Covalent Trapping and Bacterial Resistance to Ceftazidime

Levitt et al. (1) suggest that “covalent trapping” explains the increased ceftazidime resistance of cells producing the R164S mutant of KPC-2. This hypothesis appears to be unrealistic. The rate of diffusion of the antibiotic into the periplasm at an external concentration corresponding to the minimum inhibitory concentration (MIC) is $v_{in} = PA (MIC - Ipl)$ where $P$ (cm s$^{-1}$) is the permeability coefficient, $A$ is the surface area (132 cm$^2$/mg dry weight), and $Ipl$ is the “lethal antibiotic periplasmic concentration.” With *Escherichia coli*, the value of $Ipl$ is well approximated by the MIC of a strain devoid of $\beta$-lactamase. MIC and $Ipl$ are in $\mu M$ and $v_{in}$ is in nanomoles s$^{-1}$ (mg dry weight)$^{-1}$. The value of $P$ for ceftazidime has been measured by two different methods yielding 16 and $96 \times 10^{-7}$ cm s$^{-1}$ (for a detailed review see Docquier et al. (2)).

The MICs for the nonproducing and the R164S-producing strains are 0.4 and 200 $\mu M$, respectively. With an intermediate value of $P$ ($40 \times 10^{-7}$ cm s$^{-1}$), $v_{in} = 0.132$ nmol s$^{-1}$ (mg dry weight)$^{-1}$. If covalent trapping contributes to the MIC, the rate of $\beta$-lactamase synthesis should be similar to $v_{in}$, and the cells corresponding to 1 mg of dry weight must synthesize about 1 mg of $\beta$-lactamase over a period of 5 min. Moreover, at the lethal periplasmic concentration (0.4 $\mu M$), the acyl-enzyme represents at most 0.22% of the total enzyme because the $K_m$ of the R164S mutant for ceftazidime is 180 $\mu M$. Clearly, the amount of $\beta$-lactamase necessary for covalent trapping to be significant is impossible to reach.

Jean-Marie A. Frère

Centre d’Ingénierie des Protéines, CIP, University of Liège, Liège 4000, Belgium


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E-mail: jmfreere@ulg.ac.be