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The use of silver nitrate for chemical de-epithelialization and urothelialization of intestine in a rabbit model of augmentation cystoplasty

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Abstract Urinary tract reconstruction using bowel segments can result in complications such as electrolyte abnormalities, infections, stones and cancer. Intestinal mucosa is the primary site responsible for these complications. De-epithelialization of the mucosa and subsequent urothelialization might alleviate these problems. We recently reported our success in de-epithelialization and subsequent uroepithelialization of intestinal segments using 10 g/100 ml $AgNO_3$ solution in a rat model of augmentation. In this study, chemical de-epithelialization of a colonic segment was attempted using 10 g/100 ml $AgNO_3$ solution in a rabbit model of augmentation. Sigmoid cystoplasty was performed in 20 male New Zealand rabbits using a 6 cm patch of sigmoid colon. There were two groups, including one group of five rabbits (control, group 1) that underwent augmentation alone, while another group (15 rabbits, treatment group or group 2) was treated with 10g/100 ml $AgNO_3$ solution before augmentation. Control rabbits were killed at the week 8 of experimentation. Treatment rabbits were killed at 2-, 4-, 6- and 8-week intervals. Immediately before augmentation and at the end of the 8 week experimental period each rabbit underwent cystometry. De-epithelialization of the bowel epithelium without urothelialization was apparent in the treatment rabbits killed at 2 and 4 weeks. Histological analysis revealed almost complete urothelialization of the augmented sections treated with 10 g/100 ml $AgNO_3$ solution at the end of the 6 and 8 week of experimental periods. The preoperative and postoperative bladder capacities increased substantially in all groups. There was no obvious histologic difference in the amount of collagen present in the augmented tissues in any of the experimental groups. The present study confirmed that the treatment of intestinal segments with 10g/100 ml $AgNO_3$ solution led to chemical de-epithelialization and urothelialization of the augmented segments. This procedure could, theoretically, have applications to human surgery.

Keywords Bladder · Augmentation · Intestinal de-epithelialization · Silver nitrate · Rabbits

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Introduction

Enterocystoplasty is a common procedure in both adult and pediatric urologic practice. Segments from almost all parts of the gastrointestinal tract have been used to enlarged the bladder [7]. As experience with the use of various segments of the gastrointestinal tract in genitourinary reconstructive surgery has increased, an array of short- and long-term complications associated with these procedures has become evident [9, 26]. Most of these complications are intestinal or gastrocystoplastic; namely, infection, electrolyte imbalance, lithiasis, mucus production, alterations in the native bladder, and potential carcinogenesis. These are related to the presence of gastric or intestinal mucous with the absorptive and secretory properties of each segment in contact with the urine and the remaining urothelium.

Enterocystoplasty without bowel mucosa would be useful in preventing most complications related to the presence of bowel mucosa in the urinary tract. We recently reported our success with the use of 10 g/100 ml $AgNO_3$ solution out of a variety of different concentrations of $AgNO_3$ for intestinal de-epithelialization and

subsequent urothelialization in the rat model of augmentation [5]. The following study was performed using rabbits to further investigate this finding.

Materials and methods

Twenty New Zealand male rabbits (weighing 3,200–3,400 g) underwent augmentation cystoplasty. The rabbits were divided into two groups. Five rabbits underwent augmentation cystoplasty alone (group 1, control). The treatment groups of fifteen rabbits underwent bladder augmentation after application of 10 g/100 ml $AgNO_3$ solution (group 2, treatment).

All rabbits received nothing by mouth (except for water) for 24 h prior to surgery. They were also given an intramuscular injection of 100 ml/kg of ceftriaxone prior to operative procedures. This was continued 10 days postoperatively. The rabbits were than anesthetised with a mixture of ketamine (35 mg/kg) and xylasine (5 mg/kg), which was repeated every 30–45 min during the operation. The rabbits were placed in a supine position on the operating table. An 8 F catheter (pediatric feeding tube) was passed through the urethra and into the bladder of each male rabbit at the start of the operation. A lower midline incision was used in all rabbits and a 6-cm segment of large intestine was isolated on its vascular pedicle. The continuity of the bowel was restored using end-to-end anastomosis in a one layer closure. Before anastomosis, colonic segment samples were removed from the anastomotic edges for histologic examination and hydroxyproline determination. The isolated segment of the intestine was irrigated with 0.5 N saline. The isolated segment was detubularized by an incision in its antimesenteric border. The bladder was opened in the sagittal plane and the patch anastomosed to the bladder with a single layer running suture of 5-zero polyglactin. The bladder was distended by filling to near capacity via the catheter, and small leaks were repaired. No drains or cystostomy tubes were left in any of experimental rabbits. The wound was closed in two layers and dressed appropriately.

In the treatment group, 10 g/100 ml silver nitrate solution was applied to the patch surface using a soaked microtampon for 1 min. Following application, the patch surface was irrigated with saline and wiped before augmentation.

Immediately before augmentation, and at the end of the 8 week experimental period, each rabbit underwent urodynamic evaluation, consisting of maximal bladder capacity and urine leakage pressure measurements. For the urodynamic investigations, a computarized Synectics system was used. The bladder was filled at a rate of 5 ml/min through an 8 F urethral catheter. Maximal bladder capacity and urine leakage pressure were defined as the volume and pressure, respectively, at which urine started to leak from urethra.

Control rabbits were killed at the week 8 of the experiment. Treatment rabbits were killed 2 (group 2a, $n=3$), 4 (group 2b, $n=3$), 6 (group 2c, $n=3$) and 8 (group 2d, $n=6$) weeks postoperatively. The augmented bladders were carefully removed. Before fixation, a 1×1 cm portion of the grafted colonic segment was removed and kept at −40°C for determination of hydroxyproline levels (from group1 and 2d). Tissue hydroxyproline levels were determined by using Woessner's [35] method. The other parts of the specimens were fixed in 10% formaldehyde and routine procedures were performed. Paraffin sections were cut including the transitional zone between the graft and bladder, random areas of the graft and a cross section of the vascular pedicle. The slides were then stained with hematoxylin and eosine (H and E), periodic acid-Schiff (PAS) and Masson's trichrome stain. Each slide was viewed for intestinal de-epithelialization and migration of the urothelium, presence or absence of remaining mucous glands, and collagen deposition.

Results

Six rabbits died in the first week after the surgery, one from group 1 and the others from group 2. The cause of the deaths was vascular insult and ischemic necrosis of the grafting colonic segment resulting in anastomatic dehiscence, peritonitis and sepsis. Those that died during the first week following operation were replaced.

Within 8 weeks, bladder stones occurred in 3 of 5 (60%) control rabbits and in 5 of 6 (83%) treated rabbits (group 2d). The composition was struvite (100%) in all of the cases.

Table 1 summarizes the results on bladder capacities and urine leakage pressures. All animals presented a significant increase in maximal bladder capacities postoperatively (Table 1, Fig. 1). Leakage pressures were practically identical in all animals at 8 weeks after surgery (Table 1).

At the end of the 2- and 4-week experimental period, the augmented colonic segments indicated that complete de-epithelialization had occurred in the treatment group (2a and 2b) (Table 2). The epithelial layer of the grafted colonic segments was desquamated (Figs. 2, 3). There was minimal transitional epithelialization in some areas of the specimens. In the treatment group, complete

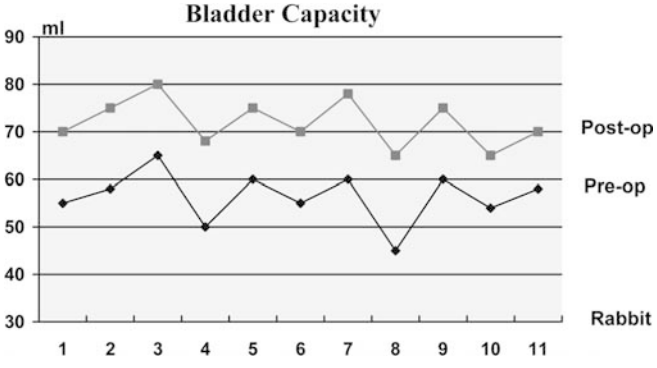

Fig. 1 Preoperative and postoperative bladder capacity for 11 rabbits. Controls from *1* to *5*, treatment group from *6* to *11* (group 2d). There was a net increase in maximal bladder capacity at leakage pressure after augmentation in each animal

Table 1 Urodynamic findings. Values are means ± SEM

	Maximal bladder capacity (ml)		Urine leakage pressure (cm H_2O)	
	Preoperative	Post-mortem	Preoperative	Post-mortem
Group 1 (control) ($n=5$)	57.6 ± 2.5	73.6 ± 2.11	71 ± 1.97	70.4 ± 1.63
Group 2d (treatment) (killed at 8 weeks, $n=6$)	55.33 ± 2.3	70.5 ± 2.14	69.83 ± 1.66	69.16 ± 1.62

Table 2 Histologic and biochemical findings. Values are means ± SEM

	Group1 (killed at 8 weeks, $n=5$)	Group 2a (killed at 2 weeks, $n=3$)	Group 2b (killed at 4 weeks, $n=3$)	Group 2c (killed at 6 weeks, $n=3$)	Group 2d (killed at 8 weeks, $n=6$)
De-epithelialization	–	+	+	+	+
Migration of urothelium	–	–	±	+	+
Collagen deposition (Masson tricrome)	+	+	+	+	+
Mucous gland	+	+	±	±	±
Hydroxyproline level (mg/gr)	13.58 ± 0.42	–	–	–	14.68 ± 0.45

Fig. 2 A section of a colonic patch showing complete de-epithelialization 2 weeks after silver application and augmentation. Masson's trichrome ×200

Fig. 3 Another section of colonic patch showing complete de-epithelialization 2 weeks after silver application and augmentation. Masson's trichrome ×400

urothelial coverage was achieved in all rabbits at the end of the 6- and 8-week experimental periods in group 2c and d (Figs. 4, 5). No foci of residual colonic epithelia on the graft surfaces could be identified microscopically (Figs. 4, 5). The mean thickness of the proliferating transitional epithelium ranged between three and five cells over the submucosa (Fig. 6). In the normal portions of the bladder, the mucosa was six to seven cell layers thick.

Collagen deposition was minimal to moderate in all groups (Masson's trichrome stain and hydroxyproline levels) with no apparent differences between the groups (Table 2, Fig. 7). Augmented segments stained positively with PAS (indicating the presence of a well-defined mucus coating as well as a normal number of goblet cells) in group 1 rabbits and partially in group 2 rabbits (Fig. 8). All augmented colonic segments were inflamed. Inflammatory cells were mostly lymphocytes and these were distributed throughout the mucosal and submucosal tissue of the augmented colonic segment (Fig. 9).

Discussion

In an attempt to circumvent the non-physiological interface between urine and bowel mucosa, several techniques of bladder augmentation with de-epithelialized bowel patches have been assessed in experimental and clinical studies. It is clear from animal models that augmentation with demucosalized bowel works well in small animals [4, 29] but results in fibrosis and contracture in large animals [24, 27, 28, 31]. Furthermore, if the mucosa is not entirely removed, the segment re-epithelializes with normal intestinal mucosa [24, 27, 28]. Therefore, new promising techniques of preserving urothelium have emerged, such as bladder auto-augmentation, seromuscular colocystoplasty lined urothelium and detubularized megaureter augmentation [2, 3, 11, 31, 33]. Unfortunately, these procedures cannot be used when bladder epithelium is scarce.

Numerous investigators have also attempted the application of alternative materials and tissues for bladder replacement. These include matrices for tissue regeneration and tissue engineering using cell transplantation [8].

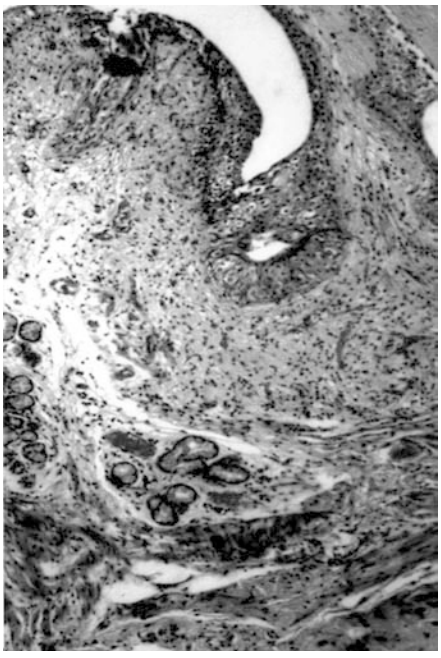

Fig. 4 At 8 weeks, augmented colonic surfaces showed complete urothelialization with no foci of residual colonic epithelia. H and E ×200

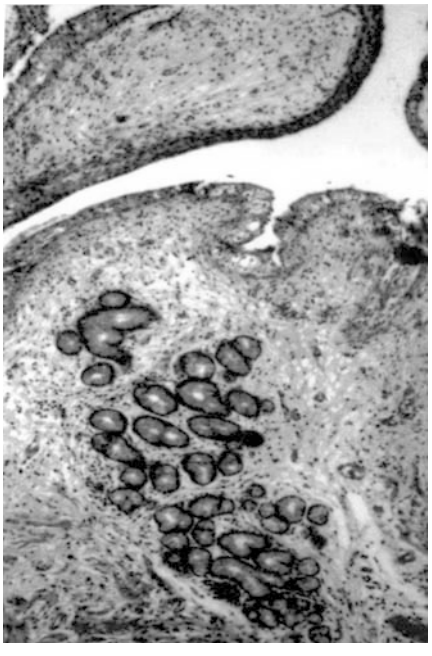

Fig. 5 Another micrograph at 8 weeks, augmented colonic surfaces showed complete urothelialization with no foci of residual colonic epithelia. H and E ×200

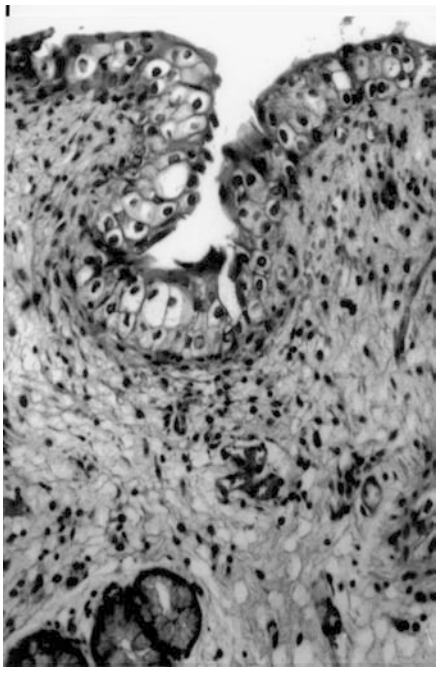

Fig. 6 The mean thickness of the proliferating transitional epithelium ranged between three and five cells over the colonic submucosa. H and E ×400

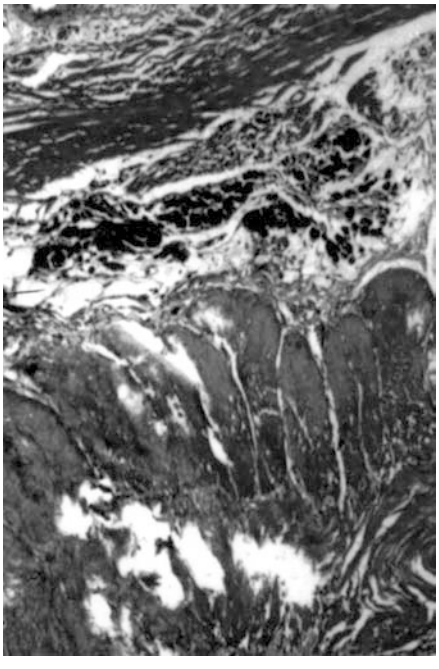

Fig. 7 A section of colonic patch showing moderate collagen deposition in the submucosal layer. Masson's trichrome ×200

Matrices for tissue regeneration can be divided into the two groups of synthetic materials designed to replace resected tissue, and organic absorbable materials designed to act as a scaffold for bladder healing, with eventual replacement by host tissue. Synthetic materials include polyvinyl sponge, tetrafluoroethylene, collagen and vicryl matrices, and silicone [1, 10, 25, 30]. In general, results have been disappointing, with infection, leakage, encrustation and extrusion of the prosthetic material. There is also a large body of literature on the use of absorbable biomaterials for bladder augmentation, including autogenous fascia, alcohol preserved fascia, human dura,

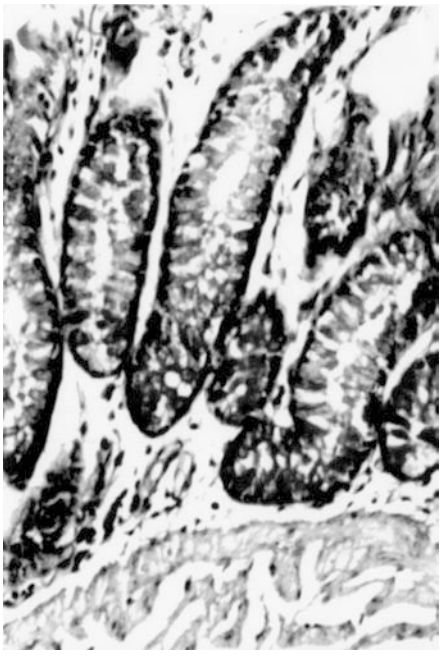

Fig. 8 A section of colonic patch of a group 2 rabbit with PAS ± mucin-secreting cells. PAS ×400

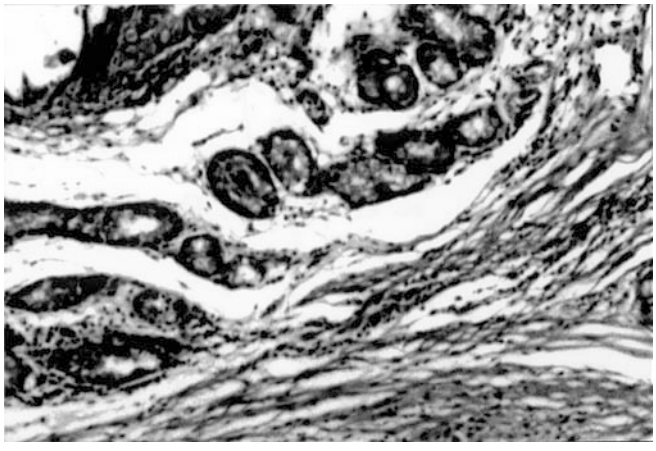

Fig. 9 A section of colonic patch of a group 2 rabbit showing lymphocyte infiltration distributed throughout the mucosal and submucosal tissue. H and E ×200

preserved bladder allograft, pericardium, collagen films and small intestinal submucosa [6, 18, 19, 20, 21, 32, 34, 38]. Results with these experimental models have been variable but superior to those achieved with synthetic materials. The growth of urothelial cells in vitro has been achieved by several groups [8]. Authors demonstrated the feasibility of culturing urothelium and smooth myocytes on a polyglactin scaffold and transferring them back into the animal [8]. While these early results of ''tissue engineering'' are fascinating, they have yet to see clinical application.

Photochemical or chemical de-epithelialization of intestinal mucosa for bladder augmentation has been offered as an alternative to the aforementioned models [12, 28]. These authors think that removing the bowel epithelium chemically or photochemically would prevent mechanical trauma and possible ischemia, which is proposed to be responsible for any subsequent fibrosis when the mucosa is physically stripped. Although the initial efforts to achieve intestinal de-epithelialization and urothelialization by chemical or photochemical means were encouraging, the intestinal mucosa was associated with urothelial cells in these studies.

We recently reported our success in de-epithelialization and subsequent uroepithelialization of intestinal mucosa using 10 g/100 ml $AgNO_3$ solution in a rat model of augmentation [5]. Encouraged by the promising results in rats, we decided to investigate the outcome of silver nitrate application in the rabbit model of augmentation. The two colocystoplasty groups were designed to assess the urodynamic and histologic outcomes of this application. Histologic results showed that 10g/100 ml $AgNO_3$ applied to the bowel surface before augmentation leads to complete de-epithelialization and partial urothelialization of the colonic mucosa within 4 weeks. Over an 8-week period, the transitional epithelium completely lined the de-epithelialized colonic segments in that group of rabbits (Figs. 4, 5). At the end of an 8 week experimental period, no foci of colonic mucosa were found in the treatment group (Figs. 4, 5). Collagen deposition was moderate in all groups of rabbits and there was no obvious difference in the level of collagen deposition in the grafted colonic segments in all rabbits killed at the end of the 8 week experimental period (Table 2). No significant differences were observed in maximal bladder capacity or leakage pressure measurements in any of the experimental groups (Table 2).

Silver ions precipitate protein and are strongly bactericidal [13, 15, 22, 36, 37]. The cytotoxicity mechanism of silver ions has been shown in several in vitro studies. Silver salts in different concentrations exert an inhibitory effect on the proliferation and differentiation of several cell lines, such as bone marrow cells, keratinocytes, hepatocytes, lymphocytes and leukocytes [14, 16, 17, 23]. Inhibition of DNA synthesis is a major cytotoxicity mechanism of $AgNO_3$ [15]. Another mechanism by which silver ions cause cell injury is mitochondrial dysfunction which leads to the inhibition of ATP synthesis [15]. This toxic effect may be the result of the blockage of the electron transport system, depolarization or altered permeability of the mitochondrial membrane, or mitochondrial DNA damage. We think that these toxic effects of silver ions play a major role in the chemical de-epithelialization of intestinal mucosa. In our study, histologic examination showed that the underlying submucosa, muscle and serosa were left intact and not affected by silver cytotoxicity. We also think that the intact submucosal layer provides a scaffold for urothelial re-epithelialization and prevents fibrosis or contraction of the de-epithelialized augmented segment.

Our results confirm that the application 10 g/100 ml $AgNO_3$ solution leads to the de-epithelialization and subsequent urothelialization of colonic mucosa with the preservation of the underlying submucosa, muscularis, and serosa in a rabbit model of augmentation. Although this technique might be applicable to other intestinal segments, thus far our experiments have been limited to sigmoid colon because this is the segment most commonly used in our practice. The long-term outcome of this application, although promising in view of the short-term findings, will need to be tested experimentally. If long-term experimental studies substantiate our results, this procedure could have applications to human surgery.

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