Urol Res (2001) 29:29–33

ORIGINAL PAPER

Savaş Demirbilek · Gazi Aydin · H. Ilyas Özardali
Serhat Baykara

Chemically induced intestinal de-epithelialization using silver nitrate for bladder augmentation

Received: 23 March 2000 / Accepted: 11 September 2000

Abstract The use of bowel segments for bladder augmentation has been associated with metabolic complications, infections, stones, and cancer at the vesicoenteric anastomosis. The establishment of a transitional epithelium over the de-epithelialized surface of a segment of intestine might alleviate these complications. In this study, chemical de-epithelialization and urothelial re-epithelialization were attempted using silver nitrate ($AgNO_3$) solutions at different concentrations. Augmentation cystoplasty was performed in 55 female Swiss-Albino rats using a 1.5-cm detubularized segment of sigmoid. Forty-one rats survived and were killed 12 weeks postoperatively. There were four groups, including one group of eight rats that underwent augmentation alone (group 1, control), while the other three groups were treated with 1 g/100 ml (11 rats), 5 g/100 ml (10 rats), and 10 g/100 ml (12 rats) $AgNO_3$ solutions, respectively, before augmentation. Histopathological analysis demonstrated almost complete de-epithelialization and urothelialization of the sigmoid segment treated with 10 g/100 ml $AgNO_3$ solution, which did not occur in the other groups. Postoperative bladder capacities were increased in all groups. There was no obvious histological difference in the level of collagen deposition and/or fibrosis in the augmented tissues in any of the experimental groups. We conclude that 10 g/100 ml $AgNO_3$ solution can be successfully used for chemical de-epithelialization and urothelial re-epithelialization of augmented intestinal segments, and are worthy of further investigation.

Key words Bladder · Augmentation · Intestinal de-epithelialization · Silver nitrate · Rats

S. Demirbilek · G. Aydin · S. Baykara
Department of Pediatric Surgery,
Harran University Faculty of Medicine,
Sanliurfa, Turkey

H. Ilyas Özardali
Department of Pathology,
Harran University Faculty of Medicine,
Sanliurfa, Turkey

S. Demirbilek (✉)
Imam Bakir Mah. Karacadag Sok. No. 38,
Ferahkent, 63300,
Sanliurfa, Turkey
e-mail: sdemirbilek@ixir.com
Tel.: +90-532-6539699; Fax: +90-414-3140265

Introduction

The detection of various risks and multiple short- and long-term complications resulting from the use of gastrointestinal segments for augmentation, substitution or replacement of the urinary bladder [1, 2, 7, 8, 18, 20, 28] gave rise to alternative approaches. Removal of the bowel mucosa has been advocated. The most frequently used approach has been the mechanical stripping of the mucosa, leaving the underlying structures to be repopulated with transitional epithelium migrating from the adjacent bladder [3, 5, 21, 24, 26, 27]. However, retraction and fibrosis of the intestinal segment may occur with little or no increase in bladder capacity [3–5, 9, 19, 21, 26]. Furthermore, if the mucosa is not entirely removed, the segment re-epithelializes with normal intestinal mucosa. In an effort to overcome the complications associated with this method, some authors described successful results with the use of photodynamic therapy and protamine sulfate and urea solution to de-epithelialize an intestinal segment before augmentation [10, 22].

Silver nitrate ($AgNO_3$) is a widely used substance and has been applied topically for cauterizing bleeding and healing wounds. Inorganic silver salts in solution are strongly bactericidal [16, 29, 30]. Silver ions precipitate protein and give rise silver-dependent cell loss [12]. The inhibitory action on deoxyribonucleic acid (DNA) synthesis is the primary event in $AgNO_3$ cytotoxicity and is associated with a significant loss of cell protein. A concentration- and time-dependent depletion of intracellular adenosine triphosphate (ATP) content is also caused by ionic silver [12]. Taking the cytodestructive effects of

silver ions into consideration, it would be possible to de-epithelialize the segment of sigmoid prior to augmentation cystoplasty, and this may promote epithelialization of the augmented intestinal segment by transitional epithelium. In this study, chemical de-epithelialization and urothelial re-epithelialization of the bowel segment for bladder augmentation were attempted using $AgNO_3$ solutions at different concentrations. Success with this procedure could theoretically have implications for human surgery.

Materials and methods

Fifty-five female Swiss-Albino rats weighing between 195 and 210 g were used in this study. Fourteen rats died in the first postoperative week secondary to surgery-related causes. The remaining 41 rats were divided into one control group and three treatment groups. The control group, comprising eight rats (2 of the 10 died), underwent augmentation alone (group 1, control). The first treatment group of 11 rats (4 of the 15 died) underwent bladder augmentation after the application of 1 g/100 ml $AgNO_3$ solution (group 2); the second treatment group of 10 rats (5 of the 15 died) underwent augmentation after the application of 5 g/100 ml $AgNO_3$ solution (group 3); and the third treatment group of 12 rats (3 of the 15 died) underwent augmentation after the application of 10 g/100 ml $AgNO_3$ solution (group 4).

All of the animals were anesthetized with ketamine before the operation. A lower midline incision was used in all cases. The bladder was opened in the sagittal plane. A 1.5-cm segment of sigmoid colon was isolated, keeping its mesentery intact. The intestinal continuity was re-established by an end-to-end anastomosis with a single layer running suture of 6-zero polyglactin. The isolated segment was detubularized by an incision in its antimesenteric border. The patch was anastomosed to the bladder with a single layer running suture of 6-zero polyglactin. In the treatment groups, $AgNO_3$ solution was applied to the patch surface using a soaked microtampon for 1 min. Following application of the $AgNO_3$ solution, the patch surface was irrigated with saline and wiped before augmentation.

The preoperative bladder capacity and end-filling pressures were measured in each group of animals. At the 12th week of the experiment all of the animals were killed under anesthesia. Before death, the bladder capacity and end-filling pressures were measured again under ketamine anesthesia. A needle (20 gauge) was inserted into the bladder to facilitate these measurements. Complete filling and bladder capacity were defined as notification of an initial drop of urine from the urethra. Bladder capacity and end-filling pressure were measured with a water manometer connected to the needle.

For the histological examination, augmented bladders were carefully removed and fixed in 10% formalin. Portions of the specimen, including the transitional zone between the graft and bladder, random areas of the graft, and a cross-section of the vascular pedicle, were embedded in paraffin. The resulting paraffin blocks were sectioned (5 μm) and the slides stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), and Masson's trichrome stain. Each slide was reviewed by the study pathologist (HIÖ) for the following: (1) intestinal de-epithelialization and migration of the urothelium; (2) presence or absence of remaining mucous glands; (3) thickness of epithelium by number of cell layers; and 4) presence or absence of fibrous scar tissue. A semiquantitative analysis for collagen deposition was done by staining full-thickness sections of both the control and the treatment rat specimens with Masson's trichrome stain. A grading scale was established from 0 to 3+, as presented in Table 1.

Results

The pre- and postoperative bladder capacity and end-filling pressure levels are summarized in Table 2. The increase in bladder capacity was statistically significant in all groups. No statistically significant differences were observed in end-filling pressure measurements in any of the rat groups. At the end of the 12-week experimental period, the augmented segment of the intestine indicated that varying degrees of de-epithelialization occurred in the treatment groups (Table 3). There was no de-epithelialization and transitional epithelialization in group 2 rats. Group 3 rats indicated some degree of de-epithelialization, ranging from a minimum of approximately 30% to a maximum of 70% (mean = 45%). Almost complete de-epithelialization and urothelialization were observed in the rats treated with 10 g/100 ml $AgNO_3$ solution (Fig. 1). Microscopical foci of residual colonic epithelia representing less than 5% of the graft surface area were identified in only 3 of the 11 animals in group 4. Collagen deposition (fibrosis) was minimal in all groups (Masson's trichrome stain) with no apparent differences between the groups (Fig. 2; Table 3). Augmented segments stained positively with PAS (indicating the presence of a well-defined mucus coating as well as a normal number of goblet cells) completely in group 1 and 2 rats, partially in group 3 rats (Fig. 3), but not in group 4 rats. All augmented sigmoid segments were inflamed. Inflammatory cells were mostly lymphocytes and plasmacytes, and these were distributed throughout

Table 1 A grading scale for collagen deposition

Criterion	Score
None	0
Submucosal collagen at least twice the thickness of the muscularis mucosa	1+
Submucosal collagen more than twice the thickness of the muscularis mucosa	2+
Collagen deposition around the smooth muscle fibers	3+

Table 2 Bladder capacity and end-filling pressure values pre- and postoperatively in each group of rats (mean ± standard deviation)

	Bladder capacity (ml)		End-filling pressure (cm/H_2O)	
	Preoperation	Postoperation	Preoperation	Postoperation
Group 1	2.4 ± 0.2	3.5 ± 0.2	14.5 ± 1.4	15.4 ± 1.4
Group 2	2.3 ± 0.15	3.3 ± 0.15	14.1 ± 1.1	14.9 ± 1.1
Group 3	2.3 ± 0.1	3.4 ± 0.1	13.9 ± 1.2	14.9 ± 1.2
Group 4	2.4 ± 0.15	3.4 ± 0.15	14.1 ± 1.6	15.2 ± 0.8

Table 3 Histological findings for each group of rats. *PAS* periodic acid-Schiff

	Group 1	Group 2	Group 3	Group 4
De-epithelialization	–	–	30–70% (mean = 45%)	95–100% (mean = 98%)
Migration of urothelium	–	–	30–70%	95–100%
Collagen deposition (Masson's trichrome stain)	0.875 ± 0.35	0.833 ± 0.54	1 ± 0.47	1.083 ± 0.51
Mucous glands (PAS)	+	+	–/+	–

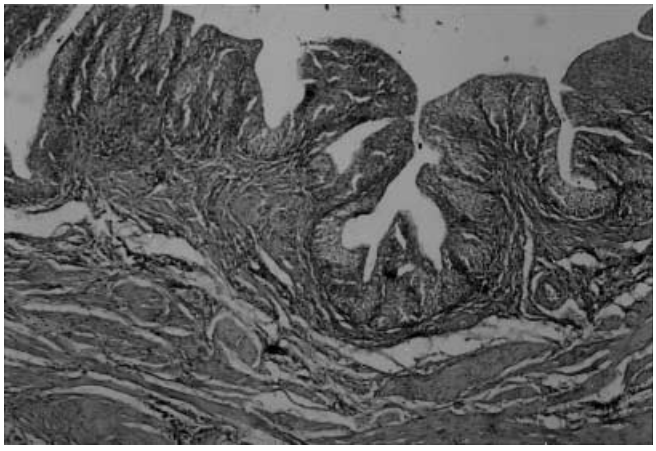

Fig. 1 A section of the sigmoid patch of a group 3 rat demonstrates complete transitional cell epithelialization of the mucosa with no microscopical foci of residual mucosa. H&E, ×40

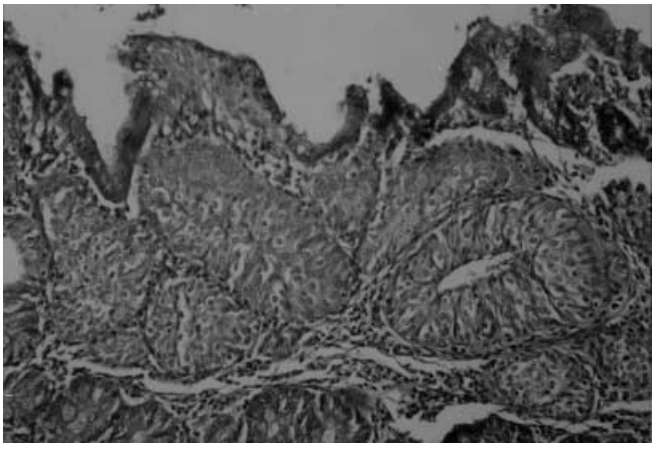

Fig. 3 A section of the sigmoid patch of a group B rat demonstrates incomplete transitional cell epithelialization of the mucosa with PAS (+) mucin-secreting cells. PAS, ×10

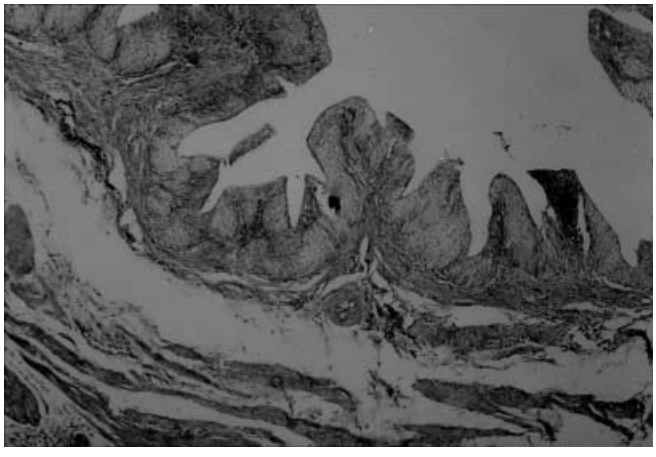

Fig. 2 A section of the sigmoid patch of a group 3 rat demonstrates complete urothelialization of the mucosa and minimal collagen deposition in the submucosa. Masson's trichrome stain, ×40

the mucosal and submucosal tissue of the augmented intestine. The mean thickness of the proliferating transitional epithelium ranged between three and four cells over the submucosa. In the normal portions of the bladder, the mucosa was six to seven cell layers thick.

In summary, chemical de-epithelialization and urothelialization of the augmented intestine with the use of 10 g/100 ml $AgNO_3$ solution preserved the underlying bowel structure such that pseudovilli covered with transitional epithelium were observed.

Discussion

Bowel segments proved to be a very useful source for the reconstruction of the urinary tract in the management of various problems [11, 25]. Almost all parts of the intestinal system have been successfully incorporated into the urinary tract for purposes of urinary diversion, bladder augmentation or substitution in the damaged urinary structure [6]. Despite the successful use of bowel segments for bladder augmentation many complications have been reported. Metabolic complications are among the most important of these undesired effects, mainly as a result of a functional enteric epithelium that is capable of normal absorptive and secretary activities. Mucus production, chronic bacteriuria, infection, stone formation, perforation, and malignant transformation at the vesicoenteric anastomosis are some of the major drawbacks of these surgical procedures [1, 2, 7, 8, 18, 20, 23, 28].

To obviate these problems, removal of the bowel mucosa has been advocated [3–5, 9, 19, 21, 24, 26, 27]. The rationale behind this approach is that repopulation of the augmented bladder with transitional cells will provide a more physiological lining and, thus, fewer electrolyte abnormalities and decreased mucin production. The most frequently used approach has been the mechanical stripping of the mucosa, leaving the underlying structures to be repopulated with transitional epithelium migrating from the adjacent bladder. However,

in animal models, surgical de-epithelialization results in retraction and fibrosis of the intestinal segment with little or no increase in bladder capacity [3–5, 19, 21, 26]. Some authors think that removing the bowel epithelium chemically or enzymatically would prevent mechanical trauma and possible ischamia, which is purposed to be responsible for any subsequent fibrosis when the mucosa is physically stripped. Recently, cytodestruction of the bladder epithelium and large intestine epithelium using protamine sulfate and urea has been reported. Although the initial effort to achieve intestinal de-epithelialization by chemical means was encouraging, the intestinal mucosa was associated with urothelial cells in this study [22].

Silver is one of several chemical agents currently being used as a local antiseptic in daily clinical practice. Silver ions precipitate protein and also interfere with essential metabolic activities of microbial cells. Inorganic silver salts in solution are strongly bactericidal. It has been shown that silver salts exert an inhibitory effect on proliferation and differentiation of several cell lines: bone marrow cells and keratinocytes [13], hepatocytes [17], lymphocytes, and leukocytes [14, 15]. After 24 h of exposure to $AgNO_3$, all assayed antiseptic concentrations markedly affected DNA synthesis, thereby demonstrating that this is a major cytotoxic mechanism [12]. Another pathway by which silver ions may cause cell injury is through mitochondrial dysfunction, which leads to inhibition of ATP synthesis. Silver ions result in intracellular ATP depletion of fibroblasts after 8 h of exposure [12]. This toxic effect may be the result of blockage of the electron transport system, depolarization or altered permeability of the mitochondrial membrane, or mitochondrial DNA damage [12]. In summary, the toxic effects of silver ions result from basal cytotoxic mechanisms that affect basic metabolic functions common to all mammalian cells. This study evaluated the potential usefulness of $AgNO_3$ solutions for chemical ablation of intestinal mucosa before augmentation in rats.

Our results confirm that the application of $AgNO_3$ at a concentration of 10 g/100 ml leads to sloughing of the sigmoid mucosa with preservation of the underlying submucosa, muscle, and serosa in the rat model (Fig. 1). Over the 12-week period, the transitional epithelium almost completely lined the intestinal augmented segment in that group of rats (Figs. 1 and 2). Minute foci of intestinal mucosa were found in 3 of the 11 rats. Unlike previous attempts to remove surgically the intestinal mucosa, chemical de-epithelialization with $AgNO_3$ solution (10 g/100 ml) did not result in shrinkage or contracture of the augmented segment. No statistically significant differences were observed in bladder capacity or end-filling pressure measurements in any of the rat groups (Table 2). There was no obvious histological difference in the level of collagen deposition in the augmented tissues in any of the experimental groups (Table 3).

The use of chemical therapy with 10 g/100 ml $AgNO_3$ solution as an adjunct to enterocystoplasty appears to be promising. Removing the bowel epithelium chemically could prevent the mechanical trauma and possible ischemia that is purposed to be responsible for any subsequent fibrosis when the mucosa is physically stripped. The ability to replace the intestinal mucosa of the augumented bladder with transitional epithelium using $AgNO_3$ solution at a concentration of 10 g/100 ml could obviate many of the complications associated with standard enterocystoplasty. Further experimentation is required in larger animal models to substantiate our results.

References

1. Blyth B, Ewalt DH, Duckett JW, Synder HM (1992) Lithogenic properties of enterocystoplasty. J Urol 148: 575
2. Blyth B, Ewalt DH, Duckett JW, Synder HM (1992) Lithogenic properties of enterocystoplasty. J Urol 148: 575
3. Bodiola F, Manivel JC, Gonzalez R (1991) Seromuscular enterocystoplasty in rats. J Urol 146: 559
4. Dewan PA, Chacko J, Ashwood P (1995) Urothelium in seromuscular colocystoplasty. Urology 46: 443
5. Dewan PA, Lorenz C, Stefanek W, Byard RW (1994) Urothelial lined colocystoplasty in a sheep model. Eur Urol 26: 240
6. Duel BP, Gonzalez R, Barthold JS (1998) Alternative techniques for augmentation cystoplasty. J Urol 159: 998
7. Filmer RB, Spencer JR (1990) Malignancies in bladder augmentations and intestinal conduits. J Urol 143: 671
8. Flood HD, Malhotra SJ, O'Connell HE, Ritchey MJ, Bloom DA, McGuire EJ (1995) Long-term results and complications using augmentation cystoplasty in reconstructive urology. Neurourol Urodyn 14: 297
9. Frey P, Lutz N, Neubo AL (1996) Augmentation cystoplasty using pedicled and de-epithelialized gastric patches in the minipig model. J Urol 156(2 Pt 2): 608
10. Haselhuhn GD, Kropp KA, Keck RW, Selman SH (1994) Photochemical ablation of intestinal mucosa for bladder augmentation. J Urol 152: 2267
11. Hendren WH, Hendren RB (1990) Bladder augmentation: experience with 129 children and young adults. J Urol 144: 445
12. Hidalgo E, Dominguez C (1998) Study of cytotoxicity mechanisms of silver nitrate in human dermal fibroblasts. Toxicol Lett 15: 169
13. Hollinger MA (1996) Toxicological aspect of topical silver pharmaceuticals. Crit Rev Toxicol 26: 255
14. Hussain S, Anner RM, Anner BM (1992) Cysteine protects Na, K-ATPase and isolated human lymphocytes from silver toxicity. Biochem Biophys Res Commun 189: 1444
15. Jansson G, Harms-Ringdahl M (1993) Stimulating effects of mercuric and silver ions on the superoxide anion production in human polymorphonuclear leucocytes. Free Radic Res Commun 18: 87
16. Lansdown AB, Sampson B, Laupattarakasem P, Vuttivirojana A (1997) Silver aids healing in the sterile skin wound: experimental studies in laboratory rat. Br J Dermatol 137: 728
17. Liu J, Kershaw WC, Klassen CD (1991) The protective effect of metallothionein on the toxicity various metals in rat primary hepatocyte culture. Toxicol Appl Pharmacol 107: 27
18. Lockhart JL, Davies R, Persky L, Figueroa TE, Ramirez G (1994) Acid-base changes following urinary tract reconstruction for continent diversion and orthotopic bladder replacement. J Urol 152: 338
19. Lutz N, Frey P (1995) Enterocystoplasty using modified pedicled, detubularized, de-epithelialized sigmoid patches in the mini-pig model. J Urol 154: 893
20. McDougol WSM (1992) Metabolic complications of urinary intestinal diversion. J Urol 147: 1199
21. Motley RC, Montgomery BT, Zolman PE, Halley KE, Kramer SA (1990) Augmentation cystoplasty utilizing de-epithelialized sigmoid colon: a preliminary study. J Urol 143: 1257

22. Niku SD, Scherz HC, Stein PC, Parsons CL (1995) Intestinal de-epithelialization and augmentation cystoplasty: an animal model. Urology 46: 36
23. Nurse DE, Mundy AR (1989) Metabolic complications of cystoplasty. Br J Urol 63: 165
24. Oesch I (1988) Neourethelium in bladder augmentation. An experimental study in rats. Eur Urol 14: 328
25. Robertson AS, Davies JB, Webb RJ, Neal DE (1990) Bladder augmentation and replacement. Urodynamic and clinical review of 25 patients. Br J Urol 68: 590
26. Salle JL, Fraga CS, Lucib A, Lampertz M, Jobim G, Jobim G, Putten A (1990) Seromuscular enterocystoplasty in dogs. J Urol 144: 454
27. Shoemaker WC (1995) The experimental use of seromuscular grafts in bladder reconstruction. Preliminary report. J Urol 73: 314
28. Steiner NS, Morton RA, Marshall FF (1993) Vitamin B12 deficiency in patients with ileocolic neobladders. J Urol 149: 255
29. Wright JB, Lam K, Burrell RE (1998) Wound management in an era of increasing bacterial antibiotic resistance: a role for topical silver treatment. Am J Infect Control 26: 572
30. Wright JB, Lam K, Hansen D, Burrel RE (1999) Efficacy of topical silver against fungal burn wound pathogens. Am J Infect Control 27: 344