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Orally Administered Ketoconazole: Route of Delivery to the Human Stratum Corneum

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Delivery of ketoconazole to human stratum corneum was studied. Thirteen healthy volunteers, three patients with chronic fungal disease and one patient with palmar-plantar hyperhidrosis were given 400 mg of ketoconazole daily for various lengths of time. The ketoconazole content of palmar stratum corneum, eccrine sweat, sebum, and serum was measured by high-pressure liquid chromatography (sensitivity, 0.005 to 0.010 μg/ml). Palmar stratum corneum obtained after 7 and 14 days of daily administration contained up to 14 μg of ketoconazole per g. Ketoconazole was not found in sebum after 7 or 14 days of daily ingestion of the antimycotic agent. Sebum from three patients with chronic fungal infection treated for >9 months contained ketoconazole ($\bar{x}$, 4.7 μg/g). Thermogenic whole body eccrine sweat contained a mean of 0.059 μg/ml on day 7 and 0.084 μg/ml on day 14 of daily administration. Ketoconazole appeared in thermogenic whole body eccrine sweat and palmar hyperhidrotic sweat within 1 h after a single oral dose. Partition studies of ketoconazole containing eccrine sweat demonstrated a 10-fold greater concentration in the sediment phase (desquamated keratinocytes) compared with the clear supernatant phase. In vitro studies with [^{3}H]ketoconazole-supplemented supernatant sweat revealed preferential binding to stratum corneum, hair, and nails and its partitioning to lipid-rich sebum. We conclude that eccrine sweat rapidly transports ketoconazole across the blood-skin barrier, where it may bind or partition to keratinocytes and surface lipids.

Ketoconazole is a synthetic oral antifungal antibiotic which in vitro has a broad spectrum of activity. In vivo, ketoconazole is highly effective against a variety of mycotic infections (8), including dermal forms of candidiasis (10), dermatophytosis (7), and tinea versicolor (15). Dermatophytosis and tinea versicolor are noninvasive, superficial infections of the nonviable stratum corneum and hard keratin structures (6).

Ketoconazole, most frequently administered as either a 200-mg or a 400-mg dose, is readily absorbed after conversion to the water-soluble salt by gastric acid. This dosage results in plasma levels of 1 to 12 μg/ml within 1 to 2 h, with 84% bound to plasma proteins and 14% bound to the formed elements of blood. Less than 1% is free in the plasma. The blood half-life, which is dose dependent, ranges from 1 to 11 h (9). After a single oral dose of 200 mg in humans, detectable levels have been found in the cerebrospinal fluid, urine, saliva, cerumen, and sebum (5).

Because ketoconazole has been detected in sebum, it has been suggested that sebum secretion is the route by which the antimycotic agent reaches the stratum corneum. Alternatively, ketoconazole may reach the stratum corneum by becoming incorporated into basal cells that move toward the skin surface, eventually becoming the stratum corneum. Passive diffusion from the plasma compartment into the stratum corneum or delivery in sweat to the body surface must also be considered.

The initial clinical response of noninflammatory extensive chronic dermatophytosis of the glabrous skin to ketoconazole is rapid (7 to 10 days) (7, 12). The excretion rate of sebum and the movement of cells from the basal layer into the stratum corneum is considerably slower (3 to 4 weeks) (3, 16, 17). This raises some question as to the importance of sebum or basal cell movement toward the surface in the initial delivery of ketoconazole to the human stratum corneum.

The purpose of this investigation was to identify the route(s) by which ketoconazole reaches the human stratum corneum. Our results indicate that eccrine sweat is one pathway by which ketoconazole rapidly reaches the stratum corneum.

MATERIALS AND METHODS

Ketoconazole. Ketoconazole was provided by Janssen Pharmaceutica. Ketoconazole tablets (200 mg; lot

no. 81 A 09/F02; expiration date January 1984) were used in vivo. Pure ketoconazole base powder (R41400, lot no. C 6401) was used for the preparation of seeded sweat and sebum control samples. A stock solution was prepared by dissolving 500 mg in 10 ml of 0.4 N HCl. The pH was adjusted to 7.0 by the addition of 0.1 N NaOH. The desired working solutions were prepared by serial dilution in sterile deionized water.

Tritiated ketoconazole (lot no. J2H-13-12) was provided as a base (specific activity, 0.839 μCi/μg; radiochemical purity, 98% as determined by thin-layer chromatography in three solvent systems) and was converted to the water-soluble salt by dissolving 3.02 mg in 5 ml of 0.4 N HCl and adjusting the pH to 7.0 with 0.1 N NaOH. A stock solution of 6 μg/ml was prepared by dilution in deionized water. The specific activity was decreased to 0.0839 μCi/μg by mixing 1 part of labeled ketoconazole (6 μg/ml) with 9 parts of unlabeled ketoconazole (6 μg/ml). A final working solution of 0.1 μg/ml was prepared by adding 1 ml of the isotope solution (specific activity, 0.0839 μCi/μg) to 59 ml of ketoconazole-free supernatant sweat.

Human subjects. Thirteen normal, healthy, male volunteers who were free of infection and taking no medication were selected for participation in the portion of the study requiring 14 days of daily ingestion of ketoconazole or a single dose. Their age and weight ranged between 20 and 35 years and 150 and 200 pounds, respectively.

Three adult patients with cutaneous mycotic infections (two having widespread, chronic dermatophytosis and one having chronic mucocutaneous candidiasis) were also studied. These patients had received a minimum of 9 months of continuous ketoconazole therapy and were ingesting 400 mg/day in a divided dose at the time of this study. A 15-year-old girl who suffered from palmar-plantar hyperhidrosis participated in the study on a single day when she ingested a single 400-mg dose of ketoconazole. After the nature of the experiment and the potential risks to their health and safety were discussed, each subject signed a university-approved consent form.

Serum collection. Blood (20 ml) was collected by venipuncture and allowed to clot in glass tubes at room temperature for 3 h. The serum was decanted, clarified by centrifugation, and stored at −70°C.

Sebum collection. All subjects were instructed to wash their face two hours before sebum collection and to avoid activity that would induce sweating to minimize contamination of sebum with sweat. Sebum was then collected by the method of Ramasastry et al. (11). Briefly, a polyurethane sponge (4 cm long and 1 cm in diameter attached to a 15-cm stainless steel wire) soaked in hexane was used to swab the forehead, nose, and cheeks. After swabbing, the sponge was placed into a previously dessicated and weighed 1.5- by 15-cm glass test tube that contained 5 ml of hexane. After soaking for 2 h, the hexane was squeezed from the sponge, and the sponge was discarded. The hexane was evaporated from the tube with a light stream of nitrogen directed at the surface. The hexane-free, sebum-containing tubes were dessicated for 18 h at 4°C and weighed to determine the amount of sebum collected. After weighing, the sebum samples were stored at −70°C.

Sweat collection. Thermogenic sweat was induced in a sauna bath operating at 60°C. The resulting sweat was collected by placing the subjects within a plastic body bag, which was loosely fastened around the neck. Usually 100 to 200 ml of sweat was collected within 15 to 30 min. Before inducing sweat, each subject took a shower bath and used soap to remove body surface dirt and lipids.

The collected sweat was divided into two portions. The first, whole sweat, was stored at −70°C. The remainder was centrifuged at 25,000 × *g* for 15 min at 4°C, which produced a clarified supernatant sweat phase and a turbid sweat sediment phase. The volume of the supernatant and the sediment was measured. The sweat sediment phase was stored at −70°C.

Measured quantities (usually 35 ml) of whole sweat and supernatant sweat were placed into 50-ml 3033 Falcon tubes, frozen, and lyophilized to dryness with a shelf-type freeze dryer (Freezemobile; The VirTis Co., Inc., Gardiner, N.Y.). The lyophilized material was resolubilized with 2 to 5 ml of deionized water, and the concentration factor was noted. The samples were stored at −70°C.

Stratum corneum collection. The stratum corneum was curetted from the palmar surfaces with a surgical steel open dermal curette. Only superficial and midlevel stratum corneum, as defined by Epstein et al. (4), was collected. The stratum corneum samples were dessicated in preweighed tubes for 18 h at 4°C, and their dry weight was determined. The weighed, dessicated samples were stored at −70°C.

Control samples. Sweat and sebum were obtained from the normal volunteers before their initial ingestion of ketoconazole. A portion of the resulting ketoconazole-free native sweat was used in the in vitro studies with [^{3}H]ketoconazole. These sweat samples were also used to prepare negative ketoconazole controls (no added ketoconazole) and positive controls (spiked with a known quantity of ketoconazole).

High-pressure liquid chromatographic determinations of ketoconazole. All assays for ketoconazole were performed by the Department of Drug Metabolism and Pharmacokinetics at the Janssen Pharmaceutica Co. Laboratories, Beerse, Belgium, under the supervision of Joseph Heykants and Robert Woestenborgh (R. Woestenborgh, W. Lorreyne, and J. Heykants, 1982, Preclinical research report R 41400/42; on file at Janssen Pharmaceutica Co.). Before shipping, all samples were randomized and coded. Blinded, seeded control samples were included. All samples were first shipped to the Janssen Pharmaceutica Co. in New Brunswick, N.J., and then transported by courier to the Belgium laboratory. The average transit time from Atlanta to Belgium was approximately 72 h.

A high-pressure liquid chromatographic assay modified from the method of Alton (1) was used for detection of ketoconazole. The sensitivity of the assay was 0.005 to 0.010 μg of ketoconazole per ml. Briefly, all samples were spiked with an internal standard (terconazole). Samples were adjusted to pH 9.0 by the addition of 0.01 N NaOH, and both the internal standard and ketoconazole were extracted with heptane-isoamyl alcohol (95:5, vol/vol). Stratum corneum and sebum samples were ultrasonically homogenized in methanol, adjusted to pH 9.0 with 0.01 N NaOH, and similarly extracted with heptane-isoamyl alcohol. The extracts were evaporated, and the resulting residue was redissolved in acetonitrile-water-diethylamine (70:30:0.05, vol/vol/vol). This solution was used as the

mobile phase in a high-pressure liquid chromatographic system with a reversed-phase column. Ketoconazole and the internal standard were detected by monitoring absorbance at 254 nm.

Different series of the calibration samples were spiked with the internal standard and submitted to extraction and high-pressure liquid chromatography. For the internal standard concentration, calibration curves were constructed by plotting the peak area ratio of ketoconazole to the internal standard (log transformed) against the ketoconazole concentration (log transformed). The linear regression parameters from these calibration curves were used to calculate unknown concentrations of ketoconazole. This calculation was accomplished with the aid of an integrating computer system coupled in line with the spectrophotometer.

Experimental design. Ten normal male volunteers were given 400 mg of ketoconazole as a single morning dose for 14 days. Venous blood for serum, sweat, and palmar stratum corneum were collected on days 7 and 14. Sebum was collected on days 3, 7, 10, and 14. Blood, sebum, and stratum corneum were obtained approximately 2 h after the daily ketoconazole dose. Palmar stratum corneum was obtained from the right hand on day 7 and from the left hand on day 14. Thermogenic, whole body eccrine sweat was obtained on days 7 and 14, 4 h after the ingestion of ketoconazole.

Three additional male volunteers were given a single oral dose of 400 mg of ketoconazole. Thermogenic, whole body eccrine sweat was obtained before and at 1, 3, 5, and 7 h after ketoconazole ingestion. Palmar stratum corneum was obtained from two of the three subjects 7.5 h after the ketoconazole dose.

A single female subject with hyperhidrosis was induced to sweat by requesting her to rapidly solve a difficult mathematical problem without use of pen, paper, or calculator. Sweating occurred only on the palmar aspects of her fingers and hands and was collected from the fingertips, from which it dripped freely. Collections were made before and at 1 and 3 h after a single 400-mg dose of ketoconazole. Immediately after the last sweat collection, stratum corneum was obtained from both palms. Before collection of the stratum corneum, the right hand was air dried with an electric fan, and the left palm was washed three times with soap and water and air dried.

Sebum was collected from the noninfected facial skin of the three patients with chronic fungal disease who had been treated with ketoconazole for ≥9 months. Sebum and stratum corneum were also collected from an area of dermatophyte-infected skin on the back of one patient. Palmar stratum corneum, serum, and thermogenic, whole body sweat were also collected from this subject. With the exception of the sweat, all samples were collected 2 h after the last dose of ketoconazole. Thermogenic eccrine sweat was collected 5 h after the last ketoconazole dose.

Partitioning of tritiated ketoconazole from eccrine sweat. Known weights of palmar stratum corneum, sebum, hair, hexane-delipidated hair, pulverized fingernail clippings, and turbid sweat sediment (keratinocytes) were added to 3.0-ml samples of supernatant sweat supplemented with [^{3}H]ketoconazole as described above. These mixtures were kept at 6°C for 5 days. The supernatant was removed by decantation, and the solid phase was washed three times with deionized water by centrifugation. The radioactivity of the supernatant and solid phases was determined by liquid scintillation counting, using Beckman Ready-Solv HP high-performance liquid scintillation cocktail and a Packard Tri-Carb liquid scintillation spectrometer, model 3330.

The ketoconazole-partitioning coefficient (KPC) was calculated from the counts per minute (cpm) and weights (in milligrams) in the following manner: KPC = [(cpm of solid phase)/(total cpm of supernatant + cpm of solid phase)]/[(solid phase weight)/(supernatant weight + solid phase weight)].

The assumption was made that the specific gravity of supernatant sweat was approximately equal to 1.0. Therefore, 1 μl of supernatant was considered equal to 1.0 mg. Ketoconazole-partitioning coefficients of >1.0 indicate the partitioning of ketoconazole into the solid phase (sweat sediment, stratum corneum, nail, hair). The experiment was repeated two times.

Statistics. The correlation coefficient between various samples was computed by standard calculation. The significance of the difference between various sample means was computed by Student's paired *t* test. Mean values are presented ± the standard deviations.

RESULTS

Ketoconazole content of stratum corneum. Palmar stratum corneum was collected 2 h after the daily dose of ketoconazole from the right hand on day 7 and the left hand on day 14 from the 10 subjects given the antimycotic agent daily for 14 days. The mean dessicated weight of the samples was 22.0 ± 19.3 mg. Five of the 10 samples collected on day 7 and 4 of the 10 samples collected on day 14 contained detectable levels of ketoconazole (Table 1). Only subject 4 had detectable levels on both days. Ketoconazole was not detectable in palmar stratum corneum at either collection time in subjects 9 and 10. The mean concentration for those samples containing detectable ketoconazole was 4.59 ± 2.21 μg/g for day 7 and 5.18 ± 6.02 μg/g for day 14. The mean serum level of ketoconazole at the time of stratum corneum collection was 6.63 ± 3.85 μg/ml on day 7 and 7.87 ± 4.72 μg/ml on day 14. There was no correlation between serum levels and stratum corneum levels at either collection time.

Palmar stratum corneum and midback stratum corneum from one patient undergoing prolonged therapy for widespread chronic dermatophytosis was also collected and analyzed. This patient had a surface area involvement of approximately 45%. After 9 months of treatment, the extent of infection had been reduced by 80%. Noninfected palmar stratum corneum from this patient contained 6.94 μg of ketoconazole per g, and the infected midback stratum corneum contained 2.69 μg/g.

Ketoconazole content of sebum. Facial sebum was collected 2 h after the daily dose of ketocon-

TABLE 1. Ketoconazole content of palmar stratum corneum collected on days 7 and 14 during a 14-day period of daily ketoconazole treatment[a]

Subject no.	Ketoconazole content			
	Stratum corneum (μg/g)		Serum (μg/ml)	
	Day 7	Day 14	Day 7	Day 14
1	ND[b]	3.12	6.76	9.21
2	ND	0.952	1.15	1.78
3	ND	14.1	7.52	4.48
4	2.07	2.53	10.90	14.50
5	3.89	ND	8.24	2.17
6	4.29	ND	2.69	9.12
7	4.54	ND	8.68	8.80
8	8.15	ND	9.36	7.37
9	ND	ND	0.38	5.39
10	ND	ND	10.60	15.90
Mean[c]	4.59	5.18	6.63	7.87
SD[c]	2.21	6.02	3.85	4.72

[a] Blood and stratum corneum were collected 2 h after the daily morning oral dosage of 400 mg.

[b] ND, None detected.

[c] The mean and standard deviation for the stratum corneum were calculated only from samples where ketoconazole was detected.

azole on days 3, 7, 10, and 14 from the 10 subjects given the antimycotic agent daily for 14 days. The mean sample weight collected was 10.5 ± 4.0 mg. Ketoconazole was not detected in any of the 40 sebum samples analyzed. Ketoconazole was detected in seeded sebum control samples. Recovery from these controls ranged from 76 to 104% of the amount added to the sample.

Sebum was also collected from three patients on prolonged (≥9 months) ketoconazole therapy for chronic fungal infection. The facial sebum collected from noninfected skin contained 3.8 to 5.7 μg/g. Sebum collected from infected mid-back skin of one patient with chronic dermatophytosis contained 3.2 μg/mg.

Ketoconazole content of thermogenic eccrine sweat. Total body thermogenic eccrine sweat was collected 4 h after the daily ketoconazole dose on days 7 and 14 from the 10 subjects given the antimycotic agent daily for 14 days. Whole thermogenic eccrine sweat from all 10 subjects at both collection times contained ketoconazole (Table 2). The quantity of ketoconazole in the sweat varied from subject to subject. The range on day 7 was 0.007 to 0.176 μg/ml, and on day 14 it was 0.008 to 0.323 μg/ml. Comparing day 7 with day 14, there was no significant difference in the mean ketoconazole content of sweat. There was also no correlation between serum levels (Table 1) and sweat levels on either day 7 or day 14.

Freshly collected whole sweat from all subjects was noticeably turbid. Microscopic examination revealed that this was the result of suspended, desquamated keratinocytes. A portion of each whole sample was, therefore, fractionated by centrifugation into the clear supernatant and keratinocyte-containing sediment phase. The quantity of keratinocyte sediment produced from 50 ml of whole sweat was approximately 1 to 2 ml (packed volume) for all samples. The ketoconazole content of both phases was measured to determine whether the presence of keratinocytes contributed to the ketoconazole content of unfractionated whole sweat. The results for each sweat sample are listed in Table 2. The sediment phase of the day 7 sweat samples, when compared on a volume-to-volume basis with the supernatant phase, contained a 30-fold greater concentration of ketoconazole. The day 14 sediment phases contained a 12-fold elevation of the ketoconazole concentration above the supernatant phase. The difference between the mean ketoconazole content of the whole sweat and supernatant sweat on day 7 was significant ($P > 0.01$). The difference was not statistically significant on day 14. The day 7 supernatant level was 41% of the whole sweat level, whereas the day 14 supernatant level was 90% of the whole sweat level. The ketoconazole content of unfractionated whole sweat at both collection times correlated directly with the content measured in the supernatant and sediment phases. Interestingly, normal, unfractionated, whole sweat samples seeded with ketoconazole, used as positive controls, revealed a distribution of the antimycotic agent to sediment and supernatant phases similar to that described above.

TABLE 2. Ketoconazole content of unfractionated and fractionated thermogenic eccrine sweat collected during a 14-day period of daily ketoconazole ingestion[a]

Subject no.	Ketoconazole content (μg/ml)					
	Whole sweat		Supernatant sweat		Sweat sediment	
	Day 7	Day 14	Day 7	Day 14	Day 7	Day 14
1	0.031	0.039	0.015	0.023	0.098	0.510
2	0.007	0.008	0.022	0.004	0.046	0.042
3	0.063	0.033	0.029	0.017	0.382	0.385
4	0.018	0.057	0.008	0.047	0.104	0.748
5	0.058	0.048	0.023	0.016	0.250	0.283
6	0.176	0.323	0.055	0.348	2.810	1.770
7	0.110	0.072	0.055	0.066	1.120	0.511
8	0.014	0.018	0.005	0.014	0.237	0.164
9	0.057	0.106	0.021	0.126	0.890	2.940
10	0.054	0.134	0.007	0.101	1.230	2.200
Mean	0.059	0.084	0.024	0.076	0.717	0.960
SD	0.051	0.093	0.018	0.104	0.857	0.990

[a] Ketoconazole was given as a single 400-mg oral dose each morning, and sweat was collected 4 h later. Whole sweat was fractionated into supernatant and sediment phases by centrifugation.

Ketoconazole recovery from these seeded control samples ranged from 81 to 84% of the amount added to the sample. Ketoconazole was not detected in any of the whole sweat samples obtained from untreated subjects. These results indicate that ketoconazole contained in the keratinocyte sediment phase contributes to the levels found in whole sweat and raises the possibility that ketoconazole may partition from the clear supernatant phase to the keratinocyte sediment phase.

To determine how rapidly ketoconazole appeared in thermogenic eccrine sweat, three normal subjects were each given a single 400-mg dose of the antimycotic agent, and their sweat was collected 1, 3, 5, and 7 h later. Ketoconazole appeared in sweat ≤1 h after ingestion of the antimycotic agent (Table 3). Peak sweat levels occurred between 3 and 5 h. The mean serum level at 2 h for these three subjects was 4.51 ± 1.19 μg/ml.

Palmar stratum corneum was sampled from subjects 11 and 12 after the last collection of their sweat. Before the samples were collected, the subjects were rested in a cool room to halt sweating, and their hands were thoroughly washed with water and air dried to eliminate possible contamination from residual ketoconazole-containing sweat. The palmar stratum corneum content of ketoconazole for subjects 11 and 12 was 8.93 and 12.30 μg/g, respectively.

Ketoconazole content of palmar hyperhidrotic sweat. Palmar skin is free of sebaceous glands, and therefore ketoconazole-containing sweat collected from this region should be free of any contribution from sebum. Furthermore, palmar eccrine sweat can be obtained free from contamination by thermogenic eccrine sweat originating from other body surfaces from patients with palmar hyperhidrosis.

A single 400-mg oral dose of ketoconazole was given to a subject with hyperhidrosis, and psychogenically induced palmar sweat was collected at 1 and 3 h after ingestion of the antimycotic agent. The quantity collected at each time point was approximately 3.0 ml. The ketoconazole content at 1 h was 0.135 μg/ml; at 3 h it was 0.110 μg/ml. These sweat samples, unlike whole body thermogenic sweat samples, were not turbid, indicating that they were nearly free of desquamated keratinocytes.

Immediately after the collection of palmar hyperhidrotic sweat, palmar stratum corneum samples were obtained. To determine whether washing extracted ketoconazole from the stratum corneum, the left hand was vigorously washed with soap and water. The right hand was not washed or rinsed. Both hands were air dried before the samples were obtained. The quantity of ketoconazole in the sample obtained from the washed left hand was 0.94 μg/g. The unwashed right hand sample contained 0.90 μg/g, suggesting that washing the hand did not reduce the ketoconazole content of the palmar stratum corneum.

TABLE 3. Ketoconazole appearance in thermogenic eccrine sweat after a single 400-mg oral dose

Time (h)	Ketoconazole (μg/ml)[a]		
	Subject 11	Subject 12	Subject 13
0	0	0	0
1	0.205	0.027	0.013
3	0.238	0.030	0.036
5	0.210	0.039	0.033
7	0.100	0.028	0.019

[a] Values are for whole, unfractionated sweat.

Partitioning of ketoconazole from eccrine sweat into stratum corneum, formed elements of the skin, and sebum. The possibility that ketoconazole readily partitions from eccrine sweat to keratinocytes, sebum, and other keratinized structures was investigated by seeding the supernatant fraction derived from whole sweat with [^{3}H]ketoconazole and measuring the accumulation of the radiolabeled antimycotic agent into the sebum and the various formed structures of the skin. The results of a single experiment are presented in Table 4. A replicate experiment yielded almost identical data. Ketoconazole partitioning coefficient values of greater than 1.0 indicate positive partitioning into the solid phase of the system. All formed elements and sebum had positive ketoconazole partitioning coefficient values. On a weight basis, curetted stratum corneum and sebum had the largest values. These results indicate that the formed elements of skin and sebum have the capacity to absorb and concentrate ketoconazole from eccrine sweat.

DISCUSSION

Unlike the systemic mycoses, which infect and invade deep tissues that are in direct contact with plasma and tissue fluid electrolyte compartments, the dermatophytic fungi invade only the dead outer stratum corneum layer of the body and its associated keratinized structures (15). These keratinized components of the skin, hair, and nails are not in direct contact with the body's fluid and electrolyte compartments. The rapid response of chronic dermatophytosis to ketoconazole suggests, however, that the antimycotic agents moves in some unknown way rapidly from the bloodstream to the stratum corneum.

There are four possible routes by which ketoconazole may reach the stratum corneum of glabrous and palmar plantar skin from the blood-

TABLE 4. Partitioning of ketoconazole from eccrine sweat into stratum corneum, formed elements of the skin, and sebum[a]

Sample	Weight (mg)	Solid phase		Supernatant phase		KPC[b]
		Total cpm	Measured cpm/mg	% cpm/200 mg	Measured cpm/mg	
Supernatant sweat	200			10,429	52	
Stratum corneum	1.2	37,596	31,330	6,717	34	680
Sebum	10.4	111,154	10,688	3,406	17	198
Pulverized nail	2.6	10,654	4,098	9,538	48	79.3
Hair	10.4	37,526	3,608	6,866	34	77.4
Delipidated hair	10.5	18,241	1,737	8,302	42	36.7
Sweat sediment	79.8	43,172	548	7,415	37	10.8

[a] The assumption was made that the specific gravity of supernatant phase sweat was approximately 1.0; therefore, 1 μl of supernatant was taken as equivalent to 1.0 mg.

[b] KPC, Ketoconazole-partitioning coefficient. Values of >1.0 indicate partitioning of ketoconazole into the solid phase.

stream. These can be divided into slow and rapid. The first slow route is by incorporation into the cells of the basal layer of the epidermis. These cells, over a period of 3 to 4 weeks, move toward the surface, keratinize, and cornify to become the stratum corneum (17). The second slow route is through the excretion of sebum. The length of time required for sebum to be produced and move from the base of the sebaceous gland is also 3 to 4 weeks (3, 16).

Rapid appearance of ketoconazole in the stratum corneum may be explained by passive diffusion from the bloodstream across the dermal-epidermal barrier, through the nonvascular lower layers of the epidermis, and upward into the stratum corneum. Alternatively, ketoconazole may move from the bloodstream into the eccrine sweat apparatus, where it becomes a component of newly formed sweat and is secreted onto the skin surface. Once on the surface, the antimycotic agent could then be absorbed by the stratum corneum.

In the present study, we first measured the ketoconazole content of palmar stratum corneum. Stratum corneum collected from this site is relatively uncontaminated by sebum, since palmar skin does not contain sebaceous glands. During 2 weeks of daily ingestion of the antimycotic agent, ketoconazole was not consistently found in palmar stratum corneum. Furthermore, the subjects who had detectable ketoconazole at the 7-day sampling time did not necessarily have it at the 14-day sampling time (Table 1). Additionally, two persons given a single dose of ketoconazole revealed demonstrable ketoconazole in their palmar stratum corneum within 7 h after ingestion of the antimycotic agent.

We interpret these results to indicate that ketoconazole was not deposited by the epidermal basal cell incorporation route because of the rapidity and inconsistency with which it appeared. The possibility that ketoconazole was extracted by washing, however, may explain why the antimycotic agent was variably present. The single experiment involving the hyperhydrotic subject, however, would suggest that ketoconazole is not readily extracted by washing.

Delivery of ketoconazole to the palmar stratum corneum is also not explained by sebum excretion, because palmar skin does not contain sebaceous glands, and the rapid appearance in palmar stratum corneum exceeds by weeks the rate of sebum excretion. Furthermore, facial sebum collected from these subjects after 7 and 14 days of ketoconazole ingestion was not found to contain ketoconazole, indicating that this route is not important in the rapid delivery of ketoconazole to glabrous skin. Sebum collected from the three patients who were on prolonged ketoconazole therapy, however, did contain the antimycotic agent, indicating that it can be secreted through the sebaceous gland apparatus (or perhaps partition to the sebum from another source).

Very little is known about the equilibrium or passive diffusion of low-molecular-weight substances between the plasma compartment and the stratum corneum. Most studies have been concerned with the movement of topically applied substances from the stratum corneum into the plasma compartment (13). Movement in the opposite direction by passive diffusion has been limited to lanthanum tracer studies, which clearly indicate that the normal epidermis prevents the loss of low-molecular-weight substances from the plasma compartment (2). Nonetheless, the possibility that ketoconazole reaches the stratum corneum by passive diffusion must be considered.

Although the present investigation did not directly address this possibility, there are several facts that mitigate against the passive diffusion route. First, >99% of the ketoconazole in the bloodstream is bound to plasma proteins and formed elements of the blood and, along with

other small molecules in the plasma, is not readily available for simple passive diffusion into the stratum corneum. Unless the epidermis has an exaggerated avidity for the antimycotic agent compared with the plasma proteins, the movement of ketoconazole into the stratum corneum by passive diffusion should be minimal. Second, we did not consistently find ketoconazole in palmar stratum corneum, despite good blood levels. However, we sampled only the upper and middle stratum corneum levels and, therefore, may have missed ketoconazole in the lower levels. The demonstration that ketoconazole was consistently present in the lower levels of the stratum corneum shortly after ingestion of the antimycotic agent would suggest that ketoconazole could readily pass through the dermal-epidermal barrier by passive diffusion. The possibility that the palmar stratum corneum may not accurately reflect how the antimycotic agent reaches glabrous stratum corneum must also be considered.

Similar to the findings for griseofulvin reported by Shah et al. (14), the data presented in Tables 2 and 3 clearly indicate that the eccrine sweat apparatus can deliver ketoconazole to the skin surface. The rapidity with which ketoconazole appeared in thermogenic eccrine sweat after a single oral dose ($\leq$1 h) is consistent with the rapid response of chronic dermatophytosis to the antimycotic. The results from the hyperhidrotic patient, where psychogenic, palmar eccrine sweat minimally contaminated by desquamated keratinocytes and free of sebum contained significant levels of ketoconazole, confirm the finding that ketoconazole appears rapidly in sweat.

Perhaps the most interesting observation is that ketoconazole readily partitioned from the fluid phase of sweat to keratinocytes, hair, nails, and sebum (Tables 2 and 4). The avidity of these structures for ketoconazole would be expected to facilitate and prolong the activity of the antimycotic agent in these structures. Differences in avidity for ketoconazole of stratum corneum from one part of the body to another may affect the activity of the antimycotic agent. There is some suggestion that griseofulvin may not readily partition into the stratum corneum (4, 14).

We conclude from the present investigation that the eccrine sweat apparatus is a major route by which ketoconazole rapidly reaches the human stratum corneum. However, we have not eliminated the possibility that ketoconazole reaches the stratum corneum by rapid passive diffusion. Delivery of ketoconazole to the skin surface by sebum under conditions of prolonged therapy may also be important.

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