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Original Article

Activation of pyruvate dehydrogenase by dichloroacetate has the potential to induce epigenetic remodeling in the heart

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ABSTRACT

Dichloroacetate (DCA) promotes pyruvate entry into the Krebs cycle by inhibiting pyruvate dehydrogenase (PDH) kinase and thereby maintaining PDH in the active dephosphorylated state. DCA has recently gained attention as a potential metabolic-targeting therapy for heart failure but the molecular basis of the therapeutic effect of DCA in the heart remains a mystery. Once-daily oral administration of DCA alleviates pressure overload-induced left ventricular remodeling. We examined changes in the metabolic fate of pyruvate carbon (derived from glucose) entering the Krebs cycle by metabolic interventions of DCA. $^{13}C_6$-glucose pathway tracing analysis revealed that instead of being completely oxidized in the mitochondria for ATP production, DCA-mediated PDH dephosphorylation results in an increased acetyl-CoA pool both in control and pressure-overloaded hearts. DCA induces hyperacetylation of histone H3K9 and H4 in a dose-dependent manner in parallel to the dephosphorylation of PDH in cultured cardiomyocytes. DCA administration increases histone H3K9 acetylation in *in vivo* mouse heart. Interestingly, DCA-dependent histone acetylation was associated with an up-regulation of 2.3% of genes (545 out of 23,474 examined). Gene ontology analysis revealed that these genes are highly enriched in transcription-related categories. This evidence suggests that sustained activation of PDH by DCA results in an overproduction of acetyl-CoA, which exceeds oxidation in the Krebs cycle and results in histone acetylation. We propose that DCA-mediated PDH activation has the potential to induce epigenetic remodeling in the heart, which, at least in part, forms the molecular basis for the therapeutic effect of DCA in the heart.

1. Introduction

The viability and activity of cardiomyocytes and the maintenance of cardiac pump function are tightly coupled with intracellular metabolism, which responds dynamically to changes in the external or internal environment. Chronic hemodynamic stress causes a homeostatic change in the intracellular metabolism of the heart. Such a change could be involved in the progression of pathological cardiac remodeling and cardiac dysfunction. Therefore, metabolic modulation presents a new therapeutic approach in the treatment of heart failure.

Healthy hearts spare glucose and rely heavily on fatty acid oxidation for energy needs. The fate of fatty acids is largely complete oxidation in the Krebs cycle. By contrast, uptake and oxidation of glucose and lactate are strongly inhibited by the high rate of fatty acid oxidation. Only 20–25% of myocardial glucose uptake is oxidized at rest with approximately 13% released as lactate [1]. Glucose becomes the predominant metabolic fuel under conditions of stress. The rate of glycolysis is accelerated in pressure-overloaded hearts, but the rate of pyruvate oxidation does not rise according to the rate of pyruvate generation [2]. As a consequence, only 10–11% of glucose is oxidized in pressure-overloaded hearts. Hence, glucose uptake exceeds myocardial glucose oxidation both at rest and under pressure overload.

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The flux control of pyruvate entry into the Krebs cycle is mediated by pyruvate dehydrogenase (PDH). The activity of PDH is regulated by reversible phosphorylation and its end products, acetyl-CoA and $NADH_2$. The phosphorylation status of PDH is determined by the opposing actions of pyruvate dehydrogenase kinases and pyruvate dehydrogenase phosphatases.

Dichloroacetate (DCA) is a generic, orally available, small-molecule metabolic modulator that promotes the activity of PDH by inhibiting its repressor protein, pyruvate dehydrogenase kinase. Previous studies have demonstrated that short-term administration of DCA improves cardiac output and left ventricular (LV) mechanical efficiency under conditions of myocardial ischemia or failure [3]. Moreover, long-term use of DCA ameliorated LV hypertrophy and preserved contractile function in the TAC model [4,5], and prevented the transition from cardiac hypertrophy to heart failure in Dahl salt-sensitive rats fed a high-salt diet [6]. Therefore, DCA has gained attention recently as a potential metabolic-targeting therapy for heart failure. The potential known mechanism for DCA-induced cardioprotection is as follows. By increasing the provision of acetyl-CoA for mitochondrial utilization, DCA-mediated activation of the PDH complex overcomes the inertia in mitochondrial ATP production [7] and thus normalizes excess H^+ production arising from high lactate production and low glucose oxidation rates [8], reverses electrical remodeling due to restoration of ventricular Kv channel expression [9,10], and suppresses oxidative stress *via* activation of the pentose phosphate pathway and glutathione redox cycle [6]. However, knowledge remains limited regarding the changes in the metabolic fate of pyruvate carbon (derived from glucose) entering the Krebs cycle by metabolic intervention with DCA.

We have examined the direct effect of DCA on the intracellular metabolism in the normal working heart at rest and exposed to a pressure overload.

2. Methods

2.1. Mice

Twelve-week-old male C57BL6/J mice weighing an average of 22.5 g were used in this study. All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee at the Keio University School of Medicine and Gunma University Graduate School of Medicine. Mice were housed in a rodent facility under a 12:12 h light–dark cycle during this study. DCA (Sigma-Aldrich Co, St. Louis, Mo) was administered once daily by gavage.

2.2. Transverse aortic constriction (TAC)

The animal procedures were performed conforming to NIH guidelines. Mice were fully anesthetized with 1.0–1.5% isoflurane gas while being mechanically ventilated with a rodent respirator. The chest cavity was opened *via* left thoracotomy to expose the heart. Following identification of the transverse aorta, a silk suture was placed between the innominate and left carotid arteries, and a loose knot was formed. A 27-gauge needle was placed parallel to the transverse aorta, and the knot was quickly tied against the needle, followed by prompt removal of the needle to yield a constriction of 0.4 mm in diameter [11]. After TAC, the thorax was closed. In sham control mice, the entire procedure was identical except for ligation of the aorta. The adequacy of the anesthesia was monitored during TAC by testing of rear foot reflexes before any incision was made, and continual observation of responsiveness to manipulations throughout the procedure. Analgesia (Ketoprofen, 5 mg/kg, subcutaneous) was given before mice recovered from anesthesia (and 24 and 48 h later). Echocardiographic measurement with the Vevo 770 echocardiography system was taken by a blinded operator with mice placed on a heating pad under light anesthesia with 1.0–1.5% isoflurane gas adjusted to obtain a target heart rate of 500 ± 50 bpm. Mice were deeply anesthetized with isoflurane gas and killed by cervical dislocation and the hearts were removed.

2.3. Biodistribution of ^{125}I-BMIPP (15-(p-iodophenyl)-3-(R,S)-methyl pentadecanoic acid) and ^{18}F-FDG (2-fluorodeoxyglucose)

The biodistribution of ^{125}I-BMIPP and ^{18}F-FDG was determined as described previously [12,13]. Mice received intravenous injections of ^{125}I-BMIPP (5 kBq) and ^{18}F-FDG (100 kBq) *via* the lateral tail vein in a volume of 100 μl. ^{125}I-BMIPP was a gift from Nihon Medi-Physics Co. Ltd. and ^{18}F-FDG was obtained from batches prepared for clinical positron emission tomography (PET) imaging in Gunma University. The animals were sacrificed 2 h after injection. The relevant tissues were isolated and weighed before analysis in a well-type gamma counter (ARC-7001, ALOKA). PET was performed with a small-animal PET scanner (Inveon, Siemens) 2 h after the intravenous injection of 18 F-FDG (10 MBq).

2.4. Combined metabolomics-pathway tracing studies by CE-MS

The metabolome analyses were carried out as described previously [12–14]. Fully labeled $^{13}C_6$-glucose (glucose labeled with ^{13}C in all six positions) was administered at 1 mg/g body weight *via* intravenous injection. After 15 min, the heart was harvested and flash-frozen in liquid nitrogen.

Frozen heart tissue for metabolite extraction was plunged into methanol (1 ml) containing the internal standards (300 μM each of methionine sulfone for cations and 2-morpholinoethanesulfonic acid for anions) and homogenized (30 s × 3 times) using a Multi-Beads Shocker (Yasui Kikai, Osaka, Japan) to inactivate enzymes. Then, deionized water (500 μl) was added, and 600 μl of the solution was transferred to another tube, to which 400 μl of chloroform was added. The mixture was mixed thoroughly and centrifuged at 12,000 *g* for 15 min at 4 °C, and the 300-μl upper aqueous layer was centrifugally filtered through a Millipore 5-kDa cutoff filter to remove proteins. The filtrate was lyophilized and dissolved in 50 μl of Milli-Q water containing reference compounds (200 μM each of 3-aminopyrrolidine and trimesate) prior to capillary electrophoresis-mass spectrometry (CE-MS) analysis.

2.5. Metabolic standards

All chemical standards were obtained from common commercial sources and dissolved in Milli-Q (Millipore) water, 0.1 M HCL, or 0.1 M NaOH to obtain 10 mM or 100 mM stock solutions. Working standard mixtures were prepared by diluting stock solutions with Milli-Q water just prior to injection into the CE-MS. The chemicals used were of analytical or reagent grade.

2.6. Instrumentation

All CE-MS experiments were performed using an Agilent CE Capillary Electrophoresis System equipped with an air pressure pump, an Agilent 1100 series MSD mass spectrometer, and an Agilent 1100 series isocratic high-performance liquid chromatography pump, a G1603A Agilent CE-MS adapter kit, and a G1607A Agilent CE-MS sprayer kit (Agilent Technologies). System control, data acquisition, and MSD data evaluation were performed using the G2201AA Agilent Chem Station software for CE-MS.

2.7. CE-MS conditions for cationic metabolites

Separations were carried out in a fused silica capillary (50 μm inner diameter × 100 cm total length) filled with 1 M formic acid as the electrolyte. Approximately 3 nl of sample solution was injected at 50 mbar for 3 s, and a voltage of 30 kV was applied. Electrospray ionization MS (ESI-MS) was conducted in the positive ion mode, with the capillary

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