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Ultrastructural Evaluation of Murine Bladder Epithelium Exposed to Verapamil

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Summary. The effect of single or multiple instillations of high verapamil concentrations on the cytoarchitecture of the bladder epithelium was assessed by electron microscopy. Ruthenium red was used to evaluate the surface mucopolysaccharide coats and the integrity of junctional complexes between luminal cells. No significant cytoplasmic change in the luminal or nonluminal cells was found in any experimental animals, nor was there a breakdown of the junctional complexes between luminal cells. These data suggest that verapamil may be safely used intravesically as adjunct to standard chemotherapy.

Key words: Urothelium, Electron microscopy, Verapamil.

Introduction

The development of drug resistance in treated tumor cells contributes to failures of chemotherapy. Verapamil, a calcium channel blocker, has been shown to counteract the resistance of leukemic cells to Adriamycin [8, 10, 11]. Similar effects have been reported with certain ovarian and bladder tumor cell lines [7, 13].

Except for alopecia, Simpson et al. [7] reported no significant systemic influence in mice receiving intravesical instillations of verapamil. However, morphological documentation of the response of the bladder mucosa to verapamil has not been reported. In this study, the cytoarchitecture of the urothelium and the integrity of its junctional complexes were evaluated by transmission electron microscopy and ruthenium red cytochemistry. The findings indicated minimal structural alteration, consistant with the lack of systemic influence of verapamil. Our results suggest the use of verapamil as an adjunct to intravesical chemotherapy.

Materials and Methods

Adult female C57BL mice were maintained on a 12 h light – 12 h dark cycle and received food and water ad lib. Animals were randomly assigned to two groups of five and received 0.5 ml of phosphate buffered saline or 10^{-5} M verapamil solution intravesically via an Interdermic PE10 catheter under pentobarbital anesthesia. Anesthesia was maintained for a minimum of one hour to prevent premature voiding. For acute study, mice were killed 1 h after verapamil or saline instillation and their bladders removed and fixed. To study the effects of multiple exposures, treatments were repeated weekly for three weeks in two additional groups of five mice. Animals were killed one week after the last instillation. At sacrifice, bladders were filled with paraformaldehyde-glutaraldehyde fixative, removed and bisected longitudinally. One half of each bladder was transferred to fixative containing 0.02% ruthenium red [6]. Tissues from both halves were cut into small pieces and kept in the paraformaldehyde-glutaraldehyde fixative for 90 min at 4 °C before being stored in 1% cacodylate buffer overnight. The next day tissues were postfixed in 1% aqueous osmium tetroxide, dehydrated in ascending concentrations of ethanol, embedded in Araldite 502 and polymerized at 60 °C for 48 h. Samples for the evaluation of the integrity of junctional complexes were exposed to 0.01% ruthenium red in cacodylate buffer and osmium tetroxide. Sections were cut and stained, then examined in a Phillips 300 electron microscope.

Results

The bladders of animals treated with saline were lined with typical transitional epithelium consisting of a surface layer of differentiated cells distinct from the underlying two or three layers of polygonal cells. The superficial cells contained numerous fusiform vesicles. Randomly scattered among these were elongate mitochondria, Golgi complexes, short strands of rough endoplasmic reticulum and a few electron-opaque lysosomes. The nuclei displayed dispersed chromatin in which were embedded one or more spherical nucleolar complexes (Fig. 1a). The underlying cells were less well differentiated, exhibited a higher nuclear-cytoplasmic ratio, had fewer cytoplasmic organelles, and did not contain fusiform vesicles. They appeared to be poorly

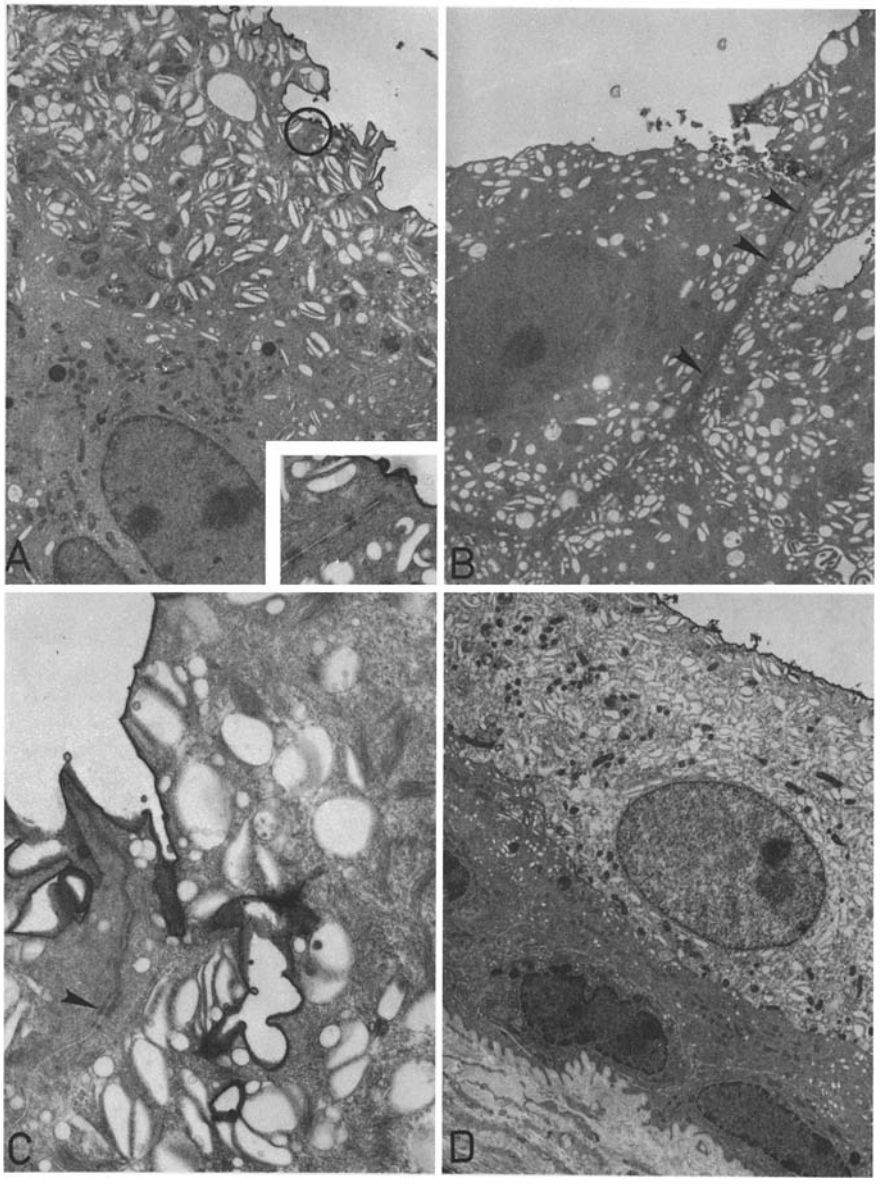

Fig. 1a–d. Bladder mucosa (a) in control showing ruthenium red staining of the glycocalyx (x3,145). *Insert* shows enlargement of circles. Note the presence of desmosomes and the absence of stain on the nonluminal surface (x12,325). b Exfoliating luminal cell. These cells exhibit an increased cytoplasmic opacity. *Arrows* indicate ruthenium red-stained nonluminal cell surfaces (x3,485). c High power view of two adjacent luminal cells from a single verapamil-exposed bladder. Note the presence of microfilament bundles and the intact junctional complex (*arrow*) (x13,600). d Bladder mucosa after multiple exposure to verapamil. Some luminal cells exhibit paler cytoplasm, but the pattern of ruthenium red staining is similar to that of controls (x2,975)

anchored to one another and displayed numerous intercellular spaces. The epithelium was separated from the underlying connective tissue by a thin basal lamina and varying amounts of intercellular matrix rich in collagen fibers.

By binding to the acid mucopolysaccharide moiety of the cell membrane [12], ruthenium red provided a continuous electron-dense covering on the free surface of the luminal cells in the saline-instilled bladders (Fig. 1a). Structurally intact junctional complexes between contiguous cells selectively inhibit movement of macromolecules across the epithelium. Except for sites of urothelial exfoliation, no ruthenium red staining of cells below the surface layer was found. The cytoplasm of cells undergoing renewal usually appeared more electron opaque and their lateral surfaces are stained by ruthenium red (Fig. 1b).

The urothelium of bladders treated with a single dose of verapamil showed no qualitative changes in either luminal or nonluminal cells. Junctional complexes between luminal cells were unaltered, since ruthenium red-stained surfaces were not evident between the cells (Fig. 1c). Moreover, the mucosal connective tissue showed neither dilated vessels nor inflammatory cells.

Examination of the urothelium after repeated saline instillations revealed superficial cells identical to those previously described overlying a layer of less well differentiated cells. There was no alteration in nuclear morphology that would suggest trauma to the cells.

Chronic verapamil exposure did not alter the cytoarchitecture of the murine bladder epithelium. A urothelium of well-differentiated superficial cells similar to those in control animals, and a multilayered array of nonluminal epithelium cells limited by an intact basement membrane were seen. Ruthenium red was seen on the luminal surface but failed to penetrate the subsurface layers. No degenerative

changes such as myelin body formation or nucleolar fragmentation were noted in urothelial cells repeatedly exposed to verapamil (Fig. 1d).

Discussion

Calcium channel blocking agents such as verapamil have been used to treat cardiovascular disorders [1, 2, 4, 15, 16]. The ability of those agents to enhance the chemosensitivity of certain tumors [10, 11] has prompted us to explore their usefulness in the management of bladder cancer.

We have previously reported that exposure to verapamil of an established human bladder cancer cell line T-24 for up to 72 h does not significantly alter the growth of these cells in vitro [7]. Nevertheless, enhanced cytotoxic effects were observed in cases where verapamil was given after cultures had initially been treated with thio-tepa. In another study we demonstrated that verapamil in combination with antineoplastic agents appears to pose no more of a threat to animals than do the antineoplatic agents alone [6]. Our findings are consistent with the results of verapamil studies on other forms of cancer [8, 11, 13].

If verapamil is to be used effectively as an adjunct to intravesical chemotherapy it is essential to show that verapamil itself is not harmful to the bladder mucosa. Structural damage to the urothelium can be produced by instillation of N-methyl-N-nitrosourea [16]. Soloway and Master [9] showed that injured urothelium favors tumor implantation. Surgical incision, freezing-ulceration or formalin instillation also result in specific mucosal lesions that ranged from focal ulceration to diffuse damage [3]. Scanning electron microscopic data from the same study indicated that most lesions were reversible; the hyperplastic regions were covered by either uniform short or pleomorphic microvilli. Our findings clearly indicate that in contrast to these studies, the instillation of verapamil does not cause sufficient insult to the murine urothelium either to open a direct access to the vascular compartment or to increase the permeability of the urothelium.

Enhancement of drug efficacy by verapamil appears to rest upon retarding the efflux of internalized drugs [12]. Since the glycocalyx of uroepithelial surface cells appeared unaltered and the junctional complexes between luminal cells remained functional, it seems likely that even after multiple instillations of verapamil the effects of verapamil on bladder mucosa are nontoxic. This finding provides justification for further testing of calcium antagonists as an adjunct to the treatment of tumors. Baring major cross-species differences between murine and human bladder, exploration of the responses of human urothelium to verapamil enhanced chemotherapy should be initiated.

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