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Role of dichloroacetate in the treatment of genetic mitochondrial diseases ☆

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ABSTRACT

Dichloroacetate (DCA) is an investigational drug for the treatment of genetic mitochondrial diseases. Its primary site of action is the pyruvate dehydrogenase (PDH) complex, which it stimulates by altering its phosphorylation state and stability. DCA is metabolized by and inhibits the bifunctional zeta-1 family isoform of glutathione transferase/maleylacetoacetate isomerase. Polymorphic variants of this enzyme differ in their kinetic properties toward DCA, thereby influencing its biotransformation and toxicity, both of which are also influenced by subject age. Results from open label studies and controlled clinical trials suggest chronic oral DCA is generally well-tolerated by young children and may be particularly effective in patients with PDH deficiency. Recent in vitro data indicate that a combined DCA and gene therapy approach may also hold promise for the treatment of this devastating condition.

Contents

1. Introduction

Clinical investigations of dichloroacetate (DCA), administered as an ionic complex with sodium or other cations, have spanned 50 years [1]. Interest has grown over the last two decades about the potential utility of DCA in the treatment of genetic mitochondrial diseases that has culminated in the recent publication of the primary outcome results of two controlled clinical trials of the drug in affected children [2] and adults [3]. These studies and related translational research on DCA have raised important questions about its place in the treatment of these devastating conditions.

This review summarizes new information on the pharmacology of DCA most relevant to its potential in congenital mitochondrial disorders in which age, disease category and pharmacogenetics emerge as criteria that may define the selection of future patients for chronic drug exposure. These findings, together with mechanistic insight into the downstream biochemical consequences of pyruvate dehydrogenase (PDH) deficiency, provide support for the concept that

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children with mutations in PDH and perhaps in other components of the PDH complex may be particularly responsive to and tolerant of long-term DCA when administered alone or in combination with other treatment modalities.

2. Site and mechanism of action

Pyruvate plays a central role in carbohydrate and energy metabolism [Fig. 1]. Its reversible decarboxylation to acetyl coenzyme A (CoA) by the PDH complex and its decarboxylation to oxaloacetate by pyruvate carboxylase provides entry of glucose-derived carbon atoms to the tricarboxylic acid (TCA) cycle. Reducing equivalents (reduced nicotinamide adenine dinucleotide, NADH; reduced flavin adenine dinucleotide, $FADH_2$) generated by the PDH-catalyzed step and by various reactions of the TCA cycle provide electrons for the mitochondrial electron transport system and for the ultimate synthesis of ATP from ADP and inorganic phosphate by Complex V (ATP synthase) of the respiratory chain.

Under aerobic conditions, the activity of the PDH complex is rate-limiting for the mitochondrial oxidation of glucose and pyruvate and for lactate and alanine, which are in equilibrium with pyruvate. It thus serves a critical role as a determinant of the efficient conversion of carbohydrate fuel into energy. Rapid regulation of the PDH multi-enzyme complex is controlled primarily by reversible phosphorylation of serine residues located in the α subunit of the heterotetrameric ($\alpha_2\beta_2$) first (E1) component of the complex. When the E1α subunit is unphosphorylated, PDH functions as an α-ketoacid decarboxylase in the presence of the obligate cofactor thiamine pyrophosphate to oxidize pyruvate. It also catalyzes the subsequent reductive acetylation of the lipoyl moiety of dihydrolipoamide transacetylase [E2; Fig. 2]. In humans, four isoforms of pyruvate dehydrogenase kinase (PDK 1–4) phosphorylate E1α and render the entire complex inactive. PDK activity is inhibited by pyruvate and by an increase in the ADP+Pi/ATP ratio and is stimulated by an increase in the ratio of NADH/NAD+ or of acetyl CoA/CoA. Dephosphorylation and activation of E1α is mediated in humans by two isoforms of PDH phosphase (PDP 1 and 2), which are themselves dependent upon calcium and magnesium ions for activity.

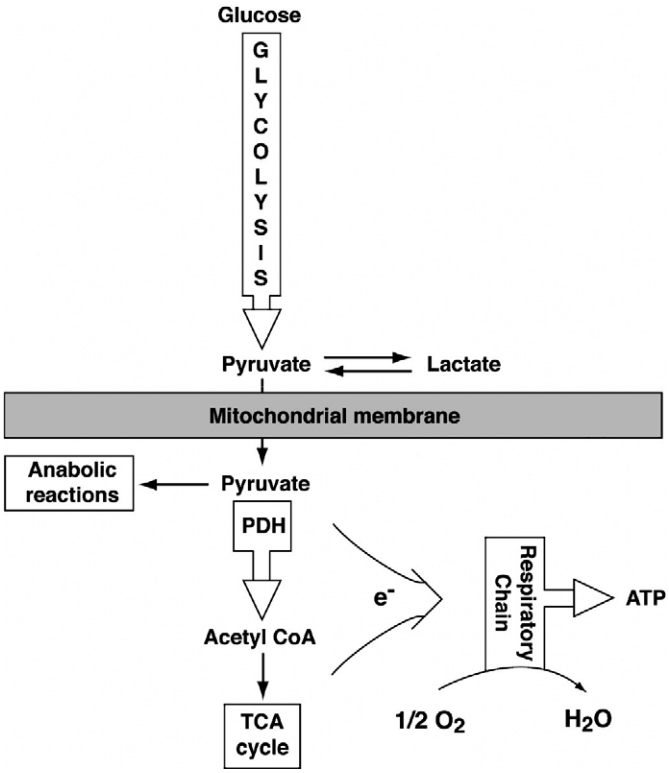

Fig. 1. Pathways of pyruvate metabolism and oxidative phosphorylation. Pyruvate may be reduced to lactate in the cytoplasm or may be transported into the mitochondria for anabolic reactions, such as gluconeogenesis and lipogenesis, or for oxidation to acetyl CoA by the pyruvate dehydrogenase (PDH) complex (PDC). Reducing equivalents (NADH, FADH) are generated by reactions catalyzed by the PDC and the tricarboxylic acid cycle and donate electrons (e^-) that enter the respiratory chain at NADH ubiquinone oxidoreductase (complex I) or at succinate ubiquinone oxidoreductase (complex II). Cytochrome *c* oxidase (complex IV) catalyses the reduction of molecular oxygen to water and ATP synthase (complex V) generates ATP from ADP.

DCA is an analog of pyruvate. It enters the circulation rapidly after an oral dose and has an oral bioavailability approaching unity [4]. The drug is transported across cell membranes (including the blood-brain barrier) by the monocarboxylate transport system for which lactate, pyruvate and ketone bodies are natural substrates [5]. DCA also competes with pyruvate for entry into mitochondria via the mitochondrial pyruvate transporter. Stimulation of PDH activity generally occurs within minutes following oral or parenteral administration and is reflected by a reduction in blood lactate concentration, the magnitude of which is dependent upon the pretreatment lactate level and the dose of DCA [6]. Careful examination of the crystal structure of PDK2 (which has the widest tissue expression among the kinases) and its interaction with natural and synthetic ligands has demonstrated that DCA and pyruvate share a common binding site in the center of the N-terminal regulatory domain [7]. DCA, in the presence of ADP, induces changes in the active site that lead to uncompetitive inhibition of PDK2 and subsequent activation of the PDH complex. The relative sensitivity of PDKs to DCA inhibition is reported to be: PDK2 ≈ PDK4 > PDK1 ≫ PDK3 [8].

For many years, the ability of DCA to rapidly activate the PDH complex by inhibiting PDK activity was considered the primary mechanism that rationalized its use in acquired or congenital conditions in which the efficient conversion of carbohydrate-derived fuel into energy was perturbed. However, as we shall review later in this article, a second mode of action of DCA on the PDH complex may underlie its sustained pharmacodynamic effects during chronic exposure.

3. Biotransformation and kinetics

The primary site of DCA metabolism is the liver, where the zeta-1 family isoform of glutathione transferase (GSTz1) dechlorinates DCA to glyoxylate in a cytoplasmic reaction that requires glutathione but does not consume it [Fig. 3; Ref. [5]]. A very minor route of DCA metabolism that accounts for <1% of an administered dose is reductive dehalogenation to monochloracetate, which may occur within the plasma compartment [9]. Monochloracetate is potentially highly neuro-toxic [10], although the site and mechanism responsible for its toxicity is unknown. The primacy of the liver in metabolizing DCA was strikingly demonstrated in patients undergoing liver transplantation, in whom clearance (Cl) of DCA from the plasma was virtually eliminated during the anhepatic phase of surgery [11].

GSTz1 is a bifunctional enzyme. As maleylacetoacetate isomerase (MAAI), it also catalyzes the penultimate step in tyrosine catabolism by converting maleylacetoacetate and maleylacetone to fumarylacetoacetate and fumarylacetone, respectively [Fig. 4; Ref. [5]]. Several important inborn errors of metabolism are associated with the tyrosine catabolic pathway. Loss-of-function mutations in fumarylacetoacetate hydrolase, the terminal enzyme in the pathway, leads to hereditary tyrosinemia type I. As a result of hydrolase inhibition, fumarylacetoacetate and maleylacetoacetate accumulate as do their ketone analogs, fumarylacetone and maleylacetone. These intermediaries possess reactive groups that make them potent alkylating agents and putative renal and hepatic toxins. Hydrolase inhibition also diverts carbon precursors to form succinylacetoacetate and succinylacetone, which inhibits a proximate step in heme synthesis and causes accumulation of the heme precursor δ-aminolevulinate (δ-