2 Influence of Tumor pH on Therapeutic Response

Chang W. Song, PhD, Robert Griffin, PhD, and Heon Joo Park, MD, PhD,

CONTENTS

SUMMARY

The intratumor microenvironment is intrinsically acidic due mainly to accumulation of lactic acid as a result of increased aerobic and anaerobic glycolysis by the tumor cells. In general, the extracellular pH (pHe) in human tumors is below 7.0, whereas the intracellular pH (pHi) is maintained at neutral range, i.e., >7.0, by powerful pHi control mechanisms. The low pHe and the significant gradients between pHe and pHi affect markedly the response of tumors to various treatments such as chemotherapy, radiotherapy and hyperthermia. For instance, the acidic pHe increases the cellular uptake of weakly acidic drugs such as cyclophosphamide and cisplatin and thus increases the effect of the drugs, whereas the acidic pHe retards the uptake of weakly basic drug such as doxorubicin and vinblastine, thereby reducing the effect of the drugs. The radiation-induced apoptosis is suppressed by an acidic environment, whereas the hyperthermia-induced cell death is potentiated by an acidic environment. Better understanding of the control mechanisms of pHe and pHi in tumors may lead to device effective treatment strategy of human tumors.

From: *Cancer Drug Discovery and Development: Cancer Drug Resistance*
Edited by: B. Teicher © Humana Press Inc., Totowa, NJ

Key Words: Intratumor pH; extracellular pH; intracellular pH; intratumor pH control mechanism; chemotherapy; radiotherapy; hyperthermia.

1. INTRODUCTION

The environmental acidity or pH of living cells and tissues is one of the major factors that influence molecular processes involved in cell cycle progression, cell proliferation, and differentiation. Likewise, oncogenesis, malignant transformation, metastasis, and angiogenesis are greatly influenced by environmental acidity. The environmental acidity also greatly influences the response of cancer cells to various treatments. The vascular network in tumors is inhomogeneous, causing insufficient oxygen supply to parts of tumors. The resultant hypoxia forces glucose metabolism through the glycolytic pathway instead of respiration, thereby resulting in the formation of lactic acid *(1–7)*. In addition, tumor cells convert glucose and other substrates preferentially to lactic acid and other acidic metabolites even under aerobic conditions, leading to acidification of the intratumor environment *(5,8,9)*. Whereas the interstitial or extracellular environment in tumors is acidic, the intracellular pH (pHi) in tumors has been found to be at neutral range, i.e.,< 7.0, similar to the pHi of normal tissues *(1,2,5–12)*. This intracellular and extracellular pH (pHe) gradient in tumor cells is maintained by sophisticated biophysical mechanisms *(1,2,13)*. It has been demonstrated that the gradient between pHe and pHi of tumor cells renders the cells resistant to weakly basic drugs by hindering the cellular uptake of the drugs, whereas the same pH gradient increases the uptake of weakly acidic drugs. The influence of tumor acidity on the thermosensitivity of tumor cells has been extensively investigated. On the other hand, relatively little has been revealed on the effect of acidic intratumor environment on the response of tumor cells to radiotherapy. In this chapter, we review the pHi control mechanisms and the implications of tumor pH and that of the pH gradient between the outside and inside of tumor cells on the response of tumor cells to various treatments.

2. TUMOR pH

It has long been known that the microenvironment in tumors of both animal and human is acidic as compared with that in normal tissues because of elevated in anaerobic as well as aerobic glycolysis in tumors *(1–6)*. As tumor nodules are formed, neovascularization begins from host venules stimulated by a number of angiogenic factors secreted by the tumor cells as well as adjacent normal cells. The newly formed tumor vascular beds are characterized by a heterogeneous distribution of dilated, irregularly bulged, constricted, twisted, and sharply bent capillary-like blood vessels *(14–23)*. Consequently, tumor blood perfusion is sluggish, resulting insufficient supply of various nutrients, including oxygen, to tumor cells. As the tumor grows larger, the intercapillary distance progressively increases, and areas beyond oxygen diffusion distance from capillaries, i.e., about 150 μm, become hypoxic *(24)*. In addition, probably because of progressively increasing interstitial pressure caused by the increasing tumor cell population *(25)*, tumor blood vessels are compressed, and the blood perfusion ceases intermittently or permanently resulting in intermittent or permanent hypoxia *(20–23)*. Hypoxia upregulates various transcription factors including hypoxia-inducible factor-1 (HIF-1), which activates the transcription of numerous genes whose protein products facilitate adaptation to hypoxia, driving the tumor toward a more malignant phenotype *(26–28)*. A well-known response of cells to hypoxia is an increase in hyperglycolytic activity characterized by increased

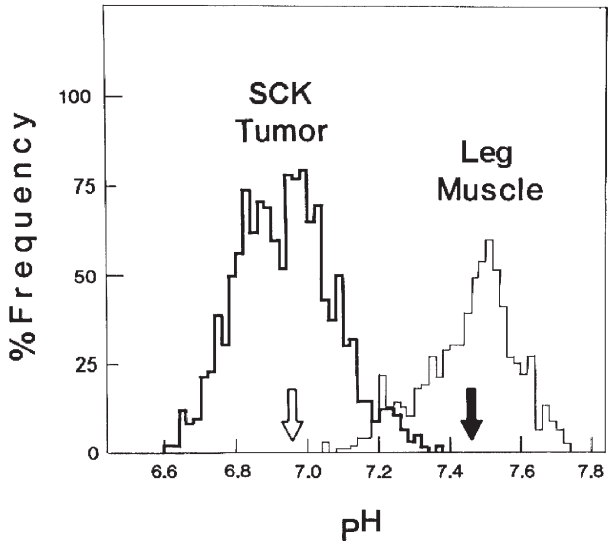

Fig. 1. Histograms of interstitial pH in the leg muscle of A/J mice and that in SCK tumors grown subcutaneously in the leg of A/J mice. The tumor diameters were 7–9 mm. The pH was measured with glass microelectrodes 50–80 mm in diameter *(3)*.

glucose uptake and formation of lactic acid, resulting in acidification of intratumor environment *(5–7)*. It has also been demonstrated that hypoxia activates carbonic anhydrase, thereby causing hydration of CO_2 molecules to carbonic acid *(7)*. Hulikova et al. *(29)* reported that tumor-associated carbonic anhydrase IX isoform is the most likely candidate involved in the formation of carbonic acid under hypoxic conditions. Hydrolysis of ATP is also a significant contributor to acidosis in tumors during acute hypoxia *(30)*. It should be pointed out, however, that tumor acidification can occur independent of hypoxia. It was shown in the early part of the last century that tumor cells metabolize glucose preferentially through glycolysis, even in the presence of oxygen *(8)*. It is believed that the endogenous acidification is an integral property of tumor cells that may have evolved to provide tumor cells with a competitive advantage over stromal cells *(5,8)*. Elstrom et al. *(31)* reported that the high rate of aerobic glycolysis in cancer cells is because of upregulation of the serine/threonine kinase Akt *(9)*.

2.1. pH in Tumors vs Normal Tissues

Until recent years, pH of animal and human tumors was determined by glass or fiber optic pH electrodes *(3,4,32–35)*. Because the diameters of the electrodes are larger than the diameters of cells and the tissue damage caused by the electrodes can be substantial, the pH values obtained with microelectrodes represent mainly pHe. Despite the technical difficulties, important information on tumor pH has been accumulated during the last several decades. Figure 1 shows histograms of pHe in SCK mammary carcinoma and that of the leg muscle of A/J mice obtained with glass microelectrodes *(3)*. It is demonstrated that the pHe in SCK tumors ranged from 6.60 to 7.38, with a mean value of 6.96, whereas that of the muscle ranged from 7.05 to 7.72, with a mean value of 7.45. This difference between mean tumor pH and mean muscle pH of as much as 0.5 pH units means that the concentration of the active H^+ ions in the interstitial space in SCK tumors was five times

greater than that in the muscle. Wike-Hooley et al. *(4)* reviewed a number of reports on the pHe value in tumors and normal tissues of animals and concluded that the tumor pHe ranged from 5.8 to 7.68, with an average of 7.09, and that the pHe in normal tissues such as muscle and liver was about 0.5 pH units higher than that in tumors. Vaupel et al. *(36)* reported that whereas average pHe in a C3H murine mammary carcinoma was 6.7, the pHe in some microareas was as low as 5.8–6.3. On the other hand, the pHe measured in extensively necrotic areas was higher than that in normal tissues, probably because of lack of formation of acidic metabolites as a consequence of previous cell death. Jahde et al. *(37)* observed that the pHe in neuroectodermal TV1A tumors grown subcutaneously in the flank of BDIX rats ranged from 6.8 to 7.1, with a mean of 7.0. Interestingly, the pHe values in the brain and kidney of BDIX rats were similar to that measured in brain tumors of the same animal.

Meyer et al. *(38)* reported as early as 1948 that the pHe of human tumors was lower than that in normal tissues, and other investigators subsequently reported similar results *(4,32,39–49)*. Wike-Hooley et al. *(4)* also reviewed the distribution of pHe in human tumors and normal tissues. The tumor pHe ranged 6.0–7.6, with a median pHe of 7.1, whereas the subcutis/muscle pHe ranged 7.3–7.8, with a median pHe of 7.55. It has been reported that, in general, the range of pH values in tumors is much greater than that the normal tissues, probably because the distribution of the vascular supply and blood perfusion in tumors are heterogeneous *(3,4)*. In this regard, the intertumor pHe variance was more striking than the intratumor pHe variance *(4)*. Based on numerous reports, Wike-Hooley et al. *(4)* concluded that the pH values in human tumors were not related to the tumor histology, degree of differentiation, tumor size, patient age, or treatment histology. However, the pHe values in metastases were higher than those in the primary tumors of a given patent.

2.2. Intracellular pH

It has become increasingly evident in recent years that pHi is not equal to pHe in tumor cells. We have studied the pHi of tumor cells in vitro using the pH-sensitive dye BCECF *(1,2)*, as shown in Fig. 2. The pHi remained at about 7.4 when the medium pH, i.e., pHe, was in the 7.0–7.4 range. As the pHe was lowered, the pHi also decreased, but only slightly. For example, at pHe 7.0 and 6.0, the pHi was 7.4 and 6.7, respectively. This in vitro study demonstrated clearly that pHi of tumor cells in a low-pHe environment remains near the neutral pH range. It has become possible in recent years to determine pHi of tumor cells *in situ* by virtue of impressive progress in magnetic resonance spectroscopy (MRS) technology. The pHi of tumors has been measured with ^{31}P-nuclear magnetic resonance (NMR), which determines the shifts in intracellular inorganic phosphate and phosphocreatine *(10,50–54)*. It is now possible to determine pHe using ^{1}H-MRS and also simultaneously determine pHi and pHe by incorporating a pHe indicator, 3-aminopropyl phosphonate, into ^{31}P-NMR *(6)*. The pHe and pHi in the same tumor cells can also be determined with ^{19}F-MRS using 6-fluropyridoxol, a vitamin B6 analog (6-fluoropyridoxol and 6-fluoropyridoxamine) or 3-[*N*-(4-fluor-2-trifluoromethyl-phenyl)-sulphamoyl]propionic acid (ZK-150471). Gillis et al. *(6)* reviewed reports on the differences between pHi and pHe in animal and human tumors determined with MRS methods and reported that the pHi was usually higher than pHe in human tumors *in situ*. In Fig. 3, we further analyzed the relationship between the pHe and pHi in human, murine,

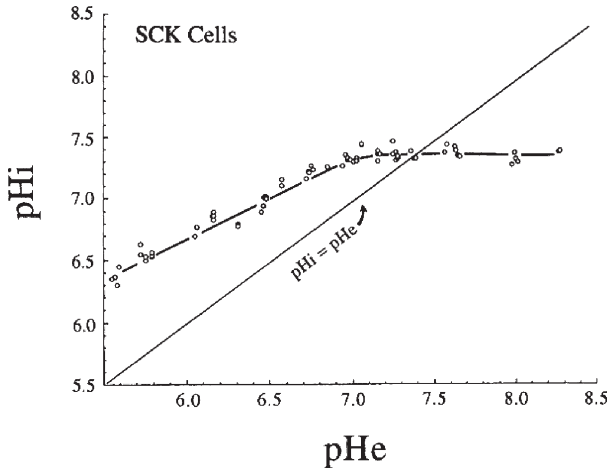

Fig. 2. Relationship between extracellular pH (pHe) and intracellular pH (pHi) of SCK tumor cells in vitro. The cells were maintained at pHe 7.2 before exposure to a new pHe. The pHi was measured using the pH-sensitive dye BCECF method 20–30 min after exposure to new pHe.

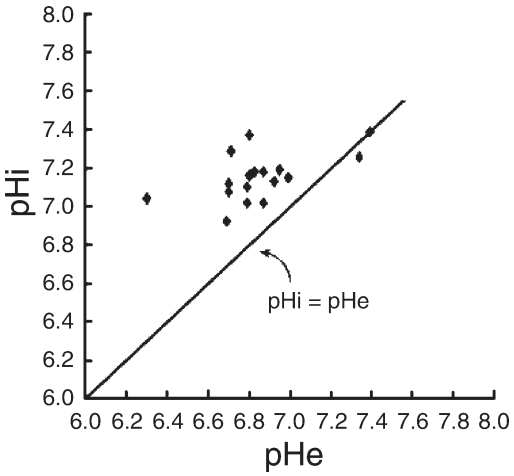

Fig. 3. The relationship between extracellular pH (pHe) and intracellular pH (pHi) in human, rat and mouse tumors *in situ*. Both pHe and pHi in the same tumors were determined with magnetic resonance imaging/magnetic resonance spectroscopy method. Data reported by ref. *6* were used to construct this figure.

and rat tumors determined with MRS/magnetic resonance imaging method and reviewed by Gilles *(6)*. It can be seen that the pHi values are higher than the pHe values in the same tumors in all tumors studied. The pHe values were correlated with phenotype, and the pHe values in larger tumors were lower than that in smaller tumors, probably because of poorer blood perfusion in larger tumors and more accumulation of acidic byproducts of glycolysis *(6)*. In conclusion, all available evidence indicates that the intracellular environment in tumor cells is less acidic as compared with extracellular environment in vitro as well as in vivo.

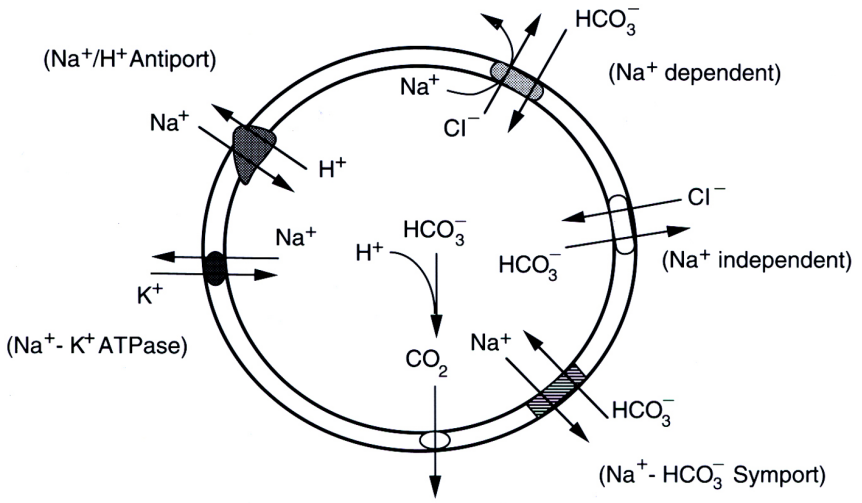

Fig. 4. Most common membrane-based intracellular pH regulatory mechanisms in mammalian cells.

3. MECHANISM OF pHi CONTROL

The fact that pHi is significantly higher than pHe in tumors demonstrates the existence of powerful mechanisms to prevent acidification of the intracellular environment *(13,55–61)*. Such significant gradient between pHe and pHi has been attributed to existence of short-term and long-term mechanisms for pHi control *(13)*. The short-term mechanisms are essentially rapid buffering responses against an acute acid load in the cytosol of cells. The most important short-term regulatory mechanism is the physiochemical buffering of the acids. Other rapid mechanisms include metabolic consumption of nonvolatile acids and transfer of acids from the cytosol to the organelles. These three mechanisms are only for rapid consumption of H^+ ions to minimize rapid acidification in the cells; therefore, their capacity to maintain the intracellular environment at neutral pH for a prolonged period is limited. Almost all mammalian cells that have been investigated thus far possess powerful systems to regulate pHi using several long-term mechanisms *(13)*. The most important mechanism for long-term pHi regulation is the exchange of Na^+ ions for H^+ ions using the Na^+/H^+ antiport, an ion exchanger in the plasma membrane (Fig. 4) *(56,57)*. This process is believed to occur by the binding of intracellular H^+ ions to the cytoplasmic surface of the exchanger and the binding of Na^+ ions to the cell surface of the exchanger. However, indications are that the exchange of Na^+ ions and H^+ ions is not a simple one-for-one exchange. It has been postulated that there might be a second cytoplasmic H^+ binding site that allosterically activates the antiport *(56,59)*. The influx of Na^+ ions and efflux of H^+ ions by this antiport is driven by a Na^+ gradient across the cell membrane. However, even in the presence of large Na^+ gradient energy, the exudation of H^+ ions from the cells is limited, and the pHi is stabilized at neutral values. This fact indicates that although the Na^+ gradient is important for the Na^+/H^+ exchange, it is not the only factor that controls the pHi. The antiport may become inactive when the pHi reaches a certain level, even though the Na^+ gradient remains large *(56)*. When the extracellular Na^+ ion concentration is low, the Na^+ gradient is reversed, and H^+ ions will enter the cells *(56)*. The Na^+ ions that enter the cells are extruded from cells driven by ATP hydrolysis. The

activity of the Na^+/H^+ antiport is partially reduced under hypoxic conditions, which may be attributed to the reduction of ATP content *(13,60)*. There is evidence that the Na^+/H^+ antiport system is secondarily dependent on the Na^+/K^+-ATPase *(60)*. A number of compounds have been demonstrated to interfere with the Na^+/H^+ antiport. Amiloride, a diuretic drug and weak base, and many of its analogs inhibit the Na^+/H^+ antiport activity by competing with Na^+ ions for the Na^+ channel *(9,11,13,57,58,61)*. Ethylisopropylamiloride, an analog of amiloride, is a more specific inhibitor of Na^+/H^+ antiport than amiloride, and as such, ethylisopropylamiloride is a much more potent inhibitor of Na^+/H^+ antiport than amiloride *(12,57)*.

The intracellular acidity is also regulated by bicarbonate-linked mechanisms, namely (1) Na^+-dependent Cl^-/HCO_3^- exchange, (2) Na^+-independent Cl^-/HCO_3^- exchange, and (3) Na^+/HCO_3^- symport (*see* Fig. 4) *(11,13,55,57,58)*. All three mechanisms are not always present in all types of cells. Usually, various combinations of the three mechanisms are found in different cell types. Among these, Na^+-dependent Cl^-/HCO_3^- exchange is probably the most important bicarbonate-linked mechanism for pHi control in mammalian cells. It responds only to acid challenge and neutralizes the intracellular environment by exchanging the negatively charged intracellular Cl^- with the extracellular Na^+/HCO_3^- complex *(62,63)*. The exchange is believed to be driven by Na^+ gradient and in some circumstances, by an additional inward-directed HCO_3^- gradient. The Na^+-independent Cl^-/HCO_3^- exchange is involved in protecting the cells from relatively rare occurrences of cell alkalinization. In this case, HCO_3^- ions are extruded from the cells, and Cl^- ions are transported into the cells to prevent the pHi from rising to an abnormally high level *(62,63)*. The Na^+/HCO_3^- symport is electrogenic, unlike the other two mechanisms *(64,65)*. A sudden reduction of Na^+ and Cl^- ions activates this mechanism to transport these two ions. Whereas this mechanism may be important for specialized acid-secreting cells, its role in regulating pHi in other mammalian cells is uncertain. All of the bicarbonate-dependent transporting mechanisms are inhibited by 4,4'-diisothiocyanostilbene 2,2'-disulfonic acids (DIDS), and 4-acetamindo-4' - isothiocyantostilbene 2,2' -disulfonic acids *(13,57,66–68)*. Ethacarynic acid inhibits the Na^+-independent Cl^-/HCO_3^- antiporter without affecting the Na^+-independent one, and picrylsulfonic acid has the opposite effect *(69)*. The Na^+-coupled CI^-/HCO_3^- exchange is also inhibited by depletion of ATP, and the Na^+-independent Cl^-/HCO_3^- exchange is inactivated by a low-pH environment *(62,70)*.

The relative importance in maintaining pHi at neutral range of the different mechanisms mentioned varies markedly in different cell types and under different conditions. The lactate $/H^+$ symport, which is inhibited by the bioflavionoids quercetin and others, is one of the most active exchange in the regulation of pHi in tumor cells *(71,72)*. However, under hypoxic conditions the lactate extrusion is reduced, and so this exchange has little effect on resting pHi in the hypoxic cells *(5)*. In the gastric glands, the Na^+/H^+ antiporter plays the dominant role, whereas in the neighboring oxyntic cells, the Cl^-/HCO_3^- exchange plays the dominant role for the pHi regulation *(57)*. Three types of ATP-driven H^+ pumps have also been identified *(57)*. One of these is an ATPase-linked H^+ pump found in some specialized epithelial cells. It has been reported that one of the mechanisms to maintain the cytosolic pH at physiological level is sequestration of cytosolic protons into acidic cellular vesicles such as endoplasmic reticulum, endosomes, and lysosomes. Interestingly, the ATPase-linked H^+ pump has been identified in a number of intracellular organelles, indicating that the ATPase-linked H^+ pump plays an important role in regulating pH in the vesicles and cytosol. The other two mechanisms are a H^+-translocating

Table 1
Genes Activated by Low pH

AP-1	VEGF
NFκB	bFGF
p53	PDECGF
p21	IL-8
MTIIA	Cyclines
GRPs	HSPs
Bax	NQO1

ATPase and a K^+/H^+ exchange ATPase, which can be suppressed by nigericin. Further understanding of the pHi control mechanisms may enable us to control the response of cells to internal as well as external stresses including various cancer treatments.

4. EFFECT OF pH ON ANGIOGENESIS AND METASTASIS

It has been established that hypoxic environment upregulates a number of transcription factors such as HIF-1, nuclear factor κB, and activator protein 1 *(73–76)*. HIF-1 has been demonstrated to activate transcription of as many as 70 genes including glucose transporters and glycolytic enzymes, which may account for the increased anaerobic glycolysis and resultant acidification of tumors under a hypoxic environment *(26–28)*. Like hypoxia, acidosis also upregulates transcription factors and activates a number of genes *(77–79)*. We have observed that exposure of tumor cells to a low pH medium elevates significantly p53 expression and p21 expression *(77)*. When the low pH medium was replaced with neutral pH medium, the expression of p53 and p21 promptly returned to normal level. Table 1 shows some of the genes or their products upregulated by an acidic environment. Note that many of the genes activated by acidosis are the same genes activated by hypoxia. For example, the angiogenic factors such as vascular endothelial growth factor, basic fibroblast growth factor, platelet-derived endothelial cell growth factor, and interleukin 8 are upregulated by both hypoxia and acidosis. In view of the fact that many hypoxic cells in tumors are in an acidic environment, how the hypoxia and acidosis interact in promoting the angiogenic process remains an important avenue to be elucidated.

The metastatic potential of tumors has been demonstrated to be related closely to the environmental acidity. The ability of murine tumor cells to form lung metastases after intravenous injection increased significantly when the cells were cultured in acidic medium before the injection *(79,80)*. Deliberate exposure of mice bearing tumors to cyclic low-oxygen breathing (12 cycles of 5% oxygen breathing for 10 min interspersed with 10 min of air breathing) every day doubled the incidence of lung metastases *(81)*. It appeared that acidosis in combination with hypoxia induced by the low-oxygen breathing enhanced the incidence of metastasis. However, acidification of murine tumors by daily administration of metaiodobenzylguanidine and/or glucose without lowering tumor pO_2 did not enhance the spontaneous metastasis potential of tumor cells in the same model *(82)*. In addition to angiogenesis, induction of genomic instability or epigenetic regulation of gene expression may be involved in the increase in metastasis in acidic and hypoxic environments *(83)*. It is likely that the cells that survive the acidic and hypoxic hostile intratumor environment are more aggressive and metastatic as compared with

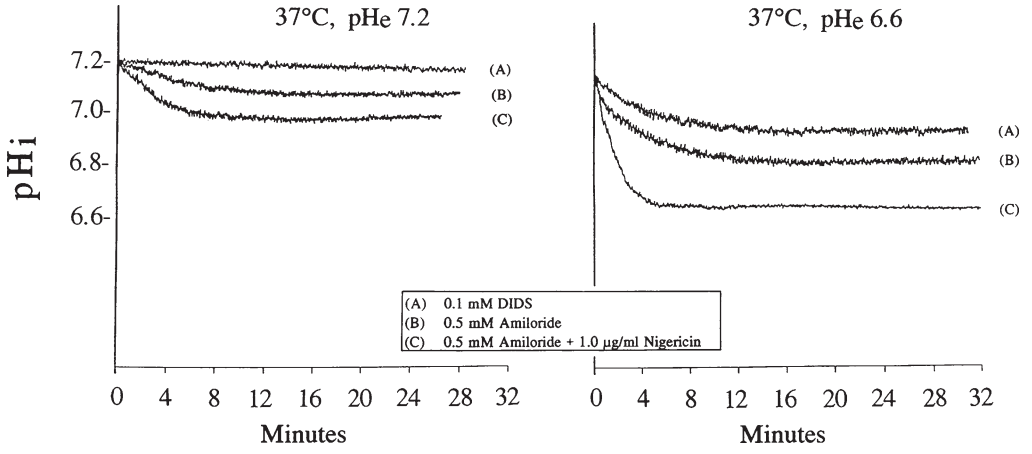

Fig. 5. Changes in pHi (BCECF intensity) in SCK tumor cells upon treating the cells with inhibitors of intracellular pH (pHi) regulatory mechanisms in extracellular pH (pHe) 7.2 or pHe 6.6. The decline in pHi caused by the inhibitors was much greater at pHe 6.6 than that at pHe.7.2.

cells in a less hostile environment. We have observed that when cells in culture were exposed to relatively mild acidic medium, cell cycle progression is slowed and thus, cell proliferation is slowed initially *(84)*. However, cells adapt eventually to the low-pH environment, and the proliferation rate is restored. It is conceivable that cells adapted to low pH are able to survive and form metastatic foci on distributing to other potentially suboptimal locations in the body.

5. THERAPEUTIC POTENTIAL OF INTRACELLULAR ACIDIFICATION

Indications are that acidification of intracellular environment is cytotoxic to tumor cells *(1,2,58,85–88)*. We have reported that the magnitude of decrease in pHi by inhibitors of pHi regulation is significantly greater in an acidic pHe environment than in neutral pHe environment. For instance, as shown in Fig. 5, a combination of amiloride, DIDS, and nigericin reduced pHi of SCK tumor cells to 6.9 and 6.4 in pH 7.2 and pH 6.6 media, respectively. Rotin et al. *(85)* reported that lowering pHi of tumor cells to 6.5 or lower with nigericin, a K^+/H^+ ionophore, was cytotoxic. Inhibition of the Na^+/H^+ antiport with amiloride or inhibition of the Cl^-/HCO_3^- exchange with DIDS alone was not toxic to the cells, even when the pHe was as low as 6.0. However, combination of amiloride or DIDS with nigericin was toxic to cells at pHe 6.5–6.8. Likewise, carbonylcyanide-3-chlorophenylhydrazine, which transports H^+ into cells, was toxic to tumor cells at pHe lower than 6.5, and its toxicity was greatly enhanced by amiloride or DIDS *(88)*. Apoptosis occurred in human leukemia HL-60 cells when pHi was lowered to 7.2–6.7 by inhibiting pHi regulation *(86,87)*. Increasing in intracellular Ca^{2+} with 4 µ*M* ionomycin, a Ca^{2+} ionophore, further increased the acid-induced apoptosis of HL-60 human leukemia cells. Importantly, the toxicity of various inhibitors of pHi regulation was observed to markedly increase when the cells were heated at 42–44°C *(1,2,9,11,89–97)*.

The direct mechanisms responsible for the cell death caused by low pHi is unclear. We have observed that an exposure of HL-60 human leukemia cells and other tumor cells to an acidic medium induces cell death through apoptosis of cells in G_1 phase *(86,87)*. The

acid-induced apoptosis could be further increased when the pHi regulatory mechanisms were inhibited *(86)*. Detailed analysis indicated that a low-pHi environment first upregulates proapoptotic protein Bax, thereby activating caspases followed by poly(ADP-ribose) polymerase cleavage and DNA fragmentation *(87)*. Interestingly, exposing cells to pH 6.2 medium was less effective than exposing to pH 6.4 or pH 6.6 medium in causing apoptosis in HL-60 cells *(87)*. It was concluded that there are optimal pH values for the major events in the apoptosis cascade such as Bax activation, caspase activation and activity, poly(ADP-ribose) polymerase cleavage, and DNA fragmentation so that an extremely acidic environment such as pH 6.2 was less effective than a pH 6.4–6.6 environment in inducing cell death via apoptosis. Recent studies *(98,99)* have indicated that cell death caused by certain chemotherapy drugs was attributable to an acidification of cells as a result of inhibition of pHi regulation mechanisms caused by H_2O_2 produced by mitochondria. These results demonstrate that the pHi regulatory mechanism may be an effective therapy target, because inhibition of pHi regulation will cause a reduction of pHi preferentially in tumor cells in acidic extracellular environment relative to normal cells and thus cause damage preferentially in tumor cells.

6. EFFECT OF pH ON RADIATION DAMAGE

Unlike extensive studies on the effects of hypoxia on radiosensitivity in the past, little has been studied in regard to the effects of acidic pH on radiosensitivity. In a series of studies, we observed that acidic environments markedly prolong radiation-induced G_2 arrest in cancer cells *(84,100–103)*. For example, when RKO human colorectal cancer cells were irradiated with 12 Gy in pH 7.5 medium, the G_2 arrest peaked at 12–16 h, and then the cells progressed into G_1 phase or died of apoptosis. On the other hand, when RKO cells were irradiated with 12 Gy and maintained in pH 6.6 medium, significant portions of cells were still in G_2 arrest 72 h after irradiation (Fig. 6). Interestingly, the radiation-induced G_2 arrest in acidic pH medium rapidly decayed as soon as the acidic pH medium was replaced with neutral pH medium *(100)*. Importantly, the apoptosis and clonogenic cell death caused by irradiation were significantly less in acidic medium than in neutral pH medium (Fig. 7). It appeared that the increase in radioresistance in acidic pH environment resulted from an increased DNA damage repair during the prolonged G_2 arrest. Similar increases in radioresistance in low extracellular pH environment have been reported by others *(104–106)*. Importantly, the environmental pH had to be reduced after treatment in order to confer resistance *(104)*.

Our studies indicate that the prolonged G_2 arrest after irradiation in an acidic pH medium was due, at least in part, to activation of CDC2, which is known to inhibit cyclin B1-CDC2 kinase activity responsible for the progression of cells through G_2/M phase *(101)*. Because the radiation-induced changes in cell cycle progression, apoptosis, and clonogenic cell death are intimately related to p53 expression, we have investigated the effect of pH on the kinetics of p53 expression *(107)*. We found that acidic environments significantly enhance the radiation-induced expression of p53, partly by increasing the formation of p53 and also partly by slowing down the degradation of p53 through inhibition of p53–murine double minute 2 (p53–MdM2) complex formation.

7. EFFECTS OF pH ON HYPERTHERMIA DAMAGE

It is well established that an acidic environment markedly increases thermal damage *(108–114)*. Detailed studies by a number of investigators using different cell lines dem-

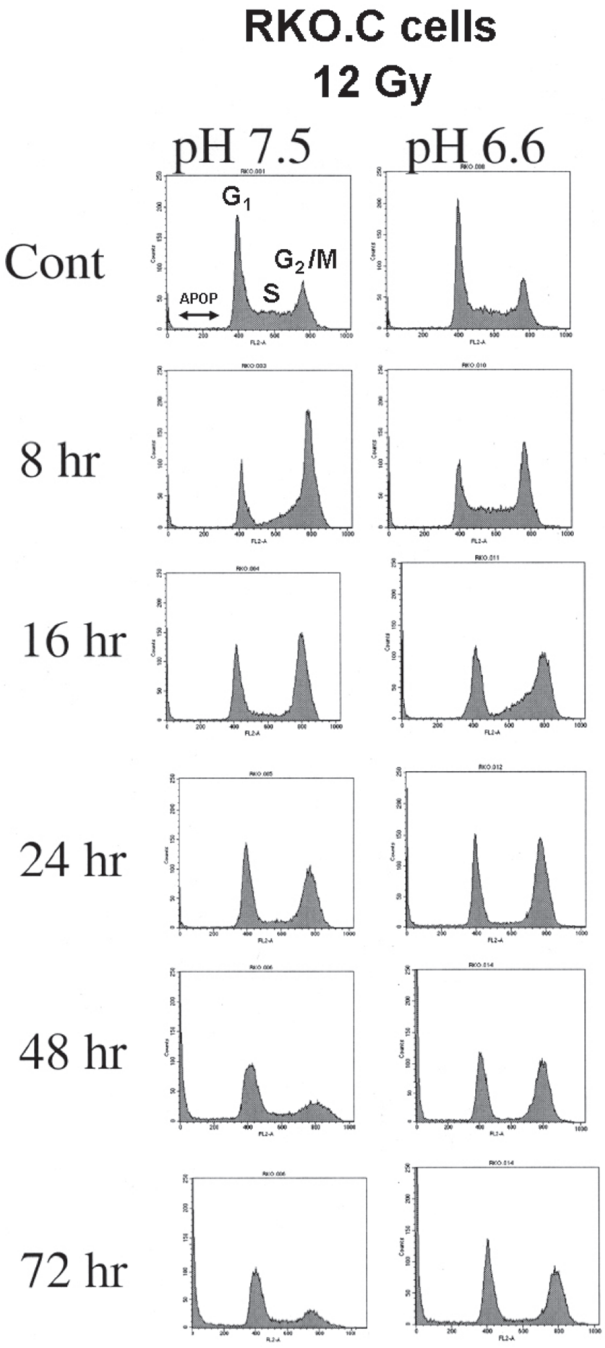

Fig. 6. Cell cycle progression and apoptosis, as demonstrated with flow cytometry, of RKO human colorectal cancer cells after irradiation with 12 Gy in pH 7.5 or pH 6.6 medium. The cell cycle progression was delayed particularly at G_2/M phase after irradiation in pH 6.6 medium compared to the delay caused in pH 7.5 medium.

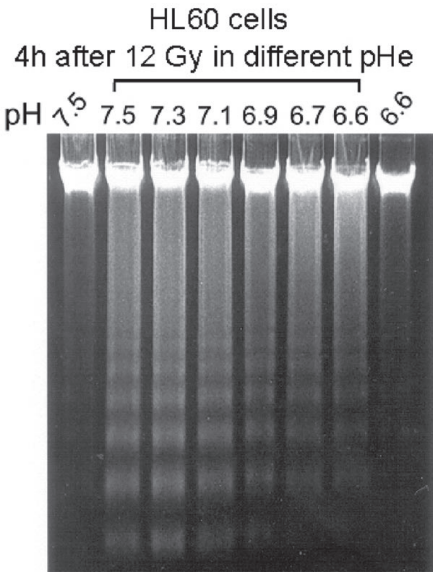

Fig. 7. Apoptotic fragmentation of DNA from HL-60 cells 4 h after irradiation with 12 Gy in different pH media. The radiation-induced apoptosis was markedly suppressed as the medium pH was lowered.

onstrated that pHi, and not pHe, is the determinant of the thermosensitivity *(113,114)*. Hahn and Shiu *(115)* reported that Chinese hamster ovary cells maintained in acidic medium for prolonged periods were not as heat-sensitive as the cells exposed to acidic medium shortly before heating, and concluded that adaptation to a low-pH environment rendered the cells resistant to heat. Cook and Fox *(116)*, and Chu and Dewey *(114)* found that the pHi of the cells that adapted to a low pH environment was significantly higher than the pHi of the unadapted cells. Furthermore, the thermal survival curves of cells adapted and unadapted to a low-pH environment were identical when the survival curves were plotted against pHi instead of pHe. Chu and Dewey *(114)* therefore concluded that the increase in pHi was the reason why cells that adapted to a low-pH environment were resistant to heat at low pHe relative to the unadapted cells. van der Berg et al. *(117)* reported that the thermosensitivity of human tumors showing an acidic interstitial pH was not necessarily greater than the thermosensitivity of tumors showing neutral interstitial pH. They concluded, therefore, that the cells in human tumors adapted to an acidic environment, and thus, the tumor cells were not heat-sensitive despite the low intratumor pH. Conceivably, an acute acid loading to cells adapted to a certain pHe would still render the areas heat-sensitive.

The intratumor pH has been observed to decrease markedly during heating of tumors, probably owing to vascular damage *(3,14,15,20,118)* and to the resultant increase in the accumulation of lactic acid *(119)*. Acute build-up of acidity during heating, particularly in the nonacidic areas, would be expected to sensitize tumor cells to heat. We observed that tumor cells in vivo were far more thermosensitive than the same cells in vitro, and we concluded that the acidic intratumor environment and the further increase in acidity during heating enhanced the thermal damage to the tumor cells *(14,119)*. The vasculature in human tumors has been reported to be more heat-resistant than the vasculature in rodent

tumors, and thus, the pH in human tumors may not drop as severely as in animal tumors on heating *(32,120)*. However, it is also quite possible that the human tumors in these previous studies were not adequately heated, and thus, the blood flow as well as the tumor pH remained unchanged. It is likely that both in human tumors or animal tumors, the pH will decrease on application of hyperthermia if heating temperature is high enough to cause vascular damage and induce hypoxia. Along these lines, attempts are being made to sensitize human tumors to hyperthermia by acidifying the tumors using hyperglycemia *(121)*.

8. EFFECT OF pH ON CHEMOTHERAPY

It is known that the influx of drugs into tumor cells will be greatly affected by the pK_a value of the drugs. The acidic extracellular environment in tumors traps weakly basic drugs, thereby hindering the influx of the drugs into cells, whereas it enhances the influx of weakly acidic drugs into cells. Furthermore, the pH gradient between the vesicular compartments and the cytosol of cells has been known to be considerable. Therefore, it is conceivable that weakly basic drugs may be trapped inside the acidic compartments, thereby limiting their cytotoxicity *(122,123)*. It follows that cells containing a larger number of acidic vesicles may be resistant to weakly basic drugs, whereas they may be sensitive to weakly acidic drugs. In addition, intracellular pH may affect the molecular interaction between drugs and their targets such as various intracellular organelles, DNA, RNA, proteins involved in cell cycle progression and cell division, and signals involved in apoptosis. The effect of pH on commonly used anticancer drugs is briefly addressed in this section.

8.1. At Normal Temperatures

Table 2 shows the relative cytotoxicity of various anticancer drugs in acidic (pH < 7.0), neutral (pH 7.0–7.4), and alkaline (pH >7.4) environments. The cytotoxicity of *bis*-chloroethylating agents such as cyclophosphamide and its derivatives, e.g. mafosfamide, nor-nitrogen mustard, melphalan, and chlorambucil, was reported to be significantly increased in acidic pH environments *(124,125)*. Cyclophosphamide is a prodrug, and a low-pH environment accelerated its bioactivation. On the other hand, the cytotoxicity of ifosfamide, an oxazaphosphorine analog of cyclophosphamide, was unaffected by the environmental acidity *(124)*. In ifosfamide, one of the chloroethryl side chains is shifted from an amino nitrogen to a ring nitrogen. Therefore, it was concluded that the *bis*-chloroethyl amine group may be a critical determinant for the H^+ ion-mediated enhancement of cytotoxicity in this group of agents *(124)*. The cytotoxicity of mafosfamide could be enhanced markedly by increasing intracellular acidity with nigericin (K^+/H^+ ionophore) in acidic medium *(124,125)*. Jahde et al. *(124)* concluded that the increase in the cytotoxicity of cyclophospamide and its derivatives in an acidic pH environments were because of an increase in the cellular uptake of the drugs and also to an increase in the monofunctional alkalinization of DNA. It was further concluded that the phase of DNA crosslink formation and that of crosslink removal were relatively independent of the environmental pH. Skarsgard et al. *(126)* reported that a low-pH environment potentiated the cytotoxicity of melphalan and chlorambucil by increasing the uptake of the drugs. Methylmethane sulphonate, a monofunctional alkylate, was reported to be independent of environmental acidity *(115)*. The alkylating potency of *bis*-chloroethylnitrosurea (BCNU) was also independent of environmental acidity *(115)*, whereas that of cyclohexyl-chloroethylnitrosourea was reported to decrease in acidic environment *(127)*.

Table 2
Activity of Drugs Under Various Acidities (pH) and Temperatures

	37°C			*Hyperthermia*[a]			
Compounds	*<7.0*	*7.0–7.4*	*>7.4*	*<7.0*	*7.0–7.4*	*>7.4*	*Ref.*
Cyclophosphamide	++	+					*122, 124, 125*
4-Hydroperoxycyclophosphamide	++	+					*124*
Mafosamide	++	+					*124, 125*
Mechlorethamine	++	+					*124*
Melphalan	++	+					*124, 126*
Chlorambucil	++	–					*122, 124, 126*
nor-Nitrogen mustard	++	+					*124*
Ifosfamide	+	+					*124*
Triethylenemelamine	++	+					*129*
Thiophosphamide	++	+					*130*
MMS	+	+	+	+++	++		*115*
BCNU	+	+	+	++++	++	+	*115, 131*
CCNU	+	++					*127*
Thiotepa	++	+					*127*
Methotrexate	+	+	+	++	+	+	*131*
5-Flourouracil	++	+					*122*
Bleomycin	+	+	+	++++	++	+	*115, 131, 134*
Mitomycin C	++	+					*133*
Amphotericin B	+	+	+	+++	++	+++	*4, 115, 131*
Doxorubicin	+	++					*122, 134*
Mitoxantrone	+	++					*122*
Vinblastine	+	++					*137*
$PtC1_4(Fast\ Black)_2$	++	+		+++	++		*141*
Cisplatin	+++	+		++++	++	+	*115, 139, 141*
Plato	+	+		+	++		*139*
Plant	++	+		++	+		*139*
Paclitaxel	+	+		+	++		*122*

MMS, methylmethane sulfonate, BCNU, *bis*-chloroethylnitrosourea; CCNU, cyclophexylcholroethyl-nitrosourea; Plato, (1,2,-diamono-r-nitrobenzene) dichloroplatinum(II); Plant, trans-*bis(*2-amino-5-nitrothiazole)dichloroplatinum(II).

[a]The hyperthermia was 42°–44°C.

The cytotoxicity of thiotepa, another alkylating agent, increased when the environment was made acidic *(128)*. The cytotoxicity of both triethylenemelamine *(129)* and thiophosphamide *(130)*, alkylating agents, against transplanted rodent tumors was found to be increased when the intratumor environment was made acidic by induction of hyperglycemic. On the other hand, the effect of methotrexate, an antimetabolite known to be very effective against certain cancers, was independent of pH in vitro *(131)*. 5-Fluorouracil is a prodrug and becomes an antimetabolite after intracellular conversion. 5-fluorouracil is a weak acid, and thus, acidic pH environment increases its cellular uptake *(132)*. Mitomycin C, bleomycin, amphotericin B, and doxorubicin (Adriamycin™) are naturally occurring anticancer agents. The cytotoxicity of mitomycin C, a bioreductive alkylating agent, slightly increased when the environmental pH was lowered *(133)*. The increase in mitomycin C cytotoxicity in an acidic environment appeared to be because of an increase in the DNA crosslinking. The cytotoxicity of bleomycin *(115,131,134)* and amphotericin B *(115,131,135)* was unchanged, whereas doxorubicine toxicity declined in an acidic pH environment *(134,136)*. Doxorubicin has a primary amine with a basic pK_a, and thus, its cellular uptake may be reduced in an acidic medium. Indeed, the uptake of doxorubicin at pH 6.6 environment was only one half of that at a pH 7.4 environment *(122,123)*. Furthermore, doxorubicin is trapped and sequestrated in acidic vesicles within the cytoplasm, which prevents the interaction of the drug with its target. A number of agents have been used to enhance the cytotoxicity of doxorubicin by inhibiting the formation of acidic vesicles, thereby releasing the doxorubicin into the cytoplasm *(122,123)*. Vinblastine and vincristine are also naturally occurring anticancer drugs. The uptake of these alkaloids has been reported to decline in an acidic environment *(137)*. The pK_a of vinblastine and vincristine are 5.0–5.5 and 7.4 at physiological pH, respectively. We have reported previously that intracellular acidification alone is able to activate caspases, thereby triggering apoptosis *(86,87)*. Interestingly, apoptosis in cancer cells caused by certain chemotherapy drugs has been attributed to intracellular acidosis caused by the drugs. As mentioned previously, Hirpara et al. *(98)* reported recently that chemotherapy drugs trigger production of H_2O_2 by mitochondria, which then inhibit the Na^+/H^+ exchanger, resulting in intracellular acidification. The resultant intracellular acidification causes mitochondrial recruitment of Bax and release of cytochrome *c* from mitochondria, thereby activating the caspase cascade leading to apoptosis *(98,99)*. Lastly, paclitaxel is one of the taxanes extracted from yew trees and a common chemotherapeutic. It is highly lipophilic and devoid of any ionizable groups, with pK_a values in the physiological range *(139)*. Therefore, the cellular uptake of this drug is independent of pHe. In all, these results clearly indicate that efficacy of many, but not all, anticancer drugs may be significantly increased by altering intratumor pH based on the pK_a value of the drugs.

8.2. At Elevated Temperatures

Although the effect of methylmethane sulphonate *(115)*, BCNU *(115,131)*, methotrexate *(137)*, bleomycin *(115,131,134)*, and amphotericin B *(4,115,131)* were independent of the environmental pH at 37°C, their cytotoxicity increased in a low-pH environment if the cells were heated (*see* Table 2). Interestingly, the cytotoxicity of amphotericin B also increased when the environment was made alkaline at elevated temperatures *(4,115)*. Hahn *(138)* suggested that heat may increase the cellular uptake of certain drugs or inhibit the repair of damage caused by drugs, and the acidic environment accentuates these processes. Related to this, Hahn and Shiu *(115)* reported that the low-

pH-adapted cells were resistant to thermochemotherapy with bleomycin, amphotericin B, and cisplatin, but not with BCNU. Thus, it was concluded that the pH dependence of cytotoxicity for some drugs at elevated temperature is affected by the pH history of the target cells.

Cisplatin is platinum complex with potent anticancer activity. The cytotoxicity of this drug increases with an increase in the environmental acidity *(115,139,140)*. Herman et al. *(84)* demonstrated that heating caused a greater increase in the cytotoxicity of cisplatin in an acidic pH environment than in a neutral pH environment. At 37°C and pHe 7.4, no difference in the sensitivity to cisplatin was observed between oxic cells and hypoxic cells. When cells were heated in pH 7.4 medium, the sensitivity of oxic cells to cisplatin markedly increased, whereas that of hypoxic cells remained unchanged. On the other hand, in pH 6.45 medium, the sensitivity of both oxic and hypoxic cells to cisplatin increased on heating. Herman et al. *(139)* also studied the cytotoxicity of analogs of cisplatin such as (1,2-diamino-4-nitrobenzene)dichloroplatinum(II) (Plato) and *trans-bis*-(2-amino-5-nitro-thiazole)dichloroplatinum(II) (Plant) under various conditions. When the environmental acidity was increased, the cytotoxicity of Plato decreased and that of Plant increased. Unlike cisplatin, Plato and Plant were more toxic toward hypoxic cells than oxic cells, but the cytotoxicity of these drugs did not increase with an increase in temperature. Teicher et al. *(140)* reported that the cytotoxicity of PtCl4(Fast Black)$_2$, an analog of cisplatin, was greater in an acidic than in a neutral environment at 37°C, and heating increased the cytotoxicity of this drug in both acidic and neutral pH environments. Oxic cells and hypoxic cells were equally sensitive to this drug at 37°C. However, when heated, oxic cells were slightly more sensitive to this drug in pH 7.4 medium, whereas hypoxic cells were slightly more sensitive to this drug in pH 6.45 medium. Teicher et al. *(141)* observed that the changes in the concentration of cisplatin and $PtCl_4$(Fast Black)$_2$ in the cells after the environmental pH and temperature were changed did not correlate with the changes in the cytotoxicity, and concluded that an increase in the reaction of the drugs with DNA was the direct cause of the increase in the cytotoxicity of the drugs in a low-pH medium at elevated temperatures. It was also suggested that metabolic changes that must occur to maintain neutral pHi in acidic environment may increase directly or indirectly the response of the cells in an acidic environment to the drugs.

9. ACIDIFICATION AND ALKALINIZATION OF TUMORS

Because an acidic intratumor environment increases the response of tumors to certain chemotherapeutic drugs and also to hyperthermic treatment, various attempts have been made to acidify the intratumor environment. It has long been known that tumors can be acidified by induction of hyperglycemia by administration of excess glucose *(10,142–144)*. It was initially proposed that the decline in the intratumor pH by hyperglycemia resulted from an increase in glucose metabolism by aerobic glycolysis *(144)*. However, indications are that the decline in intratumor pH by hyperglycemia results not only from an increase in aerobic glycolysis, but also from an increase in anaerobic glycolysis as a consequence of blood flow decline and ensuing hypoxia. The mechanisms for the decline in tumor blood flow by hyperglycemia are complicated. A serious problem in using hyperglycemia for induction of acidosis in human tumors is that tumor acidification requires a large dose of glucose exceeding the tolerable level for most patients. Furthermore, the reduction in blood flow by hyperglycemia may decrease the drug delivery to tumor cells. Acidification of rodent tumors by hyperglycemia could be enhanced by

concomitant administration of metaiodobenzylguanidine *(82,121)*, which inhibits mitochondrial respiration at complex I of the electron transport chain, resulting in an increase in lactic acid formation.

Hydralazine, a vasodilator, also decreases tumor blood flow *(145–147)*, and thus, it may increase tumor acidity. As with hyperglycemia, tumor acidification by hydralazine may not be useful to enhance the effects of drugs, because drug delivery to tumors will be reduced owing to the decrease in tumor blood flow that occurs. Furthermore, the effect of hydralazine is strongly dependent on the location of the tumor in the body, and it can reduce blood flow in many normal tissues as well *(146,147)*.

Conversely, the cellular uptake of weakly basic chemotherapy drugs may be enhanced if pHe is raised to alkaline range. Indeed, treatment of tumor-bearing mice with sodium bicarbonate has been demonstrated to cause tumor-specific alkalinization of extracellular pH and increase the antitumor effect of the weakly basic drug, mitoxantrone, which has two ionizable amines with pK_a values of 8.3–8.6 *(123)*. This strategy appears to have limited use because of the dangers of affecting blood chemistry and pH with buffering agents.

10. CONCLUSION

The intratumor environment is acidic because of elevated production of lactic acid and other acidic metabolities as a result of high aerobic and anaerobic glycolysis. However, the pHi of tumor cells is maintained at neutral range despite the acidic pHe by virtue of powerful pHi regulatory mechanisms. Lowering the pHi by inhibitors of pHi regulation is cytotoxic, particularly in a low-pHe environment. The acidic pHe and the gradient between pHe and pHi greatly affect the response of tumor cells to chemotherapy drugs, radiotherapy, and hyperthermia. The feasibility of controlling pHe and pHi by various means with the goal of increasing the response of tumor cells to various treatments is being investigated.

ACKNOWLEDGMENTS

This work was supported by RO1CA-44114 from NIH/NCI awarded to C.W.S and the 2003 Korean National Cancer Grant 03203002-2 and the National Nuclear Technology Program grant from KISTEP awarded to H.J.P. We thank Mr. Brent Williams for his help arranging the figures and tables.

REFERENCES

1. Song CW, Lyon JC, Luo Y. Intra- and extracellular pH in solid tumors: influence on therapeutic response. In: Teicher BV, ed. Drug resistance in oncology. New York: Marcel Dekker, 1993:25–51.
2. Song CW, Park HJ, Ross BD. Intra- and extracellular pH in solid tumors. In Teicher BV, ed. Antiangiogenic adnets in cancer therapy. Totowa: Humana Press, 1998:51–64.
3. Rhee JG, Kim TH, Levitt SH, Song CW. Changes in acidity of mouse tumors by hyperthermia. Int J Radiat Oncol Biol Phys 1985; 10:393–399.
4. Wike-Hooley JL, Haveman J, Reinhold HS. The relevance of tumour pH to the treatment of malignant disease. Radiother Oncol 1984; 2:343–366.
5. Webb SD, Sherratt JA, Fish RG. Mathematical modeling of tumour acidity: regulation of intracellular pH. J Theor Biol 1999; 196:237–250.
6. Gilles RJ, Raghunand N, Karczmar GS, et al. MRI of the tumor microenvironment. J Magn Reson Imaging 2002; 16:430–450.
7. Svastove E, Hulikova A, Rafajova M, et al. Hypoxia activates the capacity of tumor-associated carbonic anhydrase IX to acidify extracellular pH. FEBS Lett 2004; 19:439–435.

8. Aisenberg AC. The glycolysis and respiration of tumors. New York: Academic, 1961.
9. Kim GE, Lyons JC, Levitt SH, Song CW. Effects of amiloride on intracellular pH and thermosensitivity. Int J Radiat Oncol Biol Phys 1991; 20:541–549.
10. Gerweck LE, Rhee JG, Koutcher JA, Song CW, Urano M. Regulation of pH in murine tumor and muscle. Radiat Res 1991; 126:206–209.
11. Lyons JC, Kim GE, Song CW. Modification of intracellular pH and thermosensitivity. Radiat Res 1992; 129:79–87.
12. Lyons JC, Ross B, Song CW. Enhancement of hyperthermia effect in vivo by amiloride and DIDS. Int J Radiat Oncol Biol Phys 1993; 25:95–103.
13. Roos A, Baron WF. Intracellular pH. Physiol Rev 1981; 61:296–434.
14. Song, CW. Effect of local hyperthermia in blood flow and microenvironment: a review. Cancer Res 1984; 44(Suppl):4721s–4730s.
15. Vaupel P, Muller Klieser W, Otte J, Manz R, Kallinowski F. Blood flow, tissue oxygenation and pH distribution in malignant tumors upon localized hyperthermia. Strahlentherapie 1983; 159:73–81.
16. Hetzel FW. Biological rationale for hyperthermia. Radiol Clin North Am 1987; 27:499–508.
17. Dewhirst MW, Gross JF, Sim D, Arnold P, Boyer D. The effect of rate of heating or cooling prior to heating on tumor and normal tissue microcirculatory blood flow. Biorheology 1984; 21:539–558.
18. Jain RK, Ward-Hartely K. Tumor blood flow: characterization, modifications and role in hyperthermia. IEEE Trans Son Ultrason 1984; SU-31:504–526.
19. Song CW. Tumor blood flow response to heat. Funktionsanal Biol Syst 1991; 20:123–141.
20. Reinhold HS, Endrich B. Tumour microcirculation as a target for hyperthermia. Int J Hypertherm 1986; 2:111–137.
21. Jain RK. Determinant of tumor blood flow. A review. Cancer Res 1988; 48:2641–2658.
22. Eddy HA. Microangiographic techniques in the study of normal and tumor tissue vascular systems. Microvasc Res 1976; 11:391–413.
23. Peterson HI. Tumor blood circulation: Angiogenesis, vascular morphology and blood flow of experimental and human tumors. Boca Raton: CRC Press, 1978.
24. Thomlinson RH, Gray LH. The histological structure of some human lung cancers and possible implications for radiotherapy. Br J Cancer 1955; 9:539–549.
25. Jain RK. Physiological barriers to delivery of monoclonal antibodies and other macromolecules in tumors. Cancer Res 1990; 50(Suppl):814s–819s.
26. Hong SS, Lee H, Kim KW. HIF-1a: a valid therapeutic target for tumor therapy. Cancer Res Treat 2004; 36:344–353.
27. Mazure NM, Brahimi-Horn MC, Berta MA, et al. HIF-1: master and commander of the hypoxic world. A pharmacological approach to its regulation by siRNAs. Biochem Pharmacol 2004; 68:971–980.
28. Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003; 3:721–732.
29. Griffiths JR, McIntyre DJ, Howe FA, Stubbs M. Why are cancers acidic? A carrier-mediated diffusion model for H+ transport in the interstitial fluid. Novartis Found Symp 2001; 240:46–62.
30. Busa WB, Nuccitelli R. Metabolic regulation via intracellular pH. Am J Physiol 1984; 246:R409–R438.
31. Schornack PA, Gillies RJ. Contribution of cell metabolisms and H+ diffusion to the acidic pH of tumors. Neoplasia 2002; 5:135–145.
32 van der Berg AP. Tissue pH of human tumors and its variation upon therapy in tumor blood supply and metabolic microenvironment. Funktionsanal Biol Syst 1991; 20:234–235.
33. Hinke JA. Ction-selective microelectrodes for intracellular use. In: Eiserman G, ed. Glass electrodes for hydrogen and other cations. New York: Marcel Dekker, 1967:474–477.
34. Thomas RC. New design of a sodium-sensitive glass microelectrode. J Physiol 1970; 210:829–839.
35. Lin J-C, Levitt SH, Song CW. Relationship between vascular thermotolerance and intratumor pH. Int J Radiat Oncol Biol Phys 1991; 22:123–129.
36. Vaupel PW, Frinak S, Bicher HI. Heterogenous oxygen partial pressure and pH distribution in C3H mouse mammary adenocarcinoma. Cancer Res 1981; 41:2008–2013.
37. Ja'hde E, Rajewsky MF, Ba'umgart H. pH distribution in transplanted neural tumors and normal tissues of BDIX rats as measured with pH microelectrodes. Cancer Res 1982; 42:1498–1504.
38. Meyer KA, Kammerling EM, Amtan L, et al. pH studies of malignant tissues in human being. 1948; 8:513–518.
39. Pampus F. Die Wasserstoffionenkonzentration des Hirngewebes bei raumfordernden intracraniellen Prozessen. Acta Neurochir 1963; 11:305–318.

40. Ashby BS. pH studies in human malignant tumours. Lancet 1966; 2:312–315.
41. Thistlethwaite AJ, Leeper DB, Moylan DJ, et al. pH distribution in human tumors. Int J Radiat Oncol Biol Phys 1985; 11:1647–1652.
42. Wike-Hooley JL, van den Berg AP, van der Zee J, Reinhold HS. Human tumour pH and its variation. Eur J Cancer Clin Oncol 1985; 21:785–791.
43. van den Berg AP, Wike-Hooley JL, van den Berg-Blok AE, et al. Tumour pH in human mammary carcinoma. Eur J Cancer Clin Oncol 1982; 18:457–462.
44. Inch WR, Direct current potential and pH of several varieties of skin neoplasms. Can J Biochem Physiol 1954; 32:519–525.
45. Millet H. Measurements of the pH of normal, fetal, and neoplastic tissues by means of the glass electrode. J Biol Chem 1923; 78:281–288.
46. Naeslund J, Senson KE. Investigations on the pH of malignant tumors in mice and humans after the administration of glucose. Acta Obstet Gynecol Scand 1953; 32:359–367.
47. Vaupel P, Kallinowski F, Okunieff P. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumours: a review. Cancer Res 1989; 49:6449–6465.
48. Griffiths JR. Are cancer cells acidic? Br J Cancer 1991; 64:425–427.
49. Engin K, Leeper DB, Cater JR, et al. Extracellular pH distribution in human tumours. Int J Hyperthermia 1995; 11:211–216.
50. Eden M, Haines B, Kahler H. The pH of rat tumors measured in vivo. J Natl Cancer Inst 1955; 16:541–556.
51. Evanochko WT, Ng TC, Lilly, MB, et al. In vivo ^{31}P-NMR study of the metabolism of murine mammary 16/C adenocarcinoma and its response to chemotherapy, x-irradiation and hyperthermia. Proc Natl Acad Sci U S A 1983; 80:334–338.
52. Gillies RJ, Ogina T, Shulman RG, Ward DC. ^{31}P nuclear magnetic resonance evidence for the regulation of intracellular pH by Ehrlich ascites tumor cells. J Cell Biol 1982; 95:24–28.
53. Evelhoch JL, Sapareto SA, Jick DEL, Ackerman JJH. In vivo metabolic effects of hyperglycemia in murine radiation induced fibrosarcoma: a ^{31}P-NMR investigation. Proc Natl Acad Sci U S A 1984; 81:6496–6500.
54. Okuneiff PG, Koutcher JA, Gerweck L, et al. Tumor size dependent metabolic changes in a murine fibrosarcoma: use of Fourier transformed ^{31}P-NMR to evaluate energy metabolism. Int J Radiat Oncol Biol Phys 1986; 12:793–799.
55. Madshus IH. Regulation of intracellular pH in eukaryotic cells. J Biochem 1988; 250:1–8.
56. Grinstien S, Rothstein S. Mechanisms of regulation of the Na^+/H^+ exchanger. J Membrane Biol 1986; 90:1–12.
57. Frelin C, Vigne P, Ladoux A, Lazdunski M. The regulation of the intracellular pH in cells from vertebrates. Eur J Biochem 1988; 174: 3–14.
58. Tannock, IF, Rotin D. Acid pH in tumors and its potential for therapeutic exploitation. Cancer Res 1989; 49:4373–4384.
59. Aronson PS. Kinetic properties of the plasma membrane Na^+/H^+ exchange. Annu Rev Physiol 1985; 47:545–560.
60. Cassel D, Katz M, Rotman M. Depletion of cellular ATP inhibits Na^+/H^+ antiport in cultured human cells. Modulation of the regulatory effect of intracellular protons on the antiporter activity. J Biol Chem 1986; 261:5460–5466.
61. Zhung YX, Cragoe EJ Jr, Glaser L, Cassel D. Characterization of potent Na^+/H^+ exchange inhibitor from the aniloride series in A431 cells. Biochemistry 1984; 23:4481–4488.
62. Cassel D, Scharf O, Rotman M, et al. Characterization of Na^+-linked and Na^+-independent $C1^-/HCO_3^-$ exchange systems in Chinese hamster lung fibroblasts. J Biol Chem 1988; 263:6122–6127.
63. Boron WF. Intracellular pH regulation in epithelial cells. Am Rev Physiol 1986; 43:377–388.
64. Jentsch TJ, Matthes H, Keller SK, Wiederholt M. Carrier-mediated reabsorption of small peptides in renal proximal tribute. Am J Physiol 1986; 251:F945–F968.
65. Grassl SM, Aronson PS. Na^+/CHO_3^- Co-transport in basolateral membrane vesicles isolated from rabbit renal cortex. J Biol Chem 1986; 26:8778–8783.
66. Hutton JC. The internal pH and membrane potential of the insulin-secretory granule. J Biochem 1982; 204:171–178.
67. Thomas RC. Ionic mechanism of the H^+ pump in a snail neurone. Nature 1976; 262: 54–55.
68. Russell JM, Boron WF. Role of chloride transport in regulation of intracellular pH. Nature 1976; 264:73–74.

69. Boron WF, Hogan E., Russell JM. pH-sensitive activation of the intracellular-pH regulation system in squid axons by ATP-γ-S. Nature 1988: 332:2672–265.
70. Moolenaar WH, Tertoolen LGL, de Laat SW. The regulation of cytoplasmic pH in human fibroblasts. J Biol Chem 1984; 259:7563–7569.
71. Belt JA, Thomas JA, Buchsbaum RN, Racker E. Inhibition of lactate transport and glycolysis in Ehrlich ascites tumor cells by bioflavanoids. Biochemistry 1979; 18:3506–3511.
72. Kim JH, Kim SH, ALdieri AA, Young CW. Quercetin, an inhibitor of lactate transport and hyperthermic sensitizer of HeLa cells. Cancer Res 1984; 44:102–106.
73. Turpaev KT. Reactive oxygen species and regulation of gene expression. Biochemistry (Mosc) 2002; 67:281–292.
74. Subarsky P, Hill RP. The hypoxic tumour microencironment and metastatic progression. Clin Exp Metastasis 2003; 20:237–250.
75. Le QT, Denko NC, Giaccia AJ. Hypoxic gene expression and metastasis. Cancer Metastasis Rev 2004; 23:293–310.
76. Vaupel P, Kelleher DK, Hockel M. Oxygen status of malignant tumors: pathogenesis of hypoxia and significance for tumor therapy. Semin Oncol 2001; 28:29–35.
77. Ohtsubo T, Wang X, Takahashi A, et al. p53-dependent induction of WAF1 by low pH culture condition in human glioblastoma cells. Cancer Res 1997; 57:3910–3913.
78. Griffiths L, Dachs GU, Bicknell R, et al. Influence of oxygen tension and pH on the expression of platelet-derived endothelial cells growth factor/thymidine phosphorylase in human breast tumor cells growth in vitro and in vitro. Cancer Res 1997; 57:570–572.
79. Schlappack OK, Zimmermann A, Hill RP. Glucose starvation and acidosis: effect on experimental metastatic potential, DNA content and MTX resistance of murine tumour cells. Br J Cancer 1991; 64:663–670.
80. Jang A, Hill RP. An examination of the effects of hypoxia, acidosis, and glucose starvation on the expression of metastasis-associated genes in murine tumor cells. Clin Exp Metastasis 1997; 15:469–483
81. Hill RP, Jaeger KD. Jang A, Cairns R. pH, hypoxia and metastasis. The tumor microenvironment: causes and consequences of hypoxia and acidity. Chichester: Wiley, 2001:154–168.
82. Kalliomaki T, Hill RP. Effects of tumour acidification with glucose+MIBG on the spontaneous metastatic potential of two murine cell lines. Br J Cancer 2004: 90:1842–1849
83. Rofstad EK. Microenvironment-induced cancer metastasis. Int J Radiat Biol 2000; 76:589–605.
84. Park HJ, Lyons JC, Griffin RJ, Lim BU, Song CW. Apoptosis and cell cycle progression in an acidic environment after irradiation. Radiat Res 2000; 153:295–304.
85. Rotin D, Wan P, Grinstien S, Tannock I. Cytotoxicity of compounds that interfere with the regulation of intracellular pH: a potential new class of anticancer drugs. Cancer Res 1987; 47:1497–1505.
86. Park HJ, Makepeace CM, Lyons JC, Song CW. Effect of intracellular acidity and ionomycin on apoptosis in HL-60 cells. Eur J Cancer 1996; 32A:540–546.
87. Park HJ, Lyons JC, Ohtsubo T, Song CW. Acidic environment causes apoptosis by increasing caspase activity. Br J Cancer 1999; 80:1892–1897.
88. Newell KT, Tannock I. Reduction of intracellular pH as a possible mechanism for killing cells in acidic regions of solid tumors: effect of carbonylcyanide03-chrophgenylhydrazone. Cancer Res 1989; 49:4477–4482.
89. Haveman J. The pH of the cytoplasm as an important factor in the survival of vitro cultured malignant cells after hyperthermia. Effects of carbonylcyanide-3-chlorophenylhyrazone. Eur J Cancer 1979; 15:1281–1288.
90. Miyakoshi J, Oda W, Harata M, et al. Effects of amiloride on thermosensitivity of Chinese hamster cells under neutral and acidic pH. Cancer Res 1986; 46:1840–1843.
91. Ruifrok ACC, Konings AWTR. Effects of amiloride on hyperthermic cell killing of normal and thermotolerant mouse fibroblast LM cells. Int J Radiat Biol 1987; 52:385–392.
92. Varnes Me, Glazver KG, Gray C. pH-dependent effects of the ionophore nigericin on response of mammalian cells to radiation and heat treatment. Radiat Res 1989; 117:285–292.
93. Song CW, Lyons JC, Griffin RJ, et al. Increase in thermosensitivity of tumor cells by lowering intracellular pH. Cancer Res 1993; 53:1599–1601.
94. Song CW, Lyons JC, Griffin RJ, Makepeace CM. Thermosensitization by lowering intracellular pH with EIPA. Radiother Oncol 1993; 27:252–258.
95. Song CW, Lyons JC, Makepeace CM, et al. Effects of HMA, an analog of amiloride, on the thermosensitivity of tumors in vivo. Int J Radiat Oncol Biol Phys 1994; 30:133–139.

96. Song CW, Kim GE, Lyons JC, et al. Thermosensitization by increasing intracellular acidity with amiloride and its analogs. Int. J Radiat Oncol Biol Phys 1994; 30:1161–1169.
97. Takasu T, Lyons JC, Park HJ, Song CW. Apoptosis and perturbation of cell cycle progression by hyperthermia in an acidic environment. Cancer Res 1998; 58:2504–2508.
98. Hirpara JL, Clements MV, Pervaiz A. Intracellular acidification triggered by mitochondrial-derived hydrogen peroxide is an effector mechanism for drug-induced apoptosis in tumor cells. J Biol Chem 2001; 276:514–521.
99. Ahmad KS, Iskandar KB, Hirpara JL, et al. Hydrogen peroxide-mediated cytosolic acidification is a signal for mitochondrial translocation of Bax during drug-induced apoptosis of tumor cells. Cancer Res 2004; 64:7867–7878.
100. Lee H-S, Park HJ, Lyons JC, et al. Radiation-induced apoptosis in different pH environments in vitro. Int J Radiat Oncol Biol Phys 1997; 38:1079–1087.
101. Park HJ, Lyons JC, Ohtsubo T, Song CW. Cell cycle progression and apoptosis after irradiation in an acidic environment. Cell Death Differ 2000; 7:729–738.
102. Ohtsubo T, Igawa H, Saito T, et al. Acidic environment modifies heat- or radiation-induced apoptosis in human maxillary cancer cells. Int J Radiation Oncology Biol Phys 2001; 49:1391–1399.
103. Park HJ, Lee SH, Chung H, et al. Influence of environmental pH on G2-phase arrest caused by ionizing radiation. Radiat Res 2003; 159:86–93.
104. Freeman ML, Sierra E. An acidic extracellular environment reduces the fixation of DNA damage. Radiat Res 1984; 97:154–161.
105. Holahan EV. Stuart PK, Dewey WC. Enhancement of survival of CHO cells by acidic pH after X-irradiation. Radiat Res 1982; 89:433–435.
106. Haveman J. The influence of pH on the survival after X-irradiation of cultured malignant cells. Effects of carbonylbyanide 3-chlorophenylhydrazone. Int J Radiat Biol 1980; 37:201–205.
107. Choi EK, Robert K, Gfiffin RJ, et al. Effect of pH on radiation-induced p53 expression. Int J Radiat Oncol Biol Phys 2004; 60:1264–1271.
108. Freeman ML, Dewey WC, Hopewood LE. Effect of pH on hyperthermic cell killing: brief communication. J Natl Cancer Inst 1977; 58:1837–1839.
109. Gerweck LE. Modification of cell lethality at elevated temperatures. Radiat Res 1977; 70:224–235.
110. Gerweck LE, Dahlberg WK, Greco B. Effect of pH on single or fractionated heat treatment at 42–45°C. Cancer Res 1983; 43:1163–1167.
111. Nlsen OS, Overgaard J. Effect of extracellular pH on thermotolerance and recovery of hyperthermia damage in vitro. Cancer Res 1979; 39:2772–2778.
112. Goldin EM, Leeper DB. The effect of reduced pH on the induction of thermotolerance. Radiology 1981: 141:505–508.
113. KG Hofer, Mivichi NF. Tumor cell sensitivity to hyperthermia as a function of extracellular and intracellular pH. J Natl Cancer Inst 1980; 65:621–625.
114. Chu GL, Dewey WC. The role of low intracellular or extracellular pH in sensitization of hyperthermia. Radiat Res 1988; 11:4154–167.
115 Hahn GM, Shiu E. Adaptation of low pH modified thermal and thermochemical response of mammalian cells. Int J Hypertherm 1986; 2:379–387.
116. Cook JA, Fox MH. Effects of acute pH 6.6 and 42.0°C heating on the intracellular pH of Chinese hamster cells. Cancer Res 1988; 48:497–502.
117. van den Berg A, Wike-Hooley JL, Broekmayer-Reurink MP, van der Zee J, Reinhold HS. The relationship between the unmodified initial tissue pH of human tumors and the response to combined radiotherapy and local hyperthermia treatment. Eur J Cancer Clin Oncol 1989; 25:73–78.
118. Hetzel FW, Avery K, Chopp M. Hyperthermic "dose" dependent changes in intralesional pH. Int J Radiat Oncol Biol Phys 1989; 16:183–186.
119. Kang, MS, Song CW, Levitt SH. The role of vascular function in the response of tumors in vivo to hyperthermia. Cancer Res 1980; 40:1130–1135.
120. Thistlethwaite AJ, Leeper DB, Moylan DJ, Nerlinger RE. pH distribution in human tumors. Int J Radiat Oncol Biol Phys 1985; 11:1647–1652.
121. Canter RJ, Zhou R, Kesmodel SB, et al. Metaiodobenzylguanidine and hyperglycemia augment tumor response to isolated limb perfusion in a rodent model of human melanoma. Ann Surg Oncol 2004; 11:265–273.
122. Mahoney BP, Raghunand N, Baggett B, et al. Tumor acidity, ion trapping and chemotherapeutics. I. Acid pH affects the distribution of chemotherapeutic agents in vitro. Biochem Pharmacol 2003; 66:1207–1218.

123. Raghunand N, Mahoney BP, Gilles RJ. Tumor acidity, ion trapping and chemotherapeutics. II. pH-dependent partition coefficients predict importance of ion trapping on pharmacokinetics of weakly basic chemotherapeutic agents. Biochem Pharmacol 2003; 66:1219–1229.
124. Jahde E, Glusenkamp KH, Klunder I, Hulser DF, Tietze LF, Rajewsky MF. Hydrogen ion-mediated enhancement of cytotoxicity of bis-chlorethylating drugs in rat mammary carcinoma cells in vitro. Cancer Res 1989; 49:2965–2972.
125. Jahde E, Glusenkamp KH, Rajewsky MF. Nigericin enhances mafosfamide cytotoxicity at a low extracellular pH. Cancer Chemother Pharmcol 1991; 27:440–444.
126. Skarsgard LD, Chaplin DJ, Wilson DJ, et al. The effect of hypoxia and low pH on the cytotoxicity of melphalan and chlorambucil in vitro (abstract 23). Proceedings of the 7th International Conference on Chemical Modifiers of Cancer Treatment, Clearwarter, Florida, 2–5 Feb 1992.
127. Kwok TT, Twentyman PR. Effects of changes in oxygen tension, pH and glucose concentration on the response to CCNU and EMT6 mouse tumor monolayer cells and multicellular spheroids. Int J Radiat Oncol Biol Phys 1988; 14:1221–1229.
128. Euler J, Sauerman G, Priesching A. Wirkung von temperature, pH und thiotepa auf angehraten und thymodineinbau von aszitestumorzellen. Wien Klin Wocheschr 1974; 86:211–219.
129. Connors TA, Mitchley BCV, Rosenoer VM, Ross WCJ. The effect of glucose pretreatment on the cardinostatic and toxic activities of some alkylating agents. Biochem Pharm 1964; 13:395–400.
130. Oskinsky S, Bubnovskyja L, Sergienko T. Tumor pH under induced hyperglycemia and efficacy of chemotherapy. Anticancer Res 1987; 7:199–202.
131. Hahn GM, Shiu EC. Effect of pH and elevated temperatures on cytotoxicity of some chemotherapeutic agents on Chinese hamster cells in vitro. Cancer Res 1983; 43:5789–5791.
132. Ojugo AS, McSheehy PM, Stubbs M, et al. Influence of pH on the uptake of 5-fluorouracil into isolated tumour cells. Br J Cancer 1998; 77:873–879.
133. Kennedy KA, McGurl JD, Leondaridis L, Alabaster O. pH dependent of mitomycin C-induced cross linking activity in MET6 tumor cells. Cancer Res 1985; 45:3541–3547.
134. Urano M, Kahn J, Kenton LA. Effect of bleomycin on murine tumor cells at elevated temperatures and two different pH values. Cancer Res 1988; 48:616–619.
135. Born R, Eicholtz-Wirth H. Effect of different physiological conditions on the action of Adriamycin on Chinese hamster cells in vitro. Br J Cancer 1981; 44:241–246.
136. Hindenberg AA, Stewart VJ, Baker MA, Taub RN. Effect of pH on cellular accumulation of duanorubicvin. Am Assoc Cancer Res 1987; 28:261 (abstract no. 1031).
137. Ferguson PJ, Phillips JR, Selner M, Case CE. Differential activity of vincristine and vinblastine against cultured cells. Cancer Res 1984; 44:3307–3312.
138. Vukovic V, Tannock IF. Influence of low pH on cytotoxicity of paclitaxel mitoxantrone and topotecan. Br J Cancer 1997; 75:1167–1172.
139. Hahn GM. Hyperthermia to enhance drug delivery. In: Rational basis for chemotherapy. New York: Alan R. Liss, 1983:427–436.
140. Herman TS, Teicher BA, Collins LS. Effect of hypoxia and acidosis on the cytotoxicity of four platinum complexes at normal and hyperthermic temperatures. Cancer Res 1988; 48:2342–2347.
141. Teicher BA, Herman TS, Pfeffer MR, et al. Interaction of $PtCl_4$(Fast Black)$_2$ with hyperthermia. Cancer Res 1989; 49:6208–6219.
142. Ward JH, DipPette DJ, Held TN, Jain RK. Effect of intravenous versus intraperitoneal glucose injection on systemic hemodynamics and blood flow rate in normal and tumor tissues in rats. Cancer Res 1991; 51:3612–3616.
143. Vaupel PW, Okunieff PG. Role of hypovolemic hemoconcentration in dose-dependent flow decline observed in murine tumors after interperitoneal administration of glucose or mannitol. Cancer Res 1988; 48:7102–7106.
144. Calderwood SK, Dickson JA. Effect of hyperglycemia on blood flow, pH and response to hyperthermia (42°C) of the Yoshida sarcoma in the rat. Cancer Res 1980; 40:4728–4733.
145. Voorhees WD, Babbs CF. Hydralazine-enhanced selective heating of transmissible venereal tumor implanted in dogs. Eur J Cancer Clin Oncol 1982; 19:1027–1033.
146. Lin J-C, Song CW. Effects of hydralazine on the blood flow in RIF-1 tumors and normal tissues of mice. Radiat Res 1990; 124:171–177.
147. Hasegawa T, Song CW. Effect of hydralazine on the blood flow in tumors and normal tissues of rats. Int J Radiat Oncol Biol Phys 1991; 20:1001–1007.

http://www.springer.com/978-1-58829-530-9

Cancer Drug Resistance
Teicher, B.A. (Ed.)
2006, XVI, 617 p., Hardcover
ISBN: 978-1-58829-530-9
A product of Humana Press