Assay of sitamaquine in Leishmania donovani strains by LC-MS/MS

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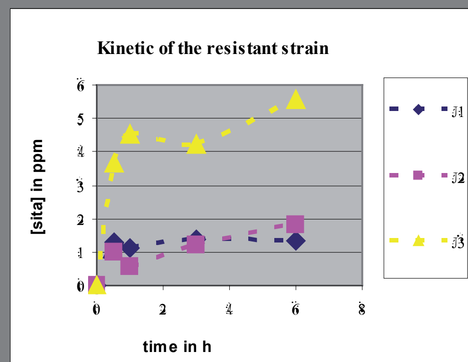
Introduction

Sitamaquine is a treatment against visceral Leishmaniosis, which is a parasitic disease. Leishmaniosis is spread worldwide and about 500 000 new cases of visceral leishmaniosis, the most severe form of the disease, appear each year : all the substances against this disease are toxic for humans and the parasite develops a resistance. The action mechanism of sitamaquine is unknown. In order to determine where sitamaquine acts in sensitive or resistant strains, it is necessary to understand the distribution of sitamaquine inside the micro-organism.

Kinetic graph of sitamaquine enter the resistant strain of Leishmania donovani



Experimental Conditions

High performance liquid chromatography coupled with mass spectrometry was used for this study. The system consisted of a Dionex Ultimate 3000 pump equipped with a Dionex WPS-3000PL autosampler automatic injector. Liquid chromatography was performed in the isocratic mode with a Utisphere ODSB column (interchim C18 250mm×2.1mm×5µm). The mobile phase was a methanol/buffer 53/47 mixture. The buffer solution consisted of acetic acid and ammoniac mixture with pH adjusted to 3.3. The flow rate was fixed at 300µL/min. The sample volumes injected were 10µL.

The mass spectrometer was a Waters-Micromass Quattro Ultima with an electrospray interface and a triple quadrupole analyser. The acquisition mode used was Multiple Reaction Monitoring (MRM). The precursor ion was at m/z = 344.4 ([M+H]+), and the fragment ion at 271.2. The collision energy was set to 25eV.

The calibration mode was external. It was set at twice (once at the beginning and once at the end of the acquisition sequence) with seven standards (concentration 0.05; 0.1; 0.5; 1; 2.5; 5;10ppm).

Results and discussion

J1, J2, J3 are different days when sitamaquine and parasitic strains were in contact to see the reproducibility of this experiment. Indeed, the same evolution was observed. The graph represents the kinetic of the sitamaquine entering in the resistant strain. It could be drawn because the quantity of parasites in the same day was similar. On the graph a dramatic expansion is observed and then the curve stops rising and remains steady. With the sensitive strain, a similar graph was obtained. Therefore the graphs show that sitamaquine entered fast in the different strains. Consequently, the resistant strain allowed sitamaquine to enter, however the resistant parasite must have a mechanism that stops this antibiotic.

Conclusion

For these assays, the liquid chromatography coupled with the mass spectrometry is a very good method because it is very selective and sensitive. Indeed, in a unicellular parasite there are billions of molecules that can interfere with the measurement of sitamaquine. Mass spectrometry is used like a detector; this allows sitamaquine to be detected alone.

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