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Glucose Metabolism in Cancer and Ischemia: Possible Therapeutic Consequences of the Warburg Effect

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ABSTRACT

The Warburg effect states that the main source of energy for cancer cells is not aerobic respiration, but glycolysis—even in normoxia. The shift from one to the other is governed by mutually counteracting enzymes: pyruvate dehydrogenase and pyruvate dehydrogenase kinase (PDK). Anaerobic metabolism of cancer cells promotes cell proliferation, local tissue immunosuppression, resistance to hypoxic conditions, and metastatic processes. By switching glucose back to oxidative metabolism, these effects might be reversed. This can be achieved using PDK inhibitors, such as dichloroacetate. Patients suffering from ischemic conditions might benefit from this effect. On the other hand, the β-blockers (adrenergic β-antagonists) often used in these patients appear to improve cancer-specific survival, and nonselective β-blockers have been shown to promote glucose oxidation. Might there be a link?

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Introduction

The interest in tumor metabolism is probably as old as oncology itself, and so is the realization that the metabolism of cancerous tissues differs significantly from that of their healthy counterparts. One of the hallmarks of tumor metabolism is the so-called Warburg effect, which was named after its discoverer, the German physiologist and physician Otto Heinrich Warburg. As early as 1924, Warburg hypothesized that the main source of energy for cancer cells was not aerobic cell respiration, but glycolysis, which is also known as glucose fermentation (1). This immediately seems to be counterintuitive—it is well known that oxidative metabolism accounts for the majority of energy produced in the cell, through oxidative decarboxylation of pyruvate and the entry of the resulting products into the Krebs cycle. Assuming that the reduced coenzymes are oxidized by the electron transport chain and used for oxidative phosphorylation, the 28 out of 30 molecules of ATP yielded from one molecule of glucose through the entirety of cell respiration come from its oxidative phases. The decarboxylation of pyruvate, which results in the formation of acetyl-CoA, NADH, and CO_2, is an irreversible reaction due to its highly negative energy delta; therefore, this must be very tightly regulated, as is any irreversible reaction in physiology. The major site of this regulation is the pyruvate dehydrogenase (PDH) enzyme complex, which regulates the decarboxylation of pyruvate and its conversion to acetyl-CoA and CO_2. PDH is activated through dephosphorylation by PDH phosphatase and inactivated through phosphorylation by the enzyme pyruvate dehydrogenase kinase (PDK), which comes in four known isoforms: PDK1, PDK2, PDK3, and PDK4. These isoforms show varying activities and abundance in different tissues of the body (2), and their ratios also appear to be altered in cancer cells (3). The interactions between these enzyme complexes are what ultimately

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determine the fate of the glycolysis products, as fuel for oxidative metabolism or for the use of the glycolytic intermediates in the synthesis of nucleotides, lipids, amino acids, and NADPH—the building blocks of new cells. This is precisely the switch a cancer cell must make: from the production of energy for physiological processes in the service of the body in which they reside to furthering their own agenda of limitless growth and proliferation that is so typical of cancer cells. An anaerobic metabolism is typical for cancer cells; however, this feature does not only concern them. Indeed, anaerobic metabolism is typical of many physiological and pathological states in which the oxygen supply is insufficient. This ranges from perfect physiological conditions, such as those in skeletal muscle during exercise, to diseases that lead to tissue ischemia, such as heart failure (4), peripheral vascular disease (5), and many others. It is crucial to differentiate between the Warburg effect that leads to anaerobic metabolism even in the presence of ample oxygen and to anaerobic metabolism that is caused by a lack of oxygen. However, many molecular mechanisms are shared between these processes and can be, to some extent, influenced in similar ways. Although we still think of the Warburg effect as specific to cancer, some of the more recent research has shown that it might also occur in normal cells that are undergoing accelerated proliferation (6).

The Warburg Effect in Cancer

It has long been observed, and even used for diagnostic purposes, that glucose uptake by cancer cells is greatly increased. Positron emission tomography (PET), scanning with the tracer fluorine-*18* (F-18) fluorodeoxyglucose (FDG) (18-F FDG PET), evaluates the uptake of glucose by cancer cells and thus allows the imaging of malignant tumors (7). This insatiable hunger for glucose enables such cells not only to generate enough energy in spite of the low efficiency of glycolysis, but also to amass a great abundance of molecules that are needed to produce more cells. This promotes their proliferation potential, which is something that all oncologists know about and attempt to combat, with still far too limited results. It is therefore important to understand how the Warburg effect works in cancer cells, to identify possible therapeutic targets.

Due to the anatomical properties of tumors, their microenvironment is often hypoxic. Rapid tumor growth is often not accompanied by sufficient angioneogenesis, and therefore, parts of tumors are often left without sufficient oxygen supply. Although the idea that tumor cells are naturally selected for their ability to thrive without oxygen offers an attractive explanation for the Warburg effect, this does not explain why even cancer cells that are exposed to ample oxygen during tumorigenesis, such as those in lung tumors (8) and leukemia (9), appear to prefer glycolytic metabolism. These changes do not appear to happen by chance. It has been shown that activation of proto-oncogenes [e.g., MYC, NF-κB, AKT, tyrosine kinases (10)], signaling pathways (e.g., PI3K), and transcription factors [e.g., hypoxia-inducible factor (HIF-1)], as well as inactivation of tumor suppressors (e.g., p53, PTEN) (11) and changes in enzyme isoform ratios [e.g., pyruvate kinase, hexokinase, lactate dehydrogenase (12)] can induce the Warburg effect in cancer cells. The increased production of lactate leads to an acidic tumor microenvironment that inhibits T-cell functions (13) and reduces cell adherence (14), which results in immunosuppression and promotes tumor metastasization. The Warburg effect and the molecular pathways that lead to it are relatively specific to cancer cells. Additionally, these appear to have devastating consequences on tumor and metastasis propagation, and can be seen as one of the causes. All of this makes the Warburg effect an interesting target for novel therapeutic strategies. Some such strategies already exist, and others are still in development.

Inducing Oxidative Metabolism

Dichloroacetate

Dichloroacetate (DCA) has been used in the treatment of hereditary lactic acidosis. Its short-term side effects are minimal, even in children in whom it has mainly been used (15). At chronically administered higher doses ($\geq$25 mg/kg/day taken orally), there is increased risk of several reversible toxicities, including especially peripheral neuropathy, neurotoxicity, and gait disturbance (16). DCA is a small molecule of about 150 Da that is highly bioavailable, and it readily crosses the blood-brain barrier. Its works through inhibition of PDK, which stimulates PDH activity. This causes the products of glycolysis to enter the mitochondria and undergo oxidative metabolism, instead of undergoing fermentation and producing lactate (16). Proapoptotic mediators, like cytochrome C and apoptosis-inducing factor, are contained inside the mitochondria. If there is entry of pyruvate into the mitochondria and thus suppression of the production of acetyl-CoA, the mitochondrial transition pore will not open and the proapoptotic mediators will remain locked inside the mitochondria (17). Stimulation of the entry of pyruvate into the mitochondria by DCA reverses this process and causes apoptosis. Indeed, the evidence that DCA actually kills cancer cells and inhibits tumor growth by inducing apoptosis is very strong. This has been demonstrated in breast and colorectal cancers

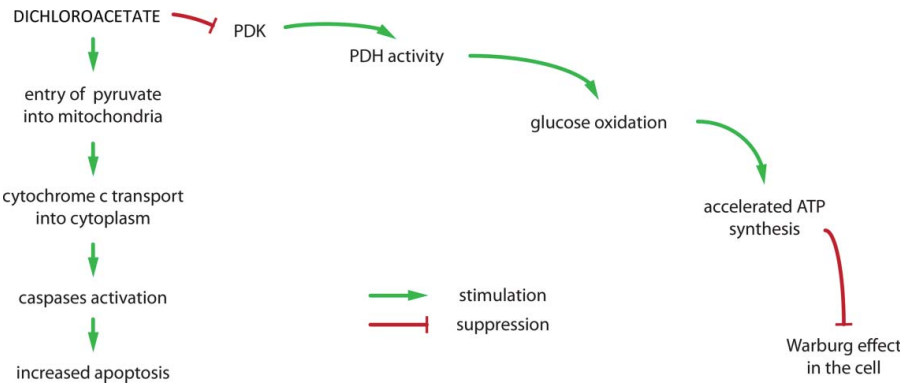

Figure 1. Possible mechanisms of dichloroacetate in the inhibition of carcinogenesis. PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase.

and in glioblastoma and other forms of cancer (18–25). The effects of DCA on apoptosis and cancer cell metabolism are shown in Fig. 1.

One of the well-known "disadvantages" of DCA is that it is a simple molecule that was discovered in the 19th century, and as such it cannot be patented [although its use as an anticancer agent has been patented (26)], so it offers poor prospects of profit for drug manufacturers. In addition to promising results in stage II clinical trials, there was a case report of a patient whose non-Hodgkin lymphoma relapsed after therapy with rituximab-CHOP (chemotherapy is made up of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone). The patient was treated with DCA 1,000 mg per day monotherapy, as one daily dose. At 71 days into the DCA protocol, complete resolution of all systemic symptoms had occurred, and the patient reported no significant side effects. Complete remission was documented by FDG PET, and this continued though the entire follow-up period of 4 yr without any therapeutic intervention other than continued use of DCA, although at a lower dosing frequency (20). It should also be mentioned that although the results of some phase II clinical trials of DCA have been mixed, the candidate selection for these studies was often limited to the terminally ill, whose nutritional and general conditions might have interfered with their survival, even if the cancer therapy they received was ideally successful for the eradication of the disease. As a very cheap, easy to produce, safe, and well-tolerated drug, DCA should definitely be further investigated as a possible cancer treatment, both in monotherapy and as part of multidrug regimens, which have proven to generally be the most successful treatment strategies in oncology.

However, if DCA improves lactate consumption and promotes oxidative metabolism, might it also be used in the treatment of conditions that result from chronic ischemia, such as chronic heart failure? Most studies that have investigated this question were performed in the 1990s, and more modern studies are lacking. One of these concluded that DCA stimulates myocardial lactate consumption, improves left ventricular mechanical efficiency, and significantly increases forward stroke volume and left ventricular minute work, with simultaneous reduction in myocardial oxygen consumption (27). On the other hand, short-term infusion of DCA did not improve noninvasively assessed left ventricular function (28) or reduce muscular fatigue during exercise in chronic heart failure patients (29). A metabolic study showed that pharmacological PDH activation (through PDK inhibition) accelerated the rate of mitochondrial ATP re-synthesis and improved the maintenance of contractile function throughout the rest-to-work transition, under both ischemic and nonischemic conditions in canine and human muscle (5). These results suggested that DCA might be useful in the treatment of chronic ischemic conditions, although its long-term use in such patients has not been studied. In addition, since the 90-day toxicity study performed in beagle dogs in 1991 that failed to establish a "no-adverse-effect level," DCA has mostly been treated as an experimental laboratory drug. With the addition of the lack of economic incentive for the manufacture of such a low-cost compound, this is hardly surprising. However, with the emergence of novel, tissue-specific, PDK inhibitor targets (2,30), safer and also more patentable drugs that take advantage of these mechanisms might be produced.

β-Blockers

β-Adrenergic receptor antagonists, which are better known as *β*-blockers, have been used for decades in the treatment of arterial hypertension, arrhythmia, and heart failure, and for secondary prevention of myocardial infarction and numerous other (mainly cardiovascular) conditions. Although the blockage of *β*-adrenergic receptor signaling has been shown hemodynamically and also energetically beneficial in the treatment of myocardial failure, the effects of *β*-blockers on the Warburg effect are still unclear. In this regard, numerous studies have shown that

β-blockers appear to improve cancer-specific survival (31–37). It has been demonstrated that selective and nonselective β-blockers reduce the resting metabolic rate, which is also known as the energy production rate (38). Nonselective β-blockers appear to shift total body substrate use from fatty acid to glucose oxidation (4). In this regard, Wallhaus et al. (39) demonstrated a 57% reduction in myocardial free fatty acids uptake following treatment with carvedilol, a nonselective β-blocker, in patients with heart failure. However, neither mean myocardial uptake of labeled glucose tracers nor the rate of glucose utilization increased significantly in this relatively small study. In another study, a possible effect of β-blockers on substrate metabolism in mouse C2C12 cells has been evaluated. Carvedilol inhibited palmitate oxidation and increased glycolysis by nearly 50% (40). As less oxygen is needed for the oxidation of glucose than for the oxidation of fatty acids, this has a favorable effect on myocardial oxygen demand in heart failure. In a recent clinical study, Contenti et al. (41) hypothesized that the activation of glycolysis through β-adrenergic stimulation by endogenous catecholamines plays an important role in lactate production and that long-term β-blocker therapy could affect the lactate concentration in patients with severe sepsis and septic shock. Authors concluded that long-term therapy with β-blockers decreased blood lactate concentration of severely ill septic patients. In another study, selective β-blocker metoprolol was shown to increase lactate uptake in heart failure patients, which is consistent with an increase in carbohydrate oxidation (42). Sharma et al. demonstrated that short-term perfusion with metoprolol inhibited fatty acid oxidation and produced marked stimulation of glucose oxidation in both healthy and diabetic hearts in rats and was associated with a decrease in lactate production, reflecting a marked improvement in glycolytic/glucose oxidation coupling and an increase in tissue ATP levels (43). The molecular mechanisms by which nonselective β-blockers promote glucose oxidation are not known, but it has been demonstrated in mice that the receptor NOR-1 (orphan nuclear receptor-1), which is a target of β-adrenergic signaling, regulates the expression of genes that encode proteins that control oxidative metabolism, such as PGC-1α (peroxisome proliferator-activated receptor gamma coactivator *1*-alpha), lipin-1α, FOXO1 (forkhead box O1), and PDK4 (44). This last, PDK4, is an isoform of the aforementioned PDK that is directly involved in the regulation of the entry of glycolysis products into oxidative metabolism. This is also one possible explanation why only the nonselective β-blockers appear to influence the shift of metabolism to glucose oxidation—because they do not only interact with the target β1-adrenergic receptors.

According to Levine and Puzio-Kuter (10), 9 of the 10 glycolytic enzymes are among HIF-1α-regulated genes. HIF-1α inhibits mitochondrial oxygen consumption by inducing PDK (45). Since HIF-1α is a substrate of Von Hippel–Lindau tumor suppressor, this ubiquitin ligase contributes to the regulation of glucose metabolism in tumor cells under hypoxia conditions. It was demonstrated that propranolol, nonselective blocker of the β-adrenergic receptor, significantly decreased the expression of the HIF-1α in serum and urine, as well as in hemangioma tissues in infantile hemangioma patients. Similarly, in vitro analysis revealed that propranolol reduces the expression of HIF-1α in hemangioma cells in a dose- and time-dependent manner, mainly by acting on β2-adrenergic receptor (46). Data of the same authors showed that propranolol inhibited the signal transducer and activator of transcription 3 (STAT3)—a critical oncogenic signaling molecule, the antiapoptotic protein Bcl-2, and VEGF. A key glycolytic enzyme in cancer cells is the phosphofructokinase isoform PFKFB3. This enzyme represents an oncogenic factor that stimulates both glycolysis and cancer cell proliferation. Based on experimental evidence mentioned below, the blockade of β-adrenergic receptor function can suppress the activity of PFKFB3 through the inhibition of HIF-1α and thus manifest anticancer action. Telang et al. (47) suggested that inhibition of the PFKFB3 gene would be useful in inhibiting the growth of cancer cells. Similarly, Calvo et al. (48) reported that silencing the PFKFB3 gene decreased glycolysis and inhibited growth of HeLa cells.

Although adrenergic stimulation has been shown to increase glucose oxidation rates, β-blockers have been documented to inhibit fat oxidation and result in an increase in glucose oxidation. This effect could be attributed to the inhibition of carnitine palmitoyltransferase (CPT)-1 (49). It is unclear whether the repression of transcriptional master regulator PGC-1α, most likely occurring as a consequence of the improved heart function, is unique to β-blockers, although repression of CPT-1 has not been reported with other drugs which improve function. The inhibition of CPT-1 would be expected to decrease the utilization of fatty acids from all sources. In the heart, the major mechanism by which CPT-1 is regulated is through modulation of malonyl-CoA levels. CPT-1 stably interacts and is directly controlled by phosphorylation induced by cAMP-protein kinase A (PKA) pathway as an effector of the β1-adrenergic receptor signaling (50). Further research from the same laboratory revealed a range of covalent modifications, which can regulate CPT-1 directly through a signaling at the level of the mitochondria, moreover, an important interaction between β-adrenergic signaling and caveolins (51). Molecular targets of β-blockers in cancer cell metabolism are summarized in Fig. 2.

Based on preclinical and clinical data, it can be concluded that the blockers of β-adrenergic receptors could

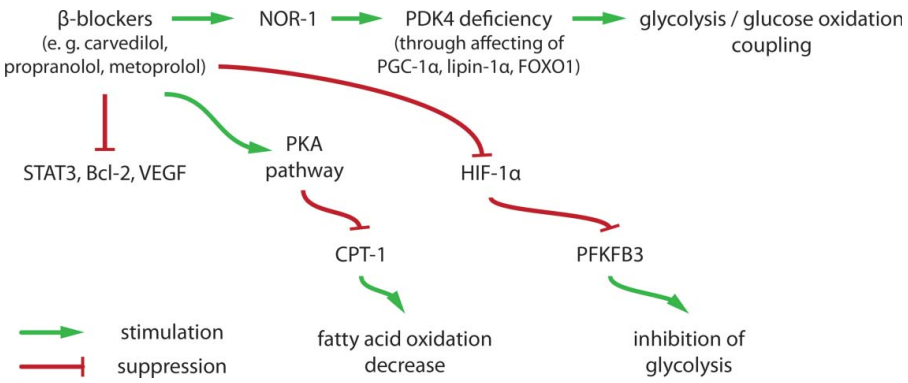

Figure 2. Molecular targets of β-blockers in the metabolism of cancer cells. Bcl-2, anti-apoptotic protein (B-cell lymphoma); CPT-1, carnitine palmitoyltransferase; FOXO1, forkhead box O1; HIF-1a, hypoxia-inducible factor; NOR-1, orphan nuclear receptor; PDK4, pyruvate dehydrogenase kinase; PFKFB3, phosphofructokinase isoform; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator; PKA, protein kinase A; STAT3, signal transducer and activator of transcription; VEGF, vascular endothelial growth factor.

have significant implications in the treatment and/or prevention strategies of cancer disease (52). However, the precise molecular mechanisms are far from being fully understood. Precise identifying of the β-adrenergic receptor system signal pathways relevant to carcinogenesis is the tool through which the mechanisms of β-blockers used for cancer treatment can be understood.

Discussion

We have only covered the very basics of the molecular mechanisms that account for the Warburg effect in cancer. There have been several reviews written on the subject that go much further into the detail of the molecular mechanisms and their implications (12,53–56). However, the purpose of this paper was to investigate the potential of the two chosen agents, DCA and β-blockers, as treatments for cancer and chronic ischemic conditions.

In contrast to DCA, the pharmacodynamics of β-blockers that relate to oxidative metabolism are poorly understood, although their effects on cancer-specific survival appear demonstrable. Of course, this does not automatically suggest that their mechanisms for the improvement of cancer survival have anything to do with oxidative metabolism. However, our results show that β-blockers have an influence not only on the total energy production rate, but, in the case of a nonselective β-blocker, also on substrate utilization (4). Namely, they promote the oxidation of glucose. It is possible that through the promotion of oxidation of glucose, β-blockers can partially reverse the Warburg effect or some of its consequences, the effect of which would be the exact opposite. To demonstrate this, the molecular mechanisms of β-blocker action on energy metabolism have to be investigated further.

The story of DCA is completely different. Although its molecular mechanisms of action in oxidative metabolism are well understood and documented, certain safety concerns that arose in animal studies and its lack of interest for the pharmaceutical industry will most likely keep it out of larger-scale clinical trials for a long time to come. The results of studies that have investigated the use of DCA under chronic ischemic conditions, such as chronic heart failure, have been promising, although not conclusive, and it is likely to stay this way due to the safety concerns about chronic DCA use in humans.

However, tissue-specific PDK inhibition appears to be an interesting opportunity and a mechanism that new, safer, more specific, and more economically viable drugs could target. This might apply to cancer therapy as well as to therapies for chronic ischemic conditions—two of the main causes of morbidity and mortality in the developed world.

Hypothesis

While the mechanisms of action of DCA on oxidative metabolism are better known than those of β-blockers, it is improbable that the same mechanism is shared between these drugs. Therefore, there is a possibility that simultaneous treatment with both DCA and nonselective β-blockers can produce synergistic effects with respect to specific cancer survival, and perhaps even harbor curative potential. As both of these drug types have relatively rich histories of documented use in clinical practice, such a trial would evade many obstacles of new drug trials. As the Warburg effect is shared among most types of cancer, these therapies, if successful, might be very broadly applicable. Both of these drug types are cheap and widely available, which increases the attractiveness of this possibility.

Conclusion

Alterations to glucose metabolism are definitely a pivotal feature of both ischemic and malignant disease. When the normally precise regulation of the entry of glycolysis

products into oxidative metabolism fails, the results can be devastating. These include a shortage of energy in cells (which results in markedly increased glucose uptake), a sudden increase in lactate that causes local tissue acidosis (which results in inactivation of immune functions), breakdown of the extracellular matrix, overabundance of other metabolic intermediates (which facilitates uncontrolled cell division), shut-off of apoptosis mediators from their sites of action, resistance of cells to hypoxic conditions, and many more effects. This is why reversing this process would not only be favorable in the treatment of cancer but also perhaps even be necessary. The benefits of shifting from fatty acid to glucose metabolism under chronic ischemic conditions mainly stem from decreased tissue oxygen consumption, which is definitely a favorable effect in ischemic tissues. However, considering the effects of β-blockers on cancer-specific survival, is it possible that they can also promote oxidation of glucose in cancer cells? And could a compound that promote oxidation of glucose in cancer cells, such as DCA, also benefit patients with chronic ischemic conditions? Due to a lack of conclusive clinical trials, these questions remain unanswered. However, due to the described demonstrable molecular mechanisms involved, these questions have been raised.

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Conflict of interest statement

The authors declare no conflict of interest.

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