Dichloroacetate (DCA) as an Oncology Chemotherapeutic Agent?

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Key Words: DCA, mitochondria, solid-tumors, oncology, chemotherapy, Warburg Effect

Abstract

Cancer remains as one of the most challenging diseases to treat. However, this era has commenced with the introduction of novel drug treatments that are safer, and less toxic. Thus, today many clinicians are changing their clinical practices by opting for targeted and/or ancillary drug treatments that kill the tumor cell populations while sparing healthy cells, thus affording the patient a valuable quality-of-life.

It is known that greater than 70% of all cancer types rely on aerobic glycolysis for energy production, which is an inefficient means of generating ATP, a feature that becomes an advantageous biomarker. Aerobic glycolysis is a result of that exists in tumors and is due to malfunctioning/hyperpolarized mitochondria. Cancer cells generally express increased aerobic glycolysis in the cytosol (Warburg Effect/lactic acid fermentation)\(^1\) rather than oxidative phosphorylation (normal cells) for energy production (the Pasteur Effect),\(^2\) thus producing excessive lactate while at the same time consuming oxygen via mitochondrial respiration.\(^3\)

In 2007, Drs. Archer and Michelakis from the University of Alberta, Canada,\(^4\) decreed the use of Dichloroacetate (DCA) as a general use chemotherapeutic agent that could reverse this mitochondrial hyperpolarized state thus inducing cancer cells to undergo apoptosis, thereby impeding cancer growth.

They recognized the ability of DCA to decrease lactate production which has been used for more than 30 years in the treatment of lactic acidosis in inherited mitochondrial diseases in humans.\(^5\) Lactic acidosis typically occurs when cells become hypoxic thus impairing cellular respiration, forcing cells to metabolize glucose anaerobically, which leads to lactate formation and thus lower pH levels.\(^6\) The characteristics of human mitochondrial diseases are virtually identical to the process of tumorigenesis, complete with the inefficient bioenergetic mitochondria.

The generic drug sodium dichloroacetate (DCA) is an orally bioavailable small molecule that, by inhibiting pyruvate dehydrogenase kinase (PDK), increases the flux of pyruvate into the mitochondria, thereby promoting glucose oxidation. This reversal suppresses mitochondrial apoptosis in cancer cells resulting in suppression of tumor growth \textit{in vitro} and \textit{in vivo}.\(^4\) Thus, it would be reasonable to propose that cells with mitochondrial defects, or cells in a high glycolytic and hypoxic environment would likely be more sensitive to glycolytic inhibition by DCA. Therefore, a prospective study of the efficacy of DCA as a potential chemotherapeutic agent was conducted.

Materials and Methods: A variety of fresh solid tumor specimens (27) were procured from patients of a private clinic, Medicor Cancer Centres Inc. (Toronto, Ontario, Canada) The tumor specimens were either obtained from biopsies of superficial metastases, superficial lymph nodes infiltrated with metastases, or at the time of major cancer surgery. The live tumor samples obtained were mechanically disaggregated to obtain single-cell heterogenates (SCH). The SCH were then incubated at 36\(^\circ\)C / 5% CO\(_2\) for 48 hours in a humidified chamber to allow for equilibration. Following incubation, the SCH were washed, counted, and a small aliquot stained with trypan blue, to assess initial viability. Twenty thousand cells were added per analysis tube. The chemotherapeutic agents were added at peak plasma concentrations (Cmax), plus/minus DCA (at peak plasma concentration/Cmax), and incubated for 72 hours. After 72 hours, the SCH were washed and tagged...
with green fluorescein LIVE/DEAD® Fixable Stains for Flow Cytometry (Molecular Probes). The reactive dye can permeate the compromised membranes of dead cells and react with free amines on the interior and exterior of the cell, whereas only membrane-exterior free amines of viable cells are available to react with the dye, resulting in intense or dim staining, respectively. SCH in vitro chemotherapy response was determined using a Becton Dickinson FACScan flow cytometer* and SCH analyzed for percentage of live versus dead cell populations against a live non-drug control. A dead cell control was also used consisting of SCH placed at 56°C for 1 hour. *All specimens were high grade / metastatic tumors unless noted; no tumor was naïve; no tumor was a primary. 10,000 events were counted for each SCH aliquot.

Results: 27 solid-tumors were studied; 3 of 27 exhibited high*/intermediate sensitivity to DCA as a single agent; 7 of 27 exhibited high*/intermediate sensitivity to DCA in combination with chemotherapeutic agent(s). 9 of 27 exhibited no sensitivity to DCA as a single agent or in combination.

Results: Twenty seven solid-tumors were studied: 6 of 27 (22%) exhibited high*/intermediate sensitivity to DCA as a single agent; 7 of 27 (26%) exhibited high*/intermediate sensitivity to DCA in combination with chemotherapeutic agent(s). 9 of 27 (33%) exhibited low sensitivity to DCA as a single agent or in combination:

- 11% efficacy (HDS) as a single agent - 15% synergy (HDS) when in combination
- 15% efficacy (IDS) as a single agent - 22% synergy (IDS) when in combination
- 33% exhibited no sensitivity (LDS) to DCA as a single agent or in combination

Breast – 11/27 solid-tumors: 24% efficacy (HDS) as a single agent - 15% synergy (HDS) when in combination 6% efficacy (IDS) as a single agent - 24% synergy (IDS) when in combination 33% exhibited no sensitivity (LDS) to DCA as a single agent or in combination

Colon – 4/27 solid-tumors: 0% efficacy (HDS) as a single agent - 25% synergy (HDS) when in combination 25% efficacy (IDS) as a single agent - 50% synergy (IDS) when in combination

FIGURES INSERTED HERE

Results/Discussion: Early carcinogenesis occurs in a hypoxic microenvironment and thus the transformed cells initially have to rely on glycolysis for energy production. However, this early metabolic adaptation appears to also offer a proliferative advantage, suppressing apoptosis. Furthermore, the byproducts of glycolysis (i.e., lactate and acidosis) contribute to the breakdown of the extracellular matrix, facilitate cell mobility, and increase the metastatic potential. Therefore, even though the tumors eventually become vascularized and O₂ levels increase, the glycolytic phenotype persists, resulting in the “paradox” of glycolysis during aerobic conditions, the Warburg effect.; Even in the presence of oxygen, most tumor cells induce glycolysis and lactic acid production as their main energy source rather than mitochondrial oxidative phosphorylation without producing reactive oxygen species. It has also been shown that the Warburg effect is also involved in the avoidance of apoptosis. Alternatively and paradoxically, the Warburg effect might serve to increase the biomass to provide nucleotides and lipid material necessary for rapidly dividing cells. This theory is supported by the fact that signaling pathways like AKT/mTOR, are known to play a role in biomass production, which also control aspects of the Warburg effect. But, Michelakis et al, further demonstrates that this metabolic-electrical remodeling is an adaptive response and thus reversible.

The metabolic and the apoptotic pathways converge in the mitochondria and thus not independent from each other and therefore the glycolytic phenotype is associated with a state of apoptosis resistance. For example, many glycolytic enzymes have been recognized to also regulate apoptosis, and several oncoproteins induce the expression of glycolytic enzymes; AKT, stimulates glycolysis and induces resistance to apoptosis, via activation of hexokinase, an enzyme catalyzing the first and irreversible step in glycolysis. AKT also induces
the translocation of hexokinase to the mitochondrial membrane where it binds to the voltage-dependent anion channel (VDAC), suppressing apoptosis.\(^\text{11}\)

DCA’s mechanism of action is as an inhibitor of (pyruvate dehydrogenase kinase) PDK, therefore able to shift tumor cellular metabolism from glycolysis and lactate production to glucose oxidation in the mitochondria. It does so by reversing the inhibition and down-regulation of Kv1.5 in most cancers, but not normal cells. The result is efflux of K\(^+\), with a resultant decrease in intracellular K\(^+\), promoting the pro-apoptotic effects of DCA.\(^\text{4}\) We show that, as predicted, DCA does change the metabolism of cancer cells and thus effectively decreases tumor growth in vitro and in vivo in some of the tumors tested.

DCA also enters the cellswitching cancer promoting/inhibiting genes on or off, including mtDNA,\(^\text{However, DCA requires an ectopically expressed membrane transporter protein, SLC5A8, which is normally silenced.}\(^\text{12}\) SLC5A8 mediates acetate transport in a Na\(^+\)-coupled manner, with the affinity of dichloroacetate for the transporter ~45-fold higher than that of Na\(^+\),\(^\text{(dichloroacetate/ Na\(^+\)}\) stoichiometry for the transport process is 2:1.\(^\text{12}\) When it does so, it restores mitochondrial function by reversing the ionic remodeling of hyperpolarized mitochondria, thus restoring apoptosis. Several studies have indeed shown that DCA induces apoptosis, in a variety of cancer cell lines.\(^\text{13-14}\) However, a recent investigation was not able to confirm these findings.\(^\text{15}\) In correlation with our pilot studies we also observed that even though DCA was able to induce mitochondrial depolarization, we observed variable induction of apoptosis or necrosis when DCA was used as a single agent, or even as a chemosensitizer. Nonetheless, long and continuous in vivo exposure may be required as demonstrated by Khan’s unpublished data and as demonstrated by Bradford and Khan.\(^\text{16}\) Also, DCA may cause cell growth inhibition without causing apoptosis.\(^\text{17}\) Moreover, Stockwin et al\(^\text{15}\) demonstrate that a very high concentration of the compound (≥25 mM) was required to induce apoptosis, wherein our studies incorporated peak plasma concentrations.

A 1982 and 1988 paper by Chen, et al. show that rhodamine 123 accumulates by various cancers and normal cells. The rhodamine 123 molecule, carries a net positive charge, and as such is accumulated and retained in areas of the cell that are more negatively charged in greater amounts and for longer periods of time than in less negatively charged areas.\(^\text{18,19}\) Thus, retention of rh123 in the mitochondria of many carcinomas suggests that the mitochondria in such cells are hyperpolarized. Due to this biochemical property, Chen points to two types of cancer that do not retain rh123, sarcoma and oat cell lung cancer (SCLC). The 1988 paper also mentions as exceptions “large cell carcinomas of the lung” and “poorly differentiated carcinoma of the colon.” This is not definitive since there is certainly much variation among all types of cancer cells, but in light of the data contained in the Chen papers, and given the importance of the normalization of mitochondrial membrane potential to the apoptosis-inducing mechanism described by Michelakis,\(^\text{4}\) it seems reasonable to assume that sarcomas, and small cell lung cancers are unlikely to respond to DCA and perhaps partially explain the results of our current study. However, in clinical practice using DCA for over 7 years, Khan has observed both sarcoma and small cell lung cancers respond well, again highlighting the variability of individual tumor behaviors and the need to individualize therapy.

Our data indicated that DCA inhibited the sensitivity of many of the conventional agents used including those used on breast and brain tissue that we hypothesized would be effective as noted above by other research groups. It should also be noted that although we analyzed “fresh” tumor tissue and any components associated with the micromilieu, many research groups tested DCA on human cells cultured as well as cell lines.\(^\text{13-15}\) The issue of using fresh tissue versus cells lines / cells in cultures always presents with concern and relevance. Cell lines are homogeneous rather than representing the heterogenic milieu of the tumor mass. As such, results for a given therapeutic agent(s) may not represent the individual’s specific response and actually may
reflect false positive or false negative effects. Further, allowing cells to proliferate in vitro does not represent the original tumor mass and thus not reflect in vivo response dynamics.

As mentioned, not all of the tumors responded to DCA as a single agent or in combination with conventional agents. There are several possible explanations for this. It is possible that the resistant tumors do not express the membrane transporter protein SLC5A8, it is known to be silenced in many tumors and not ectopic, which has been shown to be required for DCA entry into the cancer cells. The tumor specimens analyzed were high grade / metastatic tumors and hence had prior exposure, if not multiple exposures, to drugs and radiation prior to analyses. It is also possible that the tumors have developed cross resistance to DCA as a result of prior treatment with multiple cytotoxic chemotherapy agents (induction of cross resistance) and hence down-regulation of the SLC5A8 transporter protein. Further, it has been shown that when the tumor bulk has not been effectively eradicated, the risk of recurrence and metastasis is high, and thus, the efficacy of DCA may be higher when it is administered in patients with low tumor burden. Thus, as mentioned above, most of our patient population was of high tumor burden explaining our subdued results. Another conjecture is that certain tumors may be able to utilize alternate fuels to generate ATP when glycolysis is shut down by DCA (e.g. ketone bodies or free fatty acids). Also, when using DCA the exposure time may have to be increased beyond the 72 hrs as shown by Khan. Finally, if DCA is cytostatic (growth inhibition without apoptosis), instead of cytolytic/cytotoxic a cell death assay will not detect this effect.

**Conclusion:** Our findings indicate a potential role for DCA in oncology therapeutics in a wide range of cancer types. However, the diversity of the tumor responses among organ-specific cancer types underscores the necessity to conduct clinical studies on an individual basis rather than with a “one-size-fits-all” approach. It is apparent that empirically selected chemotherapy has tremendous room for improvement, since the published response rates are low in many types of cancers, especially if metastatic, as toxicity is the main reason for the high failure rate (40-50%) of chemotherapeutics interventions. Personalized treatment has to be the focus. Since DCA had been used for years to treat rare metabolic disorders, it is known to be relatively safe, has a well-characterized pharmacodynamic profile with low side effects, and is low cost, making it an ideal candidate for development as an effective anticancer agent. The identification and stratification of patients to predict DCA benefit and response can easily be performed in vitro, prior to in vivo administration. However, randomized controlled clinical trials must be designed to further correlate and validate this preliminary pilot study in the oncology setting and to fully appreciate the impact of DCA on cancer recurrence, response rates and survival rates.

**REFERENCES**

7. Pfeffer, G; Majamaa, K; Turnbull, DM; Thorburn, D; Chinnery, PF. “Treatment for mitochondrial disorders”. In Chinnery, Patrick F. The Cochrane database of systematic reviews.4: 2012.


**Unless Noted:** DCA inhibited the conventional therapeutic drug; or no synergy was noted with the conventional therapeutic drug; or if synergy was LDS; or inhibition of both agents when combined. This is noted by the Dark Colored Histograms; Red Colored Histograms (X); Synergy (HDS) when conventional chemotherapeutic drug was combined with DCA; Blue Colored Histograms (X); Synergy (IDS) when conventional chemotherapeutic drug was combined with DCA.

**ABBREVIATIONS:** IDC = invasive ductal carcinoma, NSCLC = non-small cell lung cancer, *chlor-Chlorambucil; ix-Ixempra; lap-Lapatinib; lom-Lomustine; TMZ-Temozolomide; eto-Etoposide; met-Metformin; riba-Ribvirin; rapa-Rapammune; tam-Tamoxifen; cis-Cisplatin; tar-Tarceva; MTX-Methotrexate; dox-Doxorubicin; tax-Taxol; fem-Femara; chlor-Chloroquine; FU-Fluorouracil; mito-Mitomycin; vin-vinblastine; carbo-Carboplatin; gem-Gemcitabine; nav-Navelbine; iri-Irinotecan; oxi-Oxilaplatin; MTX-Methotrexate; dox-Doxorubicin; tax-Taxol; fem-Femara; chlor-Chloroquine; FU-Fluorouracil; mito-Mitomycin; vin-vinblastine; carbo-Carboplatin; gem-Gemcitabine; nav-Navelbine; iri-Irinotecan; oxi-Oxilaplatin; HD-High Drug Concentration = 10X Peak Plasma Concentration; LD = Low Drug Concentration = 50% Peak Plasma Concentration

**Our Un-published in vivo Data:**

1. 32 year old male, leg melanoma, treated with wide excision and inguinal node dissection, local recurrence and progressive inguinal lymphadenopathy post-op while receiving natural therapy only, CT proven partial response for over 1.5 years with oral DCA, and no concurrent conventional therapies.
2. 63 year old female, non-Hodgkins lymphoma treated with standard chemotherapy, marrow injury from chemo (stopped), progression while off treatment, CT-proven stable disease for 2 years while taking oral DCA and no concurrent conventional therapy
3. 80 year old male with transitional cell bladder carcinoma, recurrent disease after multiple resections and BCG, cysto proven tumour shrinkage with short course of oral DCA (6 weeks), re-treated after 1 year, delayed radical cystectomy for 4 years.

Dr. Khan illustrates that although our in vitro data expand 72 hours, in vivo, the clinical outcomes, reveal that as a single agent or in combination, time may be a more significant determinant of in vivo efficacy. Nonetheless in vitro predetermination of efficacy proved to be an invaluable in vivo indicator.

Many of the patients however, could not be followed

To determine if the in vivo responses matched the invitro results noted above.

The reasons were:

a) the patient's condition changed, and they were unable to take chemotherapy,
b) the patient's oncologist refused to prescribe the assay-guided therapy,
c) the patient was lost to follow-up.

**Maintrac CTC Assay (cells/ml of blood )**
50 year old female, lumpectomy for newly diagnosed breast cancer.

Pathology: invasive ductal carcinoma with lobular features, lymphovascular invasion, 3/5 sentinel node positive (2 macrometastases, 1 micrometastases)

Sample sent at that time for ChemoFit/CR assay; Patient was offered radiotherapy and AC-Taxol followed by tamoxifen—DECLINED

Treatment monitored with circulating tumor cell (CTC) count (Maintrac™ Bayreuth, Germany—laser microfluorimetry for human epithelial cell antigen-positive cells)

TREATMENT 1—oral DCA + oral MET; large reduction of CTC count (effective therapy); stopped due to grade 2 peripheral neuropathy (known side effect of DCA)

Neuropathy resolved, re-treated (2) with DCA 3750 mg iv 2x/wk + MET (DCA changed to iv for decreased neuropathy risk); rapid reduction of CTC count (effective therapy)

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CLINICAL RESULTS

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<th>DRUG% Killed/Sensitivity</th>
<th>3% LDS</th>
<th>18% LDS</th>
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<tr>
<td>Taxol</td>
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<td>Doxorubicin (Dox)</td>
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<td>Navelbine (Nav)</td>
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<td>Gemcitabine (Gem)</td>
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<td>Tamoxifen (Tam)</td>
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<td>DCA</td>
<td>21% LDS</td>
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<td></td>
<td></td>
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<tr>
<td>Dox / Taxol</td>
<td>0% LDS</td>
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<tr>
<td>Dox / Taxotere</td>
<td>3% LDS</td>
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<tr>
<td>5-FU / 0% LDS</td>
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**DCA / Metformin 98% HDS (strong synergism)**

**DCA / Tam 26% LDS**

**DCA / Carboplatin 93% HDS (strong synergism)**

**DCA / Cisplatin 12% LDS**

**DCA / Metformin / Tam 30% LDS (Tam inhibits DCA + metformin)**

**Carbo / Metformin 26% LDS**

**Gem / Metformin 0% LDS**

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**Maintrac CTC Assay (cells/ml of blood)**

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<td>Nov 13</td>
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DCA + LHRH + AI  
DCA + MET + LHRH + AI
AUTHOR'S BIOGRAPHY

Dr. Bradford has a PhD in Medical Biochemistry-Oncology from the Roswell Park Cancer Institute, Division of SUNY at Buffalo School of Medical and Biomedical Sciences. She is the founder of AccuTheranostics and the inventor of the various patents. AccuTheranostics is an affiliate of the University of NY at Buffalo. She was awarded the Award for Excellence in Research from the American Federation for Clinical Research and Roswell Park Cancer Institute, has authored many scientific papers and book chapters, and been an invited speaker at various international scientific meetings. She is a member of many major professional cancer organizations including, ASCO, AACR and AAAS. As Chief Scientific Officer, she guides the day-to-day research directives as well as the clinical laboratory operations. She specializes in the field of cancer research and treatment, determined to make a meaningful difference in the lives of those faced with the disease. Her overall vision is to develop a state-of-the-art clinical laboratory to empower patients in determining and deciding their course of therapy eventuating into a positive outcome concurrent with quality-of-life; demonstrated through her initiatives of research individualized personal medicine assessment technologies.

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