Development of a method by CL-HRMS to evaluate the pharmacokinetic profile and toxicity by untargeted metabolomics of an anti-prion prototype

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The prion protein is the only agent responsible for spongiform encephalopathies (EE), which are a group of fatal neurodegenerative disorders that occur due to the conversion of the cellular prion protein into its form called scrapie. Most prion protein-related diseases occur spontaneously and affect elderly individuals, however, the transmissible form of the disease also occurs in a smaller proportion. As this disorder is fatal and still without a cure, there is a demand for the development of compounds with anti-prion activity. Several aromatic organic compounds have been studied for anti-prion activity. Still, few of these were able to cross the blood-brain barrier and showed satisfactory pharmacokinetic profiles. Compound J8 is a trimethoxychalcone with anti-prion activity confirmed with *in vitro* tests and safety confirmed through *in vivo* tests. The objective of this project is to study the permeation by the blood-brain barrier and toxicity by untargeted metabolomics of the new prototype antiprion, compound J8, in plasma and mouse brain by liquid chromatography associated with mass spectrometry and metabolomics strategies.

The method was developed and validated using liquid chromatography coupled to high-resolution mass spectrometry (CL-HRMS). Plasma and brain extractions were performed using organic solvent, agitation and centrifugation steps, to allow analysis in a liquid chromatograph coupled to a high-resolution hybrid Quadrupole-Orbitrap (Thermo Q-exactive) mass spectrometer with an electrospray ionization source operating in positive mode. The identification of the compound was based on high accuracy m/z data (error ? 5 ppm) and analysis of the fragmentation spectra. Data treatment was performed using TraceFinder 4.1 and GraphPad Prisma 8.0. Quantification was performed by constructing an analytical curve covering concentrations from 0.05 to 5.0 ng/mL. The analytical method was validated using the parameters matrix effect, linearity, limit of detection, limit of quantification, recovery and intermediate precision. The method did not present a matrix effect. The J8 prototype showed maximum concentration in the brains of mice one hour after administration and can be detected in the tissue for up to six hours. In plasma, the maximum concentration was reached soon after administration of the compound. Therefore, we can conclude that the J8 compound is capable of crossing the blood-brain barrier of mice and can be considered a candidate drug for the treatment of EE. Untargeted metabolomics is being used to support the toxicity study in the plasma and spleen of mice treated with J8.

Agradecimentos: