormone binders might exist in *P. brasiliensis* was investigated [14]. By developing a new method for quicker binding of rapidly dissociated steroids, Loose, et al., demonstrated a high affinity binding system, specific for 17 B-estradiol. Other estrogens (estrone, estradiol and progesterone) had about 25% of the affinity of estradiol and the synthetic estrogen (diethylstilbestrol, andro-gens and corticosteroids) had low affinity [15]. In the same study it was demonstrated that estradiol inhibits the morphogenesis of mycelial-to-yeast transfor-mation which is considered to be the first step in the establishment of the disease [9]. They postulated but did not test for the presence of an endogenous ligand. Loose, et al., mentions that although in vitro and in vivo bioactivity of hormones is not exact [8], these finding support that "pathogenic fungi have capacity to respond to the hormonal environment of the host through receptor-like steroid binding proteins". In a follow-up study [11] it was shown that the inhibition of the yeast phase of *P. brasiliensis* was specific for estrogens and not evident for other steroids (testosterone, 17 alpha-estradiol and corticosterone). The low capacity of this binding system was noted in that lower concentrations (10^{10}) inhibited the transfor-mation by 71% compared to a higher concentration (10^{5}) which was 19% inhibition. This study compared three strains and slight differences were noted and this was concerned mainly in the degree of transformation in the controls which could vary. This study hypothesizes that the ability of estrogen to inhibit the infective stage of *P. brasiliensis* represents the molecular site of action for resistance of females to paracoccidioido-mycosis. Also this transition might occur in the lungs because this is believed to be the primary portal of entry of this agent. This delay of the fungus in the mycelia phase may allow time for the female host to initiate an immune response or to simply delay the progress of the disease by inhibiting morphogenesis.

Coccidioidomycosis

Coccidioidomycosis is most likely to disseminate in men than women expect in pregnancy. This increased susceptibility might

be related to the host environment. Recent evidence supports this theory: 1) the growth rate of *Coccidioides immitis* and subsequent release of endospoes (In vitro) is greatly stimulated by the presence of 17 B-estradiol, testosterone, and progesterone, 2) Levels of progesterone and estrogen greatly increase during pregnancy and this might accelerate the fungus in vivo resulting in increased release of endospores [6]. The latest finding in this research is that progestin, 17 beta-estradiol and androgens have low affinity binding activity and are present in the cytosol of five strains of *C. immitis* [6,14]. 17 beta-Estradiol was highly stimulatory for the growth of endospores of all three strains and for the arthroconidia of *C. immitis*. However they tested other fungi (*P. brasiliensis*, *Blastomyces dermataitidis*, *Cryptococcus neoformans*, non-*albicans Candida* and *Torulopsis glabrata*) and found low or inconsistent binding for estrogens and androgens. This data might be due to error in the technique used for binding receptors and will be explained in more detail later.

Saccharomyces cerevisiae

Saccharomyces cerevisiae (brewer's or baker's yeast) is a common colonizer of human mucosal surfaces and mostly considered to be an occasional digestive commensal, but its role as a clinically important pathogen is controversial. However, since the 1990s, there have been a growing number of reports about its implication as an etiologic agent of invasive infection. *Saccharomyces* organisms should now be added to the growing list of emerging fungal pathogens. A particular feature of such infections is their association with a probiotic preparation of *Saccharomyces* for treatment various diarrheal disorders [16-19]. In *Saccharomyces cerevisiae* researchers were found estrogen specific receptors and they identified the endogenous fungal ligand as 17 B-estradiol, identical to the mammalian hormone [7]. The protein receptor not only was of high affinity but stereo-specific for17 alpha-estradiol. This fungal hormone was also able to exhibit estrogenic activity in rat uterine tissue and in human breast cancer cells [5, 17]. Louvion, et al., [20] showed that the hormone-binding domain (HBD) of the human estrogen receptor (ER) can function as an autonomous regulatory domain in the budding yeast, *Saccharomyces cerevisiae*. Although this yeast is not a pathogen, it is in wide use in the food and beverage industries. This raises a question of biohazard if some of these foods are contaminated with fungal estrogen.

Phialophora verrucosa

The effects of sex steroid hormones on the growth of an aetiologic agent of chromoblastomycosis, the dematiaceous fungus *Phialophora verrucosa* was determined by Hernández-Hernández, et al., [21]. The in vitro growth of *Phialophora verrucosa* on culture media including either testosterone, progesterone or estradiol at various concentrations was valued. They showed that both progesterone and testosterone inhibited the growth of *P. verrucosa*, whereas estradiol did not. They suggested that the growth of *P. verrucosa* is regulated by steroid hormones and that the effect of progesterone could be mediated through fungal intracellular progesterone receptors.

Dermatophytes

Specific binding of [3H] progesterone to cytosol of Trichophyton mentagrophytes was demonstrated by Schär, et al., [22]. They hypothesized that the functional effect of the hormone may be related to the observed resistance of females to dermatophytosis. They demonstrated that escape from growth inhibition is related to the enzymatic transformation of progesterone to polar metabolites. Two of these metabolites were determined to be 1-dehydroprogesterone and 11 alpha-hydroxyprogesterone, and the third metabolite was a 1-dehydro-hydroxypro-gesterone, but the location of the hydroxyl group could not be determined clearly [23]. Clemones, et al., [24] showed Trichophyton mentagrophytes contains a cytoplasmic macromolecule which specifically binds progesterone, and it is also an effective inhibitor of growth of the fungus.

The Methods applied for these studies

The methods for all these studies reviewed before, are similar and consist of the following steps:

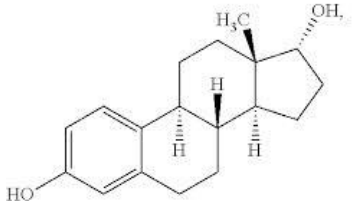
Preparation of cytosol

Cultures of fungi are grown in liquid or on agar media and washed, centrifuged, and mechanically disrupted by vortexing with glass beads. The result is termed a cytosol. This method is important when working with biohazards such as the pathogenic fungi. The cytosol is composed mainly of the protoplasm of the cells and is non- infective [14]. It should be kept in mind that the use of this cytosol may introduce unknown variables in the test system because the activity of these steroids might differ in the complete organism.

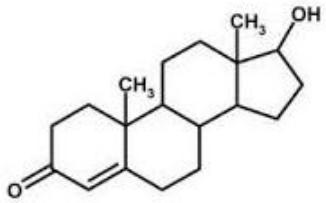
Binding of hormones

Labeled (radioactive) hormones like 17 alpha-Estradiol, 17/3-estradiol, testosterone, progesterone [Figure 1] are incubated with the fungus for up to three hours at zero degrees Centigrade. If any of the labeled hormone binds to the fungal receptor protein it will be separated from free steroid by silica gel chromatography [4].

(a)



(b)



(c)

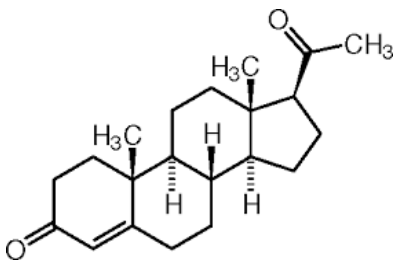


Figure 1. 17 alpha-Estradiol (a), testosterone (b), and progesterone (c)

Characterization of binder

The cytosol is subjected to various treatments to determine the character of the hormone binder; 1) activity at different temperatures, 2) enzyme treatments (trypsin, RNase, DNase, phospholipase, etc.), 3) molecular weight determination, 4) affinity, capacity of system, and specificity for the various hormones.

Production of endogenous ligan

Ethanolic extracts of fungal cultures (indicating lipids) are compared with a series of radioinert steroids for its ability to compete with labeled hormone in mammalian cell tissue cultures, including rat uterine and thymic tissue and human breast cancer cells (MCF-7). These methods are supplemented by other purification techniques (high pressure liquid chromatography). These methods suggest steroid functions in certain fungi and although they use the term receptor, they actually have not characterized the function of this system in the fungi.

Dimorphism in pathogenic fungi

Some fungi, especially those involved in the systemic mycoses exhibit dimorphism. Romano defined dimorphism as an environmentally controlled reversible interconversion of the yeast and mycelia morphologies [25]. The recent evidence presented here of possible hormonal control of mycelia to yeast transformation in *P. brasiliensis* warrants a review of this subject to see if these new theories could intergraded with the information that is presently know about the phenomenon of dimorphism. The study of dimorphism has generally been restricted to some agents of mycoses, especially in of *Mucor* spp. [26]. The study of this system is important for many reasons including: 1) use as a model for the study of eukaryotic development and cellular differentiation, 2) prevalence of dimorphism in fungal pathogens of animals, plants, and insects, 3) recent evidence presented in this paper on host interactions with pathogens [9]. Rippon, 1980 provides an excellent review of this subject of which will be summarize some areas relevant to the subject of this paper. Most studies of dimorphism have been concerned with defining the chemical differences in cell wall

composition between the mycelial (M) andyeast (Y) phases. The most extensive studies have been done on *P. brasiliensis* with the emphasis on the ultra-structural and biochemical differences.

Yeast-Phase

1) Two distinct wall layers, inner chitin layer and outer amorphous layer composed of mostly alpha-glucan and scattered islets of beta- glucan.
2) Budding arises from these islets of beta-glucan.
3) Less beta-gluconase activity.
4) More chitin than mycelial phase, 45%:11%.
5) 5× increase in protein disulfide reductase and less disulfide linkages than mycelial phase.

Mycelial-phase

1) One layer with chitin interwoven with mostly beta-glucan.
2) Alpha glucan varies with strains, 0-60%.
3) Galactomannan abundant.
4) Less protein disulfide reductase, more disulfide links than yeast.

Some of these distinct differences are evident in other dimorphic fungi but not as clear cut as in It *P. brasiliensis*. It was proposed that the transfer from 25 to 37 C° is one of the most consistent factors initiates Y-M transformation. This possibly initiates the activation of some component of cell wall synthesis. In light of the recent hormone data this theory needs integration with the function of estrogen or other steroids in this transformation. San-Blas, et.al., 1982, and Hallack, et.al., 1982, have hypothesized that there is variability in the cell wall composition of different strains of *P. brasiliences* (including lab-induced mutants) [27, 28]. They related low levels of alpha-glucan (0- 60%) in the M phase and an increase in galactomannan (0- 40%) with a decrease in virulence. This could be reversed by the transfer of yeast cells through animal or by incubation in fetal calf serum. They suggested that these factors could determine which isolates of *P. brasiliensis* survive and invade human tissue. This also will need reevaluation in light of the presence of hormone- receptor systems. Rippon states that "the ultimate determinant of the process of morphogenesis is a chemical pathway within the fungal cytoplasm, but the key metabolic process remains elusive" [9]. Sulfur containing compounds are very important for two reasons:

1) They include essential metabolites like amino acids
2) They can change the internal redox potential in the cell

The Y-phase in general has a higher metabolic rate than the M-phase and maintains a higher oxidation- reduction (OR) potential. This appears to be related to the contribution of sulfur in reducing the enzyme protein disulfide reductase. The use of sulfhydryl blocking agents causes a shift from Y-M phase [29, 30]. Temperature is important in governing the redox potentials of cell membranes especially cysteine permease. If this enzyme is activated (by higher temperature) then the level of cyclic AMP drops and the Y-phase is initiated. The M-phase has more cyclic AMP than Y-phase and at 37 C° the addition of theophyllin or dibutyryl cyclic AMP (inhibit breakdown of cyclic AMP) will initiate the M-phase. At 25 C° a "broth effect" is seen where an increase in the amount of fungus will reduce the OR potential and there is some conversion of M to Y phase. Carbon dioxide can also influence morphogenesis in. *P. brasiliensis, C. immitis, B. dermatitidis* and *Sporothrix schenckii* [31, 32]. This also might be related to the “broth effect”. In summary, although the phenomenon of dimorphism is not widely understood the addition of steroid-receptor systems will bring research closer to that elusive key. This research also adds to a better understanding of fungal diseases and possibly will aid in finding more effective treatment, especially for the systemic mycoses. For example, one of the imidazole antifungal agents, ketoconazole has an effect on the activity of certain steroids in mammals and in fungi. In conventional doses, ketoconazole has been shown to inhibit sterol synthesis in fungi [33]. It also binds the corticosteroid- binding protein of *C. albicans* [34-36]. Reports of gynecomastia in males treated with ketoconazole led to the discovery that this drug interferes with the adrenal

response (blocks cortisol) and can also block testosterone synthesis [33, 37]. These effects are reversed when therapy is discontinued. These side effects may be useful in certain clinical situations such as cancer patients where the suppression of these hormones might be therapeutic. As more information is reported on steroids in fungi, the use of ketoconazole and other drugs (estrogen?) may be used to halt systemic mycoses by promoting a focus on blocking molecular events in their life cycle. The study of steroid-receptor system in fungi also has implications for tracing the appearance and evolution of hormones in eukaryotic systems. The structural similarities of these hormones in both mammalian and fungal organisms represent conservation throughout the evolution of these molecules [11, 38]. In addition, these systems might relate to the sexual cycle of not only *P. brasiliensis* but other fungi as well.

Conclusion

Some of the mycotic infections show increased sex susceptibility and some steroids have been known to influence the immune response, but until recently no firm evidence was available to link specific hormonal evidence in this regard. Therefore, it should be an effort to make mycologists more aware of this research as a stimulus for the explore of new ideas and design further research in this area of mycology.

Authors' contributions

R.M. contributed in acquisition of data and wrote the draft and edited the final manuscript, M.C. edited the draft and the final manuscript.

Financial Disclosure

No financial interests related to the material of this manuscript have been declared.

Conflicts of interest

The authors announce no conflicts of interest.

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