DRUG EVALUATION

Drugs 49 (1): 103-120, 1995
0012-6667/95/0001-0103/$18.00/0

Amorolfine

A Review of its Pharmacological Properties and Therapeutic Potential in the Treatment of Onychomycosis and Other Superficial Fungal Infections

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Summary

Synopsis

Amorolfine is a structurally unique, topically active antifungal agent, which possesses both fungistatic and fungicidal activity in vitro. *Its spectrum of* in vitro

activity includes dermatophyte, dimorphic, some dematiaceous and filamentous fungi, and some yeasts. In clinical trials, application of amorolfine 5% nail lacquer once or twice weekly for up to 6 months produced mycological and clinical cure in approximately 40 to 55% of patients with mild onychomycosis 3 months after cessation of therapy. Overall cure and improvement was observed in approximately 85 to 90% of patients with superficial dermatomycoses following treatment with amorolfine 0.25% cream for up to 6 weeks. However, few controlled, comparative trials are available for these different mycoses, and only small numbers of patients have been evaluated to date. Both preparations appear to be well tolerated; only minor localised adverse events have been reported in clinical trials.

At present, the major potential indication for topical amorolfine appears to be onychomycosis. Within this clinical setting, amorolfine should be reserved for patients with mild infection without nail matrix involvement. Systemic therapy, however, remains essential for patients with severe intractable onychomycosis involving the nail bed. Evidence to date does not clarify whether the use of adjuvant topical amorolfine reduces the need for systemic therapy in patients with severely infected nails, or whether amorolfine is beneficial in individuals unresponsive to other treatment options.

Antifungal Activity

Amorolfine is a morpholine derivative which is chemically distinct from other currently available antifungal agents. It acts primarily by inhibiting ergosterol biosynthesis, a component of fungal cell membrane, and possesses both fungistatic and fungicidal activity. Despite *in vitro* activity against various fungi, animal test models indicate that amorolfine is inactive when given systemically for life-threatening mycoses.

Conventional *in vitro* susceptibility tests indicate that amorolfine has greatest fungistatic activity against dermatophyte and dimorphic fungi. It is also active against some dematiaceous and filamentous fungi, and against some yeasts. Fungicidal activity is also highest against *Trichophyton mentagrophytes*.

In the guinea-pig, topical amorolfine 0.01% was effective against cutaneous infection induced by *T. mentagrophytes*. On a concentration basis, amorolfine showed greater activity in clearing fungal lesions in this model than naftifine, oxiconazole, ketoconazole, bifonazole and clotrimazole, but lower activity than terbinafine. In rats, a dose-dependent log reduction in vaginal yeast cell counts was also observed with topical amorolfine starting at concentrations of 0.01%; vaginal candidiasis was completely cleared at a concentration of 1%.

Pharmacokinetic Properties

Amorolfine penetration through human nail follows an exponential relationship between drug concentration and nail layer. *In vitro* data suggest that soft diseased nails will retain less drug than hard compact nails. After a single application of nail lacquer (formulated with methylene chloride), permeation of [^{3}H]amorolfine 5% through the thumbnail ranged from 20 to 100 μg/L/h.

Mean percutaneous absorption of amorolfine through healthy human skin following a single application of 0.25% cream did not exceed 10% of the total administered dose. Systemically absorbed radioactive amorolfine was slowly excreted via urine and faeces over 3 weeks; plasma concentrations of <0.5 μg/L were detected in all samples. Further studies in volunteers indicate that active concentrations of amorolfine may be retained in healthy skin for 2 or 3 days after single applications of 0.5% cream or alcohol solution, respectively.

Therapeutic Potential

The therapeutic efficacy of amorolfine has been investigated in patients with onychomycosis, dermatomycoses and vulvovaginal candidiasis. In total, only small patient numbers have been evaluated, and comparative data are minimal.

Available noncomparative data in approximately 600 patients suggest that amorolfine 5% nail lacquer applied once or twice weekly for up to 6 months may be effective in mild onychomycosis without nail matrix involvement. Mycological and clinical cure rates of about 40 to 55% were observed in treated patients 3 months after cessation of therapy; fingernails responded consistently better than toenails. Preliminary data in patients with mild disease suggest that topical amorolfine may also be useful in conjunction with oral griseofulvin to enhance cure; further double-blind studies are, however, required to ascertain the potential benefits of combined therapy.

Amorolfine 0.25% cream may be beneficial in patients with other superficial skin infections; in 208 patients, it appeared to be comparable in efficacy with bifonazole 1% cream. Amorolfine alcohol aerosol solutions 0.5 and 2% may also be useful in patients with foot mycoses. Similarly, a single vaginal dose of amorolfine 50 or 100mg appeared to be as effective as one clotrimazole 500mg pessary in 118 women with vulvovaginal candidiasis.

Tolerability

In clinical trials, topical amorolfine (5% nail lacquer and 0.25% cream) appeared to be well tolerated, with up to 5% of patients reporting minor symptoms. In patients using the nail lacquer, these events included burning, itching, redness and local pain which were tolerable and confined to the site of application. Additional events of scaling, weeping, blistering and oedema were also described for the cream. A similar adverse event profile was reported for both the alcohol solution and vaginal tablets.

Dosage and Administration

Amorolfine 5% nail lacquer should be applied to the affected nail once or twice weekly. Treatment should be continued without interruption until the nail has regenerated and affected areas are cured. This may require up to 6 months of treatment for fingernails and between 9 and 12 months for toenails.

Amorolfine 0.25% cream should be applied to affected skin areas once daily for up to 6 weeks. Therapy should be continued until clinical cure is achieved, and for several days thereafter.

Amorolfine is a morpholine derivative which is structurally distinct from other currently available antifungals (fig. 1). It has been developed as a topical agent for the treatment of onychomycosis and other superficial mycoses. Amorolfine acts primarily by inhibiting ergosterol biosynthesis, a component of fungal cell membrane, and possesses both fungistatic and fungicidal activity. Throughout this review the nomenclature used for the various fungal infections discussed follows the recommendations of the International Society for Human and Animal Mycology (1992).[1]

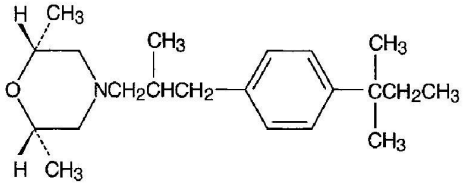

Fig. 1. Structural formula of amorolfine.

1. Antifungal Activity

1.1 *In Vitro* Studies

1.1.1 Fungistatic Activity

Antifungal susceptibility tests generally provide limited clinically useful data regarding the

susceptibility of an isolated organism from a patient to a particular antifungal agent.[2] The reliability of *in vitro* testing is hampered by various factors including the lack of interlaboratory reproducibility. Minimum inhibitory concentration (MIC) values depend heavily on experimental conditions such as the medium used, pH, inoculum size and duration of incubation. Moreover, correlation between *in vitro* susceptibility and clinical efficacy is extremely variable.[3] Nevertheless, *in vitro* tests may provide preliminary data on antifungal activity, although the results of comparative studies should be interpreted cautiously.

Traditional susceptibility tests show that amorolfine possesses *in vitro* activity against a wide range of pathogenic fungi (table I). Available data have been collated from various studies using a variety of experimental conditions. Of the species tested, the most susceptible are dermatophyte and dimorphic fungi. The activity of amorolfine against yeasts and moulds is more variable, and it is generally less active against these organisms. Although various studies have directly compared the activity of amorolfine with other antifungal agents, relevant comparisons of *in vitro* activity between all the agents tested cannot be made from summarised MIC values alone.

The combination of amorolfine with griseofulvin, ketoconazole, itraconazole or terbinafine resulted in a slight increase in fungistatic activity (as assessed by MIC values) in comparison with amorolfine alone. Of the organisms tested, this finding was consistent only for dermatophytes, but not yeasts.[11]

The ability of fungi such as *Candida albicans* to exhibit structural dimorphism, i.e. to grow either as a budding yeast or as an elongated mycelium, is of importance when considering its pathogenicity. Of the 2 available morphological forms, the hyphal form appears to possess greater virulence than the yeast form, although both are generally present within an infected lesion. *In vitro* MIC assessments of antifungal activity during germ tube formation (the primary stage in the development of hyphae) may reflect *in vivo* efficacy more closely than conventional MIC values.[12] These latter tests primarily target yeast forms and do not take the dimorphic nature of this organism into account. In this respect, the antifungal activity of amorolfine ap-

Table I. Summary of the *in vitro* activity of amorolfine against pathogenic and opportunistic fungi[4-9]

Organism	MIC range (mg/L)[a]
Dermatophyte fungi	
Trichophyton mentagrophytes	0.001-0.13
T. rubrum	<0.001-0.13
Epidermophyton floccosum	0.003-6.2
Microsporum canis	0.001-0.13
M. gypseum	0.01-0.13
Filamentous fungi (moulds)	
Aspergillus fumigatus	16->128
A. flavus	30->128
A. niger	3->100
A. nidulans	3->100
Acremonium spp.	0.25-2
Fusarium spp.	0.3-100
Scopulariopsis brevicaulis	0.03-5
Scytalidium spp.[b]	0.1-1
Pathogenic yeasts	
Candida albicans	0.001->100
C. glabrata (T. glabrata)	0.06->100
C. guilliermondii	0.1-2
C. krusei	0.05-10
C. parapsilosis	0.02-100
C. tropicalis	0.001->100
Cryptococcus neoformans	<0.001-8
Pityrosporum spp. (*Malassezia* spp.)	0.005-0.5
Dimorphic fungi	
Blastomyces dermatitidis	0.13-0.5
Histoplasma capsulatum	0.063
Sporothrix schenckii	0.63-0.5
Dematiaceous fungi	
Phaeohyphomycosis complex[c]	0.63-0.25
Chromoblastomycosis complex[d]	0.13->128

a Commonly used culture media for antifungal tests include Casitone agar/broth,[4,5,7] Kimmig's agar,[6,8] Sabourand's agar[9] and Dixon's agar[9]. MIC values generally determined by agar dilution methods. Incubated at 28, 30 or 37°C for 2 to 7 days.

b Data taken from a review by Polak.[10]

c *Exophiala jeanselmei, Wangiella dermatitidis, Cladosporium bantianum.*

d *Fonsecaea pedrosoi, Phialophora verrucosa.*

Abbreviation: MIC = minimum inhibitory concentration.

peared greater than that of terbinafine or fluconazole, but comparable to itraconazole, miconazole or econazole, and lower than clotrimazole or ketoconazole.[12]

To improve both the predictive value and reproducibility of classical *in vitro* tests, various other methods have also been proposed, all of which use criteria other than MIC values to assess antifungal susceptibility.[2] One method calculates the area of a given sector under an antifungal dose-response curve and expresses this as a percentage of the dose-response curve of a theoretical noninhibitory drug; the value obtained is the relative inhibition factor (RIF).[13] For drugs that are noninhibitory this value approaches 100%, but for highly active agents RIF approaches 0%. Antifungal RIF values are calculated by measuring fungal adenosine triphosphate (ATP) concentrations in tissue culture mediums.[14] Under these conditions, amorolfine showed greater activity against dermatophytes than *Candida* or *Aspergillus* spp. with mean respective RIF values of 29, 67 and 93%.[13] In general, these values were comparable with those obtained for topical azole antifungals (including clotrimazole, econazole, miconazole, bifonazole) in terms of activity against *Candida* and dermatophytes; amorolfine, however, was less active than the azole antifungals against *Aspergillus* spp.

Spectrophotometric assays of yeast growth relative to control growth also produce highly reproducible values which may allow the clinical susceptibility of an organism to an antifungal to be predicted. Relative growth is inversely related to the relative susceptibility of an organism to an inhibitor, such that the greater the percentage value for a given isolate the lower the susceptibility of that isolate to the agent tested.[2] Using *in vitro* microdilution plate cultures with amorolfine at a concentration of 13 mg/L, preliminary findings indicate that of the *Candida* spp. assessed, *C. albicans* was the least susceptible to amorolfine (relative growth ≈ 70%) whereas *C. krusei* was the most susceptible (≈10%). Furthermore, relative growth data thus obtained correlated well with MIC values.

The susceptibility of yeasts to antifungal agents may be influenced by changes in incubation temperature. In the case of amorolfine, an increase in temperature from 25 to 37°C lowered the susceptibility of *C. albicans* and *C. tropicalis*, but not of *C. glabrata*, *C. krusei* or *C. tropicalis* as assessed by relative growth values.[15] These *in vitro* findings may reflect *in vivo* clinical efficacy; certainly the topical efficacy of amorolfine is greater than its systemic efficacy in deep organs at temperatures of 37°C.[16]

1.1.2 Fungicidal Activity

The fungicidal activity of an agent (i.e. the ability of that drug to kill fungi) depends on both the concentration of drug and the duration of contact; lower drug concentrations may be fungicidal with longer contact. Of the organisms tested (*Trichophyton mentagrophytes*, *C. albicans*, *Histoplasma capsulatum* and *Cryptococcus neoformans*), amorolfine showed greatest fungicidal activity against T. mentagrophytes; respective concentrations of amorolfine required for 90% killing of these fungi after a 48-hour incubation period on casitone medium were 0.001, 1, 1.7 and 30 mg/L. At 24 hours, 90% killing was achieved with corresponding concentrations of 3, 3, 10 and 100 mg/L.[16]

1.2 *In Vivo* Studies

The therapeutic efficacy of amorolfine is limited to superficial fungal infections such as dermatomycoses and vaginal candidiasis. Despite its *in vitro* activity against various fungi, animal test models indicate that amorolfine is inactive when given systemically for life-threatening mycoses.[16] This lack of systemic activity has been postulated to result from rapid metabolism or extensive protein binding.[16] Other factors such as higher internal temperatures of 37°C may also lower the susceptibility of some organisms to amorolfine (section 1.1.1).

In guinea-pigs cutaneously infected with *T. mentagrophytes*, fungal foci were cleared by topical application of amorolfine for 11 days.[16] Efficacy was assessed by 2 parameters: a reduction in lesion severity or an increase in the number of my-

cosis-free animals. On a concentration basis, amorolfine showed greater activity than naftifine, oxiconazole, ketoconazole, bifonazole and clotrimazole, but lower activity than terbinafine when treatment was initiated 6 hours after infection (before fungal foci are visible); at respective amorolfine and terbinafine concentrations of 0.01 and 0.001%, all animals were free of fungal lesions. Higher concentrations ranging from 0.03 to 1% were required to produce comparable effects for the other agents tested above. Similar results were also observed when treatment was delayed to 3 days after infection (as lesions became visible); although foci were completely cleared by topical amorolfine 0.03% and terbinafine 0.1%, the latter was more effective in reducing the lesion score.[10,16]

Studies in the rat indicate that topical amorolfine may also be useful in the treatment of vaginal candidiasis. Efficacy was assessed by reductions in vaginal yeast cell counts of *C. albicans*. Twice daily intravaginal application of amorolfine, with concentrations starting at 0.01% for 3 days, produced a dose dependent log reduction in cell count; a concentration of 1% cleared the vagina of *C. albicans* completely.[10,16]

1.3 Other Effects

Modifications in processes such as phagocytosis and intracellular killing of fungi by antifungal agents may be favourable and enhance fungal death. Using a neutrophil monolayer assay, *in vitro* data suggest that amorolfine does not affect neutrophil phagocytosis, or affect fungal cell wall biosynthesis in a manner which enhances attachment of fungi to neutrophil membranes.[17] Activation of other neutrophil functions such random migration and chemotaxis were, however, inhibited in another study.[18]

The ability of fungi such as *C. albicans* to adhere to host cells, prosthetic devices or catheters is a major factor in the pathogenesis of infection. Incubation of *C. albicans* for 18 to 24 hours with amorolfine 2.5 mg/L produced a significant dose dependent reduction in adherence properties; this effect was not observed after shorter incubation times of 10 minutes to 2 hours. At these respective times, adherence values of 13 and 52% were noted for *C. albicans*.[18,19]

1.4 Mechanism of Action

The fungistatic or fungicidal activity of amorolfine depends primarily on its ability to inhibit the formation of ergosterol, a component of the fungal cell membrane.[10] Alterations in membrane sterol content lead to changes in membrane permeability which subsequently affect fungal metabolic processes. Within this sterol biosynthetic pathway, amorolfine interferes with 2 enzymes, Δ^{14}-reductase and $\Delta^7\Delta^8$-isomerase, leading to depletion of ergosterol (fig. 2).[20] These effects are both time and concentration dependent; observed changes in sterol patterns correlate with inhibition of fungal growth. Although amorolfine exhibits significantly higher affinity for the isomerase than the reductase enzyme as seen by their respective IC_{50} (concentration producing 50% inhibition) values of 0.0018 versus 2.93 μmol/L in *Saccharomyces cerevisiae* (a nonpathogenic yeast), the earlier position of the reductase enzyme within this pathway gives it greater importance. At a molecular level, amorolfine does not primarily affect cellular respiration or synthesis of DNA, RNA, protein or carbohydrate at inhibitory drug concentrations.[10]

Secondary damage within fungal cell ultrastructure arising from membrane disruption by inhibition of ergosterol formation has been demonstrated *in vitro* by electron microscopy. At concentrations of 0.1 to 100 mg/L, amorolfine produces varying degrees of damage within nuclei, mitochondria, cytoplasm, cytoplasmic membrane and cell wall of both *T. mentagrophytes* and *C. albicans*.[21] The resulting loss of essential physiological activity may ultimately lead to inhibition of growth or cell death. In *T. mentagrophytes* these effects were apparent at lower concentrations of amorolfine 0.08 mg/L.[22] Similar effects have also been described for other agents such as terbinafine[23] and oxiconazole.[24]

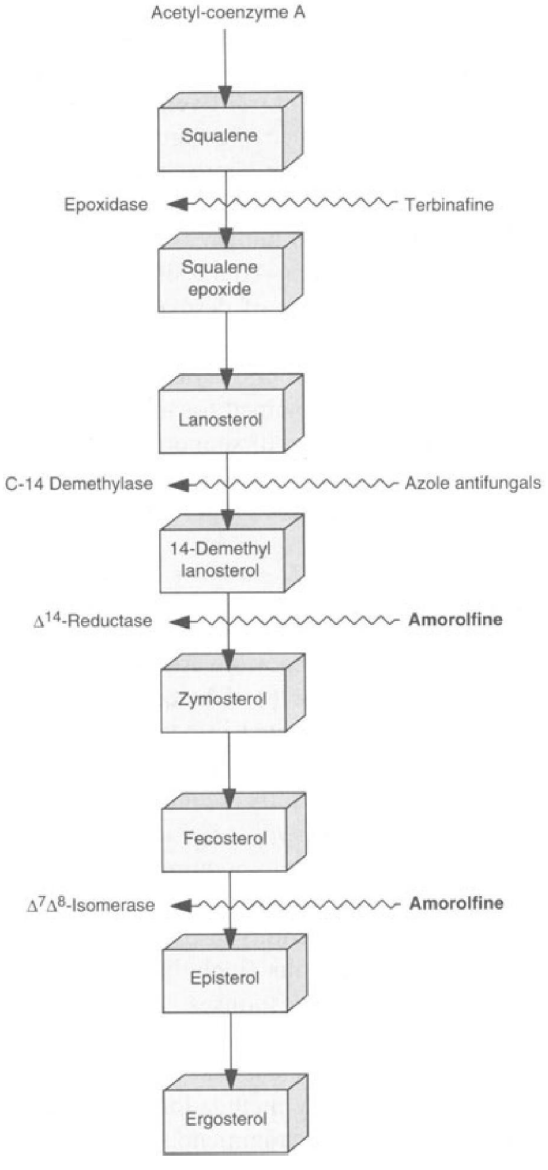

Fig. 2. Sterol biosynthetic pathway in fungi, showing the steps at which amorolfine, terbinafine and azole antifungals inhibit enzymes.

2. Pharmacokinetic Properties

At present, only two topical preparations of amorolfine (5% nail lacquer and 0.25% cream) are commercially available for the treatment of onychomycosis and superficial dermatomycoses. Although the efficacy of an alcohol aerosol solution and vaginal tablets has been assessed in small patient numbers, pharmacokinetic data are not available for these preparations.

2.1 Nail Tissue Penetration

Historically, topical therapy for onychomycosis has met with little success, due in part, to poor penetration of the antifungal agent into nail tissue. In humans, the pharmacokinetics of amorolfine penetration follow an exponential relationship between drug concentration and nail layer. Although highest *in vitro* amorolfine concentrations (0.96 to 6.68 μg/mg) were observed in the uppermost layer of the nail, some microbiological activity (>0.06 μg/mg) was also measurable in lower nail layers.[25] Amorolfine concentrations in pretreated nail slices, measured by inhibiting the growth of *C. albicans*, further demonstrate that its penetration through human nail is dependent on the nail condition, such that soft diseased nails will retain less drug than hard compact nails.[25] While the amount of amorolfine present within the nail may be readily measured using this technique, these results do not indicate whether the concentration measured is freely available to inhibit fungi or whether it is bound to keratinised tissue.[25]

In vitro permeation of amorolfine through human nail has also been assessed by measuring amorolfine flux through nails mounted within diffusion chambers.[26] Results thus obtained suggest that sufficient concentrations of amorolfine are available in both the nail plate and bed to give fungistatic and fungicidal concentrations against some species, such as dermatophyte and dimorphic fungi (sections 1.1 and 1.2). After a single application of nail lacquer (formulated with methylene chloride), permeation rates of [^{3}H]amorolfine 5% through the thumbnail ranged from 20 to 100 μg/L/h (*vs* 100 to 200 μg/L/h through human trunk skin); peak rates of 100 μg/L/h occurred between 5 to 25 hours after a single application.[26] A comparable profile was observed after multiple applications over a period of 8 days. Although toenails were assessed in

this study, available data do not clarify whether similar concentrations to those permeating through thumbnails also penetrate through toenails.

Penetration rates through the nail may also be vehicle dependent. *In vitro* permeation of amorolfine was consistently lower with an ethanol vehicle in comparison with methylene chloride, but greater with penetration enhancers such as dimethyl sulfoxide (DMSO).[26] These differences, however, were not apparent *in vivo*. The use of two amorolfine 5% lacquer formulations (ethanol *vs* methylene chloride) in 34 patients with mild disease produced comparable responses in terms of fungal inhibition zones.[27] Whether these preparations will have a similar effect on infections deeply seated in the nail plate is, however, unclear. It should be noted that commercially available amorolfine 5% nail lacquer is formulated with a methylene chloride base.

2.2 Percutaneous Absorption and Cutaneous Retention

Available data suggest that the mean percutaneous absorption of [^{14}C]amorolfine 0.25% cream applied to healthy skin (left intact or stripped) is unlikely to exceed 10%.[28] Following a single application in 12 volunteers, recovery of radioactive amorolfine from occlusive dressings and skin strippings were comparable in all subjects. In addition, the highest levels of radioactivity were observed within the topmost layer of the stratum corneum. After application, absorbed radioactive amorolfine was slowly eliminated via the urine and faeces over a period of 3 weeks; in all plasma samples, measurable quantities of intact drug were <0.5 μg/L. Thus, although only small amounts of amorolfine appear to be absorbed through the skin, it is uncertain whether multiple doses will significantly affect this profile.

In the guinea-pig, the cutaneous retention time of a single topical dose of amorolfine cream 0.5% was measured by infecting pretreated skin with fungus spores after 24 to 96 hours. The protective effect of amorolfine increased as the interval between pretreatment and fungal challenge decreased. Within this experimental model, the appearance of fungal foci were delayed for 16 days when fungal spores were administered 24 hours after amorolfine treatment.[29] Even after 96 hours, the cutaneous persistence of amorolfine prevented the appearance of foci for approximately 10 days.

The persistence of amorolfine 0.5% cream or alcohol solution within human skin has also been assessed in 6 volunteers. After a single application of the cream, inhibition of dermatophyte growth (*Trichophyton* spp.) was observed in treated skin strippings for up to 48 hours; a corresponding value of up to 3 days was obtained for the alcohol solution.[30] While these results support once daily topical application of amorolfine, the effect of diseased skin on the retention properties of amorolfine requires investigation.

3. Therapeutic Potential

The therapeutic efficacy of various topical preparations of amorolfine (nail lacquer, cream and spray) have been evaluated in patients with onychomycosis and superficial dermatomycoses. Topical agents are generally recommended only for superficial localised mycoses; widespread or intractable fungal infections require systemic therapy. Additionally, systemic therapy is usually required for fungal infections of the nail and scalp, whereas other infections (including tinea pedis) may show adequate responses to topical therapy.[31,32] In this context, it is important to bear in mind that available clinical trial data within these therapeutic areas rarely include long term follow-up, and thus current recommendations are based primarily upon clinical experience.[31] Other topical amorolfine preparations such as vaginal tablets have also been assessed in patients with vulvovaginal candidiasis.

3.1 Onychomycosis

Fungal infections of the nail account for more than 90% of nail infections and represent about 30% of all superficial fungal infections.[33] Onychomycosis is especially prevalent in adults and the elderly but relatively infrequent in children. In-

Table II. Various clinical presentations of onychomycosis[34-36]

Form of onychomycosis	Clinical features	Causative organism
Distal and lateral subungual	Commonest form. Invasion of the undersurface of nail plate starts at distal edge and side of nail plate, and progresses to proximal end of nail. The nail plate thickens and becomes friable as the infection becomes established	*Trichophyton* spp., *E. floccosum*, *S. brevicaulis*, *S. dimidiatum*
Superficial white	Uncommon and mainly seen in toenails. Fungal invasion of the superficial surface of the nail plate alone occurs. White 'islands' of infection may be easily removed by scraping nail	*T. mentagrophytes*, *Acremonium* spp.
Proximal subungual	Uncommon. Proximal invasion of the nail plate occurs usually secondary to chronic paronychia. In dermatophyte infections the subungual nail plate is also affected; fungal invasion may extend distally and involve all nail layers	*C. albicans*, *T. rubrum*
Total dystrophic	Occurs as a result of any of the above 3 types, or may be associated with chronic mucocutaneous candidiasis. The entire nail plate is destroyed, leaving a thickened and abnormal nail bed	

fections may arise from invasion of healthy nail or may be secondary to pre-existing nail disease or tinea pedis (athlete's foot). Clinical onychomycosis is defined by the method in which fungal invasion of the nail occurs; 4 main types are recognised (table II). Of the organisms implicated, dermatophytes (*Trichophyton* spp. and *Epidermophyton floccosum*) are the most common, yeasts (in particular *C. albicans*) are the second most frequent pathogen, whereas nondermatophyte infections from moulds such as *Scopulariopsis brevicaulis*, *Scytalidium dimidiatum* (previously known as *Hendersonula toruloidea*), *S. hyalinum*, *Aspergillus* or *Fusarium* spp. are relatively rare and these organisms are generally considered to be secondary invaders of previously damaged nails (table III). The prevalence of specific organisms is also dependent upon geographical location.[34,35,38]

Although not considered a serious health risk, onychomycosis is a chronic infection and difficult to eradicate completely. Cure rates for toenail infections are especially low (10 to 40%) even after prolonged systemic use of antifungal agents such as griseofulvin.[39,40] Fingernail infections generally respond better to therapy, but long term treatment is still necessary.[41] Treatment responses depend on both the extent of affected area and nail growth rate;[37,42] in extensive toenail infections outgrowth of the affected nail from the distal end may take up to 12 months, but this is generally reduced to 3 to 4 months in fingernail infections.

Over the last 30 years the mainstay of treatment for onychomycosis has been griseofulvin, despite its lack of activity against *C. albicans*. Even with prolonged courses of griseofulvin, the overall cure rate for toenail infections has been less than 40%. Increasing resistance of dermatophytes to griseofulvin has also been reported.[43] Another conventional agent ketoconazole, while active against *Candida* spp., rarely may cause liver dysfunction and is thus not recommended as a first-line agent.[44] More recently, agents such as itraconazole,[45] terbinafine[46] and fluconazole[47] have shown good efficacy for dermatophyte onychomycosis with cure rates >70%. A more detailed discussion on the treatment of onychomycosis may be found in a review by Hay.[48]

Laboratory confirmation of onychomycosis by microscopy, culture and/or histological evaluation of nail clippings and subungual debris is mandatory before initiating therapy, since the various in-

Table III. Pathogens isolated at baseline in patients with onychomycosis of toenails (n = 358) and/or fingernails (n = 88)[37]

Pathogen	% isolated	
	toenails	fingernails
Trichophyton rubrum	56	35
Trichophyton mentagrophytes	19	11
Other dermatophytes	2	3
Candida albicans	11	30
Other yeasts	10	19
Moulds	2	2

Table IV. Summary of studies evaluating the efficacy of amorolfine nail lacquer (AMO) for the treatment of onychomycosis. Treatment duration was 6 months

Reference	Site of infection (≈ % of toenail assessments)	Study design	Treatment (no. of patients)	Patient response (%)[a]			
				overall cure[b]	mycological cure[c]	negative culture	clinical cure[d]
Dose-response studies							
Lauharanta[50]	FN/TN (85)	db,r,pg,mc	AMO 2% ow (49)	12			
			AMO 5% ow (51)	38*			
Reinel & Clarke[49]	FN/TN (75)	nb,r,pg,mc	AMO 5% ow (156)	46		71	
			AMO 5% bw (161)	52		75	
Noncomparative study							
Zaug[37]	FN/TN (80)	nc,nb,mc	AMO 5% ow (446)		FN (55)	FN (73)	FN (52)
					TN (54)	TN (67)	TN (41)
Combination therapy							
Lauharanta et al.[51]	FN/TN (90)	nb,r,pg	Phase 1 (2 months)[e]				
			AMO 5% bw + GRI 1g od (87)		42		
			GRI 1g od (85)		13		
			Phase 2 (4 months)				
			AMO 5% bw (87)		67		FN (32)
							TN (22)
			GRI 500mg od (85)		45		FN (15)
							TN (11)

a Response at follow-up (i.e. 3 months after stopping therapy).

b Assessed by negative fungal cultures with clinical recovery (≤10% nail affected).

c Assessed by negative microscopy and negative culture.

d Assessed visually by clinical improvement scales. Parameters measured include nail thickening, splitting, and discoloration.

e Efficacy assessed at 2 and 6 months. Findings after 1 year's therapy are currently awaited.

Abbreviations and symbols: bw = twice weekly; db = double-blind; FN = fingernail; GRI = griseofulvin; mc = multicentre; nb = nonblind; nc = noncomparative; od = once daily; ow = once weekly; pg = parallel group; r = randomised; TN = toenail; * significant difference between treatment options ($p < 0.05$).

vading organisms respond to different therapies.[34] Histological examination reveals not only the fungal elements present but also the depth of their penetration into the nail plate.[48]

The efficacy of amorolfine nail lacquer has been evaluated in 3 multicentre studies comprising centres from Europe, Latin America, Asia and New Zealand (sections 3.1.1 and 3.1.2). Only patients with mild onychomycosis (with less than 80% affected nail surface, intact matrix and lunula) were recruited into these studies; the main indications being distal subungual onychomycosis (75%), with superficial white onychomycosis (10%) and other mixed forms (15%) representing the remainder.[37] Despite the high proportion of patients with toenail infections requiring prolonged therapy, maximum treatment duration in these studies was only 6 months. Of the 2 most common invading organisms, the prevalence of *T. rubrum* was greater than that of *T. mentagrophytes* (table III). Concomitant fungal infections such as tinea pedis were simultaneously treated with amorolfine 0.5% cream.

Treatment of onychomycosis is generally continued until clinical and mycological cure is achieved.[36] In the studies discussed, efficacy was based upon separate mycological (direct microscopy and cultures) and clinical (subjective improvement scale) measures at treatment end and at follow-up (3 months post-treatment), or upon an overall assessment of both culture results and clinical changes at follow-up. The criteria for overall cure included negative fungal cultures with clinical

recovery (≤10% nail affected), whereas for improvement negative cultures were accompanied by clinical improvement (at least 20% reduction of total affected area compared with baseline). Positive fungal cultures were defined as treatment failures.[42] It is important to note that although negative fungal cultures provide evidence of drug efficacy, they do not indicate permanent eradication of the organism. Furthermore, visually assessed clinical improvement scales (which examine parameters such as nail thickening, splitting and discolouration) only estimate the percentage of clinically healthy to clinically diseased nail plate.[49]

Recent proposals by the American Food and Drug Administration (September 1994), however, state that successful treatment of onychomycosis may be defined by clinical parameters alone; although a negative mycological culture is not required in clinical practice these data should be collected in clinical trials. Mycological confirmation of the presence of a causative fungal agent at the beginning of a study is still necessary.

3.1.1 Dose-Response Studies

Dose-response studies in patients with mild onychomycosis, mainly of the toenail, have evaluated the efficacy of amorolfine nail lacquer at 2 different concentrations (2 *vs* 5%)[50] and with 2 different application regimens (once weekly *vs* twice weekly)[49] [table IV]. Although overall cure rates were greatest when the 5% lacquer was applied twice weekly, these were not significantly higher than those values obtained with a once weekly application of 5% lacquer. Fingernails showed a better response with the twice weekly regimen, but this difference was not evident for toenails.[49] Treatment failure occurred in approximately 30% of patients irrespective of the treatment option used.[49,50] Data from these studies further indicate that cure is related to the duration of onychomycosis; overall cure was higher when the duration of onychomycosis was up to, rather than over, 5 years (51 *vs* 19%).[42]

3.1.2 Noncomparative Study

The majority of patients (62%) undergoing therapy with amorolfine nail lacquer have been treated with the 5% strength applied once weekly for a period of 6 months (table IV).[37] Generally, eradication rates for *T. rubrum* were slightly lower than for *T. mentagrophytes* or *C. albicans*; within one cohort, respective mycological cures were achieved in 75, 90 and 86% of patients with fingernail infections and 64, 84 and 73% of patients with toenail onychomycosis, 3 months post-therapy.[37] In terms of onset of clinical cure, amorolfine was consistently more effective in fingernail than in toenail infections (fig. 3), regardless of whether invading organisms were dermatophytes or yeasts.[37,42] However, despite the observed increase in cure rates between treatment cessation

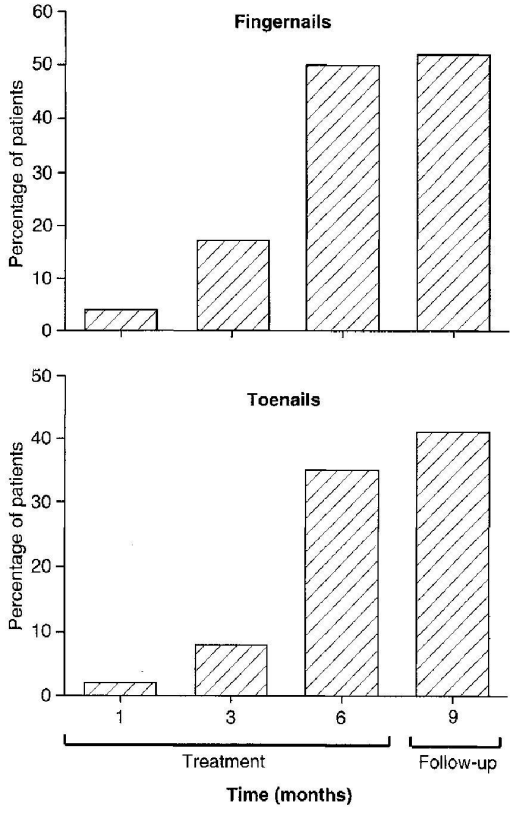

Fig. 3. Progressive clinical cure observed in patients with onychomycosis of the toenail (n = 358) and fingernail (n = 88) treated with 5% amorolfine nail lacquer once weekly for 6 months.[37]

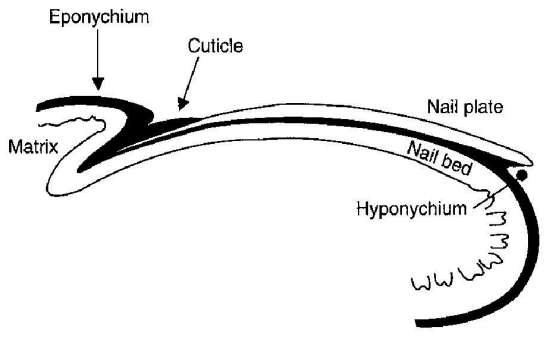

Fig. 4. Anatomy of the nail (reproduced from Goodman,[36] with permission).

and follow-up (fig. 3), amorolfine therapy should be maintained until complete cure is achieved to prevent relapses.[42]

3.1.3 Combination Therapy

In severe onychomycosis with matrix involvement oral therapy is essential. Growth of the nail plate occurs from the root matrix which extends from beneath the nailfold to the edge of the lunula (fig. 4). Orally administered antifungal agents are thought to penetrate the nail plate via the matrix, and from the nail bed by diffusion,[52] such that cure of the plate is achieved slowly. This is illustrated by the low mycological cure rate of 13% seen in patients with predominantly toenail infection receiving oral griseofulvin for 2 months.[51] The addition of a topical antifungal agent to an oral drug may, therefore, enhance cure and reduce overall treatment duration.

Preliminary nonblind data suggest that the combination of oral griseofulvin with topical amorolfine may be more effective than griseofulvin monotherapy (table IV).[51] After 2 months of therapy, mycological cure was enhanced in patients receiving combined treatment (42 *vs* 13%); subsequent monotherapy with each agent for an additional 4 months produced higher cure rates for amorolfine (67 *vs* 45%). The use of topical amorolfine alone or in conjunction with oral griseofulvin for the entire duration of the study was, however, not investigated; certainly these comparators need to be included in future double-blind studies. Although patients with extensive onychomycosis were recruited into the study, the majority (and most of the responders) had no matrix involvement; however, it was not specified what differences were observed between these subsets. In addition, the design of this study did not allow any conclusions to be made regarding the need for shorter term systemic treatment with adjuvant topical therapy. Findings after 1 year's therapy are currently awaited.

3.2 Dermatomycoses

Superficial skin infections may frequently occur at sites such as the feet, groin and scalp. Dermatomycoses are generally caused by *Trichophyton*, *Microsporum* and *Epidermophyton* spp., although the prevalence of specific organisms depends on geographical location. In one analysis of 527 patients recruited from Europe and Latin America, of the 533 pathogens isolated, *T. rubrum* was the most common pathogen occurring in 60% of patients. Less common were *T. mentagrophytes* (16%), *E. floccosum* (8%), *Microsporum canis* (8%) and *C. albicans* (5%).[53] As with onychomycosis, diagnosis of dermatomycoses should be confirmed by microscopy and culture of skin scrapings.[54]

Topical therapy with antifungals (creams, lotions) such as clotrimazole,[55,56] econazole[55] and miconazole[57] is well established and effective for the treatment of most superficial mycoses; response rates of 70 to 90% are generally observed. Other drugs such as bifonazole,[58-60] tioconazole,[57] ciclopiroxolamine,[56] naftifine,[61] and terbinafine[61] have also produced comparable response rates. While older remedies such as compound benzoic acid ointment (Whitfield's ointment) are useful, they are cosmetically less acceptable.[31,32] Although a variety of agents are available, few studies have compared their relative efficacies. In clinical practice, treatment failure rates may be high and result from a variety of factors, including the difficulty of treating wide areas of skin, inappropriate use of topical agents and poor compliance.[31,44]

The efficacy of amorolfine cream and alcohol spray for dermatomycoses has been investigated in

3 multicentre studies. Assessments of efficacy were generally based on combined mycological (microscopy and culture) response and resolution of clinical signs and symptoms (itching, erythema and scaling), the former being the main efficacy parameter. Mycological cure was defined as negative culture and microscopy. At follow-up (1 to 3 weeks after the end of treatment), overall efficacy was also assessed as: cure (negative mycological findings and absence of clinical signs and symptoms), improvement (negative mycological findings and improved clinical signs and symptoms) or failure (positive mycological result regardless of clinical picture). Affected areas were generally treated once daily for up to 6 weeks.

3.2.1 Clinical Studies

In a total of 735 patients treated with amorolfine cream, approximately half the patients (40 to 50%) presented with tinea pedis. Other frequently diagnosed mycoses were tinea corporis (≈15%) and tinea cruris (≈20%).[42] The duration of fungal infection ranged from 3 days to 40 years. Double-blind comparative data evaluating 3 concentrations of amorolfine cream (0.125, 0.25 and 0.5%) in these patients indicated that all preparations possessed similar efficacy with regard to both cure/improvement rates and activity against invading pathogens.[53,58]

Within one cohort of 527 patients, mycological cure (negative microscopy and culture) was observed in a total of 380 (72%) patients, 1 week after amorolfine therapy;[53] overall assessments of efficacy appeared to be slightly greater with the 0.5% cream, while increasing doses of the 3 concentrations produced respective cure/improvement rates of 86, 86, and 89%. Despite alleviation of most symptoms, residual signs of erythema and scaling were still present in up to 30% of patients. Although statistically significant differences were not observed between groups in the eradication of *T. rubrum*, *T. mentagrophytes*, *E. floccosum*, *M. canis*, *C. albicans* and other pathogens, *M. canis* responded less favourably to treatment than other organisms. Similarly, subgroup analysis of the various body areas treated (feet, large body areas, skin folds) revealed no statistically significant differences between these cure rates regardless of the concentration used; nevertheless, mycoses of the feet and skin folds showed a more favourable response. On the basis of these and tolerability data (section 4), amorolfine 0.25% cream was subsequently chosen for clinical usage.

The results of a second study evaluating 208 patients confirm these findings.[58] In addition, all concentrations of amorolfine cream (0.125, 0.25 and 0.5%) were comparable in efficacy with bifonazole 1% cream. Mycological cure (negative culture and microscopy) was noted in 86% of patients regardless of agent or concentration used, while overall assessments of cure/improvement were >90% for each group.

In the specific treatment of fungal infections of the foot, the efficacy of an alcohol solution of amorolfine sprayed on to the affected area has recently been investigated. Aerosols may be especially useful for moist areas such as the feet since they leave little residue and have a drying effect. Of the 348 patients investigated, approximately 70% presented with tinea pedis; intertriginous mycosis was the most prevalent clinical form observed. At follow-up, after 3 to 6 weeks' treatment with amorolfine 0.5 or 2% spray used once daily, mycological cure (microscopy and culture) was observed in approximately 95% of patients irrespective of treatment group.[62] Cure/improvement as assessed by overall efficacy measures was also comparable between groups (>96%). Moreover, mycological and clinical failure were not observed in 16 patients with plantar ('moccasin') tinea pedis, a form particularly difficult to treat. As with amorolfine cream, eradication of invading pathogens (*T. rubrum*, *T. mentagrophytes*, and *C. albicans*) was favourable for both concentrations used.

3.3 Vulvovaginal Candidiasis

Vulvovaginal candidiasis is a common gynaecological complaint; it has been estimated that up to 75% of women experience at least one episode of vulvovaginal candidiasis during their life-

time.[63] Of the typical organisms implicated, *C. albicans* accounts for up to 80% of infections.[63] Symptom severity is not directly dependent upon the number of yeasts present; some organisms, however, are associated with a higher rate of recurrence after standard therapy. Factors leading to reinfection such as antibiotic therapy, oral contraceptive use, pregnancy, diabetes mellitus and poor compliance with antifungal therapy are important considerations when assessing recurrence of infection. In this respect, partners may also need to be treated.[32,63]

Vulvovaginal candidiasis is usually treated with topical antifungal agents. Of the available preparations, the imidazoles (e.g. clotrimazole, miconazole, econazole) generally appear superior to nystatin in terms of cure rates; shorter imidazole courses of between 1 to 7 days provide similar efficacy rates to older 14-day regimens.[63] In those individuals with resistant or recurrent infection, oral agents such as fluconazole and itraconazole may be preferred to ketoconazole which is rarely associated with hepatotoxicity.

In one double-blind study evaluating 118 nonpregnant women, comparable cure rates were observed in patients treated with single vaginal doses of either amorolfine 50mg, amorolfine 100mg or clotrimazole 500mg.[64] One vaginal tablet was inserted at night and concomitant vulvitis or balanitis of the partner was simultaneously treated with the corresponding cream. Clinical cure was observed in approximately 90 to 95% of patients 1 week after treatment, irrespective of the treatment option. At 4 weeks post-therapy, however, cure was observed in 80, 84 and 68% of patients using amorolfine 50mg, amorolfine 100mg and clotrimazole 500mg; corresponding relapse rates were 10, 10 and 25%. In a smaller study (n = 40) comparing the efficacy of low amorolfine dosages of 10, 25 and 50mg, respective cure rates of 77, 54 and 57% with higher relapse rates of 15, 31 and 21% were observed 4 weeks post-therapy.[65] Antifungal therapy should aim to achieve negative cultures since relapse rates were closely associated with a positive culture at week 1.[64,65] Moreover, *C. glabrata* was notably linked with both treatment failures and a persisting carrier state.[64]

4. Tolerability

The various topical preparations of amorolfine (nail lacquer, cream, alcohol aerosol solution and vaginal tablet) appear to be well tolerated in the limited number of patients assessed in clinical trials to date.

In one analysis of 727 patients treated with amorolfine nail lacquer for 6 months, 7 (1%) patients experienced local adverse events.[42] Symptoms of burning, itching, redness and local pain were tolerable and confined to the site of application; higher concentrations of amorolfine (5 *vs* 2%) applied more frequently (twice *vs* once weekly) were not associated with a greater incidence of adverse events.[49,50]

In a further 882 patients treated with amorolfine cream (0.125, 0.25, or 0.5%), 47 (5%) reported one or more of the following events: itching, burning, erythema, scaling, weeping, exudation, oedema, blistering, dermatitis (2 patients) and eczematous reaction (1 patient).[53,58] In one study, 19 of 714 (3%) patients discontinued amorolfine therapy because of intolerance of treatment.[53] Significant differences in adverse events between the 3 concentrations of amorolfine (0.125, 0.25, 0.5%) cream were not observed, although lower concentrations of amorolfine 0.125 and 0.25% were better tolerated than 0.5% when the cream was applied to skin folds.[53] A similar adverse event profile was also described for the alcohol aerosol solution[62] and the vaginal tablets.[64]

5. Dosage and Administration

Amorolfine 5% nail lacquer should be applied to affected fingernails or toenails once or twice weekly. Nails should be filed down and cleaned before application, and allowed to dry for about 3 minutes after application of lacquer. Treatment should be continued without interruption until the nail is regenerated and affected areas are finally cured. In general, 6 months treatment is required for fingernails and 9 to 12 months for toenails, with

regular reviews every 3 months. The use of cosmetic lacquer or artificial nails is contraindicated during amorolfine treatment.

Amorolfine 0.25% cream should be applied to affected skin areas once daily in the evenings for at least 2 to 3 weeks (up to 6 weeks for foot mycosis). Therapy should be continued without interruption until clinical cure is achieved, and for several days thereafter.

Although amorolfine alcohol aerosol solution and vaginal tablets have been used in clinical trials, these preparations are not commercially available.

6. Therapeutic Potential of Amorolfine

Until recently, the management of some superficial fungal infections, particularly onychomycosis, was hampered by a lack of effective therapeutic options. Even today, available antifungal agents may not be effective against recalcitrant infections such as 'moccasin type' tinea pedis due to *T. rubrum*, or those due to *S. brevicaulis* or *S. dimidiatum*.[31,41,66] Despite the introduction of newer agents, adequate comparative data are notably lacking, and in most instances the choice of therapy is based primarily on clinical experience or educated guesswork.

At present, the main indication for topical amorolfine appears to be for the treatment of onychomycosis. In general, fingernails respond more favourably to treatment than toenails. With the previous limited availability of effective oral agents and the low treatment success rates seen for toenail infections, many dermatologists in the past were reluctant to treat toenail onychomycosis at all, and long term systemic therapy was usually reserved for younger individuals.[31] With the advent of effective newer oral agents, these prescribing trends are likely to change. Topical antifungal therapy provides an attractive option for the treatment of onychomycosis in terms of limiting adverse effects, increasing patient acceptability, and offering an alternative treatment option for those individuals unable to take oral agents. Previous results with other topical agents such as bifonazole 1%/urea 40%[67] and tioconazole 28%[68] have, however, been disappointing with cure rates ranging from 12 to 46%. The inherent difficulty of treating nail infections (especially toenails), coupled with the disappointing clinical responses observed despite good antifungal penetration into nail keratin, suggests that these agents may only be suitable for mild infections, as adjunctive therapy to oral agents or after chemical or surgical nail avulsion. There is no evidence at present to support the continuous use of a topical antifungal following oral treatment in patients with residual onycholysis, to prevent reinfection.[48]

Although the *in vitro* antifungal activity of amorolfine against moulds is more variable than against dermatophytes, topical amorolfine may offer potential in the treatment of nail infections arising from *Scopulariopsis*. Extensive nail destruction resulting from invasion of other organisms such as *Scytalidium* is, however, unlikely to respond to topical therapy. In this context it is important to note that clinical trials performed to date have mainly recruited patients with dermatophyte infections; future studies are certainly warranted to determine the value of amorolfine in nondermatophyte infections.

Available noncomparative data indicate that topical amorolfine may provide some benefit in the treatment of mild onychomycosis of 5 years or less in duration. In a total of approximately 600 patients treated with amorolfine 5% nail lacquer once or twice weekly for up to 6 months, clinical and mycological cure was observed in approximately 40 to 55% of individuals 3 months after cessation of therapy; amorolfine was consistently more effective in fingernail than in toenail infections. Although these results are encouraging, only patients with early mild nail infections affecting less than 80% of the nail surface area and without nail matrix involvement were recruited. This is important, since spontaneous fluctuations in the natural course of infections such as superficial white onychomycosis may in itself clear the condition.[31] Placebo-controlled studies are, thus, necessary to obtain a more accurate indication of efficacy. Interpretation of data was further complicated by the

use of short 3-month follow-up periods which do not permit an accurate assessment of cure rate since relapse rates are especially high for this condition.

Preliminary data in patients with onychomycosis also suggest that the additional use of topical amorolfine nail lacquer with conventional griseofulvin therapy may enhance cure in comparison with griseofulvin monotherapy. Adequate comparators were, however, not used in this study. In addition, of those patients responding to therapy, the majority had no nail matrix involvement. Whether these findings can be extended to patients with severe onychomycosis or whether topical amorolfine lessens the need for systemic therapy in severely infected nails remains unclear.

In the treatment of superficial skin infections or vulvovaginal candidiasis, respective therapy with amorolfine 0.25% cream or amorolfine 50 or 100mg vaginal tablets may be beneficial. As with onychomycosis, small patient numbers and lack of good comparative data preclude definitive conclusions to be made with respect to these indications. Further investigations in patients with other skin conditions such as pityriasis vesicolor, erythrasma, and seborrhoeic dermatitis may well extend the role of amorolfine;[69] certainly it may be useful in those individuals sensitised to imidazoles. The prophylactic use of amorolfine in patients with recurrent and chronic vulvovaginal candidiasis also warrants investigation.

In clinical trials evaluating amorolfine 5% nail lacquer and 0.25% cream, adverse events were reported by up to 5% of patients. These were minor and localised to site of application.

On present evidence, the use of amorolfine nail lacquer should be limited to patients with mild onychomycosis without nail matrix involvement. Systemic therapy, however, is essential for severe intractable nail infections with nail bed involvement. Although it may be anticipated that concomitant use of topical amorolfine with other oral antifungal agents may enhance cure of severely infected nails, it is unclear whether the need for systemic therapy may be reduced by adopting these measures, or whether patients unresponsive to other therapeutic options may benefit from topical amorolfine.

References

1. Odds FC, Arai T, Disalvo AF, et al. Nomenclature of fungal diseases: a report and recommendations from a Sub-Committee of the International Society for Human and Animal Mycology (ISHAM). J Med Vet Mycol 1992; 30: 1-10
2. Odds FC. Antifungal susceptibility testing of *Candida* spp. by relative growth measurement at single concentrations of antifungal agents. Antimicrob Agents Chemother 1992; 36: 1727-37
3. Galgiani JN. Antifungal susceptibility tests. Antimicrob Agents Chemother 1987; 31: 1867-70
4. Clayton YM. Relevance of broad-spectrum and fungicidal activity of antifungals in the treatment of dermatomycoses. Br J Dermatol 1994 Apr; 130 Suppl. 43: 7-8
5. Martin E, Aretio R, Parras P, et al. Antifungal activity of amorolfine hydrochloride *in vitro* against clinical isolates of yeasts. J Mycol Med 1992; 2: 152-3
6. Espinel-Ingroff A, Shadomy S, Gebhart RJ. In vitro studies with R 51,211 (itraconazole). Antimicrob Agents Chemother 1984; 26: 5-9
7. Polak A. Antifungal activity *in vitro* of Ro 14-4767/002, a phenylpropyl-morpholine. Sabouraudia 1983; 21: 205-13
8. Shadomy S, Espinel-Ingroff A, Kerkering TM. In-vitro studies with four new antifungal agents: BAY n 7133, bifonazole (BAY h 4502), ICI 153,066 and Ro 14-4767/002. Sabouraudia 1984; 22: 7-15
9. Regli P, Ferrari H. *In vitro* activity of a new antifungal agent derived from morpholine: amorolfine [in French]. Pathol Biol Paris 1989; 37 (5 Pt 2): 617-20
10. Polak AM. Preclinical data and mode of action of amorolfine. Clin Exp Dermatol 1992; 17 Suppl. 1: 8-12
11. Polak A. Combination of amorolfine with various antifungal drugs in dermatophytosis. Mycoses 1993; 36: 43-9
12. Schaude M, Ackerbauer H, Mieth H. Inhibitory effect of antifungal agents on germ tube formation in Candida albicans. Mykosen 1987; 30: 281-7
13. Odds FC, Webster CE, Abbott AB. Antifungal relative inhibition factors: BAY 1-9139, bifonazole, butoconazole, isoconazole, itraconazole (R 51211), oxiconazole, Ro 14-4767/002, sulconazole, terconazole and vibunazole (BAY n-7133) compared *in vitro* with nine established antifungal agents. J Antimicrob Chemother 1984; 14: 105-14
14. Odds FC, Abbott AB. Relative inhibition factors – a novel approach to the assessment of antifungal antibiotics *in vitro*. J Antimicrob Chemother 1984; 13: 31-43
15. Odds FC. Effects of temperature on anti-*Candida* activities of antifungal antibiotics. Antimicrob Agents Chemother 1993; 37: 685-91
16. Polak A, Dixon DM. Antifungal activity of amorolfine (Ro 14-4767/002) in vitro and in vivo. In: Fromtling RA, editor. Recent trends in the discovery, development and evaluation of antifungal agents. Basel: J.R Prous Science Publishers, 1987
17. Richardson MD, Shankland GS, Gray CA. Effect of amorolfine (Ro 14-4767/002) on in vitro phagocytosis and intracellular killing of *Candida albicans* by human neutrophils. Mycoses 1989; 32: 245-9
18. Vuddhakul V, McCormack JG, Seow WK, et al. Effects of the newer antifungal agents (bifonazole, ICI 195, 739 and amorolfin) on *in vitro* phagocytic, lymphocytic and natural-killer cell responses. Int J Immunopharmacol 1989; 11: 817-28

19. Vuddhakul V, McCormack JG, Seow WK, et al. Inhibition of adherence of *Candida albicans* by conventional and experimental antifungal drugs. J Antimicrob Chemother 1988; 21: 755-63
20. Polak-Wyss A, Lengsfeld H, Oesterhelt G. Effect of oxiconazole and Ro 14-4767/002 on sterol pattern in *Candida albicans*. Sabouraudia 1985; 23: 433-42
21. Müller J, Polak-Wyss A, Melchinger W. Influence of amorolfine on the morphology of Candida albicans and Trichophyton mentagrophytes. Clin Exp Dermatol 1992 Sep; 17 Suppl 1: 18-25
22. Nishiyama Y, Asagi Y, Hiratani T, et al. Morphological changes associated with growth inhibition of *Trichophyton mentagrophytes* by amorolfine. Clin Exp Dermatol 1992; 17 Suppl. 1: 13-7
23. Nishiyama Y, Asagi Y, Hiratani T, et al. Effect of two different classes of antifungal agents, terbinafine and amorolfine, on the ultrastructure of *Trichophyton mentagrophytes* [abstract P-B-41]. J Electron Microsc 1989; 38: 311
24. Melchinger W, Polak A, Müller J. Die Wirkung von Amorolfin und Oxiconazol auf die Ultrastruktur von *Trichophyton mentagrophytes* in Vergleich. Mycoses 1988; 33: 393-404
25. Polak A. Kinetics of amorolfine in human nails. Mycoses 1993; 36: 101-3
26. Franz TJ. Absorption of amorolfine through human nail. Dermatology 1992; 184 Suppl. 1: 18-20
27. Mensing H, Polak-Wyss A, Splanemann V. Determination of the subungual antifungal activity of amorolfine after 1 month's treatment in patients with onychomycosis: comparison of two nail lacquer formulations. Clin Exp Dermatol 1992 Sep; 17 Suppl. 1: 29-32
28. Roncari G, Ponelle C, Zumbrunnen R, et al. Percutaneous absorption of amorolfine following a single topical application of an amorolfine cream formulation. Clin Exp Dermatol 1992; 17 Suppl. 1: 33-6
29. Polak A. Antifungal activity of four antifungal drugs in the cutaneous retention time test. Sabouraudia 1984; 22: 501-3
30. Gip L. *In vitro* studies of the antifungal activity of amorolfine, a phenylpropyl morpholine. Adv Ther 1989; 6: 26-38
31. Hay RJ, Roberts SOB, Mackenzie DWR. Mycology. In: Champion RH, Burton JL, Ebling FJG, editors. Textbook of dermatology. 5th ed. Vol. 2. Oxford: Blackwell Scientific Publications, 1992: 1127-216
32. Anonymous. Drugs acting on the skin. In: Prasad AB, editor. British National Formulary. 27th ed. London: British Medical Association and Royal Pharmaceutical Society of Great Britain, 1994: 407-51
33. Arikian SR, Einarson TR, Kobelt-Nguyen G, et al. A multinational pharmacoeconomic analysis of oral therapies for onychomycosis. Br J Dermatol 1994; 130 Suppl. 43: 35-44
34. Clayton YM. Clinical and mycological aspects of onychomycosis. JAMA South East Asia 1993; 9 Suppl. 4: 7-10
35. Zaias N. Clinical manifestations of onychomycosis. Clin Exp Dermatol 1992; 17 Suppl. 1: 6-7
36. Goodman GJ. Contemporary treatment of onychomycosis. Persistence is the 'name of the game'. Curr Ther 1986; 28 (10): 43-55
37. Zaug M. Amorolfine nail lacquer: once-weekly application in onychomycosis. JAMA South East Asia 1993; 9 Suppl. 4: 19-22
38. Adams JD. My approach to fungal nail disorders. Curr Ther 1990; 31: 108-13
39. Davies RR, Everall JD, Hamilton E. Mycological and clinical evaluation of griseofulvin for chronic onychomycosis. BMJ 1967; 3: 464-8
40. Kaden R. Klinische und Mykologische Nachuntersuchungen Griseofulvin Behandelter Onychomykosen [in German]. Mycopathol Mycol Appl 1965; 25: 351-60
41. Hay RJ, Clayton YM, del Palacio A, et al. Amorolfine: an innovation in antimycotic therapy. Introduction. Clin Exp Dermatol 1992; 17 Suppl. 1: 1
42. Zaug M, Bergstraesser M. Amorolfine in the treatment of onychomycoses and dermatomycoses (an overview). Clin Exp Dermatol 1992; 17 Suppl. 1: 61-70
43. Higgins G. Utilising newer, more effective agents *vs* onychomycosis [meeting report]. Inpharma 1993 (912): 15-6
44. Hay RJ. Treatment of dermatomycoses and onychomycoses--state of the art. Clin Exp Dermatol 1992; 17 Suppl 1: 2-5
45. Hay RJ, Clayton YM, Moore MK, et al. An evaluation of itraconazole in the management of onychomycosis. Br J Dermatol 1988; 119: 359-66
46. Goodfield MJD, Andrew L, Evans EGV. Short term treatment of dermatophyte onychomycosis with terbinafine. BMJ 1992; 304: 1151-4
47. Kuokkanen K, Alava S. Fluconazole in the treatment of onychomycosis caused by dermatophytes. J Dermatol Treat 1992; 3: 115-7
48. Hay RJ. Onychomycosis. Agents of choice. Dermatol Clin 1993; 11: 161-9
49. Reinel D, Clarke C. Comparative efficacy and safety of amorolfine nail lacquer 5% in onychomycosis, once-weekly versus twice-weekly. Clin Exp Dermatol 1992 Sep; 17 Suppl. 1: 44-9
50. Lauharanta J. Comparative efficacy and safety of amorolfine nail lacquer 2% versus 5% once weekly. Clin Exp Dermatol 1992 Sep; 17 Suppl. 1: 41-3
51. Lauharanta J, Zaug M, Polak A, et al. Combination of amorolfine with griseofulvin: *in vitro* activity and clinical results in onychomycosis. JAMA South East Asia 1993; 9 Suppl. 4: 23-7
52. Munro CS, Rees JL, Shuster S. The unexpectedly rapid response of fungal nail infection to short duration therapy. Acta Derm Venereol 1992; 72: 131-3
53. del Palacio A, Gip L, Bergstraesser M, et al. Dose-finding study of amorolfine cream (0.125%, 0.25% and 0.5%) in the treatment of dermatomycoses. Clin Exp Dermatol 1992; 17 Suppl. 1: 50-5
54. Clayton YM. Clinical and mycological diagnostic aspects of onychomycoses and dermatomycoses. Clin Exp Dermatol 1992; 17 Suppl. 1: 37-40
55. Cullen SI, Rex IH, Thorne EG. A comparison of a new antifungal agent, 1 percent econazole nitrate (Spectazole™) cream versus 1 percent clotrimazole cream in the treatment of intertriginous candidosis. Curr Ther Res 1984; 35: 606-9
56. Bogaert H, Cordero C, Ollague W, et al. Multicentre double-blind clinical trials of ciclopirox olamine cream 1% in the treatment of tinea corporis and tinea cruris. J Int Med Res 1986; 14: 210-6
57. Fredriksson T. Treatment of dermatomycoses with topical tioconazole and miconazole. Dermatologica 1983; 166 Suppl. 1: 14-9
58. Nolting S, Semig G, Friedrich HK, et al. Double-blind comparison of amorolfine and bifonazole in the treatment of dermatomycoses. Clin Exp Dermatol 1992; 17 Suppl. 1: 56-60

59. Hernández-Pérez E. Bifonazole cream: once-a-day application every second day in tinea cruris and tinea corporis. Dermatologica 1984; 169 Suppl. 1: 93-8
60. Soyinka F. Bifonazole in the treatment of fungal skin infections in the tropics: a clinical and mycological study. Curr Med Res Opin 1987; 10: 390-6
61. Jones TC. Treatment of dermatomycoses with topically applied allylamines: naftifine and terbinafine. J Dermatol Treat 1990; 1 Suppl. 2: 29-32
62. Nolting S, Reinel D, Semig G, et al. Amorolfine spray in the treatment of foot mycoses (a dose-finding study). Br J Dermatol 1993; 129: 170-4
63. Rein MF. Vulvovaginitis and cervicitis. In: Mandell GL, Douglas Jr RG, Bennett JE, editors. Principles and practice of infectious diseases. 3rd ed. New York: Churchill Livingstone, 1990: 953-65
64. del Palacio A, Sanz F, Garcia-Bravo M, et al. Single dose treatment of vaginal candidosis – randomised comparison of amorolfine (50 mg and 100 mg) and clotrimazole (500 mg) in patients with vulvovaginal candidosis. Mycoses 1991; 34: 85-91
65. del Palacio-Hernanz A, Sanz Sanz F, Gimeno Fernández C, et al. Randomized parallel double blind study with amorolfine monodose tablets (10, 25, 50mg) in vaginal candidoses [in Spanish]. Rev Iber Micol 1991; 8: 104-10
66. Hay RJ. Amorolfine, a breakthrough in topical antimycotic therapy. Introduction. Dermatology 1992; 184 Suppl 1: 1-2
67. Hay RJ, Roberts DT, Doherty VR, et al. The topical treatment of onychomycosis using a new combined urea/imidazole preparation. Clin Exp Dermatol 1988; 13: 164-7
68. Hay RJ, Mackie RM, Clayton YM. Tioconazole nail solution - an open study of its efficacy in onychomycosis. Clin Exp Dermatol 1985; 10: 111-5
69. del Palacio A. Clinical experiences with amorolfine. In: Rippon JW, Fromtling RA, editors. Cutaneous antifungal agents. Selected compounds in clinical practice and development. New York: Marcel Dekker, Inc., 1993: 59-77

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