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Research letters

Temporal characterization of cardiac expression of glucose transporters SGLT and GLUT in an experimental model of myocardial infarction

Introduction

Myocardium uses free fatty acids as its principal energy substrate in healthy hearts, with glucose representing 25–30% of the total energy source. However, in the presence of ischaemia, glucose becomes the main adenosine triphosphate generator, acting through anaerobic glycolysis processes [1]. Sodium-glucose cotransporters (SGLT) and passive glucose transporters (GLUT) are responsible for glucose reaching cellular cytosol. The SGLT2 isoform is predominant in the kidneys and the SGLT1 isoform is predominant in the intestines, although the latter has recently been described in the heart as well [2,3]. GLUT receptors are ubiquitous, with the GLUT4 isoform predominating in cardiomyocytes, although other receptors (1, 3, 8, 10, 11, 12) are also found in myocardium [4]. In cases of ischaemia-reperfusion damage, contractile function recovery depends on an increase in glucose concentration, and over-expression of those receptors has been suggested as being cardioprotective in the presence of ischaemia [5].

Recently, inhibition of SGLT2 receptors in diabetes patients has been shown to prevent heart failure and reduce cardiovascular mortality [6]. Acute myocardial infarction (AMI) is a frequent complication in diabetes patients and is associated with high mortality. In experimental models of AMI, an early increase in GLUT1 receptors has been described, with a later increase in SGLT1 [7,8]. However, the response of SGLT2 receptors to AMI is still unknown, yet it is of particular interest as drugs are used to inhibit these receptors.

The aim of the present study was to describe the expression of SGLT and GLUT transporters in myocardium in both the short- and long-term after AMI, while differentiating between infarcted and non-infarcted myocardium.

Methods

The experiment protocols were approved by the University of Murcia Committee on the Ethics of Animal Experiments (permit number: A13150105). The animals were housed under standard laboratory conditions in a pathogen-free facility with environmentally controlled rooms (22 °C, 60% humidity, 12 h light/dark cycle), and fed ad libitum. After a 7 days adaptation period, 50 male Wistar rats (weight: 220–250 g) were subjected to AMI by permanent ligation of the anterior descending coronary artery, and randomly assigned to five groups (10 rats per group) according to time from surgery to sacrifice: 1 week; 2 weeks; 4 weeks; 12 weeks; and 24 weeks. Animals were anaesthetized with intraperitoneal ketamine (75 mg/kg) and medetomidine (0.5 mg/kg). In parallel, eight animals were subjected to surgery with no ligation (sham group).

All rats underwent echocardiography examination 24 h after surgery and on the day before sacrifice (HD11 XE, Philips, Amsterdam, The Netherlands). Standard two-dimensional (2D) echocardiography images of the left ventricle were obtained in both long- and short-axis views, as previously described [9]. Myocardial infarct size was determined from right parasternal short-axis views at the mitral valve, papillary muscles and apical planes, using a 12-segment system and the mean of three measurements. In brief, after sacrifice, 20 mg of fresh cardiac tissue from both infarcted regions and non-infarcted myocardium was washed with cold Dulbecco's phosphate-buffered saline (DPBS), followed by use of the Dynabeads mRNA DIRECT Kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA), as per manufacturer's instructions. First-strand complementary DNA (cDNA) was synthesized using the GeneAmp RNA PCR Core Kit (Hoffmann-La Roche, Basel, Switzerland). Real-time polymerase chain reaction (RT-PCR) was performed with SYBR Premix Ex Taq (TaKaRa Biotechnology [Dalian] Co., Ltd., Dalian, Liaoning Province, China), following manufacturer's instructions, and a LightCycler 480 System (Hoffmann-La Roche).

Efficiency of each primer pair was assessed by serial dilutions of cDNA. Subsequently, 0.5–3 μL of template cDNA was amplified by 50 cycles of RT-PCR (10 s at 95 °C, 10 s at 60 °C and 15 s at 72 °C), followed by a dissociation stage to ensure that only one product was amplified. The genes studied were for SGLT1, SGTL2, GLUT1, GLUT2 and GLUT4 (glucose transporters), as well as collagen I, fibrosis markers transforming growth factor (TGF)-β and α-smooth muscle actin (α-SMA), and inflammation markers interleukin (IL)-6, tumour necrosis factor (TNF)-α and monocyte chemoattractant protein (MCP)-1. The gene for glyceraldehyde-3-phosphate dehydrogenase (GADPH) was used as the housekeeping gene. Primer sequences were: SGLT1, forward 5′-GAC TGA TTC TCG GCT TCC TG-3′, reverse 5′-GTG AGG AGG GAG ATG ACC AA-3′; SGLT2, forward 5′-CCT GCT GCG TGA CCC TGT GA-3′, reverse 5′-ACA TGG GCA TCA GCT TCA GGT-3′; GLUT1, forward 5′-ACT CCA TGC TGA TGA TGA ACC-3′, reverse 5′-CAG GCC ACA GTA CAC TCC AAT-3′; GLUT2, forward 5′-CTT GGT TCA TGG TTG CTG AAT-3′, reverse 5′-GAA GTC CGC AAT GTA CTG GAA-3′; and GLUT4, forward 5′-CTC TCA GGC ATC AAT GCT GTT-3′, reverse 5′-GAG ACC AAC GTG AAG ACG GTA-3′. Primer sequences for other markers have been previously described elsewhere [9].

Statistical analysis

Data were expressed as means ± standard error of the mean (SEM). Normality was tested using the Kolmogorov-Smirnov test. Differences between all groups were tested by a Kruskal-Wallis test. For multiple comparisons with the sham group, the Siegel-Castellan test was used. Non-parametric correlations were studied only in infarcted animals according to Kendall's method. Statistical signifi-

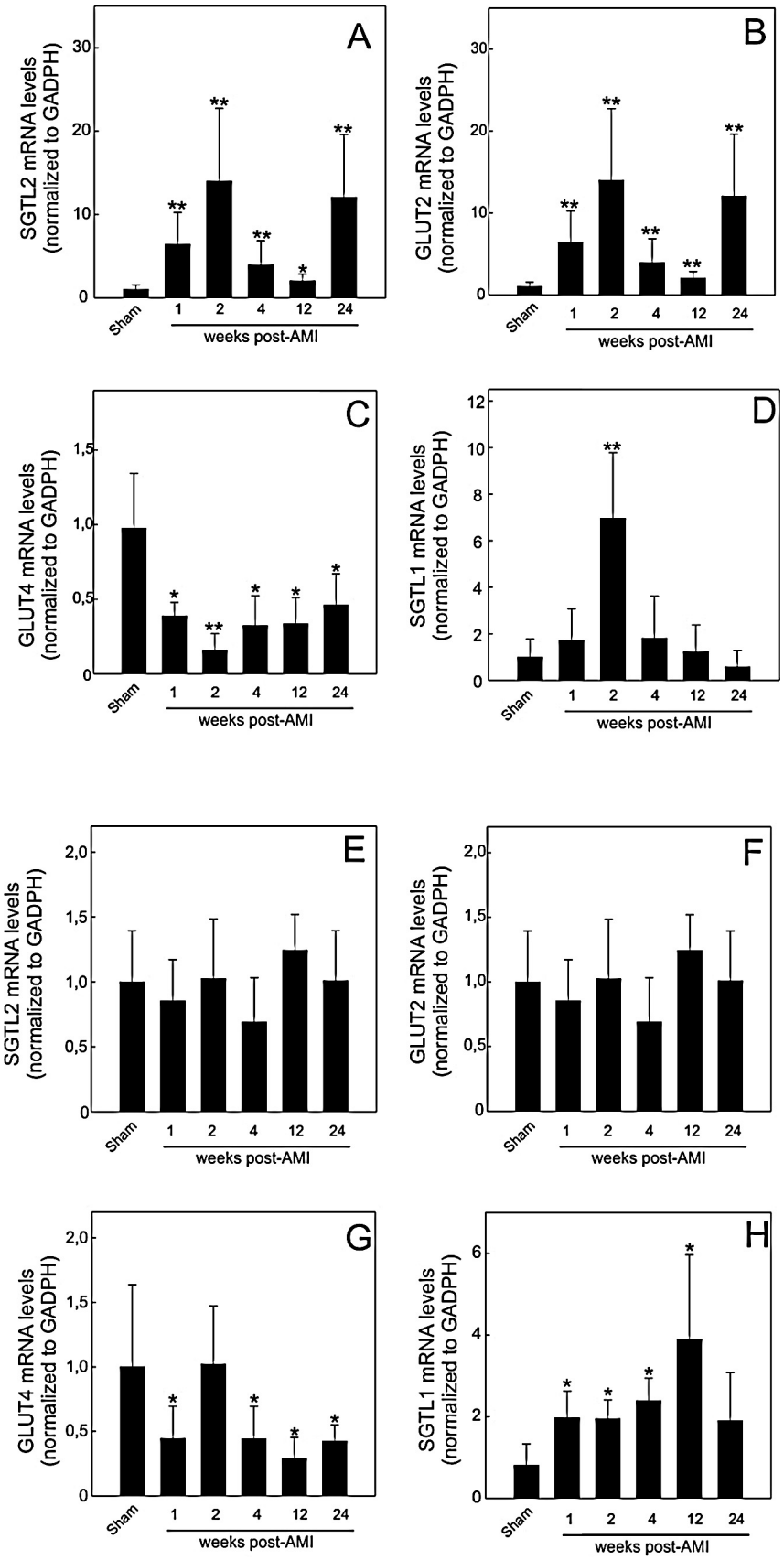

Fig. 1. Quantitative reverse-transcription polymerase chain reaction analysis of SGLT2 (A), GLUT2 (B), GLUT4 (C) and SGLT1 (D) in infarcted and non-infarcted (sham) myocardium. Data for the same receptors, normalized against GADPH, are compared with the sham myocardium (E–H). *: $P < 0.05$, **: $P < 0.01$ vs. sham group.

cance was assumed at $P < 0.05$. Data were statistically analyzed using IBM SPSS Statistics V22.0 software (IBM Corp., Armonk, NY, USA).

Results

Surgical mortality was 22% during the first 48 h, and no deaths occurred thereafter. The presence of AMI was confirmed by echocardiography performed 24 h post-AMI, and showed no differences in infarct size between AMI groups ($P = 0.318$). Compared with the sham group, AMI rats exhibited a significant decrease in left ventricular ejection fraction, and an increase in left ventricular volume and diameter, both of which reached maximum at 24 weeks ($P < 0.001$).

In infarcted myocardium (Fig. 1A–D), SGLT2 expression was significantly higher than in the sham group, with peaks of expression at 2 and 24 weeks post-AMI (Fig. 1A). GLUT2 expression was similar to that observed for SGLT2 (Fig. 1B). In contrast, GLUT4 expression was significantly lower after the first week (Fig. 1 C). SGLT1 expression was significantly higher at 2 weeks post-AMI compared with the sham group (Fig. 1D).

In non-infarcted myocardium (Fig. 1E–H), an early increase in SGLT1 expression was followed by maximum expression at week 12 (Fig. 1H), but with no significant differences regarding the rest of the SGLT and GLUT expression levels (Fig. 1E–G). There was a positive correlation between SGLT2 and GLUT2 expression ($r = 0.827$, $P < 0.001$), whereas GLUT4 was negatively correlated with SGLT2 expression ($r = 0.352$, $P = 0.04$) and GLUT2 expression ($r = 0.374$, $P = 0.027$). A weak but significant correlation was observed between SGLT2 and GLUT2 receptors in infarcted myocardium and in inflammation markers ($P < 0.05$ and $r < 0.25$ for IL-6, TNF-α and MCP-1), but no significant correlation was seen with fibrosis markers.

Discussion

These data clearly point to an increase in SGLT2 expression in infarcted myocardium, which has never been previously described in the literature. Also, GLUT2 expression appears to be a possible adaptive mechanism, as it was seen to behave in a similar way to SGLT2. In addition, this was the first-ever study to identify the early and sustained increase in SGLT2 and GLUT2 transporters in response to persistent myocardial ischaemia in an AMI model.

Previously in a similar model, Rosenblatt-Velin et al. [8] found an early increase in GLUT1 expression in the peri-infarct zone of myocardium. Banerjee et al. [7] showed an increase in mRNA SGLT1 expression at 30 days post-AMI, but did not differentiate between infarcted and non-infarcted myocardium. However, in their study, SGLT1 expression was observed in biopsies taken from patients with chronic ischaemic cardiomyopathy. In our present model, an increase in SGLT1 was also observed that mainly affected non-infarcted myocardium. In contrast, in infarcted myocardium, the main increase in glucose transporters was of SGLT2 and GLUT2. This parallel increase in the two transporters might be explained by the glucose absorption mechanism that, similar to what is found in the kidneys, requires the presence of both receptors [2]. The inverse relationship observed with GLUT4 might be explained as a shift towards SGLT2 and GLUT2 transporters, which are more efficient in hypoxic situations [10]. A correlation with inflammatory markers was also observed, but the limitations of our study design meant it was not possible to elucidate whether there is any causal or direct relationship between the two responses to AMI.

Recently, the use of SGLT2 inhibitors has provided relevant clinical benefits by reducing mortality and cardiovascular complications [6]. Our present study shows an increase in SGLT2 and GLUT2 transporters in response to AMI, which could represent a protective mechanism for maintenance of glucose homoeostasis in persistently hypoxic myocardium. In this context, and albeit speculative, SGLT2 inhibition might not be beneficial. In fact, in a similar scenario to persistent peripheral ischaemia, the Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes (CANVAS) Study recently found an increase in the number of ischaemic amputations in patients taking an SGLT2 inhibitor [11].

Nevertheless, the applicability of our present findings is limited by the animal model used, as such regulation may differ in humans, and the fact that our study was restricted to the setting of acute myocardial ischaemia with myocardial necrosis. Indeed, no SGLT2 expression was observed in the myocardial biopsies taken from chronic ischaemic cardiomyopathy patients [12], although the study included patients with acute ischaemia as AMI patients. The detection of protein levels would have strengthened the findings.

In conclusion, the present study has characterized the temporal changes that take place in the myocardial expression of glucose transporters in response to AMI. In this context, GLUT2 and SGLT2 expression was significantly increased in infarcted myocardium, which could have implications considering their inhibition in clinical scenarios. Further studies are now needed to assess the effect of SGLT2 inhibitors on myocardial remodeling processes in the setting of AMI.

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Disclosure of interest

The authors declare that they have no competing interest.

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J. Sánchez-Más[a,1], E. Saura-Guillén[b,1], M.C. Asensio-López[c,1], Á. Soriano-Filiu[c,1], M. Carmen Sánchez-Pérez[c,1] A.M. Hernandez-Martinez[b,1], A. Lax[c,*,1], D. Pascual-Figal[c,d,e,**,1]

[a]*Departamento de Ciencias Biomédicas, Facultad de Ciencias de la Salud, Universidad CEU Cardenal Herrera, Moncada, Valencia, Spain*
[b]*Servicio de Endocrinología, Hospital Universitario Virgen de la Arrixaca, Murcia, Spain*
[c]*IMIB-Arrixaca, Facultad de Medicina, Universidad de Murcia, Muricia, Spain*
[d]*Servicio de Cardiología, Hospital Universitario Virgen de la Arrixaca, Murcia, Spain*
[e]*CIBER-CV, Instituto de Salud Carlos III, Madrid, Spain*

*Corresponding author. Biomedical Research Institute Virgen de la Arrixaca (IMIB-Arrixaca), University of Murcia, Ctra. Madrid-Cartagena s/n, 30120 Murcia, Spain. Tel.: +34-868885016; fax: +34-968369662
**Co-corresponding author. Cardiology Department, University of Murcia, LAIB, room 2.52, Av. Buenavista s/n, 30120 Murcia, Spain. Tel.: +34-868888136; fax: +34-968369662
E-mail addresses: alax@um.es (A. Lax Perez), dpascual@um.es (D. Pascual-Figal).

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Gender differences in cardiovascular risk profiles and diabetes care among adults with type 2 diabetes in Germany

The cardioprotective effects of female *vs.* male gender are lost or even reversed in the presence of diabetes mellitus [1]. Yet, investigating gender differences in cardiovascular risk profiles and indicators of diabetes care among adults with diabetes can help to clarify such differences in diabetes-related complications [2]. A study using data from general-practice clinics across southwest Germany found that women with type 2 diabetes (T2D) were less likely to receive medications for treatment of coronary heart disease (CHD) than men with T2D in the presence of CHD [3]. However, data from large population-based studies are scarce, particularly in Germany [4]. Thus, the present report on gender differences in cardiovascular risk profiles and diabetes care is based on a nationwide representative sample of adults with T2D in Germany, using data from the most recent German National Health Interview and Examination Survey (DEGS1 2008–2011).

Of the 7115 DEGS1 participants aged 18–79 years, a total of 591 (309 men, 282 women) had diabetes as defined by a history of physician-diagnosed diabetes or current use of antidiabetic drugs [5]. After excluding those with type 1 diabetes ($n = 8$) and women with gestational diabetes ($n = 42$) as described previously elsewhere [5], as well as patients with T2D aged < 40 years ($n = 15$), the present study was finally based on data from 526 T2D patients (302 men, 224 women) aged 40–79 years (Table 1). As previously described in detail [6], data collection comprised:

- sociodemographic factors and health-related behaviours using self-administered questionnaires;
- personal history of physician-diagnosed diabetes, cardiovascular disease (CVD), hypertension and hyperlipidaemia, age at diagnosis of diabetes, diabetes-specific complications, diabetes-related self-management and diabetes care indicators based on computer-assisted personal interviews;
- detailed review of all medications used within the past 7 days;
- and highly standardized anthropometric, blood pressure (BP) and laboratory measures, including glycated haemoglobin A_{1c} (HbA_{1c}), serum total cholesterol and high-density lipoprotein (HDL) cholesterol, serum creatinine and semiquantitative measures of albuminuria.

DEGS1 was approved by the Federal and State Commissioners for Data Protection and the Charité-Universitätsmedizin Berlin ethics committee (No. EA2/047/08). Survey participants provided their written informed consent prior to the interviews and examinations.

Gender-specific prevalence estimates and 95% confidence intervals (CIs) were calculated for cardiovascular risk variables and indicators of diabetes care as per the current clinical guideline recommendations for primary and secondary prevention of CVD in patients with diabetes [7]. Absolute differences in prevalence estimates among women compared with men were also calculated and adjusted for current age, diabetes duration (the difference between age at diagnosis and current age) and potential confounders, including region of residence, community size, living alone and educational attainment (classified as primary, middle and high), according to the international Comparative Analysis of Social Mobility in Industrial Nations (CASMIN) [6]. Stata SE 14 software (StataCorp LLC, College Station, TX, USA) was used for all statistical analyses.

Among the eligible adults diagnosed with T2D, men and women did not differ significantly in mean age (65.1 years *vs.* 66.6 years), age at diabetes diagnosis (54.7 years *vs.* 54.3 years), diabetes duration (10.2 years *vs.* 12.1 years), prevalence of physician-diagnosed hypertension (76.8% *vs.* 83.0%) or hyperlipidaemia (58.0% *vs.* 55.2%), or region of residence or community size (data not shown). Compared with men, women with T2D were less likely to be highly educated (4.6% *vs.* 13.2%) and more likely to be living alone (30.0% *vs.* 16.2%, respectively).

As for cardiovascular risk profiles, women with T2D had a higher prevalence of elevated non-HDL cholesterol and central obesity, but a lower CVD prevalence compared with men (Table 1). In both genders, $> 90\%$ of adults with T2D had at least one classic CVD risk factor. Women performed better than men in some indicators related to self-management (glucose self-monitoring, eye examination within the past 12 months). Most notably, among insulin users, nearly all men and women reported self-monitoring of blood glucose (data not shown). Moreover, women with T2D overall were significantly and consistently less likely than men to use guideline-recommended medications for CVD prevention [statins, antithrombotic medications ($> 90\%$ aspirin)], although these differences were less pronounced and not statistically significant among those diagnosed with CVD. Women were also significantly less likely to use first-line antihypertensive medications such as angiotensin-converting enzyme inhibitors (ACEIs)/