Comparison of *In Vitro* Activity of Undecylenic Acid and Tolnaftate against Athlete's Foot Fungi

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Received July 17, 1978, from the *Pharmaceutical Division, Pennwalt Corporation, Rochester, NY 14623.* Accepted for publication August 28, 1978. [*]Present address: Rochester General Hospital, Rochester, N.Y. [‡]Pharmaceutical Research Associates, Waltham, Mass.

Abstract □ Undecylenic acid and tolnaftate were tested in an *in vitro* test system to evaluate their relative "killing time" efficacy against *Trichophyton mentagrophytes, Trichophyton rubrum,* and *Epidermophyton floccosum.* Commercial products containing these active agents were tested similarly. The pure active agents were equivalent in activity. The commercial product containing undecylenic acid appeared to be more effective against the test organisms than did the product containing tolnaftate.

Keyphrases □ Undecylenic acid—*in vitro* comparison to tolnaftate against athlete's foot fungi, drugs and formulations □ Tolnaftate—*in vitro* comparison to undecylenic acid against athlete's foot fungi, drugs and formulations □ Athlete's foot—*in vitro* comparison of effectiveness of undecylenic acid and tolnaftate as pure drug and commercial formulations

Athlete's foot or *Tinea pedis* is probably the most common superficial fungus infection in humans. A number of causative agents are usually present, including *Trichophyton mentagrophytes,* which normally infects the interdigital spaces of the toes, *Trichophyton rubrum,* which resides on the plantar surface, and *Epidermophyton floccosum* (1).

Numerous preparations are available for the treatment of *T. pedis.* Underwood *et al.* (2) estimated that over 100 proprietary products were available in 1946. The latest estimate (1977) shows more than 50 products available on a nonprescription basis (3). Oral and topical prescription products raise this number significantly. However, relatively few active ingredients are used in these products. Typically, a product contains a keratolytic, an antifungal agent, and, perhaps, an antibacterial agent.

Fatty acids and their salts possess antifungal activity. The antifungal activity of products containing undecylenic acid and its salts was demonstrated some time ago (4). Until the 1960's, it was probably the therapy of choice. Recent human efficacy studies confirmed this earlier work (5). Since 1960, other compounds have been synthesized for topical relief of athlete's foot. Among these is tolnaftate.

To evaluate the relative efficacy of the undecylenates and tolnaftate, a series of clinical studies was performed[1]. The current study was designed to evaluate *in vitro* the relative "killing time" of undecylenic acid and tolnaftate as well as commercial products incorporating these active ingredients.

EXPERIMENTAL

Fresh cultures of *Trichophyton mentagrophytes* (ATCC 9533), *Trichophyton rubrum* (ATCC 10218), and *Epidermophyton floccosum* (ATCC 10227) were used. After 1 month of culturing and subculturing on Mycosel agar, a sufficient quantity of mycelial growth was obtained to begin testing. Plugs of agar containing mycelial growth were cut out using a sterile No. 2 cork borer with an outside diameter of 7 mm. Plugs were cut for replicate experiments and controls. The cut plugs were placed in sterile plastic petri dishes (60 × 15 mm). Each dish contained the necessary number of plugs for one replicate study. All excess agar was trimmed away from the mycelium using a sterile transfer loop and was discarded.

Table I—Comparison of Times Required to Kill Three Fungi by Suspensions of Undecylenic Acid (2%) and Tolnaftate (1%)—First Study

Organism	Contact Time, min	Number Killed of Seven Replicates: Undecylenic Acid	Tolnaftate
T. mentagrophytes	5	0	0
	10	0	0
	15	0	0
	20	0	1
	30	3	2
	60	7	7
T. rubrum	5	0	0
	10	0	1
	15	0	0
	20	4	3
	30	7	6
	60	7	7
E. floccosum	5	0	0
	10	0	0
	15	0	0
	20	0	0
	30	1	0
	60[a]	7	0

[a] Values significantly different ($p = 0.0003$); all others not significant.

Materials tested for antifungal activity included undecylenic acid[2], tolnaftate[3], and commercial powders containing these active ingredients[4]. The commercial powders were prepared and tested as 20% aqueous suspensions. Undecylenic acid was tested at a 2% concentration (aqueous suspension), and tolnaftate was tested at a 1% suspension concentration. All suspensions contained 0.1% polysorbate 20 as a dispersant.

All test mycelial plugs were immersed in the test product contained in a covered sterile dish. The positive control replicate was removed almost immediately, and the beaker was then placed on a mechanical rotator to keep the product in suspension and in good contact with the plugs. At each predetermined contact time (5, 10, 15, 20, 30, and 60 min for the pure actives and 60, 90, 120, 180, and 240 min for the commercial products), a set of seven replicate plugs was removed and the beaker was replaced on the rotator.

The treated plugs were carried through the following rinse cycle:

1. Placed in a culture tube containing 20 ml of the "first rinse" sterile solution, vortexed for 1 min, and allowed to remain in the rinse solution for 1 hr.
2. Transferred to the "second rinse" tube (10 ml), vortexed for 1 min, and soaked for an additional 30 min.
3. Transferred to the "third rinse" tube (10 ml), vortexed for 1 min, and allowed to soak overnight.

The rinsing solutions were prepared as follows. The first rinse was 1% peptone, 0.1% polysorbate 20, and sodium bicarbonate and distilled water

[1] To be published.

[2] Pennwalt receiving No. 05294.

[3] Code 56500, received from Schering Co.

[4] Desenex Powder, lot 3107 (also contains 20% zinc undecylenate), Pennwalt. Aftate Powder, lot 5VY, Plough.

0022-3549/79/0300-0384$01.00/0

Table II—Comparison of Times Required to Kill Three Fungi by Suspensions of Commercial Antifungal Products—First Study

Organism	Contact Time, min	Number Killed of Seven Replicates: Product A[a]	Product B[b]	Statistical Conclusion
T. mentagrophytes	60	3	1	N.S.[c]
	90	6	0	Significant ($p = 0.002$)
	120	7	2	Significant ($p = 0.01$)
	180	7	5	N.S.
	240	7	7	N.S.
T. rubrum	60	5	0	Significant ($p = 0.01$)
	90	7	4	Significant ($p = 0.09$)
	120	7	7	N.S.
	180	7	7	N.S.
	240	7	7	N.S.
E. floccosum	60	0	0	N.S.
	90	1	0	N.S.
	120	6	0	Significant ($p = 0.002$)
	180	7	2	Significant ($p = 0.01$)
	240	7	3	Significant ($p = 0.03$)

[a] A 20% aqueous suspension of Desenex powder. [b] A 20% aqueous suspension of Aftate powder. [c] Not significantly different.

to volume, with the pH adjusted to 7.6. The second and third rinses were 1% peptone, 0.1% polysorbate 20, and distilled water. On the following day, the plugs were resuspended by vortexing and aseptically transferred to the surface of Mycosel agar (BBL) plates. All seven replicates were placed in a single agar plate. The plates were incubated at ambient room temperature (24–28°) for 7 days for *T. mentagrophytes*, 10 days for *T. rubrum*, and 12 days for *E. floccosum*, and the number of replicate plugs showing growth in each set was recorded.

The described studies (seven replicates for each organism, test product, and time interval) were performed twice by different investigators approximately 1 year apart. The second investigator, however, utilized test dermatophytes that were recent clinical isolates maintained in his laboratory culture collection rather than the ATCC cultures.

RESULTS AND DISCUSSION

The results of the *in vitro* testing are shown in Tables I–IV. Tables I and III and II and IV compare the results obtained for the pure material and commercial products, respectively, by the two investigators. When the activity of pure undecylenic acid was compared to that of pure tolnaftate, no significant differences were found (except for one organism, at one time, and by only one investigator and not deemed experimentally significant) in the time required to kill the test fungi. All analyses were conducted using Fisher's Exact Test (6). Comparison of commercial powders containing these active agents showed interesting results. The first investigator (Table II) showed equivalence between both products in about half of the test instances and superiority of the undecylenate-containing product in the other half. These significant differences indicate that the undecylenate product (as tested by this investigator) killed the test organisms more rapidly than the tolnaftate-containing product.

Table III—Comparison of Times Required to Kill Three Fungi by Suspensions of Undecylenic Acid (2%) and Tolnaftate (1%) —Second Study

Organism	Contact Time, min	Number Killed of Seven Replicates[a]: Undecylenic Acid	Tolnaftate
T. mentagrophytes	5	0	0
	10	0	0
	15	0	0
	20	0	0
	30	2	2
	60	7	7
T. rubrum	5	0	0
	10	0	0
	15	0	0
	20	0	2
	30	2	2
	60	6	5
E. floccosum	5	0	0
	10	0	0
	15	0	0
	20	0	0
	30	1	0
	60	2	2

[a] Values not significantly different.

Table IV—Comparison of Times Required to Kill Three Fungi by Suspensions of Commercial Antifungal Products—Second Study

Organism	Contact Time, min	Number Killed of Seven Replicates: Product A[a]	Product B[b]
T. mentagrophytes	60	0	0
	90	0	0
	120	0	0
	180	1	0
	240[c]	5	0
T. rubrum	60	0	0
	90	0	0
	120	0	0
	180	1	0
	240	3	0
E. floccosum	60	0	0
	90	0	0
	120	5	2
	180	7	5
	240	7	7

[a] A 20% aqueous suspension of Desenex powder. [b] A 20% aqueous suspension of Aftate powder. [c] Values significantly different ($p = 0.01$); all others not significant.

The data in Tables III and IV are the results of the equivalent study by another investigator. No significant differences were found between the two active ingredients, essentially confirming the first study. When the two commercial products were tested, results were again essentially equivalent to the first study. There was a different time interval where statistical significance was observed compared to the initial study. However, on a global basis, one can conclude that undecylenates are possibly more effective in *in vitro* killing time than tolnaftate alone. Moreover, this finding would probably apply to the commercial powders containing these active agents.

The concentrations of active ingredients used differed for the two agents tested to simulate the commercial products available. Thus, undecylenic acid was used in a concentration of 2% and tolnaftate was used at 1%. Although the concentrations of active agents varied in the commercial products tested, the undecylenates are probably more effective (as determined by killing time) than tolnaftate.

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