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Novel approaches in the rational design of antifungal agents of low toxicity

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Abstract

This paper presents an overview of studies on novel strategies for the rational design of antifungal agents of low toxicity and overcoming the multidrug resistance (MDR) of fungi. This goal was achieved both due to the introduction of a novel target, glucosamine-6-phosphate synthase, as well as to the recognition of molecular basis of selectivity of action of amphotericin B derivatives.

Keywords: Antifungal agents; Glucosamine-6-phosphate synthase; Amphotericin B derivatives

Control of eucaryotic systems constitutes one of the most challenging problems in chemotherapy. Among the still unsolved clinical needs is the chemotherapy of systemic fungal infections. Systemic candidiasis, aspergillosis, histoplasmosis and cryptococcosis constitute still serious and of growing importance problem in medicine, that has been deepened during the last decades due to the increase of a number of immunocompromised patients mainly because of the use of immunosuppressive agents and the spread of HIV infections. In recent years a new threat has emerged due to the appearance of fungal strains with multi-drug resistance (MDR).

The ideal antifungal agent should exhibit high antifungal activity, broad antifungal spectrum, fungicidal action, reluctance to induce resistance, the ability to overcome multi-drug resistance and low animal toxicity. None of the available clinical antifungal agents fulfill all these requirements.

In our multidisciplinary studies on the rational design of novel antifungal agents we developed a program based on two strategies.

1. Strategy 1: novel target

The enzyme glucosamine-6-phosphate synthase, EC 2.6.1.16, has been identified as a novel target for antifungal agents. Highly selective irreversible potent inhibitors of the enzyme, novel glutamine analogs, have been obtained. These studies comprised purification to homogeneity of *C. albicans* enzyme overexpressed in *S. cerevisiae* and molecular studies on its active center [1], studies on the mechanism of enzyme action, which together with data of other authors allowed us to elucidate the molecular mechanism of enzyme catalysis [2–7], and studies on molecular modelling of selectivity of the enzyme–inhibitor interaction [8]. The latter approach allowed us to identify the structural factors of glutamine analogs decisive for the high selectivity of inhibitor in regard to glucosamine-6-phosphate synthase. It appeared that the specific conformation of the inhibitor molecule is crucial in selectivity effect.

A group of selectively acting glutamine analogs has been synthesized, of which N^3-4-methoxyfumaroyl-L-2,3-diaminopropanoic acid (FNMP) and *N′-trans*-epoxy-succinamoyl-L-2,3-diaminopropanoic acid (EADP) were the most interesting [9–11]. Both inhibitors covalently inactivated the enzyme. The elucidated mechanism of enzyme inactivation showed that FMDP and EADP act as active site directed inactivators [12–14].

FMDP and EADP, being amino acid (glutamine) analogs, could not be efficiently transported to cells via highly specific amino acid permeases. To eliminate this difficulty we applied the portage transport concept. Inhibitors built into oligopeptide structures were very well transported by peptide permeases and cleaved by

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intracellular peptidases, generating in situ the 'warhead' which cannot escape the cell and unable to be exported by specific amino acid permeases. Both types of peptides exhibit excellent antifungal activity, broad antifungal spectrum and fungicidal action [15–18]. FMDP peptides also demonstrated promising in vitro activity against multi-drug resistant *S. cerevisiae*. Lys-Nva-FMDP and Nva-EADP were selected as optimal for development studies. The first compound did not show toxicity to mammalian cells in vitro or to mice and was effective in experimental candidiasis [19,20]. Tripeptides are superior to dipeptides from the point of view of transport characteristics. In *C. albicans* they are taken up by two permeases: di-tripeptide and oligopeptide permeases, which ensures better uptake and slows down the development of resistance [21].

2. Strategy II: new face of an old drug

This strategy was based on the recognition of the molecular basis of very poor selective toxicity of polyene macrolide antibiotics (PMA) otherwise exhibiting excellent properties and removal of this discriminating feature upon rational modification. High toxicity of PMA results from the similarity of their mammalian and fungal membrane located targets: cholesterol and ergosterol, respectively. Somewhat higher affinity of the antibiotics to ergosterol hardly enables their practical application. The interaction of PMA with plasma membrane of sensitive organisms impairs the barrier function of the membrane, leading to the lethal effects due to uncontrolled diffusion of ions and small metabolites, change in membrane fluidity, and modification of the activity of membrane located enzymes. In spite of high animal toxicity and water insolubility, PMA are of great interest due to their unique properties among antifungal agents. They exhibit: (1) very high antifungal activity; (2) a very broad antifungal spectrum; (3) a fungicidal type of action, and (4) a reluctance to induce resistance. Much effort was made to lower the toxicity and to improve the water solubility of PMA. These aims were not achieved by the screening of the new PMAs and only partially solved by the development of the new delivery systems. In consequence, the rational chemical modification of PMA is expected to be the most appropriate to reach the goal.

The selection of an optimal candidate from the PMA group, amphotericin B (AmB), for the rational modification was done on the basis of characteristics: relation between the structural types of PMA and the kind of membrane changes induced [22], selective toxicity characteristics [23], reversibility (repair) of membrane changes induced in microbial and mammalian cells [24,25], metabolic requirements for the interaction of PMA with cells [26] and permeabilizing activities of PMA in mammalian and fungal cells [27–30] and in lipidic vesicles [31,32].

The water insolubility is due to the tendency of AmB molecules to aggregate [33]. Our molecular dynamics simulations pointed to the interactions occurring in the selfassociation [34]. Such association can be hindered if the antibiotic molecule bears the net electric charge [35]. AmB derivatives of that type are perfectly water soluble.

The identification of structural factors of AmB and its derivatives essential for their interaction with sterols, sterols containing lipidic, vesicles and model fungal and mammalian cells as well as for membrane permeabilizing activity and characteristics of permeability pathways induced pointed to the essential role of ionisable functional groups in the 'polar head' of the antibiotic molecule [28,32,36–40]. The ability to protonate the nitrogen atom at the mycosamine moiety is indispensable for high membrane activity [32,41]; however, the position of this atom can be shifted by modification [42].

A molecular model of the primary sterols–antibiotic complex comprising interaction characteristics and mechanism of differential affinity to cholesterol and ergosterol has been designed [32,43] on the basis of the above experimental data, supplemented by molecular modelling comprising molecular mechanics, molecular dynamics and quantum chemical calculations [35,41,44–48].

Rational design of water soluble, with improved selective toxicity, derivatives of AmB was based on the above findings. Two generations of derivatives were designed and synthesized. First generation derivatives comprise compounds modified at the carboxyl group. In these compounds the improvement of selective toxicity is based on the disturbance of the hydrogen bonds network in the complex with sterols. The most interesting compound of this group is AmB-*N*,*N*′-dimethylaminopropylamide (AMA) [49,50], exhibiting few fold improved selective toxicity, and due to the positive charge of the molecule, good water solubility of its salts. Dramatic improvement of selective toxicity was achieved however, in second generation derivatives due to the introduction of bulky substituent inducing appropriate steric hindrance effect, which essentially disturbs the interaction with cholesterol but not with ergosterol. The most interesting compound of this group is *N*-methyl-*N*-fructosyl-AmB methyl ester (MFAmE) [51]. The compound exhibits good antifungal activity, 100-fold diminished animal toxicity, compared with AmB, and excellent water solubility of its salts. Moreover, the compound exhibited high activity against the MDR-type *S. cerevisiae* strain.

The development of AmB derivatives with much improved properties was based on the rationale concerning the recognition of the molecular nature of the antibiotic–sterol primary complex, which indeed con-

tributes essentially to biological properties of active compound. However, the biological effects of AmB and of its derivatives could also depend on the behavior of the supramolecular structures involved. Thus far the structure of the channel formed by AmB in cholesterol-containing membranes has been calculated using molecular modelling methods [52].

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