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Electropermeabilization in bladder cancer chemotherapy

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Abstract *Purpose*: Electropermeabilization has been used for the introduction of genes into cells. Using this technique, we introduced the cytotoxic drug bleomycin (BLM) into cells and examined whether the technique might be useful for the treatment of bladder cancer. *Materials and methods*: For electropermeabilization in vitro, we used YTS-1 cells, a human transitional cell carcinoma line. Aliquots of cell suspension were mixed with a solution of BLM and immediately exposed to electric pulses. A high-power pulse generator was used to supply square-shaped pulses of 1250 V/cm (100 μs, eight pulses). After a 2-h post-shock incubation, cells were washed and incubated for one further hour. Then the concentration of BLM in the cells was measured using a bioassay. For electropermeabilization of tissue, we used normal male Wistar rats. The bladder was exposed and 10 mg/kg BLM was injected into the caudal vein. A series of eight pulses with a time constant of 100 μs at an electric field intensity of 1000 V/cm was applied. The bladder, liver and lungs were extracted 1 h later and prepared for quantification of the BLM concentration using the bioassay. *Results*: Electrotreated cells contained significantly higher concentrations of BLM than nonelectrotreated cells. The concentration of BLM 1 h after electrotreatment in bladder tissue was 2.7 times higher than that in nonelectrotreated bladder tissue. *Conclusion*: The electropermeabilization technique has the potential to serve as a new and effective modality for the treatment of bladder cancer.

Key words Bladder cancer · Chemotherapy · Electropermeabilization · Drug delivery

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Introduction

It has been reported that the application of a strong electric field across a cell results in the formation and expansion of membrane pores [7, 8, 11, 24]. This process technique can readily be used to induce an increase in transmembrane permeability in many types of cells, and has been used to introduce cytotoxic drugs [16], foreign materials [4, 15] and genes [14, 23] into cells. The aim of this investigation was to determine whether electropermeabilization could be used to introduce a cytotoxic drug (bleomycin, BLM) into human bladder cancer cells in culture and into bladder tissue in vivo.

Materials and methods

Cell line and culture techniques

YTS-1 carcinoma cells, originally derived from a line of human transitional carcinoma cells [13], were used. Cells were maintained as monolayers in plastic culture flasks in complete medium that consisted of RPMI-1640 (Sigma, St. Louis, Mo.) supplemented with 10% fetal calf serum in a humidified incubator in an atmosphere of 5% CO_2 in air. Experiments were performed using cells in the logarithmic phase of growth.

Electropermeabilization of cultured cells

After trypsinization, cells were washed twice with phosphate-buffered saline (PBS) and resuspended in PBS of 37 °C at a density of $5–10 \times 10^7$ cells/ml. Aliquots of 0.4 ml of this suspension were mixed with 0.4 ml of a two-fold-concentrated solution of BLM, and immediately exposed to an electric field. A high-power pulse generator (BTX T820; BTX, San Diego, Colif.) was used to supply square-shaped high-voltage pulses. The efficiency of pulse generation was checked with an oscilloscope connected to the generator. A polystyrene cuvette with two aluminum electrodes was used for delivery of the electric pulses. The capacity of the cuvette was 0.8 ml and the gap between the electrodes was 4 mm.

Determination of the appropriate intensity of the electric field

In order to determine the optimal electric-shock conditions, we used the Lucifer yellow (LY) test [17]. Cells were shocked at different field strengths in the presence of 1 m*M* LY and then incubated at 37 °C for 20 min. After four successive washes with PBS, the percentages of living fluorescent cells, living nonfluorescent cells and granular fluorescent cells were determined (Fig. 1). The cells were observed using a fluorescence microscope (BX60-43-FLBD1; Olympus, Tokyo) equipped with BP 400–440 and BA475 filters (Olympus, Tokyo).

Quantification of bleomycin retained in cultured cells

A series of eight pulses with a time constant of 100 μs at an intensity of 1250 V/cm was applied to the cells in the presence of BLM at the clinically obtainable concentrations of 10 μg/ml and 100 μg/ml [5]. After 2 h of post-shock incubation, cells were washed and centrifuged twice with PBS, and combined pellets were resuspended and incubated for 1 h in complete medium. After this second incubation, the concentration of BLM was measured using a bioassay with *Bacillus subtilis* PC1-219 [6]. The concentrations of BLM in the electrotreated cells were compared with those in nonelectrotreated cells which had been subjected to the same procedures with omission of the electrotreatment. The statistical significance of the differences between electrotreated cells and nonelectrotreated cells were assessed using Student's *t*-test.

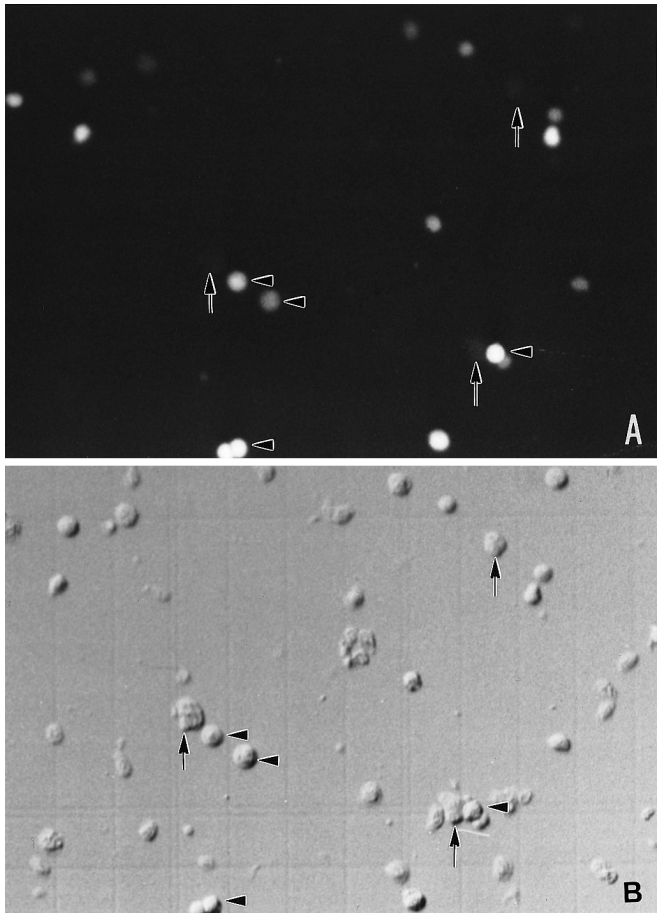

Fig. 1 A, B Microscopic views of cells shocked at 1250 V/cm (a series of eight pulses with a time constant of 100 μs). **A** Very bright spots (*arrowheads*) indicate electropermeabilized living cells. Indistinct, weak spots (*arrows*) indicate dead permeabilized cells (Lucifer yellow, fluorescent image). **B** Dead cells, observed with an interference microscope, are granular and flat (× 100, Olympus BX 50–34 DIC)

Electropermeabilization of tissue

Male Wistar rats weighing 250 g were used. Rats were anesthetized with intraperitoneal pentobarbital (40 mg/kg) and the peritoneal cavity was entered via a lower abdominal midline incision. The urinary bladder was exposed. A series of eight pulses with a time constant of 100 μs at an intensity of 1000 V/cm was applied. For the delivery of electric pulses, a high-power pulse generator BTX 820 with two plane parallel electrodes was used.

Quantification of bleomycin retained in the bladder tissue

With rats under pentobarbital anesthesia as described above, BLM was injected into the caudal vein at 10 mg/kg. Immediately after the injection, square-shaped electric pulses were applied, as described above. Organs were extracted 1 h after the injection and prepared for quantification of BLM using the *B subtilis* bioassay. The concentrations of BLM in the tissues of electrotreated rats were compared with those in tissues of nonelectrotreated rats which had been subjected to the same procedures with the omission of electrotreatment. The statistical significance of differences between tissues from electrotreated and nonelectrotreated rats was assessed using Student's *t*-test.

Results

Determination of the appropriate intensity of the electric field

The LY test revealed that the highest percentage of cultured cells was permeabilized and survived and the lowest percentage of cells was killed at intensities between 1000 and 1250 V/cm (Fig. 2).

Quantification of bleomycin retained in the cultured cells

The concentration of BLM in cells subjected to electric shock in 100 μg/ml BLM was 1.16 ± 0.13 μg/g, whereas that in control cells was 0.63 ± 0.07 μg/g. The concentration of BLM in cells subjected to electric shock in 10 μg/ml BLM was 0.69 ± 0.23 μg/g, whereas that in control cells was 0.27 ± 0.05 μg/g. Electrotreated cells contained 1.9 (100 μg/ml BLM) and 2.5 (10 μg/ml BLM) times higher concentrations of BLM than nonelectrotreated cells (Fig. 3). The amounts of BLM in electrotreated cells were significantly higher than those in nonelectrotreated cells.

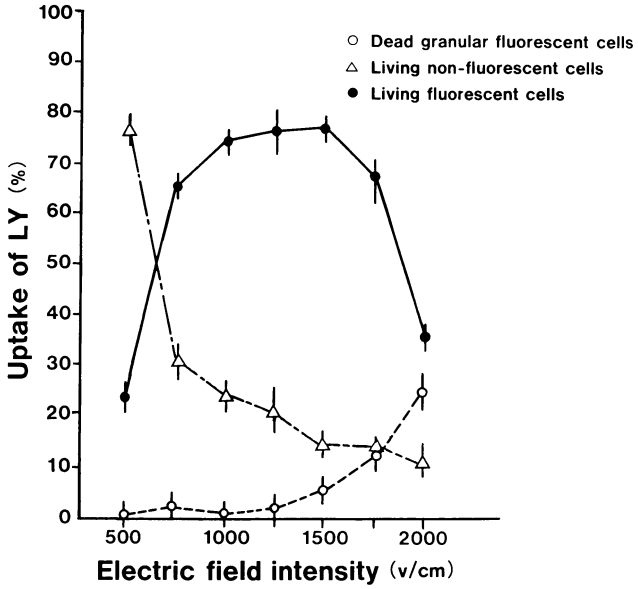

Fig. 2 After exposure to an electric field of 1250 V/cm (a series of eight pulses with a time constant of 100 μs), 77% of cells were electropermeabilized and 1.5% of cells were dead. With stronger the electric fields, the higher percentage of dead cells (○ dead granular fluorescent cells), △ living nonfluorescent cells, ● living fluorescent cells. Each point is the mean ± SD (*bar*) of the results from five samples

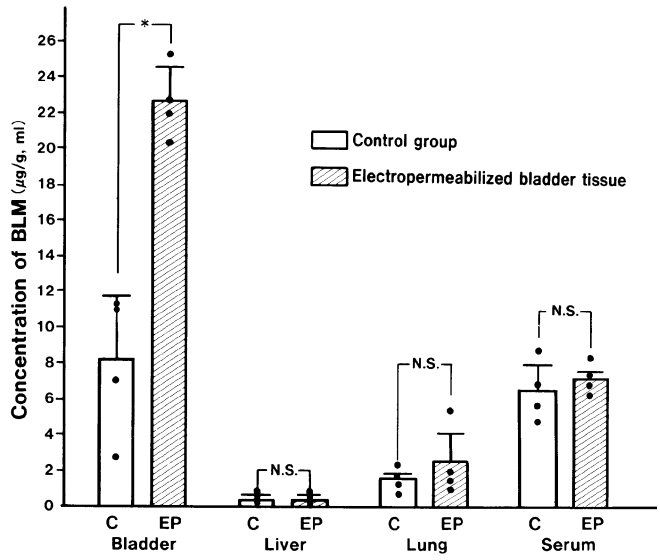

Fig. 4 Concentrations of BLM in electropermeabilized and control bladder tissue after injection of BLM at 10 mg/kg. Concentrations in serum, liver and lung tissue are also shown. The results are means ± SD from four rats (*C* control, *EP* electropermeabilized bladder tissue, *N.S.* not significant). $*P < 0.05$

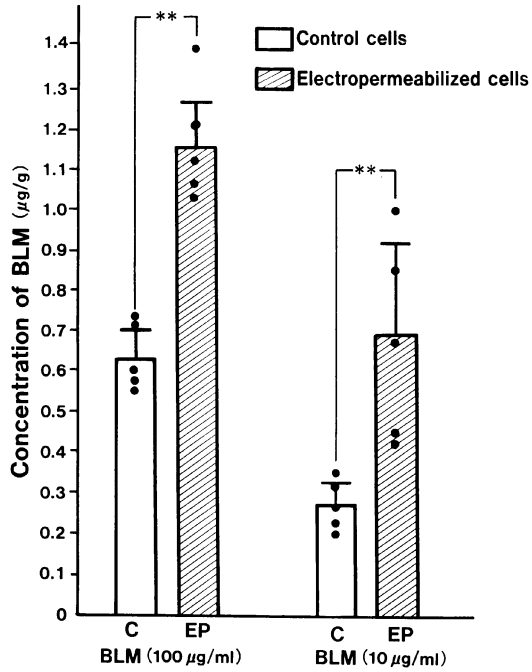

Fig. 3 Concentrations of BLM in cells subjected to electric shock and control cells in 100 μg/ml BLM and 10 μg/ml BLM. Columns show the means ± SD from five samples (*C* control, *EP* electropermeabilized cells). $**P < 0.01$

Quantification of bleomycin retained in bladder tissue

Concentrations of BLM in electropermeabilized and control bladder tissue after injection of BLM at 10 mg/kg were 22.7 ± 1.8 μg/g and 8.2 ± 3.6 μg/g, respectively. The concentration of BLM in the electrotreated bladder tissue was 2.7 times higher than that in nonelectrotreated bladder tissue (Fig. 4). In the case of the serum, liver and lung, the respective concentrations of BLM in the two groups of rats were very similar and far below those in the bladder tissue of electrotreated rats.

Discussion

The recommended treatment for patients with muscle-invasive bladder cancer is usually radical cystectomy. However, because of the diversion of the urine, the patient's quality of life after radical cystectomy is severely reduced. Recently, therapeutic efforts have focused on preservation of the bladder [9, 10, 20, 21]. In order to eradicate the cancer and maintain adequate bladder function, the development of more effective local treatment modalities is clearly needed.

BLM has been reported to be an effective drug for the treatment of bladder cancer [2, 18]. Its antineoplastic and antibiotic effects have been attributed to the induction of double-strand breaks in DNA [3]. Some reports indicate that, even though BLM can perturb plasma membranes, this effect is not correlated with its cytotoxicity [1], and we can assume that therapeutically relevant targets of BLM are, therefore, intracellular. The cytotoxicity of BLM is clearly increased with increasing concentrations of the drug in cells [22]. Thus, by introducing more BLM into

cells through the plasma membrane, we can obtain higher cytotoxicity.

The enhancement of the cytotoxicity of three antineoplastic drugs, namely cisplatin [16], bleomycin [19] and fluorodeoxyuridylate [12], using electropermeabilization in vitro has been reported. In most cases, it was postulated that this enhancement was caused by increased uptake of the drug by the cells. Melvik et al. [16] confirmed this hypothesis by direct measurements of the amount of cisplatin in electropermeabilized cells and nonelectropermeabilized cells. While the exact mechanisms responsible for the passage of BLM from the extracellular environment to the cytoplasm are unknown, our results suggest that, even under normal conditions, BLM is able to cross the plasma membrane and that electropermeabilization allows internalization of 2–2.5 times more of the drug than normal diffusion.

Most previous experiments involving chemotherapy in vitro using electropermeabilization were performed under low-temperature conditions that are difficult to achieve in a clinical setting. In our experiments, we performed all the procedures at 37 °C and at clinically relevant concentrations of the drug [6]. And we found that 2.7 times more BLM could be introduced into bladder tissue by electropermeabilization under physiological conditions than was introduced without this treatment.

Thus, electropermeabilization seems to have potential as a new clinically effective modality for local treatment of bladder cancer. Nevertheless, the clinical utility of electropermeabilization to enhance drug uptake into bladder tissue will depend on the long-term effect of the electrical treatment. There is also the issue of how the altered permeability resulting from electric treatment would affect the long-term health of the tissue as well as its normal function, including the barrier function of the urotherium to limit the absorption of potentially toxic compounds concentrated in the urine.

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