Selective cellular acidification and toxicity of weak organic acids in an acidic microenvironment

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Summary
The mean extracellular pH (pHe) within solid tumours has been found to be lower than in normal tissues. Agents which cause intracellular acidification at low pHe might have selective toxicity towards cells in tumours. Weak acids (or their anions) with pKa values in the range of 4-6 have a higher proportion of their molecules in the uncharged form at low pHe and can diffuse more rapidly into cells. The effects of organic acids including succinate, monomethyl succinate and malonate to acidify cells have been evaluated under conditions of different pHe in the acidic range. These weak acids caused intracellular acidification of murine EMT-6 and human MGH-U1 cells in a concentration and pHe dependent fashion. At concentrations of 10 mM and above, these acids also caused intracellular cytotoxicity to these cells at low pHe (<6.5). The rate and extent of cellular acidification caused by these weak acids and their cytotoxicity at low pHe, were enhanced by exposure to amiloride and 5-(N-ethyl-N-isopropyl)amiloride (EIPA), agents which inhibit Na+/H+ exchange, and hence the regulation of intracellular pH. Acid dependent cytotoxicity was also investigated in a murine solid tumour using the endpoints of growth delay and colony formation in vitro following treatment in vivo. Agents were tested alone or with 15 Gy X-rays to select a population of hypoxic (and presumably acidic) cells. Achievable serum concentrations of succinate were about 1 mM and no antitumour activity of succinate was detected when used in this way. It is concluded that weak acids are selectively taken up into cells, and can cause selective cellular acidification and toxicity, at low pHe in culture. Weak acids that are normal cellular metabolites are not toxic in vivo, but weak acids carrying cytotoxic groups offer the potential for selective uptake and toxicity under the conditions of low pHe that exist in many solid tumours.

Materials and methods

Reagents
Succinic acid, malonic acid, succinic acid monomethyl ester, butyric acid, propionic acid, ATP-bioluminescent assay kit and antimycin A were obtained from the Sigma Chemical Co., St. Louis, MO, USA. 2',7'-bis(2-carboxyethyl)-5 (and 6-) carboxyfluorescein acetylamethyl ester (BCECF-AM) was purchased from Molecular Probes, Eugene, Oregon, USA. Radiolabelled 2,3-4C-succinic acid (Sp. activity = 56 mCi mmol-1) was obtained from New England Nuclear, Mississauga, Ontario, Canada.

Cells
The murine mammary sarcoma cell line EMT-6 (obtained originally from Dr R. Sutherland, Rochester, NY, USA) and the human bladder carcinoma cell line MGH-U1 (obtained originally from the Urology Research Laboratory, Massachusetts General Hospital, Boston, MA, USA) were maintained in α-minimum essential medium (α-MEM) supplemented with 0.1 mg ml-1 of kanamycin and 5% foetal bovine serum (FBS). These cell lines were chosen because their mechanisms of regulation of pH have been studied in detail and because they allow comparison of effects against human and rodent cell lines (Boyer & Tannock, 1992). Both cell lines were reestablished from frozen stock at about 3

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month intervals and were tested routinely for mycoplasma and were not contaminated. Cells were grown as monolayers in tissue culture flasks and were detached from flasks with 0.05% trypsin and 0.53 mM ethylenediaminetetraacetic acid (EDTA) (Gibco, Grand Island, NY, USA). All experiments were performed with exponentially growing cells.

Measurement of intracellular pH

Intracellular pH (pHi) was determined by using the pH sensitive fluorescent probe BCECF-AM (Rink et al., 1982). Cells at a concentration of 1.5 × 10^6 cells/mL in N-2-hydroxyethylpiperazine-N-ethanesulfonic acid (Hapes) buffered medium at pH 7.4 were loaded with the esterified form of the fluorochrome, BCECF-AM, by incubation in the dark with 2 µg/mL for 20 min at 37°C. Cells were washed by centrifugation and 80 µL of the cell suspension containing ~5 × 10^5 cells was then added to a polystyrene cuvette containing NMG (140 mM N-Methyl-D-glucamine) or Na^+ (140 mM NaCl) each containing 1 mM KCl, 1 mM CaCl2, 1 mM MgCl2 and 5 mM glucose, buffered to different values of pHe with 20 mM Tris/Mes. The cuvette was temperature controlled (37°C) and stirred continuously using a magnetic flea. The uncharged form of BCECF-AM diffuses into the cell where it is cleaved by nonspecific esterases to form the charged, impermeant BCECF. Fluorescence was monitored with excitation and emission wavelengths set at 495 nm and 525 nm respectively. Weak acids (or their anionic forms) were titrated to desired values of pHe with KOH and were added to the cuvette in varying concentrations, and change in pHi was monitored with time. Calibration of pHi was performed after each experiment by adding 2 µg/mL nigericin to a cuvette of BCECF-containing cells in K^+ buffer (identical to the Na buffer with isoosmotic replacement of KCl for NaCl). Nigericin sets pHi equal to pHe under these conditions and fluorescence was measured at varying pHe after sequential addition of aliquots of 1 mL solutions of Mes(2-N-morpholino)ethanesulfonic acid (Mes) or Tris. This method of calibration has been supplemented in some experiments by lysing the cells and comparing fluorescence with measurements of pHe, with an appropriate correction for intracellular quenching of BCECF. There was an approximately linear relationship between pHi and fluorescence intensity in the range of pH 6.0 to 7.5. The activity of the Na^+ / H^+ exchanger was estimated from the rate of change of pHi following addition of 100 mM Na^+ to acid-loaded cells suspended in Na^+ free (NMG) solution (Madshus, 1988; Tannock & Rotin, 1989).

Assessment of cytotoxicity in vitro

Toxicity of weak acids at varying pHe was assessed by using the endpoint of colony formation. Five mL aliquots of a suspension containing 10^6 cells mL^-1 in α-MEM plus FBS buffered to the required pH were added to small glass vials. The cells were stirred continuously at 37°C, and humidified air (plus 5% CO2) flowed through the vials, as described previously (Rotin et al., 1987). Weak acids were buffered to the same pH and added to the same aliquots of the cell suspension 15 min later at the desired times after adding weak acids. 0.5 mL samples of the cell suspension were withdrawn by passing a long needle attached to a syringe through the gas outlet tube. The cells were washed and resuspended in α-MEM plus 5% FBS at pH 7.3, diluted, and plated in triplicate petri dishes. Colonies were stained with methylene blue and counted 9 to 13 days later. Surviving fraction was calculated as the ratio of plating efficiencies of treated and control cells plated at the same pH. Plating efficiency for control plates incubated at pH 6.0 or above was always greater than 50%.

Uptake of radiolabelled succinate

Experiments involving uptake of radioactive succinate into EMT-6 cells were carried out in medium containing 140 mM NaCl, 1 mM KCl, 1 mM MgCl2, 1 mM CaCl2 which was buffered with Mes/Tris at 37°C to the desired pHe; mixing was performed with a magnetic flea. Exponentially growing cells were trypsinised and washed with buffer solution at different pH without succinate. Cells were centrifuged and resuspended in isotonic solution at different pH containing 2 µM antimycin A to inhibit the oxidation of succinate (Spencer, 1976). After preincubation for 20 min, cells (10^6 mL^-1) were exposed to buffer at different pH which also contained 2 µM antimycin A, 5 mM unlabelled succinate and labelled succinate at a concentration 4 µCi mL^-1 (2 µCi mL^-1 in a repeat experiment). After different time periods, 0.3 ml aliquots were removed and nigericin was added to a final concentration of 5 µM; nigericin has been shown previously to cause almost complete inhibition of succinate transport (Spencer, 1976). Cells were centrifuged through a dibutyphthalate/corn oil (10:3) mixture to separate them from the buffer solution. Radioactivity was counted in the pellet using a liquid scintillation counter (LS 330; Beckman).

Effects of weak acids on tumour growth

Maximum tolerable doses of weak acids were determined in preliminary experiments by injecting different doses intraperitoneally into small groups of three mice. We determined the maximum dose at which animals survived with no abnormal behaviour, and with minimal weight loss. This dose was then used in subsequent experiments to study effects on growth of the KHT fibrosarcoma in syngeneic C3H/HeJ mice. This tumour is known to develop an acidic microenvironment with a mean value of pHe of 6.84 ± 0.06, and methods of transplantation and assessment of growth delay have been described previously (Newell et al., 1992).

Appropriate dilutions of organic acids were titrated with NaOH to neutral pHe and were injected intraperitoneally in volumes of 0.1 to 0.03 mL per gram body weight. Groups of mice bearing tumours with a mean diameter of 8.5 mm received either a single injection of succinate or monomethyl succinate or a course of four injections given at hourly intervals. To seek evidence for selective toxicity to hypoxic cells, a dose of 15 Gy was given immediately prior to a single injection, or between the second and third injections when multiple doses of the acid were given. Most cells which survive this radiation dose are expected to be hypoxic. Local tumour irradiation was delivered by using a specially designed 100 KV X-ray machine at a dose rate of 11.4 Gy min^-1 (Newell et al., 1992). For assessment of the effect of treatment on tumour growth, tumour diameter was estimated on coded mice every second day by passing the tumour-bearing leg through a series of graded holes drilled in lucite. Mice were killed humanely when their tumours attained a mean diameter of 14 to 15 mm. Tumour growth curves were constructed from a previously defined calibration curve relating tumour weight to medium diameter.

Effects of succinate on tumour cell survival in vivo

We assessed clonogenic survival following treatment of the EMT-6 tumour, which is also known to develop an acidic microenvironment (mean pH of 6.75 ± 0.06; Newell et al., 1992). Balb/c mice were inoculated with 20–24 µg Na-succinate or 5 × 10^5 EMT-6 cells into the hind flank. The tumours were allowed to grow for 7 days at which time the animals were randomised into groups to receive treatment. Treatments included four intraperitoneal injections of 0.01 ml g^-1 body weight of 500 mM Na-succinate or saline (controls) given at 1 h intervals; 15 Gy local X-irradiation given alone or between the second and third injections with Na-succinate; 5-(N-ethyl-N-isopropyl) amiloride (EIPA, 20 µM kg^-1 body weight) injected immediately after irradiation together with the same schedule of succinate injections; and hydralazine (10 mg kg^-1 body weight) injected with EIPA in a similar schedule. Hydralazine was given to try to inhibit tumour blood flow and thereby lower tumour pH (Lin & Song, 1990; Newell et al., 1992; Thomas et al., 1992). Tumours were removed from the mice 24 h after the last treatment and cell
survival was estimated by using an in vitro clonogenic assay as described previously (Thomson & Rauth, 1974).

**Measurement of succinate in murine serum**

Succinate was assayed enzymatically by using a Boehringer Mannheim assay kit. Briefly, blood samples were collected from anaesthetised mice by puncturing the heart and serum was prepared from the blood by centrifugation. Measurement of the concentration of succinic acid in the serum utilises the following principle. Succinate is converted by the enzyme succinyl-CoA synthetase in the presence of inosine-5'-triphosphate (ITP) and Coenzyme A (CoA) to succinyl CoA with simultaneous formation of inosine-5'-diphosphate (IDP) and inorganic phosphate. Inosine-5'-diphosphate reacts with phosphoenol pyruvate in the presence of pyruvate kinase to pyruvate and ITP. Pyruvate is then reduced by NADH in the presence of lactate dehydrogenase (LDH). The amount of NADH oxidised in the above reaction is proportional to the amount of succinic acid originally present. Change in concentration of NADH was measured by its absorbance at 340 nm.

**Results**

**Cellular acidification**

Experiments which characterise the effects of weak acids to cause intracellular acidification are described in Figures 1–3 and in Table I. All experiments were repeated to ensure reproducibility.

When mammalian cells grown at physiological pH (7.3) are added to medium buffered to a lower pH there is a slow fall in pHi to a new equilibrium value; for EMT-6 cells at pH 6.5 this equilibrium is about 6.9 (Table I and Figure 1). The monocarboxylic acids propionic acid, butyric acid and monomethyl succinic acid, and the dicarboxylic acids malonic acid and succinic acid all caused a marked increase in acidification of EMT-6 cells (Table I) and MGH-U1 cells (data not shown) at pHi 6.5. When cells, formerly grown at pH 7.3 in a-MEM, were added to buffers containing these acids the rate of acidification was higher in NMG-containing buffer than in Na⁺-containing buffer (Figures 1a and b) presumably because the presence of Na⁺ allowed cellular acidification to be opposed by the activation of the Na⁺/H⁺ exchanger. Acidification of cells in the presence of Na⁺ and the Na⁺/H⁺ inhibitor EIPA was more rapid than in Na⁺-buffer, and only slightly slower than that observed in Na⁺-free NMG containing buffer (Figure 1c).

The rate of acidification was dependent on concentration and on pH for all acids used (Figures 1 and 2). The decrease in pHi was most rapid for cells exposed to monocarboxylic acids such as propionic acid, butyric acid and monomethyl succinic acid in NMG containing buffer (Table I) and these acids were able to acidify cells at neutral pH 7.1; dicarboxylic acids did not cause cellular acidification at pH 7.1 (Figure 2).

All mammalian cells express the Na⁺/H⁺ exchanger which allows exchange of extracellular Na⁺ for intracellular H⁺ under conditions when cells becomes acidified (Madshus, 1988; Tannock & Rotin, 1989). In order to measure Na⁺-dependent pHi recovery from intracellular acidification by these weak acids, cells containing BCECF were exposed to different concentrations of weak acids in NMG-containing buffer at different pHs. When pHi had fallen to a low value, 100 mM NaCl was added, and Na⁺/H⁺ exchanger activity was measured by the rate of alkalisation. Recoverability of pHi after acidification by these weak acids is concentration and pH dependent but pHi returned to neutral in the presence of NaCl after addition of monocarboxylic acids at normal pH (Figure 2). Exposure of cells to dicarboxylic acids (or their anions) such as succinate or malonate caused them to have slower recovery of pH than exposure to monocarboxylic acids. At a concentration of 50 mM succinate there was almost complete inhibition of the Na⁺/H⁺ exchanger at pH 6.1.

The above experiments have shown that organic acids increase the rate and extent of acidification when cells are placed acutely in medium at low pH which contains them. However cells in an acidic microenvironment in tumours may be chronically adapted to low pH. We have therefore performed additional experiments to determine whether organic acids were able to acidify cells that were adapted to low pH.

When EMT-6 cells were exposed to Na⁺ buffer at different pHs in the acidic range the cytoplasmic pHi equilibrated after about 30 min at a value which was dependent on (but higher than) pH (Figure 3a). Addition of succinate or

![Figure 1](image1.png)

**Figure 1** Changes in intracellular pH (pHi) of EMT-6 cells (previously grown in α-minimum essential medium (αMEM) at extracellular pH (pHe) 7.3) as a function of time after adding cells to N-methyl-D-glucamine (NMG) buffer a, or Na⁺ buffer b and c, at pHe 6.5; the buffer contained double-distilled water (DDW) or different concentrations of succinate a–c, with or without 5-(N-ethyl-N-isopropyl) amiloride (EIPA), 10 μM; c).
monomethyl succinate (data not shown) to cells that were adapted to acidic conditions caused acidification, and inhibition of the residual Na\(^+\)/H\(^+\) activity by amiloride or EIPA led to further acidification (Figure 3a). The relationship between pH\(i\) and pH\(e\) under equilibrium conditions is shown in Figure 3b; cells are able to maintain values of pH\(i\) above pH\(e\) under acidic conditions. Addition of weak acids shifted the equilibrium between pH\(e\) and pH\(i\), such that the cells maintained a smaller pH gradient across the cell membrane under acidic conditions and this effect was enhanced in the presence of EIPA (Figure 3b).

### Uptake of radioactive succinic acid

It has been reported previously that the rate of cellular uptake of succinate is increased at low pH\(e\) (Spencer, 1976). We measured the uptake of \(^{14}\)C-succinate at intervals from 1 to 10 min after adding it to medium at pH 6.13 and 7.15 (Figure 4). Our experiments confirm that the rate of uptake of radiolabelled succinate is increased at low pH\(e\).

### Acids cause in vitro cytotoxicity

Exposure of exponentially-growing EMT-6 cells to succinate, monomethyl succinate or malonate caused cytotoxicity that was dependent on the duration of exposure, the concentration of acids used, and on pH\(e\) (Figure 5a and b). Toxicity was observed at pH\(e\) 6.5 and below, and cell killing increased as pH\(e\) was reduced.

Since amiloride and EIPA increase cellular acidification (Figures 1 and 3), presumably by inhibiting Na\(^+\)/H\(^+\) exchange.

### Table 1

| Organic acids | pKa | Structures                  | Minimum pH\(i\) (mean ± s.e.m.) | ΔpH\(i\) in 1 min (mean ± s.e.m.) |
|---------------|-----|-----------------------------|---------------------------------|-----------------------------------|
| A > Monocarboxylic acids | Propionic acid | 4.87 | CH\(_3\)CH\(_2\)COOH | 6.54 ± 0.08 | 0.45 ± 0.04 |
|               | Butyric acid  | 4.82 | CH\(_3\)CH\(_3\)CH2COOH | 6.56 ± 0.05 | 0.40 ± 0.06 |
|               | Monomethyl    | 4.21 | HOOCCH\(_3\)CH\(_2\)COOH | 6.43 ± 0.06 | 0.54 ± 0.07 |
| B > Dicarboxylic acids | Malonic acid  | 2.83 & 5.69 | HOOCCH\(_3\)COOH | 6.66 ± 0.04 | 0.10 ± 0.02 |
|               | Succinic acid | 4.21 & 5.64 | HOOCCH\(_3\)CH\(_2\)COOH | 6.58 ± 0.06 | 0.11 ± 0.03 |
| C > Buffer only (Control) | - | - | 6.95 ± 0.03 | 0.03 ± 0.02 |

pHi, intracellular pH.
exchange, we studied the cytotoxicity of weak acids in combination with amiloride or EIPA (Figure 6). Amiloride or EIPA showed minimal toxicity when used alone in the range of pH 6.0–7.0. When 20 mM succinate or monomethyl succinate were used in combination with 100 μM amiloride or 10 μM EIPA at pH 6.1, there was enhancement in cytotoxicity due to the weak acids and effects were much greater when using the potent analogue EIPA (Figure 6). Thus although Na⁺/H⁺ exchange activity is inhibited at low pH in the presence of succinate (Figure 2b), residual activity appears to be important in protecting the cells from cytotoxicity.

Responses of tumours to in vivo treatment

Untreated KHT tumours grew from a mean diameter of 8.5 mm (±0.25 g) to 12.5 mm (±1.0 g) in 2–3 days and single or multiple doses of succinate or monomethyl succinate had at most minimal effects to influence the rate of tumour growth. Representative data for multiple doses of succinate are shown in Figure 7. Irradiation of KHT tumours with 15 Gy X-rays produced growth delay of about 10 days. This growth delay in irradiated mice was not increased significantly by administration of single or multiple doses of succinate (Figure 7) or monomethyl succinate (data not shown) at maximal tolerated doses.

Since hydralazine can reduce tumour blood flow and mean pH we assessed the effects of succinate (± EIPA) with or without radiation on clonogenic survival of EMT-6 tumours in Balb/c mice that had received hydralazine. Tumour cell survival was measured directly using an in vitro clonogenic assay. Only X-radiation produced a cytotoxic effect on EMT-6 cells, and there was no additional effect of succinate (data not shown).

Concentration of succinate in serum

The serum concentration of succinate was assayed after injection of a single dose as used in the above experiments. As shown in Table II, the maximum serum concentration of succinate was about ~1.5 mM, and this is not in the range which leads to cytotoxicity in tissue culture.

Discussion

In the present studies we have investigated the potential of weak acids to cause intracellular acidification and cytotoxicity in an acidic microenvironment as can be found in some regions of solid tumours. The results indicate that weak acids alone or in combination with agents that inhibit the Na⁺/H⁺ antiporter (and hence pH regulation) cause intracellular acidification and cytotoxicity at low pH (<6.5) but exert little or no cytotoxicity at physiological pH. Unfortunately, the relative low concentrations achievable in vivo make it unlikely that this mechanism will lead to antitumour effects at tolerated doses when using the organic acids which were studied here.
The presumed mechanism of intracellular acidification by weak acids is increased diffusion of a weak acid into cells at low pH, when a greater proportion is in the protonated and uncharged form than at pH 7.0. Once inside the cell, the pH is well above pKa, leading to dissociation of the protonated form and acidification. For example, there are three forms of the divalent acid succinate which exist in solution, i.e. HAH, HA– and A2– (where A represents the succinate anion). The relative proportions of each component at any level of pH may be calculated from the pKa values using the Henderson-Hasselbalch equation, as illustrated in Table III. If the uptake of succinic acid into the cell is proportional to the extracellular concentration of the un-ionised form, the rate of entry should be 10-fold higher at pH 6.5, and 86-fold higher at pH 6.0, as compared to pH 7.0. Our experimental measurements of uptake of radiolabelled succinate show that this is indeed dependent on pH, but with a rate of uptake that is 2–3 times higher at pH 6.13 compared with pH 7.15. This lower ratio of uptake rates is probably due to higher levels of protonated acids inside the cell at lower levels of pH (and pHe) with a consequent reduction of transmem-
brane gradient for the protonated acid. Monocarboxylic acids are expected to cause more rapid acidification than dicarboxylic acids, since a higher proportion is in the neutral form, and we found that they could acidify cells in the physiological range of pH in NMG buffer. Also the presence of a monocarboxylic acid transporter in the plasma membrane (Deuticke et al., 1982) may contribute to uptake of monocarboxylic acids, and to the partially ionised form (HA⁻) of dicarboxylic acids.

We found that weak acids were toxic to cultured cells only at low pH, and cell death was probably caused by cellular acidification. Low pH can interfere with a variety of cellular processes including energy metabolism, and persistence of low pH may lead to irreversible injury to the cell. However, measurement of ATP (using chemoluminescence) as an indicator of energy metabolism after 2 or 4 h exposure to succinate (50 mM) at pH 6.2 or 7.2 showed no significant difference as compared to control (data not shown).

Cells are able to resist intracellular acidification by using intracellular buffers and by activating the Na⁺/H⁺ antipor and the Na⁺ dependent HCO₃⁻/Cl⁻ exchanger (Madshus, 1988; Tannock & Rotin, 1989). We showed that Na⁺/H⁺ exchange activity contributed substantially to recovery of pH, except when a high concentration of succinate was applied at low pH (6.1). Cells which were exposed to succinate or monomethyl succinate in the presence of the Na⁺/ H⁺ exchange inhibitor amiloride, or its more potent analogue EIPA had greater and more sustained acidification and greater cytotoxicity below pH 6.5; thus even low residual Na⁺/H⁺ exchange activity appear to protect cells against cytotoxicity due to cellular acidification. Similar results were observed previously when cells were exposed to the ionophores nigerin or CCCP at low extracellular pH (Newell & Tannock, 1989; Rotin et al., 1987).

The present experiments suggest that, at low pH, weak acids can cause lethal injury to cultured cells. In solid tumours, the mean value of pH is often low (Wike-Hooley et al., 1984; Vaupel et al., 1989). Values of pH are likely to be lowest in nutrient deprived (presumably hypoxic) regions of tumours because of production of lactic acid and hydrolysis of ATP, and insufficient clearance of metabolically produced acids. We have studied the in vivo effects of weak acids by using them alone, or with radiation to select a target population of hypoxic and poorly nourished cells. We have also employed hydralazine, which has been reported to decrease pH in tumours by inhibiting blood flow (Lin & Song, 1990), with maximum tolerable doses of succinate and EIPA, but found no toxicity following in vivo treatment with weak acids for any of these conditions. Failure to achieve toxicity in vivo is most likely due to failure to achieve an adequate concentration in plasma. The maximum concentration of succinate following a single injection of a maximally tolerated dose was approximately 1.5 mM which is below the toxic range. Since the buffering capacity of cells is high (20–30 mM/pH unit; Boyer & Tannock, 1992), use of weak acids which transport protons into cells together with their anions will probably require millimolar concentrations to achieve cellular acidification.

Although weak acids such as succinate, which are normal cellular metabolites, are unlikely to have therapeutic effects, the present work emphasises the potential for using the low pH in tumours to obtain selective toxicity. Toxicity might be induced by intracellular acidification, but the strategy of using ionophores, which remain in the cell membrane and which can acidify cells at micromolar concentrations, remains the more promising approach (Maidorn et al., 1993). The selective uptake of weak acids at low pH (Figure 4) has therapeutic potential if the acids carry cytotoxic moieties that act against intracellular targets. Increased activity at low pH has been reported for some conventional anticancer drugs that are inactive at neutral pH such as chlorambucil and mephalan (Jahde et al., 1989; Mikkelsen & Wallach, 1982), although the success of the approach will depend on the intrinsic toxicity of the drug that is concentrated in cells under acidic conditions. Some previous investigators have argued for the selective activity of cytotoxic agents that are weak bases, but since values of pH in tumours are normal whereas values of pH are often low, it is the specific design of cytotoxic weak acids which is likely to offer therapeutic advantage through the mechanism of selective uptake into tumour cells.

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