Molecular approaches and imaging of antibiotic travel through bacterial envelope

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Bacterial drug resistance is an increasingly serious threat to public health that requires a better understanding of the various mechanisms used by the bacterial cells. In Gram negative bacteria, the outer membrane barrier is a key step for drug influx. Monitoring the kinetics and the drug amount which is able to enter into the bacteria is important for understanding the translocation across the membrane. Fluorescent antibiotics are a good way to follow their accumulation in bacteria with spectrofluorometry or microfluorimetry. Previously, Cinquin *et al.* [1] have studied the fluoroquinolones accumulation in bacteria.

This study is focused on β-lactam antibiotics that target the peptidoglycan assembly in periplasmic space. Two fluorescent ceftazidime (CAZ) molecules were derived from the CAZ, a cephalosporin of the third generation. CAZ- 1 is always fluorescent, and CAZ -2 becomes fluorescent only when it cleaved by β-lactamases. The two fluorescent CAZ-1 and CAZ-2 show antibacterial activity. Some studies have presented the use of β-lactamase inhibitors with CAZ as a potential measure to circumvent the bacterial enzymatic resistance. The accumulation of these two modified-CAZs was monitored in ARS108, a clinical isolate which is porin- and products a high level of β-lactamases.

To follow the accumulation of the fluorescent compounds inside bacterial population, the total lysate was analyzed with spectrofluorometer while the individual bacteria were observed under DUV microscope.

The results show that the accumulation of CAZ-1 is effective in ARS108 only with a membrane permeabilizer that facilitates the drug permeation. To visualize the accumulation of CAZ-2, the combination of a membrane permeabilization and β-lactamase activity is necessary.

CAZ-1 is a good probe to display the permeabilization of the bacterial membrane barrier. Since the CAZ-2 fluorescent signal occurs only with β-lactamase activity, and β-lactamases are only localized in the periplasm, so it highlights the periplasmic location of the compound.

Moreover, the permeation kinetics and the location of the ß-lactam in the periplasmic space will be the future objective. Alongside we will correlate, in real time, the local concentration of antibiotics and the activity on the target for a better definition of efficient drug profile.

[1] B. Cinquin, L. Maigre, E. Pinet, J. Chevalier, R.A. Stavenger, S. Mills, M. Réfrégiers, J.M. Pagès, Scientific Reports 5, Article number: 17968 (2015)