Boosting the pentose phosphate pathway restores cardiac progenitor cell availability in diabetes

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Aims
Diabetes impinges upon mechanisms of cardiovascular repair. However, the biochemical adaptation of cardiac stem cells to sustained hyperglycaemia remains largely unknown. Here, we investigate the molecular targets of high glucose-induced damage in cardiac progenitor cells (CPCs) from murine and human hearts and attempt safeguarding CPC viability and function through reactivation of the pentose phosphate pathway.

Methods and results
Type-1 diabetes was induced by streptozotocin. CPC abundance was determined by flow cytometry. Proliferating CPCs were identified in situ by immunostaining for the proliferation marker Ki67. Diabetic hearts showed marked reduction in CPC abundance and proliferation when compared with controls. Moreover, Sca-1pos CPCs isolated from hearts of diabetic mice displayed reduced activity of key enzymes of the pentose phosphate pathway, glucose-6-phosphate dehydrogenase (G6PD), and transketolase, increased levels of superoxide and advanced glucose end-products (AGE), and inhibition of the Akt/Pim-1/Bcl-2 signalling pathway. Similarly, culture of murine CPCs or human CD105pos progenitor cells in high glucose inhibits the pentose phosphate and pro-survival signalling pathways, leading to the activation of apoptosis. In vivo and in vitro supplementation with benfotiamine reactivates the pentose phosphate pathway and rescues CPC availability and function. This benefit is abrogated by either G6PD silencing by small interfering RNA (siRNA) or Akt inhibition by dominant-negative Akt.

Conclusion
We provide new evidence of the negative impact of diabetes and high glucose on mechanisms controlling CPC redox state and survival. Boosting the pentose phosphate pathway might represent a novel mechanistic target for protection of CPC integrity.

Keywords
Diabetes • Cardiac progenitor cells • Oxidative stress • Glucose

1. Introduction
Diabetes mellitus (DM) is a major risk factor for coronary artery disease and heart failure. Furthermore, growing evidence indicates that non-ischaemic diabetic cardiomyopathy has a relevant clinical impact and requires specific therapy.1–3 One distinct feature of diabetic cardiomyopathy consists of the progressive loss of cardiac cells by apoptosis and necrosis, due to the activation of oxidative stress.5–8 DM also impinges upon mechanisms of cardiovascular repair. However, the biochemical adaptation of cardiac stem cells to sustained hyperglycaemia remains largely unknown. Here, we investigate the molecular targets of high glucose-induced damage in cardiac progenitor cells (CPCs) from murine and human hearts and attempt safeguarding CPC viability and function through reactivation of the pentose phosphate pathway.

6–9 The pentose phosphate pathway contributes to the maintenance of cardiomyocyte redox state and contractility, by generating the reducing pyridine nucleotide NADPH and acting as an anabolic alternative of glycolysis.11 We recently showed that the activity of glucose-6-phosphate dehydrogenase (G6PD) and transketolase, two pivotal enzymes of the pentose phosphate pathway, is reduced in hearts of diabetic mice, leading to the accumulation of advanced glycation end-products (AGE) and O-N-acetylglucosamine modification and inactivation of Akt and consequent inhibition of the pro-survival Pim-1/Bcl-2

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signalling pathway. Inhibition of transketolase activity in DM was ascribed to deficit of its coenzyme thiamine. Of note, supplementation with benfotiamine (BFT), a lipid-soluble analogue of thiamine, reduces oxidative stress and AGE accumulation, protects cardiomyocytes from apoptosis and attenuates the development of cardiomyopathy and post-ischaemic heart failure in diabetic murine models. 

The biochemistry of CPCs remains largely unknown. Seminal studies showed the dependency of stem cells on anaerobic glycolysis for basal energetic requirements and on the pentose phosphate pathway for protection from oxidative damage. In line, G6PD gene deletion increases the vulnerability of embryonic stem cells to oxidative stress resulting in increased cell death, while decreased transketolase activity inhibits the proliferation and the migration of neuronal progenitor cells. We hypothesize that the pentose phosphate pathway is crucial for the maintenance of CPC redox state and that its inhibition contributes in CPC depletion during DM. We used Sca-1+ CPCs from murine hearts and CD105+ progenitor cells from atrial appendages of patients undergoing on-pump bypass cardiac surgery to test these hypotheses. Results show for the first time that DM inhibits crucial steps of the pentose phosphate pathway in CPCs, resulting in the accumulation of superoxide and suppression of the pro-survival signalling pathway. Importantly, the restoration of G6PD and transketolase activity by BFT supplementation safeguards viability and function of progenitor cells from murine and human hearts. G6PD silencing abrogates BFT-induced benefit, thereby confirming that the pentose phosphate pathway is a promising target for interventions aimed at preserving the integrity of resident CPCs.

2. Methods

2.1 Ethics

Investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and with the approval of the British Home Office and the University of Bristol. Studies on human CPCs complied with the ethical principles stated in the ‘Declaration of Helsinki’. Informed written consent was obtained from each patient and the protocol was approved by the University of Bristol Hospitals Trust (UBHT).

2.2 In vivo studies

Type-1 DM was induced in male CD1 mice (Charles River, UK) by the injection of streptozotocin (STZ; 40 mg/kg body weight ip per day for 5 days). Age-matched animals that received STZ-vehicle served as non-diabetic controls.

To rescue the deficit of the pentose phosphate pathway, mice were randomly assigned to receive BFT (70 mg/kg body weight/day) or vehicle (1 mM/L HCl) in drinking water from 4 weeks after DM induction. This dose of BFT reportedly produces a four-fold increase in plasma thiamine. Non-diabetic age-matched control mice entered the same treatment protocol.

2.3 Flow cytometry quantification of murine CPC

Hearts were obtained from diabetic (20 weeks from DM induction) and age-matched non-diabetic mice (n = 4 per group) sacrificed by cervical dislocation. After repeated washing to remove the circulating blood cells, a ‘myocyte-depleted’ cardiac cell population was prepared by incubating minced left ventricle (LV) myocardium in a mixture containing 0.02% collagenase II and 0.02% collagenase IV (both from Worthington), which is lethal to adult mouse cardiomyocytes. Cells were then filtered through a 40-μm mesh and used for flow cytometry characterization and quantification, as described earlier. Briefly, the single cell suspension was incubated for 30 min in the dark at 4°C using a combination of phycoerythrin-conjugated anti-mouse stem cell antigen (Sca-1) and allophycocyanin-conjugated anti-lineage markers (both from BD Bioscience Pharmigen). Propidium iodide (PI) staining was used to identify non-viable cells. Unstained samples and single stained samples were used as controls.

2.4 In situ assessment of cardiac cell proliferation and viability

Cardiomyocyte and CPC proliferation was assessed by immunohistochemistry on 5 μm-thick paraffin embedded sections after deparaffinization and antigen retrieval (n = 5 controls, n = 6 vehicle-treated diabetic and n = 5 BFT-treated diabetic mice). Proliferating cells were identified by the presence of the nuclear marker Ki67. Alfa-sarcomeric actin (ASA) identified cardiac precursor cells and myocytes. Staining for the cell surface marker c-Kit was used to recognize CPCs and the tryptase marker to exclude c-Kit+ mastocytes from the analysis. Results obtained in mice showed that the majority of cardiac c-Kit+ cells coexpress either Sca-1-like or Sca-1 antigen. Likewise, a large fraction of Sca-1+ cells coexpress c-Kit antigen. Viability of the c-Kit+ cells was recognized with survivin, an inhibitor of apoptosis, that regulates both apoptosis and cell cycle. Recent studies showed decreased survivin expression in diabetic endothelial cells. The frequency of proliferating CPCs in the heart is expressed as the number of ki-67+ CPCs per 100 CPCs (recognized as c-Kit+ Tryptase- cells) and the viable CPCs were expressed as the number of c-Kit+survivin+ cells per cm2. Total of three sections were scored from the middle portion of the each mouse heart. Last, sections were stained with 4′,6-diamidino-2-phenylindole to recognize the nuclei.

Alexa Fluor and DyLight fluorochromes conjugated secondary antibodies were used to detect primary antibodies (see Supplementary material online, Table S1). Images were taken using a Leica TCS-SP2 confocal laser microscope (×63 oil immersion objective—numerical aperture: 1.40).

2.5 CPC expansion for functional and molecular characterization

Functional and molecular analyses were performed on primary cultures of murine and human CPCs. Hearts were collected from mice (diabetic and age-matched controls) after cervical dislocation, minced, pooled, and enzymatically digested as described above. CPCs were then enriched by differential centrifugation using a CPC isolation kit (Millipore). Purity was confirmed by flow cytometry analysis of Sca-1. The extraction yielded 1 × 105 Sca-1+ cells from a pool of five hearts. CPCs were selected for Sca-1 based on earlier publications demonstrating the potential of Sca-1 positive cells to differentiate into cardiomyogenic lineages. Moreover, recent studies demonstrated increased cardiac dysfunction and poor adaptation to stress in Sca-1 knockout mice, while CPCs knocked out for Sca-1 showed retarded ex vivo expansion.

Progenitor cells were also isolated from human hearts (n = 12). Briefly, atrial appendages were obtained from patients undergoing on-pump cardiac surgery for correction of aortic valve stenosis. A small piece of the right atrium appendage, which is routinely amputated and discarded during the insertion of the two stage venous cannula, prior to establishing cardiopulmonary bypass, was collected in ice-cold isolation buffer. The tissue was minced and digested with collagenase and CPCs were then enriched by gradient separation using the Millipore kit. Flow cytometry showed that the isolated cells highly express the mesenchymal stem cell markers CD105 and CD90. This antigenic profile reportedly identifies a subpopulation of cardiac progenitors that can be expanded in culture to...
generate millions of cells endowed of cardiomyogenic and vasculogenic capacities.27–30
To confirm the capacity of murine and human CPCs to give rise to mature cardiomyocytes, the isolated cells were exposed to differentiation medium (Millipore) for 1 week, after which they were stained for cardiac specific markers such as ASA, connexin-43, and GATA-4.

2.6 Functional assays on CPCs from murine diabetic hearts
CPCs from hearts of diabetic mice, given BFT or vehicle, were cultured for 1 week in stem cell maintenance medium (Millipore), containing 5 mM glucose. Then, cells were used in the 5-bromo-2-deoxyuridine (BrdU) proliferation assay (Roche, in triplicates) and the caspase-3/7 activity assay (Caspase-Glo® 3/7, Promega, six wells per each condition). Moreover, samples were processed for the measurement of G6PD and transketolase activity and western blotting analyses (n = 4 per group).

2.7 Pro-survival effect of BFT on CPCs exposed to high glucose in vitro
To simulate the diabetic condition in vitro, non-diabetic murine or human CPCs isolated according to the methodology described above were cultured in the presence of high d-glucose (HG, 30 mM) or normal glucose (5 mM) added with 25 mM of α-mannitol for osmotic control. After 72 h, cells were supplemented with either BFT (150 μM) or vehicle (1 mM HCl) for further 24 h. Optimal in vitro concentration of BFT was decided on the basis of our earlier studies on cultured adult cardiomyocytes.6 At the end of experiments, samples were collected for the caspase-3/7 activity assay (six wells per each condition) and western blot analyses (n = 4 samples per group).
To study the effect of BFT on proliferation, cells (5 × 10^3/well) were seeded in a 96-well plate and cultured for 24 h in HG. The medium was then supplemented with BFT or vehicle plus BrdU (10 μM). BrdU incorporation was measured 24 h later. Each experiment was performed in triplicate and repeated three times.

2.8 Knockdown experiments
CPCs were exposed to HG or α-mannitol for 24 h. Then, they were transfected with small interfering RNA (siRNA-G6PD, Dharmacon Technologies) to silence G6PD or the relevant scrambled siRNA (20 pM/μL) as a control, using lipofectamine 2000 (Invitrogen) and cultured in the same conditions for further 48 h. Effective silencing was confirmed by assessing G6PD activity in separate experiments. Akt activity was silenced using a dominant negative mutated form of Akt (Ad.DN-Akt) and the empty vector (Ad.Null) was used as a control (both at 100 MOI). Transfected cells were exposed to BFT for 48 h and used for functional studies and western blotting analyses (n = 4 biologic replicates per each group).

2.9 Western blot analyses
Proteins were extracted from murine or human cells using the standard technique. Following separation of whole cell extracts (50 μg) on SDS–polyacrylamide gels, proteins were transferred to polyvinylidene difluoride membranes (Amersham-Pharmacia) and probed with specific antibodies (see Supplementary material online).

2.10 Immunocytochemistry
Cells were plated on an eight-chamber multi-well slide and underwent different treatment protocols. Then, they were fixed with freshly prepared 4% PFA, followed by probing the cells with specific antibodies (see Supplementary material online, ). Images were taken using the fluorescence microscope (Olympus).

2.11 In situ detection of reactive oxygen species
Superoxide production in cultured cells was determined using the fluorescent dye dihydroethidium (DHE, Invitrogen, Molecular probes). Cells were incubated with 5 μM/L DHE, at 37 °C for 30 min. Images (× 100 magnification) were captured with a fluorescence camera (Olympus), using an imaging software (Media Cybernetics) and the mean DHE fluorescence intensity of nuclei was calculated by dividing the combined fluorescence value of the pixels by the total number of pixels in 15 randomly selected field using Image-Pro advanced software.31

2.12 Statistical analysis
All results are represented as mean ± SEM. Differences between multiple groups were compared by the analysis of variance (ANOVA) followed by a Holm–Sidak multiple comparison test. Two-group analysis was performed by the t-test (paired or unpaired as appropriate).

3. Results
3.1 Reduction of resident CPCs in diabetic hearts
Flow cytometry analysis of single-cell extracts was used to compare the abundance of Sca-1pos CPCs in hearts of diabetic and non-diabetic mice (Figure 1A). Staining for CD45 and PI was used to exclude hematopoietic cells and non-viable cells from the analysis. Results indicate reduction in PInegCD45negSca-1pos cells in hearts of diabetic mice (12 ± 2 vs. 23 ± 4% of the myocyte-free single-cell digest in nondiabetic controls, P < 0.05, Figure 1B). Of note, BFT supplementation prevented the reductive effect of DM on CPC abundance (26 ± 5%, P < 0.05 vs. vehicle and NS vs. non-diabetic controls, Figure 1B). A mild increase in CPC number was observed in the hearts of non-diabetic mice given BFT, although this change did not reach statistical significance (P = 0.06 vs. vehicle).

We next analysed the number of proliferating cells by immunohistochemistry (Figure 1C and D). DM reduced the number of Ki67pos cardiomyocytes (72 ± 9 vs. 136 ± 8 per 10^5 nuclei in controls, P < 0.01) and Ki67poscKitpos CPCs (9 ± 1 vs. 29 ± 3 per 10^5 nuclei in controls, P < 0.01). The deficit in CPC number and proliferative activity was attenuated by BFT (104 ± 10 per 10^5 nuclei and 24 ± 6 per 10^5 nuclei, respectively, P < 0.05 vs. vehicle for both comparisons). DM also reduced the number of cKitpossurvivinpos cells (3.4 ± 1.5 vs. 7.6 ± 2.4 cells/cm^2 in a control, P < 0.01), suggesting that diabetic CPCs are more prone to apoptotic cell death (Figure 1E). As expected, BFT prevented this deficit (14.5 ± 3.4 cells/cm^2, P < 0.01 vs. vehicle).

3.2 Diabetes-induced CPC dysfunction
We next compared biochemical and functional features of diabetic and non-diabetic CPCs. The isolation procedure yielded a highly enriched population of Sca-1pos cells (see Supplementary material online, Figure S1). When grown in stem cell maintenance medium, CPCs conserved their stem cell characteristics even after three passages (see Supplementary material online, Figure S2). Conversely, when exposed to differentiation medium, they acquired typical cardiomyocyte antigens, such as ASA, connexin 43, and GATA-4 (Figure 2A, upper panel). Importantly, diabetic CPCs showed impaired differentiation when exposed to differentiation medium (Figure 2A, lower panel and see Supplementary material online, Figure S3).
Figure 1 Diabetes reduces the abundance of CPCs. (A) Gating procedure for the identification of Pl<sup>neg</sup>CD45<sup>neg</sup>Sca-1<sup>pos</sup> CPCs. CD45<sup>neg</sup> cells were gated from total population extracted from the left ventricle and then the percentage of Sca-1<sup>pos</sup> cell population was analysed. (B) Bar graph shows the percentage of Sca-1<sup>pos</sup> cells in the CD45<sup>neg</sup> fraction. NDV, non-diabetic mice treated with vehicle; NDB, non-diabetic mice treated with BFT; DV, diabetic mice treated with vehicle; DB, diabetic mice treated with BFT. Values are mean ± SEM. *P, 0.05 vs. NDV and #P, 0.05 vs. DV. Each group consisted of four mice. (C and D) Confocal microscopy images and bar graphs showing α-sarcomeric actin positive (ASA) myocytes (C) or c-Kit positive cells (D) expressing Ki67. Scale bars: 20 μm. (E) Confocal microscopy image and bar graphs showing c-Kit positive cells expressing survivin. Scale bars: 25 μm. Values are mean ± SEM. *P < 0.01 vs. NDV and #P < 0.01 vs. DV. Each group consists of five mice.
Sca-1\textsuperscript{pos} CPCs from hearts of diabetic mice showed reduced transketolase (Figure 2B) and G6PD activity (Figure 2C) (\(P < 0.01\) vs. non-diabetic for both comparisons) and increased AGE levels (Figure 2D) (\(P < 0.001\) vs. non-diabetic). Inhibition of the pentose phosphate pathway might increase CPC vulnerability to oxidative stress. In line, diabetic CPCs showed high superoxide levels (see Supplementary material online, Figure S4, \(P < 0.01\) vs. non-diabetic), activation of effector caspase 3/7 (Figure 2E, \(P < 0.01\)), and reduced proliferation (Figure 2F, \(P < 0.001\)). In line with functional tests, western blot analysis showed marked down-regulation of the pro-survival signalling pathway encompassing Akt (Figure 2G), Pim-1 (Figure 2H), and Bcl-2 (Figure 2I) in diabetic CPCs (\(P < 0.01\) vs. non-diabetic for all comparisons).

In vivo treatment of diabetic mice with BFT restored transketolase and G6PD activity, reduced AGE and superoxide levels and corrected the functional and expressional deficits of CPCs (Figure 2B–I and see Supplementary material online, Figure S3). Moreover, BFT treatment resulted in the activation of the pentose phosphate pathway and an increased proliferation in CPCs of non-diabetic controls.
3.3 Rescue of HG-challenged CPCs by BFT

To verify the role of glucotoxicity in DM-induced CPC damage, we simulated the diabetic environment in vitro by exposing CPCs isolated from hearts of non-diabetic mice to HG. As expected, HG drastically increased caspase activity (Figure 3A) and inhibited the Akt/Pim-1/Bcl-2 pathway (Figure 3B–E) ($P < 0.01$ vs. NG for all comparisons). HG also caused a 33% reduction in CPC proliferation (Figure 3F) ($P < 0.001$ vs. NG). Of note, in vitro supplementation with BFT restored proliferation and pro-survival signalling and suppressed HG-induced activation of caspase-3/7 (Figure 3A–F) ($P < 0.01$ vs. HG).

To strengthen the translational implications of our study, we performed similar experiments in progenitor cells isolated from human hearts. The isolation procedure yielded a highly purified population of CD90<sup>pos</sup>CD105<sup>pos</sup> cells (see Supplementary material online, Figure S5A). Human CPCs maintain high levels of the two antigens after three passages (see Supplementary material online, Figure S5B), but acquire typical cardiomyocyte antigens when cultured in differentiation medium (Figure 4A, upper panel), which was impaired in cells exposed to HG (Figure 4B, lower panel and see Supplementary material online, Figure S5A). Human CPCs maintain high levels of the two antigens after three passages (see Supplementary material online, Figure S5B), but acquire typical cardiomyocyte antigens when cultured in differentiation medium (Figure 4A, upper panel), which was impaired in cells exposed to HG (Figure 4B, lower panel and see Supplementary material online, Figure S5A).

3.4 Silencing of G6PD abrogates the benefit of BFT on CPCs

To verify the molecular mechanism underlying BFT-induced effects, additional studies were performed on G6PD knocked-down CPCs. Silencing approach resulted in remarkable reduction in G6PD activity (Figure 5A and H), leading to attenuation of BFT-induced effects on the survival and the proliferation of both murine (Figure 5B–G) and human CPCs (Figure 5I–N) ($P < 0.01$ vs. scrambled). Similarly, Akt inhibition by dominant-negative Akt resulted in abrogation of BFT-induced improvement of CPC survival and proliferation and Pim-1/Bcl-2 activation (Figure 6) ($P < 0.01$ vs. Ad.Null).

4. Discussion

This study provides novel mechanistic insights into the detrimental effect of DM on CPC viability and function. We confirm that CPCs are reduced in diabetic hearts and manifest defective proliferative capacity. Furthermore, CPCs isolated from diabetic hearts or de novo exposed to HG show reduced activity of two key enzymes of the pentose phosphate pathway, G6PD, and transketolase. Failure to dispose reactive oxidative species and AGE through the pentose phosphate pathway results in CPC loss by apoptosis. Boosting the pentose phosphate pathway by BFT prevents CPC deficits, thus...
Figure 4  High glucose induces functional alterations in human CPCs. (A) Immunocytochemistry microphotographs of cardiomyocytes derived from CD90<sup>pos</sup>CD105<sup>pos</sup> cells following culture in differentiation medium. Scale bars: 50 μm. (B–D) Transketolase activity (B), G6PD activity (C), and AGE levels (D) in human CPCs exposed to HG. (E–J) Bar graphs showing the levels of activated caspase-3/7 (E), pAkt (F), Pim-1 (G), Bcl-2 (H) and pBad (I), and proliferation (J) of CPCs from human hearts. Symbols are the same as in Figure 3. Each experiment was repeated three times in triplicate.
Figure 5: Silencing of G6PD abrogates the benefit of BFT. (A–N) Bar graphs showing G6PD activity (A and H), pAkt (B and I), Pim-1 (C and J), pBad (D and K), Bcl-2 (E and L), activated caspase-3/7 levels (F and M), and BrdU incorporation (G and N) in cultured murine (A–G) and human CPCs (H–N) after G6PD silencing. CPCs cultured in normal (NG) or high glucose (HG) was transfected with siRNA-G6PD or scrambled siRNA and then exposed to BFT or vehicle. Values are mean ± SEM. *P < 0.01 and **P < 0.001 vs. NG within siRNA-G6PD or scrambled siRNA groups; #P < 0.01 and ##P < 0.001 vs. HG within siRNA-G6PD or scrambled siRNA groups; $P < 0.01 vs. the corresponding treatment of the scrambled siRNA group. Each experiment was repeated four times in triplicate except caspase 3/7 activity, which was performed in six wells per each condition and repeated three times.
Figure 6 Silencing of Akt abrogates the benefit of BFT. (A–L) Bar graphs show the levels of pAkt (A and G), Pim-1 (B and H), pBad (C and I), Bcl-2 (D and J), activated caspase-3/7 (E and K), and BrdU incorporation (F and L) in murine (A–F) and human (G–L) CPCs. CPCs cultured in high glucose (HG) or normal glucose (NG) were infected with Ad.DN-Akt or Ad.Null followed by another 24 h culture in the presence of BFT or vehicle. Each experiment was performed in triplicate and repeated three times. For caspase-3/7 activity, assay was performed in six wells per each condition and repeated three times. *P < 0.01 and **P < 0.001 vs. NG within Ad.DN-Akt or Ad.Null groups; #P < 0.01 and ##P < 0.001 vs. HG within Ad.DN-Akt or Ad.Null groups. $P < 0.01 vs. corresponding treatment of the Ad.Null group.
sugesting that this mechanism could represent a therapeutic target to restore endogenous regenerative potential in diabetic cardiac disease.

The shift of cardiomyocyte biochemistry in DM has been the focus of intense research (reviewed in Boudina and Abel1). Under normal conditions, the heart is capable of utilizing different classes of substrates, such as fatty acids and carbohydrates, for energy production. This flexibility in substrate utilization is lost in DM. Moreover, decreased adenosine triphosphate production, mitochondrial uncoupling, overproduction of reactive oxygen species, and accumulation of lipid and glucose intermediates altogether impair cardiomyocyte survival and LV function. The bioenergetics of cardiac stem cells remains, however, largely unknown, especially in terms of adaptation to stress conditions, such as chronic hyperglycaemia. Here, we newly show that DM remarkably reduces the activity of key enzymes of the pentose phosphate pathway in CPCs, resulting in decreased anti-oxidant defence mechanisms, accumulation of glucose intermediates, and activation of apoptosis. These effects are seemingly due to a direct action of hyperglycaemia, since in vitro exposure of non-diabetic human or murine CPCs to HG results in similar biochemical and functional deficits. Of note, DM-induced inhibition of the pentose phosphate pathway was documented after culture of CPCs in NG medium, in line with a concept of metabolic memory of prior glycaemic exposure.32 The underlying epigenetic mechanism remains to be elucidated.

Importantly, in vivo and in vitro supplementation with BFT prevents the DM-induced deficit in CPC viability. Since BFT also restores proper activity of transketolase and G6PD, we propose that boosting the pentose phosphate activity could be an effective means for CPC protection. However, thiamine pyrophosphate also acts as a cofactor for several enzymes that link glycolysis to the citric acid cycle, like pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase. To verify the mechanism behind BFT-induced protection, we silenced G6PD which is the first and rate-limiting enzyme of the pentose phosphate pathway. The results show that G6PD knock-down abrogates the effect of BFT on CPC survival and proliferation. This inhibitory effect was mimicked by interfering with pro-survival signalling through Akt silencing. Altogether, these data support a functional association of the pentose phosphate pathway and pro-survival signalling in CPCs.

In conclusion, our study provides novel information on mechanisms linking glucose metabolism and cardiac stem cell homoeostasis and highlights the important role of the pentose phosphate pathway in maintaining the proper redox state of CPCs. Furthermore, we show the feasibility of pampering these precious cells by supplements of vitamin B1. We have previously shown that BFT prevents the evolution of diabetic cardiomyopathy.5 Hence, BFT might represent a potential means for global protection of the diabetic heart. The present study might have important implications for stem cell therapy. Preliminary results from the SCIPIO and CADUCEUS cell therapy trials using c-Kit+ and CD105+ progenitor cells indicate safety and efficacy in patients with myocardial ischaemia.33,34 As the two trials are still ongoing, it is not possible to determine whether diabetic patients received the same benefit of non-diabetic peers. Ex vivo preconditioning with agents that restore cell functionality might prove utilitarian in CPC-based regenerative therapies in patients with diabetes.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

Conflicts of interest

none declared.

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