JPPT

Clinical Investigation

Griseofulvin Only Modestly Diminishes Persistence of *Trichophyton tonsurans* on the Scalp of Carriers

Susan M. Abdel-Rahman, PharmD,[1,2] Krista J. Wright, LPN,[1] and Harriett C. Navarre, RN, PNP[2]

[1]Division of Pediatric Clinical Pharmacology and Medical Toxicology, The Children's Mercy Hospital; [2]Department of Pediatrics, University of Missouri-Kansas City School of Medicine, Kansas City, Missouri

OBJECTIVES Using genetic strain typing, we previously identified a high rate of *T. tonsurans* carriage among preschool-aged children attending an urban daycare center. No treatment was provided as part of the observational study; however, children when symptomatic were treated in accordance with daycare policies. This retrospective investigation examines antifungal drug therapy received during the previous investigation and characterizes the impact of treatment on persistence of the fungus on the scalp.

METHODS Children in whom serial typeable isolates of *T. tonsurans* were recovered were eligible for evaluation. Clinic charts were reviewed and dispensing records obtained from the primary pharmacies serving the daycare. Infection patterns were examined before and after treatment.

RESULTS We identified 72 dispensing records for 53 children, all of whom received griseofulvin. Nine children could not be evaluated because treatment was coincident with their last study visit. Thus, 63 treatment events in 44 children with 331 discrete infection events remained. After a single course of griseofulvin, 22.7% of children became culture negative, 6.8% acquired another strain of *T. tonsurans* and, 70.5% remained persistently positive with the same strain carried prior to treatment. Among those receiving a second course of therapy, 54% remained positive and the cumulative percent of children that became culture negative increased to 36.4%. If children subsequently acquiring a different strain are considered together with those that became culture negative, cumulative strain clearance was observed in 43% of children. Neither the griseofulvin dose nor the duration of time over which children were infected prior to treatment differed between those that remained positive and those that became negative.

CONCLUSIONS Griseofulvin eradicates dermatophyte scalp carriage in less than one-half of preschool-aged children receiving between one and four 4-week courses of the drug.

KEYWORDS antifungal, carrier state, dermatophyte, tinea, *Trichophyton*

J Pediatr Pharmacol Ther 2009;14:94–99

INTRODUCTION

The baseline rate of dermatophyte infections in the general population varies by infection type (i.e., the afflicted tissue site) and is heavily influenced by the demographic characteristics of the population, the geographic region from where the individuals hail and the species of dermatophyte that are endemic to that region. Among children in developed countries prevalence rates of the most common infection types (e.g., *tinea capitis*, *tinea corporis*) rarely exceed 10%, even in high risk populations.[1-6] However, population surveys conducted in undeveloped and developing countries reveal clinical infection rates that exceed 1 in every 4 children.[7-13] The prevalence of asymptomatic carriage also varies widely based on the aforementioned criteria. Apart from the knowledge that carrier rates increase above basal levels in populations that are in close contact with an index case (e.g., families, sporting teams, long-term health care facilities),[14-19] the relationship between symptomatic infection and asymptomatic carriage remains poorly understood.

Address correspondence to: Susan Abdel-Rahman, PharmD, Professor of Pediatrics, Division of Clinical Pharmacology and Medical Toxicology, Children's Mercy Hospitals and Clinics, 2401 Gillham Rd., Suite 0411, Kansas City, MO 64108, email: srahman@cmh.edu

In a recent two-year investigation conducted at an urban daycare center we observed *Trichophyton tonsurans* scalp infections to occur in no fewer than 1 of every 5 children in attendance with infection rates climbing to 1 in every 2 children in some months.[20] The majority of these children were asymptomatic, and by means of multilocus, molecular strain typing we confirmed that the vast majority were persistent carriers of the same genetic strain of *T. tonsurans*. Notably, the likelihood of experiencing symptoms varied markedly by the pattern of fungal acquisition. Children who predominantly or exclusively carried a single strain of *T. tonsurans* experienced a 2- to 4-fold greater risk of developing symptomatic disease than children who transiently acquired and lost random strains of the pathogen.

In light of these findings, we theorized that the relatively recalcitrant nature of dermatophyte scalp infections may reflect the failure of current treatment regimens to adequately resolve persistent infection rather than represent re-infection from the environment wherein there exists a large fungal burden (e.g., the daycare center). In this paper we revisit the children enrolled in the previous investigation, explore the treatment patterns and examine whether systemic antifungal therapy appeared to alter the course of their infection.

MATERIALS AND METHODS

The children described in this investigation derive from those enrolled in a prospective epidemiologic investigation previously reported on by the investigators.[20] In brief, the longitudinal investigation took place at an urban child care center that serves the needs of over 600 preschool and school-aged children in the Kansas City metropolitan area. All children aged 2 to 6 years were followed monthly over the course of 2 years (106 to 174 children per month) and each child in attendance was examined for clinical indicators of infection. Fungal scalp samples were concurrently obtained from each child during these visits, irrespective of whether symptoms were manifest, contributing a total of 3,541 cultures. Fungal material was harvested from all positive cultures, the genomic DNA isolated and the organisms genetically strain typed using 12 sequence variations in 2 gene loci. Carrier status was determined for children in whom typeable *T. tonsurans* isolates were recovered on more than one occasion and it is these children that were eligible for this evaluation. This retrospective evaluation was reviewed and approved by the Pediatric Institutional Review Board at Children's Mercy Hospital.

Clinic charts were retrospectively reviewed and dispensing records obtained from the two primary pharmacies that serve the children enrolled at this daycare center. Any oral antifungal that was dispensed for a dermatophyte infection was recorded along with the prescribed dose and duration of therapy. Patient weight was obtained from the clinic charts for visits corresponding with the prescription. Only children for whom a prescription was dispensed by the pharmacy were included in this analysis.

The infection status of each child was evaluated prior and subsequent to therapy, along with the nature of the infecting strains. Treatment response was examined using standard descriptive statistics. Differences in the course of infection between children that reverted to a culture negative status and those that remained positive were evaluated using an unpaired two-tailed Student's t-test. Statistical analyses were performed with SPSS version 12 (SPSS, Chicago, IL). The significance limit accepted for all statistical analyses was $p = 0.05$.

RESULTS

As detailed previously, 446 children were enrolled over the course of two years. Of the 3,541 scalp cultures that were collected, 1,390 were positive for growth consistent with *T. tonsurans* and 1,048 demonstrated a sufficient quantity of DNA to strain type the isolate. A single typeable isolate of *T. tonsurans* could be recovered in 264 children with 173 children demonstrating more than one typeable isolate for comparison. Among these 173 children, we could identify 72 pharmacy dispensing records for 53 children. In 9 children, the impact of drug therapy on fungal carrier status could not be evaluated because treatment was coincident with their last study visit. Thus, 44 children with 331 typeable isolates (average 7.5 per child) and 63 discrete treatment events remained from which to evaluate the impact of treatment on carrier status.

The 44 children included in this investigation were followed on average 528 ± 182 days (me-

dian: 608 days, range: 133-713 days). An average 176 days elapsed between the time of the first positive culture and the time children became symptomatic prompting treatment (median 153 days; range 1-425 days). In all cases children were treated with griseofulvin at doses ranging from 7 to 22 mg/kg daily (average: 11.3 ± 3.5 mg/kg). In all but 2 cases children were treated for 4 weeks, with one child each treated for 6 and 8 weeks.

After the first course of therapy 34 children remained culture positive, 31 with the same strain of *T. tonsurans* and 3 with a different strain. The remaining 10 children became culture negative. The prescribed dose of griseofulvin did not appear to influence whether *T. tonsurans* was retained on or cleared from the scalp (p=0.869). In addition, the duration of time that children harbored the pathogen prior to treatment did not influence outcome after the first course of therapy. Children who remained culture positive had been infected an average of 155 ± 125 days, as compared to 184 ± 144 days in children who became culture negative (p = 0.454). Notably, there was no difference in the time of follow up after completion of the first course of therapy between these 2 groups (213 ± 143 vs. 232 ± 136, p = 0.466) suggesting that children were not inadvertently classified as culture negative owing to inadequate follow-up. Given the large variations in fungal genetic diversity observed in this population, we were not able to identify whether specific *T. tonsurans* strains were more susceptible to eradication.

In the majority of children who remained culture positive after treatment there was no delay between the end of treatment and the recurrence of positive cultures. In 22 of the 34 children, cultures were positive at the first study visit after completing treatment. Six children were positive by their second visit and 4 were positive by their third study visit after completing treatment. In one child each it did take 5 and 7 visits before they reappeared as culture positive. Notably, it was in these latter two children, along with one child experiencing a 3 month delay, who were re-infected with a different strain type. The remaining children were persistent carriers of the same strain of *T. tonsurans* observed before treatment was initiated.

Fourteen children went on to receive a second course of therapy. One was a child that reverted to culture negative after the first course of therapy but remained symptomatic. Of the remaining 13 for whom there were strain type data prior to the second course of treatment, an equal number reverted to negative (n=6) or remained positive (n = 7). As was observed after the first course of therapy, the time of follow up did not appear to influence the classification of these children. The duration of follow-up in children who became culture negative (208 ± 133 days) did not differ significantly from the children who remained culture positive (115 ± 63 days, p = 0.437). Of the 7 children that remained culture positive, 3 received a third course of therapy, one of whom received an additional fourth course of therapy. All of these children remained persistently culture positive despite treatment.

DISCUSSION

Clinical trials that evaluate the therapeutic efficacy of anti-infective agents typically offer a single point estimate of the fraction of individuals administered that drug that are either successfully treated or fail to respond. For many drugs these data are wholly adequate; however, the interpretation of results from trials of oral antifungal agents used in the management of chronic dermatophytoses are met with additional challenges. Estimates of clinical cure can be skewed by clinical indicators of infection that may persist long after eradication of the pathogen (e.g., hyperkeratosis, onycholysis, alopecia) while estimates of mycological response often fail to provide information on long-term clearance of the pathogen. Unfortunately, when longitudinal analyses are integrated into treatment trials, the evaluation of serial isolates is often restricted to characterization at the species level, limiting the ability to distinguish relapse from re-infection.

Genetic strain typing, which drills down beyond the species level, affords the opportunity to track the persistence of unique pathogen strains within a patient or a population.[21] For pathogens which cause chronic infections that are relatively recalcitrant to treatment (e.g. the dermatophytes responsible for tinea capitis and onychomycosis), strain typing of serial samples is currently the best way to discriminate whether recurrent infections represent the acquisition of a new strain or recrudescence of an infection that has not been adequately treated.[22-24] We previously followed a group of 446 children over 2 years to gain insight

into the natural course of dermatophyte acquisition and transmission in the preschool-aged population.[20] By means of genetic strain typing we identified that the majority of children did not transiently acquire and lose random strains of *T. tonsurans*. Once a child in this urban day-care environment acquired the pathogen they retained the strain on their scalp for a period of time exceeding that of the study.[20]

In the current investigation we detail a subset of those children (n = 44) that were followed an average of 17.6 months through 331 discrete infection events. Carriage was confirmed by the presence of serial isolates (in the absence of clinical indicators of disease) that displayed the same genetic strain type as the isolate recovered when the child manifested symptoms prompting treatment. Pursuant to treatment, griseofulvin eradicated carriage in fewer than 30% of children when those that became culture negative were considered together with those that acquired a different strain of *T. tonsurans*. The cumulative percent of children who became culture negative approached 43% after two courses of therapy. No additional increase in the fraction of children reverting to a culture negative status was conferred by 3 or 4 courses of therapy; however, there was an insufficient number of children to accurately assess the impact of additional treatment courses on *T. tonsurans* carriage.

We attempted to minimize the uncertainty in our estimates of carrier state eradication by restricting evaluation to those children for whom a prescription was dispensed. However, we were unable to assess medication adherence in this retrospective investigation. As such, the data at best offer a reflection of the anticipated change in infection status that might be expected in a practice setting. Notably, there were children that appeared to receive doses below those that are routinely used for the management of tinea capitis,[25] although the recipients were no more likely to retain the pathogen than children who successfully cleared the organism.

Our findings are supported by a single case report and are surprisingly consistent with 2 small-scale investigations that similarly applied strain typing to serial dermatophyte isolates. Cordeiro and colleagues were able to recover the same genetic strain type of *T. rubrum* from their patient on nine separate occasions over the course of 2-years, despite multiple courses of oral antifungal therapy.[22] In 2 additional studies, serial isolates derived from infected toenails prior and subsequent to treatment were subjected to genetic strain typing. In these studies, approximately two-thirds of the participants demonstrated persistence of the same strain type before and after treatment with sampling durations that spanned 2-20 months.[23,24] In contrast to our investigation, none of the aforementioned reports established the presence of the infecting isolate prior to the appearance of symptoms, nor did their patients appear to resolve the symptoms of infection over the course of the study. Consequently, they were able to establish the failure of oral antimycotics to clear the infecting strain type but were unable draw any conclusions as to the impact of treatment on carrier status.

It is important to emphasize that strain typing is not universally informative.[26] Typing strategies with low discriminatory power may attribute the presence of a single strain type to persistent carriage, when in fact the assay is not sufficiently sensitive to detect minor variations between strains.[26] Moreover, typing strategies that have not been validated for use in longitudinal studies (e.g. those that have not verified the stability of the loci under investigation or those that incorporate rapidly mutable markers) may incorrectly attribute observed changes in genetic strain type to the acquisition of a new strain. These techniques are also of limited utility when there does not exist a significant degree of genetic heterogeneity for the organism in a given geographic region. As above, one may erroneously attribute recovery of the same genetic strain type to pathogen persistence when, in fact, this cannot be distinguished from re-infection. By extension, these data are difficult to interpret in populations and/or geographic regions where the baseline degree of variability is unknown.

We had extensive knowledge of the baseline rate of genetic variation within North American isolates of *T. tonsurans* through studies undertaken prior to conducting the prospective epidemiologic study that served as the basis for this investigation.[27,28] Moreover, we verified that the sequence variations incorporated into the typing scheme were stable on serial passage of the organisms for a period of time exceeding the duration of the longitudinal investigation. As such, we are reasonably confident that these observations accurately represent pathogen per-

sistence within the population.

When cure rates experienced in clinical practice fall short of those observed in clinical trials, attempts to explain the disparate findings include questionable medication adherence, inter-individual pharmacokinetic variability and changing susceptibility profiles.[29] Rarely do we consider the environmental or host specific factors that influence long-term persistence of the pathogen within a population. The data from this investigation demonstrate that children can establish a relationship with their infecting dermatophyte strain long before symptoms appear and suggest that the typical course of griseofulvin can disrupt this relationship in only a minority of cases. We would be remiss not to point out that the drug regimens typically employed in clinical practice (as evidenced in this investigation) are often smaller and shorter than those evaluated in clinical trials wherein higher efficacy rates are established.[30] However, the majority of clinical trials do not establish the nature of infection prior to enrollment (i.e. acute infection vs. persistent carriage) and they often fail to follow children for protracted durations after trial completion.

Gaining insight into the biological bases underlying dermatophyte persistence in children will be necessary to guide the development of management strategies that offer the best chance of eradicating infection.

DISCLOSURE The authors declare no conflicts or financial interest in any product or service mentioned in the manuscript, including grants, equipment, medications, employment, gifts, and honoraria.

ACKNOWLEDGEMENTS This work was supported by grant AR053234 from the National Institute of Arthritis, Musculoskeletal and Skin Diseases and the Henson Endowed Fund for Pediatric Research.

REFERENCES

1. Suganami M, Hirose N, Shiraki Y, et al. *Trichophyton tonsurans* infection among judo practitioners who attended the National Junior High School Judo Tournament in Japan (2005): incidence and therapeutic response. Nippon Ishinkin Gakkai Zasshi 2006;47:319-324.
2. Triviño-Duran L, Torres-Rodriguez JM, Martinez-Roig A, et al. Prevalence of tinea capitis and tinea pedis in Barcelona schoolchildren. Pediatr Infect Dis J 2005;24:137-141.
3. Williams JV, Honig PJ, McGinley KJ, Leyden JJ. Semiquantitative study of tinea capitis and the asymptomatic carrier state in inner city school children. Pediatrics 1995;96:265-267.
4. Sharma V, Hall JC, Knapp JF, et al. Scalp colonization by *Trichophyton tonsurans* in an urban pediatric clinic. Arch Dermatol 1988;124:1511-1513.
5. Bocobo FC, Eadie GA, Miedler LJ. Epidemiologic study of tinea capitis caused by *T. tonsurans* and *M. audouinii*. Public Health Rep 1965;80:891-898.
6. Terreni AA. Tinea capitis survey in Charleston, SC. Arch Dermatol 1961;83:88-91.
7. Woldeamanuel Y, Mengistu Y, Chryssanthou E, Petrini B. Dermatophytosis in Tulugudu Island, Ethiopia. Med Mycol 2005;43:79-82.
8. Woldeamanuel Y, Leekassa R, Chryssanthou E, et al. Prevalence of tinea capitis in Ethiopian schoolchildren. Mycoses 2005;48:137-141.
9. Lohoue Petmy J, Lando AJ, Kaptue L, et al. Superficial mycoses and HIV infection in Yaounde. J Eur Acad Dermatol Venereol 2004;18:301-304.
10. Awoderu VA, Nebo GC, Otudero VO. Occurrence of dermatomycoses and in-vitro therapeutic efficacy of three antifungal drugs on the growth of *Epidermophyton floccosum*. Mycopathologia 2003;156:295-301.
11. Chandrasekaran N, Kamalam A, Thambiah AS. Tinea capitis in an Anglo-Indian school. Trop Geogr Med 1981;33:253-255.
12. Kamalam A, Thambiah AS. Trichophyton violaceum infection in an Indian school. Int J Dermatol 1976;15:136-139.
13. Omar AA. Ringworm of the scalp in primary-school children in Alexandria: infection and carriage. East Mediterr Health J 2000;6:961-967.
14. Poisson DM, Rousseau D, Defo D, Esteve E. Outbreak of tinea corporis gladiatorum, a fungal skin infection due to *Trichophyton tonsurans*, in a French high level judo team. Euro Surveill 2005;10:187-190.

15. Nyawalo JO, Bwire M. Single dose and intermittent griseofulvin regimens in the treatment of tinea capitis in Kenya. Mycoses 1988;31:229-234.
16. Ergin S, Ergin C, Erdogan BS, Kaleli I, Evliyaoglu D. An experience from an outbreak of tinea capitis gladiatorum due to *Trichophyton tonsurans*. Clin Exp Dermatol 2006;31:212-214.
17. Viguié-Vallanet C, Serre M, Masliah L, Tourte-Schaefer C. Épidémie de teignes à *Trichophyton tonsurans* dans une école maternelle de la région parisienne. Ann Dermatol Venereol 2005;132:432-438.
18. Lewis SM, Lewis BG. Nosocomial transmission of *Trichophyton tonsurans* tinea corporis in a rehabilitation hospital. Infect Control Hosp Epidemiol 1997;18:322-325.
19. Shiraki Y, Hiruma M, Hirose N, et al. A. nationwide survey of *Trichophyton tonsurans* infection among combat sport club members in Japan using a questionnaire form and the hairbrush method. J Am Acad Dermatol 2006;54:622-626.
20. Abdel-Rahman SM, Simon S, Wright KJ, et al. Tracking *Trichophyton tonsurans* through a large urban childcare center: defining infection prevalence and transmission patterns by molecular stain typing. Pediatrics 2006;118:2365-2373.
21. Zaidi N, Konstantinou K, Zevros M. The role of molecular biology and nucleic acid technology in the study of human infection and epidemiology. Arch Pathol Lab Med 2003;127 1098-1105.
22. Cordeiro RA, Brilhante RSN, Rocha MFG, et al. Antifungal susceptibility and genetic similarity of sequential isolates of *Trichophyton rubrum* from an immunocompetent patient with chronic dermatophytosis. Clin Exp Dermatol 2006;31:122-124.
23. Gupta AK, Kohli Y, Summerbell RC. Variation in restriction fragment length polymorphisms among serial isolates from patients with *Trichophyton rubrum* infection. J Clin Microbiol 2001;39:3260-3266.
24. de Assis Santos D, de Carvalho Araujo RA, Kohler LM, et al. Molecular typing and antifungal susceptibility of *Trichophyton rubrum* isolates from patients with onychomycosis pre- and post-treatment. Int J Antimicrob Agents 2007;29:563-569.
25. American Academy of Pediatrics. Tinea Capitis (Ringworm of the Scalp). In: Pickering LK, Baker CJ, Long SS, McMillan JA, eds. Red Book: 2006 Report of the Committee on Infectious Diseases. 27th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2006:655.
26. Abdel-Rahman SM. Strain Differentiation of Dermatophytes. Mycopathologia. 2008;166(5-6):319-333
27. Gaedigk A, Gaedigk R, Abdel-Rahman SM. Genetic Heterogeneity in the ribosomal RNA gene locus of *Trichophyton tonsurans*. J Clin Microbiol 2003;41:5478-5487.
28. Bhathena A, Gaedigk R, Abdel-Rahman SM. Characterization of the *ALP1* gene locus of *Trichophyton tonsurans*. Mycopathologia 2005;160:265-272.
29. Abdel-Rahman SM, Nahata MC, Powell DA. Response to Initial Griseofulvin therapy pediatric patients with tinea capitis. Ann Pharmacother 1997;31:406-410.
30. Higgins EM, Fuller LC, Smith CH. Guidelines for the management of tinea capitis. British association of dermatologists. Br J Dermatol 2000;143:53-58.