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[^{11}C]-Acetoacetate PET imaging: a potential early marker for cardiac heart failure

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ABSTRACT

The ketone body acetoacetate could be used as an alternate nutrient for the heart, and it also has the potential to improve cardiac function in an ischemic–reperfusion model or reduce the mitochondrial production of oxidative stress involved in cardiotoxicity. In this study, [^{11}C]-acetoacetate was investigated as an early marker of intracellular damage in heart failure.

Methods: A rat cardiotoxicity heart failure model was induced by doxorubicin, Dox(+). [^{14}C]-Acetoacetate, a non-positron ($\beta-$) emitting radiotracer, was used to characterize the arterial blood input function and myocardial mitochondrial uptake. Afterward, [^{11}C]-acetoacetate ($\beta+$) myocardial PET images were obtained for kinetic analysis and heart function assessment in control Dox(−) (n = 15) and treated Dox(+) (n = 6) rats. The uptake rate (K_1) and myocardial clearance rate (k_2 *or* k_{mono}) were extracted.

Results: [^{14}C]-Acetoacetate in the blood was increased in Dox(+), from 2 min post-injection until the last withdrawal point when the heart was harvested, as well as the uptake in the heart and myocardial mitochondria (unpaired *t*-test, $p<0.05$). PET kinetic analysis of [^{11}C]-acetoacetate showed that rate constants K_1, k_2 and k_{mono} were decreased in Dox(+) ($p<0.05$) combined with a reduction of 24% of the left ventricular ejection fraction ($p<0.001$).

Conclusion: Radioactive acetoacetate *ex vivo* analysis [^{14}C], and *in vivo* kinetic [^{11}C] studies provided evidence that [^{11}C]-acetoacetate can assess heart failure Dox(+). Contrary to myocardial flow reserve (rest–stress protocol), [^{11}C]-acetoacetate can be used to assess reduced kinetic rate constants without requirement of hyperemic stress response. The proposed [^{11}C]-acetoacetate cardiac radiotracer in the investigation of heart disease is novel and paves the way to a potential role for [^{11}C]-acetoacetate in cardiac pathophysiology.

1. Introduction

Heart failure is highly prevalent in the elderly population and leads to poor outcomes. If the heart is not able to respond to the blood supply demand of the body, alteration of its function will progress. Doxorubicin, a potent chemotherapy agent, causes cardiac toxicity leading to dose-dependent dilated cardiomyopathy and heart failure [1]. Reduction of oxidative phosphorylation through mitochondrial respiration is one of the major characteristics of heart failure [1].

The ketone body radiotracer [^{11}C]-acetoacetate is expected to have some similarity to [^{11}C]-acetate, a well-known myocardial positron emission tomography (PET) radiotracer [2,3], which can measure indices of myocardial blood flow (MBF) and oxidative metabolism (MVO_2), as well as reliably evaluate heart function [4–8]. For comparison, acetate is directly metabolized to acetyl-CoA by acetyl-CoA synthetase in mitochondria [9], by which it enters the Krebs cycle. Acetyl-CoA can return to the cytoplasm where it can be incorporated into sterol and fatty acid synthesis by the action of the ATP-citrate lyase (Fig. 1). Acetoacetate, the ketone body, crosses the myocardial membrane using the same monocarboxylic transporter [10] as acetate and is also a substrate of the Krebs cycle. The mitochondrial oxidation of acetoacetate as an energy substrate first requires its conversion into acetoacetyl-CoA by succynyl-CoA-3-ketoacid transferase [11] and then into acetyl-CoA by acetoacetyl-CoA thiolase [9]. Cytosolic acetoacetate has the potential to be directly incorporated into the lipogenesis pathway.

The goal of this study was to assess the pharmacokinetic properties of [^{11}C]-acetoacetate in heart failure Dox(+). The heart pathology was induced by doxorubicin, which adversely affects mitochondrial

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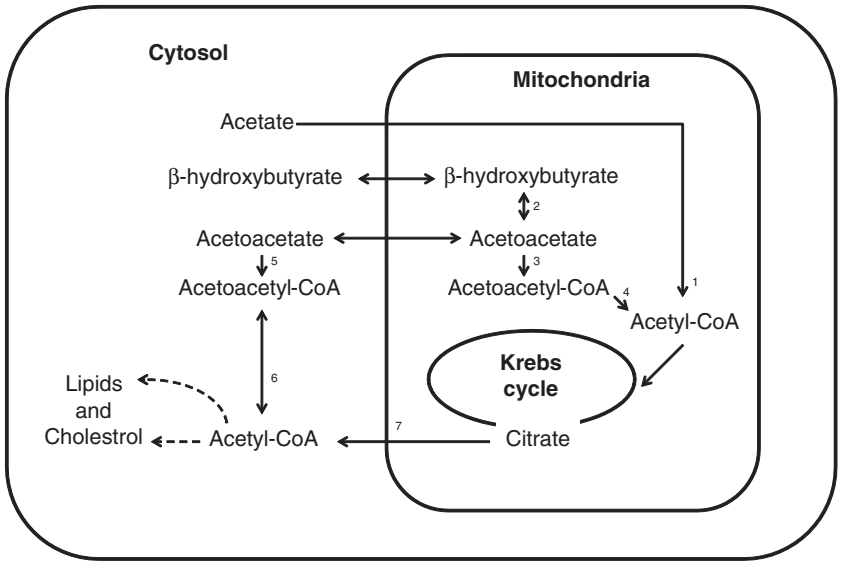

Fig. 1. Schematic representation of the metabolic pathways of acetate, beta-hydroxybutyrate and acetoacetate in the heart. Key to the enzymes involved: (1) acetyl-CoA synthetase; (2) Beta-hydroxybutyrate dehydrogenase; (3) succinyl-CoA 3-oxoacid CoA transferase; (4) acetoacetyl-CoA thiolase (mitochondrial); (5) acetoacetyl-CoA synthetase; (6) acetoacetyl-CoA thiolase (cytoplasmic); (7) ATP citrate lyase.

membrane integrity, stimulating apoptosis of the myocardial cells, and decreases heart function [12]. [^{14}C]-Acetoacetate was used to obtain supplemental information characterizing blood metabolites and myocardial cell localisation. Several consuming time steps are required to analyse blood metabolites or isolate the mitochondrial fraction, thus using the longer-lived [^{14}C] isotope [13] was helpful. Compartmental model analysis of myocardial uptake and clearance rates of [^{11}C]-acetoacetate was investigated. The uptake rate constant obtained from the [^{14}C]-acetoacetate blood information with a one tissue compartment model and the clearance rate constant from a monoexponential single fit were compared against the data from the 3-compartment kinetic model [14]. Further investigation of myocardial acetoacetate metabolism in the cardiotoxic heart failure model could be used to evaluate cardioprotective pathways or novel treatments.

2. Materials and methods

2.1. Animal experiments

Twelve male Fisher rats received 2 mg/kg of doxorubicin i.v. weekly for 6 weeks to induce heart failure [15]. Dosage for the chemotherapy treatment was established from previous studies and observation of a significant decrease in left ventricular ejection fraction (LVEF) [1,8]. The [^{14}C]-acetoacetate blood input function and heart biodistribution were measured in healthy control male Fisher rats (Dox(−), n = 6, 239 ± 62 g) and heart failure model rats (Dox(+), n = 6, 218 ± 33 g). Dynamic ECG-gated cardiac PET image acquisitions with [^{11}C]-acetoacetate were conducted in Dox(−) (N = 15, 290 ± 38 g) and Dox(+) (N = 6, 211 ± 34 g). All animals were given free access to food and water before the experiments. All procedures used isoflurane anesthesia (Abbott Laboratories, Montreal, Canada) at a concentration of 1.0%–1.5% with 1–1.5 L/min oxygen flow, catheter was inserted into the tail vein for injection and blood β-hydroxybutyrate concentration was measured before (PrecisionXtra, Abbott Laboratories) to exclude rats that were fasted (>0.3 mmol/L were excluded). Body temperature, respiration and heart rates were monitored for duration of the experiment (SA Instruments Inc., New York, USA). Recommendations from the Canadian Council on Animal Care and the Ethics Committee for Animal Experiments at our institution were strictly followed in this study.

2.2. Radiotracers

Using an excess of NaOH, [3-^{14}C]-acetoacetate was obtained from the alkaline hydrolysis of [^{14}C]-ethylacetoacetate (1.9 GBq/mL; American Radiolabeled Chemicals, St. Louis, USA), purified on an anion exchange resin chromatography column (AG1X8; 200–400 mesh, Biorad, Montreal, Canada), and eluted with citrate solution at pH 5.0 [16]. The eluate was divided into 200 μL samples and the [^{14}C]-acetoacetate fraction was collected and pooled. [1-^{11}C]-Acetoacetate was synthesized by carboxylation of the lithium isopropenolate anion, produced by the addition of isopropenyl acetate to methyllithium [17]. For injection, [^{11}C]-acetoacetate was formulated in a citrate-NaCl buffer at a final pH of 5.5.

2.3. [^{14}C]-Acetoacetate blood characterization

[^{14}C]-Acetoacetate (0.1 μmol/300 μL) non-positron emitter (β−), was injected i.v. over 1 min into the tail vein of the rats using an automatic injection pump. Blood samples (75 ± 25 μL) were collected manually from the femoral artery at 20, 40, 60, 90, 120, 150, 300, 600, 900 and 1200 s post-injection. At the end of the experiment (last withdrawal point), the animals were exsanguinated, the heart was harvested, washed with 0.9% NaCl, and kept on ice for further processing.

Plasma samples were divided into two fractions; the first was used to measure the concentration of ^{14}C in the blood, while the second plasma fraction was dried by speed vacuum and used for metabolite analysis. Before drying, the samples were brought to a pH of 5.0 to promote the formation of acetoacetate salts ($pKa = 3.77$) and evaporation of water and carbon dioxide ([^{14}C]-CO_2). Our analysis of the blood was restricted to carbon dioxide since it is the major product of acetoacetate beta-oxidation. For each time point, the percentage of the radioactivity remaining in the plasma as intact [^{14}C]-acetoacetate was obtained as follows [18]:

$$\frac{\begin{array}{c}Blood\ volume\ (mL) = 0.06 \times body\ weight\ (g) + 0.77\\ (Plasma\ radioactivity)/hematocrit \times Blood\ volume\end{array}}{Radioactivity\ injected} = \%\ of\ blood\left[^{14}\mathrm{C}\right]\mathrm{acetoacetate} \quad (1)$$

This equation assumes a hematocrit level of 42.7% [18]. The time–activity curves (TAC) of [^{14}C] and [^{14}C]-acetoacetate were calculated