Abstract. The anticancer efficacy of the pyruvate analog 3-bromopyruvate has been demonstrated in multiple tumor models. The chief principle underlying the antitumor effects of 3-bromopyruvate is its ability to effectively target the energy metabolism of cancer cells. Biochemically, the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been identified as the primary target of 3-bromopyruvate. Its inhibition results in the depletion of intracellular ATP, causing cell death. Several reports have also demonstrated that in addition to GAPDH inhibition, the induction of cellular stress also contributes to 3-bromopyruvate treatment-dependent apoptosis. Furthermore, recent evidence shows that 3-bromopyruvate is taken up selectively by tumor cells via the monocarboxylate transporters (MCTs) that are frequently overexpressed in cancer cells (for the export of lactate produced during aerobic glycolysis). The preferential uptake of 3-bromopyruvate via MCTs facilitates selective targeting of tumor cells while leaving healthy and non-malignant tissue untouched. Taken together, the specificity of molecular (GAPDH) targeting and selective uptake by tumor cells underlines the potential of 3-bromopyruvate as a potent and promising anticancer agent. In this review, we highlight the mechanistic characteristics of 3-bromopyruvate and discuss its potential for translation into the clinic.

Tumors are characterized by a profound dependency on glucose metabolism for energy generation. This increased demand for glucose is the basis for tumor imaging with 18F-fluorodeoxyglucose (an analog of glucose) positron-emission tomography (FDG-PET), which is clinically widely used in the diagnosis of human cancer. When combined with computed tomography (CT), PET/CT imaging is a powerful diagnostic tool with a high sensitivity and specificity (>90%) (1). The success of PET imaging as a cancer diagnostic tool has opened the door to the possibility of exploiting glucose metabolism as a target for therapy. Published pre-clinical data and reviews have revealed the therapeutic potential of targeting glucose metabolism in cancer (2-4). However, despite tremendous progress in our understanding over the molecular mechanisms regulating tumor metabolism which has led to the development of several antiglycolytic agents, the translation of such agents to the clinic has not yet been successful.

The glucose analog 2-deoxyglucose was one of the first known inhibitors of glucose metabolism tested for anticancer effects (5). Its mechanisms of action is rather simple; 2-deoxyglucose competes with glucose in the first step of glucose metabolism for phosphorylation by the enzyme type II hexokinase (HK II) resulting in the conversion of glucose to glucose-6-phosphate. 2-Deoxyglucose is converted to deoxyglucose-6-phosphate, a molecule that cannot be metabolized any further in the glycolytic pathway, thus causing an inhibition of glycolysis. 2-Deoxyglucose has been shown to inhibit glycolysis and cause cancer cell death in vitro. However, further testing in pre-clinical animal studies and human clinical trials showed no significant tumor response to 2-deoxyglucose used as a single anticancer agent. This could be due to two reasons. Firstly, 2-deoxyglucose does not chemically bind with the target enzyme to inactivate it; therefore, the enzyme is able to continue catalyzing any glucose available in an in vivo system. Secondly, the existence of feeder pathways into the glycolytic pathway enables carbohydrate molecules such as sucrose, maltose and lactose to enter the pathway subsequent to the step catalyzed by hexokinase, and keeps the energy-producing ability of the cell patent. In addition, the possibility of the emergence of a chemoresistant phenotype also exists (6). In particular, aggressive or
hypoxic (known to be more glycolytic) tumors exhibited resistance to 2-deoxyglucose. Recent data showed that the effect of 2-deoxyglucose-mediated cancer cell death is primarily due to the inhibition of glycosylation, a process that adds glycol moieties to proteins, thus necessitating further investigation to understand the principal molecular mechanisms underlying the therapeutic efficacy of 2-deoxyglucose (6).

Lactate dehydrogenase (LDH), another glycolytic enzyme, has attracted considerable interest as the production of lactate during glycolysis essentially relies on LDH activity. Compelling in vitro and in vivo data demonstrated that LDH could indeed be a potential therapeutic target (7, 8). However, the challenge possibly limiting the usefulness of LDH as a therapeutic target lies in the fact that tumor cells can switch to oxidative phosphorylation (mitochondrial respiration), thereby evading the effect of LDH inhibition. Perhaps the recent developments targeting glutamine metabolism along with glucose metabolism could profoundly affect cancer cells (9, 10).

Chemoresistance and systemic toxicities are two of the several factors that can affect the successful translation of drugs to the clinic. Thus, from a clinical perspective, in order to be successful, a potential drug candidate (inhibitor of a target) must possess key attributes, such as (i) the ability to selectively target tumor cells and (ii) molecular specificity resulting in the direct potent inhibition of the target. One such agent, the pyruvate analog, 3-bromopyruvate fulfills these requirements and has generated significant interest due to its remarkable anticancer effects in various tumor types. During the past decade, since its discovery as an anticancer agent, impressive data have emerged providing tremendous insight into the therapeutic efficacy and mechanistic aspects of 3-bromopyruvate action.

3-Bromopyruvate: Selective Targeting of Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

The first reports on the cytotoxic effects of 3-bromopyruvate (Figure 1) in cancer cells demonstrated a massive depletion of intra-cellular ATP in a dose-dependent manner (11, 12). Further analysis of 3-bromopyruvate-treated cells revealed the release of cytochrome c, a well-studied apoptotic marker, thereby suggesting disruption of the mitochondrial membrane. The combination of ATP depletion, synonymous with impaired glucose metabolism, and disruption or depolarization of the mitochondrial membrane made HK II the likely target since it is a mitochondrial-membrane bound enzyme. Treatment of cancer cells with 3-bromopyruvate at 5 mM resulted in the inhibition of HK II activity, implying that HK II was indeed the target of 3-bromopyruvate (11). In addition, since cancer cells are also characterized by up-
regulation of HK II, it was thought that inhibiting HK II underlies 3-bromopyruvate-mediated cytotoxicity in cancer cells. However, recent studies in several laboratories demonstrated that at a cytotoxic dose, 3-bromopyruvate did not affect HK II activity (Figure 2A and B) and that the dose required for HK II inhibition was much higher. For example in HepG2 cells, 3-bromopyruvate was able to induce cell death at a concentration of 100 μM, whereas HK II activity remained unaffected at a dose 10- to 50-times higher (13), confirming that HK II inhibition is unlikely to be the prime target of 3-bromopyruvate. On the other hand, what these results revealed was the fact that another or possibly several other molecular targets were involved.

Tracer studies using radiolabeled (14C)-3-bromopyruvate clearly demonstrated that 3-bromopyruvate primarily inhibits the enzyme GAPDH (Figure 2C) (14, 15). Other investigators have also confirmed this finding, thus establishing once and for all that the antiglycolytic effect of 3-bromopyruvate is primarily mediated through direct inhibition of GAPDH (Figures 3-5). A recent report showed that the propyl ester derivative of 3-bromopyruvate also primarily targets GAPDH (16). In addition to directly inhibiting GAPDH, it appears that 3-bromopyruvate also significantly increased the level of intracellular reactive oxygen species (ROS) (17). In turn, the disruption of glycolysis and/or increase in intracellular ROS caused endoplasmic reticulum (ER) stress and translation inhibition (18) that directly led to cancer cell death. It has therefore become abundantly clear that 3-bromopyruvate inflicts a multipronged lethal assault on cancer cells leading to apoptotic cell death. While it is evident that ER stress and the accumulation of ROS are established consequences of the interaction of 3-bromopyruvate and its target GAPDH, what remains unknown is whether the sole blockade of GAPDH and resultant inhibition of glycolysis is sufficient to induce tumor cell death. Or is it possible that all these elements, i.e. increased level of ROS, chronic ER stress and finally glycolysis inhibition, are required to be present for the cancer cells to die? Recent data using shRNA specifically targeting GAPDH showed that the GAPDH-targeting property of 3-bromopyruvate is sufficient to promote apoptosis. This was true both in vitro and in vivo (19). GAPDH is therefore the selective preferred target of 3-bromopyruvate and its blockade by 3-bromopyruvate is sufficient to promote antitumor effects.

3-Bromopyruvate: Beyond Molecular Specificity

Drug-related toxicity constitutes one of the major challenges of drug therapy. This is also true when considering a drug candidate that inhibits a specific target. After having demonstrated the ability of 3-bromopyruvate to selectively bind GAPDH, it became critical to understand whether it would bind to similar targets in the blood and plasma, since such interactions could easily be responsible for major systemic toxicities. Unpublished data from our laboratory demonstrate that 3-bromopyruvate exhibits strikingly selective molecular targeting in vivo. Although antineoplastic, alkylating agents such as metallo-drugs (e.g. cisplatin and oxaliplat) have been known to bind with albumin (20, 21), systemic administration of 3-bromopyruvate to Sprague-Dawley rats showed no evidence of albumin as the primary
target of 3-bromopyruvate (unpublished data). Thus, the
guiding principle underlying the selective and specific
binding of 3-bromopyruvate with its molecular target remains
to be characterized.

Unique Opportunities

Safety and selective uptake. Selectivity is the critical element
required for the successful developmental outcome of
compounds that bind irreversibly to their target. 3-
Bromopyruvate, a highly reactive alkylating agent selectively
binds with GAPDH. Intriguingly, similar alkylators currently
in clinical practice, shown to bind with genomic DNA and
mitochondria have been known to cause neurotoxicity,
nephrotoxicity and cardiotoxicity (22, 23). Fortunately, and
surprisingly, 3-bromopyruvate has so far demonstrated a safer
toxicity profile than any of these agents, at least in various pre-
clinical animal models. In a rabbit model of liver tumor,
intravenous administration of 3-bromopyruvate (at 1.75 mM
and higher doses) resulted in no considerable accumulation in
major vital organs or toxicity (24). Similarly, toxicology studies
on rats showed that intravenous delivery of therapeutic dose of
3-bromopyruvate (1.75 mM) did not cross the blood-brain
barrier, thus preventing any potential brain toxicity
(unpublished data). The findings, to date, reveal that a
locoregional approach for delivering 3-bromopyruvate to
tumors is the preferred method because it is therapeutically
highly effective, without causing any systemic toxicity. Even if
the drug were to escape into the systemic circulation during
locoregional administration, it would not be a significant
problem since no systemic toxicity was evident when the drug
was given systemically.

As already mentioned, specific inhibition of the target is
clearly a desirable property of any anticancer agent, but
unfortunately it alone is not sufficient for therapeutic success
in the clinical setting. Indeed, other essential characteristics are
required for successful translation of any potential therapeutic
agent. These are (a) selective targeting of tumors and (b)
minimal or absence of systemic toxicity. 3-Bromopyruvate, despite being a small molecule (molecular mass=166.96), shows marked selectivity in targeting tumors because of the preferential and specific uptake of 3-bromopyruvate by tumor cells. Recent research has shed light on the process of tumor selectivity, which appears to be dependent on the expression of monocarboxylate transporters (MCTs) that are overexpressed in the majority of cancer types (25, 26). Thangaraju et al. (27) and Matsumoto et al. (28) have demonstrated that MCT-1 is the primary transporter used by 3-bromopyruvate to penetrate inside cancer cells, thereby facilitating the initiation of its anticancer effects. Thus, the cancer-related overexpression of MCTs and the selective uptake of 3-bromopyruvate through them provide a novel paradigm to specifically target cancer (Figure 6).

Combination therapy. Emerging data also indicate that resistance to chemotherapy can be overcome by combining current anticancer agents with 3-bromopyruvate. Studies of several in vivo and in vitro models of different tumor types have shown that combination therapy with 3-bromopyruvate enhances tumor response to therapy better than the response obtained with the use of single agent alone. Moreover, the therapeutic efficacy of combination therapy with 3-bromopyruvate has been proven to be valid for different classes of anticancer agents (29-35). It is noteworthy that 3-bromopyruvate-dependent increased efficacy of other anticancer agents involves various mechanistic principles depending upon the nature of the drug combined with 3-bromopyruvate. In a recent report, Nakano et al. (36) showed that the anticancer effects of daunorubicin were improved greatly in the presence of 3-bromopyruvate. The underlying mechanism for this enhanced effect in the presence of 3-bromopyruvate has been attributed to the increased retention of daunorubicin by 3-bromopyruvate-mediated inhibition of ATP-binding cassette transporters, eventually blocking the drug efflux. Furthermore, as 3-bromopyruvate causes ATP depletion, and the intracellular ATP level directly affects chemoresistance (37), 3-bromopyruvate can therefore promote the retention within cancer cells of chemotherapeutic agents that are frequently pumped-out via efflux mechanisms during chemoresistance. On the other hand, when combined with platinum-based drugs, 5-fluorouracil, daunorubicin and mitoxantrone, the increase in therapeutic efficacy has been found to be due to the simultaneous inhibition of proliferation (by drug) and the induction of oxidative stress, and energy depletion (by 3-bromopyruvate) (17, 36). Thus, combining 3-bromopyruvate with other agents certainly demonstrates increased therapeutic efficacy and is valid for multiple agents targeting different tumor types.

The heterogeneity and versatility of tumor cells largely contribute to the differential response of tumors to therapy. Understandably, if any single pathway or mechanism is blocked or disrupted, cancer cells can compensate for this loss of function via alternative feeder pathways or rescue mechanisms. For example, in the case of the glycolytic
pathway, if glycolysis is blocked, the pentose phosphate pathway can play a role in the survival and growth of tumors. Conversely, targeting such rescue mechanism(s) simultaneously while blocking glycolysis could yield better results in terms of killing cancer cells. Fortunately, as shown in Figure 6, 3-bromopyruvate-mediated anticancer effects not only involve direct targeting of glucose metabolism but also activation of chronic cellular and ER stress, thereby creating a lethal multipronged attack on tumor cells.

In summary, administration of the therapeutic dose of 3-bromopyruvate as a single agent has profound anticancer effects, and at lower doses it has been shown to enhance the efficacy of other chemotherapeutic agents. Finally, to advance the prospect of 3-bromopyruvate as a therapeutic agent for cancer, it will be necessary to develop a delivery method/vehicle for its systemic administration and characterize the optimal treatment regimen for mono- and combination therapies.

Acknowledgements

We gratefully acknowledge the support by the Charles Wallace Pratt Research Fund and Abdulrahman Abdulmalik Research Fund. The authors declare that there is no conflict of interests with regard to this article.

References


