REVIEW OF CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS, INTERNATIONAL
EDITION 18: 59 (2004)

Molecular Modeling of the Interactions of a Purine Transporter with Different Substrate Analogues: A first Step towards the Systematic Development of Antifungal Drugs

George Diallinas

Faculty of Biology, Department of Botany, University of Athens, Panepistimioupolis, Athens 15781, Greece. e-mail: diallina@biol.uoa.gr, fax: 30210-7274702

SUMMARY

UapA, a highly specific uric acid-xanthine transporter in *Aspergillus nidulans,* is a member of a large family of nucleobase-ascorbate transporters (NAT) conserved from bacteria to humans (1). Making use of a biochemical approach, which converts competitive inhibition constants (K_i) of several nucleobases into ΔG, we estimated the contribution of different positions in the purine ring in specific interactions with UapA. We propose that purines bind to specific amino acid residues of UapA via at least three hydrogen bonds. In particular, two ubiquitous hydrogen bonds with O2 and O6 of the purine ring and a third, more flexible bond, with a hydrogen acceptor (N7, N9 or O8) in the imidazole ring. The flexibility of the third interaction provides a rationale for the differential binding affinities and of various analogues including allopurinol. A similar but not identical model has been developed for the UapA homologue of the human pathogen *Candida albicans.*

To further investigate the molecular nature of the proposed interactions we studied several altered specificity or affinity UapA mutants (2-4). Our results strongly suggest that residues Q449 and N450, located in the vicinity of transmembrane segment 8 or 9 (TMS8 or TMS9) might be involved in purine binding. In particular, our evidence strongly suggests that residue Q449 interacts with positions O8 or N9 of the purine ring. Interestingly, while residue F569 located in TMS12 of the wild-type transporter, does not seem to be involved directly in interactions with purine substrates, specific substitutions of the aromatic Phe with polar or small hydrophobic residues (Ser, Thr, Ala) generate UapA molecules with enlarged specificity. Such substitutions can fully or partially suppress the effects caused by the loss or the modification of interactions of UapA with purines due to different substitutions in residue Q449. It seems that a molecular cross-talk between specific residues at positions 449 and 569 in UapA is key for the genetic design of transporter molecules with altered specificities and kinetics.

These results put the basis for the development, without the use of crystallography or NMR, of molecular models describing detailed structure-function relationships in the active site of UapA and other NAT transporters. Given that NAT purine transporters are ubiquitous in fungi (1), including first line pathogens such as *C. albicans* and *A. fumigatus*, and that analogous human purine transporter recognize their substrates in a different way (1,5), such models should allow the orthological design of novel drugs or therapies.

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