Reduction of Intracellular pH as a Possible Mechanism for Killing Cells in Acidic Regions of Solid Tumors: Effects of Carbonylcyanide-3-chlorophenylhydrazone

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ABSTRACT

The environment of cells within solid tumors is known to be acidic relative to that in normal tissue, and the viability of tumor cells may depend on mechanisms which maintain intracellular pH (pHi) above the extracellular pH (pHe). It has been shown therefore the toxicity in vitro of the proton ionophore carbonylcyanide-3-chlorophenylhydrazone (CCCP), since this agent has been reported to be capable of transporting H\(^+\) equivalents through artificial lipid bilayers and mitochondrial membranes. CCCP was toxic to the human bladder carcinoma cell line MGH-U1 and to the murine mammary sarcoma cell line EMT-6 only at pHe < 6.5. CCCP transported H\(^+\) equivalents through cell membranes at physiological (7.35) and low pHi (6.20). Cell lines were found to have steady-state pHi values approximately 0.1 to 0.2 units above pHe at pHe < 6.50. Addition of CCCP led to a decrease in steady-state pHi values as compared to untreated cells at pHe < 6.50, whereas there was no apparent effect of CCCP on steady-state pHi values at pHi > 6.50. The CCCP-induced reduction in steady-state pHi combined with the uncoupling of oxidative phosphorylation by CCCP appeared to be the major mechanisms leading to cell death at pHi < 6.50. The toxicity of CCCP under acidic conditions was enhanced by amiloride and 4,4'-diisothiocyanostilbene-2,2-disulfonic acid, agents which are known to inhibit membrane-based ion exchange mechanisms which regulate pHi under acidic conditions. When both agents were combined with CCCP, cell killing was observed at pHi < 7.30. Our results suggest that mechanisms which regulate pHi under acidic conditions which occur in solid tumors may represent targets for new forms of tumor-specific therapy.

INTRODUCTION

Microelectrode measurements of pHi in solid tumors in both humans and animals have suggested that tumor tissue is on average 0.5 pHi units more acidic than normal tissue (1). Thus, representative values of pHi in tumor tissue are usually in the range of pHe 6.5 to 7.0, whereas values of pHi in normal tissue are usually in the range of pHe 7.1 to 7.4. Nutrient deprivation due to poor vascularization within solid tumors is probably the major cause of this acidity (2, 3). In particular, cells which are deprived of oxygen become dependent on glycolysis for energy metabolism, and the resulting production of lactic acid in combination with hydrolysis of ATP results in the net generation of H\(^+\) equivalents (4). In an acidic environment it has been observed that cells maintain pHe\(^+\) above pHe, primarily by the action of membrane-based ion exchange mechanisms. Major mechanisms include the Na\(^+\)/H\(^+\) antiporter, which is inhibited by amiloride and its analogues (5), and the Na\(^+\)-dependent HCO\(_3\(^-\)/Cl\(^-\) exchanger, which is inhibited by stibene derivatives such as DIDS (6). Ion exchange mechanisms which regulate pHi may be important for tumor formation and growth since variant cells which lack Na\(^+\)/H\(^+\) exchange activity have been found to have reduced tumorigenicity in vivo (7, 8).

Cells in nutrient-deprived regions of solid tumors may be resistant to radiation due to hypoxia. Also, these cells may be resistant to chemotherapy due to their reduced proliferative rate and/or because of poor penetration of some anticancer drugs to these cells. Hence, the presence of nutrient-deprived cells may be one explanation for the failure of conventional therapy. A large amount of research has been aimed at the development of compounds which will increase the sensitivity of nutrient-deprived hypoxic cells to radiation; however, to date clinical trials with radiosensitizers have been largely unsuccessful (9). An alternative approach to kill nutrient-deprived tumor cells may be to use the acidity which exists in their microenvironment to cause intracellular acidification. This might be achieved by using (a) agents which transport H\(^+\) equivalents into cells, and/or (b) compounds which inhibit the membrane-based exchangers responsible for maintaining pHi above pHe.

Previous studies in this laboratory have shown that the ionophore nigericin, which exchanges K\(^+\) for H\(^+\), was cytotoxic in vitro to two cell lines at pHi < 6.5 (10). Furthermore, the inhibitors amiloride and DIDS, when used in the presence of nigericin, caused killing of cells in the range of pHi (6.5 to 7.0) which is commonly found in solid tumors (10) The proton ionophore, CCCP, has been shown to uncouple oxidative phosphorylation (11), and it has been reported that CCCP is capable of transporting H\(^+\) equivalents through artificial lipid bilayers and mitochondrial membranes (12). Due to its proton-conducting capability and its enhancement of the effects of radiation and hyperthermia in vitro (13, 14), CCCP was a logical choice to test whether reduction in pHi could lead to selective killing of tumor cells at low pHi. The objectives of this in vitro study were to determine whether the nonelectroneutral protonophore CCCP would cause pH-dependent cell killing similar to that observed for the electroneutral ionophore nigericin (10) and to determine if cell killing was related to the transport of H\(^+\) through cell membranes.

MATERIALS AND METHODS

Cells. The murine mammary sarcoma cell line EMT-6 (obtained from Dr. R. Sutherland, Cancer Center for Experimental Therapeutics, Rochester School of Medicine and Dentistry, Rochester, NY) and the human bladder carcinoma cell line MGH-U1 (obtained from Dr. G. Prout, Urology Research Laboratory, Massachusetts General Hospital, Boston, MA) were maintained in a-MEM supplemented with 0.1% fetal bovine serum, 0.25% nonessential amino acids, 10 mM HEPES, N,N-hydroxyethylpiperazine-N\(^-\)2-ethanesulfonic acid, K\(^+\)\(_{\text{in}}\), intracellular K\(^+\); Mes, 2-(N-morpholino)ethanesulfonic acid; Na\(^+\), intracellular Na\(^+\); PCA, perchloric acid; Tris, tris(hydroxymethyl)aminomethane.

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all other chemicals were purchased from Sigma (St. Louis, MO).

Assessment of Cell Survival. Cell suspensions at a concentration of 10⁶ cells/ml in α-MEM plus 5% FCS were added to glass vials and stirred continuously at 37°C. A humidified gas mixture of 5% CO₂ in air was passed through the vials over the cell suspension as described previously (16). To maintain the desired pH, separate solutions of α-MEM plus 5% FCS were prepared containing 25 mM HEPES or 25 mM bicarbonate. By mixing these solutions, medium of the required pH was obtained which had a constant 25 mM buffer concentration. The pH of prepared medium was relatively stable over a 6-h incubation period with a small acidification of 0.05 to 0.1 pH units being observed in most experiments.

After 30 min of gassing in the appropriate pH medium, the required volume of CCCP dissolved in 100% ethanol was added to the exposure vials from a 0.02 M stock solution of CCCP. Control vials were exposed to the same volume of 100% ethanol, which did not exceed 0.2%, a dose which was not toxic for either cell line. At the appropriate times aliquots were removed from the vials, and cells were washed and resuspended in fresh α-MEM plus 5% FCS at pH 7.30. Cells were then counted, diluted, and plated in triplicate in Petri dishes. Colonies were stained and counted 11 days later.

Measurement of Intracellular pH. The fluorescent dye BCECF was used to measure pHᵢ as described elsewhere (17). Briefly, cells are exposed initially to the membrane permeant tetraacetoxymethyl ester of BCECF. After diffusing into the cytoplasm, nonspecific cellular esterases cleave BCECF-AM, thereby liberating the poorly permeant, highly fluorescent BCECF which has an excitation peak at 495 nm and an emission peak at 525 nm. BCECF has an approximate pKᵢ of 7.0, making it suitable for monitoring pHᵢ. The relationship between pHᵢ and fluorescence intensity is approximately linear in the range of pHᵢ 6.0 to 7.5.

For individual experiments, cells were initially exposed to 2 μg/ml of BCECF-AM in α-MEM without serum for 30 min at 37°C, pelleted by mild centrifugation, and resuspended in fresh α-MEM without serum at pH 7.30 to a final concentration of 6.0 x 10⁶ cells/ml. To examine the short-term regulation of pHᵢ (<5 min), alliquots of 4.8 x 10⁵ cells were then added to a cuvet containing NMG medium (10 mM glucose, 1 mM KCl, 1 mM MgCl₂, and 140 mM N-methyl-d-glucamine buffered to various pHᵢ with 20 mM Mes/Tris). In experiments to monitor the long-term regulation of pHᵢ (>30 min), cells were resuspended in fresh α-MEM minus phenol red plus 5% FCS at pH 7.30. Alliquots of 4.8 x 10⁵ cells were then added to a cuvet containing α-MEM minus phenol red plus 5% FCS buffered to various pHᵢ with bicarbonate/HEPES (25 mM). Measurements of pHᵢ were carried out in a Perkin Elmer LS3 fluorescence spectrophotometer.

Calibration of pHᵢ versus fluorescence intensity was performed on the same batch of cells using two methods. Cells were placed in K⁺ medium (same as NMG with isoosmotic replacement of N-methyl-d-glucamine with KCl, or α-MEM minus phenol red plus 5% FCS with isoosmotic replacement of Na⁺ with K⁺) which contained nigericin. The ionophore nigericin exchanges K⁺ for H⁺ and sets H⁺/K⁺ = K⁺/K⁺; thus, when cells are placed in K⁺ medium with approximately the same concentration of K⁺ as in the cytoplasm (approximately 140 mM), pHᵢ will equalize with plasma when the medium is titrated with concentrated Tris or Mes. Alternatively, calibration was performed at the end of each experiment by disrupting cell membranes with 0.05% Triton X-100 followed by direct titration of the medium with concentrated Tris or Mes. To account for the observed red shift in BCECF fluorescence when the dye is released from cells, a correction factor was determined by using the K⁺-nigericin calibration technique (18).

Measurement of Glucose, Lactate, and ATP. The consumption of glucose and the production of l-lactate in the incubation medium was determined by using commercial kits (Sigma) with a spectrophotometer (Cary 219; Varian) (19). After acid extraction of cells with 0.4 M PCA at 0°C followed by neutralization with 1 M K₂CO₃, ATP levels were measured using a luciferine-luciferase ATP assay kit (Sigma) with a liquid scintillation counter (Beckman; LS 330) (20).

Measurement of Intracellular Na⁺ and K⁺. Na⁺ and K⁺ were determined by methods described previously (17). At various times during exposure to CCCP, 2.5 x 10⁶ cells were removed from vials and washed twice with ice-cold choline buffer (140 mM choline chloride, 10 mM glucose, 1 mM CaCl₂, 1 mM MgCl₂, 20 mM Tris-Mes, pH 7.3). The buffer was aspirated, and the pellet was resuspended in 1 ml of Li⁺-standard solution (3 meq/liter; Radiometer, Copenhagen, Denmark) and mixed vigorously. The cell suspension was stored overnight at ~70°C, thawed, and vortexed to rupture the cells. After sedimentation of debris, Na⁺ and K⁺ concentrations in the supernatant were determined by flame photometry (Model FLM3; Radiometer). Based on cell volumes measured electronically at each time point (channelizer manufactured by the electronics department at the Ontario Cancer Institute), the volume-corrected Na⁺ and K⁺ were calculated.

RESULTS

Effect of CCCP and Low pHᵢ on Cell Survival. MGH-U1 and EMT-6 cells exposed to CCCP (15 μM, 5 μM, respectively) and low pHᵢ (6.10) showed a marked decrease in cell survival after 6 h, whereas cells exposed to either CCCP at physiological pHᵢ (7.30) or to low pHᵢ alone (6.10) had no decrease in colony-forming ability (Fig. 1). The observed cytotoxicity was dependent on the duration of exposure (Fig. 1, left) and on the concentration of CCCP (Fig. 1, right). At low pHᵢ (6.10), EMT-6 cells were more sensitive to a given concentration of CCCP than MGH-U1 cells; to produce the same decrease in relative plating efficiency, approximately 3 times the concentration of
CCCP was required for MGH-U1 cells as compared to EMT-6 cells.

The cytotoxicity of CCCP in the range of pH, 6.00 to 7.40 is shown in Fig. 2. For both cell lines, a sharp decrease in survival was observed at pH, below 6.50 in the presence of CCCP (Fig. 2), and there was no cytotoxicity at pH, > 6.50.

Effect of CCCP on Regulation of pHi. In an attempt to elucidate the mechanisms responsible for the cytotoxicity of the combination of low pH, and CCCP, we examined the effect of CCCP on the regulation of pHi. Fluorescence traces to examine the short-term regulation of pHi (<5 min) showed that, when MGH-U1 cells were exposed to CCCP at physiological pH, (7.35), intracellular acidification occurred (Fig. 3). CCCP also produced intracellular acidification in cells exposed to low pH, (6.20), and the rate of acidification observed in the first minute at low pH, was significantly higher than at physiological pH,.

At physiological and low pH, the rate of acidification observed in the first minute was dependent on the concentration of CCCP.

To examine the long-term regulation of pHi, the relationship between pHi and pH, was determined after cells had equilibrated for at least 30 min in the presence or absence of CCCP. At pH, < 6.5, CCCP caused an intracellular acidification such that pHi was slightly below pH, whereas cells not exposed to the drug maintained pHi approximately 0.1 to 0.2 pH units above pH, (Fig. 4). CCCP did not appear to have any effect on steady-state pH, at pH, > 6.5. Using the relationship between pHi and pH, (Fig. 4), we examined cell survival as a function of pHi for cells in the presence or absence of CCCP (Fig. 5). Although there was a small reduction in cell survival in the range of pH, 6.0 to 6.5 in the absence of CCCP, there was a significantly larger decrease in relative plating efficiency when CCCP was present.

![Fig. 2. Dependence on pH, for cell killing of MGH-U1 (A) and EMT-6 (B) cells exposed for 4 h to 20.0 μM and 7.5 μM CCCP, respectively. Points, mean of triplicate plates; bars, range.](image)

![Fig. 3. Fluorescent traces showing the fall in pHi for MGH-U1 cells exposed to CCCP at physiological or low pH, Qualitatively similar results were obtained for EMT-6 cells. Values represent mean and standard deviation from three experiments.](image)

![Fig. 4. Relationship between pH, and pH, for cells in the presence (10 μM) or absence of CCCP. Cells were allowed to equilibrate for at least 30 min at the indicated pH, value before determination of pH.](image)

![Fig. 5. Survival of MGH-U1 cells as a function of pH, in the presence (15.0 μm) or absence of CCCP after 4 h. Qualitatively similar results were obtained for EMT-6 cells. Points, mean of triplicate plates; bars, range.](image)
present. Experiments using EMT-6 cells gave qualitatively similar results.

Effect of CCCP and Low pH on Energy Metabolism and Ion Distribution. CCCP is a known uncoupler of oxidative phosphorylation (11), and low pH is known to inhibit glycolysis (21). We therefore performed experiments to determine the effects of CCCP at various pH on glucose consumption, lactate production, and cellular concentration of ATP. Glucose consumption remained unchanged, and lactate production was slightly increased for cells exposed to CCCP at pH 7.30 for 4 h (Fig. 6). Glycolysis was inhibited when cells were exposed to low pH (6.10) in the presence or absence of CCCP as glucose consumption and lactate production were dramatically reduced.

Effects of CCCP and low pH on ATP concentration after 4-h incubations are summarized in Table 1. There were differences in ATP levels of MGH-U1 and EMT-6 cells at pH 7.30 in the presence of CCCP, 46% and 103% of controls, respectively, and at low pH (6.10) in the absence of CCCP, 36% and 63% of controls, respectively. Both cell lines when placed at low pH, in the presence of CCCP had ATP levels reduced to approximately 30% of controls.

Experiments to determine the influence of CCCP on the distribution of intracellular Na+ and K+ are summarized in Fig. 7. Cells incubated at pH 7.30 in the presence or absence of CCCP (15.0 μM) for 4 h had constant Na+, and K+ concentrations of 8.0 and 123.0 meq/liter, respectively. Exposure of cells to pH 6.10 led to no change in Na+, whereas there was a slow decrease in K+ to 93.0 meq/liter after 4 h. However, the combination of CCCP and pH 6.10 led to an increase in Na+ to 33.0 meq/liter and a reduction in K+ to 81.0 meq/liter. Qualitatively similar results were obtained for EMT-6 cells.

Table 1. Relative concentrations of ATP for MGH-U1 and EMT-6 cells incubated for 4 h at the indicated pH, in the presence of 15.0 and 5.0 μM CCCP, respectively.

<table>
<thead>
<tr>
<th>pH</th>
<th>MGH-U1 % of ATP</th>
<th>EMT-6 % of ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7.00</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>pH 7.00 + CCCP</td>
<td>46</td>
<td>103</td>
</tr>
<tr>
<td>pH 6.10</td>
<td>36</td>
<td>63</td>
</tr>
<tr>
<td>pH 6.10 + CCCP</td>
<td>31</td>
<td>30</td>
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*ATP levels are expressed as percentage of controls to account for any loss of ATP which may have occurred prior to extraction of cells with PCA. Values are representative from two experiments.

![Fig. 6](image_url)

Fig. 6. Lactate production and glucose consumption by MGH-U1 and EMT-6 cells exposed to CCCP (15.0 μM), at various pH values (pH 7.30 and pH 6.10). Columns, mean from three experiments; bars, SD.

![Fig. 7](image_url)

Fig. 7. Changes in Na+ and K+ concentrations for MGH-U1 cells in the presence of CCCP (15.0 μM) and low pH (6.10) or physiological pH (7.30). Qualitatively similar results were obtained for EMT-6 cells.

![Fig. 8](image_url)

Fig. 8. A, colony-forming ability of MGH-U1 cells exposed to CCCP (15.0 μM) or amiloride and DIDS (both 0.1 mM) at various pH values (pH 7.30, pH 6.10), or the combination of all three at pH 6.45. Controls, which showed no reduction in relative plating efficiency, include cells exposed to the above combination of the three agents at pH 7.30 ( ), or pH 6.10 ( ). In the absence of drugs, B, killing of MGH-U1 cells exposed to the combination of CCCP, amiloride, and DIDS at various values of pH (dark symbols). Controls were exposed to pH 7.30 in the absence of drugs (open symbols). Qualitatively similar results were obtained for EMT-6 cells. Points, mean of triplicate plates; bars, range.

Fig. 9. Change in Na+, K+, and pH in the presence of CCCP at various pH values (pH 7.30, pH 6.10), or exposure to pH 7.30 ( ) or 6.45 ( ) in the absence of drugs. A, Na+ levels. B, K+ levels. C, pH levels. Qualitatively similar results were obtained for EMT-6 cells. Points, mean of triplicate plates; bars, SD.

![Fig. 9](image_url)

Fig. 9. Changes in Na+, K+, and pH levels for MGH-U1 cells exposed to CCCP (15.0 μM) and low pH (6.10) or physiological pH (7.30). Qualitatively similar results were obtained for EMT-6 cells.
DISCUSSION

The results from this study indicate that CCCP alone, or in combination with agents that inhibit the membrane-based exchangers responsible for the regulation of pH$_i$, can produce large cytotoxic effects at low pH$_i$ in vitro, without toxicity at physiological pH$_i$.

Cell killing by CCCP was limited to pH$_i$ < 6.5 (Fig. 2). This dependence on pH$_i$ was similar to that observed for CCCP by Haveman (13) and in our laboratory with nigericin (10). We observed some variability in colony-forming ability of cells exposed to low pH$_i$ and CCCP which was probably a result of the critical dependence of cell survival on pH$_i$ and on the concentration of CCCP used (compare Figs. 1 and 2). Furthermore, the observation that the murine tumor cell line was more sensitive to CCCP at low pH$_i$ as compared to the human tumor cell line is not surprising as others have found species-dependent variation in response to anticancer drugs (22).

Haveman suggested that the ability of CCCP to kill cells and to sensitize them to heat and radiation may have been due to the equilibration of pH$_i$ and pH$_h$ caused by the known H$^+$ conducting properties of CCCP (13, 14). We observed that CCCP caused an increased rate of H$^+$ flux through cell membranes at physiological and low pH$_i$ (Fig. 3). The acidification observed at physiological pH$_h$ is assumed to be a result of the electrical potential across the cell membrane, whereas at low pH$_h$, the acidification was most likely due to the combination of an H$^+$ gradient into cells and the electrical potential across the cell membrane. We observed that exposure to CCCP resulted in a reduction in steady-state pH$_i$ as compared to unexposed cells only at pH$_i$ < 6.5 (Fig. 4), which parallels the observed cytotoxicity at pH$_i$ < 6.5 (Fig. 2); however, CCCP did not cause equilibration of pH$_i$ and pH$_h$ over the range of pH$_i$ 6.0 to 7.4. It should be noted that the reduction in pH$_i$ in the presence of CCCP at pH$_i$ < 6.5 may have been partially a result of a decrease in pH$_i$ within mitochondria. The observed cell killing by CCCP at pH$_i$ < 6.5 may therefore have been due to an increase in H$^+$ flux into cells leading to a reduction in steady-state values of pH$_h$. Additional mechanisms leading to cell death are suggested by the finding that cell survival at low pH$_i$ was significantly reduced in the presence of CCCP in comparison to its absence (Fig. 5).

Influences of CCCP and low pH$_i$ on energy metabolism of cells may have contributed to cytotoxicity. At pH$_i$, 7.30 in the presence of CCCP, there was no evident increase in lactate production (Fig. 6) or reduction in ATP (Table 1) as compared to pH$_i$, 7.30 alone for EMT-6 cells. This result suggests that either (a) the concentration of CCCP used did not uncouple the mitochondria or (b) these cells preferentially use glycolysis. Since previous research has shown that 110 nM CCCP is capable of uncoupling mitochondria (12) and in this study μM concentrations of CCCP were used, the latter appears to be more likely. Hence it would appear that EMT-6 cells have enhanced rates of aerobic glycolysis, a phenomenon which has been previously documented for other tumor cell lines (23). MGH-U1 cells at pH$_i$, 7.30 in the presence of CCCP had no increase in lactate production; however, unlike EMT-6 cells, MGH-U1 cells had a reduction in ATP (Table 1) as compared to controls. Therefore, MGH-U1 cells utilize oxidative phosphorylation, and our results suggest that these cells do not have enhanced rates of aerobic glycolysis. Since there was no difference in survival between MGH-U1 and EMT-6 cells at pH$_i$, 7.30 in the presence of CCCP (Fig. 1), our results imply that the uncoupling effect of CCCP did not have a major impact on cell survival at physiological pH$_h$.

At low pH$_h$, lactate production was inhibited for both cell lines in the presence or absence of CCCP (Fig. 6), confirming the previously reported sensitivity of phosphofructokinase to low pH (21). For both cell lines, there was also a decrease in ATP content at low pH$_h$ in the presence of CCCP (Table 1). This decrease in ATP content was paralleled by a marked reduction in survival for both cell lines. These results suggest that the major mechanism leading to cell killing at low pH$_h$ in the presence of CCCP was the reduction in pH$_h$, combined with the uncoupling effect of CCCP.

Coinciding with a decrease in energy production by cells exposed to the combination of low pH$_h$ and CCCP, both cell lines were found to have perturbations in Na$^+$, and K$^+$ concentrations which probably reflect inhibition of the Na$^+$/K$^+-$ATPase (Fig. 7). Given the high affinity of the Na$^+$/K$^+$-ATPase for ATP (24), inhibition of the exchanger was most likely due to the reduction in pH$_h$ (25), rather than to a decrease in ATP content. Furthermore, no attempt has been made to determine the possible contribution of the activation of lysosomal proteases at low pH$_i$ to cell killing (26). Overall, our results would seem to indicate that, at pH$_i$, < 6.50, CCCP produced intracellular acidification which led to cell death by causing alterations in cellular energy metabolism and ion gradients.

Cells which were exposed to CCCP in the presence of the ion-exchange inhibitors amiloride and DIDS had cytotoxicity in a higher range of pH$_h$ (<7.3) than following exposure to CCCP alone (pH$_i$, < 6.5) (Fig. 8). This cytotoxicity may have been a result of intracellular acidification by CCCP combined with inhibition of the Na$^+$/H$^+$ and Na$^+$-dependent HCO$_3^-$/Cl$^-$ exchangers by amiloride and DIDS, thus preventing cells from extruding H$^+$ equivalents. Interestingly, the observed cell killing by CCCP (alone or in combination with amiloride and DIDS) was qualitatively similar to that previously observed for nigericin (10). Amiloride has been shown to inhibit Na$^+$/Ca$^{2+}$ exchange, Na$^+$/K$^+$/ATPase activity, and protein synthesis (27), and DIDS inhibits the transport of several anions (28). Although amiloride and DIDS were not toxic when used alone, the nonspecific effects of these drugs may also have contributed to the enhanced cell killing when they were used in combination with CCCP.

Our studies have demonstrated that it is possible to induce selective killing of tumor cells in vitro at reduced pH$_i$, < 7.3 by using agents which interfere with the regulation of pH$_h$ by (a) acidifying the cytoplasm (CCCP or nigericin) and/or by (b) inhibiting the Na$^+$/H$^+$ or Na$^+$-dependent HCO$_3^-$/Cl$^-$ exchangers (amiloride and DIDS). These results are provocative in that it may be possible to kill selectively nutrient-deprived cells in solid tumors by taking advantage of the existing acidic conditions. To further test the usefulness of this strategy, experiments should be performed to determine whether there is any cytotoxicity of these combinations of agents at physiological pH to normal tissue (fibroblasts) in vitro, and whether therapeutic effects can be achieved against solid tumors in rodents.

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REFERENCES

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