Morphological changes and induction of antifungal resistance in *Aspergillus fumigatus* due to different CO₂ concentrations

Sima Darabian¹, Sassan Rezaie², Sadegh Khodavaissy², Somayeh Sharifynia³

1.Department of Medical Mycology and Parasitology, School of Public Health, International Campus, Tehran University of Medical Sciences, Tehran, Iran.

2.Department of Medical Mycology and Parasitology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

3.Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Disease (NRITLD), Shahid Beheshti University of Medical sciences, Tehran, Iran.

Objectives: Aspergillosis is one of the most common opportunistic fungal diseases in immunocompromised and neutropenic patients. *Aspergillus fumigatus* is the most common cause of this infection. Due to changes in CO₂ concentration that some pathogens face during the infection process and for understand roles of CO₂ as a carbon basic, in this study the effects of variable CO₂ concentration as one of the environmental factors were examine in the morphological changes and induction of antifungal resistance in *A. fumigatus*.

Methods: *A. fumigatus* strains were cultured and incubated under the 1%, 3%, 5% and 12% of CO₂ concentrations, each time in 1, 2, and 4 weeks. Control culture was maintained for 1 week without CO₂ concentration. Morphological changes were investigated and antifungal susceptibility test was performed according to recommendations stated in the Clinical and Laboratory Standards Institute (CLSI) M38-A2 document. All tests of different CO₂ concentrations were compared with control sample. In order to assess the stability of the developed MIC, the strains were sub-cultured for an additional 3 week in the absence of CO₂ concentrations. Along the in vitro induction process, microscopic and macroscopic cultural observations as well were registered.

Results: The results indicated morphological changes, included of shallow dark green, smooth, crisp to powdery colonies and reduced growth as macroscopic, short conidiophores, deformed vesicles and declined conidia as microscopic changes were seen. Maximum this changes observed in 1, 3, 5 and 12% of the CO₂ concentration in the maximum incubation period (4 weeks). Antifungal susceptibility pattern of susceptible strains to itraconazole, voriconazole and amphotericin B increased after expose with 12% concentration of CO₂ and 4 weeks incubation. Cross-resistance to all tested azoles was observed. MIC value for itraconazole, voriconazole and amphotericin B were 16 µg/ml, 1 µg/ml and 16 µg/ml, respectively. These values for control groups were 0.125 µg/ml, 0.125 µg/ml and 2 µg/ml, respectively.

Figure1.(Photographs of macroscopic morphological changes of *A. fumigatus* following exposure to different levels of CO₂ after four weeks. (A) Macroscopic examination with the original colonie; (B) microscopic examination with the normal features of conidiation; (C, D) inception of changes were observed under 1% CO₂ atmosphere, (E, F) 3% CO₂ atmosphere, (G, H) 5% CO₂ atmosphere, and (I, J) the most changes occurred under 12% CO₂ atmosphere.

Conclusions: Different CO₂ conditions exposure induced *A. fumigatus* morphological changes and an evident increase of MIC values as well as the development of cross-resistance with, itraconazole and voriconazole.

Table 1. In vitro susceptibility of *A. fumigatus* to itraconazole, voriconazole, and amphotericin B under different CO₂ atmospheres

Strain	CO ₂ level	Time (week)	Minimum inhibitory concentration (µg/ml)		
			ITR	VOR	AMB
<i>A. fumigatus</i> (ATCC 1028)	1% CO ₂	1	0.125	0.5	4
		2	0.5	0.125	4
		4	1	0.125	8
	3% CO ₂	1	1	0.5	8
		2	1	0.125	8
		4	1	0.5	8
	5% CO ₂	1	0.5	0.5	8
		2	2	0.5	8
		4	8	0.5	16
	12% CO ₂	1	1	0.5	8
		2	2	0.5	8
		4	16	1	16
Control cultures		0.125	0.125	1	

Abbreviations: ITR: itraconazole; VOR: voriconazole; AMB: amphotericin B

1. Abdolrasouli A, Rhodes J, Beale MA, Hagen F, Rogers TR, Chowdhary A, et al. Genomic context of azole resistance mutations in *Aspergillus fumigatus* determined using whole-genome sequencing. *MBio*. 2015; 6(3):e00536-15.

2. Tobal JM, Balieiro ME. Role of carbonic anhydrases in pathogenic micro-organisms: a focus on *Aspergillus fumigatus*. *J med microbiol*. 2014; 63(1):15-27.

3. Sasani E, Khodavaissy S, Agha Kuchak Afshari S, Darabian S, Aala F, Rezaie S. Pseudohyphae formation in *Candida glabrata* due to CO₂ exposure. *Curr Med Mycol*. 2016; 2(4):49-52.