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Intracutaneous Transport of Orally Administered Fluconazole to the Stratum Corneum

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Fluconazole administered at 150 mg/week for 1–5 weeks is effective orally against dermatophytes and yeast in stratum corneum. Clinical and mycological cure rates approach 90%, but the precise distribution of the drug within various layers of skin is uncertain. We administered fluconazole at 150 mg/week for 2 weeks to 5 volunteers. Distribution of fluconazole in biopsies of skin was imaged by energy dispersive analysis of X-rays (EDX) and transmission electron microscopy, and in cells by electron energy-loss spectroscopy (EELS). Eight hours after a second dose, EDX showed fluconazole highest and homogeneously distributed in stratum corneum, lower in the rest of the epidermis, and lowest in dermis. The highest fluconazole levels detected by EELS were in cytoplasmic inclusions of sweat and sebaceous glands and less in keratinocytes and dermal collagen. We conclude that fluconazole delivered to stratum corneum by direct diffusion from capillaries and in sweat is also in all likelihood transported in sebum.

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Progressively invasive superficial mycoses may cause serious fungal disease, especially in immunocompromised AIDS and cancer patients. Topical treatment of these infections is often inadequate because locally applied agents fail to penetrate the stratum corneum at concentrations that inhibit or kill intracutaneous fungi and yeast. Orally administered antimycotics have been used against invasive dermatophytes and yeast but with variable results (1, 2). One azole derivative, fluconazole – a bis-triazole derivative containing two atoms of fluorine – achieves clinical and mycotic cure rates approaching 90% in patients with superficial and progressively invasive dermatomycoses (3). However, the delivery of antimycotic to sites of infection has not been fully defined. Multiple routes for the transport of orally administered fluconazole to intracutaneous sites of dermatomycoses have been postulated following chemical analyses of various antimycotics in eccrine sweat, serum, surgically separated stratum corneum, epidermis and dermis (4). However, the *in situ* distribution of fluconazole in skin has not been reported. In this report, we describe the use of energy dispersive analysis of X-rays (EDX) and electron energy-loss spectroscopy (EELS) to visualize fluorine equivalents of fluconazole *in situ* in skin from normal human volunteers, who received a 150-mg capsule of fluconazole once a week for 2 weeks.

MATERIALS AND METHODS

Subjects

Twelve healthy male volunteers (mean age 30 years; range 22–49) received fluconazole (Diflucan®, Pfizer) 150 mg once a week for 2 weeks. They had all given their informed consent, and the study was approved by the Ethical Committee of the University of Gothenburg.

Drug administration

Fluconazole was administered orally as a 150-mg capsule weekly for 2 weeks.

Collection of skin specimens

A 4-mm punch biopsy was taken from the skin of the back of each subject. Subcutaneous tissue was separated and the biopsy transferred to a sterile tube for subsequent processing.

Electron energy loss spectroscopy (EELS)

Specimens of skin were fixed in a solution of 2.5% glutaraldehyde in 0.175 PIPES buffer containing dimethylformamide at 4°C and prepared for ultramicrotomy. Others were rapidly frozen and cryosectioned (5). Thin sections of skin were prepared for examination in a Zeiss CEM902 energy filtering electron microscope, as previously described (5, 6).

Energy dispersive analysis of X-rays (EDX)

Millimeter-thick sections of some specimens were flat-embedded in epoxy resin and thin-sectioned for examination in a Phillips 301 transmission electron microscope. The remainder of the specimen was critical point dried and mounted on a stub. The face of the specimen next to the mm-thick section taken for TEM was studied in an Amray scanning electron microscope equipped with a collecting spectrometer (7). The surface of the stub-mounted skin specimen was scanned in a raster within the prescribed energy window of the collecting spectrometer to acquire a qualitative pixel map of fluorine distributed in the sample (8).

Reconstruction of TEM and EDX images

The tissue and cellular distribution of fluconazole was delineated by photographically superimposing the pixel map of fluorine equivalents of the antimycotic over the corresponding TEM of the adjacent section of skin.

RESULTS

Fig. 1 illustrates the distribution of orally administered fluconazole in human skin. The composite photomicrograph was created by superimposing a pixel map of fluorine – imaged by EDX – on a transmission electron photomicrograph of the same cross section of skin from fluconazole-treated subjects. The blue-coloured pixel equivalents of fluconazole overlay – from upper left to lower right, the surface of the skin (upper left), stratum corneum (black), epidermis (pink/cellular) and dermis (pink).

The skin biopsy was taken 7 days after a single oral 150-mg dose of fluconazole. The pixel images of fluconazole equivalents acquired by energy dispersive analysis of fluorine show that fluconazole accumulated homogeneously on the surface and

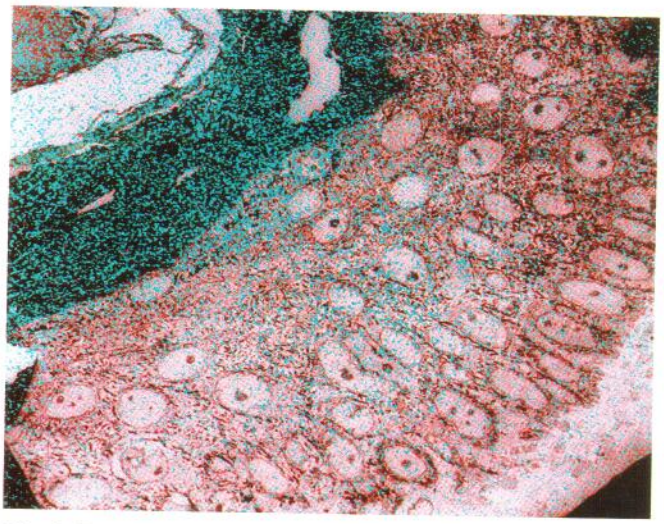

Fig. 1. Compositional image of fluconazole distributed on the surface and among layers of a skin biopsy 7 days after a single oral 150 mg dose. The blue-coloured pixels are fluorine equivalents of fluconazole imaged by energy dispersive analysis of X-rays. This pixel map was superimposed over a transmission electron photomicrograph of a section of skin adjacent to the surface assayed by energy dispersive analysis of X-rays. ×1,500.

in each skin layer but varied in concentrations among the surface, stratum corneum, epidermis and dermis. Levels of fluconazole are highest in stratum corneum, lower in the rest of the epidermis and lowest in the dermis. These images correspond to data in companion electron capture detection studies that found fluconazole higher in stratum corneum (~ 20 μg/g) than in epidermis/dermis (~ 4.5 μg/g). *In vitro* data suggest that levels of fluconazole <2 μg/g may be effective *in vivo* against yeast and dermatophytes (4, 9).

Fluconazole was also seen in cytoplasmic inclusions in thin sections of skin from the same biopsies used to produce the EDX images. These images of fluconazole observed by EELS correlate with levels of the antimycotic assayed by gas chromatography and electron capture detection (4). In Fig. 2, pixels (arrows) within cytoplasmic vacuoles represent fluconazole equivalents detected in multiple observations of dense cytoplasmic inclusions and extracellular (luminal) space throughout sebaceous glands. Fluconazole equivalents were also observed in cytoplasmic inclusions and luminal spaces of sweat glands, within cells of stratum corneum, keratinocytes, epidermal cells, dermal collagen and in vascular elements. In these thin sections that do not have a large excitation volume of cell substance beneath the surface, EELS can detect as little as 10^{-12} gram of material in a surface area of only 10 square nanometers (10, 11). This favours high spatial resolution of elemental fluorine equivalents of fluconazole within the skin specimen but images only a small area and requires long image acquisition times. Moreover, detection protocols set to exclude background image few pixels in any single plane of view.

DISCUSSION

The tissue distribution of fluconazole equivalents (fluconazole) in human skin biopsies from subjects who received a single oral 150-mg dose was observed using a scanning electron microscope equipped with a collecting spectrometer. This methods makes possible the observation of fluconazole throughout sectioned skin biopsies. The images show fluconazole distributed throughout the skin with the highest concentrations in the stratum corneum and rest of the epidermis. The cellular localization of fluconazole was imaged with an energy-filtering electron microscope by EELS. EELS can detect less than 10^{-20} gram of fluorine in an area of 10 square nanometers and localize fluorinated molecules of fluconazole (5). By this method we imaged fluconazole in deep cellular elements of sweat glands and along the duct to the stratum corneum. Our observation of fluconazole in these loci is consistent with a report (13) that eccrine sweat collected from human subjects administered a 150-mg dose of fluconazole once weekly for 2 weeks had a concentration of fluconazole equal to 3.77 μg/ml within 4 h after the second dose. This level of fluconazole compares with concentrations of the antimycotic in epidermis/dermis (4.62 μg/g) and in stratum corneum (23.4 μg/g) (4).

We also observed fluconazole in dense cytoplasmic elements of sebaceous glands. Pixel equivalents of fluconazole were detected within cells deep in the gland next to capillaries, in follicular cells and duct lumina. This is the first reported observation *in situ* of fluconazole within cells of sebaceous glands. While fluconazole in sebaceous cells may represent a station on a route of transfer of the drug from dermal capillaries to the stratum corneum, no pharmacokinetic data on the drug's levels in sebum have been reported. Moreover, fluconazole is predominantly hydrophilic and considerably less lipophilic compared with itraconazole and terbinafine (4). It has been reported earlier that water soluble substances may be transported from the stratum corneum to the hair follicles by water (14). However, in that study histamine was applied to the skin and the reaction was observed with a binocular dissecting microscope. It is unlkikely

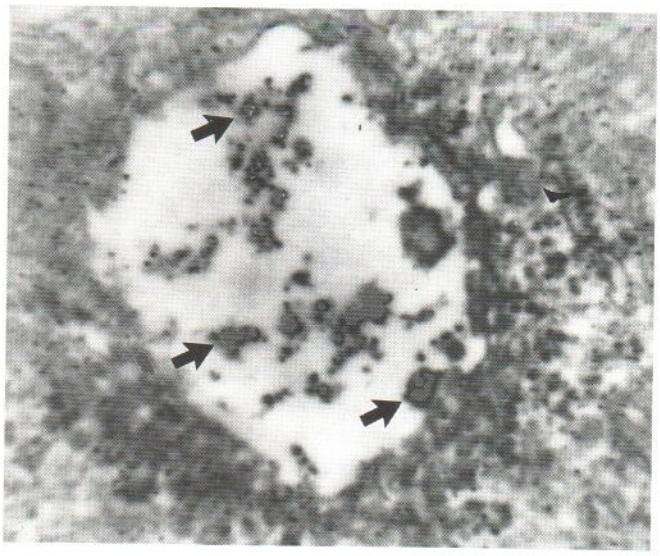

Fig. 2. Grey-coloured pixels (*arrows*) imaged by electron energy loss spectroscopy represent cellular accumulations of fluconazole detected in multiple observations of cytoplasmic inclusions within sebaceous glands. ×7,500.

that this mechanism can explain the presence of fluconazole within cytoplasmic elements of sebaceous glands.

Our observations support the conclusions that fluconazole and other antimycotics are transported into the stratum corneum and to the surface of skin through different routes (4, 12). These include diffusion from capillaries and through the dermis-epidermis, accumulation in epidermal basal cells that migrate to the surface in the normal turnover of keratinocytes and secretion in sweat and sebum. Other cellular transfer routes of fluconazole *in vivo* have also been have suggested. Human polymorphonuclear leukocytes and monocytes achieve high cellular concentrations of fluconazole (cellular: extracellular ≥ 2.2) in vitro (13). Phagocytes that achieve comparable levels of drug *in vivo* can migrate to cutaneous sites of infection, where phagocytosis and intracellular killing of dermatophytes may be enhanced by cellular levels of antimycotic (15).

We conclude that high concentrations of fluconazole in the stratum corneum and levels in epidermis and dermis that exceed MICs for most yeast and dermatophytes probably are achieved through multiple routes of delivery of active antimycotic through the skin to the surface. Transport of high concentrations of fluconazole in stratum corneum, epidermis, and sweat and accumulation of the antimycotic in dermis and in sebaceous cells ensures achievement of therapeutic levels of drug at sites of fungal infections at the surface of the stratum corneum and also levels of fluconazole sufficient against invasive fungi.

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