

Manipulating Tumor Acidification as a Cancer Treatment Strategy

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Abstract

Manipulation of the extracellular and/or intracellular pH of tumors may have considerable potential in cancer therapy. The extracellular space of most tumors is mildly acidic, owing to exuberant production of lactic acid. Aerobic glycolysis – attributable largely to chronic activation of hypoxia-inducible factor-1 (HIF-1) – as well as tumor hypoxia, are chiefly responsible for this phenomenon. Tumor acidity tends to correlate with cancer aggressiveness; in part, this reflects the ability of HIF-1 to promote invasiveness and angiogenesis. But there is growing evidence that extracellular acidity *per se* boosts the invasiveness and metastatic capacity of cancer cells; moreover, this acidity renders cancer cells relatively resistant to the high proportion of chemotherapeutic drugs that are mildly basic, and may impede immune rejection of tumors. Thus, practical strategies for raising the extracellular pH of tumors may have therapeutic utility. In rodents, oral administration of sodium bicarbonate can raise the extracellular pH of tumors, an effect associated with inhibition of metastasis and improved responsiveness to certain cytotoxic agents; clinical application of this strategy appears feasible. As an alternative approach, drugs that inhibit proton pumps in cancer cells may alleviate extracellular tumor acidity while lowering the intracellular pH of cancer cells; reduction of intracellular pH slows proliferation and promotes apoptosis in various cancer cell lines. Well-tolerated doses of the proton pump inhibitor esomeprazole have markedly impeded tumor growth and prolonged survival in nude mice implanted with a human melanoma. Finally, it may prove feasible to exploit the aerobic glycolysis of cancers in hyperacidification therapies; intense intracellular acidification of cancer cells achieved by induced hyperglycemia, concurrent administration of proton pump inhibitor drugs, and possibly dinitrophenol, may have the potential to kill cancer cells directly, or to potentiate their responsiveness to adjunctive measures. A similar strategy, but without proton pump inhibition, could be employed to maximize extracellular tumor acidity, enabling tumor-selective release of cytotoxic drugs encased in pH-sensitive nanoparticles.

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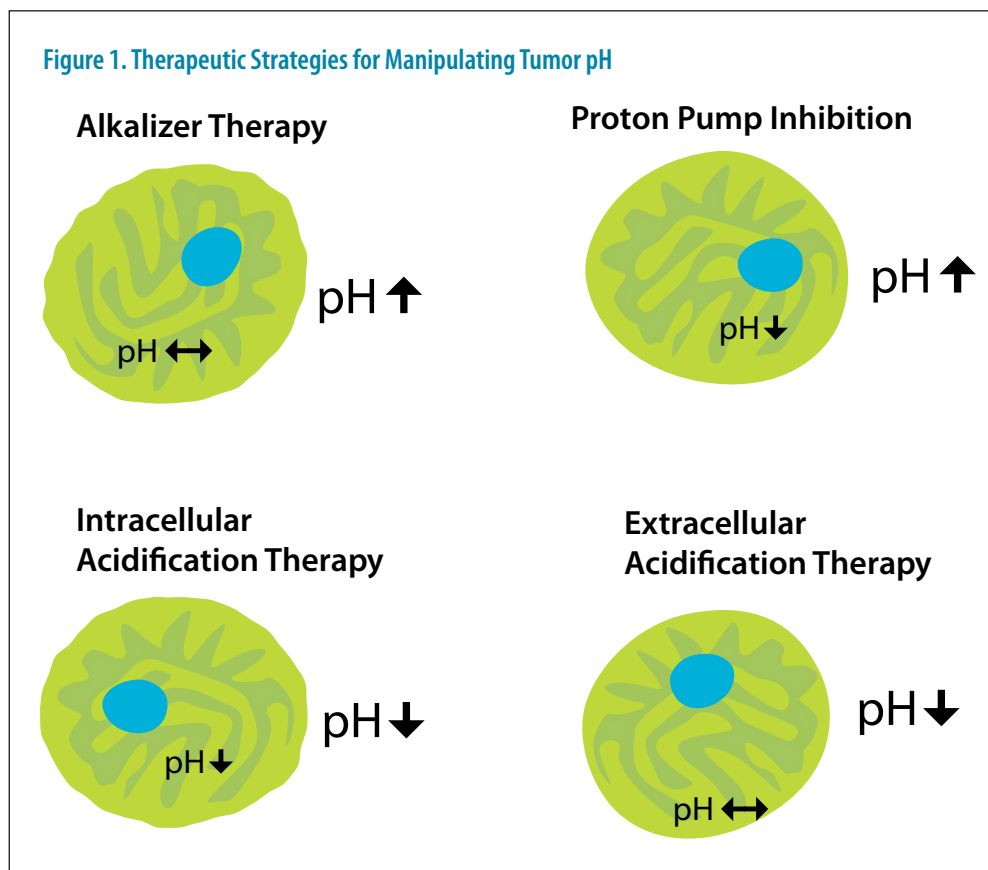
Introduction

Manipulation of the extracellular and/or intracellular pH of tumors may have considerable potential in cancer therapy. Four distinct strategies (Figure 1) can be envisioned: (1) alkalizer therapy that increases the pH of the extracellular space; (2) proton pump inhibition that decreases the intracellular pH, while increasing the extracellular pH; (3) acute intracellular acidification that kills cancer cells directly or potentiates their sensitivity to adjuvant measures; and (4) acute extracellular acidification that enables tumor-selective release of cytotoxic drugs encased in pH-sensitive nanoparticles.

Extracellular Acidity – A Marker for and Mediator of Cancer Aggressiveness

As originally reported by Otto Warburg, most cancers are characterized by aerobic glycolysis – wasteful glycolytic conversion of glucose to lactic acid, even when sufficient oxygen is available to support efficient mitochondrial respiration. The extent to which this phenomenon is expressed tends to correlate with tumor aggressiveness. Recent studies have clarified that the aerobic glycolysis of cancer cells is commonly attributable to chronic overactivation of the transcription factor hypoxia-inducible factor-1 (HIF-1), which boosts expression of a range of glycolytic enzymes and pyruvate dehydrogenase kinase-1 (which functions to inhibit pyruvate dehydrogenase and thus expedite conversion of pyruvate to lactate) and promotes mitochondrial autophagy.¹⁻⁴ Tumor production of lactic acid is also driven by anaerobic glycolysis in tumor regions that are hypoxic. Owing to avid production of lactic acid, the extracellular space of most tumors is mildly acidic, with the greatest degree of acidity encountered in the tumor core. Cancer cells, however, usually maintain a normal intracellular pH, owing to proton pumps and intracellular buffers.⁵⁻⁷

Key words: cancer, tumor, bicarbonate, pH, acidification, acidify, alkaline, alkaliizer, alkalization, metastatic, metastasis, proton pump, buffer, trisodium citrate, hyperacidification, hyperglycemia, cytotoxic, acid



The degree to which pH is depressed in tumors – as mirrored by their lactate levels – tends to correlate with prognosis, the more acidic tumors being associated with poorer outcome.⁸⁻¹⁰ In part, this phenomenon may reflect the fact that tumor acidity is serving as a marker for HIF-1 activation, which works in a variety of complementary ways to boost tumor capacity for invasion, metastasis, angiogenesis, and chemoresistance.^{11,12} However, there is increasing evidence that extracellular acidity *per se* contributes to the aggressiveness of cancer cells, boosting extracellular proteolytic activities, expression of pro-angiogenic factors, and metastatic capacity.^{7,10} Homeostatically, this makes good sense – extracellular acidity, like hypoxia, is a typical consequence of suboptimal perfusion, and it is not surprising that cells have evolved to sense this acidity and take appropriate countermeasures.

Cultivation of various types of cancer cells under the mildly acidic conditions that prevail in many tumors has been reported to boost transcription of the angiogenic factors vascular endothelial growth factor (VEGF) and interleukin(IL)-8, increase extracellular release and/or expression of key proteases such as cathepsin B, matrix

metalloproteinases -2 and -9, and to amplify the invasiveness and metastatic capacity of cancer cells, *in vitro* and *in vivo*.¹³⁻²³ In one particularly striking study, incubation of various human melanoma cell lines at pH 6.8 (compared with 7.4) for 48 hours approximately doubled the yield of lung metastases following their intravenous administration in nude mice.²¹ An analogous impact of prolonged exposure to extracellular acidity on the invasiveness and migratory activity of human melanoma cells *in vitro* has also been reported.^{22,23}

The effect of extracellular acidity on HIF-1 activity appears so far to have received little study. Activated transcription of VEGF and IL-8 under acid conditions has been traced to increased activity of nuclear factor-kappaB and activator protein-1 in certain cancer cell lines.^{14,16} Increased extracellular proteolytic activity appears to reflect, in part, an increased tendency of lysosomes to migrate to the cell periphery and discharge their contents via exocytosis.^{18,24}

Acidification of the extracellular space in tumors can also contribute to chemoresistance. Since many cytotoxic cancer drugs are mildly basic, their increased protonation in the extracellular space of

tumors would be expected to impede their transit through cell membranes, rendering cancer cells less susceptible to their effects.²⁵⁻²⁷ Moreover, extracellular lactic acid can suppress the tumoricidal activity of cytotoxic T lymphocytes and natural killer cells; it also inhibits lymphocyte proliferation and dendritic cell maturation.²⁸⁻³⁶ These immunosuppressive effects appear not to be mediated by acidity *per se*, but by influx of lactic acid via a lactate/H⁺ co-transporter that under neutral conditions functions to remove lactic acid from leukocytes.

Since tumor acidity appears to make a meaningful contribution to cancer aggressiveness, chemoresistance, and evasion of immune rejection, measures for normalizing the pH of tumors may have therapeutic utility. Aerobic glycolysis and tumor acidification could be suppressed by measures that inhibit the activity of HIF-1. Various practical strategies for achieving this may be currently available, and new drugs are being developed that target this transcription factor.^{12,37} However, alternative measures for ameliorating the extracellular acidity of tumors have been proposed. Novel strategies for exploiting the aerobic glycolysis of tumors in cancer therapy may also prove feasible.

Systemic Buffering: Increasing Extracellular pH

Gillies et al have shown that dietary measures that boost the bicarbonate level of plasma can elevate the subnormal pH of tumors to some degree, without notably influencing the pH of blood or healthy tissues.^{25,26} The failure of oral bicarbonate to influence the pH of plasma presumably reflects the fact that a physiological buffer tends to drive pH to the physiological value of 7.4.⁷ Furthermore, they demonstrated that this strategy may have clinical potential. In nude mice implanted with a human breast cancer, chronic oral administration of sodium bicarbonate, while not influencing the expansion of primary tumors, markedly reduced the number and size of metastases in lung, visceral organs, and lymph nodes.³⁸ These findings thus raise the possibility that systemic buffering, achieved by oral administration of high doses of agents such as sodium bicarbonate or trisodium citrate³⁹ – or possibly even a natural diet of low-to-moderate protein content, but high in potassium-rich fruits, vegetables, and juices⁴⁰⁻⁴² – could dampen the aggressiveness of certain cancers by partially alleviating their extracellular acidity.⁷ Whether this strategy would influence transcriptional activity of HIF-1 is unclear, but it

evidently would tend to counteract one of the key pathogenic downstream consequences of HIF-1 overactivation. It is curious to note that alkalizing diets have long been recommended by naturopathic physicians as a strategy for slowing cancer spread, and that oral administration of sodium bicarbonate – albeit in doses that likely are clinically suboptimal – has also recently gained popularity as an alternative cancer therapy.

In the sodium bicarbonate breast cancer study, mice received about 0.84 mEq Na daily; assuming that the mice weighed about 20 g, and normalizing by the 3/4th power of relative weights,⁴³ the equivalent dose in a 70 kg human would be 378 mEq, which corresponds to a daily dose of 31.75 g sodium bicarbonate or 32.5 g trisodium citrate. At the Whitaker Wellness Institute, the authors are currently implementing an alkalinizing therapy with cancer patients. Large acute doses of either sodium bicarbonate or trisodium citrate (which have been studied as aids to exercise performance, usually at 40-60 mg/kg) can induce temporary nausea and diarrhea;³⁹ it therefore is prudent to administer the daily dose gradually throughout the day. Moreover, gradual administration should more aptly mimic the mouse study, in which sodium bicarbonate was administered in drinking water, and thus was consumed continuously during waking hours, presumably achieving a more even elevation of tumor extracellular pH than could be achieved with bolus doses. The protocols currently employed are: add 500 mL (about 2 cups) of water to a small teapot and stir in two rounded teaspoons (about 12 g) of sodium bicarbonate or trisodium citrate plus one packet of sweetener of choice. Patients who are “on the go” can prepare this beverage in a 500-mL water bottle. This is to be consumed gradually over an hour or more. If this procedure is followed three times daily (e.g., in mid-morning, mid-afternoon, and late evening), about 36 g of sodium bicarbonate or trisodium citrate will be provided daily. If desired, this fluid can be heated to make tea or herb teas, or can be flavored with a Crystal Light™-type product. Sodium bicarbonate has the advantage that it is quite inexpensive and readily available; trisodium citrate may be less likely to promote intestinal gas.

Exploiting Proton Pump Inhibitors: Decreasing Intracellular pH

As an alternative or adjunctive strategy for correcting the extracellular acidity of tumors, a number of researchers have explored inhibition of the membrane ion pumps that maintain an

alkaline intracellular pH by extruding protons or importing bicarbonate ions. Many cancers express extracellular forms of carbonic anhydrase – CAIX and CAII – which acidify the extracellular space while generating bicarbonate that can be imported.^{44,45} A Na^+/H^+ exchanger (NHE), of which several isoforms exist,^{46,47} is a prominent mediator of proton extrusion; the NHE1 isoform is ubiquitously expressed.⁴⁸ Proton extrusion is also achieved by the vacuolar H^+ -ATPase (V-ATPase), which hydrolyzes ATP to drive proton pumping.⁴⁹ Although the chief physiological role of this pump is to acidify intracellular vacuoles such as lysosomes and endosomes, it is also expressed in the plasma membrane of many cancer cells, particularly those with metastatic capacity.⁵⁰⁻⁵⁴ Moreover, the protons pumped into vacuoles often reach the extracellular space when these vacuoles fuse with the plasma membrane and extrude their contents. A Na^+ -dependent Cl/HCO_3^- exchanger also functions to maintain an alkaline intracellular pH.

Proton pump inhibition tends to decrease intracellular pH, as it raises that of the extracellular space. The ameliorative impact on extracellular acidity tends to be sustained (despite the fact that the rate of lactate generation must ultimately match the rate of lactate export), presumably because intracellular acidity tends to suppress the efficiency of glycolysis.⁵² The intracellular acidification associated with proton pump inhibition can have an impact on cancer cell behavior, independent of that of the associated elevation of extracellular pH. Indeed, proton pump inhibitors exert anti-proliferative and pro-apoptotic effects on certain cancer cell lines;⁵⁵⁻⁶⁰ furthermore, intracellular acidification has been shown to enhance the killing efficacy of hyperthermia (42°C) as well as the apoptotic response to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL).⁶¹⁻⁶⁴

Although a number of different agents have been employed as proton pump inhibitors in cell culture or rodent studies, few of these have been used clinically. Notable exceptions are the proton pump inhibitor drugs (PPIs; e.g., omeprazole, esomeprazole) used clinically to suppress gastric acidity. When activated by mildly acidic conditions, these drugs can inhibit the V-ATPase by a covalent interaction.^{27,65} As emphasized by De Milito et al, the particular merit of these drugs is that their impact can be tumor-specific, as they are activated in the mildly acidic extracellular space of tumors.^{27,52,56} Other agents, such as bafilomycin, that act to inhibit V-ATPase, are tissue non-selective

and have been found to have unacceptable systemic toxicity. *In vitro*, V-ATPase inhibitors have exerted anti-proliferative, pro-apoptotic, and thermosensitizing effects.^{56,57,59,60,63,66} *In vivo*, non-toxic doses of PPIs, analogous to those used clinically in Zollinger-Ellison syndrome, have been shown to suppress the growth of a human melanoma in nude mice, an effect associated with a near-doubling of survival time.⁶⁷ Knockdown of V-ATPase expression achieved via small interfering RNA in a human hepatocellular carcinoma was found to markedly slow the growth and impede the metastatic spread of this cancer in nude mice.⁶⁸ And another agent inhibitory to V-ATPase has been reported to suppress the formation of spontaneous lung metastases in mice transplanted with a human non-small cell lung cancer.⁶⁹ Pre-administration of omeprazole notably potentiated the growth-retardant impact of cisplatin on a human melanoma in nude mice, presumably at least in part because cisplatin is a mildly basic drug whose intracellular uptake is impaired by the acidic extracellular milieu of tumors.²⁷

Another key mediator of proton extrusion is NHE1. This pump can be inhibited by supra-clinical concentrations of the diuretic amiloride. A derivative of amiloride, EIPA, is about 200 times more potent in this regard, but has never been developed for clinical use.^{70,71} More recent studies have employed the NHE1 inhibitor cariporide, which has been taken to phase III trials as a drug for decreasing risk of myocardial infarction (MI) subsequent to coronary bypass surgery.^{72,73} Unfortunately, this agent is unlikely to be approved for this purpose, as it was found to modestly increase cerebrovascular mortality while decreasing MI risk.⁷³ Nonetheless, a well-tolerated dosage schedule has been established, so it is conceivable that this drug could be developed as a cancer therapeutic if its utility in this regard could be established in rodent studies. A number of studies have established that this agent, often used in conjunction with an inhibitor of the Cl/HCO_3^- exchanger, can lower the intracellular pH of cancer cells.^{58,74-76} In a human pancreatic adenocarcinoma cell line, inhibitors of V-ATPase and NHE1 have been shown to have an additive impact on intracellular pH and on thermosensitization; this suggests the desirability of evaluating joint administration of PPIs and cariporide in rodents.⁶³

Intracellular Hyperacidification Therapy: Acutely Maximizing Intracellular Acidity

A sufficiently large reduction in intracellular pH can promote apoptosis in cancer cells,⁵⁵⁻⁶⁰ could be used to achieve tumor-specific uptake or activation of certain drugs whose effects are pH-sensitive,^{27,75,77,78} and can potentiate the cytotoxic impact of local hyperthermia (~42° C) and TRAIL.⁶¹⁻⁶⁴ This raises the interesting prospect that acute intracellular tumor acidification could be developed as a strategy for achieving rapid substantial tumor kill – perhaps when used in conjunction with other cytodisruptive measures whose activity is greater at acidic pH. Indeed, some researchers have suggested the utility of such a strategy for potentiating the efficacy of concurrent local hyperthermia or chemotherapy.

An increase in tumor-specific acidification can be achieved if the rate of tumor glycolysis is maximized. Substrate availability for glycolysis is usually suboptimal in tumors, owing to the fact that, especially in aggressive tumors with high glycolytic capacity, tumor glucose levels tend to be relatively low owing to avid uptake of glucose for glycolysis and inefficient tumor perfusion.⁷⁹ Hence, induced hyperglycemia tends to boost tumor glycolysis and decrease extracellular tumor pH by elevating tumor glucose levels; indeed, there are many reports that induced hyperglycemia tends to lower the extracellular pH of tumors, both in rodents and in human cancers *in situ*.^{74,79-88}

A further boost in glycolysis could be achieved by suppressing mitochondrial ATP generation; inhibitors or uncouplers of electron transport could be employed for this purpose. In many rodent and cell culture studies, an inhibitor of mitochondrial complex I, meta-iodobenzylguanidine, has been shown to amplify the depression of tumor intracellular pH achieved with hyperglycemia and/or proton extrusion inhibitors.^{74,89-92} This agent, in radioiodinated form, has been used in the treatment of neuroendocrine tumors⁹³ – but the doses employed for this purpose are lower than those required for effective mitochondrial inhibition. A more practical and intriguing prospect in this regard is offered by the uncoupling agent dinitrophenol (DNP). DNP is mildly acidic, and its uncoupling activity is reported to be six-fold greater at pH 6.4 than at pH 7.4;⁹⁴ thus, its impact might be amplified in acidified tumors.⁹⁵

Moreover, DNP was employed clinically in the

1930s as an anti-obesity agent.⁹⁶ Although in excess it can give rise to lethal hyperthermia, it seems to be reasonably well tolerated in a daily dose of 3-5 mg/kg – a sufficient dose to raise the metabolic rate and promote substantial weight loss. In human melanoma cell cultures, addition of DNP (0.1 mM) very markedly potentiated the increase in glycolysis achieved with high glucose exposure.⁹¹ Sub-millimolar concentrations of DNP have been reported to slow proliferation, induce apoptosis, and exert a pro-oxidant effect in a human lung adenocarcinoma cell line;⁹⁷ albeit the concentrations employed in this study are somewhat higher than would be systemically tolerable.

These considerations suggest that it might be appropriate to examine the joint impact of hyperglycemia (achieved by sustained high-dose intravenous glucose infusion), PPIs, cariporide, and physiologically-tolerable concentrations of DNP on intracellular pH and survival of cancer cells *in vitro* and in rodents. Such a strategy could also be assessed as an adjuvant to local hyperthermia or to administration of cytotoxic agents that are more active at acidic intracellular pH. If this strategy showed good efficacy in rodents, it could rapidly be translated to clinical application, as each of the drugs involved has already received substantial clinical evaluation and is known to be reasonably safe within defined dose levels. Owing to the requirement for hyperglycemia, this approach could only be used episodically. Conceivably, measures that raise extracellular tumor pH could be employed in the intervals between treatments.

Extracellular Hyperacidification Therapy: Acutely Maximizing Extracellular Acidity

An alternative approach to hyperacidification therapy would be to maximize the acidity of the tumor extracellular space (as with hyperglycemia and DNP, in the absence of proton pump inhibitors), with the intent of achieving tumor-selective delivery of cytotoxic drugs that are activated by acidity. In particular, nanoparticles that break down or fuse with cell membranes under mildly acidic conditions are being developed for selective drug delivery to acidified tumors.⁹⁸⁻¹⁰¹ Concurrent administration of proton pump inhibitors would be expected to impede optimal extracellular acidification, since the resulting suppression of intracellular pH would act as a brake on glycolysis, slowing the rate of lactate generation.

Summing Up

Manipulation of the extracellular and/or intracellular pH of tumors may have considerable potential in cancer therapy. At least four distinct strategies merit evaluation in this regard: (1) alkalizer therapy that increases the pH of the extracellular space; (2) proton pump inhibition that decreases the intracellular pH, while increasing the extracellular pH; (3) acute intracellular acidification that may be directly cytotoxic or that potentiates the lethality of adjuvant measures; and (4) acute extracellular acidification that enables tumor-selective release of cytotoxic drugs from acid-labile nanoparticles.

The extracellular acidity that characterizes most tumors – reflecting aerobic glycolysis induced by HIF-1 overactivity, as well as hypoxia in some tumor regions – tends to correlate negatively with cancer prognosis and is now known to be more than an epiphenomenon. Extracellular acidity can increase the invasive spread of cancer cells, while protecting them from immune attack and from the many cytotoxic agents that are mildly basic. Fortunately, feasible doses of safe alkalizing agents, such as sodium bicarbonate, have the potential to alleviate tumor acidification to some degree; in cancer-bearing mice, this strategy has been found to suppress metastatic spread and improve response to chemotherapy. The extracellular acidity of tumors can also be corrected with proton pump-inhibitory drugs that are selectively activated in an acidic milieu. This approach has the ancillary advantage that it promotes the intracellular acidification of cancer cells; intracellular acidity tends to slow cellular proliferation while boosting apoptosis. Finally, inasmuch as intense intracellular acidification can be lethal, or can potentiate the lethality of other agents, acute hyperacidification therapies can be envisioned, in which measures that maximize cancer glycolysis (temporary induced hyperglycemia and possibly dinitrophenol) are employed concurrently with proton pump inhibitors. A variant approach would be to acutely amplify extracellular tumor acidity by maximizing tumor glycolysis in the absence of proton pump inhibitors, so as to induce selective uptake of concurrently administered drugs enclosed in acid-labile nanoparticles.

The Warburg phenomenon, with the attendant acidification of the extracellular milieu of tumors, has fascinated cancer scientists for many decades, but the modulation of these phenomena has yet to earn a role in orthodox cancer therapy. This situation may be on the brink of changing, as

molecular biologists are now defining the ways in which extracellular or intracellular acidity can influence cancer behavior and are devising novel and clinically feasible strategies for manipulating tumor acidity. Clearly, a number of these strategies have intriguing potential and deserve further exploration in pre-clinical and clinical studies.

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