NMR molecular profiling of human blood plasma in induced myocardial ischemia

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Purpose/Introduction:

Management of myocardium ischemia and necrosis takes most of the time in the clinical context of coronary syndromes in cardiology departments. The protocols of management of patients entering emergency rooms because thoracic pain of potential ischemic origin without necrosis include serial measurement s of biomarkers, stress tests and, in many cases, unnecessary hospitalization. In this context, detection of metabolic markers, which represent dynamic changes in just a few minutes after ischemia, is an attractive option both for diagnosis precision and for rapid and efficient management. High resolution NMR allows measuring a great deal of low molecular weight metabolites which constitute what is called metabolic fingerprint characteristic of the system state at the moment of sample taking [1]. In this communication, we used NMR metabolic profiling to characterize metabolically blood plasma of patients pre and post angioplasty.

Subjects and methods

We recorded 1D pre-saturation 1H spectra in a 600 MHz spectrometer of blood plasma from 12 patients with underwent angioplasty and 4 controls. Plasma was taken before and 10 minutes after intervention or just with 10 minutes time difference for controls. The amount of blood plasma analyzed for each subject was 500 uL. The spectra were recorded in a Bruker-AVANCE600 spectrometer. All measurements were performed at a temperature of 37C. NMR 1D-presaturated single pulse spectra were obtained for all samples. Additionally, 2D experiments were collected in selected samples for assignment purposes. All spectra were preprocessed with 0.3Hz line broadening. Alanine doublet was used for spectral referencing. The chemical shift region including resonances between 0.5 and 4.7 ppm was investigated.

Statistical analysis was performed using in-house MATLAB scripts and the PLS Toolbox statistical multivariate analysis library. Principal components chosen explained at least 70% of the variance. Metabolite quantification was achieved by in-house peak-fitting routine over most relevant signals.

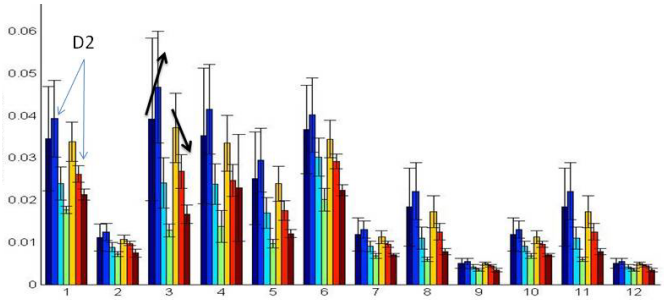

Figure 1. Relative levels of most relevant spectral regions for differentiating between NMR plasma profiles pre and post angioplasty in patients (cold colors) and controls (warm colors).

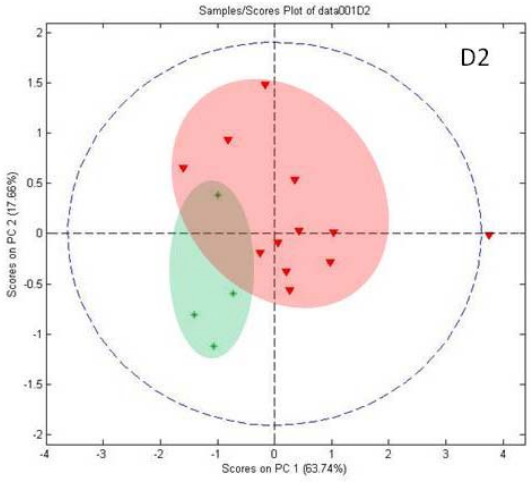

Figure 2. PCA scores plot of plasma NMR metabolic profiles of patients (red) and controls (green) in samples taken 10 minutes after intervention (patients) or 10 minutes after first sample taking (controls).

Results and discussion

Our NMR spectra of blood plasma from patients and controls showed narrow signals and adequate SNR with well resolved multiplicities. Direct comparison of the spectra demonstrated that plasma from patients and controls are metabolically similar. The blood plasma metabolic profile determined here was highly consistent with previous studies. However, subsequent statistical multivariate analysis showed differences in signals belonging mainly to ketonic bodies and fatty acids. The differential profile detected here between plasma of ischemic and non ischemic human subjects may provide the basis for new diagnosis methods of the myocardial ischemia.

References

1. Brindle JT, Antti H, Holmes E, Tranter G, Nicholson JK, Bethell HW, Clarke S, Schofield PM, McKilligin E, Mosedale DE, Grainger DJ. Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based metabonomics. Nat Med. 2002 Dec;8(12):1439-44.

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