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Metabolomic Profile of Human Myocardial Ischemia by Nuclear Magnetic Resonance Spectroscopy of Peripheral Blood Serum

A Translational Study Based on Transient Coronary Occlusion Models

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Objectives The aim of this study was to investigate the metabolomic profile of acute myocardial ischemia (MIS) using nuclear magnetic resonance spectroscopy of peripheral blood serum of swine and patients undergoing angioplasty balloon–induced transient coronary occlusion.

Background Biochemical detection of MIS is a major challenge. The validation of novel biosignatures is of utmost importance.

Methods High-resolution nuclear magnetic resonance spectroscopy was used to profile 32 blood serum metabolites obtained (before and after controlled ischemia) from swine (n = 9) and patients (n = 20) undergoing transitory MIS in the setting of planned coronary angioplasty. Additionally, blood serum of control patients (n = 10) was sequentially profiled. Preliminary clinical validation of the developed metabolomic biosignature was undertaken in patients with spontaneous acute chest pain (n = 30).

Results Striking differences were detected in the blood profiles of swine and patients immediately after MIS. MIS induced early increases (10 min) of circulating glucose, lactate, glutamine, glycine, glycerol, phenylalanine, tyrosine, and phosphoethanolamine; decreases in choline-containing compounds and triacylglycerols; and a change in the pattern of total, esterified, and nonesterified fatty acids. Creatine increased 2 h after ischemia. Using multivariate analyses, a biosignature was developed that accurately detected patients with MIS both in the setting of angioplasty-related MIS (area under the curve 0.94) and in patients with acute chest pain (negative predictive value 95%).

Conclusions This study reports, to the authors' knowledge, the first metabolic biosignature of acute MIS developed under highly controlled coronary flow restriction. Metabolic profiling of blood plasma appears to be a promising approach for the early detection of MIS in patients. (J Am Coll Cardiol 2012;59:1629–41) © 2012 by the American College of Cardiology Foundation

Decision making in patients with suspected acute myocardial ischemia (MIS), especially in those with normal results in the current standard of care based on electrocardiographic results and troponin levels, is among the most challenging tasks doctors face in emergency departments (1). In this scenario, biomarkers or biosignatures with the ability to reliably discriminate ischemic from nonischemic patients would be highly appreciated, and they could have important clinical implications in daily practice to reduce the use of

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Abbreviations and Acronyms

FA = fatty acid

MIS = myocardial ischemia

NMR = nuclear magnetic resonance

PCA = principal component analysis

PLS-DA = projection to latent structures for discriminant analysis

unnecessary resources in the workup of patients and to avoid inappropriate discharges. Several studies have reported potential biomarkers of MIS, although so far, none has reached routine practice, because of insufficient accuracy (2–4).

Altered cardiac metabolism is the primary consequence of MIS. Metabolomics permits a quantitative measurement of the multiparametric metabolic responses of living systems to pathophysiological stimuli by simultaneously examining dynamic changes in hundreds of low–molecular weight metabolites in tissues or biofluids (5). However, human metabolomics studies are at risk for potential clinical confounders such as interindividual variability, diet, drug effects, age, sex, and comorbidities. Serial sampling performed in patients both before and after a controlled perturbation may circumvent these problems, allowing each patient to serve as his or her own biological control (5).

High-resolution nuclear magnetic resonance (NMR) spectroscopy combined with advanced multivariate analyses methodologies poses several advantages over classical biochemical assays. First, it represents a powerful, reproducible, and inexpensive technique for investigating the metabolome in the area of disease diagnosis without extensive sample preparation. Second, simultaneous measurements of changes in multiple metabolic parameters are detectable in as little as 20 μl (just a couple of drops) of peripheral blood plasma. And last but not least, NMR spectra provide information not only about the amounts of certain metabolites but also about their physicochemical status (6–8).

Transient angioplasty balloon–induced coronary occlusion is ideally suited for metabolomic studies. Not only does it allow a highly controlled model of MIS (9), but it also avoids the confusing effects of metabolic responses of large muscle groups. The latter inevitably occurs in exercise-induced ischemia (7) and could potentially interfere with the interpretation of the effects of MIS on the peripheral blood plasma metabolomic profile.

The aim of the present study was to develop a metabolic profile of acute MIS in peripheral blood serum. For this purpose, we used, in swine and patients, highly controlled models of MIS based on transient angioplasty balloon–induced coronary occlusion. The role of metabolomics to detect MIS in such a controlled fashion has not been previously evaluated. A preliminary clinical validation of the developed biosignature was carried out in patients with spontaneous acute chest pain and normal electrocardiographic results and troponin values.

Methods

Chemometric analysis of high-resolution NMR spectroscopic findings for blood serum metabolic profiling was used throughout. The 3 steps of the present study are detailed as follows and in Figure 1.

Experimental study on animal models. After fasting for at least 12 h, 9 juvenile domestic female pigs weighing 25 to 30 kg were sedated and anesthetized, and transient angioplasty balloon–induced MIS in the proximal left anterior descending coronary artery was induced, as we have previously described (10). Coronary artery occlusion was confirmed by contrast injection and by electrocardiographic ST-segment deviation. After 5 min, the balloon was deflated, and restoration of normal coronary flow was documented by angiography.

Once diagnostic angiography had been performed, blood samples were drawn before and 10 and 120 min after ischemia. Samples were prepared and frozen for subsequent analyses. The animals were allowed to recover. For all metabolomic analyses, the state before ischemia was used as the own control in each experiment. No deaths, significant complications, coronary dissection, or closure was detected.

The study was approved by the Animal Care and Use Committee of the University of Valencia and conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Controlled model of ischemia in patients. A group of 21 ambulatory patients without acute coronary syndromes scheduled to undergo percutaneous coronary intervention for stable angina were enrolled. Patients were treated with heparin (100 U/kg). Medical treatment was not stopped and was left at the discretion of the attending cardiologists. Patients fasted for 12 h before and after the procedure. A 6-F sheath was introduced into the left radial artery to engage the culprit coronary artery. Appropriate catheters and angioplasty wires were used. Ischemia was induced by inflating the appropriate balloon in each case. At least 1 min of uninterrupted total coronary occlusion was confirmed in all patients by contrast injection and ST-segment deviation. Stents were successfully implanted in all patients. No deaths or significant complications were detected. Troponin I (Dade Behring, Newark, Delaware) was serially measured at 6 and 12 h after angioplasty. One patient was excluded because of an asymptomatic post-procedural troponin I increase. Accordingly, the study group comprised 20 patients. Once diagnostic angiography had been carried out, peripheral blood samples for metabolomic analyses were drawn before and 10 and 120 min after angioplasty. Samples were prepared and frozen for subsequent analyses.

A control group made up of 10 patients studied with coronary angiography but with normal coronary arteries was included. The same protocol applied in patients was used in controls. Blood samples for metabolomic analyses were