PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **182,** 325–327 (1986)

Stimulation of Rat Bladder Epithelial DNA Synthesis by Intravesical Instillation of Distilled Water (42347)

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Abstract. Two commonly used cystoscopic infusion fluids were examined to determine whether their infusion stimulates DNA synthesis of the bladder epithelium. Following a single intravesical dose of 0.5 ml of distilled water or 1.5% L-glycine solution, rats were killed periodically up to 1 week. A transient but significant increase in epithelial cell [^{3}H]thymidine labeling was observed at 48 hr after distilled water instillation. Glycine solution did not stimulate DNA synthesis.

Cystoscopy and the intravesical instillation of fluids are procedures which are used routinely for the diagnosis and therapy of urinary tract disease. Patients with histories of carcinomas of the urinary bladder are followed with cystoscopies every 3 to 6 months. Ideally, cystoscopy procedures including bladder distension under hydrostatic pressure and exposure of the epithelium to instillation fluids should be noninjurious since carcinoma induction may be enhanced by methods or agents which stimulate cell proliferation. Conventional cystoscopy fluids are nonphysiologic. Distilled water, the most commonly used during cystoscopy, produces histological changes in the epithelium (1), and 1.5% L-glycine, an alternative cystoscopy fluid, lacks electrolytes. In this manuscript, we describe the effects of exposure of rat bladder epithelium to several cystoscopy fluids, using a controlled infusion technique which minimizes changes that may be related to vesical distension or rapid dilation (2). The results show that distilled water per se can induce DNA synthesis in the rat urinary bladder.

Materials and Methods. Female Fischer rats (Charles River Breeding Laboratory, Wilmington, Mass.) weighing 125–150 g were used. Under methohexital sodium anesthesia (5 mg/100 g body wt), external genitalia were thoroughly cleaned with 70% ethanol and bladders were emptied by gentle massage of the suprapubic region. Test fluids were instilled via a polyethylene tubing (0.28 mm i.d., 0.61 mm o.d.; Clay Adams, Parsippany, N.J.) attached to a 25-gauge needle, and were delivered at 0.1 ml/min using an electrically driven infusion pump (Harvard Apparatus Co., Inc., Millis, Mass.). Caution was excercised so that the tip of the catheter remained at the bladder neck to avoid trauma to the mucosa. Following completion of infusion, the urethral meatus was ligated gently for 5 min to prevent loss of instilled fluid. Fluids tested were distilled water (group 1), 1.5% L-glycine solution (Travenol Laboratories, Deerfield, Ill.) (group 2) and 0.9% NaCl solution (group 3). Group 4 rats received only manipulation of the meatal area with a pair of forceps without teeth under anesthesia and group 5 rats served as an untreated control. Four rats of each group were killed 24, 48, and 72 hr and 1 week after intravesical treatment. One hour before killing each rat was injected ip with [^{3}H]thymidine (2 Ci/mmol; Amersham Co., Arlington Heights, Ill.) at 1 μCi/g body wt. Animals were killed between 2:00 and 5:00 PM to avoid any effect of circadian rhythm.

Histological sections were coated with NTB liquid emulsion (Eastman Kodak, Rochester, N.Y.), exposed for 2 weeks, and were stained with hematoxylin–eosin. Numbers of labeled cells per 1000 epithelial cells were counted. Counting was repeated 10 times for each bladder, using different fields.

Results. Microscopic examination showed no significant abnormality except for mild submucosal edema which was noted in bladders of rats treated with distilled water and killed at 24 and 48 hr. None of the bladders revealed evidence of rupture of the mucosa.

An increase in urothelial labeling indices

0037-9727/86 $1.50

TABLE I. [^{3}H]THYMIDINE LABELING INDICES PER 1000 UROTHELIAL CELLS

Group	Day 1	Day 2	Day 3	Day 7
1. Distilled water	0.53 ± 0.72 (4)	3.68 ± 2.10 (4)*	0.43 ± 0.38 (4)	0.33 ± 0.40 (4)
2. Glycine	0.50 ± 0.29 (4)	0.47 ± 0.06 (3)	0.30 ± 0.08 (4)	0.10 ± 0.14 (4)
3. Saline	0.18 ± 0.15 (4)	0.20 ± 0.08 (4)	—	0.23 ± 0.15 (3)
4. Sham	0.13 ± 0.12 (3)	0.35 ± 0.39 (4)	0.33 ± 0.32 (4)	0.10 ± 0.08 (4)

Note. [^{3}H]Thymidine labeling indices are expressed as averages of labeled cells in ten 1000-cell counts. For the three untreated control rats, an average of 0.33 was obtained with the range from 0.1 to 0.5. Values are means ± SD. Numbers of rats studied are shown within parentheses.

* Significantly different from the saline group ($P < 0.03$) and the sham group ($P < 0.05$), two-sided (unadjusted for multiple comparisons) Wilcoxon test.

was noted only in bladders treated with distilled water and only at 48 hr (Table I). The increase was significant when compared with the saline group ($P < 0.03$) or the sham-operated group (group 4) ($P < 0.05$). The rise in the labeling index was, however, transient, and returned to an insignificant level by 72 hr. Epithelial cells labeled were mostly basal cells. Occasional labeled cells, however, were found in intermediate and surface cells. Those bladders which showed more labeled epithelial cells also showed more labeled stromal cells located immediately beneath the epithelium; they were mostly fibroblasts with occasional endothelial cells.

Discussion. Observations by several investigators have suggested that cystoscopy procedures are not entirely without risk (3–5). In a previous study done by one of us (R.S.W.) and his colleagues (2), irrigation fluid was infused rapidly in an attempt to duplicate conventional cystoscopy procedure in humans. A marked increase in the thymidine labeling index and focal necrosis was produced following infusion of distilled water or physiological saline.

In the present study, we evaluated the effects of cystoscopy fluids on the bladder epithelium by substituting controlled slow infusion of the test solution so that the effects of rapid expansion could be avoided. Under these experimental conditions, instillation of distilled water resulted in significant increase in [^{3}H]thymidine-labeled basal cells. The rise was transient with the peak at 48 hr. Other fluids tested, 1.5% glycine and 0.9% NaCl solution, did not result in a statistically significant increase in thymidine incorporation. The stimulating effect of distilled water is most probably due to its hypoosmolality. This is consistent with Tannenbaum's observation on the morphology of the "water effect" in human urothelium (1). It is not known if the results in rat can be extrapolated directly to humans. Based on morphological considerations, it is entirely possible that human urothelium may be more sensitive to injury by both distilled water exposure and rapid increase in volume (4). The results of this study suggest that distilled water used as an infusion fluid has the added risk of acting as a possible tumor promoter. One of the important roles of tumor promoters is the stimulation of cell proliferation, as clearly demonstrated at various tumor sites including urinary bladder (5). Recently, Wallace *et al.* reported enhancement of rat urinary bladder carcinogenesis by repeated intravesical instillation of distilled water but not by 1.5% L-glycine solution (6).

Patients with histories of bladder cancer are frequently subjected to repeated cystoscopic examinations using distilled water as an irrigation fluid. Urologists should keep in mind the possible untoward effect associated with the use of distilled water. In this regard, 1.5% glycine solution is a nonstimulating irrigation fluid and may be better suited for routine use.

This study was supported by NCI Grants CA 14649, 25034, 33510, and 34074. We acknowledge the kind assistance of Dr. Joan S. Chmiel and Mr. Carl-Bertil Wallemark of the Northwestern University Cancer Center Biometry Section for the statistical analysis.

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Received February 13, 1986. P.S.E.B.M. 1986, Vol. 182.
Accepted March 26, 1986.