Chemico-Biological Interactions 178 (2009) 303–309

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Aspects of the steroid response in fungi

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ARTICLE INFO

Article history:
Available online 13 November 2008

Keywords:
Fungi
Steroid response
Gene regulation
Biotransformation
Steroid extrusion
Drug resistance

ABSTRACT

The number of fungal infections is increasing due to higher numbers of immunocompromised patients. Unfortunately, drug resistance represents a major additional problem in clinical praxis. Therefore factors contributing to infection by opportunistic pathogens, and to their growth and drug resistance are of major importance.

It has been known for some time that mammalian steroid hormones are toxic to fungi. In this paper the response of fungi to the presence of steroid hormones will be discussed at different levels. First, the effect of steroid hormones on fungal growth, morphology and virulence will be considered. Processes affecting steroid intracellular concentration will be discussed; steroid uptake and, even more, steroid extrusion are currently of special interest. The role of biotransformation in the detoxification of active steroids will be taken into consideration and phases of steroid metabolism in fungal cells will be compared to phases of classical xenobiotic metabolism. Steroid signaling in fungi is presently not yet clear. It results in a global response of fungi to steroid hormones. Some of the genes differentially expressed in fungi as the result of exposure to steroid hormones may contribute to fungal drug resistance.

1. Introduction

Steroid hormones are important signaling molecules in higher organisms which, after fulfilling their task, are deactivated, conjugated with glucuronic acid or sulfate, and excreted as inactive conjugates via bile or urine [1]. In the host, opportunistic pathogens inhabiting the digestive system, vagina, skin etc., are exposed to steroid hormones [2,3]. The concentration of these varies depending on the tissue and other characteristics of the host. In addition, steroids can be prescribed for treatment of various diseases, and they can be obtained from the environment or taken intentionally as food supplements, anabolic steroids or, as dehydroepiandrosterone (DHEA), to slow the aging process. Normal flora as well as pathogens are exposed to them.

Microorganisms in the gastrointestinal tract, mostly bacteria, constitute a diverse community that processes undigested food to the benefit of the host [4,5]. It is capable of biotransforming steroid hormones secreted via bile into the intestine [6–9]. Steroids may be deconjugated and otherwise metabolized, and some of the modified steroids enter enterohepatic circulation [9]. Finally, steroid hormones produced by animals, in manure and in wildlife, and by humans are excreted into the environment through sewage discharge and animal waste [10,11]. They are in a free form or as conjugates which can easily be converted into the free form. Those produced by humans are collected in the sewage system and end up in waste water treatment plants, where various processes are applied [10,12]. Steroid hormones can still be detected in the effluents of the sewage treatment plants and in surface water. Their concentration is lowered due to biosorption and biotransformation/mineralization [13].

Estrogens are of special concern in the environment; they exert their physiological effects at lower concentrations than other steroids and their concentrations are sufficiently high to interfere with health in man, livestock and wildlife [14–16].

Research on exposure to steroid hormones of fungi *in vivo* and *in vitro* is important for several reasons. The number of fungal infections is increasing due to larger numbers of immunocompromised patients because of AIDS, organ transplants and other reasons [17]. Steroid hormones have been found to play a role in the host/fungus relationship, affecting either the host and/or the fungus during fungal infection. Their effect on fungal growth, morphology and virulence has been suggested. In bacteria they were found to affect not only growth but also antimicrobial susceptibility [18,19]. Pleiotropic drug resistance states have been found to be induced by steroids in *Candida albicans* and *Saccharomyces cerevisiae* [20].

In this paper some of the cell compensatory mechanisms that are provoked in fungi in the presence of steroid hormones will be discussed. Special interest will be focused on the protection of cells by

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doi:10.1016/j.cbi.2008.11.002

detoxification of steroid hormones and/or their extrusion from the cells. The third possible protective mechanism, rearrangement of the cell wall, will be mentioned. All these processes are made possible by steroid signaling, resulting in induction of protein expression and/or protein activation.

2. Steroid hormones: effects on fungal growth, morphology, virulence and drug resistance

Steroid hormones are toxic to fungi. Their effects will be discussed at the three levels and some representative examples will be given to illustrate the role of the steroids. The effect of steroid hormones on fungal growth will be considered first. Inhibition and, to a lesser extent, stimulation of fungal growth has been observed, the effect of steroid hormones being strain specific. Secondly the effect of steroids on fungal and yeast morphology will be discussed. Depending on the microorganism, the transformation of microorganisms from yeast to hyphal form or *vice versa* has been connected with increased virulence. Thirdly, the steroid effect on fungal drug resistance will be mentioned, drug resistance being a major problem in the treatment of fungal infections. Finally, the well established effect of steroid hormones on the susceptibility of hosts to fungal infections has to be mentioned [21,22], although it will not be discussed in the present context.

Several examples of the inhibitory action of steroid hormones on fungal growth *in vitro* can be found: androstenedione was found to inhibit the growth of *Schizosaccharomyces pombe* [23], progesterone and some other steroids the growth of the fungus *Rhizopus nigricans* [24,25] and of the halophilic black yeast *Hortaea werneckii* [26,27]. In the skin, which is an independent endocrine organ, androgens are metabolized within pilosebaceous units and have direct effects on dermatophytes. Hashemi et al. investigated the relationship between serum concentrations of androgens and the susceptibility of the host to dermatophytosis. Significantly lower levels of testosterone were detected in patients with this disease caused by *Epidermophyton floccosum*, suggesting an inhibitory effect of higher testosterone concentrations on fungal growth [28,29]. In addition *E. floccosum* is capable of steroid biotransformation; biotransformation of DHEA by this fungus leads to the nearly complete conversion of the substrate into multiple products. In this way the fungus has been suggested to influence the hormone regulated cutaneous defense mechanisms [30].

To our knowledge estrogens are the only steroids which have been found to stimulate fungal growth, as shown for *C. albicans*. This microorganism is an opportunistic pathogen located on the mucosal surfaces of the gastrointestinal and reproductive tracts and has rarely been isolated from the environment. It lives in the majority of the human population with no harmful effects, although overgrowth results in candidiasis. The latter has become a human disease of increasing importance, and stimulatory effects of estrogens on the growth of *C. albicans* were therefore considered. Elevated estrogen levels and the incidence of vaginal candidiasis were found to be positively correlated. Vaginal colonization with *C. albicans* was found to be increased during pregnancy as a result of elevated estrogen levels [31]. In addition, ovarian hormones were suggested to have a significant influence on experimental candidiasis [32–34].

Fungi causing diseases in mammals and, particularly, in humans, frequently require morphogenetic transitions to survive and invade the host [35]. To infect host tissue, the usual unicellular yeast-like form of *C. albicans* has to switch to an invasive, multicellular filamentous form. Invasion correlates with changes such as those of host immunity, competition from other saprophytes and other perturbations of its niche [36,37]. The presence of estrogens was found to affect the morphology of the yeast, the number of cells forming germ tubes and germ tube length [31]. Estrogens promote the conversion of yeast to hyphal forms and by such action they may enhance virulence of the organism [38].

In contrast to the opportunistic pathogen *C. albicans*, *Paracoccidioides brasiliensis* can be found as a mold in the soil [39]. After its spores are inhaled, exposure to the new environment in the host, particularly increased temperature, triggers changes in fungal morphogenesis. The pathogen acquires the yeast form in patients and experimental animals [39,40]. Paracoccidioidomycosis, more frequent in developing countries of Latin America, occurs mostly in males. The progress of the disease is evidently hormonally regulated. Estrogens were found to inhibit the transformation of the mycelial form to yeast, thus preventing the disease [40,41]. These results suggest that *P. brasiliensis*, as well as *C. albicans*, prefers mycelial form at higher concentrations of estrogens in the host environment.

Finally, steroid hormones can influence fungal susceptibility to drugs, with potential consequences for treatment of fungal infections. Their contributions to drug resistance at the molecular level remain largely unknown [20].

Various antifungal drugs are currently available that act on a limited number of targets [42]. For a drug to be successful, it has to enter the microbial cell and attain a sufficiently high intracellular concentration. Cellular targets have to be sensitive to the actions of the antimycotic. Fungal pathogens, on the other side, use several mechanisms to circumvent the inhibitory actions of antifungals; for example, they change the amount or sensitivity of the target enzyme, like cytochrome P450 lanosterol 14α-demethylase or (1,3)-D-β-glucan synthase. Other possibilities are to use an alternative pathway to the one that is inhibited and/or to lower the intracellular concentration of the drug by its efficient extrusion [43,44].

Steroid response may make the cells less vulnerable to other stresses, like the presence of the antimycotic. This could be accomplished by upregulation of genes involved in drug resistance and drug resistance associated genes, stress response genes like Hsp 70 and Hsp 90 or genes connected with biofilm formation [45–47]. Steroids might in principle increase the concentration of the targets by, for example, inducing the synthesis of the enzymes from the ergosterol biosynthetic pathway. Upregulation of *ERG 11* gene coding for lanosterol 14α-demethylase and other ERG genes has been documented in the progesterone response in *S. cerevisiae* [20]. Steroids can affect the concentration of the antimycotic in the fungus by stimulating its efficient extrusion. The induction of the efflux pumps in the presence of steroids has been documented for *S. cerevisiae* and *C. albicans* [45,48].

Recently developed antifungals, the echinocandins, target cell wall biosynthesis by inhibiting (1,3)-D-β-glucan synthase. Progesterone treatment was found to increase expression of several genes involved in the biogenesis of the cell wall [48]. In addition transcription of the Hsp 90 gene was found to be responsive to progesterone in *S. cerevisiae* and to progesterone and estradiol in *C. albicans* [38,45]. Hsp 90's role in drug resistance is, in combination with calcineurin, to enable responses to specific stresses, like changes in the composition of the cell membranes and cell wall.

In vitro experiments showed that, in the presence of progesterone and estradiol, *C. albicans* cells became more resistant to fluconazole and ketoconazole [45]. But, it is important to keep in mind that the effect of steroids is dependent on the concentration and time of exposure of microbial cells to steroids. To what extent steroid hormones contribute to clinical resistance is presently unknown.

The effects of steroid hormones have not been investigated only in humans and fungi but also in plants. They were found to affect plant growth and influence their generative development [49].