Enhancing 3-Bromopyruvate Toxicity in Tumor Cells By Inducing Hyperglycemia

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ABSTRACT
Background: The pyruvate analog 3-bromopyruvate has attracted much interest in recent years as a potential anticancer agent due to this compound's capacity to drastically reduce the cell's energy level. The compound's cytotoxic action seems to be directed particularly against cancer cells. Monocarboxylate transporters have been suggested and shown, in a few cases, to drive the compound inside the cells but there is still no general consensus on their upregulation or expression in cancers. In principle, a free diffusion mechanism might also provide the means for the compound to be taken up specifically by cancer cells, exploiting the pH gradient existing across the cell membrane in solid tumors as consequence of their overactivated glycolysis. With the aim of improving the effectiveness of 3-bromopyruvate as anticancer drug, in view of future pre-clinical and clinical trials, the specificity of this compound's action against cancer cells is discussed, examining the mechanism by which the drug can be directed inside the cancer cells and suggesting a way to facilitate this process. Results: A critical analysis of studies performed on the mechanism by which the above drug enters cancer cells suggests that the pH gradient in solid tumors between the environment and the cell cytoplasm may be crucial to this mechanism. There are also studies supporting the conviction that this pH gradient can be enhanced in vivo by glucose infusion. The role of monocarboxylate transporters in the transfer of the drug inside cancer cells is not straightforward, however and there is still no general consensus on it's over expression in cancers.
Conclusion: Given existing data on the acidic pH of the environment in solid tumors and the feasibility of lowering the pH of the tumor environment by administering a hyperglycemia-inducing treatment, administering glucose in excess in the setting of a potential cancer therapy with 3-bromopyruvate could have the effect of driving more of this compound inside the cancer cells, thereby enhancing its toxic effect on the tumor.

Key words: Cancer therapy, 3-bromopyruvate, warburg effect, tumor pH, tumor acidification, Induced hyperglycemia


INTRODUCTION
Unlike other diseases, despite recent advances cancer therapy remains an unsolved problem and new approaches are continuously being proposed, some of them returning to old concepts such as the Warburg effect. This study suggest the possibility of improving the effectiveness of a possible cancer therapy using with 3-bromopyruvate, exploiting a consequence of the Warburg effect, i.e., the acidification of the tumor environment caused by the over activated glycolysis. Although it was discovered by Warburg already 90 years ago (Warburg et al., 1924), the anomalous energy metabolism of cancer (overactivated glycolysis and abundant lactate production, also in the presence of oxygen) has attracted much renewed interest in recent years, as documented by the numerous reviews continuously appearing on this matter Kim and Dang, 2006; Heiden et al., 2009; DeBerardinis and Thompson, 2012; Hsu and Sabatini, 2008).

The are several reasons for this renewal of interest in cancer energy metabolism but the most important are probably: The widespread clinical use of positron emission tomography with fludeoxyglucose (FDG-PET) which relies on the Warburg effect, for imaging cancerous tissues; the finding that pathways that are modified in cancer cells (due to activated oncogenes or mutated tumor suppressors) also involve the reprogramming of energy metabolism (Levine and Puffo-Kuter, 2010; Cairns et al., 2011); and as mentioned above, the poor clinical outcomes achieved with even the more recent therapies based on advances in our understanding of the genetic causes of cancer, such as those targeting dysregulated signal transduction and/or tumor suppressor pathways.

The idea of exploiting the anomalous energy metabolism of cancer cells in the development of drugs designed to kill the cancer cells is by no means new (Aft et al., 2002) but has gained an increasing number of supporters in recent years, making the targeting of glycolysis a promising therapeutic strategy in oncology.
(Pellicano et al., 2006; Pedersen, 2007; Gatenby and Gillies, 2007; Birsoy et al., 2012), to mention just a few of a number of similar proposals.

For more than ten years, 3-bromopyruvate has been studied intensively as an inhibitor of energy metabolism in cells and of glycolysis in particular. It has supplanted the classical inhibitor 2-deoxyglucose in the interest of researchers in this area (Ko et al., 2001), since, it was found capable of eradicating advanced liver cancers in the rat with no apparent systemic toxic effects (Geschwind et al. 2002; Ko et al., 2004). This finding prompted a number of studies both in vivo and in isolated cells or subcellular preparations (for a review see Ganapathy-Kanniappan et al., 2010; Shoshan, 2012) and 3-bromopyruvate is now a candidate drug undergoing clinical development (Ko et al., 2012).

As for the compound’s targets, it was recently established (and has been generally accepted) that the main target along the glycolytic pathway is the enzyme glyceraldehyde 3-phosphate dehydrogenase (Pereira et al., 2009; Dell’Antone, 2009; Ganapathy-Kanniappan et al., 2010; Birsoy et al., 2013), although other enzymes like the vacuolar H⁺-ATPase are inhibited too (Dell’Antone, 2006; for other references, see Birsoy et al., 2013). Right from the early studies (Ko et al., 2004) conducted on preparations obtained from liver tumor tissues, oxidative phosphorylation was found to be inhibited by this pyruvate analog too and succinate dehydrogenase later emerged as another possible target of its inhibitory action (Dell’Antone, 2009; Pereira et al., 2009).

Following an analysis of the mechanism of monocarboxylate uptake by cancer cells, the present study suggests that inducing hyperglycemia in vivo should ameliorate the outcomes of cancer therapy using said drug.

MONOCARBOXYLATE TRANSPORT INTO CELLS

Monocarboxylates such as pyruvate, lactate and others, are transported inside the cells by members of the SLC16 A gene family, composed of sixteen members (Halestrap, 2012), the most studied of which are the H⁺-coupled monocarboxylate transporters (MCTs) 1-4, while other transporters in the family are Na⁺-coupled.

In KBM7 mutagenized cells, transportation of the pyruvate analog 3-bromopyruvate inside the cells has been shown to depend strictly on the expression of MCT1 (Birsoy et al., 2013), an H⁺-coupled transporter involved in lactate excretion from cells. Overexpression of this (or other transporters) might thus explain the specificity of 3-bromopyruvate toxicity in cancer (Pedersen, 2007). If MCT1 expression is essential (as reported in the above-mentioned study) for 3-bromopyruvate to enter the cell and induce its death, given that neither of these events occurred in MCT1-null cells, then at first glance a free diffusion mechanism for the drug’s entry would not appear to be a reliable mechanism. In the system tested in the above-mentioned study at least, this would also mean that MCT1 was the only monocarboxylate transporter to be activated. Other possible 3-bromopyruvate transporters, like the sodium-coupled transporters SLC5A8 (Thangaraju et al., 2009) were presumably not active if the drug had no effect in MCT1-null cells. An alternative explanation for this important result is discussed below.

As cited above (Thangaraju et al., 2009), the sodium-coupled monocarboxylate transporter SMCT1 (SLC5A8) has also been shown to transport 3-bromopyruvate inside breast cancer cells but following the transporter’s ectopic expression.

MONOCARBOXYLATE TRANSPORTERS IN CANCERS

Expression of the above-mentioned or other sodium-coupled transporters is silenced not only in breast cancer but also in cancers of various systemic organs (Thangaraju et al., 2009; Cothankingaswamy et al., 2013; Ganapathy-Kanniappan et al., 2009). It is thought to avoid butyrate accumulation in colon cancer cells, or of pyruvate in other cancer cells, since, these metabolites would have a role as tumor suppressors by inhibiting histone deacetylases (Thangaraju et al., 2009; Cothankingaswamy et al., 2013; Ganapathy et al., 2009).

Monocarboxylate transporters have become attractive targets in cancer treatment (Fang et al., 2006; Pinheiro et al., 2012; Kennedy and Dewhirst, 2010), although their expression or upregulation in many cancers remains uncertain (Ganapathy et al., 2009; Fang et al., 2006; Pinheiro et al., 2012). Some studies have found the H⁺-coupled MCTs 1-4 upregulated in cancer cells and several researchers have reported their overexpression in a subset of cancers (Birsoy et al., 2013) but others have failed to confirm such findings (Ganapathy et al., 2009; Fang et al., 2006; Pinheiro et al., 2012).

The need for MCT1 to be expressed in order for 3-bromopyruvate to enter cancer cells (Birsoy et al., 2013) leads to the question of whether the drug could be effective in most cancers with no other pathway available for its transportation inside the cancer cells, as discussed below.

ACID pH IN TUMORS

Many studies over the last few decades have confirmed that the pH of the environment is acidic in
many solid tumors, averaging around 6.7, or less-as opposed to the 7.2-7.4 of normal tissues-while intracellular pH is maintained within a range of 7.0-7.2 (Tannock and Rotin, 1989; Zhang et al., 2010; Estrella et al., 2013; Neri and Supuran, 2011). This is generally believed to be due to the high lactate production in cancer cells as a consequence of glycolysis being overactivated (more than 30-fold) and oxidative phosphorylation being downregulated (Kennedy and Dewhurst, 2010; Helmlinger et al., 2008).

To avoid any deleterious cytoplasm acidification resulting from this overactive glycolysis and abundant lactate production, lactate (into which most pyruvate, the end product of glucose metabolism, is converted) is transported outside the cell together with a proton. Other mechanisms are also at work to expel protons, however, such as the sodium-hydrogen exchange or a plasma lemmia vacuolar ATPase expressed in cancer (Zhang et al., 2010).

**pH GRADIENT-DRIVEN MONOCARBOXYLATE TRANSPORT INTO CELLS**

Monocarboxylates may be taken up by transporters, as discussed above, or they may enter cells via a free diffusion. In this case (based on mass law equations), the concentration of the monocarboxylate from inside to outside the cell should equal to the $H^+/\alpha H^+$ ratio, assuming that the diffusion involves the uncharged acid form (Deuticke, 1989; Poole and Halestrap, 1993; Halestrap, 2012). The same applies to the monocarboxylates carried inside the cells by $H^+$-coupled transporters (Halestrap and Price, 1999). In this framework, an alternative explanation as to why MCT1 was essential to 3-bromopyruvate uptake and its cytotoxic efficacy in the system considered in the above-cited study at least (Birsoy et al., 2013) might be that the free diffusion mechanism only becomes important when the extracellular pH is more acidic than the intracellular pH. The phenomenon would therefore only be observable when there is a pH gradient, a condition not met in cultures because the external pH is kept under control at near neutral values. Since, MCT1 is the only transporter responsible for exporting $H^+$ and lactate (as can be inferred from the findings with 3-bromopyruvate), an acidification of the cytoplasm can be expected in MCT1-null cells. This would naturally prevent any 3-bromopyruvate entry via a free diffusion mechanism, as well as interfering with the success of any other pH-dependent, transporter-mediated entry. It is also hardly conceivable that such a cytoplasm acidification and lactate overload would not impair glycolysis; if $H^+$ could be extruded via different pathways, however, lactate overload would negatively affect glycolytic activity and cell survival.

If a free diffusion mechanism dependent on the pH gradient can drive the entry of 3-bromopyruvate into cancer cells, then the enhanced expression of $H^+$-coupled transporters may not be a necessary condition for 3-bromopyruvate to enter and take effect.

In conclusion, the pH gradient existing in tumor cells would enhance a free diffusion mechanism or a monocarboxylate-mediated transport and this might explain the specificity of 3-bromopyruvate’s action in cancer, as previously suggested (Dell’Antone, 2012).

In ancestral cells, when glycolysis was the principal and perhaps the sole pathway to food metabolism for cell survival, the external acidic pH might have been the only mechanism driving nutrients inside the cells. In pluricellular and more complex organisms, the advantages of keeping the cell environment pH neutral (to avoid acidity-induced membrane damage, for instance) compel cells to endow themselves with a more ad hoc way to take up the same metabolites even in the absence of a pH gradient.

**MANIPULATING TUMOR pH AS A CANCER TREATMENT STRATEGY**

Manipulating the extracellular and/or intracellular pH has been proposed as a cancer therapy on several occasions (McCarty and Whitaker, 2010). There is a growing body of evidence that extracellular acidity per se boosts the invasiveness and metastatic capacity of cancer cells (Estrella et al., 2013) and this has prompted practical strategies to raise the extracellular pH. The results of oral administration of sodium bicarbonate in rodents support the feasibility of clinical applications of this strategy (McCarty and Whitaker, 2010).

Inhibiting proton pumps can alleviate extracellular tumor acidity while lowering the tumor cells’ intracellular pH which is deleterious to their survival. On the other hand, the over-regulated glycolysis in cancer could be exploited in hyperacidification therapies: The intense intracellular acidification induced by hyperglycemia and the concurrent administration of proton pump inhibitors may have the potential to kill cancer cells directly or, without proton pump inhibitors, to maximize extracellular tumor acidity and enable the tumor-selective release of cytotoxic drugs (McCarty and Whitaker 2010).

It has been reported that intravenously administering glucose in tumors from several xenograft lines, raising blood glucose to only 2.5 times the normal value, sufficed to reduce the mean tumor pH to 6.4 (Volk et al. 1993). Previous studies also reported a lowering of the extracellular pH of tumors, in both rodent and human
cancers in situ (Jahde and Rajewsky, 1982; McCarty and Whitaker 2010). In a recent study on pancreatic cancer cells, hyperglycemic treatment with excess glucose stimulated glucose metabolism and therefore also ATP content by inducing hypoxia-inducible factor 1α (Liu et al., 2013). So what might be the consequences of hyperglycemic treatments for normal tissues? Very importantly, this treatment affected normal tissue pH only very slightly or not at all (Volk et al., 1993).

The concomitant administration of glucose and 3-bromopyruvate should drive an approximately ten-fold accumulation of the drug in cancer cells by comparison with the compound's circulating levels which would thus be many times higher than in normal cells.

CONCLUSION

The transportation of 3-bromopyruvate inside some cancer cells is mediated, according to some reports, by H+ -coupled or sodium-coupled monocarboxylate transporters and MCT1 in particular has proved essential to the drug's cytotoxic action. There is still no agreement, however, concerning the expression of H+ -coupled transporters in many cancers and a possible lack of upregulation of these transporters could make the drug less effective in such cases, unless a free acid diffusion mechanism is operative.

A free acid diffusion mechanism is naturally relevant when there is a pH gradient across the cell plasmalema, as in the case of solid tumors and this could explain the specificity of the action of 3-bromopyruvate against tumor cells. That some studies found monocarboxylate transporters expression as a prerequisite for the detection of 3-bromopyruvate transport inside the cells may be because the experimental conditions did not contemplate a pH gradient across the cell plasmalema.

If the free diffusion mechanism is also operative, as believed, then transporter overexpression may not be a necessary condition for the drug to have its toxic effect in cancer. Whatever the mechanism involved in the drug's transportation, hyperacidification of the tumor cell microenvironment could enhance the accumulation of 3-bromopyruvate inside cancer cells. This hyperacidification could be achieved by administering glucose in excess, exploiting the high capacity for glycolysis and lactate production in cancer (Warburg). Because high-glucose-driven hyperacidification is tumor-specific, inducing hyperglycemia could enhance 3-bromopyruvate toxicity in tumors again with minimal effects in normal tissues.

REFERENCES


