REVIEW ARTICLE

Clotrimazole as a pharmaceutical: past, present and future.

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Summary

Clotrimazole is a broad-spectrum antimycotic drug mainly used for the treatment of *Candida albicans* and other fungal infections. A synthetic, azole antimycotic, clotrimazole is widely used as a topical treatment for *tinea pedis* (athlete's foot), as well as vulvovaginal and oropharyngeal candidiasis. It displays fungistatic antimycotic activity by targeting the biosynthesis of ergosterol, thereby inhibiting fungal growth. As well as its antimycotic activity, clotrimazole has become a drug of interest against several other diseases such as sickle cell disease, malaria and some cancers. It has also been combined with other molecules, such as the metals, to produce clotrimazole complexes that show improved pharmacological efficacy. Moreover, several new, modified-release pharmaceutical formulations are also undergoing development. Clotrimazole is a very well-tolerated product with few side effects, although there is some drug resistance appearing among immunocompromised patients. Here, we review the pharmaceutical chemistry, application and pharmacology of clotrimazole and discuss future prospects for its further development as a chemotherapeutic agent.

Introduction

Clotrimazole is a broad-spectrum antimycotic drug that is in widespread use for the treatment of *Candida albicans* and other fungal infections. Its antimycotic properties were discovered in the late 1960s. As an active ingredient, it is marketed as a generic drug under various different trade names and by various companies worldwide. In addition to its antimycotic acitivity, clotrimazole is used in the treatment of metronidazole-resistant *Trichomoniasis* to relieve symptoms (Cudmore *et al.* 2004) and displays activity against certain Gram-positive bacteria (Alsterholm *et al.* 2010). It is a synthetic compound.

Chemical structure and molecular formula

The molecular formula of clotrimazole is $C_{22}H_{17}ClN_2$, and its molecular weight is 344·8 g mol^{-1}. The structure of clotrimazole is illustrated in Fig. 1.

Description of structural features

Clotrimazole is considered to be chemically peculiar (Fig. 1). It contains four aromatic rings bonded to a tetrahedral (sp3 hybridized) carbon atom, causing a highly steric encumbrance on this atom. One of the aromatic groups is an imidazole ring, and this is known to mediate electron transfer reactions in biological systems (Eaton and Wilkins 1978; Eaton and Wilson 1979). Its remaining aromatic rings comprise a triphenylmethyl system – a structure that is known to form and stabilize radical intermediates (Hicks 2007). One of these rings is chloro-substituted at its C2 position. Although clotrimazole is an achiral molecule, its two phenyl rings are enantiotopic, with one being pro-R and the other pro-S. These enantiotopic specificities can be differentiated by interaction with a chiral molecule (Eliel *et al.* 1994).

Computational modelling of clotrimazole in a structure-based mechanistic study yielded four stable conformers, none of which has two aromatic rings in the same plane (Navas *et al.* 2004). These computational studies indicated that the energy content of a putative coplanar conformer is very high, resulting in an extremely unstable structure, due to interactions between the substituents at the *ortho*-positions in the aromatic rings. Thus, the authors concluded that clotrimazole does not have the coplanar physical properties that are typical of many xenobiotics that act as ligands for the aryl hydrocarbon receptor, but instead has a
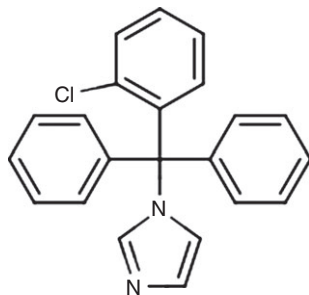
Figure 1 The chemical structure of clotrimazole (1-[(2-chlorophenyl) diphenylmethyl]-1H-imidazole).

'propeller-like' conformation. These computed models of clotrimazole's structure are supported by X-ray diffraction analysis of the crystalline form of clotrimazole (Song and Shin 1998). Navas and co-workers (Navas *et al.* 2004) also computed the molecular electrostatic potential (MEP) and dipole moment of clotrimazole. As these two parameters provide an indication of the charge distribution and electrostatic potential of a molecule, they are used to model and explain the interactions between biologically active chemicals and their biomolecular targets. MEP mapping revealed that clotrimazole possesses a peripheral electron-rich region corresponding to its nonsubstituted nitrogen atom and a region with a positive electrostatic potential that corresponds to the substituted nitrogen atom. This analysis suggested that clotrimazole would interact efficiently with acidic or electrophilic species that are present in biological target molecules via its nonsubstituted nitrogen. Its dipole moment values were typical of molecules with a high proportion of heteroatoms, low symmetry and relatively large size. For all four conformations of clotrimazole, the dipole orientates from the imidazole ring (negative end) towards the chlorine atom (positive end) and dipole moment values ranged from 3·78 to 5·58 D.

Therapeutic class and pharmaceutical use

Clotrimazole is a member of the azole class of synthetic antimycotic agents that were discovered in the 1960s. Azoles comprise the largest class of antimycotic drugs in clinical use and can be further subdivided into two classes on the basis of their chemical structure: imidazoles and triazoles. Clotrimazole falls into the imidazole subclass of azole drugs. Along with econazole and miconazole, clotrimazole is the drug of choice for the topical treatment of tinea pedis (athlete's foot), tinea cruris and tinea corporis caused by isolates of *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Microsporum canis* and *C. albicans* (Gelone and O'Donnell 2006). It is also widely used in the topical treatment of vulvovaginal and oropharyngeal candidiasis.

Brief summary of antimicrobial activity

All azole-type antimycotic drugs interfere with the biosynthesis of ergosterol, which is a major component of the fungal cytoplasmic membrane. Specifically, azoles including clotrimazole inhibit the microsomal cytochrome P450 (CYP450)-dependent event 14-α-lanosterol demethylation, which is a vital step in ergosterol biosynthesis by fungi (Hitchcock *et al.* 1990) (Fig. 2). The resultant depletion of ergosterol and its replacement with the aberrant sterol species, 14-α-methylsterol, perturb normal membrane permeability and fluidity. Downstream effects include decreased activity of membrane-bound enzymes, including those involved in cell wall synthesis, increased cell wall leakiness and leaking of cell contents. Moreover, because ergosterol directly stimulates growth of fungal cells in a hormone-like fashion, rapid onset of these events results in a dose- and time-dependent inhibition of fungal growth. Clotrimazole is generally considered to be a fungistatic rather than a fungicidal drug, although as for many antimicrobials, this distinction is not absolute as it exhibits fungicidal effects at higher concentrations.

The selectivity of azole drugs for fungal cells *in vivo* reflects their greater affinity for fungal versus mammalian cytochrome P450 enzymes. Specifically, these agents target the cytochrome P450-Erg11p or Cyp51p enzyme, which possesses monooxygenase activity and catalyses the removal of the 14-α-methyl group of lanosterol and/or eburicol in fungi. To date, only one Cyp51p crystal structure has been reported – that isolated from *Mycobacterium tuberculosis* (Podust *et al.* 2001). However, by examining this crystal structure, it was possible to identify substrate recognition sites on this protein that could account for the activity of azole antimycotics. Cyp51p proteins contain an iron protoporphyrin moiety located at their active site, and it is at this site that azole compounds bind to the iron atom, via a nitrogen atom (N-3 or N-4) in their imidazole (N-3) or triazole (N-4) ring. The other part of the azole drug binds to an apoprotein, and this interaction is dependent on the individual structure of the drug itself. The exact conformation of this active site differs widely between fungal species and among the many mammalian P450 enzymes (Marichal *et al.* 1990), and, as described below, exploiting these differences offers the possibility of rationally designing drugs with improved efficacy against certain fungal species.

Clotrimazole resistance is a problem particularly in immunocompromised patient populations (Pelletier *et al.* 2000; Sobel 2007). Resistance has been linked to overexpression of efflux pump genes such as the Candida drug resistance$_1$, Candida drug resistance$_2$ and multidrug resistance$_1$ genes (White *et al.* 2002). In *Candida glabrata*, the protein antiporter, CgTpo3, was found to increase

Figure 2 Ergosterol is a vital component of fungal plasma membranes fulfilling a similar function to cholesterol in animal cell membranes. Clotrimazole targets the enzyme lanosterol 14-α-demethylase responsible for the conversion of lanosterol to 4,4-dimenthyl-ergostrienol a common target of azole drugs. Other antifungal drugs, such as terbinafine, target squalene epoxidase.
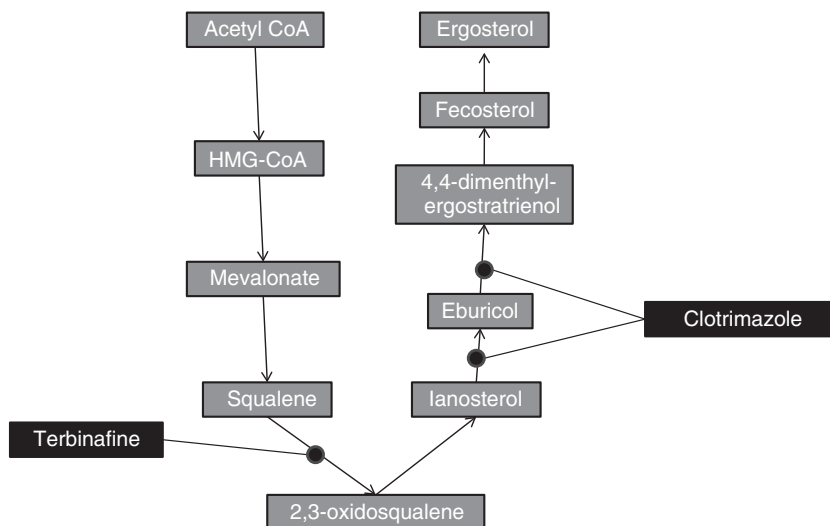
resistance to azole drugs (Costa *et al.* 2014). Changes in the drug target lanosterol 14-α-demethylase caused by mutations to, or overexpression of, the ERG11 gene may also cause resistance in some cases (Mishra *et al.* 2007). In some cases of resistance, nonazole treatments or second-generation drugs may be suitable as alternative pharmacotherapies (Valerio *et al.* 2013).

While CYP450 inhibition is accepted to primarily account for the antimycotic properties of clotrimazole, this drug also exhibits other diverse pharmacological actions. These include inhibition of sarcoplasmic reticulum Ca2+-ATPase (Bartolommei *et al.* 2006), depletion of intracellular calcium stores (Jan *et al.* 2000) and blockade of calcium-dependent potassium channels and voltage-dependent calcium channels (Rittenhouse *et al.* 1997; Wu *et al.* 1999; Shah *et al.* 2001; Tian *et al.* 2006). It is believed that the action of clotrimazole on these diverse targets accounts for biological effects of this drug that are independent of its antimycotic action. For example, clotrimazole inhibits the proliferation of several normal and cancer cell lines *in vitro* (Benzaquen *et al.* 1995) and inhibits the expression of adhesion molecules by TNF-α (Thapa *et al.* 2009), and it has been reported to exert neuroprotective effects and to modify the cytotoxicity of some metal cations (Oyama *et al.* 2006). There is much interest in the therapeutic application of clotrimazole in sickle cell disease, because its metabolite, ICA 17043, is believed to exert a beneficial effect on erythrocyte dehydration by blocking the Gardos calcium channel (Brugnara *et al.* 1996; Brugnara and De Franceschi 2006; Gbotosho *et al.* 2013). Clotrimazole also exhibits antimalarial activity *in vitro* (Tiffert *et al.* 2000), possibly by inhibiting hemoperoxidase, thereby causing oxidative stress in the parasite (Trivedi *et al.* 2005).

Similar drugs

Antimycotic azole drugs that are closely related to clotrimazole include the imidazole drugs, miconazole, econazole and ketoconazole, and the triazole drugs fluconazole and itraconazole. They are all broad-spectrum antimycotics and are generally poorly absorbed orally, with the exception of ketoconazole and itraconazole (reviewed in Odds *et al.* 2003; Gelone and O'Donnell 2006; Lorand and Kocsis 2007). In the clinical settling, miconazole and econazole are used very similarly to fluconazole in the treatment of topical fungal infections, whereas miconazole is also frequently used intravenously for the treatment of systemic infections in patients who cannot tolerate amphotericin. Unlike other azoles, itraconazole displays activity against *Aspergillus.*

Because the extent of antifungal activity for each azole drug depends largely on the exact interaction between the drug and cytochrome P450, rational drug design approaches have been employed in the design of a new generation of azole drugs that incorporate triazole structures (reviewed in Odds *et al.* 2003; Lorand and Kocsis 2007; Weig and Brown 2007; Pasqualotto *et al.* 2010). These newer drugs include voriconazole and ravuconazole, which are structurally based on fluconazole and posaconazole, which is closely related to itraconazole. In comparison with the first-generation triazole drugs, the newer triazole compounds have extended antimycotic spectrums. These drugs have, in essence, been developed to address the limitations of existing azole compounds, including the emergence of drug-resistant fungal strains. Use of these second-generation drugs is reviewed in the context of paediatric medicine in Valerio *et al.* (2013).

Two other classes of antimycotic drugs also target the ergosterol biosynthetic pathway, despite being considered chemically distinct from clotrimazole or azole compounds in general. Most notably, the allylamines, including terbinafine, inhibit the enzyme squalene epoxidase which acts earlier in the ergosterol biosynthetic pathway (Fig. 2). These compounds can have fungicidal effects and have also demonstrated activity against filamentous fungi and a few pathogenic yeasts. Thus, there is considerable interest in the possibility of formulating them in combination with azole drugs to achieve synergistic inhibitory effects on ergosterol synthesis. The phenylmorpholine class of drugs that includes amoroline affects two late events in ergosterol synthesis by inhibiting Erg24p reductase and Erg2p isomerase enzymes. It is largely used for superficial mycoses, and its targets have not been researched extensively.

Pharmaceutical dosage forms and administration

Within the European Union, clotrimazole is available in topical cream and pessary formulations under a variety of trade names. In the USA, additional formulations are available, including clotrimazole lotions, powders, lozenges, topical solutions and vaginal inserts/tablets. Clotrimazole is sometimes formulated with the steroids hydrocortisone or bethamethasone, and in some cases, these compounded preparations may be labelled as coclimasone (Sweetman 2007). Typical excipients in clotrimazole creams include benzyl alcohol, cetostearyl alcohol, medium-chain triglycerides and triceteareth-4 phosphate.

Although its availability differs slightly from country to country, monopreparations of clotrimazole are generally available over-the-counter, while combined preparations may require a prescription. In products aimed at the treatment of fungal skin infections, clotrimazole is usually formulated as a 1% cream, lotion, spray or solution. In the treatment of vulvovaginal candidiasis, the normal dosage forms are either 100 mg, 200 mg or 500 mg pessaries which are administered daily for 6, 3 or 1 days, respectively. Similar doses may be obtained by application of 1, 2 or 10% creams to the vaginal area. In the treatment of oropharyngeal candidiasis, clotrimazole lozenges are usually formulated to contain 10 mg of the active drug and these are sucked slowly until dissolved, five times daily for 14 days. In the prophylactic prevention of oropharyngeal candidiasis in immunosuppressed individuals, this dose is reduced to 10 mg, three times daily, for the duration of the immunosuppressive therapy.

According to the USP, clotrimazole should contain not less than 98·0% and not more than 102·0% clotrimazole while clotrimazole creams, lotions, lozenges, topical solutions and vaginal inserts should contain not less than 90·0% and not more than 110·10% of the labelled

amount of clotrimazole. According to the British and European Pharmacopoeias, clotrimazole should contain not less than 98·5% and not more than 100·5% of clotrimazole with reference to the dried substance.

Side effects, interactions and contraindications

Topical forms of clotrimazole are available as over-the-counter medication and are considered reasonably safe and without serious side effects. However, there have been limited case reports of contact allergic dermatitis with clotrimazole creams that are not attributable to allergies to the vehicles or excipients, but are caused by the active ingredient itself (Kalb and Grossman 1985). Intravaginal clotrimazole applied via a pessary may damage latex contraceptives (condoms) necessitating the use of additional contraceptive measures during the period of administration.

The most prominent side effects of clotrimazole preparations in current use are those associated with the use of oral lozenges for the treatment of oral candidiasis. These include nausea, vomiting, unpleasant mouth sensations, pruritus and elevation of liver enzymes (reviewed in Ellepola and Samaranayake 2000). Clotrimazole lozenges are not sold in the European Union but are widely available in the USA and other countries. Clotrimazole tablets or capsules designed for swallowing, as opposed to sucking, are no longer used as they were associated with GIT disturbances, dysuria and mental depression. Notably, because of clotrimazole's very limited water solubility and GIT toxicity, other imidazole antifungal drugs have replaced clotrimazole in oral capsule formulations. For example, oral Canesten, which is available over-the-counter in the United Kingdom, but not in Ireland, contains ketoconazole.

Because clotrimazole is not systemically absorbed, drug interactions are not a major issue with its use. It can be used safely with consumption of alcohol, does not affect driving ability, and there is no evidence of it posing a risk to the developing foetus in pregnancy. Pessaries are not recommended for use in children or infants, although the drug itself poses no special risk to this subpopulation. Clotrimazole is also safe for use in the elderly population and in breast-feeding mothers.

Concerns surrounding environmental toxicity

There are growing concerns about the abundance of chemicals in our environment for which inadequate data are available with regard to their toxicity. While pharmaceutical compounds are stringently assessed with regard to their toxicity to humans prior to their marketplace release, there is relatively little assessment of their environmental effects. Certain types of chemicals are considered to be persistent in the environment as they can

bioaccumulate in wildlife species. Aquatic wildlife is at particular risk as it is exposed to discharges from municipal waste water from households and industry. Chemicals that affect key enzymatic pathways that are evolutionarily conserved across species, such as cytochrome P450, are of particular concern as they may have unintentional ecotoxicological effects. Clotrimazole fits several of these criteria as it targets cytochrome P450 activity, is nonbiodegradable in the environment (i.e. half-life of more than 60 days) and is therefore considered to be a persistent chemical. As a consequence, clotrimazole was included on the OSPAR list of chemicals for priority action at the meeting of the Convention for the Protection of the Marine Environment of the north-east Atlantic (the OSPAR Convention) in 2002. However, after conducting a thorough environmental risk assessment of clotrimazole, it was concluded that clotrimazole does not pose a significant environmental risk to marine and aquatic life (OSPAR Commission, 2005). Despite this, some recent studies have suggested that clotrimazole may affect marine microalgae at low concentrations (Porsbring et al. 2009).

Conclusion and future directions

Invasive fungal infections have increased in frequency worldwide in recent decades and have emerged as a major cause of illness and death, especially in immunocompromised individuals (Malani and Kauffman 2007). One reason for the marked increase in these problematic infections is the growing size of the highly immunocompromised patient population. Ironically, this trend in part reflects clinical success in other areas such as treatment of human immunodeficiency virus (HIV) infection, cancer and increasing numbers of transplant recipients. Resistance to clotrimazole, which used to be a rare occurrence, is now quite common in certain patient subpopulations with candidiasis (Pelletier et al. 2000). While these epidemiological trends are unlikely to impact significantly on the widespread use of clotrimazole and related drugs in the general population, they do continue to drive the rational design of new drug entities with improved activity spectrums and these newer drugs, such as posaconazole, are likely to supersede clotrimazole for the treatment of invasive fungal infections in select, high-risk patient populations.

Nevertheless, the further development of clotrimazole as a pharmaceutical is an area of intense research at present. There are prospects both for its exploitation in new indications and for the development of new formulations. A scaffold based on clotrimazole is being used as a pharmacophore in the design and synthesis of novel antimalarial drugs that are cheap and easy to synthesize (Gemma et al. 2007, 2008). Palladium–clotrimazole complexes that exhibit enhanced cytotoxicity against tumour

cell lines, in comparison with clotrimazole alone, are under investigation as novel antineoplastic agents (Navarro et al. 2006). Indeed, several other metal–clotrimazole complexes, such as ruthenium–clotrimazole and platinum–clotrimazole, also display promising antineoplastic characteristics (Navarro et al. 2009; Robles-Escajeda et al. 2013). There is also intense interest in using clotrimazole and its metabolite as lead compounds in the strategic design of novel treatments for sickle cell disease, on the basis that they can reduce erythrocyte dehydration in vivo by inhibiting the so-called Gardos, calcium-dependent potassium channel that malfunctions in this disease (Brugnara et al. 1996; Brugnara and De Franceschi 2006; Gbotosho et al. 2013).

New approaches to formulation of clotrimazole include a buccal bioadhesive film containing clotrimazole, which was found to inhibit oral candidiasis for up to 6 h (Singh et al. 2008), and a thermosensitive vaginal gel formulation formed by complexation of clotrimazole with beta-cyclodextrin, which has been shown to reduce the release rate of clotrimazole in comparison with standard preparations (Bilensoy et al. 2006). This type of slow-release formulation may exhibit increased efficacy over other vaginal delivery systems, as traditional vaginal creams, pessaries and tablets tend to have short residency times in the vagina due to the natural cleansing process that takes place there. The use of liposomes containing clotrimazole may also provide increased residency in the vagina, thereby improving gel formulations for treatment (Vanić and Škalko-Basnet 2013). RS 100 nano-capsules have recently been studied in the treatment of C. albicans and C. glabrata, and these have been reported as more active than free clotrimazole alone (Santos et al. 2014). Given the scale of the current market for vulvovaginal clotrimazole preparations, novel formulations that can demonstrate advantage over preexisting preparations could potentially attract a large revenue stream. Nano-fibre mats for oral applications are also superior in efficacy and have reduced toxicity over lozenges and powders in current use, although further pharmaceutics investigations are needed (Tonglairoum et al. 2014).

In conclusion, clotrimazole is an effective, safe and welltolerated drug with an unusual chemistry that is widely used in the treatment of skin, vulvovaginal and oropharyngeal fungal infections. It is sold in most developed countries worldwide under a variety of trade names, and a large number of clotrimazole formulations are available. Although emerging resistance to clotrimazole may limit the future use of this drug in certain patient subpopulations, in the general population, its widespread use is likely to continue for the foreseeable future. Ongoing development of clotrimazole as a pharmaceutical is currently focused on finding new clinical indications for the drug, its use as a lead compound in structure-based drug

design studies and the optimization of formulated products to enhance drug delivery.

Conflict of Interest

No conflict of interest declared.

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Other resources

Chemical Drawing package used: MarvinSketch ver. 4.1.13. for Macintosh Downloadable from www.chemaxon.com.