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Research Paper

Real-Time and Non-invasive Monitoring of the Activation of the IRE1α-XBP1 Pathway in Individuals with Hemodynamic Impairment

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ABSTRACT

Many stressors that are encountered upon kidney injury are likely to trigger endoplasmic reticulum (ER) stress, subsequently activating transcriptional, translational and metabolic reprogramming. Monitoring early cellular adaptive responses engaged after hemodynamic impairment yields may represent a clinically relevant approach. However, a non-invasive method for detecting the ER stress response has not been developed. We combined a metabolomic approach with genetic marker analyses using urine from individuals undergoing scheduled cardiac surgery under cardiopulmonary bypass to investigate the feasibility and significance of monitoring the ER stress response in the kidney. We developed an original method based on fragment analysis that measures urinary levels of the spliced X-box binding protein 1 (sXBP1) mRNA as a proxy of inositol-requiring enzyme 1α (IRE1α) activity because sXBP1 is absolutely sensitive and specific for ER stress. The early engagement of the ER stress response after ischemic stress is critical for protecting against tissue damage, and individuals who mount a robust adaptive response are protected against AKI. The clinical consequences of our findings are of considerable importance because ER stress is involved in numerous conditions that lead to AKI and chronic kidney disease; in addition, the detection of ER stress is straightforward and immediately available in routine practice.

1. Introduction

Medical conditions that ultimately lead to ischemic tissue injury, such as ischemic acute kidney injury (AKI), are accompanied by micro-environmental alterations (nutrients, growth factors and oxygen deprivation) and disturbances in cellular homeostasis (energetic failure) in the kidney, forcing cells to engage adaptive responses to reduce or eliminate the intensity of the stressor, initiate metabolic reprogramming, regulate the fate of the cell by activating both anti- and pro-apoptotic pathways, and activate communication networks with the immune system to signal a problem (Chovatiya and Medzhitov, 2014; Kotas and Medzhitov, 2015). These molecular and cellular adaptive responses occur very early after the initiation of a stressful condition, long before cell death and the initiation of maladaptive repair processes (Bonventre and Zuk, 2004; Duffield, 2014; Ferenbach and Bonventre, 2015; Zuk and Bonventre, 2016). Therefore, the detection of their activation constitutes an opportunity for the early diagnosis of ongoing tissue injury, which is crucial for the development of preventive and therapeutic strategies in renal medicine.

Endoplasmic reticulum (ER) stress and its adaptive response, the unfolded protein response (UPR), represent an archetypal example of these adaptive stress responses. ER stress and the disruption of ER proteostasis or parts of the UPR that, for instance, affect epithelial cells, actively participate in the development of AKI and chronic kidney disease (CKD) (El Karoui et al., 2016; Inagi et al., 2014; Mami et al., 2016a; Mami et al., 2016b; Zuk and Bonventre, 2016). A wide range of cellular environments and events induce ER stress, including an autophagy deficiency, energy deprivation, limited or excess nutrients, dysregulated calcium levels, perturbations in redox homeostasis, inflammatory challenges and hypoxia, virtually all of which are encountered in medical situations that promote ischemic AKI (Walter and Ron, 2011). Under ER stress conditions, the aim of the UPR is to adapt to the changing environment and reestablish normal ER function by inducing

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transcriptional, translational, and metabolic reprogramming (Walter and Ron, 2011; Wang and Kaufman, 2016; Gao et al., 2015; Wellen and Thompson, 2010). Three major mediators are involved in the UPR: ATF6 (activated transcription factor 6), IRE1α (inositol-requiring enzyme 1α) and PERK (protein kinase RNA (PKR)-like ER kinase).

Of particular interest in the UPR is the IRE1α/X-box binding protein-1 (XBP1) axis. The ribonuclease activity of IRE1α catalyzes the unconventional splicing of a 26 nucleotide intron from the mRNA encoding the transcription factor XBP1, creating a transcriptionally active spliced XBP1 (sXBP1). XBP1 protein translated from mammalian unspliced XBP1 mRNA acts as negative regulator by heterodimerizing with the transcription factor sXBP1 to promote its degradation. Genes that are regulated by sXBP1 enhance protein folding, transport, and degradation, expand protein secretory pathways, and rewire cellular energetic metabolism (Wu et al., 2015; Cubillos-Ruiz et al., 2015; Chen et al., 2014; Hetz and Glimcher, 2009). Therefore, the activation of the IRE1α/XBP1 axis protects against ER stress. The clinical (yet unproven) consequence of the activation of this pathway would be that the robust production of sXBP1 induces (and could predict) protection against tissue damage and the detection of sXBP1 may have prognostic value. Since XBP1 splicing depends exclusively on IRE1α activity, sXBP1 is only produced if and when ER stress occurs (with a rare exception), therefore reflecting the presence of ER stress with absolute sensitivity. In some specific cases, IRE1α is not activated by the accumulation of misfolded proteins within the RE: Toll like receptors 2 and 4 activation by microbial products specifically promotes the phosphorylation of IRE1α and the activation of XBP1 (Martinon et al., 2010). In turn, a lack of sXBP1 production virtually eliminates the possibility of activating the IRE1α branch of the UPR.

We performed this proof of concept study to test and validate the hypothesis that the ER stress response to a renal ischemic insult can be non-invasively monitored and provides information about patients' risks of developing AKI. Therefore, we combined a metabolomic approach for monitoring metabolic reprogramming with an original method that measures urinary levels of the sXBP1 mRNA as a readout of IRE1α activity to dynamically and non-invasively monitor renal ER stress. This prospective pilot study was performed in a cohort of individuals undergoing scheduled cardiac surgery under extracorporeal circulation using cardiopulmonary bypass (CPB), a procedure that promotes hemodynamic impairment and ischemic stress in the kidney.

2. Methods

2.1. Patients

From 17 February 2017 to 26 April 2017, 42 patients undergoing scheduled cardiac surgery with CPB were enrolled. The exclusion criteria were: an eGFR < 30 ml/min/1.73 m^2, infusion of a radio contrast agent within the 24 h before surgery, a preoperative left ventricular ejection fraction <40%, age < 18 years, pregnancy, and the inability to provide consent. AKI was diagnosed according to the KDIGO Clinical Practice Guideline for AKI criteria (http://kdigo.org/) using serum creatinine levels and urine output after the surgery.

2.2. Study Approval

This single-center, prospective, pilot study was approved by the French ethical committee on 7 February 2017 (CPP Sud Est III n° 2016-072 B) and registered under the EudraCT n° 2016-A01871-50. All patients provided written consent for study participation and for the biological analysis before inclusion.

2.3. Surgical Procedure

All medications acting on the renin-angiotensin system and all diuretics were stopped on the day before surgery. General anesthesia was induced according to the protocols of the Anesthesiology Department. Anesthesia was induced and maintained in patients using target-controlled infusions of sufentanil and propofol. Atracurium was used for all anesthetics. Hemodynamic parameters were monitored with a radial artery catheter on a Philips Intellivue® MX 800. Tranexamic acid was injected before and after CPB. The CPB target flow rate was calculated using the following equation: flow (l/min) = 2.6 × body surface area. Heparin was infused before and during CPB to achieve and maintain an activated clotting time > 400 s. The protamine dose was calculated at a 1:1 rate of the heparin dose and infused at the end of CPB. Cardioplegia was applied at the surgeon's discretion. Lost blood was recovered using Cell Saver® Elite® (Haemonetics™, France) and re-transfused when possible. Vasotropic or inotropic agents, fluids and transfusion products were administered at the discretion of the anesthesiologist based on clinical, echocardiographic and biological findings. After surgery, all patients were transferred to the cardiovascular intensive care unit (ICU).

2.4. Prospective Sample and Data Collection

Urine samples were collected from a urinary catheter at 3 different times: (1) after the induction of anesthesia and before the start of CPB, (2) at the end of the CPB procedure, and (3) on the day after surgery in the ICU. Urine samples were collected in Corning 50-ml conical tubes and centrifuged at 2000*g* for 20 min within 4 h of collection. Cell pellets were conserved in 300 μL of RLT® buffer (Qiagen™, France) and stored until mRNA extraction. Supernatants and cell pellets were stored at −80 °C until analysis. Hemodynamic data were extracted from the Philips system using IXTREND® software version 2.1.0 FW14 (Ixellence GmbH, Germany). MAP values were recorded every 1.25 s during the CPB procedure. Clinical data were prospectively extracted from the hospital's electronic medical records. All clinical data and samples were de-identified.

2.5. ^{1}H NMR Spectroscopy

Urine samples were prepared to obtain a final volume of 600 μl (400 μl of urine; 160 μl of 200 mM phosphate buffer, pH 7.4; 1 mM trimethyl silyl propionate, sodium salt (TSP) as the NMR chemical shift reference, 6 mM NaN_3; and 40 μl of D_2O). Urine ^{1}H NMR spectra were measured at 300 K on a Bruker Avance II® 500 MHz spectrometer (Bruker Biospin GmbH, Germany) equipped with a SampleXpress® automation sample changer and a standard 5 mm broadband inverse (BBI) probe with a Z-gradient. The spectra were acquired using a 1D nuclear Overhauser effect spectroscopy (NOESY) pulse sequence with presaturation for water suppression. The parameters used for the pulse sequence were: a relaxation delay of 1 s, a mixing time of 100 ms, an acquisition time of 1.36 s and a 90° pulse length of 8 μs. Data points (32K) were collected during 64 scans with a spectral width of 20 ppm.

Preprocessing of the urine ^{1}H NMR spectra was performed with MestReNova® 8.0 software. A line-broadening factor of 0.3 Hz was applied prior to Fourier transform. The spectra were then phased, baseline corrected, and referenced to TSP. Each ^{1}H NMR spectrum (0.24 to 10.00 ppm) was reduced by an equidistant binning method with a bin width of 0.04 ppm to limit misalignment problems. The spectral regions corresponding to urea (5.52 to 6.04 ppm) and water (4.28 to 5.24 ppm) were deleted to remove variability because of the suppression of water. Integral normalization (typically a constant integral of 100) was performed on the remaining 210 bins. The resulting matrix was normalized with a commonly applied method for equidistant binned spectra, Pareto scaling, by dividing the mean-centered variables (bins) by the square root of their standard deviations.

2.6. Fragment Analysis of Urine sXBP1 and XBP1 mRNA Levels

RNA was extracted from the pellets using the RNeasy mini kit® (Qiagen™) and reverse-transcribed into cDNAs using TaqMan® Reverse

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