Different pH Dependency of Mitomycin C Activity in Monolayer and Three-Dimensional Cultures

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Purpose. Previous studies by other investigators have shown an enhancement of mitomycin C (MMC) activity at acidic extracellular pH (pH_e) in monolayer cultures of human cells. The goal of the present study was to determine if the efficacy of intravesical MMC therapy in patients treated for superficial bladder cancer can be enhanced by using acidified dosing solutions. We evaluated (a) the effect of pH_e on MMC activity in patient bladder tumors in vitro, and (b) the pH dependency of MMC activity in 2-dimensional monolayer and 3-dimensional multilayer cultures of human bladder RT4 tumor cells.
Methods. Patient bladder tumors were maintained as 3-dimensional histocultures. RT4 cells were harvested and maintained as monolayer cultures or as 3-dimensional cell pellets on a collagen gel matrix. The cell pellets were 300–450 cell layers and 4,000–5,000 μm in diameter. Tumors or cells were incubated for 2 hr with MMC-containing media at pH_e of 5, 6, and 7.4. The drug effect was measured by the inhibition of DNA precursor (thymidine) incorporation. The stability of MMC as a function of pH_e was determined. About 24% of MMC was degraded following 2 hr exposure at pH_e 5 and$\leq$ 2% at pH_e 6 and 7.4.
Results. The drug concentrations required to inhibit thymidine incorporation by 50% (IC_{50}) were corrected for the degraded MMC at acidic pH_e. The results showed no pH-dependent MMC activity in human patient bladder tumors nor in RT4 multilayer cultures; the IC_{50} values were about 10 μg/ml at all three pH_e. In contrast, the monolayer RT4 cultures showed a pH-dependent MMC cytotoxicity; the IC_{50} were 0.1, 0.8 and 1.2 μg/ml at pH_e 5, 6 and 7.4, respectively ($p < 0.05$). Pre-incubation of multilayered RT4 cultures in acidic pH medium for 8 hr enhanced the MMC activity; the IC_{50} was reduced by about 5 fold at pH_e 5 and about 3 fold at pH_e 6. Similar pH-dependent MMC activity was found when multilayers were pre-treated for 1 hr with 0.5 μg/ml nigericin, a proton ionophore known to cause the intracellular pH (pH_i) to equilibrate with pH_e.
Conclusions. These data suggest that the difference in the pH dependency of MMC activity in the monolayer and multilayer systems was due to the different experimental conditions. The time lag for pH_i to equilibrate with pH_e in the multilayer systems and the instability of MMC at low pH_e imply that the efficacy of intravesical MMC therapy is unlikely to be enhanced by using acidic dosing solution.

KEY WORDS: mitomycin C; pH effect; human bladder cancer.

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ABBREVIATIONS: MMC, mitomycin C; LI, labeling index; IC_{50}, drug concentration needed to produce a 50% inhibition of thymidine LI; pH_e, extracellular pH; pH_i, intracellular pH; CFA, colony forming assay; HPLC, high performance liquid chromatography; MEM, minimal essential medium; MES, morpholinoethane sulfonic acid; EDTA, ethylenediamino tetracetic acid.

INTRODUCTION

Mitomycin C (MMC) is a bioreductive alkylating agent and has shown activity against hypoxic cells (1). Previous studies by several groups of investigators demonstrate a 2 to 5 fold enhancement of MMC activity against monolayer cultures of human bladder RT-112, murine EMT6, human colon HCT-116 and leukemia LI210 cells when the extracellular pH (pH_e) decreased from 7.4 to 5 (2–5). In these systems, the intracellular pH (pH_i) was found to decrease in parallel with the pH_e (3,4). In contrast, no significant increase in MMC activity was found in human colon HT-29 and rat Walker RW-256 cells at pH_e of 6.6 and 5.0, respectively (6,7). The reasons for these contradicting results have not been elucidated. It has been proposed that the acidic microenvironment in solid tumors may be advantageous in achieving a selective MMC antitumor effect (2–4); the pH_e in most tumor tissues is on average 0.5 units lower than the pH_e in normal tissue (8).

Our laboratory has been investigating approaches to improve the treatment efficacy of bladder cancer by intravesical therapy. Our previous studies have shown that the variable and incomplete patient response to intravesical MMC therapy is in part due to the low chemosensitivity of some tumors and in part due to the inability to deliver sufficient drug concentrations to the tumor site (9). An ongoing clinical trial evaluates the merit of pharmacokinetic interventions that aim to increase the drug concentration at the target site (10). The present study was to investigate if the activity of MMC against bladder tumors can be enhanced by using acidic dosing solutions. The effect of pH on MMC activity in histocultures of surgical specimens of human bladder tumors, and in monolayer and multilayer cultures of human bladder cancer RT4 cells was investigated.

MATERIAL AND METHODS

Chemicals and Supplies

Sterile pigskin collagen (Spongostan Standard) was purchased from Health Designs Industries (Rochester, NY), [methyl-^{3}H]thymidine (specific activity 60 μCi/μmole) from ICN Biomedicals (Irvine CA), RT4 cells from American Type Culture Collection (Rockville, MD), tissue culture media from GIBCO Laboratories (Grand Island, NY), chamber slides from Nunc, Inc. (Naperville, IL), and nigericin and morpholinoethane sulfonic acid (MES) from Sigma Chemicals Co. (St. Louis, MO). MMC and porfiromycin were gifts from Bristol Myers Squibb Co. (Wallingford, CT) and American Cyanamid Co. (Pearl River, NY), respectively. All other regents were of HPLC grade and were used as received.

Equipment

The high performance liquid chromatography (HPLC) equipment consisted of an Applied Biosystems model 400 pump (Foster City, CA), a Hewlett-Packard model 3390 integrator (Palo Alto, CA), and a Waters 710B automated sampler and model 441 UV detector (Milford, MA). The Zeiss Axiovert 35 microscope (Thornwood, NY) was equipped with a 7×7 mm disc micrometer in the right eyepiece (Thomas Scientific, Swedesboro, NJ).

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In Vitro Stability and HPLC Analysis of MMC

Because of the instability of MMC at acidic pH (11), it was necessary to determine the change of drug concentration in media with time at pH_e 5, 6, and 7.4. Six ml of culture medium buffered to the desired pH, containing 15 μg/ml MMC, was incubated in a 6-well plate at 37°C in 5% CO_2 humidified air. Triplicate samples were used for each condition. Serial samples of 250 μl were taken over 24 hr. The pH of the culture medium was simultaneously measured using a pH electrode at each time point. Samples were stored at −70°C. MMC was analyzed by reversed-phase HPLC using a previously published assay (12). In brief, the stationary phase was a Perkin-Elmer Pecosphere C18 column (Norwalk, CT). The mobile phase was 13% acetonitrile in 2.5 mM phosphate buffer, pH 6.9. Fifty μl of the sample was mixed with 150 μl of 9.1 M phosphate buffer and 50 μl of the internal standard porfiromycin (0.75 mg/100 ml). MMC and porfiromycin in the eluate were monitored at 365 nm.

Human Bladder Tumor Histocultures

Six human bladder transitional cell carcinomas were obtained through the Tissue Procurement Service of The Ohio State University Comprehensive Cancer Center. Following surgical removal, the specimens were placed immediately in Hank's balanced salt solution, and prepared for culture within 2 to 4 hr postsurgery as described previously (13). Briefly, the non-necrotic portions of a tumor were cut into 1 mm^3 fragments. Four to five fragments were placed on a 1 cm^2 piece of collagen gel and incubated at 37°C. The maintenance medium consisted of minimal essential medium (MEM), pH 7.4, 10% fetal bovine serum, 1% non-essential amino acids, 100 μg/ml gentamicin and 95 μg/ml cefotaxime. The cultures were fed twice a week with fresh medium and were used four days after initiation.

Cell Culture

RT4 cells (passage 20 to 30) were grown in a T-150 flask to 90% confluency. The maintenance medium was similar to that for the histoculture, with the exception that McCoy medium was used instead of MEM. Cells were detached from the flask by washing twice with $Ca^{2+}-Mg^{2+}$ free Hank's balanced salt solution containing 0.016% of ethylene-diamino tetraacetic acid disodium salt (EDTA) and incubating for 3 to 5 min in 0.05% trypsin-EDTA. Thereafter, cell pellets were obtained by centrifugation at 1,000 × g for 10 min. For monolayer cultures, cells were resuspended in fresh medium. One hundred thousand cells were seeded onto each well of 8-well chamber slides pre-coated with 0.1% rat tail collagen solution and were grown for 24 hr. For multilayer cultures, 10–15 μl of cell pellets containing about 10^7 cells were pipetted onto a piece of collagen gel (1 cm^2) and were grown for 4–7 days prior to the experiment. The multilayer cell pellet consisted of 300 to 450 cell layers and was about 4,000 to 5,000 μm in diameter. Cells of the same passage were used to prepare both monolayer and multilayer cultures.

Preparation of Acidic Culture Media

For the experimentation, the maintenance media, i.e., MEM and McCoy's, were used to maintain pH at 7.4 for patient tumors and RT4 cells, respectively. For pH 5 and 6, MEM was buffered with 20 mM MES and 0.1M $NaHCO_3$ for both tumor histocultures and cells. To all media, 10% fetal bovine serum, 100 μg/ml gentamicin and 95 μg/ml cefotaxime were added. The pH of the MES-buffered MEM was adjusted by addition of 1 N HCL. Both neutral pH and acidic pH media were pre-incubated in humidified air with 5% CO_2 at 37°C overnight to achieve a stable pH_e prior to the experiment. The pH in the drug-free and MMC-containing media fluctuated within a range of 0.5 units throughout the 2-hr experiment.

Pharmacodynamic Studies

The chemosensitivity experiments were conducted at pH_e 5, 6, and 7.4. On the day of experimentation, the maintenance medium was removed and the cells or tumor histocultures were rinsed three times with the medium of desired pH_e to remove the residual maintenance medium. MMC was dissolved in buffers to give a final concentration of 0.01 to 100 μg/ml. The drug solutions were prepared freshly prior to the experiment to avoid degradation in the acidic medium. The experiment was initiated by adding 4 ml of drug-containing medium to the tumor histocultures and RT4 multilayers in 6-well plates and 0.3 ml to the monolayers in 8-well chambers. The MMC treatment was for 2 hr, which is equivalent to the duration of drug instillation during intravesical therapy in patients. After treatment, the histocultures and multilayer RT4 cultures were washed 3 times with 4 ml drug-free medium, and the monolayer cultures with 0.3 ml medium. The histocultures were incubated with [^{3}H]thymidine at a concentration of 2 μCi/ml for 4 days, whereas RT4 monolayers were incubated for 1 day and multilayers for 3 days with 1 μCi/ml. These thymidine concentration and exposure intervals were selected to ascertain maximal [^{3}H]thymidine incorporation. Thereafter, histocultures and cells were fixed, stained and processed for autoradiography. The labeling index (LI) was determined microscopically, and was defined as the number of labeled nuclei divided by the total number of nuclei within a grid at 400× magnification. For RT4 monolayers, a random field containing about 300 to 400 cells was selected for evaluation because a pilot study indicated no significant differences in labeling index between different areas of the monolayers. For RT4 multilayers and human tumor histocultures, the LI was evaluated in the outermost 3–5 cell layers and/or the most actively labeled area, respectively, to standardize the comparison.

The effect of nigericin, a proton ionophore known to cause the pH_i to equilibrate with the pH_e (14), on MMC activity was determined. RT4 multilayer cultures were pre-treated with 0.5 μg/ml nigericin for 1 hr followed by a 2-hr exposure to 5 μg/ml MMC. Drug-containing medium was removed at the end of the 2-hr MMC exposure.

Data Analysis

The concentration-effect relationship was analyzed by computer-fitting the experimental data to equation 1 (15).

$$E = E_0\left(1 - \frac{C^n}{K^n + C^n}\right) \quad (1)$$

where E is the LI, C is the drug concentration, E_0 is the baseline effect in the absence of drug, K is the drug concentration at

one-half E_0 and n is a curve shape parameter. The plot of LI, expressed as a percent of LI of untreated control, versus MMC concentration was analyzed using nonlinear least squares regression (SAS Institute Inc., Cary, NC) to obtain the MMC concentration needed to produce a 50% inhibition (IC_{50}).

The degradation rate constants of MMC at different pH_e's were calculated by Equation 2

$$Fraction\ remaining = e^{-k_d t} \quad (2)$$

where k_d is the degradation rate constant and t is the elapsed time. As shown in Figure 1, a significant fraction of MMC was degraded at pH_e 5 after 2 hr but not in pH_e 6 and 7.4 after 24 hr. Hence the IC_{50} at pH_e 5 was corrected for the degradation over 2 hr using equation 3 where IC^*_{50} is the IC_{50} determined using the initial concentration.

$$IC_{corrected} = \frac{IC^*_{50}\left(1 - e^{-2k_d}\right)}{2k_d} \quad (3)$$

Statistical Analysis

For RT4 cells, which came from a normal population with equal variances, the differences between groups were determined by one-way analysis of variance (ANOVA) and post-hoc Student's t-tests. Non-parametric randomized block ANOVA was used for histocultures because variation in IC_{50} values among patients may be in part related to pH effect.

RESULTS

In Vitro Stability of MMC

The decrease in MMC concentration in culture media, as a function of time, was pH-dependent. As shown in Figure 1, MMC concentrations decreased log-linearly with half-lives of 196, 56 and 5.1 hr and with corresponding degradation rate constants of 0.00354, 0.0124 and 0.136 hrs^{-1}, at pH_e 7.4, 6 and 5, respectively. The fraction lost in 2 hr was calculated to be insignificant at pH_e 6 and 7.4 at ≤ 2% and was significant at pH_e 5 at 24%.

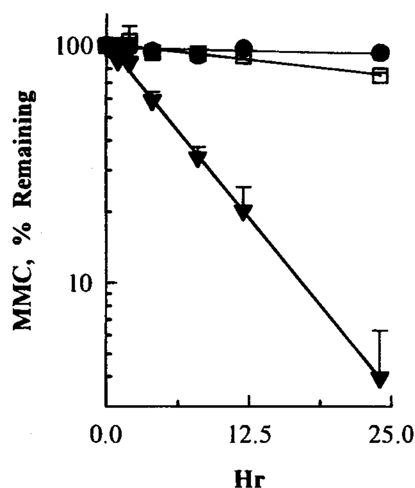

Fig. 1. Degradation of MMC in culture medium at pH_e 5(▼), pH_e 6 (□) and pH_e 7.4(●). Mean + SD (n = 3). For pH_e 6 and 7.4, the SD were smaller than the symbols. The starting concentration was 15 μg/ml MMC.

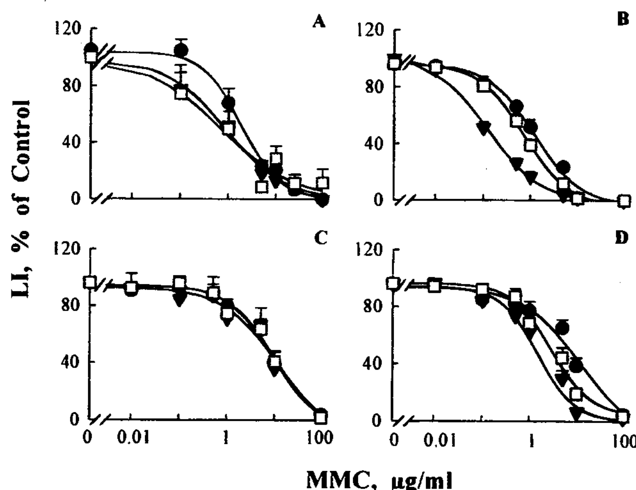

Fig. 2. Effect of pH_e on MMC activity in histocultures of a representative patient bladder tumor (A), RT4 monolayers (B), multilayers without pre-incubation (C) and with 8-hr pre-incubation (D) at pH_e 5 (▼), 6 (□) and 7.4 (●). Mean + SEM of a representative experiment (RT4 studies were performed in triplicate. For the histoculture study, LI was determined in 4 to 12 tumor fragments per data point).

Effect of pH_e on MMC Activity Against Human Bladder Tumor Histocultures

The LI of untreated human tumors following a 2 hr incubation at pH_e 5, 6, and 7.4 were 46.2 ± 10.1%, 43.8 ± 8.9% and 40.9 ± 8.5% (Mean ± SD, n = 6 each), respectively. The differences were not statistically significant, suggesting that the 2 hr exposure at acidic pH_e had no adverse effects on cell viability and proliferation. MMC produced a sigmoidal concentration-response relationship. Figure 2A shows the results of a representative experiment. Table 1 shows the mean results of

Table I. Effect of pH_e on MMC Activity in Human Bladder Tumor Histocultures and RT4 Monolayer and Multilayer Cultures[a] (Mean ± SD)

	IC_{50} (μg/ml)		
pH_e	Human Bladder Tumor Histoculture (n = 6)	RT4 Multilayers (n = 3)	RT4 Monolayers (n = 3)
5	10.2 ± 18.0[b,c]	8.9 ± 1.0[b,c]	0.1 ± 0.02[b,d]
6	7.6 ± 13.3[c]	9.3 ± 1.6[c]	0.8 ± 0.04[d]
7.4	13.8 ± 18.9	11.7 ± 1.6	1.2 ± 0.03

Note: Statistical analysis were done using non-parametric randomized block ANOVA for histocultures, and one-way ANOVA and post-hoc Student's t-test for RT4 monolayer and multilayer cultures.
[a] Cells and tumors were treated with MMC for 2 hr at the desired pH_e.
[b] Corrected for 24% degradation over 2 hr using Equation 3.
[c] Not different between pH effects and IC_{50} values, p > 0.5.
[d] Different from pH 7.4, p < 0.05.

6 experiments. A comparison of the IC_{50} of MMC at the three pH_e's did not show a significant pH-dependent drug effect ($p > 0.5$).

Comparison of MMC Activity in Monolayer and Multilayer RT4 cells

The LI of untreated controls were about 80% in monolayers and about 75% in multilayers at all 3 pH_e's. The unchanged LI indicate that the 2 hr exposure at acidic pH_e had no adverse effect on cell proliferation. MMC also produced a sigmoidal concentration-response relationship in RT4 monolayers (Figure 2B) and in RT4 multilayers (Figure 2C). The IC_{50} values at the 3 pH_e are summarized in Table 1. These data show a significant pH-dependent MMC activity in RT4 monolayers, with a 1.6- and 10-fold enhancement when pH_e decreased from 7.4 to 6 and 5 ($p < 0.05$ in both cases). In contrast, decreasing the pH_e had no effect on MMC activity in RT4 multilayers; the IC_{50} was about 10 μg/ml at all three pH_e's. The lack of pH dependency in the multilayers is similar to the observations in human bladder tumor histocultures (Table 1). At pH_e 7.4, the IC_{50} of MMC in RT4 monolayers was 10-fold lower than those in RT4 multilayers and human bladder tumor histocultures, indicating that the bladder tumor cells were more sensitive to MMC when cultured as monolayers compared to being cultured in 3-dimensional systems. This difference was more pronounced at low pH_e, as indicated by the 100-fold lower IC_{50} value at pH_e 5 in the monolayers compar to in the 3-dimensional systems (Table 1).

Effects of Pre-incubation and Nigericin on MMC Activity

Pre-incubation of RT4 multilayers for 8 hr at low pH_e produced a pH-dependent enhancement of MMC activity (Figure 2D). The IC_{50} was lowered by about 5-fold to 1.7 ± 0.3 μg/ml at pH_e 5 and by about 3-fold to 2.8 ± 0.2 μg/ml at pH_e 6, whereas the IC_{50} at pH_e 7.4 remained unchanged at about 10 μg/ml ($n = 3$). The pH-dependent enhancement of MMC sensitivity after pre-incubation was unlikely to be the result of acidic pH_e on cell viability and proliferation because a separate study showed no significant differences in the LI of the untreated controls at the three pH_e values (about 70%).

Pre-exposure of RT4 multilayers for 1 hr to 0.5 μg/ml nigericin also resulted in pH-dependent enhancement of MMC activity, similar to that observed after 8-hr incubation in acidic pH_e medium (Table 2).

Table II. Effect of Preincubation in Acidic pH_e and Pretreatment with Nigericin on pH-Dependent MMC Cytotoxicity in RT4 Multilayers[a] (Mean ± SD, n = 3 each)

	LI, % of Control		
Condition	pH_e 5	pH_e 6	pH_e 7.4
No pre-incubation	64.4 ± 2.1%	63.4 ± 2.3%	64.0 ± 2.4%
8-hr pre-incubation	28.6 ± 7.5%[b]	45.2 ± 1.9%[b]	64.9 ± 1.8%
Nigericin	20.7 ± 6.0%[b]	39.1 ± 3.3%[b]	60.1 ± 4.1%

[a] RT4 multilayers were pre-incubated with medium at desired pH_e for 8 hr or with 0.5 μg/ml nigericin for 1 hr, and then treated with 5 μg/ml MMC for 2 hr. The LI of untreated control was 70% and was unaffected by pre-incubation. The reduction in thymidine LI, as a measure of MMC effect, was expressed as % of control.

[b] Different from no pre-incubation, $p < 0.05$.

DISCUSSION

The goal of this study was to determine if the therapeutic efficacy of intravesical MMC in patients can be enhanced by using acidic instillation solutions. Previous studies (2–7), as summarized in Table 3, have shown contradictory results on the pH dependency of MMC activity; some investigators demonstrated a significant enhancement of MMC activity at acidic pH_e, whereas others did not find a significant difference. These studies were performed using monolayer cultures of tumor cell lines, and the drug effect was measured by the colony forming assay or as a reduction of cellular ATP content. The reasons for the contradictory results in the literature are not apparent, possibly due to the different susceptibility among different cell lines to the pH_e effect. The present study was designed to compare the pH-dependent MMC activity in different experimental systems, i.e., 2-dimensional monolayers versus 3-dimensional multilayers. Our data show a lack of enhancement in the activity of MMC at reduced pH_e in patient bladder tumor histocultures and RT4 multilayers, whereas RT4 monolayers were 10- to 100-fold more susceptible to the pH_e effect (Table 1). These findings indicate that the pH-dependent enhancement of MMC activity observed in monolayers was not operative in 3-dimensional culture systems.

A possible mechanism of differential sensitivity to the pH_e effect between the monolayer and 3-dimensional multilayer cultures is a difference in the rate of equilibration between pH_i and pH_e in these two systems. The pH_i in monolayers might have equilibrated with the acidic pH_e within the 2 hr treatment, whereas the pH_i in multilayers did not. This hypothesis is supported by the observations of enhanced MMC activity in RT4 multilayers after 8-hr pre-incubation in acidic media, and after pre-treatment by a proton ionophore nigericin.

It is of interest to note that at pH_e of 5 and 6, the IC_{50} in RT4 monolayers were about 17- and 4-fold, respectively, lower than IC_{50} in RT4 multilayers even after pre-incubating in the multilayers for 8 hr. It is unlikely that the lower sensitivity in the RT4 multilayers was due to a difference in drug penetration because we restricted the area for drug activity evaluation to the outermost 3–5 cell layers in the multilayer cultures. This area was readily labeled by [^{3}H]thymidine, a molecule of comparable size to MMC. A possible mechanism may be a lessened chemosensitivity of cells in multilayered systems compared to cells in monolayer cultures, presumably due to 3-dimensional cell-to-cell interaction, as has been shown by others (16,17). An alternative mechanism may be a difference in the activity of its activating enzymes. MMC is activated by several intracellular quinone reductases. The two major reductases are (a) DT diaphorase which catalyzes the two-electron reduction of MMC to hydroquinone and is the major activating enzyme under aerobic condition, and (b) NADPH cytochrome P450 reductase which catalyzes the one-electron reduction to form the semi-quinone radical and is the major activating enzyme under anaerobic conditions (18). The DT diaphorase activity is enhanced under acidic conditions (19,20), and this pH-dependent effect is more pronounced under aerobic than anaerobic conditions (5,21,22). It is conceivable that a difference in the oxygenation status in the monolayer and multilayer systems might have resulted in a different extent of MMC activation with a higher activation in the better oxygenated monolayers. Further studies are needed

Table III. Comparison of pH-Dependent MMC Activity in Monolayer and 3-Dimensional Cultured Systems

Tumor Cell	Tissue Origin	System	MMC Exposure (hr)	Activity Measurement	MMC Activity		Enhancement Ratio	References
					pH_e 7.4	Reduced pH_e		
Patient tumors	Bladder	3-dimensional	2	Thymidine labeling (IC_{50}, μg/ml)	13.80	11.70 (pH_e 5.0)	1.2	Present study
RT-4	Bladder	3-dimensional	2	Thymidine labeling (IC_{50}, μg/ml)	11.70	10.20 (pH_e 5.0)	1.0	Present study
RT-4	Bladder	Monolayer	2	Thymidine labeling (IC_{50}, μg/ml)	1.20	0.10 (pH_e 5.0)	12.0	Present study
RT-112	Bladder	Monolayer	1	CFA[a] (% reduction)	42.80	8.80 (pH_e 5.2)	4.9	2
EMT6	Mammary	Monolayer	2	CFA[a] (IC_{50}, μg/ml)	0.10	0.02[b] (pH_e 5.7)	5.0	3
HCT-116	Colon	Monolayer	1	CFA[a] (IC_{50}, μg/ml)	0.10	0.03 (pH_e 6.0)	3.3	4
LI210	Leukemia	Monolayer	4	CFA[a] (% reduction)	NA[c]	NA[c] (pH_e 6.0)	2.3	5
RW-256	Carcinosarcoma	Monolayer	24	Cellular ATP (Cell number*10^6/ml)	0.22	0.17 (pH_e 5.0)	1.3	6
HT-29	Colon	Monolayer	1	CFA[a] (% reduction)	NA[c]	NA[c] (pH_e 6.6)	1.2	7

Note: Experiments were performed under aerobic conditions. The enhancement ratio is defined as (cytotoxicity at pH_e 7.4) divided by (cytotoxicity at reduced pH_e).
[a] Colony forming assay.
[b] Estimated from the reported data.
[c] Not applicable. Only the enhancement ratio was reported.

to elucidate the mechanism(s) of the lower sensitivity of the multilayered cultures to MMC and to the effect of acidic pH_e.

In conclusion, data of the present study indicate that the pH_e effect on MMC activity was dependent on the experimental conditions, and that cells in monolayer cultures are significantly more sensitive than cells in multilayer systems at acidic and neutral pH_e. Because a prolonged exposure to acidic pH_e was necessary to enhance the MMC activity in multicellular systems, the pH-dependent MMC activity is likely to be important in solid tumors only when the acidic pH_e can be maintained for a long period, e.g., 8 hr. In well-perfused tumors where the pH_e is regulated by the perfusing blood, the pH-dependent MMC activity is less likely to have an important role. The non-enzymatic degradation of MMC at pH 5, as shown in the present and previous studies (15), further diminishes the value of using acidic MMC dosing solution for intravesical therapy of superficial bladder cancer.

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