The Warburg Effect: Why and How do Cancer Cells Activate Glycolysis in the Presence of Oxygen?

Miguel López-Lázaro*

Department of Pharmacology, Faculty of Pharmacy, University of Seville, Spain

Abstract: Cells can obtain energy through the oxygen-dependent pathway of oxidative phosphorylation (OXPHOS) and through the oxygen-independent pathway of glycolysis. Since OXPHOS is more efficient in generating ATP than glycolysis, it is recognized that the presence of oxygen results in the activation of OXPHOS and the inhibition of glycolysis (Pasteur effect). However, it has been known for many years that cancer cells and non-malignant proliferating cells can activate glycolysis in the presence of adequate oxygen levels (aerobic glycolysis or Warburg effect). Accumulating evidence suggests that the persistent activation of aerobic glycolysis in tumor cells plays a crucial role in cancer development; the inhibition of the increased glycolytic capacity of malignant cells may therefore represent a key anticancer strategy. Although some important knowledge has been gained in the last few years on this growing field of research, the basis of the Warburg effect still remains poorly understood. This communication analyzes why cancer cells switch from OXPHOS to glycolysis in the presence of adequate oxygen levels, and how these cells manage to avoid the inhibition of glycolysis induced by oxygen. Several strategies and drugs that may interfere with the glycolytic metabolism of cancer cells are also shown. This information may help develop anticancer approaches that may have clinical relevance.

Key Words: Aerobic glycolysis, glycolysis inhibitors, metabolism, dysoxic metabolism, hypoxia-inducible factor 1, reactive oxygen species, hydrogen peroxide, superoxide anion.

1. INTRODUCTION

Most of the energy that cells need to live is produced in the mitochondria through an oxygen-dependent process called oxidative phosphorylation (OXPHOS). This process couples the oxidation of NADH and FADH₂ with the phosphorylation of ADP to form ATP, the chemical energy currency of cells. OXPHOS is oxygen-dependent because the electrons resulting from the oxidation of NADH and FADH₂ need to be ultimately accepted by oxygen (O₂). Cells can also produce ATP through glycolysis, which takes place in the cytosol and does not require O₂. In the process of glycolysis, one molecule of glucose is broken down into two molecules of pyruvate, resulting in the production of ATP. This process consumes NAD⁺, which can be regenerated by the conversion of pyruvate to lactate (Fig. 1). OXPHOS is more efficient in generating ATP than glycolysis; the oxidation of one molecule of glucose gives a net yield of 30 ATPs via OXPHOS and 2 ATPs via glycolysis. It is comprehensible, therefore, that cells generate ATP through OXPHOS when they have enough O₂ levels. This was first noted by Pasteur in the late 19th century, who observed that, as the O₂ levels decreased, the generation of ATP shifted from OXPHOS to glycolysis (Pasteur effect) [1-4].

In the first half of the 20th century, Otto Warburg first observed that cancer cells had increased rates of glycolysis despite the presence of adequate O₂ levels [5]. This phenomenon, called aerobic glycolysis or Warburg effect, has repeatedly been observed in cancer cells [6-8]. Indeed, the widespread clinical use of the imaging technique positron-emission tomography using the glucose analogue tracer 18fluorodeoxyglucose (FDG PET) has demonstrated that the glycolytic phenotype is observed in most human cancers [6]. It has also been known for several decades that the metabolic switch from OXPHOS to aerobic glycolysis is not a unique feature of tumor cells, as it is also found in non-transformed proliferating cells [9-11]. These experimental observations raise two questions that have long puzzled cancer biologists. First, if OXPHOS is more efficient in generating ATP than glycolysis, why do cancer cells and non-malignant proliferating cells activate glycolysis when the O₂ levels are adequate? Second, if O₂ acts as a glycolysis inhibitor (Pasteur effect), how do these cells manage to activate glycolysis in the presence of O₂?

An understanding of the Warburg effect might be exploited therapeutically, as the sustained activation of glycolysis in tumor cells seems to play a crucial role in cancer development. For instance, recent data suggest that cancer cells may depend on glycolysis for ATP generation and that cancer cells’ dependence on glycolytic energy progressively increases as malignant transformation occurs [7, 12-14]. Furthermore, it has been demonstrated that the accumulation of glucose metabolites caused by the constitutive activation of glycolysis results in the activation of hypoxia-inducible factor 1 (HIF-1) [15, 16]. The activation of HIF-1 increases the transcription of many genes that code for proteins that favor cancer development, including proteins involved in glucose metabolism, apoptosis resistance, invasion, metastasis and angiogenesis [17-19]. Evidence suggests that the persistent activation of glycolysis can also favor tumor growth [20, 21], as well as tumor invasion and metastasis via acidification of the tumor microenvironment [22, 23]; indeed, it has been proposed recently that the glycolytic phenotype is necessary for evolution of invasive human cancers [6, 22]. The present communication analyzes why cancer cells switch from OXPHOS to aerobic glycolysis and how they avoid O₂-induced glycolysis inhibition. In addition, possible strategies aimed at inhibiting glycolysis in cancer cells are discussed, and several drugs that may interfere with the glycolytic metabolism of cancer cells are examined. This knowledge may help develop effective antitumor strategies.

2. WHY DO CANCER CELLS ACTIVATE GLYCOLYSIS IN THE PRESENCE OF O₂?

The first explanation for the phenomenon of aerobic glycolysis was given by Otto Warburg several decades ago. He proposed that cancer cells have increased glycolytic rates despite the presence of O₂ because these cells have irreversible damages to OXPHOS [5]. Despite being rejected in the beginning, recent observations seem to support this hypothesis. For instance, it has been reported that cells from the most common cancer types have decreased expression of ATP synthase [24, 25], a protein complex required for OXPHOS. Mitochondrial mutations, which may lead to malfunction in OXPHOS, have also been observed in tumor cells [7]. It has also been shown that inactivation of p53—one of the most commonly mutated genes in cancer—may trigger the Warburg effect; p53 is in-
involved in the activity of cytochrome c oxidase, a protein complex involved in OXPHOS [26]. However, recent data have shown that glycolysis inhibition in cancer cells can enhance OXPHOS activity; this seems to indicate that the Warburg effect is not caused by irreversible damages to OXPHOS [21, 27]. This last observation is supported by the fact that the Warburg effect has also been observed in non-transformed proliferating cells, which are not supposed to have irreversible damages to OXPHOS.

Based on mathematical models and empirical observations, it has been proposed recently that cancer cells activate aerobic glycolysis because the persistent activation of glycolysis leads to environmental acidosis, which is toxic to normal cells but harmless to cancer cells. The sustained activation of glycolysis would therefore provide cancer cells with a powerful growth advantage that would prevail over an efficient ATP generation through OXPHOS. The activation of glycolysis serves the key purpose of providing carbon skeletons for biosyntheses. This implies that the activation of glycolysis is essential for cell proliferation, as cell proliferation requires the synthesis of new molecules (e.g. nucleic acids, lipids, proteins) and as glycolysis provides most of the building blocks required for the synthesis of these molecules [2–4]. For instance, as represented in Fig. (2), cells need to synthesize nucleic acids in order to proliferate (S-phase of the cell cycle). The starting point for nucleotide biosynthesis is the sugar ribose-5-phosphate, which is derived from the glycolytic metabolite glucose-6-phosphate. Likewise, the glycolytic metabolites glucose 6-phosphate, 3-phosphoglycerate, phosphoenolpyruvate and pyruvate are key precursors in the biosynthesis of several amino acids (i.e. His, Ser, Cys, Gly, Tyr, Phe, Trp, Ala, Val, Leu) [4] required for the synthesis of proteins. In addition, the glycolytic metabolite dihydroxyacetone phosphate is the key precursor of glycerol, a necessary metabolite for the synthesis of lipids. Lipid formation also requires fatty acids, and experimental data support that glucose-induced cytosolic synthesis of acetyl CoA is required for the synthesis of fatty acids [27-29].

The essential role of glycolysis in cell proliferation is supported by experimental observations that have revealed that cell proliferation is inhibited in normal and cancer cells placed in glucose-deficient media containing high levels of alternative energy sources, or in cells treated with the non-metabolizable glucose analogue 2-deoxyglucose (3) [11, 30-32]. These observations support that cancer cells and non-malignant proliferating cells may activate glycolysis in the presence of $O_2$ in order to proliferate (Fig. 2). In other words, if glycolysis was always inhibited in the presence of $O_2$, cells could not keep sustained proliferative rates under aerobic conditions. It is important to note that, despite being a powerful reason, there is evidence that suggests that non-malignant proliferating cells
A decrease in H$_2$O$_2$ in the cell death induced by reactive oxygen species (ROS) such as glycolysis seems to play an important role in the protection against cancer cells do not activate aerobic glycolysis with the sole enzyme in the regulation of glycolysis.

Growing cells have an enormous demand for ATP to fuel their growth, and glycolysis seems much better suited to meet this demand [27, 38].

3. HOW DO CANCER CELLS ACTIVATE GLYCOLYSIS IN THE PRESENCE OF O$_2$?

Recent reports have shown evidence that suggests that cancer cells have an alteration in O$_2$ metabolism (dysoxia or dysoxic metabolism), which may drive tumor growth, invasion and metastasis [20, 23, 39]. This section discusses that this alteration in O$_2$ metabolism may also play a key role in the Warburg effect. In the process of OXPHOS, ATP generation is coupled with a reaction in which O$_2$ is reduced to H$_2$O via the ROS superoxide anion (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$). Fig. (3) represents that a deviation of O$_2$ metabolism from the route that generates ATP to the route that produces O$_2^-$ and H$_2$O$_2$ can activate aerobic glycolysis.

A decrease in O$_2$ metabolism via OXPHOS can activate aerobic glycolysis. It is recognized that high ATP levels can repress glycolysis via an allosteric inhibition of phosphofructokinase (PFK), a key enzyme in the regulation of glycolysis [2]. The possible basis of the Pasteur effect is that O$_2$ allows ATP synthesis through OXPHOS; this produces an allosteric inhibition of PFK resulting in glycolysis inhibition [2, 39]. It implies that glycolysis is not directly inhibited by O$_2$, but by ATP. It makes sense to think, therefore, that the presence of O$_2$ will not cause the inhibition of glycolysis when O$_2$ is not used to generate ATP. Accordingly, Fig. (3) proposes that cells manage to activate glycolysis in the presence of O$_2$ by reducing O$_2$ metabolism through OXPHOS. A decrease in O$_2$ metabolism through OXPHOS would reduce ATP generation; this would release PFK inhibition by ATP and would activate glycolysis. The activation of glycolysis would compensate the decreased ATP generation through OXPHOS.

An increase in O$_2$ metabolism through the ROS O$_2^-$ and H$_2$O$_2$ may also produce aerobic glycolysis. It is known that O$_2^-$ can produce intracellular alkalinization (pH) [40, 41] and that intracellular alkalinization can activate glycolysis by increasing the activity of PFK; the activity of this enzyme is extremely sensitive to small changes in pH in the physiological range, a high pH increasing the activity of this enzyme) [39, 42, 43]. Although the activation of the enzyme PFK is fundamental in the activation of glycolysis, cells need to increase the expression of glucose transporters (e.g. GLUTs) and glycolytic enzymes (e.g. hexokinase, PFK, pyruvate kinase) to keep sustained glycolytic rates. It is now well accepted that an increase in the cellular levels of H$_2$O$_2$ activates HIF-1 [39, 44-46]. HIF-1 activation plays a key role in the transcription of genes that code for glucose transporters and glycolytic enzymes [17, 47-49]; indeed, it has been observed that cells lacking HIF-1 exhibit decreased glycolytic capacity [48]. H$_2$O$_2$ can also activate Akt [50-52] and the oncogenes ras, src, and myc [53, 54], which are involved in the synthesis of glucose transporters and glycolytic enzymes [55, 56]. Although Akt, ras and src are known to activate...
HIF-1 [17, 55], the possibility that H$_2$O$_2$-induced transcription of glucose transporters and glycolytic enzymes may occur independently of HIF-1 cannot be excluded [57].

Experimental observations support that many situations that have been linked to the Warburg effect may be integrated in the model represented in Fig. (3). As mentioned before, the first explanation to the Warburg effect was given by Otto Warburg, who proposed that this phenomenon was caused by irreversible damages to OXPHOS [5]. While some observations support that cancer cells have alterations in proteins involved in OXPHOS [7, 24-26], others suggest that the Warburg effect is not caused by irreversible damages to OXPHOS [21, 27]. All these observations can be integrated in the model shown in Fig. (3), which proposes that any situation that produces a decrease in OXPHOS activity in the presence of O$_2$ can produce the Warburg effect.

It has been discussed recently that cancer cells have increased rates of aerobic glycolysis because of an adaptation to intermittent hypoxia [6]. It is known that intermittent hypoxia increases O$_2^-$ generation [58, 59] and, according to the model represented in Fig. (3), an increased O$_2^-$ generation would lead to the activation of aerobic glycolysis. The present model also agrees with the proposal that intracellular alkalization is involved in the Warburg effect [60-62], as intracellular alkalization may cause OXPHOS repression [39] and glycolysis activation [42, 60, 61]. Accordingly, experimental data suggest that intracellular alkalization may activate p53 [63] and p53 inactivation may cause the Warburg effect [26]. Although it has been shown that IL-3 can increase glycolysis through activation of the Akt/Pim kinases [33, 64]. IL-3-induced activation of glycolysis might also be integrated in Fig. (3), as IL-3 can increase O$_2^-$ generation [65] and the intracellular pH [66].

Factors have been linked to the activation of aerobic glycolysis [15, 17, 55, 56]. Interestingly, it has been shown that H$_2$O$_2$ can activate HIF-1 [44-46], akt [50-52], ras, src, and myc [53, 54]. The activity of the Na$^+$/K$^+$-ATPase pump has also been associated with the activation of aerobic glycolysis [67, 68], and it has been observed that H$_2$O$_2$ can activate Na$^+$/K$^+$-ATPase [69] and that catalase prevents the activation of this pump [70]. In brief, it seems that many of the situations that have been linked to the activation of aerobic glycolysis may be integrated in the model represented in Fig. (3).

HIF-1 is a heterodimeric transcription factor that consists of a constitutively expressed HIF-1α subunit and a HIF-1α subunit, the expression of which is highly regulated. HIF-1 overexpression has been observed in the most common cancer types, and is therefore considered a potential target for cancer therapy [17, 71]. The activation of HIF-1 seems to play an important role in the metabolic switch from OXPHOS to aerobic glycolysis (Warburg effect), as this transcription factor not only can activate aerobic glycolysis but also repress OXPHOS [72, 73]. Indeed, experimental data have shown that the activation of HIF-1 mediates the expression of pyruvate dehydrogenase kinase (PDK), this results in competitive dehydrogenase (PDH) inhibition, decreased conversion of pyruvate to acetyl-CoA, reduced activity of the tricarboxylic acid cycle and subsequent oxphos repression [72, 73] (Fig. 1). A recent article has reviewed that, in clear cell renal carcinoma (an in perhaps in other human cancers), HIF-1 mediates increased glucose uptake, increased lactate production, and decreased OXPHOS, thus delineating for the first time the molecular mechanisms underlying the switch from oxidative to glycolytic metabolism in human cancer [49]. Recent evidence also suggests that an alteration in O$_2$ metabolism (dysoxia) may be the main mechanism responsible for HIF-1 activation under hypoxic and aerobic conditions [39]; this supports the idea that an alteration in O$_2$ metabolism may cause the Warburg effect.

According to the model shown in Fig. (3), cancer cells would activate glycolysis in the presence of O$_2$ because of a deviation of O$_2$ metabolism from the route that generates ATP to the route that produces O$_2^-$ and H$_2$O$_2$. This alteration in O$_2$ metabolism would activate aerobic glycolysis by producing OXPHOS repression, increased generation of O$_2^-$ and H$_2$O$_2$, intracellular alkalization, and HIF-1 activation (see Fig. 3). This model is supported by experimental data that have revealed that most cancers have HIF-1 overexpression [17, 18], alkaline intracellular pH values (7.12–7.65 compared with 6.99–7.20 in normal tissues) [60, 62, 74], excessive generation of O$_2^-$ and H$_2$O$_2$ [75-78] and structural alterations in OXPHOS that may cause OXPHOS repression [7, 24-26].

4. GLYCOLYSIS INHIBITION: STRATEGIES AND DRUGS

The attenuation or inhibition of glycolysis in tumor cells may be exploited for the development of cancer chemopreventive and chemotherapeutic strategies. On the one hand, as illustrated in Fig. (2) and discussed elsewhere [20, 22, 23], evidence suggests that glycolysis is essential for cell proliferation, tumor invasion and metastasis. The reduction of the glycolytic capacity of tumor cells would restrict their ability to proliferate, invade adjacent tissues and migrate to distant organs. This suggests that the attenuation of glycolysis in tumor cells may represent a useful strategy for preventing or stopping the development of cancer (i.e. cancer chemoprevention). On the other hand, evidence supports that the activation of glycolysis protects cells from H$_2$O$_2$-induced cell death [10, 34-37], and that cancer cells are more susceptible to H$_2$O$_2$-induced cell death than normal cells [78, 79]. In addition, cancer cells are more dependent on glycolytic ATP than normal cells [7, 8, 12-14]. This suggests that the inhibition of glycolysis may produce selective killing of cancer cells and be a practical strategy for cancer chemotherapy (see ref. [105]).

Several strategies and drugs can be used to inhibit the glycolytic metabolism of cancer cells. The first and perhaps most straightforward way of inhibiting glycolysis is reducing the blood and intersti-
tial glucose levels by using insulin therapy. Mice can survive doses of human insulin that results in a decrease of blood glucose by one order of magnitude (approximately, from 240 mg/dL to 24 mg/dL). The effect of insulin on xenograft tumors is currently under investigation [80].

Inhibition of glycolytic enzymes is another strategy that may result in glycolysis inhibition in tumor cells. As illustrated in Fig. (2), hexokinase (HK), phosphofructokinase (PFK) and pyruvate kinase (PK) catalyze the three irreversible reactions of glycolysis; these enzymes are important control sites of glycolysis [2]. HK is the first and rate-limiting reaction in glycolysis and catalyzes the phosphorylation of glucose to glucose-6-phosphate (G6P). This step converts a non-ionic molecule (glucose) to an anion (G6P) that is trapped in the cells. Lonidamine (1), a derivative of indazole-3-carboxylic acid that is currently undergoing clinical trials for the treatment of benign prostatic hypertrophy, can inhibit the phosphorylation of glucose by HK. This drug is currently undergoing clinical trials in combination with other anticancer agents for the treatment of different types of cancer [7, 80]. The pyruvate analog 3-BP (3-(3-BrPA)) (2) is a HK inhibitor that has shown potent anticancer activity in preclinical studies [12, 81]. The glucose analog 2-deoxyglucose (2-DG) (3) is a competitive inhibitor of glucose metabolism that competes with glucose for HK. Once 2-DG (3) is phosphorylated by HK, it cannot be metabolized further; this leads to accumulation of 2-DG (3) within the cell and results in glycolysis inhibition [7]. This agent, however, seems to produce toxic effects when used as a primary therapy and it is currently being explored in combination with other anticancer agents [82]. Evidence suggests that HK and glucose-6-phosphate 1-dehydrogenase (G6PDH) are primary targets of imatinib (Gleevec) (4), an anticancer drug already approved for clinical use [83]. As mentioned before, the activity of PFK is extremely sensitive to small changes of pH in the physiological range, a high pH increasing the activity of this enzyme [39, 42, 43]. Evidence suggests that cancer cells have high intracellular pH values, which may activate PFK and contribute to explain the high glycolytic rates of these cells [60-62]. Activation of H+- extruders such as the Na+/H+-exchanger NHE-1 has been shown to play a key role in the elevation of the intracellular pH in cancer cells. Inhibitors of NHE-1, such as amiloride or 5,5-dimethylamiloride (DMA) (5), may therefore reduce the intracellular pH, PFK activity and glycolysis, and produce antitumor effects. Indeed, these two drugs have shown anticancer effects in vivo [60, 84, 85]. In addition, it is well known that PFK is inhibited by citrate [3]. The enzyme ATP citrate lyase (ACL) catalyzes the conversion of citrate to cytosolic acetyl-CoA. Inhibitors of ATP citrate lyase (ACL), such as SB-204990 (6), may cause citrate to build up and inhibit glycolysis [14, 29, 86]. It has been shown that SB-204990 (6) limits in vitro proliferation and survival of tumor cells displaying aerobic glycolysis and reduces in vivo tumor growth [29]. Inhibition of the enzyme pyruvate kinase (PK), which catalyzes the third irreversible reaction of glycolysis (Fig. 2), represents another strategy for inhibiting glycolysis; this enzyme seems to play an important role in tumor growth and invasion, and its inhibition may produce antitumor effects [87].

Fig. (1) shows that the enzymes lactate dehydrogenase (LDH), pyruvate dehydrogenase (PDH) and pyruvate dehydrogenase kinase (PDK) play important roles in glycolysis and OXPHOS. The enzyme LDH catalyzes a reaction by which pyruvate is reduced to lactate; this reaction permits the regeneration of NAD+, needed for glycolysis to continue (Fig. 1). Recent observations showed that attenuation of LDH reduced the glycolytic metabolism of cancer cells and produced antitumor effects in animals [21, 27]. The authors discussed that, because individuals with complete deficiency of LDH do not show any symptoms under ordinary circumstances, the inhibition of LDH activity may represent a relatively nontoxic approach to interfere with tumor growth [21]. Deck et al. synthesized several dihydroxynaphthoic acids that were potent inhibitors of LDH [88]; these drugs might inhibit glycolysis and display antitumor effects. On the other hand, recent evidence suggests that HIF-1 activation mediates the expression of PDK [72, 73]; this enzyme represses OXPHOS by inhibiting PDH (Fig. 1) and may therefore play a role in the activation of aerobic glycolysis. Inhibition of PDK may therefore attenuate glycolysis and produce anticancer effects. Several reports have shown that different groups of compounds are inhibitors of PDK [89-95]; these drugs may inhibit glycolysis and produce antitumor effects. Accordingly, a recent study has shown that the PDK inhibitor dichloroacetate (DCA) (7) produced marked antitumor effects [95]; DCA (7) is a known glycolysis inhibitor that has been shown to inhibit HIF-1 activation in a variety of preclinical studies [67-69]. The authors observed that DCA (7) in the drinking water at clinically relevant doses for up to 3 months prevented and reversed tumor growth in vivo, without apparent toxicity and without affecting hemoglobin, transaminases, or creatinine levels. They concluded that the ease of delivery, selectivity, and effectiveness make DCA (7) an attractive candidate for cancer therapy which can be rapidly translated into phase II–III clinical trials.

Another strategy for reducing the increased glycolytic rates of tumor cells is the inhibition of the synthesis of glucose transporters and glycolytic enzymes. As discussed before, the activation of HIF-1 plays a key role in the expression glucose transporters and glycolytic enzymes. Inhibition of HIF-1 may therefore reduce the persistent activation of aerobic glycolysis in tumor cells and produce antitumor effects. Preclinical studies have already shown that inhibition of HIF-1 activity has marked effects on tumor growth [17]. HIF-1 is considered a potential target for cancer chemoprevention [19] and therapy [17] and, recently, many efforts to develop new HIF-1-targeting agents have been made by both academic and pharmaceutical industry laboratories [17, 18, 96, 97]. As a result, several FDA-approved anticancer drugs (e.g. topotecan, imatinib (4), trastuzumab, NS398, celecoxib, ibuprofen) have been found to inhibit HIF-1 activity [18]. Several natural products (e.g. resveratrol, genistein, apigenin, berberin) have also been found to inhibit the activity of this transcription factor [18, 97].

Several other drugs have shown ability to inhibit glycolysis, including oxothiamine, genistein, 5-thioglucone, mannoheptulose, α-chlorohydhin, ormidazole, gluosfamide, or arsenic compounds (see references [7, 8]). Oxamate (an analogue of pyruvate that blocks the step of glycolysis that converts pyruvate to lactic acid) and isocitrate (an inhibitor of glyceraldehyde 3-phosphate dehydrogenase) may also produce antitumor effects [98-100]. Bisphosphonates [101] or tuberinib [102] may also inhibit glycolysis in cancer cells. As mentioned before, the activity of the Na+/K+ -ATPase pump has also been associated with the activation of aerobic glycolysis [67, 68]. Cardiac glycosides, such as ouabain (8) or digitoxin, are known inhibitors of the Na+/K+ -ATPase pump that have shown antitumor effects [103-105].

Finally, Fig. (3) implies that the glycolytic capacity of cancer cells may be restricted by preventing or reducing excessive cellular levels of O2− and H2O2. It has been demonstrated that the use of antioxidants, such as the enzyme catalase, prevents the activation of HIF-1 induced by hypoxia and non-hypoxic stimuli [44-46]. Since HIF-1 plays a key role in the activation of glycolysis, the use of antioxidants would prevent HIF-1 activation; this would attenuate the glycolytic capacity of tumor cells and would prevent the development of invasive cancers [23].

5. CONCLUSIONS

Cells can obtain energy through the oxygen-dependent pathway of OXPHOS and through the oxygen-independent pathway of glycolysis. Since glycolysis is less efficient in generating ATP than OXPHOS, it is comprehensible that glycolysis is inhibited in cells under aerobic conditions (Pasteur effect). It is not well understood, however, why cancer cells and non-malignant proliferating cells...
have increased rates of glycolysis in the presence of an adequate O₂ supply (aerobic glycolysis or Warburg effect). Established biochemical evidence indicates that the activation of glycolysis is essential for cell proliferation. This suggests that, if glycolysis were always inhibited in the presence of O₂, cell proliferation would be restricted under aerobic conditions. The necessity that cells proliferate under aerobic conditions seems a powerful reason to justify the presence of aerobic glycolysis in cancer cells and non-malignant proliferating cells. The possible mechanisms by which these cells evade O₂-induced glycolysis inhibition (Pasteur effect) are not well-defined. The present report has discussed evidence that supports the key mechanism involved in the activation of aerobic glycolysis may be a switch in O₂ metabolism from the route that generates ATP to the route that produces O₂⁻ and H₂O₂ (dysoxic metabolism). This switch in O₂ metabolism has already been proposed to mediate other key aspects of the carcinogenesis process, including HIF-1 activation, tumor growth, invasion and metastasis. Finally, possible strategies and drugs that may be used for reducing the increased glycolytic capacity of cancer cells have been shown. This knowledge may help develop anticancer strategies, as a growing body of research suggests that the increased glycolytic metabolism of tumor cells plays a crucial role in cancer development.

REFERENCES


**Fig. (4).** Selected glycolysis inhibitors. These drugs have been shown to inhibit glycolysis and produce anticancer effects.


