Nuclear Magnetic Resonance Spectroscopy applied to metabolomics of the crack users

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Abstract

This research aims to compare metabolomic profiles of blood serum samples of healthy people to those who are addicted to crack using nuclear magnetic resonance spectroscopy (^1H NMR). ^1H NMR data shall be then treated using chemometric tools such as principal component analysis (PCA) aiming to differentiate two groups of samples and enable identification of NMR spectral regions important for the metabolic differences. Further, biomarkers for crack dependence might be identified and thus improve clinical diagnosis and discovery of new pharmacological and therapeutic interventions in this drug dependence treatment.

Key words: Nuclear Magnetic Resonance, Metabolomics, Crack users.

Introduction

Crack is a mixture of cocaine and some base, such as sodium bicarbonate, and water. It has a huge capacity to cause addiction in human beings due to its quickly interaction with central nervous system. Since 1990's, Brazil faces the increase in the number of crack users¹ and the serious public health problem that the lack of treatment of these users causes.

Therefore, the aim of the present research proposal is to compare blood serum sample metabolites using ^1H NMR of crack users to the corresponding NMR spectra from blood serum samples of healthy people. Our aim is to find potential biomarkers that would help to improve diagnosis and therapeutics from all of the clinical manifestation caused by crack addiction.

Results and Discussion

The blood serum samples were collected and processed by our collaborators from the Department of Psychiatry from Federal University of São Paulo (UNIFESP) and stored in biofreezer at $-80\text{ }^{\circ}\text{C}$ for not more than 10 days before NMR analyses were performed.

Until the present moment, 27 blood serum samples from healthy individuals (control group) were analyzed and also 11 blood serum samples from the crack users.

The ^1H NMR analyses were made in triplicate using deuterium oxide (D_2O) as a solvent in blood serum sample preparation and recorded in a Bruker AVANCE (600 MHz) spectrometer at $25\text{ }^{\circ}\text{C}$ using the TBI probe.

For a better spectral resolution and small metabolites analysis, ^1H NMR analyses using a T_2 filter were made with a CPMG pulse sequence. Some of the obtained results are disclosed on Images 1 and 2.

Image 1. Examples of the ^1H NMR spectra (ns = 128) of blood serum samples: crack users (red) and control healthy group (blue).

Image 2. Example of the ^1H NMR spectrum using a T_2 filter (ns = 128) at δ 0.80 – 4.50 area of a blood serum sample of a crack user.

Conclusions and Perspectives

A previous study performed on mice treated with cocaine² had shown that significant metabolic changes among healthy and treated groups exist. Assuming that mice represent excellent animal models for cocaine-dependence, we intend to, in a pioneer research, execute metabolomics by ^1H NMR. And expect that in great number of samples can accomplish multivariate statistical analysis and using 2D NMR spectra, discover the principal biomarkers for this addiction.

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¹Schneider, J.; Lisboa, C.; Mallmann, C. *Psiquiatria* **2014**, 3, 24-33.

²Li Y. *et al. Neuroscience* **2012**, 218, 196-205.