Electromotive versus Passive Diffusion of Mitomycin C into Human Bladder Wall: Concentration-Depth Profiles Studies

Savino M. Di Stasi, Antonella Giannantoni, Renato Massoud, Susanna Dolci, Pierluigi Navarra, Giuseppe Vespasiani, and Robert L. Stephen

ABSTRACT

The objectives of these investigations were: (a) to make a preliminary study to assess concentration-depth profiles of mitomycin C (MMC) in the bladder wall at specified time intervals after passive diffusion (PD); and (b) to conduct a major study to compare concentration-depth profiles after PD and electromotive drug administration (EMDA) of MMC. Full thickness sections of viable human bladder wall were placed in two-chamber cells with urothelium exposed to donor compartments containing 40 mg of MMC in 100 ml of 0.96% NaCl solutions and with serosa-facing receptor compartments containing 0.9% NaCl solutions. In the preliminary study during each of nine experimental sessions, five sections of bladder wall were individually exposed to MMC for either 5, 15, 30, 45, or 60 min. In the major study, an anode and a cathode were sited in the donor and receptor compartments, and 14 paired experiments—current (20 mA)/no current—were conducted over a 30-min period. Bladder wall sections were cut serially into 40-μm slices parallel to the urothelium and analyzed by high-performance liquid chromatography for MMC concentrations (μg/g wet tissue weight). Tissue viability and morphology and MMC stability were assessed by trypan-blue exclusion test, histological examination, and mass spectrometry analysis. In the preliminary study (PD only), mean MMC concentrations (μg) at 5, 15, 30, 45, and 60 min were: (a) for urothelium, 15.3, 60.0, 58.2, 60.1, and 57.8, respectively; (b) for lamina propria, 2.2, 18.9, 19.3, 16.1, and 17.3, respectively; and (c) for muscularis, 0.4, 2.0, 1.8, 1.3, and 2.4, respectively. In the comparative study, MMC concentrations and coefficients of variation (CV) were as follows: (a) for urothelium after PD, 46.6 with CV = 69.5%, and after EMDA, 170.0 with CV = 43.6% (P < 0.0001); (b) for lamina propria after PD, 16.1, with CV = 60.6%, and after EMDA, 65.6 with CV = 29.3% (P < 0.0001); and (c) for muscularis after PD, 1.9 with CV = 82.8%, and after EMDA, 15.9 with CV = 82.2% (P < 0.0005). All of the bladder sections remained viable, and the chemical structure of MMC was unchanged. It was concluded that EMDA significantly enhances MMC transport into all of the layers of the bladder wall, and sections of viable human bladder are a reliable tool for assessing different modes of drug delivery.

INTRODUCTION

Bladder cancer accounts for approximately 261,000 new diagnoses annually and about 115,000 cancer deaths worldwide (1). Superficial transitional cell carcinomas, the most frequently diagnosed bladder tumors, are either confined to the mucosa (stage Ta), or invading the lamina propria (stage T1) or appear as carcinoma in situ (stage Tis) involving areas of the urothelium (2). TUR is the standard initial diagnostic and therapeutic step with recurrence rates (after TUR alone) reported as varying from 30% to over 90% of cases and with stage progression as varying from 5 to the 30% (3). The likelihood of recurrence and progression has led to widespread use of intravesical instillation of cytotoxic agents (4); their diffusion into the bladder wall provides optimal contact between the tumor or tissue at risk and drug while little systemic uptake occurs.

Adjuvant intravesical chemotherapy significantly reduces tumor recurrence, but the reported benefits are highly variable, ranging from −3 to 41% for thiotepa, 1 to 43% for MMC, and 5 to 39% for doxorubicin; superficial tumors seem to respond more favorably than invasive tumors (5). The following other studies have assessed conditions for optimal MMC intravesical therapy: (a) pharmacokinetics in superficial-bladder-cancer patients (6); (b) MMC penetration into the bladder wall of dogs (7); (c) MMC penetration into the bladder wall of patients undergoing radical cystectomy (8); and (d) MMC pharmacodynamics in cultured human bladder tumors (9). It was suggested that the variable response to intravesical MMC may be due: (a) to the large inter- and intra-subject variability of drug diffusion into tumor sites; (b) to inadequate drug concentrations in deep tissues; and (c) to the variable sensitivity of patients’ tumors to MMC. But unfortunately, much of the reported variability originates from large differences in the drug solutions used (primarily their osmolalities) and from the lack of standardization in the physiological preparation of experimental subjects—two factors that have profound effects on PD of drugs into the bladder wall.

Terminology involving EMDA of solutes is yet to be finalized. Iontophoresis describes the accelerated transport of ions (into tissues) by means of an electric current passed through a solution containing the ions to be administered at a rate defined by Faraday’s Law:

\[ J_i = I(\text{tr})/z F \text{ mol/s} \]  

where \( I \) is the current (amperes), \( tr \) the proportion of applied current carried by i, and z the valency; \( F \) is Faraday’s constant (11). Usually iontophoresis is associated with increased transport of water that will entrain any nonionized solutes present—a phenomenon often termed electroosmosis, a form of “solvent drag” (11). Drug transport rate (\( dD/dt \)) is the algebraic sum of that induced by PD and by EMDA (\( dD/dt = PD + EMDA \)), but, when dealing with a membrane of low permeability such as the urothelium, EMDA is so dominant that, for all practical purposes, it may be considered as the sole force manipulating drug transport. Thus, administration rates are not only markedly increased but they are controllable simply by varying the current intensity (\( dD/dt = K \cdot I \))

MMC is almost totally nonionized within the full pH range tolerated by the bladder (4.5–8.5 units); therefore, realistic clinical or laboratory investigations involve electroosmosis only. The requisite ions for this mode of EMDA come as a sodium chloride excipient in the MMC crystals supplied. When current of positive polarity is applied to a solution containing Na\(^+\)/CT/MMC, sodium ions are iontophoresed into underlying tissues, a process that includes transport of water in the form of hydration shells around Na\(^+\) (12) as well as in the free form (11). Solubilized, nonionized MMC is entrained in this water flux in quantities proportional to its concentration in solution. Appendix A supplies a derivation for iontophoretic transport rates of Na\(^+\) and leads into Appendix B, which derives electroosmotic

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transport rates of MMC within Na⁺ hydration shells. Although the calculations for MMC underestimate delivery rates many-fold (free water flux is ignored), electroosmosis is obviously less efficient than iontophoresis, but it has been applied successfully in laboratory (13) and clinical (14, 15) studies.

Recently, we reported that EMDA reduces the variability and enhances the administration rates of MMC into viable human bladder wall (13). The present laboratory investigations were designed to compare concentrations of MMC as a function of tissue depth in the bladder wall after PD and EMDA and were conducted in two phases: (a) MMC concentrations during PD measured at different time points up to 1 h; and (b) MMC concentrations after PD and EMDA after a 30-min exposure time.

MATERIALS AND METHODS

Chemical. MMC in 10-mg vials and porfiromycin (internal standard) were supplied by Kyowa Italiana Farmaceutici srl (Milan, Italy). HPLC analyses showed that MMC and porfiromycin were >99% pure. HPLC solvents and reagents were obtained from Sigma-Aldrich srl (Milan, Italy) and were of analytical grade. Frozen tissues were glued onto the cryotome object holder with Cryomatix compound (Shandon Ltd., Astmoor, United Kingdom).

Apparatus. A two-chamber polyvinylchloride diffusion cell was used for MMC delivery to bladder wall tissue samples (16). The current generator and silver spiral electrodes were supplied by Physion srl (Medolla, MO, Italy) and were of analytical grade. For all experiments, the pH values of MMC solutions were measured using a model 1001 pH meter (Sentron, Roden, the Netherlands). Frozen tissues were sectioned by cryotome (Jung 3000 CM, Leica, Buffalo, NY). Tissue weights were determined with a digital balance (Sartorius, Roden, the Netherlands). Frozen tissues were sectioned by cryotome (Jung 3000 CM, Leica, Buffalo, NY). Tissue weights were determined with a digital balance (Sartorius, Roden, the Netherlands). Frozen tissues were sectioned by cryotome (Jung 3000 CM, Leica, Buffalo, NY). Tissue weights were determined with a digital balance (Sartorius, Roden, the Netherlands).

Harvesting of Tissue Samples. From informed patients undergoing radical cystectomy for bladder cancer, full-thickness tissue samples of 1 cm in diameter were cut by a cylinder blade. No patient had been administered prior radiotherapy or chemotherapy. The surgical technique was modified to maintain blood supply for as long as possible (8), and, after the bladder was removed, tissue specimens were excised within 5 min from visually cancer-free bladder wall that had not been scarred by previous TUR. Perivesical fat was removed, and the serosal surface was exposed. The samples were placed in a cell culture medium (1× DMEM, HyClone Europe Ltd., Cramlington, United Kingdom) at 4°C and transported to the laboratory in an average time of 10 min.

Preliminary Depth- and Time-Profiles of MMC across Bladder Wall. We performed pilot investigations to determine the concentration-depth profiles of passive MMC administration with respect to time. During each experimental session, five tissue samples from a single patient were placed between two chambers of individual diffusion cells with a mirror-lake central window. MMC (200 mg) and 4800 mg of NaCl were dissolved in 500 ml of bidistilled H₂O. The pH was measured, and then the solution was divided into five volumes of 100 ml each, which were placed in the donor compartments. The receptor compartments were filled with 100 ml NaCl solution 0.9%. The dwell times of tissue in the five drug solutions were 5, 15, 30, 45, and 60 min. The experimental sessions were performed in times up to 70 min; therefore, the total time between the removal of the bladder and the completion of the experiments did not exceed 85 min.

Comparison of EMDA and PD Depth-Profiles of MMC across Bladder Wall. During each paired experiment, two tissue samples from a single patient were placed between the two chambers of individual diffusion cells. MMC (80 mg) and 1920 mg of NaCl were dissolved in 200 ml of bidistilled H₂O, the pH was measured, and then the solution was divided into two volumes of 100 ml each (40 mg of MMC in NaCl 0.96% solution), which were placed in paired PD and EMDA donor compartments. The receptor compartments were filled with 100 ml NaCl solution 0.9%. In EMDA experiments an anode was placed in the donor compartment and a cathode in the receptor compartment. The electrodes were connected to the current generator and experiments were performed with pulsed direct current of 20 mA at 2500 Hz for a duration of 30 min. No electric current was applied in PD control experiments. All of the experiments were performed in an average time of 35 min; therefore, the total time between removal of the bladder and the completion of the experiment was about 60 min.

In all of the studies and after each experiment, the pH of the donor compartments was measured, 1-ml samples of all of the donor and receptor compartment solutions were withdrawn, and MMC concentrations were determined. At the end of each MMC administration, the tissue samples were blotted with absorbent paper to remove unabsorbed drug solution, and the tissue segments exposed to MMC were cut out as circles of 0.9 mm diameter by a cylinder blade.

Cryotome Sectioning of Tissue. Each tissue segment was rapidly weighed and frozen by placement of the urothelial side on a flat stainless-steel plate cooled on liquid nitrogen; care was taken to avoid distending the tissues during the freezing process. The time interval between the completion of the experiment and the freezing of the tissue was less than 5 min. The frozen tissue was glued onto the cryotome object holder with cryoadhesive material so that the outer surface of the serosa was anchored on the cryotome object holder, and the urothelial surface was exposed for sectioning. The first 80 µm of tissue directly in contact with the MMC solution was trimmed off to avoid contamination by the dosing solution. Tissue was sectioned into 40-µm thicknesses, placed in preweighed nalgene microcentrifuge tubes, and stored frozen. For tissues between 80 and 200 µm in depth (urothelium), each 40-µm section was analyzed individually. For tissue between 200 and 1200 µm in depth (lamina propria) and beyond 1200 µm in depth (muscularis), five and ten segments, respectively, were pooled for analysis. In a normal tissue sample harvested from the same bladder, transverse frozen tissue sections were taken and stained with H&E before sectioning, to estimate the actual thickness of the bladder wall and its histological characteristics and to correlate the histological tissue type with depth.

Measurements of MMC in Tissues. MMC concentrations were standardized to wet tissue weights because this method measured both unbound and reversibly bound MMC distributed in extra- and intracellular compartments (13, 17). To the weighed tissue samples collected in nalgene microcentrifuge tubes was added a solution containing 5.0 mM phosphate buffer (pH 7.0), 10 mM 2,3-dihydroxybenzylamine, and 10 mM 2-tert-butyl-4-methylphenol in a ratio of 1:3 (v/v). The samples were homogenized at room temperature for 1 min and centrifuged at 10,000 × g for 20 min. To the resulting supernatant was added 20 µl of porfiromycin (0.5 µM) as internal standard. MMC was extracted once with ethyl acetate in a ratio of 1:2 (v/v) with mild shaking for 10 min. After centrifugation at 500 × g for 5 min, the samples were dried under N₂. The samples were reconstituted in 200 µl of HPLC mobile phase [12.5% acetonitrile and 87.5% of 5 mM phosphate buffer (pH 6.9)], and 50 µl were injected. HPLC was performed using a reversed-phase C18 column (Pecosphere, 5 µm, 83 × 4.6 mm, Perkin-Helmer Labs, Norwalk, CT). The solvent flow rate was 1 ml/min, and UV detection was at 365 nm. The elution volumes for MMC and porfiromycin were 75 and 11.7 ml, respectively. The limit of detection was 10 ng/ml, and all of the analyses were performed in duplicate.

Histological Analyses. Before (untreated samples) and after each EMDA/PD experiment, tissue sections of approximately 0.5 cm², fixed in buffered formalin (4% formaldehyde in 152 mM phosphate buffer [pH 7.3]) for 3 h, were paraffin-embedded and oriented for cross-section through the middle of the samples. The microscopic sections, stained with H&E, were examined in random sequence, and the reviewer was blinded as to the group. To avoid bias during the examination of the wall thickness, each microscopic section was arbitrarily divided into approximately equal quadrants by lines intersecting at 90°. Micrometer measurements were made wherever a line crossed the tissue sample, to a total of eight examinations per sample. At a magnification of ×100, all of the tissue layers (urothelium, lamina propria, and muscularis) were inspected for cytological changes.

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Trypan Blue Exclusion Test. Before and after each preliminary PD experimental session and each EMDA/PD experiment, normal tissue sections from the same bladders were excised and incubated in 0.04% trypan blue in nMEM for 1 h at room temperature in a rotation chamber. Control samples were fixed in buffered formalin for 2 h and then stained by trypan blue 0.04%. After incubation, tissues were washed in 152 mM phosphate buffer (pH 7.3), placed in Cryomatrix compound with 10% polyvinyl alcohol and 4% polyethylene glycol, and frozen in liquid nitrogen; 30-μm sections were cut with a cryostat, mounted in 40% glycerol in 152 mM phosphate buffer (pH 7.3), and stained by trypan blue 0.04%. Observations were with a multiple 40× objective. Labeled cells within the tissue were considered nonviable whereas unlabeled cells were regarded as viable.

Voltage Measurement. During each of the EMDA experiments, the voltage (V) across the entire circuit (leads, electrodes, MMC solution, tissue sample, and receptor compartment solution) was measured.

Mass Spectrometry Analysis. The samples of MMC obtained before and after EMDA were examined by positive-ion Fast Atom Bombardment mass spectrometry in m-nitrobenzyl alcohol. Observations were with a quadrupole instrument (VG Quattro, Fisons Inc, Manchester, United Kingdom) scanning over m/z 160 to 560 at 0.66 s/decade, resolution power 1000 (10% valley definition). Sample periods were bombarded with 10 kV C s beam from a cesium gun anode. Repetitive-scan spectra were recorded by a VG LAB-BASE data system, mass calibrated with the cluster ion spectrum of a 1:1 molar mixture of cesium iodide and sodium iodide between m/z 23 and 3500.

Data Analysis. Descriptive statistics, expressed both as individual values and as means ± SE and as CV were supplied for tissue weights (g), tissue depth (μm), MMC per wet tissue unit (μg/g), initial and final pH values in the donor compartments, and voltage (V) across the circuit. Comparisons between paired experimental groups were performed by Wilcoxon’s matched-pairs test. Differences were taken as significant if P < 0.05.

RESULTS

PD Tissue Concentration-Time Profiles. Nine experimental sessions were performed, and 45 tissue sections were analyzed. The mean tissue weight was 0.608 ± 0.012 g. The mean tissue weights of the urothelium, lamina propria, and muscularis were 0.018 ± 0.001, 0.132 ± 0.003, and 0.457 ± 0.009 g, respectively. The mean thickness of bladder wall sections was 8138 ± 245 μm. The mean thicknesses of the urothelium, lamina propria, and muscularis were 191 ± 4.8, 1186 ± 55.2, and 6761 ± 246.5 μm, respectively.

Mean bladder wall MMC concentrations (μg/g wet tissue weight) as a function of time are shown in Fig. 1. MMC concentrations in the 80–200, 200–1200, and 1200–4000-μm compartments are reported in Table 1. Comparisons among the group means of the three compartments show that MMC concentrations in the 80–200-μm compartment are at all time points significantly higher than those in both the 200–1200 and 1200–4000-μm compartments (P < 0.01 and P < 0.001, respectively). Likewise, concentrations in the 200–1200-μm compartments are always significantly higher (P < 0.01) than those in the 1200–4000-μm compartments.

MMC concentrations increased in all of the compartments during the first 15 min, with the concentrations at 5 min significantly lower than those found after 15 min at all of the tissue depths. After 15 min, a plateau was reached in all of the compartments, and MMC concentrations did not undergo any significant changes up to 60 min of incubation.

EMDA versus PD Tissue Concentration-Depth Profiles. Fourteen paired experiments were performed, and 28 tissue sections were analyzed. The results are shown in Table 2. The mean thickness of bladder wall sections was 7957 ± 84.3 μm. The mean thickness of the urothelium, lamina propria, and muscularis were 195 ± 2.4, 1193 ± 26.9, and 6571 ± 284.6 μm, respectively. The mean concentration of MMC (Cmean) transported into the bladder wall by EMDA (28.23 ± 4.0 μg/g) significantly exceeded the concentration achieved by PD (5.33 ± 0.76 μg/g), which is mirrored by the respective concentrations observed in the urothelium or C80–200 μm (170.03 ± 19.59 versus 46.57 ± 8.58 μg/g), lamina propria or C200–1200 μm (65.62 ± 5.10 versus 16.11 ± 2.58 μg/g), and superficial muscle layers or C1200–4000 μm (15.88 ± 3.47 versus 1.86 ± 0.41 μg/g). The comparison of MMC tissue distribution in bladder wall after 30 min of EMDA and PD are shown in Fig. 2. The variability of MMC delivery (CV) was markedly reduced with EMDA as compared with PD in all except the superficial muscle layer.

MMC Concentrations and pH in Cell Compartments. Measurements of MMC in the donor compartments showed no significant differences and were not sufficiently sensitive to permit mass balance studies. No MMC was detected in the receptor compartments. The

Table 1. Comparisons among the group means of the three compartments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>C80–200 μm</th>
<th>C200–1200 μm</th>
<th>C1200–4000 μm</th>
<th>Cmean ± SE</th>
<th>C80–200 μm</th>
<th>C200–1200 μm</th>
<th>C1200–4000 μm</th>
<th>Cmean ± SE</th>
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<tr>
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<td>4.11</td>
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<td>0.38</td>
<td>13.37</td>
<td>7.01</td>
<td>0.58</td>
<td>2.00</td>
</tr>
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</table>

Min.

- Fig. 1. Time course of MMC concentrations in total tissues (80–4000 μm in depth).
- Data are expressed as μg of MMC/g of wet tissue, the means ± 1 SE of nine experiments per group. *P < 0.05 versus MMC concentrations at 15 min.
initial mean pH value (6.45 ± 0.13 units) of MMC solution in all of the donor compartments did not differ significantly from the final value (mean 6.42 ± 0.17 units). As reported by others (18), MMC is stable within this pH range; therefore, nonenzymatic degradation in drug solution was not a significant factor in this study.

**Trypan Blue Exclusion Test.** All of the tissue sections that were processed demonstrated negative staining of the cells within urothelium, lamina propria, and muscularis, which indicated viability of tissues throughout the time of the experiments. All of the cell layers of the control sections that were fixed in buffered formalin and then incubated in trypan blue were intensely stained by the dye.

**Histological Findings.** All of the tissue samples examined were morphologically coherent, with orderly urothelial cells, subepithelial connective tissue, and muscle layers. There was no histological evidence of mucosal or bladder wall abnormality.

**Voltage Measurements.** During EMDA experiments the potential difference across the complete circuit was measured at 6.4 ± 0.6 V (range, 5.7–7.1 V).

**Mass Spectrometry Data.** Mass spectral analysis of MMC samples showed no chemical modification after EMDA.

**DISCUSSION**

The present laboratory investigations were designed to compare concentrations of MMC as a function of depth in viable human bladder wall after administration by PD and electroosmosis. It was deemed advisable to conduct preliminary studies of passive MMC transport as a function of both depth and times of administration, which would provide guidelines to a time frame for the comparative study. Wientjes et al. investigated MMC concentration-depth profiles, but their chosen models—living dogs (7) and humans (8)—included urine volumes and blood flow, two largely uncontrollable variables that are absent in these present laboratory experiments.

A feature of the preliminary PD studies is the apparent disappearance of a quantity of MMC. Measurements at 15, 30, 45, and 60 min showed surprisingly consistent MMC concentrations averaging 5.73 μg/g in total tissue samples (Table 1). Values for individual layers were also consistent; and with an average mass of 0.59 g/specimen, the total MMC content was ~3.4 μg/specimen. Diffusive flux of

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Concentration-depth profiles of MMC in bladder wall tissue layers after EMDA and PD</th>
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</thead>
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<tr>
<td><strong>Experiment</strong></td>
<td><strong>Urothelium</strong></td>
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<tr>
<td>1</td>
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</tr>
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<td>2</td>
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</tr>
<tr>
<td>3</td>
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<tr>
<td>12</td>
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<td>14</td>
<td>0.014</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>6.21</strong></td>
</tr>
<tr>
<td><strong>Mean</strong></td>
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<td><strong>C&lt;sub&gt;mean&lt;/sub&gt;</strong></td>
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</table>

**a C<sub>80–200 μm</sub>**, **C<sub>200–1200 μm</sub>**, and **C<sub>1200–4000 μm</sub>** are the concentrations in urothelium (in which Ta tumors are located), lamina propria (T1 tumors), and superficial muscle layer (T2a tumors). **b C<sub>mean</sub>** (mean concentration between 80 and 4000 μm) was determined as the total amount of MMC found in tissue divided by the total tissue weight.
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Fig. 2. EMDA significantly increases MMC concentrations in all of the tissue depths compared with PD. Data are expressed as g of wet tissue, the means ± 1 SE of 14 experiments per group. *P < 0.0005 and **P < 0.0001 versus PD.

MMC (JMMC) from the donor compartment into tissues may be represented as:

\[ J_{MMC} = \frac{D_{MMC}}{\Delta C_{MMC}} \cdot \Delta x \]  

where \( D_{MMC} \) is the diffusion coefficient and \( \Delta C \) is the concentration difference across a membrane (\( \Delta x \)). Transforming data from Table 1: at time 0, \( \Delta C = 400 \mu g/ml \), and at 15 min, \( \Delta C \equiv 400 - 60 \equiv 340 \mu g/ml \), making the average concentration gradient over this period approximately 370 \( \mu g/ml \). Therefore, with an average concentration gradient of 340 \( \mu g/ml \) over the time frame of 15–60 min, the quantity of MMC administered into tissues every 15 min is \((340 \div 370) \times 3.4 \equiv 3.1 \mu g/ml \). Yet nowhere are the cumulative quantities of 3.1, 6.2, and 9.3 \( \mu g/ml \) demonstrated in measurements taken at 30, 45, and 60 min, respectively. The above calculations are not precise and omit detail, but they are sufficiently substantive to indicate that, at the end of 60 min, about 9 \( \mu g/ml \) of MMC have seemingly vanished. Inflowing urine and capillary blood outflow are absent; with a detection limit of 10 ng/ml, quantities exceeding 1 \( \mu g/ml \) in the 100-ml receptor compartment should have registered, and, because they did not, some other explanation for the missing mass is required. Bio-transformation of MMC is necessary for its activation (19), and it has been reported that bioreduction occurs rapidly with the formation of DNA adducts (20). Presumably, this is what happened to the MMC demonstrated in measurements taken (22). These encouraging findings have added impetus to plans for additional laboratory studies comparing EMDA/MMC levels in cancerous and normal tissues to assess whether this visual focusing effect on cancerous areas is mirrored by increased MMC levels.

Laboratory studies with human bladder tumors have demonstrated that MMC concentrations of 16, 25, and 43 \( \mu g/ml \) are required to produce a 90% inhibition of the labeling index in Ta, T1, and T2a tumors, respectively (9). Under the conditions imposed on the present studies, urothelial levels after both PD and EMDA exceeded 16 \( \mu g/ml \) but only 3 of 14 PD lamina propria measurements (mean, 16 \( \mu g/g \)) exceeded 25 \( \mu g/g \) in contrast to all of the 14 levels after EMDA (mean, 66 \( \mu g/g \)). Disappointingly, only 1 of 14 EMDA measurements in the superficial muscle layer exceeded 43 \( \mu g/g \), and this was probably an aberration. However, cancerous bladder tissue is far more permeable to water and solutes than is normal urothelium (21); theoretically, the disorganized architecture of the tumors also indicates a lower electrical resistance than normal tissue and, hence, the focusing of applied electric current on malignant areas. In aqueous solution, electric current is the quantitative transport of ions (with water and entrained solutes) that would preferentially enter cancerous bladder areas from nonmalignant urothelium with deep blue staining, easily distinguishable on cystoscopy (22). These encouraging findings have added impetus to plans for additional laboratory studies comparing EMDA/MMC levels in cancerous and normal tissues to assess whether this visual focusing effect on cancerous areas is mirrored by increased MMC levels.

Kyowa Italiana Farmaceutici srl (Milan, Italy) supplies MMC in vials containing NaCl with a MMC:NaCl ratio of 1:24 (mg), commonly used in Europe. Vials supplied by Bristol-Myers Co. (Wallingford, CT) contain mannitol as excipient with a MMC:mannitol ratio of 1:2 (mg), commonly used in the United States. These two formulations and their varying concentrations, which are used or recommended in different studies (Table 3), cause problems in the interpretation of results. Specifically, the toxicities of solutions listed in Table 3 span 950 milliosmols, equivalent to about 16,000 mm Hg. With all due respect, this is a chaotic background for the evaluation of passive transport (and clinical efficacy) of MMC whose migration across a membrane is represented by:

\[ J_{MMC} = \frac{D_{MMC}}{\Delta C_{MMC}} \cdot \Delta x \]  

\( + C_{MMC} (1 - \sigma) J_v \)  

convective flux \( \) convective flux \( \)

where \( C \) is concentration, \( J_v \) is volume flux, \( L_p \) is hydraulic permeability, \( \sigma \) is reflection coefficient, \( \Delta P \) is transmembrane hydrostatic pressure (negligible), and \( \Delta \pi \) is the osmotic pressure differential. In the present studies, the donor solutions were close to isotonic, which made the convective flux of MMC minimal and the term “passive diffusion” (PD) technically correct. However, for the remaining investigations listed in Table 3 (7, 8, 13, 23, 24), convective flux will influence the results; the transport of drugs into cells is enhanced by hypotonic (<300-milliosmolar) solutions and retarded by hypertonic solutions, an osmotic effect that has been demonstrated both in test tubes and i.p. in living animals (25). Inflowing urine of varying

<table>
<thead>
<tr>
<th>References</th>
<th>Model</th>
<th>Dose (mg)</th>
<th>Excipient (mg)</th>
<th>Volume</th>
<th>( \sim )mosM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wientjes et al. (7)</td>
<td>Dog</td>
<td>20</td>
<td>Mannitol (40)</td>
<td>40 ml H2O</td>
<td>7</td>
</tr>
<tr>
<td>Wientjes et al. (8)</td>
<td>Humans</td>
<td>20</td>
<td>Mannitol (40)</td>
<td>40 ml H2O</td>
<td>7</td>
</tr>
<tr>
<td>Wientjes et al. (23)</td>
<td>Computer simulation</td>
<td>40</td>
<td>Mannitol (80)</td>
<td>20 ml H2O</td>
<td>28</td>
</tr>
<tr>
<td>Di Stasi et al. (13)</td>
<td>Human bladder wall</td>
<td>10</td>
<td>NaCl (240)</td>
<td>100 ml H2O</td>
<td>75</td>
</tr>
<tr>
<td>Present study</td>
<td>Human bladder wall</td>
<td>40</td>
<td>NaCl (960)</td>
<td>100 ml H2O</td>
<td>320</td>
</tr>
<tr>
<td>Wittes et al. (24)</td>
<td>Humans</td>
<td>40</td>
<td>NaCl (960)</td>
<td>50 ml NaCl 0.9%</td>
<td>960</td>
</tr>
</tbody>
</table>

* mosM, milliosmolar.

Table 3 Some formulations, volumes, and osmolalities of MMC solutions employed experimentally and clinically

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functions as a drug receptacle. A second (return) electrode solution is infused into the bladder, the target organ, which also ing an active (“treatment”) electrode is inserted, and the selected drug electrocautery procedures. A generously perforated catheter contain-setting is surprisingly simple and somewhat analogous to intravesical motic, and charge-competitive effects of inflowing urine (26).

For any solution of NaCl: \( tr_{Na} \) is the transference number for Na; \( C \) is concentration; \( \mu \) is mobility; and \( i \) is the summation index of all ions in solution (\( C_{Na} = \mu \)).

\[
tr_{Na} = \frac{C_{Na} \cdot \mu_{Na} \cdot z_{Na}}{(C_{Cl} \cdot \mu_{Cl} \cdot z_{Cl}) + (C_{Na} \cdot \mu_{Na} \cdot z_{Na})}
\]

Equation 5 states that the addition of ions will cause a decline in \( dNa/dt \). If there are sufficient NaCl ions to conduct the total applied charge, the Na concentration (\( C_{Na} \)) is not relevant.

### Appendix B: Electroosmotic Transport of MMC in Na⁺ Hydration Shells.

\[
J_i (\text{mol/s}) = \frac{I}{|z| \cdot F}
\]

\( N \) is Avogadro’s number.

If one-half of the total applied charge is carried by Na⁺ into the bladder wall:

\[
N (\text{ions/mol}) = 6.023 \times 10^{23}
\]

\( I \) is the current in milliamperes (mA).

\[
J_{Na} = 0.5 \left( \frac{60 \cdot I \cdot N}{1000 \cdot F} \right) = 1.87 \times 10^{17} \text{ ions/mA \cdot min}
\]

\( R_{Na} \) is the radius of Na⁺ (Ref. 12).

\[
R_{Na} = 1.02 \text{ Å}
\]

\( R_{hydr} \) is the radius of hydrated Na⁺.

The total amount of water (\( V \)) transported as hydration shells with Na⁺:

\[
V = J_{Na} \cdot (V_{hydr} - V_{Na})
\]

\[
= J_{Na} (1.88 \times 10^{-22}) \text{ ml/mA \cdot min} + 3.5 \times 10^{-5} \text{ ml/mA \cdot min}
\]

\[
dM/dt = (3.5 \times 10^{-5}) C_{MMC} / \text{mL} \cdot \text{min}
\]
The concentration of MMC is a major factor. Additional inflowing ions probably have a negligible effect.

REFERENCES

Electromotive versus Passive Diffusion of Mitomycin C into Human Bladder Wall: Concentration-Depth Profiles Studies


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