REVIEW ARTICLE

A 15 Year Evolution of Dichloroacetate-Based Metabolic Cancer Therapy: A Review with Case Reports

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OBJECTIVES

- Introduce and review the metabolic theory of cancer (history and background)
- Explain the application of metabolic theory to cancer therapeutics
- Introduce the concept of a metabolic multi-targeted approach to cancer therapeutics
- Use case reports to illustrate the clinical viability of the multi-targeted approach to cancer therapeutics

ABSTRACT

Despite Otto Warburg's discovery of aerobic glycolysis in cancer cells in the 1920's, the potential for developing therapeutics that targeted cancer cell metabolism was essentially ignored until 2007 when a groundbreaking publication was released from a group of Canadian researchers. Bonnet et al. (who paradoxically were not specialized in oncology) discovered that the generic drug dichloroacetate sodium ("DCA") could reverse the Warburg phenotype in cancer cells *in vitro* and *in vivo* resulting in natural cancer cell suicide and tumour shrinkage in rats. This phenomenon was previously thought to be impossible as it was believed that mitochondria in malignant cells were permanently altered and unable to trigger apoptosis. Despite the fact that no large clinical trial of DCA as a cancer therapy was ever completed, a small number of doctors in North America and Europe rapidly translated this new knowledge into clinical cancer protocols through independent observational research and creative thinking.

Since off-label drug use is permitted in most jurisdictions, clinicians initially began to use DCA in patients who had failed all conventional therapies. Over the years, further novel anti-cancer mechanisms of DCA were discovered such as angiogenesis inhibition, immune activation and cancer stem cell targeting. Around 2011, the work of Seyfried (USA) began to illuminate the importance of glutamine inhibition and suggested that a multi-energetic targeted approach was superior to glycolysis inhibition alone.

A collaborative effort of the authors incorporating Seyfried's concepts resulted in the creation of a new metabolic protocol named "MOMENTUM" (**M**etabolic, **O**ncologic, **M**ulti-**EN**ergetic **T**argeted, **U**niversal, **M**odified). In this protocol, glucose and glutamine metabolism were targeted simultaneously with a combination of multiple natural and pharmacologic agents administered intravenously. Surprising preliminary clinical results in several difficult cancer cases confirmed that metabolic multi-targeted methods are extremely promising, and more so than metabolic monotherapy. Life threatening side effects of this approach to cancer management are virtually non-existent and therapy costs are manageable.

A disappointing absence of industry funding for large clinical trials has not curtailed the development of the metabolic approach as a clinically viable methodology, proving that unadulterated medical science can conquer the ongoing push for multibillion-dollar economic reward.

Keywords: dichloroacetate; cancer; glycolysis; Warburg; glutamine; mitochondria; metabolic; apoptosis

Warburg and The Birth of Metabolic Cancer Therapy

In the 1920s Dr. Otto Warburg proposed that cancer cells exhibit mitochondrial dysfunction which results in an abnormal metabolic phenotype of glycolysis (conversion of glucose to lactate without oxygen or anaerobic respiration or fermentation) even in the presence of adequate oxygenation[1] . This phenomenon is now known as the Warburg effect. Since healthy non-cancerous cells prefer aerobic respiration (glucose oxidation or oxidative phosphorylation) due to the efficiency of adenosine triphosphate ("ATP") generation, the Warburg effect promised to be a selective method of targeting cancer cells. Unfortunately, the Warburg effect was largely ignored for decades, perhaps related to scientific inertia[2]. Instead, the oncology community chose to focus on treatments based on cytotoxic chemotherapies despite their serious side effects and poor overall success rates[3].

The Warburg effect was revisited in the 1980s when fluorodeoxyglucose positron emission tomography ("FDG PET") scan was developed[4]. The FDG PET scan took advantage of the enhanced glucose uptake of cancer cells as compared to healthy cells. Since aerobic glycolysis in cancer cells is inefficient at generating ATP (relative to oxidative phosphorylation) more glucose is required by cancer cell than normal cells[5]. This allowed cancer cells to be selectively imaged using radioactive glucose. FDG PET scans proved to be useful for almost all cancer types which suggested that metabolic cancer therapy could be similarly universal.

In 2000 Hanahan and Weinberg re-invigorated Warburg's idea of metabolic cancer targeting in their paper "Hallmarks of Cancer: The Next Generation". They referred to 6 previously-described hallmarks of cancer (sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion / metastasis), but more importantly, added two more: reprogramming of energy metabolism and evasion of immune destruction[6].

Despite successful use of FDG PET scans for cancer imaging, and the Hanahan and Weinberg publication, therapeutic use of the Warburg effect was not realized until 2007. Bonnet, Archer et al. published a paper which would become the foundation of metabolic cancer therapeutics[7]. It was demonstrated that an off-patent drug called dichloroacetate sodium ("DCA") could reverse the Warburg effect in a range of cancer types (breast, lung and brain) *in vitro* and *in vivo* (rats). The mechanism of action of DCA was proven to be inhibition of the enzyme pyruvate dehydrogenase kinase ("PDK") which effectively caused a shift from glycolysis to glucose oxidation, resulting in depolarization of the mitochondrial membrane, activation of caspases and triggering of apoptosis. Tremendous excitement was generated worldwide by this publication since it elucidated a novel systemic method of treating cancer with an old non-toxic drug that could potentially be used for any cancer type.

Unique Features of Dichloroacetate Metabolic Cancer Therapy

Immediately after the landmark 2007 publication was released, physicians began exploring the viability of DCA to treat cancer patients. DCA was a particularly promising metabolic therapy due to several unique features of this chemical:

1) it was a simple chemical resembling a fusion of table salt and vinegar, which could be easily manufactured;
2) it had been studied before in humans by Stacpoole et al. (USA) and was shown to have mild reversible side effects[8];
3) it was demonstrated safe for long-term use[9];
4) it had high oral bioavailability, close to 100%[10];
5) it was off-patent and thus relatively inexpensive;
6) it had potential for use in children due to prior published pediatric research [11] , thus raising a potential to reduce the suffering of pediatric patients and their parents who are continually looking to avoid harmful drug side effects;
7) it appeared to be far safer than cytotoxic chemotherapy, which potentially could open new options for the elderly and other patients too frail for chemo.

In early 2007, one of the authors (Khan) began using DCA for cancer patients who had exhausted all conventional therapies, with dosing based on the prior clinical trials of DCA as a treatment for congenital lactic acidosis. Systematic observation and data collection of 179 patients treated with DCA revealed over 60% of advanced-stage cancer patients had measurable short-term symptomatic and/or objective improvements. Thus, it was concluded that DCA was a viable cancer therapy in humans[12].

Almost immediately, there was backlash from the medical community. Concerns were raised that doctors and patients using unapproved DCA outside of clinical trials would somehow interfere with recruitment of patients into trials[13]. Curiously, the scientists critical of the early off-label use of DCA

seemed to be oblivious to the viewpoint of cancer patients who were given a death sentence due to exhaustion of approved therapy options. Still other scientists recognized that the discovery of DCA as a metabolic cancer therapeutic might be the "dawn of a new era"[14].

Observational Data Emerges from Around the World

Within 3 years of the Bonnet DCA publication, human data began to emerge. In 2010, the world's first clinical case report demonstrating that DCA had anti-cancer activity in humans was published (Flavin, USA/Germany).[15] It was demonstrated that metastatic medullary thyroid cancer was reversed to the point of radiologic and biochemical remission using oral DCA therapy. In 2011, the world's first clinical case report was published that demonstrated DCA's anti-neoplastic actions could demonstrably improve quality of life by dramatic and sustained pain reduction in a patient with cancer of unknown primary (Khan, Canada).[16] In 2014 the world's first case series of successful cancer therapy in a range of tumor types using intravenous DCA was published (Khan et al., Canada/USA)[17]. No doubt these exciting human publications provided an incentive to scientists around the world to continue research into DCA to learn more about its use as a cancer therapy.

Many new DCA publications began to appear which illustrated the activity of DCA in a large number of cancer types in addition to those originally identified in the 2007 Bonnet publication (breast, lung, glioblastoma). These included bile duct / cholangiocarcinoma[18], bladder[19], colon[20], gastric[21], head and neck (squamous cell)[22], leukemia[23], liver[24], lymphoma[25], medulloblastoma[26], melanoma[27], multiple myeloma[28], neuroblastoma[29], neuroendocrine[30], ovarian[31], pancreatic[32], prostate[33], renal[34], sarcoma (osteosarcoma[35], angiosarcoma[36], rhabdomyosarcoma[37], fibrosarcoma[38]), thyroid[39], uterine (endometrial[40], cervical[41]) and Wilm's tumour[42]. Further exciting data revealed synergism between DCA and radiotherapy[43,44] and also chemotherapy[45,46,47]. The first human data illustrating the potential for strong synergism of DCA and radiotherapy was published in 2012 in a case report of long-term complete remission of a patient with a rare metastatic renal squamous cell carcinoma[48].

New publications also elucidated new mechanisms of action of DCA including angiogenesis inhibition[49], immune enhancement by reduction of tumor lactate production[50] and cancer stem cell targeting[51]. It was also established that DCA could not only kill cancer cells but could act as a cytostatic agent in humans. Two cases were published (Khan et al., Canada/Australia) to illustrate this new mechanism: one case of a patient with metastatic colon cancer who stabilized for nearly 4 years[52] and one case of a metastatic melanoma patient who stabilized for over 4 years[53].

In 2012 a case report was published (Ishiguro et al., Japan) of successful treatment of a 51-year-old patient with chemo-resistant metastatic cholangiocarcinoma using DCA in combination omeprazole and tamoxifen[54]. This publication proved to be another useful addition to the body of case reports supporting the clinical benefits of DCA, illustrating potential advantages over chemotherapy in treating a rare cancer type.

The Importance of Glutaminolysis Inhibition

Despite the clinical success of the metabolic approach using DCA, the results were not perfect. Some patients were resistant to therapy from the beginning and others developed resistance over time. It was noted that resistance could develop quickly in some cases or could take years[55]. The work of Seyfried provided a clue as to one possible resistance mechanism. In addition to his work on ketogenic diet[56], Seyfried determined that glutamine targeting using the drug 6-diazo-5-oxo-L-norleucine ("DON") would be an important addition to glycolysis inhibition since cancer cells could use the amino acid glutamine as an alternate fuel source if glucose was not available[57]. DON seemed like an ideal glutaminolysis inhibitor since it had an established human safety profile based on earlier clinical trials. However, it was not readily available as a pharmaceutical grade product.

Oxaloacetate - A Natural Glutaminolysis Inhibitor

One of the authors (Ghen) became aware of new evidence of the potential for cancer cell lines to have amino acid fermentation capabilities (glutamine[58], serine[59], arginine[60]). He proposed the addition of high dose oral and/or intravenous oxaloacetate ("OA") as a potent natural glutaminolysis inhibitor. OA, also known as 3-carboxy-3-oxopropanoic acid, is an intermediate of the Krebs citric acid cycle. Oxaloacetate is ubiquitous in the mitochondria and cellular mitochondrial density is tied to oxaloacetate concentration[61]. OA has inherent capability as a glutamate scavenger[62] (glutamate being the metabolite of glutamine), making it unique for preventing amino acid fermentation to succinate, as seen in multiple cancers. OA inhibits the amino acid fermentation pathway, and reverses the Warburg

effect[63] making it an ideal adjunct to DCA. OA combines with glutamic oxaloacetic transaminase ("GOT") which breaks down glutamate into 2-keto glutamate and aspartate[64,65,66]. The glutamate inhibitory effect is dose-dependent and can sequester and significantly reduce the deleterious activity of glutamate. In addition, due to its position in the Krebs cycle, OA can increase the ratio of oxidized to reduced nicotinamide adenine dinucleotide (NAD+/NADH ratio)[67]. In experiments with lower animals, OA has been shown to increase activation of sirtuin genes with subsequent improvement in lifespan[68].

Ghen developed and began using a buffered sterile solution of OA intravenously as part of his metabolic protocols in cancer patients to reduce free glutamate levels and help to reverse the cancer Warburg metabolic phenotype. In a small clinical trial, OA has been used successfully in the treatment of brain cancer with chemotherapeutic and targeted agents including temozolomide, everolimus, cabozantinib, carboplatin, nilotinib, and bevacizumab[69,70,71]. In gliomas, gain of function mutations occur in isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) which produce D-2 hydroxyglutarate ("D-2-HG"). OA is a competitive inhibitor of D-2-HG. Use of OA is extremely safe and doses up to 3000mg/day have been shown to have no serious adverse effects in humans[72]. No toxicity of OA was observed in mice after an oral dose of 5000mg/kg. LD50 was not reached but reported conservatively as "> 5000mg/kg" (Method 425 OECD Acute Oral Toxicity Test, Oxaloacetic Acid, Test Performed May 2, 2006, Cash A, Terra Biological, San Diego, CA, USA). The OA molecule has a half-life *in vivo* of under 2 hours[73].

The MOMENTUM DCA Protocol

The use of OA proved to be an elegant solution to the lack of availability of DON, and seemed to be superior since OA is a natural product with no significant side effects. Due to a need to combine therapeutics to overcome potential resistance, the authors added nicotinamide adenine dinucleotide ("NAD+") to their metabolic therapy cocktail. NAD+ has been demonstrated to inhibit glycolysis by inhibiting PDK[74], and also to stimulate a T-cell response against malignant cells[75]. An increase of the NAD+ to NADH ratio has also been shown to reprogram cancer cell populations back to normal metabolism. Ozone therapy also appears to be a useful addition to the protocol due to its ability to enhance anti-cancer immunity[76]. The combination of DCA, OA and NAD+ was termed "MOMENTUM" DCA metabolic therapy. The derivation is as follows:

Metabolic – referring to targeting of cell metabolism
Oncologic – referring to cancer cells as the focus of metabolism inhibition
Multi-Energetic - referring to inhibiting multiple energetic pathways simultaneously
Targeted – referring to selective targeting of cancer cells (sparing healthy cells)
Universal – referring to the applicability of the therapy to potentially all cancer types
Modified – modified from the original DCA monotherapy approach

New observational findings (Khan) revealed the first 7 out of 8 consecutive patients treated with the intravenous MOMENTUM protocol (DCA 60-70mg/kg + OA 300mg + NAD+ 100mg infused 2 days per week) responded rapidly (within 2 to 6 weeks) with objective stabilization /reduction / necrosis of tumors and/or reduction of serum cancer markers, confirming the exciting potential of this protocol. Selected cases are presented to illustrate the findings:

Case 1 - 79 year old male with Stage 3 circumferential gastric cancer invading through stomach wall, declined surgery and chemotherapy due to high risk of complications, treated with MOMENTUM DCA protocol, reduction of thickness of cancer by more than 50% in under 2 months measured by pre- and post-treatment CT scans. Therapy was combined with intravenous ozonated saline and 2 anti-parasite medications that influence multiple molecular targets in cancer cells.

Case 2 – 52 year old female with stage 4 melanoma metastatic to lungs, progressing on serial CT scans during treatment with low dose naltrexone, stabilization of lung tumours after only 6 weeks of MOMENTUM DCA infusion therapy (confirmed by pre- and post-treatment CT scans).

Case 3 – 46 year old male with multifocal glioblastoma, resistant to temozolomide, MRI done immediately before starting MOMENTUM DCA therapy, MRI repeated after only 1 month of infusions showed necrosis ("treatment effect") which was attributed to radiation by the radiologist. However, the patient did not have any radiation, only MOMENTUM DCA therapy.

Case 4 - 56 year old female with left breast cancer metastatic to bone. The patient initially had sternal and rib pain secondary to the metastatic lesions. MOMENTUM DCA treatment was started in

combination with olaparib. All previous elevated cancer markers returned to normal, PET/CT scan demonstrated complete remission, and the patient became pain-free. Inflammatory markers including interleukin levels were normalized.

The current updated intravenous adult cocktail (2023) includes DCA ranging from 50-70mg/kg followed by OA 1000mg and NAD+ 200mg. Infusions are given twice per week. In summary, oxaloacetate has been shown to reverse the Warburg effect, inhibit glutamate, activate the Forkhead Box O3 tumor suppressor protein ("FOXO3") inducing differentiation, increase the NAD+/NADH ratio and synergize with NAD+ infusion.

Future Directions

The observational findings described serve to confirm Seyfried's concept that attacking multiple metabolic cancer targets is required for the best clinical outcomes. On this basis, the authors are now exploring the addition of fatty acid oxidation inhibitors (such as beta-hydroxybutyrate [77] or ranolazine[78]) to the cocktail. Beta-hydroxybutyrate is a ketone body that has been shown to inhibit fatty acid oxidation in cancer cells, and also has the potential to be a simpler alternative to the ketogenic diet. Ranolazine is an anti-anginal drug that also inhibits fatty acid metabolism.

Another aspect to be explored is the addition of drugs to enhance the activity of the membrane transporter SLC5A8 as a method to boost entry of DCA into cancer cells, and thus reduce the required serum concentrations[79]. This could allow lower doses to be used which would minimize side effects. An additional method of increasing the anti-cancer effects while reducing side effects could be the use of a nanoparticle delivery system for DCA. This could allow more selective delivery of DCA to cancer cells while reducing the exposure of nerve cells to provide protection against neuropathy, which is a dose limiting side effect of DCA. Dosing of DCA is currently calculated on a mg/kg basis. This method is limited because it does not consider the inter-individual variation in hepatic DCA metabolism by the enzyme glutathione transferase zeta ("GSTz"). Based on the work of Stacpoole[80], the authors believe it would be ideal to dose DCA based on pharmaco-genomics (identifying DNA sequences of GSTz variants) or pharmaco-epigenomics (identifying gene expression of GSTz variants).

Conclusions

Based on the theory and observational findings to date, metabolic therapy (or perhaps it is more correctly labelled "immuno-metabolic therapy") is not only a viable cancer treatment strategy, but it is the way of the future. Cancer patients should remain hopeful that ongoing clinical success and published observational data may serve as further encouragement to research scientists around the world to continue advancing this exciting field of cancer therapeutics.

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