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Dichloroacetate is an antimetabolite that antagonizes acetate and deprives cancer cells from its benefits: A novel evidence-based medical hypothesis

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ABSTRACT

Dichloroacetate (DCA) is a promising safe anticancer drug that cured a patient with chemoresistant non-Hodgkin's lymphoma and treated lactic acidosis effectively. The well-known mechanism of DCA action is through stimulating Krebs cycle (stimulating pyruvate dehydrogenase via inhibiting pyruvate dehydrogenase kinase). This prevents lactate formation (Warburg effect) depriving cancer cells of lactate-based benefits e.g. angiogenesis, chemoresistance and radioresistance. Here, we introduce novel evidence-based hypotheses to explain DCA-induced anticancer effects. On pharmacological and biochemical bases, we hypothesize that DCA is a structural antagonist of acetate competing with it for target enzymes and biological reactions. We hypothesize that DCA exerts its anticancer effects via depriving cancer of acetate benefits. We hypothesize also that acetate is an antidote of DCA capable of treating DCA toxicity. Many reports support our hypotheses. Acetate is vital for cancer cells (tumors depend on acetate) and DCA is structurally similar to acetate. DCA exerts opposite effects to acetate. Acetate caused a decrease in serum potassium, phosphorus and glucose, and an increase in serum lactate, citrate, free fatty acids and ketone bodies (serum acetoacetate and beta-hydroxybutyrate levels). Acetate decreased the proportion of active (dephosphorylated) pyruvate dehydrogenase in perfused rat heart. DCA produced quite opposite effects. Intravenous infusion of acetate produced metabolic alkalemia while DCA caused minimal effects on acid-base status. Acetate is important for cancer cells metabolism and survival as elevated acetate can drive resistance to targeted cancer treatments. Acetate is required for epidermal growth factor receptor vIII mutation in lethal brain tumors. Experimentally, DCA inhibited acetate oxidation in hearts of normal rats and reversed inhibitory effects of acetate on the oxidation of glucose. During presence of DCA with no glucose in heart perfusions with [1-14C]acetate, DCA decreased the specific radioactivity of acetyl CoA and its product citrate. This proves our hypotheses that DCA is an antimetabolite that antagonizes acetate for vital reactions in cancer cells. Acetate may be used as an antidote to combat DCA toxicity.

Introduction

Monochloroacetate (MCA), dichloroacetate (DCA), and trichloroacetate (TCA) are chlorinated acetates that are formed during water disinfection processes [1]. DCA results in daily life from water chlorination [2] and is also a promising drug treatment for treating mitochondrial diseases, pulmonary arterial hypertension and cancer [3]. In clinical oncology, DCA emerged as a promising safe anti-neoplastic agent causing cancer cells apoptosis via targeting the enzyme pyruvate dehydrogenase kinase (PDK) that regulates initiation of Krebs cycle using pyruvate [4]. Depriving cancer cells of lactate is vital as a cancer therapeutic strategy due to the numerous lactate-based benefits

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Table 1
Hypotheses points.

Hypotheses points:
1. DCA competes with acetate for target enzymes and biological reactions based on structural similarity (structural antagonism) (Fig. 1).
2. DCA deprives cancer cells of acetate benefits i.e. DCA is an antimetabolite.
3. Acetate is an antidote to DCA that can treat DCA toxicity.

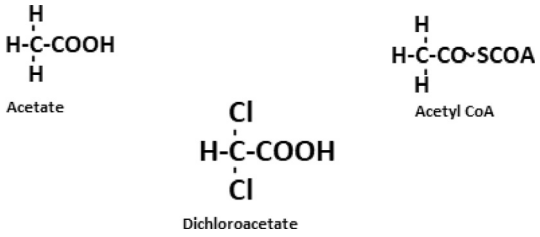

Fig. 1. Acetate is structurally similar to dichloroacetate and may act as a structural antagonist to it.

to cancer cells and tumors e.g. angiogenesis, chemoresistance and radioresistance. [5]. Interestingly, DCA was reported as a successful treatment to a young child having metabolic hyperlactacidemia [6]. A major hope in cancer treatment is based upon introduction of DCA in clinical oncology based on the promising success of DCA in treating many human patients having different types of tumors [7–9]. The promising report by Strum et al. in curing a patient having chemoresistant non-Hogkin lymphoma using DCA [10] urged us to search for potential novel anticancer mechanisms beyond DCA [11].

Herein, we introduce novel evidence-based hypotheses and mechanisms of DCA anticancer effects based on earlier findings through antagonizing acetate (Table 1, Fig. 1).

The hypothesis/theory

On evidence-based pharmacological and biochemical bases, we introduce here our novel evidence-based hypotheses regarding new anticancer mechanisms of action of DCA. We hypothesize that DCA is an antimetabolite (based on structural similarity to acetate). We hypothesize that DCA antagonizes acetate for target enzymes and biological reactions based on structural similarity (structural antagonism). DCA is structurally similar to acetate. Substitution of two hydrogens in acetate by two chloride ions produces DCA. We hypothesize that DCA is a structural analog to acetate that may antagonize its biochemical effects and compete with it for its target enzymes and biochemical reactions Evidences supporting that are summarized in Table 2 where DCA produces quite opposite effects to acetate in many situations [12].

Evaluation of the hypothesis/idea

We searched the literature extensively to formulate our suggested new concepts regarding the novel anticancer mechanisms of action of DCA. Acetate caused a decrease in serum potassium, phosphorus and glucose, and an increase in serum lactate, citrate, free fatty acids and ketone bodies (serum acetoacetate and beta-hydroxybutyrate levels). Acetate decreased the proportion of active (dephosphorylated) pyruvate dehydrogenase in perfused rat heart. DCA produced quite opposite effects [12]. Regarding the effects of intravenous acetate infusion on pH, acetate produced metabolic alkalemia while DCA infusion caused minimal effects on acid-base state and oxygen consumption [12]. All these evidences prove that DCA is a competitive antagonist of acetate.

There are many reports that support our idea that DCA works through antagonizing acetic acid (Table 2). DCA inhibited extraction of plasma free fatty acids (as acetic acid) in heart tissues from normally fed rats and also from alloxan-induced diabetic rats. In heart perfusions with glucose, insulin and acetate (stimulators of glycolysis and subsequently Krebs cycle), DCA decreased the cell citrate concentration (product of Krebs cycle). DCA was also reported not to be metabolized to dichloroacetyl-CoA, dichloroacetylcarnitine, citrate or CO(2) [13], which supports the hypothesis that DCA acts as an antagonist that inhibits the enzyme activity without being metabolized. This supports also that DCA is an enzyme inhibitor that does not undergo further metabolism. In the absence of glucose and insulin (stimulators of glycolysis and subsequently Krebs cycle), DCA inhibited glycolysis as a source of acetyl CoA causing no increase in acetyl CoA levels [13]. Taking into account that acetate is vital for many tumor types, its possible antagonism by DCA can be a novel mechanism for DCA-induced cancer cell cytotoxicity.

The vital question that arises now: is acetate itself important for cancer cells metabolism and survival?

Acetate promotes tumor growth

Acetate is a bioenergetic substrate for human glioblastoma and brain metastases. Elevated acetate levels can drive resistance to targeted cancer treatments. Treatment with sequentially increasing concentrations of acetic acid was reported to persistently enhance RNA, protein, and DNA synthesis. That was followed by extensive epidermal hyperplasia [14]. Based on that, acetate promotes synthesis of DNA and macromolecules essential for cancer cell division. Either glucose or acetate is required for the activation of epidermal growth factor receptor vIII (a common growth factor receptor mutation in the fatal brain cancer glioblastoma multiforme). Moreover, elevated energetic nutrient levels (acetate or glucose) can induce resistance to targeted cancer treatments [15]. Also, acetate is an epigenetic regulator of posttranslational protein modification, and is a carbon source for cancer cell biomass accumulation. Acetate plays a major role in protein acetylation and acetate oxidation plays an important role in carcinogenesis, cancer progression and treatment [11].

Empirical data

Acetate fuels tumor growth where acetate is a fuel for promoting the

Table 2
Comparison between effects of acetate and DCA [12]

	Acetate	DCA
Effects of intravenous infusion on pH	Metabolic alkalemia	Minimal effects on acid-base state and oxygen consumption
Effects on serum potassium and phosphorus	Marked decrease	Marked increase
Effects on serum lactate and citrate levels	Increase	Decrease
Effects on serum free fatty acids	Increase	Decrease
Effects on serum acetoacetate and beta-hydroxybutyrate levels	Increased markedly	Less increase
Effects on serum glucose level	Decreases significantly	Decreases
Effects on the proportion of active (dephosphorylated) pyruvate dehydrogenase in perfused rat heart	Decreases	Increases

growth of tumors. This was confirmed by positron emission tomography (PET) imaging studies using [11C]acetate where many tumor types are dependent on acetate as a source of carbon to get acetyl-CoA. Excessive acetate uptake was reported in prostate, lung, liver, and brain cancers [16–19]. Tumors exhibit acetate dependence. Acetate constitutes a vital supply of acetyl-CoA for feeding these tumors [20]. Interestingly, [11C]acetate PET imaging is preferred than [18F]fluorodeoxyglucose (FDG) PET imaging as [11C]acetate PET imaging is more accurate and sensitive. Acetate consumption by human tumors is evident by nuclear magnetic resonance (NMR). Acetyl coA synthase seems to be of paramount importance to consume acetate as a supply of acetyl-CoA. Primary and metastatic mouse orthotopic brain tumors have the capacity to oxidize acetate and glucose. Acetate consumption in such tumors correlated with the expression of acetyl-CoA synthetase enzyme [21].

Consequences of the hypothesis and discussion

DCA exerts potent promising anticancer effects. DCA exerted antiproliferative and pro-apoptotic effects against many cancer cells. DCA was reported to reverse the glycolytic phenotype and inhibit metastasis of breast cancer cells both *in vitro* and *in vivo* [22]. DCA treatment was reported to reduce cancer cell-induced production of lactate and to sensitize malignant cells to 5-fluorouracil in gastric cancer cells [23]. Moreover, DCA reduced cancer cells (hypoxic HeLa cells) proliferation, viability and colony counts [24]. Unlike many chemotherapeutics, hypoxia (a common criterion of cancer biology) enhances the antitumor activity of DCA [25].

Interestingly, DCA killed cancer stem cells effectively e.g. embryonal carcinoma stem cells (shares in teratocarcinoma development that exhibited strong dependence on glycolysis with decreased mitochondrial biogenesis) [26]. Till now, DCA-induced anticancer effects are looked at as through stimulating Krebs cycle entry via inhibiting PDK causing stimulation of pyruvate dehydrogenase complex where acetyl CoA starts Krebs cycle through combination with oxaloacetate to form citrate. This necessitates that glycolysis reaction becomes aerobic and not anaerobic (ending in formation of lactate, Warburg effect). Tumor glycolysis-induced pyruvate is converted into lactate (Warburg effect) that confers many lactate-based benefits to cancer cells e.g. angiogenesis, chemoresistance and radioresistance [5]. Based on that, DCA clearly reverses cancer glycolytic phenotype and exerts potent anticancer effects.

Future perspectives based on our evidence-based hypothesis include many points. Our hypotheses will guide scientific research in this direction to open a new research frontier towards many investigations based on our hypothesized points that DCA antagonizes acetate. The following practical research points and applications may benefit medical practice:

1. An experimental design to investigate acetate-induced inhibition of DCA-induced cell death.
2. An experimental biochemical design to investigate the enzyme kinetics of DCA-induced antagonistic effects of acetate and the effects of DCA on acetate-based enzyme systems e.g. acetyl CoA synthase enzyme [27].
3. Future possibility of using acetic acid-based treatments as antidotes and antagonists of DCA to combat DCA accidental toxicity and poisoning.
4. Acetate dependence of tumors can be utilized for developing sensitive diagnostic tools and radiotracers.

That started to be recently utilized e.g. 11C-acetate and 18F-fluorodeoxyglucose positron emission tomography/computed tomography dual imaging for the prediction of response and prognosis after transarterial chemoembolization.

Conclusion

We conclude that DCA is an antimetabolite that antagonizes acetate for vital reactions in cancer cells.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2018.11.012.

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