2019

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MECHANISMS OF ACTION: PHYSIOLOGICAL EFFECTS

Examination of Fluconazole-Induced Alopecia in an Animal Model and Human Cohort

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ABSTRACT Fluconazole-induced alopecia is a significant problem for patients receiving long-term therapy. We evaluated the hair cycle changes of fluconazole in a rat model and investigated potential molecular mechanisms. Plasma and tissue levels of retinoic acid were not found to be causal. Human patients with alopecia attributed to fluconazole also underwent detailed assessment and in both our murine model and human cohort fluconazole induced telogen effluvium. Future work further examining the mechanism of fluconazole-induced alopecia should be undertaken.

KEYWORDS alopecia, antifungal, fluconazole, side effect

Fluconazole is a frequently prescribed antifungal used in the treatment of cutaneous, mucosal, or invasive fungal infections. Serious toxicity is uncommon, and nausea, vomiting, anorexia, and mild liver enzyme test abnormalities are the most frequently reported adverse effects (1, 2). The development of alopecia, cheilitis, dry skin, and/or rash also may occur, and although these symptoms are generally benign they are frustrating for both patients and physicians alike.

The association of alopecia with fluconazole was noted shortly after initial approval (3), although neither the pathophysiology nor the hair-cycle-specific effects of fluconazole have been previously investigated. It has been speculated that alopecia and other "retinoid-like" toxicities (hair loss, dry skin, and cheilitis) are secondary to an unidentified P450 interaction between triazoles and endogenous retinoic acid derivatives (4), which are well known to cause hair loss (5).

We sought to determine the effects of fluconazole on hair growth and retinoic acid levels at predetermined time points to establish an animal model of long-term antifungal therapy and to confirm these findings in a clinical cohort of fluconazole-treated patients with alopecia.

Male Wistar rats weighing 200 to 220 g were obtained from Charles River Laboratories (6) and fed a standard diet (LabDiet 5001; containing 15 IU/g of vitamin A to avoid dermatologic manifestations of vitamin A deficiency). Rats were randomized into two groups (oral fluconazole 35 mg/kg/day [dosing strategy available in supplemental methods] and no fluconazole). Rats were anesthetized (3 to 5% inhaled isoflurane), followed by cardiac puncture for serum. Hair, blood, and tissue samples were obtained

Citation Thompson GR, III, Krois CR, Affolter VK, Everett AD, Varjonen EK, Sharon VR, Singapuri A, Dennis M, McHardy I, Yoo HS, Fedor DM, Wiederhold NP, Aaron PA, Gelli A, Napoli JL, White SD. 2019. Examination of fluconazole-induced alopecia in an animal model and human cohort. Antimicrob Agents Chemother 63:e01384-18. https://doi.org/10.1128/AAC.01384-18.

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Received 2 July 2018
Returned for modification 5 August 2018
Accepted 13 October 2018
Accepted manuscript posted online 19 November 2018
Published 29 January 2019

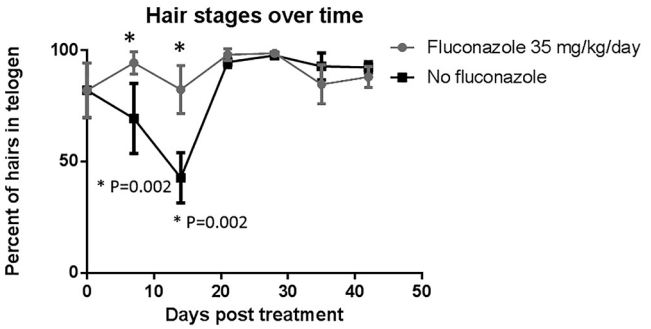

FIG 1 Percentage of hair in telogen phase over time in fluconazole-treated and nontreated animals.

from six rats in each group at each time point: days 0, 14, 21, 28, 35, and 42. Fifty hairs were plucked prior to blood and tissue sample acquisition. Skin (with hair intact), liver, and brain samples were then obtained. Tissues were frozen in liquid nitrogen and stored at −80°C until all-*trans* retinoic acid (atRA) quantification. All procedures were performed under yellow light. All animal studies were performed in accordance with the local Institutional Animal Care and Use Committee.

All rat hair and skin samples were read in blinded fashion as anagen, catagen, or telogen (for an example, see Fig. S1 in the supplemental material). The number of hairs at each stage of growth and atRA concentrations in different tissue/serum at different time points were compared by standard *t* tests and the Mann-Whitney U test as appropriate. Data were analyzed using GraphPad Prism (La Jolla, CA).

Examination of 50 hair follicles at each time point detected no differences in the number of hairs in telogen at the beginning of the study (day 0) (Fig. 1); however, by day 7 significant differences were noted. At this time point (day 7), fluconazole-treated rats had a significantly higher number of hairs in telogen (median, 48; range, 44 to 50) than those in the untreated group (median, 37.5; range, 23 to 43) ($P = 0.002$). These differences remained through day 14, with fluconazole-treated rats exhibiting a higher number of telogen hairs (median, 41; range, 33 to 47) than their untreated counterparts (median, 21.5; range, 12 to 28) ($p = 0.002$). Differences on days 21, 28, 35, and 42 were not statistically significant ($P > 0.05$ for all time points) due to the progression of the untreated rats though the normal hair cycle (normal hair cycle in rats is synchronized and ~38 days) (7).

Retinoid quantification, quantitative PCR, nucleic acid preparation, reverse transcription-PCR, histopathology, and immunofluorescence methods are included in the supplemental material. atRA in serum and skin tissue were not significantly different at any time point during the conduct of this study (Fig. 2). Interestingly, skin retinyl ester levels did differ significantly between groups at days 7 ($P = 0.011$) and 14 ($P = 0.002$), with fluconazole-treated rats exhibiting higher levels than their untreated counterparts. No difference in serum retinyl ester levels were noted at any time point. Retinol levels were different between groups, with significant differences noted at days 7 ($P = 0.002$) and 14 ($P = 0.009$) in the untreated group. However, skin retinol levels were higher in the fluconazole-treated group on day 14 ($P = 0.039$), with a trend toward higher levels on day 7 as well ($P = 0.071$).

Although serum and skin atRA levels were no different between groups, in order to evaluate the possibility of fluconazole effects on retinoic acid metabolism at the cellular level or within tissue-specific sites, we also performed molecular examination of the hair follicles and immunofluorescence using gene expression assays of the retinoid-responsive genes CYP26A1 and RARβ. There were no significant differences in expression between the fluconazole-treated and nontreated rats for either gene at day 14 (median change in expression of CYP26A1, 1.16; range, –1.75 to 2.51; median change in RARβ expression at 14 days, –1.84; range, –2.38 to 1.99). Changes were also not significant at day 28 for either gene (median change in expression of CYP26A1, 1.37; range, –1.48 to 3.18; median change in RARβ, 1.33, range, –1.04 to 1.95) (Fig. S2).

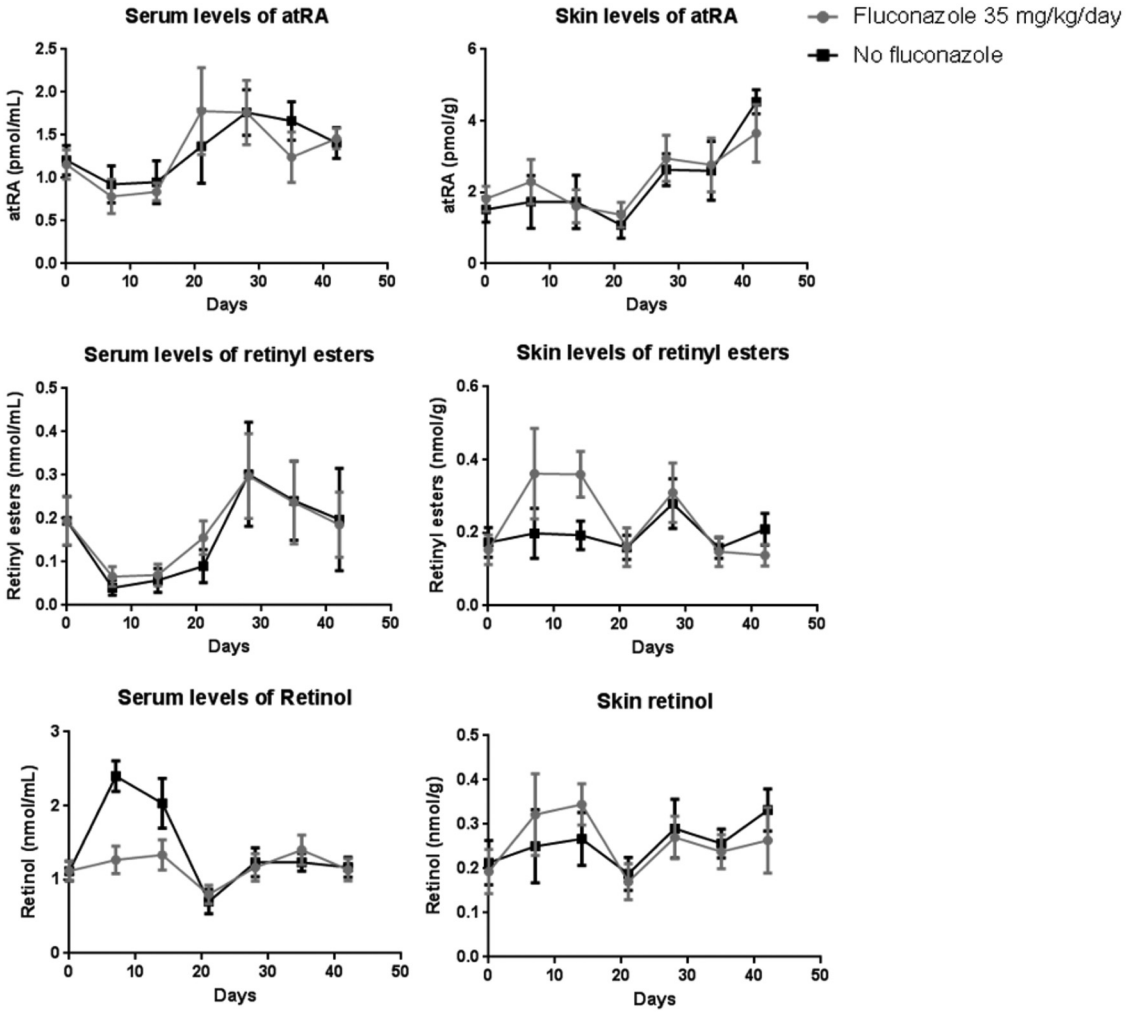

FIG 2 Serum and skin levels of atRA, retinyl esters, and retinol. *, Statistical significance. *P* values are noted over groups where differences are apparent.

RARβ expression at day 14 in hair follicles of cranial skin samples was examined by confocal immunofluorescence in control and fluconazole-treated animals. Images show that RARβ (red) is present in both control (Fig. S3A and B) and fluconazole treated (Fig. S3C and D) animals at day 14. Interestingly, RARβ expression is less abundant in samples treated with fluconazole (Fig. S3E). Similar results were obtained for follicles of caudal skin samples (data not shown). There were no differences in transforming growth factor β (TGF-β) expression in control or fluconazole-treated samples in cranial or caudal skin samples (data not shown). There was no difference in RARβ expression in hair follicles of cranial and caudal skin samples examined after fluconazole treatment for 42 days (data not shown). These findings confirm a lack of retinoid effect in the skin of rats despite the development of telogen effluvium.

Five consecutive patients receiving treatment for chronic coccidioidomycosis with fluconazole and receiving no other medications were enrolled following their initial clinical complaint of alopecia (Table 1). Patients underwent visual inspection of the scalp and microscopic examination of at least ten hair samples after the hair pull test (8), and all hairs were found to be in the telogen state, confirming telogen effluvium consistent with the animal model. Human patients enrolled in this study had fluconazole serum levels determined by liquid chromatography-tandem mass spectrometry (ARUP Laboratories, Salt Lake City, UT). Informed consent obtained from all patients enrolled. The median serum fluconazole levels were 39.8 μg/ml (range, 15.8 to 62.3 μg/

TABLE 1 Patients with development of alopecia following initiation of fluconazole therapy

Patient no.	Age (yrs)	Sex	Type of infection	Fluconazole daily dose (mg)	Serum fluconazole level (μg/ml)	Duration of fluconazole (days)	Alopecia site	Medication change after complaint of alopecia	Outcome
1	51	F	Right shoulder	800	39.8	115	Scalp	Itraconazole	Resolved at 6 mos
2	58	M	Chronic pulmonary	800	62.3	1010	Scalp	Posaconazole (solution)	Improved at 4 mos
3	72	M	Chronic pulmonary	800	55.0	288	Scalp	Itraconazole	Resolved at 6 mos
4	23	M	Chronic pulmonary	400	15.8	390	Scalp and arms	Patient refused due to cost	Continued alopecia
5	68	F	Chronic pulmonary	400	20.3	683	Scalp	Itraconazole	Improved at 4 mos

ml). At the time of enrollment, the patients had received fluconazole for a median of 390 days (range, 115 to 1,010 days). Most patients were transitioned to an alternative triazole, and hair loss abated over the ensuing three months. A single patient refused a change in treatment, and his alopecia persisted unchanged on follow-up.

Alopecia associated with fluconazole was observed soon after approval (3, 9) and in the initial description, between 12 and 25% of the patients were noted to develop substantial alopecia while receiving fluconazole. The majority of patients (29/33, 87%) received at least 400 mg of fluconazole daily, and alopecia was noted at a mean of 3.2 months (range, 2 weeks to 7 months) after starting antifungal therapy. Similarly, voriconazole, which is structurally related to fluconazole, was noted to cause alopecia following the *Exserohilum* fungal meningitis outbreak, with 82% of patients noticing alopecia after 1 month of therapy (10).

Neither of these prior reports speculated on the mechanism of triazole-induced alopecia; however, it has been suggested by others that a P450-mediated interaction between azoles and endogenous retinoids might be responsible given the retinoid-like effects of fluconazole (xerosis, alopecia, etc.) (11). Since this proposition, the metabolism of retinoic acid derivatives has undergone extensive investigation. Resistance to atRA treatment in cancer therapy has been attributed to increased systemic clearance (12, 13). All CYP26 enzymes have been shown to metabolize retinoic acid with CYP26A1 responsible for the majority of hepatic clearance (14–16). Fluconazole, voriconazole, itraconazole, and ketoconazole have been found to inhibit CYP26A1 mediated hydroxylation of retinoic acid *in vitro* at concentrations of >1 μM (15) (Fig. S4), and clinical reports have shown a 6-fold increase in plasma atRA levels immediately following the receipt of fluconazole (17). Together, these findings support the previously proposed hypothesis of triazole-induced alopecia as a consequence of endogenous retinoic acid metabolism inhibition and provide a plausible explanation for the "retinoid-like" side effect profile observed in some patients on fluconazole or voriconazole.

Increases in retinoic acid result in autoinduction of metabolism, thereby reducing overall exposure (18). Fluconazole has been found to upregulate hepatic CYP26A1 expression, thus inducing retinoic acid clearance (19), and elevated serum atRA levels following azole administration are not maintained over time (20). Extensive investigations in our animal model of long-term triazole-induced toxicity corroborates these *in vitro* findings; we observed no differences in serum and skin atRA levels over time and skin expression of known atRA responsive genes, and in fact there was a decrease in RAR in the fluconazole-treated group. Collectively, these findings suggest that fluconazole downregulates RAR expression by an unclear mechanism; however, inhibition of CYP26-mediated metabolism of atRA is not responsible for the observed changes based on similar serum and tissue levels between groups, and retinoic acid has been shown previously to increase RAR expression (21). Furthermore, our findings of RARβ downregulation in fluconazole-treated animals with no differences in TGF-β contrasts with the reported mechanism of atRA-induced hair loss. These prior reports suggest that RARβ upregulation and the increase in TGF-β transcription cease proliferation of the hair follicle and induce apoptosis (22), a key molecular difference from our findings.

Our results confirm fluconazole as a cause of alopecia and, more specifically, as a cause of telogen effluvium. Patients in our cohort presenting with fluconazole-induced alopecia experienced a return of normal hair growth following a transition to an

alternative nonstructurally related triazole (itraconazole or posaconazole), whereas the only patient continuing fluconazole therapy underwent progressive patchy alopecia. It is also of interest that azole-induced alopecia has been noted only with the structurally similar compounds fluconazole and voriconazole (23). Alopecia attributed to itraconazole or posaconazole has not been reported to our knowledge, nor was it noted in the recently completed isavuconazole phase 3 studies (24, 25). It remains unclear if alopecia does not occur with itraconazole, posaconazole, or isavuconazole, or if there is a concentration-dependent effect among triazole agents, and that this manifestation will be later described following the higher serum drug levels now seen with oral posaconazole tablets and isavuconazole (26).

In conclusion, we developed a murine model of fluconazole-induced alopecia, describe the type of hair loss as telogen effluvium in both the animal model and a human cohort, and illustrate the resolution of alopecia with a change to an alternative triazole agent. The molecular mechanisms responsible for progression from anagen to catagen/telogen, and thus the precipitation of telogen effluvium, have yet to be fully elucidated, and although our results demonstrate changes in retinoic acid metabolism, they eliminate the accumulation of retinoic acid as the primary contributor and the fundamental cause remains elusive.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01384-18.

SUPPLEMENTAL FILE 1, PDF file, 1.1 MB.

ACKNOWLEDGMENTS

We thank Ingrid Brust-Mascher, UC-Davis Health Sciences District Advanced Imaging Facility.

Existing departmental funds were used for this research.

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