De novo induction of resistance against voriconazole in Aspergillus fumigatus

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Letter to the Editor

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Sir,

The opportunistic pathogen *Aspergillus fumigatus* causes invasive aspergillosis in patients with reduced immune function [1]. Infections caused by this organism are difficult to treat, particularly in critically ill patients. Therapy prospects become very dismal when the pathogen is resistant to the various azole compounds that are the drugs of choice against this organism.

The same resistance mechanism, designated TR_{34}/L98H, was uncovered in clinical isolates from patients who had not been treated with azoles previously as in *A. fumigatus* exposed to azole fungicides used for crop protection and material preservation [2]. This observation suggests that the origin of this resistance mechanism may lie in the use of azoles in the environment, since resistant conidia can easily reach the human population transported by air or in any other way. What is not understood at present is whether azole resistance in *A. fumigatus* can be induced de novo by exposure to sublethal drug concentrations, as was shown for *Escherichia coli* [3], or whether horizontal transfer of resistance genes is essential. The development of resistance during patient therapy suggests that de novo emergence of resistance is possible [4,5], but since genetic exchange cannot entirely be ruled out, this needs to be proven under conditions that do not allow gene transfer.

An azole-susceptible strain of *A. fumigatus*, designated AZN 8196, was grown on plates as described previously [2] with sublethal concentrations of voriconazole and itraconazole. Following 7 days of incubation, the strains were transferred to (i) a plate with the same drug concentration and (ii) a plate with a two times higher level. When the plate with the higher drug concentration showed growth within 1 week, the plate with the lower level was discarded and the procedure was repeated. At every transfer, the minimum inhibitory concentration (MIC) of the strain was measured by following growth during 48 h in a set of liquid cultures having drug concentrations stepwise increasing by a factor of 2.

Exposure of *A. fumigatus* to stepwise increasing concentrations of voriconazole starting at 0.125 μg/mL resulted in a rapid increase of the MIC once the concentration of the drug exceeded the initial MIC (Fig. 1). After 5 weeks, the MIC reached 8 μg/mL, even though the concentration in the plate was only 2 μg/mL. A similar attempt to induce itraconazole resistance was not successful. The MIC for itraconazole went up only by a factor of 2, from 0.125 μg/mL to 0.25 μg/mL. The sharp increase in MIC for voriconazole suggests that a genetic mutation may play a role. This idea was investigated by sequencing PCR products of the *cyp*51A and *cyp*51B genes that contained known hotspots for azole resistance in *A. fumigatus* [5]. The wild-type and voriconazole-resistant sequence were unchanged compared with the published sequences of the *cyp*51A and *cyp*51B genes as recorded in GenBank under accession nos. AF338659 and AF338660, respectively. The only mutations found were in the *cyp*51B gene of the strains grown on plates that had an itraconazole concentration of 0.25 μg/mL; these were two silent mutations (S274S and P394P). Hence, if any mutations were involved in developing this de novo azole resistance, they were not in the *cyp*51A and *cyp*51B genes.

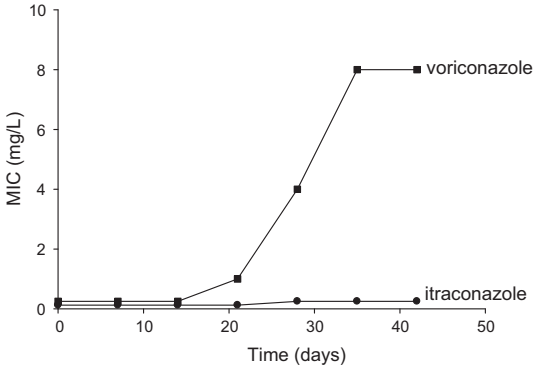

Fig. 1. Minimum inhibitory concentrations (MICs) of *Aspergillus fumigatus* for voriconazole and itraconazole as a function of culture time in days. The concentration of voriconazole in the substrate increased stepwise by a factor of 2 each time from 0.125 μg/mL to 4 μg/mL during the experiment.

On the one hand it is possible that *A. fumigatus* adjusts itself by differentially regulating expression levels to induce, for example efflux pumps, as was found for de novo acquisition of antibiotic resistance in *E. coli* [6]. Even this type of adaptation has lasting effects and is not readily reversed when the drug is no longer present. On the other hand it is equally likely that resistance to voriconazole is caused by different mutations than the well known ones in *cyp*51A and *cyp*51B. Regardless, in both cases the main conclusion is that azole resistance in *A. fumigatus* is readily induced by exposure to sublethal concentrations of these drugs. Use of a single strain may limit the general applicability of this conclusion, but the proof of principle remains valid. Especially in agricultural practice, where the application is often not overly precise, sublethal concentrations might be encountered. It is therefore essential that unnecessary exposure of *A. fumigatus* to these essential drugs for human medicine is prevented.

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Competing interests

BHtK is employed by The Netherlands Food and Consumer Product Safety Authority. All other authors declare no competing interests.

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Ethical approval

Not required.

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