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Review

Dichloroacetate and cancer: New home for an orphan drug?

Shyam Kankotia [a], Peter W. Stacpoole [a,b,*]

[a] Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, University of Florida College of Medicine, Gainesville, FL, United States
[b] Department of Biochemistry and Molecular Biology, University of Florida College of Medicine, Gainesville, FL, United States

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ABSTRACT

We reviewed the anti-cancer effects of DCA, an orphan drug long used as an investigational treatment for various acquired and congenital disorders of mitochondrial intermediary metabolism. Inhibition by DCA of mitochondrial pyruvate dehydrogenase kinases and subsequent reactivation of the pyruvate dehydrogenase complex and oxidative phosphorylation is the common mechanism accounting for the drug's anti-neoplastic effects. At least two fundamental changes in tumor metabolism are induced by DCA that antagonize tumor growth, metastases and survival: the first is the redirection of glucose metabolism from glycolysis to oxidation (reversal of the Warburg effect), leading to inhibition of proliferation and induction of caspase-mediated apoptosis. These effects have been replicated in both human cancer cell lines and in tumor implants of diverse germ line origin. The second fundamental change is the oxidative removal of lactate, via pyruvate, and the co-incident buffering of hydrogen ions by dehydrogenases located in the mitochondrial matrix. Preclinical studies demonstrate that DCA has additive or synergistic effects when used in combination with standard agents designed to modify tumor oxidative stress, vascular remodeling, DNA integrity or immunity. These findings and limited clinical results suggest that potentially fruitful areas for additional clinical trials include 1) adult and pediatric high grade astrocytomas; 2) BRAF-mutant cancers, such as melanoma, perhaps combined with other pro-oxidants; 3) tumors in which resistance to standard platinum-class drugs alone may be overcome with combination therapy; and 4) tumors of endodermal origin, in which extensive experimental research has demonstrated significant anti-proliferative, pro-apoptotic effects of DCA, leading to improved host survival.

Contents

1. Introduction

A drug acting at a site integral to a cell's survival will exhibit a spectrum of dynamics determined by the physiological or pathological state of that cell. In the case of the xenobiotic dichloroacetate (DCA), its principal pharmacological target, the mitochondrial pyruvate dehydrogenase complex (PDC), is fundamental to eukaryotic life, so it is not surprising that acquired or congenital disorders of this mega-complex have profound ramifications for human health [1].

In this article, we focus on the anti-cancer properties of DCA, first described in the seminal report in 2007 by Bonnet and coworkers [2]. Since then, over 60 peer-reviewed laboratory and clinical investigations have contributed to establish DCA as a prototype of a new class of

* Corresponding author at: PO Box 100226, JHMHC, Gainesville, FL 32610-0226, United States. Tel.: +1 352 273 9023.
E-mail address: pws@ufl.edu (P.W. Stacpoole).

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"metabolic modulators," acting to revert a cancer cell's metabolism toward a more normal phenotype, a consequence that, ironically, initiates the malignant cell's demise. To place this interesting effect of DCA in proper context, it is fitting to summarize briefly how its study as a putative anti-cancer agent arose.

2. Getting here from there

DCA is a product of water chlorination and the metabolism of a few industrial solvents and drugs, fostering both its ubiquity throughout our biosphere and interest among a spectrum of disciplines, from environmental toxicology to medicine [3]. When administered orally as the sodium salt, DCA is rapidly absorbed, has a bioavailability approaching unity, transverses the plasma and mitochondrial membranes via the monocarboxylate and pyruvate transporter systems, respectively, readily crosses the blood–brain barrier and may be concentrated in mitochondria [3,4].

The plasma half-life of an initial oral dose of 12.5–50 mg/kg DCA is ~1 h. The drug is dehalogenated to glyoxylate (which is inactive toward PDC) by cytosolic and mitochondrial glutathione transferase zeta 1 (GSTZ1). GSTZ1 is a bifunctional enzyme that, as maleylacetoacetate isomerase (MAAI), catalyzes the penultimate step in the phenylalanine/tyrosine catabolic pathway (Fig. 1). DCA is a mechanism-based (suicide) inhibitor of GSTZ1/MAAI, resulting in decreased activity and protein expression of the enzyme with repeated drug exposure and in the plasma accumulation of DCA [3]. Both subject age [5] and GSTZ1/MAAI haplotype variation [6] influence plasma DCA kinetics and toxicity. Controlled trials of chronic oral DCA in patients with mitochondrial diseases demonstrated good tolerability and safety in young children [7–9] but an increased risk of symptomatic peripheral neuropathy in older adolescents and adults [10], due to apparent oxidative stress in peripheral nerves [11]. Plasma clearance of DCA was found subsequently to be inversely related to age in humans and rats [5]. In addition, differences in *GSTZ1*/*MAAI* haplotype differentiate "slow" and "fast" drug metabolizers, independently of age [6,12]. Based on its lactate-lowering effect and with genetics-based dosing, therapeutic plasma trough levels of DCA are generally reached within a few days of administration [13].

DCA's therapeutic potential was foreshadowed by research in the 1950s and 1960s on the pharmacology of various ionic complexes of dichloroacetate [14]. These studies led to the discovery of the DCA moiety as a molecule capable of eliciting significant effects on carbohydrate and lipid metabolism in experimental diabetes [15] and, soon thereafter, to the landmark report of its stimulatory effect on the PDC [16]. These early findings led to interest in DCA as an anti-diabetic and lipid-lowering drug [17], with potential in treating these conditions as well as myocardial and cerebrovascular ischemia [4,18] and acquired [19,20] and congenital [7,8,21] forms of lactic acidosis, the latter particularly due to loss-of-function mutations in the PDC [9].

3. How it works

The multienzyme PDC is located in the mitochondrial matrix. The complex catalyzes the rate-limiting step in the aerobic oxidation of glucose, pyruvate, alanine and lactate to acetyl CoA and is thus integral to cellular energetics (Fig. 2) [22–25]. Oxidative phosphorylation (OXPHOS) is initiated by the PDC or by the fatty acyl CoA dehydrogenase-catalyzed reactions. Regardless of the inherent integrity of the more distal tricarboxylic (TCA) cycle or respiratory chain, OXPHOS would be severely impaired if the activities of the two initiating enzymes of mitochondrial glucose and fatty acid metabolism were inhibited or if further reactions in the β-oxidation pathway were blocked, so as to limit the availability of carbohydrate or lipid-derived acetyl CoA. Pyruvate, the immediate substrate for the PDC, is itself a highly regulated intermediate critical to many important cellular processes [26]. However, only the PDC irreversibly decarboxylates pyruvate to acetyl CoA, thereby assuming an intracellular "gatekeeper" role that links glycolysis in the cytoplasm to the TCA cycle and, ultimately, to OXPHOS in the mitochondrion. Consequently, loss-of-function mutations in any of the components of the complex lead to mitochondrial energy failure and create the potential for disastrous clinical consequences for the host [27,28]. About half our daily caloric intake passes through the PDC, so it is not surprising that flux through the complex is highly regulated to maintain carbohydrate and energy homeostasis [29,30]. Rapid post-transcriptional regulation of the PDC is effected mainly by reversible phosphorylation of one or more of three serine residues on the E1α subunit. In humans, a family of four pyruvate dehydrogenase kinase (PDK) isoforms reversibly phosphorylate, and inactivate, the PDC, while two pyruvate dehydrogenase phosphatase (PDP) isoforms dephosphorylate the complex and restore catalytic activity (Table 1) [30–32]. Both PDKs and PDPs are themselves regulated proteins [30–34]. Very rare congenital defects in PDP have been reported and exhibit a clinical phenotype similar to that due to mutations in the PDC per se [27]. Newly described mutations in the *PDK 3* gene appear to cause X-linked dominant Charcot–Marie–Tooth disease [35] and a splice site mutation in *PDK 4* is linked causally to dilated cardiomyopathy in the Doberman pinscher [36]. Acetylation [34], succinylation [37,38] and glycosylation [39] of the E1α subunit and phosphorylation of PDP1 [40,41] and PDK1 [42] have also been reported to exert regulatory effects on the PDC under certain experimental conditions, but their physiological significance in vivo remains to be determined.

Although traditionally considered an exclusively mitochondrial matrix protein, Hitosugi and coworkers [42] reported the presence of PDC on the outer mitochondrial membrane in human KG1a myeloid leukemia and H1299 lung cancer cells. This year, Sutendra et al. [43] made the surprising discovery that the entire mitochondrial PDC megacomplex can translocate to the nucleus of mammalian cells, a process that is sensitive to cell cycle stages, serum factors and mitochondrial stress. The physiological and pathological consequences of this fascinating finding remain to be revealed.

DCA, a structural analog of pyruvate, stimulates PDC activation by at least two mechanisms. The first, and best established, is by inhibiting PDKs, thereby maintaining the PDC in its unphosphorylated form. PDK2 is the most ubiquitously expressed kinase and is most susceptible to inhibition by DCA [25]. The molecular interactions between DCA and PDKs have been studied extensively [44–48]. In general, DCA appears to bind to a hydrophobic pocket in the N-terminal domain of PDK and, in the presence of ADP, disrupts binding of the kinase to the lipoyl (E2) domain of the PDC [49]. At least for the PDK1 isoform, DCA induces a conformational change in the protein that alters both nucleotide-binding and lipoyl-binding pockets, inhibiting the catalytic activity of the kinase [44]. PDK inhibition by DCA occurs rapidly in vivo, because the stimulatory effect of the drug on PDC activity is measurable within 15–30 min of a single oral or parenteral dose and wanes within 12–24 h after administration [50,51]. The increase in enzyme activity is associated temporally with significant decrements in circulating pyruvate and lactate concentrations [50]. The fall in blood lactate is a useful surrogate marker for DCA's effect on the PDC and is dose-dependent up to at least a 50 mg/kg dose in humans [52].

The second mechanism by which DCA increases PDC activity is by inhibiting turnover of the complex. This was first demonstrated in rat liver following repeated in vivo dosing, whereby total and unphosphorylated PDC increased as a function of dose and was not prevented by pharmacological inhibitors of transcription or translation [50]. Such an apparent increase in enzyme stability was also observed in primary cultures of fibroblasts from PDC deficient patients [53,54]. The molecular mechanism accounting for stabilization of the PDC by DCA is unknown. However, it is reasonable to postulate that it may be linked to the drug-induced change in the phosphorylation state of the complex, because there is considerable precedent for decreased turnover in response to changes in a protein's phosphorylation

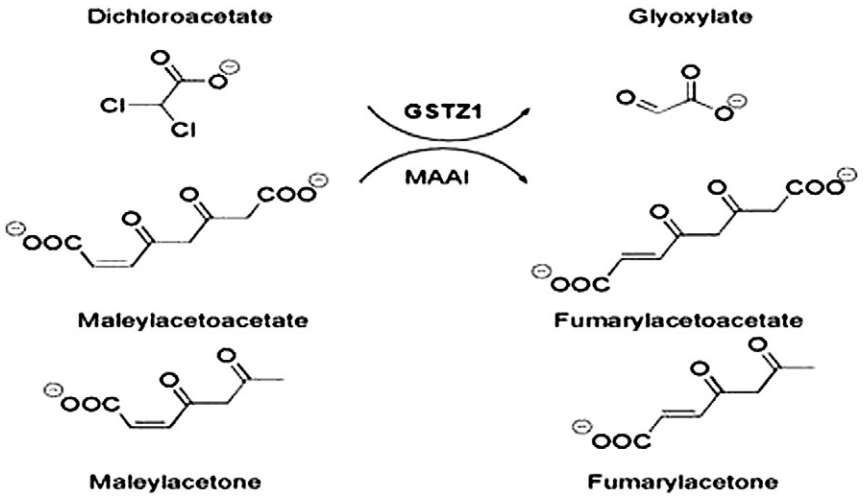

Fig. 1. Reactions catalyzed by the glutathione-dependent bifunctional enzyme GSTZ1/MAAI. DCA is dehalogenated by GSTZ1 to glyoxylate, upon which the xenobiotic enters the general carbon pool of the host. MAAI catalyzes the penultimate step in the phenylalanine/tyrosine catabolic pathway, isomerizing maleylacetoacetate and maleylacetone to fumarylacetoacetate and fumarylacetone, respectively.

state [55–60]. Regardless of the precise mechanism, stabilization of the PDC by DCA is consistent with the protracted lactate-lowering and other dynamic effects of the drug observed with its repeated administration in humans that persist long after DCA is cleared from plasma [17].

4. The PDC and cancer

Cancer cells derive much or most of their bioenergetic needs by means of glycolysis, rather than by mitochondrial oxidative metabolism, a cardinal feature of tumors first described by Otto Warburg over 80 years ago [61]. This phenomenon, known as the Warburg effect, occurs even in the presence of adequate oxygen supply (aerobic glycolysis) and leads to net lactate release by the tumor [62]. Over the last decade the Warburg effect has been reinterpreted in the light of modern biology and increasing recognition of the central role of mitochondria in the genesis and progression of cancer [63–69]. Multiple transcription factors and molecular mechanisms converge to establish the metabolic phenotype of cancer. However, hypoxia-inducible factor-1 (HIF-1) is arguably the dominant driver of these processes. HIF-1 is a dimeric molecule comprised of a constitutively expressed beta subunit and an alpha subunit susceptible to marked variation in expression. Translocation of HIF-1α to the nucleus and binding to its β subunit induces the transcription of hundreds of genes. HIF-1α controls metabolic and pH-regulating pathways in hypoxic or cancer cells, in which it is stably expressed independently of the O_2 concentration in the tumor microenvironment [70,71]. By up-regulating the expression of glucose transporters, most enzymes of glycolysis (including lactate dehydrogenase) and PDK [72–76], HIF-1α effectively induces aerobic glycolysis and inhibits the PDC. Mitochondrial O_2 consumption is therefore attenuated and the production of superoxide ($O_2^{\bullet}$), the major reactive oxygen species (ROS) resulting from electron "leakage" from the respiratory chain, is reduced. HIF-1α also directly influences mitochondrial electron transport. It alters the composition of complex IV (cytochrome c oxidase) by up-regulating a more efficient isoform (COX 4–2) and promotes proteaosomal degradation of the COX 4–1 isoform that predominates during normoxia [77]. In addition, mitochondrial biogenesis is also inhibited by HIF-1α in at least some forms of cancer [78].

Glycolysis generates lactate, not lactic acid. The accompanying H^+ derives from the hydrolysis of ATP that is generated during cytoplasmic glucose breakdown [79]. Hence, both lactate and hydrogen ions (H^+) are produced in increased amounts under HIF-1α-driven accelerated aerobic glycolysis. Moreover, HIF-1α stimulates lactate and H^+ extrusion into the extracellular space by increasing the expression of monocarboxylate co-transporter 4 (and perhaps other MCT transporters) and the Na^+/H^+ exporter NHEI [80,81]. Furthermore, because HIF1α up-regulates carbonic anhydrases IX and VII, intracellular CO_2 is converted to bicarbonate, thereby maintaining an alkaline intracellular environment while promoting acidic extracellular conditions [82].

HIF-1α also helps govern processes essential to vascularization, growth and metastasis. Many of the genes and signaling pathways involved in angiogenesis, proliferation and migration of endothelial cells and extracellular matrix metabolism are under direct HIF-1α control, including both transcriptional and post-transcriptional induction of vascular endothelial growth factors (VEGF) [73,80,81,83].

Conversion from oxidative metabolism to glycolysis by HIF-1α provokes several other metabolic shifts that stimulate DNA, membrane and organelle synthesis for cancer cell growth and maintenance, as well as increased intracellular reducing power and inhibition of caspase activity and other pro-apoptotic factors that foster cell survival. Accelerated glycolysis not only increases lactate production but also diverts glucose carbon (via glucose-6-phosphate) through the pentose phosphate pathway, which provides ribose-5-phosphate for nucleotide synthesis. Increased amounts of reduced nicotinamide adenine dinucleotide phosphate (NADPH), generated by the pentose pathway and by both cytoplasmic and TCA cycle reactions involving malic enzyme, can increase levels of reduced glutathione, further dampening production of ROS, an important stimulus for caspase-mediated apoptosis [84]. Decreased mitochondrial $O_2^{\bullet}$ concentration also leads to hyperpolarization of the mitochondrial membrane ($\Delta\Psi m$) and to closure of the mitochondrial permeability transition pore (MPTP), thus preventing egress into the cytoplasm of apoptosis-inducing factor (AIF) and cytochrome c. Superoxide is normally reduced by manganese superoxide diamutase (MnSOD) to the less reactive hydrogen peroxide (H_2O_2), but the fall in $O_2^{\bullet}$ concentration also reduces H_2O_2, which is a potent signal molecule for the redox-sensitive potassium channel Kv1.5 in the plasma membrane. Inhibition, cytochrome c release and Kv1.5 expression hypopolarize the cell, promoting voltage-dependent entry of calcium ions. The rise in intracellular Ca^{2+} fosters tonic activation of nuclear factor of activated T-cells (NFAT), which translocates to the nucleus to initiate various transcriptional programs, including those counteracting Kv1.5 expression and apoptosis and promoting cancer cell proliferation and metastasis [85,86].

Because mitochondrial PDC activity is blocked by HIF-1α-activated PDK, less acetyl CoA is available to condense with oxaloacete to form citrate. However, it has been proposed [87] that HIF-1α, in collaboration with the oncogene c-Myc, can promote an alternative pathway for citrate synthesis by reductive metabolism of glutamine to the TCA cycle intermediate α-ketoglutarate, which, in turn, provides substrate

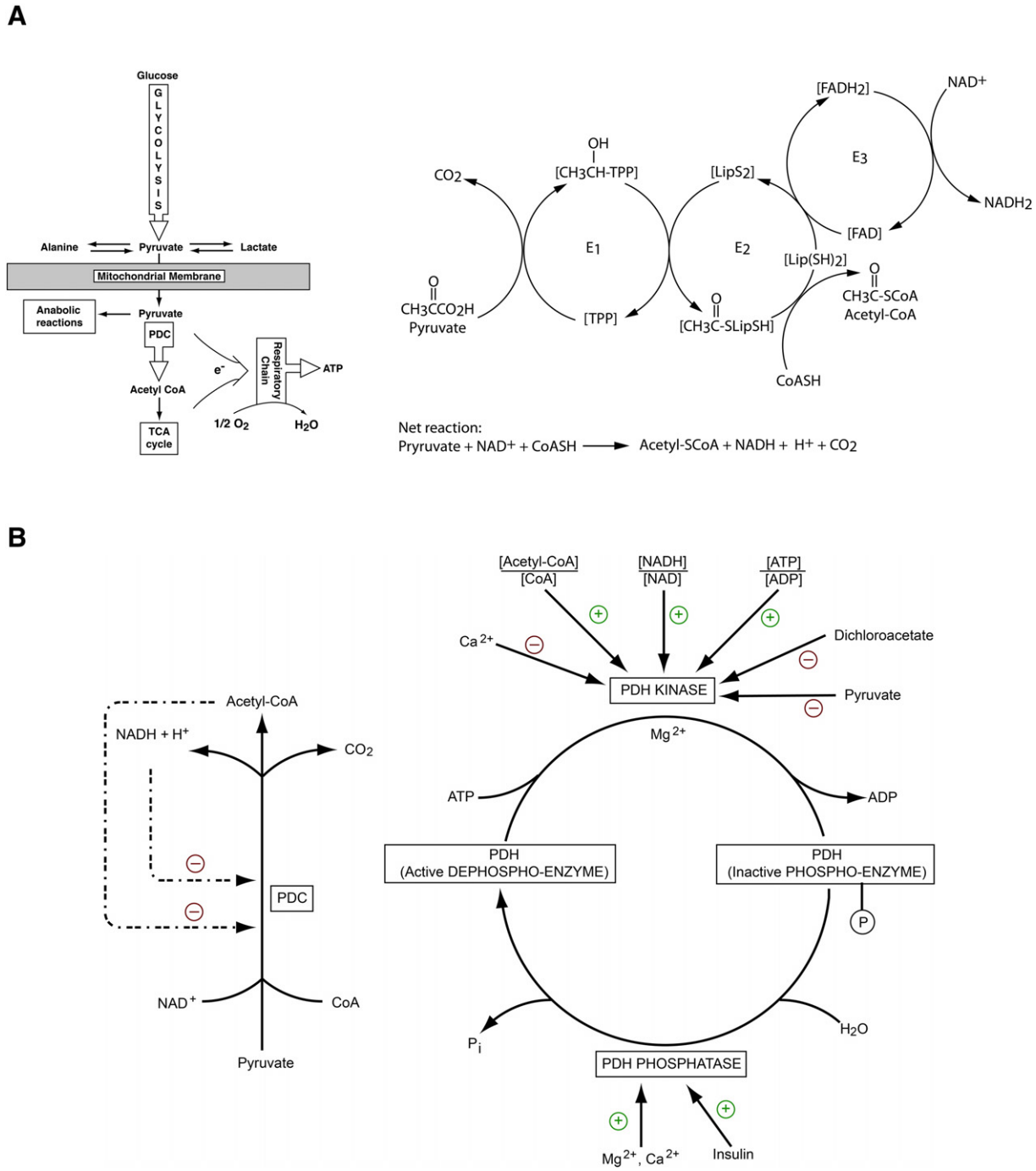

Fig. 2. Panel A: The 9.5 M Da eukaryotic complex is organized into multiple copies of three enzymatic components [22,23]. The heterotetrameric ($\alpha 2\beta 2$) pyruvate dehydrogenase (E1) decarboxylates pyruvate in the presence of thiamine pyrophosphate (TPP). Dihydrolipoamide acetyltransferase (E2) transfers the acetyl group to a lipoic acid moiety that synthesizes up to 60 molecules of acetyl CoA from reduced coenzyme A per macromolecular complex. Reduced lipoate is reoxidzed by dihydrolipoamide dehydrogenase (E3) in a coupled redox reaction in which NADH is generated. The PDC also utilizes an E3 binding protein (E3BP) to tether the E3 component to the complex's core [24]. The net reaction thus provides glucose-derived acetyl CoA for the tricarboxylic (TCA) cycle and reducing equivalents (NADH) for the respiratory chain or for anabolic reactions, such as lipid synthesis. Four requisite cofactors enable pyruvate oxidation (thiamine; B1) and the synthesis of coenzyme A (pantothenic acid; B5), acetyl CoA (lipoic acid) and NADH (riboflavin; B2 and niacin; B3). The gene for the E1α subunit is located on the X chromosome and all components of the complex are nuclear encoded. **Panel B:** Rapid regulation of the PDC is mediated primarily by reversible phosphorylation of up to three serine residues on the E1α subunit, rendering the complex inactive. Phosphorylation of E1α is facilitated by a family of four pyruvate dehydrogenase kinase isoforms (PDK 1–4), whereas two pyruvate dehydrogenase phosphatase isoforms (PDP 1 and 2) dephosphorylate, and activate, the PDC. PDKs themselves are activated by a rise in the intramitochondrial ratio of acetyl CoA:CoA and NADH:NAD^+, as well as by an increase in cellular energy charge (ATP:ADP). Pyruvate and certain structurally-related halogenated analogs, such as dichloroacetate (DCA), inhibit PDK activity. PDPs are positively regulated by insulin or magnesium ions and PDP1 can be activated by calcium ions. PDK and PDP isoforms are differentially expressed in tissues and PDK isoforms exhibit variable sensitivity to pyruvate and DCA [25].

for citrate formation. Citrate may then pass into the cytoplasm and, under the influence of the oncogene AKT, generate acetyl CoA, the building block for de novo lipid and steroid synthesis. Thus, HIF-1α plays a defining role in influencing many aspects of cancer cell biology, but the PDC/PDK axis is the pivot point that determines whether or not aerobic glycolysis and all its downstream consequences take hold. Consequently, it seems logical that a small molecule targeting this axis might have a significant impact on tumor cell metabolism and survival.

Table 1
Properties of PD kinases and PD phosphatases [23–25].

Tissue protein expression and properties of PDKs:
PDK1: heart PDK2: all tissues PDK3: testis (insensitive to pyruvate, DCA) PDK4: sk. muscle, heart (+PPARα agonists, statins, glucocorticoids)
Binding capacity to E2 lipolyl domain: PDK3 > PDK1 = PDK2 > PDK4 Specific activity: PDK3 > PDK1 = PDK4 >> PDK2 Ki DCA: PDK3 ≫ PDK1 = PDK4 ≫ PDK2 (0.2 mM)
Tissue protein expression of PDPs:
PDP1 (Ca^{2+} sens.): muscle, liver, kidney, lung, spleen PDP2 (Ca^{2+} insens.): kidney, liver, heart, brain

5. DCA and cancer

DCA has been used alone or in combination with other treatments in tumors derived from all three germ layers at widely varying doses (Table 2) [2,88–147]. In extreme cases, in vitro concentrations have been studied over a 6-log range [64]; however, most experiments in cultured cell lines derived from humans or rodents employed doses between 1 and 50 mM. In non-malignant cells, the ex vivo threshold DCA concentration for stimulating PDC activity is ≤1 mM [15 and unpublished observ.]. Thus, much higher in vitro doses may exhibit actions beyond those directly attributable to PDC activation. Similarly, DCA is reported to decrease blood lactate levels in vivo in humans at doses as low as about 5 mg/kg/24 h [4,52] and in rodents (which metabolize DCA more rapidly) [cf. 148] at doses of about 25–50 mg/kg/24 h [4].

In the initial study by Bonnet et al. [2], DCA applied to cultures of human non-small cell lung cancer (A549), glioblastoma (MO59K) and breast cancer (MCF-7) cells increased glucose oxidation and reversed the hyperpolarization of the mitochondrial membrane. Further experiments in one or more of these cell lines showed that DCA decreased lactate production and the mitochondrial membrane potential ($\Delta\Psi m$) and stimulated mitochondrial production of NADH, ROS and H_2O_2. In turn, these changes resulted in activation of plasma membrane Kv channels, decreased cellular proliferation and increased apoptosis. None of these effects was elicited in non-cancerous, healthy cells exposed to DCA. A549 cells injected into nude rats formed solid tumors. DCA administration at the time of cell injection delayed the onset of solid tumor formation, reduced tumor volume and proliferation and increased apoptosis. The findings of subsequent investigators employing diverse cell lines and in vivo animal models are broadly consistent with these results (Suppl. Tables 1 and 2) [2,88–141,142–147].

Although not directly shown in Bonnet's work [2], later reports clearly demonstrated that the stimulatory effect of DCA on tumor cell metabolism is mediated by decreasing the expression of one or more PDK isoforms [99,104]. When measured, activity or expression of HIF-1α is also decreased by DCA [90,95,104,111,120,121,132]. No experimental evidence to date implicates DCA as a direct suppressor of HIF-1α. In fact, it might be argued that DCA should have the opposite effect, because ROS is reported to stabilize HIF-1α and prevent its ubiquitination and degradation [149,150]. In addition, both the PDC and the TCA cycle enzyme α-ketoglutarate dehydrogenase can also generate $O_2^{\bullet}$ [151,152] and the oxidation of lactate to pyruvate yields NADH, which is a substrate for nitrous oxide synthase 4 that also produces ROS [153]. Furthermore, DCA's effect on the processes of OXPHOS should increase tissue oxygen consumption, exacerbating tumor hypoxia and increasing the drive for HIF-1α up-regulation [e.g., 133]. Thus, the DCA-stimulated increased flux through the PDC and, as a consequence, through the TCA cycle and respiratory chain, should promote HIF-1α expression [154]. However, countering these findings is that PDC activation by DCA decreases glycolytically-derived lactate and pyruvate concentrations, thereby diminishing the stabilizing influence of these molecules on HIF-1α expression [155,156]. Furthermore, as described below, DCA may increase expression of the tumor suppressor p53, which binds to HIF-1α and promotes its proteasomal degradation [157–159]. Alternatively, direct PDK inhibition by DCA may induce a signal for HIF-1α instability. Clearly, the relationship between DCA and HIF-1α in cancer requires further study.

Regardless of the mechanism, the DCA-associated fall in HIF-1α expression contributes to the decrease in PDK activity and, when measured, to a decrease in the expression of VEGF [95,105,119], potentially contributing to the commonly observed inhibitory effects of the drug on angiogenesis, cell proliferation and tumor growth (Suppl. Tables 1 and 2). A related potential mechanism accounting for the inhibitory action of DCA on tumor size is its ability to reduce to lactate and H^+ levels within the tumor microenvironment. Experimental induction of an acidic microenvironment up-regulates expression of proangiogenic and proinflammatory proteins, matrix metaloproteinases and other peptides associated with enhanced invasive and metastatic potential of tumor cells [160–166]. In addition, lactate accumulation in and around tumors in vivo has been associated with metastatic spread and poor clinical outcome [167–170]. Indeed, tumor-derived lactic acid is a pro-inflammatory mediator that modulates immune function, in part by altering extracellular pH [167,168]. Lactic acid is also reported to activate the IL-23/IL-17 pathway and increases expression of arginase 1 in macrophages, which inhibits T-cell proliferation and activation [171]. The acidic microenvironment leads subsequently to impaired lymphocytic cytotoxicity [171], dendritic cell function [172] and other effects [173–178] that, together, dampen the host's response to the tumor and facilitate invasion and metastasis. The relationship between lactic acid and tumor proliferation is further revealed by the finding that experimental reversal of tumor acidity by alkalization inhibits metastatic spread [179]. DCA is reported to exert immunomodulatory activity by activating the IL-R/IFN-γ pathway in a 3-methylcholanthrine fibrosarcoma cell line [180]. In addition, DCA stimulation of the PDC and, indirectly, of the various decarboxylases in the TCA cycle, leads to increased production of CO_2 that can be converted to bicarbonate by carbonic anhydrase, consequences that increase the host's natural buffering capacity and provide a potential mechanism by which the drug inhibits inflammatory mediators. Ohashi et al. [105] found that DCA alone failed to affect growth of EG7 and B16 melanoma cells implanted in mice, but synergized with poly(I:C) to suppress tumor growth. Thus, the effect of DCA on tumor and peri-tumor lactate and H^+ concentrations probably contributes to the increased survival of DCA-treated animals harboring implanted human cancers regardless of germ line origin [2,95,121,133].

Embryonal carcinoma cells, derived from pluripotent teratocarcinomas, exhibit the Warburg effect in culture and resist the cytotoxic and anti-proliferative effects of DCA [130]; however, sensitivity to the drug can be achieved by exposing the cells to a medium enriched in oxidizable substrates glutamine and pyruvate. Reversal of the glycolytic phenotype of tumors by DCA is reported to impact other pathways associated with tumor maintenance and survival. DCA induced a Bax-dependent apoptosis in cultured rat glioma stem cells [102]. This effect was associated with over-expression of FOXO3 and p53 and translocation of these transcription factors to the nucleus that, in turn, led to up-regulated pro-apoptotic BH3 proteins Bax, Noxa and Puma. The tumor suppressor p53 inhibits transcription of PDK2 [181] and, presumably, of other PDKs and the stimulatory effect of DCA on p53 expression and related pro-apoptotic factors has been widely confirmed [95,102,120,121,123,138,147]. In various primary B lymphoblastoid cell lines, DCA showed synergistic cytotoxicity in combination with Nutlin-3, a small molecular antagonist of the inhibitory action of E3 ubiquitin-protein ligase Mdm2 on p53 to increase p53 activity and increase expression of several p53-target genes, especially p21.

Induction of ROS-mediated apoptosis is the generally accepted mechanism accounting for DCA's tumoricidal action. However, Lin

Table 2
Summary of DCA preclinical studies in mammalian cancer.

Cell type	In vitro DCA dosage (mM)	In vivo DCA dosage (mg/kg)	Co-treatment or analogue	Reference #
Ectodermal				
Human breast	2.5–40		Estradiol derivative	[88]
Human breast	2.5–80	112	Bicarbonate	[89]
Human breast	5		Arsenic trioxide	[90]
Human breast	1–30			[91]
Human breast	0.25–1			[92]
Human breast	0.001–100		5-ALA, PDT	[93]
Human breast	0.5			[2]
Human breast			Mitaplatin* (in vivo)	[94]
Human breast	0.5–5	50		[95]
Human breast	10		Allopurinol	[96]
Rat breast	1–5	23–200		[97]
Human glioblastoma	10	50	Bevacizumab	[98]
Human glioblastoma	0.5			[2]
Human glioblastoma	1	100	Fl-EGFR	[99]
Rat glioma	1–128	25–125		[100]
Rat glioma		200		[101]
Rat glioma	0.25–1		Radiation, etoposide	[102]
Human medulloblastoma	0.1–10		Cisplatin	[103]
Human melanoma	0.05–20	Varied (75 mg/L of H2O)	Elesclomol	[104]
Mouse melanoma	2–10	25–100	Poly(IC) immunotherapy	[105]
Mouse melanoma		100	Capecitabine	[106]
Human neuroblastoma	0.1–10		Cisplatin	[103]
Human neuroblastoma	0.25–2			[107]
Human neuroblastoma	5–50	2.5–25		[108]
Human neuroblastoma	1–30			[91]
Human neuroblastoma	25			[109]
Human squamous	1–40		Sulindac	[110]
Human squamous	2–8			[111]
Human squamous			*N*-phe. Dichlo.* (in vitro)	[112]
Human squamous	0.001–100		5-ALA, PDT	[93]
Human squamous	0.05–0.2		Mitaplatin*, cisplatin	[113]
Mesodermal				
Human cervical	2–16		Cisplatin	[114]
Human cervical	10			[115]
Human cervical	1–40		Oncolytic AV	[116]
Human cervical	0.02		Mitaplatin*, cisplatin	[117]
Human cervical	1.6–25		2-Deoxy-D-glucose	[118]
Mouse cervical	1.6–25	25	DCA-PLA mats* (in vivo)	[119]
Human lymphoma	5–40			[120]
Mouse lymphoma	2–10	25–100	Poly(IC) immunotherapy	[105]
Mouse lymphoma	1	112	Cisplatin	[121]
Human monocytoma	5		DCA-Hb-Hp*	[122]
Human leukemia	0.1–50		Cisplatin	[103]
Human leukemia	1–30		Nutlin-3	[123]
Human myeloma	5–25	200	Bortezomib	[124]
Human myeloma	10–40		Bortezomib	[125]
Human ovarian	0.16–0.62			[126]
Human fibrosarcoma	0.45–1.35		Omeprazole, tamoxifen	[127]
Human osteosarcoma	0.1–10		Cisplatin	[103]
Human osteosarcoma	0.02		Mitaplatin*, cisplatin	[117]
Human rhabdomyosarcoma	0.1–10		Cisplatin	[103]
Human sarcoma		86		[128]
Human sarcoma	0.1–10		Cisplatin	[103]
Human testicular	0.02		Mitaplatin*, cisplatin	[117]
Endodermal				
Human pancreatic		50		[129]
Human pancreatic	1.6–25			[118]
Human pancreatic	25			[109]
Mouse carcinoma**	1–10			[130]
Human colorectal	10–100			[131]
Human colorectal	10	150		[132]
Human colorectal	2–20	50	PR-104	[133]
Human colorectal	1–40		Oncolytic AV	[116]
Human colorectal	0.1–1	150	Radiation	[134]
Human colorectal	1.2–50		3-Methyladenine, Atg7	[135]
Human colorectal	75–100	200		[136]
Mouse colorectal	1.6–25		DCA-PLA mats* (in vivo)	[137]
Human endometrial	1–10			[138]
Human gastric	10–100		5-Fluorouracil	[139]
Human gastric	20		5-Fluorouracil	[140]
Human hepatoma	1–60	100	Sorafenib	[141]
Human hepatoma			*N*-phe. Dichlo.* (in vitro)	[112]
Human hepatoma	1–40		Oncolytic AV	[116]

Table 2 *(continued)*

Cell type	In vitro DCA dosage (mM)	In vivo DCA dosage (mg/kg)	Co-treatment or analogue	Reference #
Human hepatoma	0.05–0.2		Mitaplatin*, cisplatin	[113]
Human lung	2.5–40		Sulindac	[110]
Human lung			*N*-phe. Dichlo.* (in vitro)	[142]
Human lung	10		Pt drugs*	[143]
Human lung			*N*-phe. Dichlo.* (in vitro)	[112]
Human lung			*N*-phe. Dichlo.* (in vitro)	[144]
Human lung	1–40		Oncolytic AV	[116]
Human lung	0.02		Mitaplatin*, cisplatin	[117]
Human lung	0.5	50–100		[2]
Human lung			Pt drugs* (in vitro)	[145]
Human lung	0.5–5	50		[95]
Human lung		100	Capecitabine	[106]
Human prostate	0.01–100		Radiation	[146]
Human prostate			Honokiol DCA*	[147]
Human prostate	80			[136]
32 Ectodermal				
22 Mesodermal				
33 Endodermal	74 in vitro studies	25 in vivo studies	47 Co-treatment studies, 18 analogue studies	Totals

Key: * = analogue; ** = these P19 stem cells were induced by retinoic acid and differentiated primarily into endodermal cells, however this method may have also resulted in cells of other germ layers.
5-ALA = 5-aminolevulinic Acid; PDT = photodynamic therapy; Fl-EGFR = full-length epidermal growth factor receptor; *N*-phe. Dichlo. = *N*-phenyl dichloroacetamine derivative; Oncolytic AV = oncolytic adenovirus; PLA mats = polylactide mats; DCA-Hb-Hp = DCA–hemoglobin–haptoglobin complex; PR-104 = hypoxic cytotoxin; Atg7 = Atg7 siRNA autophagy inhibitor; Pt drugs = platinum-based drugs.
Note: All in vivo studies used rodent models (mice or rats).

et al. [136] recently sowed that exposure of human colorectal and prostate carcinoma cell lines and HT29 colorectal tumor xenografts to DCA cell cycle increased arrest and expression of the autophagy marker LC3BII, in association with mitigation of the Warburg effect increased ROS production and MCT1 expression and indirect evidence of mTOR inhibition. These changes reversed upon removal of DCA. In contrast, there was minimal evidence of apoptosis or necrosis from DCA treatment. The in vitro findings might be considered suspect, because of the high (75–100 mM) drug concentrations employed. However, only 4 days of administration of 200 mg/kg DCA (a reasonable dose, given the high rate at which mice metabolize the drug) [148] also induced autophagy, but not apoptosis. These findings require independent verification but raise the possibility that DCA may cause tumor suppression that is reversible upon discontinuation of treatment.

Although yet to be applied specifically in the treatment of cancer, a combined therapeutic approach for congenital PDC deficiency using DCA and adeno-associated viral (AAV) vector technology to deliver the wild-type E1α subunit gene (*PDHA1*) demonstrated additive or supra-additive effects in primary cultures of PDC deficient fibroblasts [54]. Recent extensions of this proof-of-principal work showed that AAV-mediated delivery of *PDHA1* to human liver cancer cells increased PDC activity and caused apoptosis in the cells [182]. Such an approach could be translated into clinical trials, using DCA in combination with cell-specific AAV serotype vectors that cause high-efficiency transduction of tumor cells [183].

6. Structural analogs

"Slow release" ionic complexes and esters containing DCA were synthesized originally to modify the plasma kinetics of the drug and prolong its dynamic action on intermediary metabolism [184]. Subsequently, other structural analogs were developed with the aim of increasing their molar potency toward PDK inhibition (Suppl. Table 2) [94,112,113,117,119,122,137,142–145,147]. To date, there have been no published reports that these or any other analogs of DCA have been administered to humans. However, the emergence of DCA as a potential anti-cancer agent has rekindled enthusiasm for developing new, patentable, compounds with greater specificity and potency. The most intriguing and best studied such analog so far is "Mitaplatin," a fusion compound of two molecules of DCA and cisplatin [117]. Mitaplatin undergoes dissociation when exposed to a negative intracellular redox potential, allowing cisplatin to target the nucleus and disrupt nucleotide cross-links and DCA to target the mitochondria and reverse the Warburg effect. Several human cancer cell lines, but not healthy human cells, demonstrated collapse of the $\Delta\Psi m$ and increased necrosis and apoptosis upon exposure to mitaplatin. The IC_{50} μM range for cell lines was similar between mitaplatin and cisplatin and was much lower than IC_{50} values for DCA. Mitaplatin decreased 2-deoxyglucose uptake and activated the PDC in tumor cell lines [113]. Similar results have been obtained with other DCA-platin fusion molecules [145]. Nanoparticle encapsulation of mitaplatin prolonged platin plasma kinetics, reduced its tissue distribution in rats and enhanced its duration of anti-tumor activity in vivo [94]. There is irony in this effort to chemically combine two known peripheral neurotoxins, albeit with the hope that greater potency of the analog may enable acceptable dose reduction. In this regard, the findings with mitaplatin compare generally favorably with those from studies in which DCA and cisplatin were administered separately as combination therapy, both in vitro [114,121] and in vivo [121,185], although DCA is reported to abrogate the anti-tumor activity of cisplatin in some cancer cell lines [103].

Among the other DCA analogs that have been synthesized [112,119,123,137,142–145,147] is an interesting and potentially cell-selective conjugate in which up to 12 DCA molecules are bound to the hemoglobin tetramer, which, in turn, binds to haptoglobin to form a stable complex [122]. The complex is thought to be taken up by cells via the hemoglobin scavenger receptor CD163 and to target circulating monocytic leukemic cells, leading to apparent depolarization of the $\Delta\Psi m$ and induction of apoptosis in vitro at concentrations approximately three orders of magnitude lower than DCA. Most recently has been the synthesis of "Mito-DCA," in which a lipophilic triphenylphosphonium cation is attached through a biodegradable linker to three DCA moieties [186]. Although the rationale for developing Mito-DCA appears based on several erroneous assumptions regarding the parent drug's pharmacological properties, the analog was found to exceed DCA in potency against certain human prostate cancer cell lines.

It will be interesting to determine whether the molar superiority of analogs following brief, in vitro exposure to cancer cells can be translated to clinical situations. Although DCA is a comparatively weak PDK inhibitor, its inhibition of its own metabolism with repeated exposure [3] (Section 2) and its apparent accumulation in the mitochondrial compartment may achieve drug concentrations in the high μM range necessary to maintain tonic suppression of PDK.

7. Combination therapy

From the knowledge that aerobic glycolysis is associated with radiation and drug resistance by cancer cells [187,188], a few studies have combined DCA with X-irradiation or standard chemotherapeutics in an effort to overcome resistance to these agents (Suppl. Table 2). Liao and coworkers [189] found that the radiation-induced senescence of MDA-MB-231-2A human breast cancer cells led to increased glycolysis and acidification of the tumor microenvironment, which were reversed by exposure to DCA. Reasoning that stimulation of oxygen consumption in tumor cells could enhance the killing effect of hypoxia-specific cytotoxins, Cairns et al. [188] reported that in vitro exposure of RKO and Su. Eighty-six human tumor cells to DCA increased oxygen consumption, which subsequently led to hypoxia of the cells and increased the antitumor effectiveness of tirapazamine. Shen et al. [141] observed an inverse association between reliance on glycolysis for ATP and resistance to the angiogenic inhibitor sorafenib in a series of human hepatocellular carcinoma cell lines. Addition of DCA reversed the Warburg effect in these cells and increased their sensitivity to sorafenib-induced apoptosis, an effect recapitulated in a mouse model in vivo. Qualitatively similar results were obtained in human multiple myeloma cells [124,125], in which sensitivity to the pro-apoptic action of bortezomib was enhanced by the metabolic shift from glycolysis to mitochondrial oxidative metabolism induced by addition of DCA. Recognizing that the nonsteroidal anti-inflammatory drug sulindac made cancer cells more sensitive to oxidative stress, Ayyanthan and coworkers [110] showed that the combination of sulindac and DCA enhanced the in vitro killing of human non-small cell lung cancer and squamous cell carcinoma cells (but not normal cell lines) by a mechanism associated with mitochondrial ROS production and activation of the pro-apoptic factor Jnk.

A similar strategy of enhancing ROS-mediated killing of tumor cells was adapted by others, using a combination of DCA with either the light-activated pro-oxidant, delta-aminolevulinate (δ-ALA) in A431 squamous cell and MCF-7 breast cancer cell lines [93], allopurinol in several human breast carcinoma lines [96] or arsenic trioxide (As_2O_3; ATO) [90]. Like δ-ALA, ATO is a reactive molecule capable of covalent binding to proteins via modification of thiol groups [190] and is considered first-line therapy for new-onset acute promyelocytic leukemia [191]. ATO is reported to depolarize ΔΨm, inhibit the PDC and induce ROS-mediated apoptosis in cancer cells [192,193]. Sun and coworkers [97] found that DCA potentiated the cytotoxicity of ATO in several human breast cancer cell lines and decreased expression of c-Myc, HIF-1α and Bcl-2. DCA independently increased cellular ATP levels, an effect associated with enhanced expression of the ATP synthase (complex V) β subunit and which was opposite to the effect of ATO on the enzyme.

High tumor lactate levels are reported to correlate directly with resistance to radiotherapy [194–196]. Pre-treatment of glioma rat stem cells with DCA sensitized them to irradiation-induced Bax-dependent apoptosis [97]. Increased radiosensitivity and Bax-dependent apoptosis with DCA was also found in human prostate (PC-3-Bcl-2 and PC-3-Neo) colonic adenocarcinoma (WIDR) and glioblastoma (LN18) cell lines and in WIDR xenograft tumors in nude mice [134,146].

8. Clinical use

Three early phase, open-label trials of oral DCA in cancer patients have been published, two in patients with brain tumors [13,197] and one in patients with metastatic breast cancer or advanced non-small cell lung cancer [198]. In the first report, Michelakis et al. [197] administered 12.5 mg/kg twice daily to five adults, three with recurrent and two with newly diagnosed glioblastoma multiforme (GBM), one of whom was started on DCA alone and the other on standard treatment with radiation therapy and temozolomide (TMZ). Symptomatic peripheral neuropathy necessitated a 50% dose-reduction that reportedly was well-tolerated. One patient with recurrent disease and both patients with newly diagnosed GBM showed evidence of radiologic regression by magnetic resonance imaging. Of the three patients with recurrent disease, one died from complications of brain edema 3 months after starting DCA, another required draining of a cyst and debulking within 11 months of initiating DCA and the third showed radiologic progression on month 3 of DCA administration that resulted in further debulking and addition of radiation therapy and TMZ. All four living subjects were reported to be clinically stable at month 15 of DCA administration. Biochemical studies conducted in freshly excised GBM specimens from 49 patients to which DCA was applied ex vivo revealed that the drug depolarized the ΔΨm, increased ROS levels, activated p53 and suppressed the expression of both HIF-1α and VEGF.

Human toxicity from chronic DCA exposure is generally limited to a reversible sensory and motor peripheral neuropathy that is now known to be influenced by subject age and genotype. DCA plasma clearance is inversely associated with age in humans and rats [5]. As previously noted (Section 2) the major route of DCA biotransformation is by dehalogenation to the naturally occurring molecule, glyoxylate, by glutathione transferase zeta 1 (GSTZ1). Haplotype variability in the *GSTZ1* gene confers relatively "slow" or "fast" plasma clearance of the drug in children [12] and adults [6]. It is noteworthy that dose-limiting neuropathy in the GBM study occurred at a DCA dose identical to that which children with congenital mitochondrial diseases have received safely for many years [9], but which may cause neuropathy in adults with mitochondrial diseases [10]. Dunbar and colleagues conducted a phase 1 trial of DCA in 15 adults with recurrent World Health Organization grade III astrocytoma or GBM or with brain metastases from a primary cancer outside the central nervous system. To mitigate drug-induced neuropathy, dosing was based on haplotype variation in GSTZ1. Eight patients completed at least one 4 week cycle and daily treatment continued for up to 480 days [13 and unpubl. observ.]. No dose-limiting toxicities occurred and no subject withdrew because of lack of tolerance to DCA, when dosed at ≤12.5 mg/kg/12 h.

An open label study of DCA (6.25 mg/kg twice daily) in patients with advanced breast and lung cancer [198] was terminated prematurely after limited enrollment, because of disease progression and associated complications. A single case report [199] described complete remission over 4 years of non-Hodgkin's lymphoma in a middle-aged man orally administered 1 g/day DCA as monotherapy after relapse from standard chemotherapy.

9. Concluding remarks

Repurposing an old drug is often based on discovering new sites and mechanisms of action of the chemical. In the case of DCA, most of its dynamic effects are mediated by perturbation of the PDC/PDK axis and the resulting impact on carbohydrate metabolism and bioenergetics. Such is the basis for its reported beneficial actions in diabetes mellitus, acquired and congenital forms of lactic acidosis, myocardial and cerebral ischemia and, most recently, pulmonary arterial hypertension (PAH) and cancer [3]. It is noteworthy that many of these disorders share a common pathophysiology in which the balance between glycolysis and mitochondrial oxidative metabolism is skewed in favor of the former energetic pathway. Accelerated aerobic glycolysis, lactate and H^+ accumulation, suppression of the PDC and up-regulation of HIF-1α are all manifested in congenital PDC deficiency [200], PAH [201,202] and cancer, creating a common biochemical phenotype aimed at ensuring cell survival when the rate of the pyruvate oxidation is limiting.

At present, all evidence indicates that reactivation of the PDC is the common mechanism accounting for DCA's anti-cancer effects. As a consequence, there are at least two fundamental changes in tumor metabolism elicited by DCA that antagonize tumor growth, metastasis and survival. The first is the redirection of glucose metabolism from glycolysis to oxidation, leading to inhibition of proliferation and induction of caspase-mediated apoptosis. These effects of DCA exposure have been replicated widely in both human cancer cell lines and in tumor implants. The second major change in tumor metabolism from stimulating PDC activity is the oxidative removal of lactate, via pyruvate, and the co-incident buffering of H^+ by dehydrogenases located in the mitochondrial matrix. Although reduction in lactate and hydrogen ion concentrations is a well-established effect of the drug, its significance with regard to DCA's anti-tumor activity has only recently been appreciated.

Preclinical experiments indicate that DCA has additive or synergistic effects when used in combination with standard agents designed to modify tumor oxidative stress (irradiation, sulindac), vascular remodeling (bevaciznmab, bortezomib), DNA integrity (cisplatin, doxorubison, etoposide) or immunity (Poly (I:C)). Clinical results limited to open label studies in patients with recurrent brain tumors who have failed standard therapy suggest that it may soon be time for a controlled trial evaluating DCA in combination with standard therapies as initial treatment for high grade gliomas. Of particular merit may be the application of recent metabolomic techniques to such studies to increase understanding of the metabolic signature of gliomas [203] and other cancers [204] and the impact of DCA thereon. Trials targeting the pediatric brain tumor population may also be warranted, particularly given the safety of chronically administered DCA in children. Other potentially fruitful areas for early clinical trials that are grounded in strong preclinical research include BRAF-mutant cancers, such as melanoma [104,205], perhaps in combination with other pro-oxidants, and hematological and solid tumors of endodermal origin, in which extensive experimental research has demonstrated significant anti-proliferative, pro-apoptotic effects of DCA leading to improved host survival. Future clinical studies should incorporate a treatment strategy based on knowledge of a subject's GSTZ1 haplotype status to reduce risk of dose-limiting peripheral neuropathy.

Aerobic glycolysis and HIF-1α activation are not unique to cancer. Reliance on glycolytically-derived ATP for energy is vital to many physiological and adaptive processes, including the development of the early preimplantation conceptus [206–209] and stem cells [210–212] and other proliferative conditions, such as the response of immune cells to inflammation or infection [213,214] and wound repair [213,215]. Accordingly, chronic exposure to DCA could have a number of potentially harmful consequences by shifting cells to a more oxidative metabolism. Therefore, it is perhaps reassuring that DCA administration has been reported to stimulate murine embryonic development, viability and fetal weight [216,217], cerebral and myocardial ischemia-reperfusion injury [4,9] and other types of wound healing [4]. Given DCA's pharmacological profile and its demonstrated action against a wide variety of human cancers, it is time this orphan product had more aggressive oncological parenting.

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