MALASSEZIN – A TRYPTOPHAN METABOLITE ISOLATED FROM THE DERMAL YEAST *MALASSEZIA FURFUR* ACTS AS AN AGONIST OF THE AH RECEPTOR

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Introduction

Malassezia is a lipophilic dermal yeast and part of the residential flora of the skin of humans and warm-blooded animals [1]. At present the genus comprises seven species, e.g. *M. globosa* and *M. furfur*. *Malassezia* plays an important role in many pathological disorders associated with this group of yeast such as pityriasis versicolor, seborrhoeic dermatitis, atopic dermatitis and otitis, but the mechanisms remain still unclear [2]. The tryptophan-dependent formation of fluorochromes might be causative of pathogenesis and symptoms of the *Malassezia*-associated skin diseases such as depigmentation, reduced UV light sensitivity, skin color variations or fluorescence of the lesions [3].

The AhR is a ligand-activated basic helix-loop-helix transcription factor. It mediates most of the toxic effects of dioxin-like environmental pollutants such as polychlorinated dibenzodioxins (PCDDs) or polychlorinated biphenyls (PCBs). The prototype of AhR agonists is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), but some tryptophan-derived compounds such as indolo-[3,2-b]carbazole (ICZ) are also potent ligands of the AhR. Activation of the cytosolic AhR leads to translocation into the nucleus followed by heterodimerisation with the aryl hydrocarbon receptor nuclear translocator (ARNT). This ligand-AhR-ARNT-complex binds to xenobiotic responsive elements (XREs) in the 5'-flanking region of several genes such as CYP1A and certain UDP-glucuronosyltransferases, and turns on their transcription [4,5].

The structural similarity of malassezin (Fig. 1), one of the tryptophan metabolites produced by the lipophilic yeast *Malassezia furfur*, to known AhR agonists led us to the investigation of its CYP1A-inducing potency.

Figure 1: Structure of Malassezin

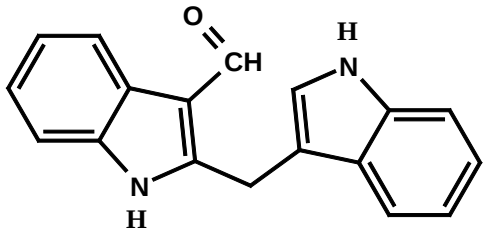
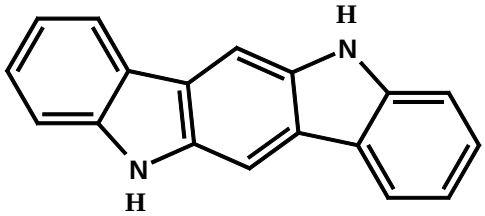


Figure 2: Structure of ICZ



Methods and Materials

Malassezin was isolated from *M. furfur*, cultivated in a minimal medium with tryptophan as the single nitrogen source. After isolation and characterization of the metabolites substances were synthesized as described before [6].

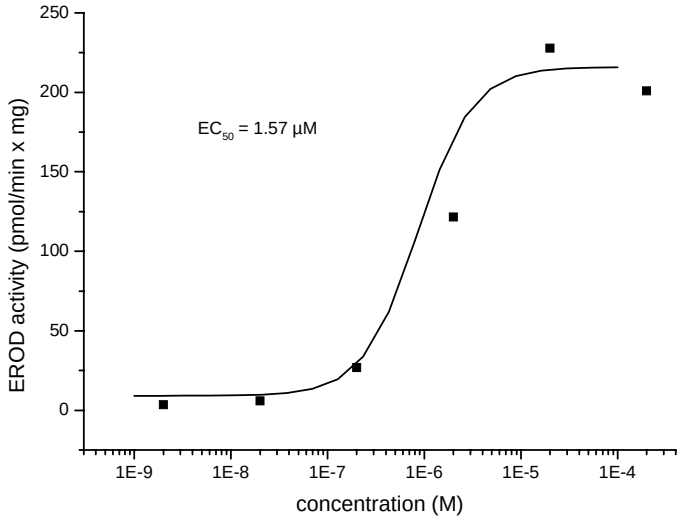
Primary rat hepatocytes were prepared from male Wistar rats weighing 150-220 g as described earlier [7]. In brief, after anesthesia of the animals, livers were perfused in a two-step procedure with a calcium-free EGTA-containing buffer, followed by perfusion with a collagenase-containing buffer. Hepatocytes were cultured in DMEM containing 20 % fetal bovine serum (FCS) and 0.1 µM dexamethasone on collagenated culture dishes (60 mm diameter). Three hours after seeding, medium was changed and malassezin dissolved in dimethyl sulfoxide (DMSO) was added. The cells were incubated at 37°C for 48 hours. TCDD at a final concentration of 1 nM and DMSO served as controls. After incubation the cells were washed with ice-cold saline and scraped off with a tris-buffered sucrose solution, pH 7.4. The cells were centrifuged and homogenized by sonication on ice [7].

The catalytic activity of CYP1A in the rat hepatocytes was measured as 7-ethoxyresorufin O-deethylase (EROD) using a spectrofluorometer (Perkin-Elmer LS-5B) according to the method of Burke and Mayer [8]. Protein contents were analyzed according to Lowry [9].

Results and Discussion

In our experiments with rat hepatocytes malassezin isolated from *M. furfur* was found to act as an AhR agonist inducing CYP1A-catalysed EROD activity (EC_{50} = 1.57 µM). Thus the potency of malassezin as an AhR ligand is in a concentration range similar to that of ICZ (EC_{50} = 0.26 µM).

Figure 3: EROD induction curve of malassezin



X-ray crystal structure investigation showed that malassezin is a non-planar molecule and does not fit into a 7 x 14 A rectangle [6]. Thus, it does not fulfill major requirements for potent AhR agonists [10]. Perhaps malassezin is a novel prototype of AhR agonists. Alternatively, it may be easily converted into a potent agonist such as ICZ. In supernatants of cell cultures no ICZ could be detected by fluorescence-HPLC analysis. However, the formation of minor amounts sufficient for AhR activation, e.g. by intracellular metabolism, cannot be excluded.

It remains to be elucidated if the yeast benefits from the biosynthesis of an AhR agonist and/or if AhR activation by malassezin contributes to the pathogenesis of pityriasis versicolor.

This is the first example for a biosynthetic product of a micro-organism relevant to human health shown to act as an AhR agonist. Our findings indicate that the AhR signalling pathway may be part of a defence system aimed at micro-organisms and/or their metabolites.

References

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