The immediate enzymatic cocktail effect on the absorptive function of neobladder made by ileocystoplasty

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Summary

The effectiveness of an enzymatic cocktail, collagenase and trypsin, on the absorptive function of ileal segment was studied to evaluate the changes within groups after performing ileocystoplasty in an animal model. Twenty-one female mixed-bred Persian dogs were randomized into 7 groups. The groups included: 1) negative control group (NCG) in which no ileocystoplasty was performed (n = 3); 2) positive control group (PCG) in which routine ileocystoplasty was performed (n = 3) and groups 3 to 7) those underwent ileocystoplasty with the ileal segment being treated with collagenase and trypsin for 5, 10, 15, 20 or 25 min (groups, 5 minutes enzymatic treatment group (5METG), 10METG, 15METG, 20METG and 25METG; each containing three dogs). To perform the glucose absorption test, 50% dextrose was instilled into the bladder five weeks after surgery. The blood glucose level (BGL) was measured in each group before the study (T1), two weeks after the surgery in PCG and other treatment groups (T2), before anaesthesia (T3), after anaesthesia at 5-minute intervals up to 25 min (T4 to T9, respectively). There was no significant difference in BGL in T1 to T3 and T4 to T9 in 25METG and NCG, however, BGL was found significantly higher in T1 to T3 and T4 to T9 in PCG, 5METG, 10METG, 15METG and 20METG. It can be concluded that collagenase and trypsin can affect absorptive function of the neobladder and are able to reduce the absorptive function, particularly in early days after the surgery. Moreover, these results also confirm that 50% dextrose instilled into the neobladder and native urinary bladder will not increase BGL in 25METG and NCG even with increasing the time of exposure of neo- or native urinary bladder to enzymatic cocktail till 25 min.

Key words: Neobladder, Absorptive function, Glucose, Dog

Introduction

Ileocystoplasty is used quite commonly for reconstruction of end-stage bladder dysfunction when other more conservative treatments fail. Almost all parts of the gastrointestinal system have been successfully incorporated into the urinary bladder for urinary diversion, increase capacity, improve compliance, abate uncontrollable detrusor contractility, or intractable interstitial cystitis (Rowland and Mitchell, 1985; Klimberg and Wajsman, 1986; Hendern and Hendern, 1987; Mitchell and Piser, 1987; Melchior *et al*., 1988). Complications include the metabolic disturbances secondary to electrolyte reabsorption, metabolic acidosis because of absorptive functions of epithelial tissue of ileal segment, asymptomatic chronic bacteriuria, urinary tract infections, vitamin B_{12} and nutritional deficiencies in pediatric patients, urinary obstruction secondary to mucus formations of goblet cells, diarrhoea following neural stretch reflexes, osteomalacia and osteopenic changes, adenocarcinoma and bladder rupture following ischaemia or scar tissue formation along the anastomosis, which may question the beneficial effects of the procedure (Rink and Adams, 1998).

Several techniques were introduced for mechanical ablation of gastrointestinal mucosa with different clinical results (Rink

and Adams, 1998). Enzymatic treatment of the ileal segment has been suggested for de-epithelialization of the mucosa and reducing its absorptive and secretive functions (Turkeri *et al*., 1996; Bakhtiari *et al*., 2005).

The objective of this study is to evaluate the effectiveness of treatment of ileal segment with collagenase and trypsin on its absorptive function in early days after the surgery and to determine whether this method can be applied to minimize the postoperative absorptive function of the ileal segment.

Materials and Methods

Twenty-one female Persian mixed-bred dogs, aged from 12 to 24 months and weighing between 15 and 24 kg, were vaccinated and received anti-helminthic drugs. Dogs received neomycin sulfate, 0.5 mg/kg, PO, for 24 hrs before operation, and were kept off feed 12 hrs before surgery. The dogs were randomized into seven groups: 1) negative control group (NCG) in which ileocystoplasty was not performed; 2) positive control group (PCG) in which routine ileocystoplasty without enzymatic treatment of the ileal segment was performed; 3-7) treatment groups in which ileocystoplasty was performed with 5, 10, 15, 20 or 25 min enzymatic treatment of ileal segment with a 0.125% enzymatic cocktail consisting of collagenase and trypsin on respective treatment groups (5METG, 10METG, 15METG, 20METG and 25METG each contained three dogs).

An intravenous line was inserted preoperatively. All dogs received 5% dextrose-lactate-Ringer's solution at 20 ml/kg/hr. For prophylaxis, cefazolin (20 mg/kg, IV) was administrated before induction of anaesthesia. The anaesthetic protocol consisted of atropine sulfate (0.02 mg/kg, SC) as premedication in addition to diazepam (0.27 mg/kg, IV) and ketamine hydrochloride (5.5 mg/kg, IV) for induction and 1% halothane for maintenance of anaesthesia.

Following surgical preparation, surgery was performed through a midline abdominal approach. A 25-cm ileal segment at least 10 cm distal to the ileocecal valve and before the ileocecal ligament with an adequate mesentery to reach the native bladder was selected. The mesentery was removed from the bowel at each end for a short distance to create a window, and the bowel was divided at these ends. Intestinal continuity was re-established with an ileoileostomy created using an end-to-end anastomosis with a single-layer simple interrupted 3-0 polyglactin 910 suture. The ileal segment for augmentation was irrigated with 0.9% normal saline and 0.25% neomycin sulfate solution. Enzymatic treatment of ileal segment with a predetermined protocol, on times stated earlier, was performed in treatment groups. The ends of intestinal segment, which had been clamped during the enzymatic treatment, were cut to ensure equal enzyme contact throughout the segment. Fifteen cm of the ileal segment was opened on its antemesenteric border, detubularized and reconstructed in a U-form shape. The bladder was incised in the sagittal plane extending near the bladder neck ventrally and near the trigone dorsally. Anastomosis of the ileal segment to the native bladder was done in two-layer fashion (an inner layer of running 3-0 polyglactin 910 suture and an outer layer of running Cushing 3-0 polyglactin 910 suture). The mesenteric window at the bowel anastomosis was closed. Water tightness of ileoileostomy and ileocystoplasty were checked with injection of 0.9% normal saline solution into the neobladder for detecting any leakage from suture lines. The abdominal cavity was irrigated with 0.9% normal saline solution. The peritoneum and abdominal muscles, and also subcutaneous tissue were separately closed with a single-layer running 3-0 polyglactin 910 suture. Skin was closed with simple interrupted 3-0 polyamide sutures. The skin was bandaged to prevent self-mutilation and contamination. Postoperatively, all dogs received intravenous fluids for 24 hrs and cefazolin (20 mg/kg, IM, q 8 h) for 3 days.

The absorptive capacity of ileal segment epithelium was determined by glucose absorption test for confirming its rate. Five weeks after surgery, 60 ml of 50% dextrose instilled through 12 French Foley catheters into bladder. The blood glucose level (BGL)

was measured in each group before study (T1), two weeks after the surgery in PCG and other treatment groups (T2), before anaesthesia (T3), after anaesthesia and from 5-minute intervals up to 25 min (T4 to T9, respectively).

Statistical analysis

A mixed-model (repeated-measures) analysis of variance (ANOVA) was used to compare BGL within each group at different times during the study. A p-value <0.05 was constituted statistically significance (Petrie and Watson, 1999; Shannon and Davenport, 2001).

Results

All animal tolerated the surgery well. This confirmed that ileocystoplasty can be an appropriate operation for treatment of bladder disorders in dogs. The quantitative data collected from evaluating of BGL revealed no significant difference between NCG and 25METG throughout two separate times (T1 to T3 and T4 to T9). Other groups, 5METG, 10METG, 15METG and 20METG, had also no significant difference with each other over the mentioned time. Although BGL following ileocystoplasty was almost similar between NCG and 25METG on T2 and T3, it was much higher among the rest groups (5METG, 10METG, 15METG and 20METG) over the same period. The important point was that BGL showed no significant difference within both groups (NCG and 25 METG) at various times in the early period. BGL in group 20METG was nearly less than 5METG, 10METG and 15METG. It, however, was significantly different from NCG and 25METG (Fig. 1).

Fig. 1: Glucose absorption with time in groups. T1: before the study; T2: two weeks after beginning of the study in PCG and other treatment groups; T3: before anaesthesia for glucose absorption test; T4 to T9: after anaesthesia for glucose absorption test at 5-min intervals up to 25 min, respectively; NCG: negative control group; PCG: positive control group; 5METG, 10METG, 15METG, 20METG and 25METG: 5, 10, 15, 20 or 25 min enzymatic treatment groups, respectively

Discussion

Use of parts of intestine is now one of the best and useful methods used for engraft of urinary bladder. These methods have known complications, some of which are harmful and severe, particularly during early days and weeks following enterocystoplasty (Rink and Adams, 1998), which could attribute to the intestinal part used (Goldwasser *et al*., 1986; Narayan *et al*., 1991). One of the useful choices is the ileal segment that was vastly used for treatment of many disorders of urinary bladder since (1889) (Rink and Adams, 1998). The researches try to introduce the best and reliable way to achieve suitable solution for reduction in immediate post-operative complications due to graft. Mechanical de-nudation of intestinal mucosa, seromuscular enterocystoplasty, chemical destruction of the intestinal mucosa by hypertonic saline solution, hemiacidrin, photochemical ablation using haematoporphyrin derivatives and red light, collagenase and trypsin in rats and dogs, protamine sulfate and urea solution, silver nitrate solution and HEPES-buffered saline (N-[2-hydroxyethel] piperazine-N'-[2-etha-nesulfonic acid) have been suggested for de-epithelialization of the ileal mucosa and reducing its absorptive and secretive functions in order to fulfill the requirements of an ideal substitute for the urinary bladder tissue (Brandell *et al*., 1994; Haselhuhn *et al*., 1994; Niku *et al*., 1995; Rink and Adams, 1998; Demirbilek *et al*., 2001; Liu *et al*., 2001; Bakhtiari *et al*., 2005).

Glucose is gradually absorbed from the neobladder with autotransplanted ileum. The absorption rate of neobladder, using standard ileocystoplasty procedure, is rapid. The malabsorption of an intestinal segment

used as the urinary bladder seems to be favorable, because the hyperchloremic metabolic acidosis caused by urine reabsorption can not occur. However, the studies have shown that carbohydrate absorption is not generally impaired (Ueno *et al.*, 2001).

Being BGL in baseline value in 25METG at the end of the second and fifth weeks showed that the enzymatic cocktail used is able to reduce absorption capability of ileal segment in early days and weeks. The high BGL observed in other groups over the same period revealed insufficiency of the enzymatic treatment between 5 and 20 min and ineffectiveness of urine on absorptive function of ileal segment during the early days and weeks. The last finding states to relief early and prematurely complications due to ileal segment which are very harmful and have unwilling outcome in early days. The change of the absorptive capability within 25METG was confirmed by Bakhtiari *et al*., (2005), with instillation of 50% dextrose into the neobladder. They found that the ileal segment of neobladder is unable to absorb glucose following 25 min of treatment of ileal segment with enzyme.

Kojima *et al*., (1998) showed that the ileal segment changes after six months resulted in its reduced absorptive function, so that urine solute is unable to decrease absorptive function within early months. We also found that the ileal segment absorptive function does not change while urine is in neobladder lumen and urinary bladder in early weeks in PCG, 5METG, 10METG, 15METG and 20METG.

Kojima *et al*., (1998), showed that electrolyte imbalance is expectable during early months, particularly in patients who being suffer from chronic renal failure. We showed that it is possible to decrease and even abolish the absorptive function of the ileal segment using enzymatic cocktail for early time post-operatively. This finding is noticeable and useful outcomes in patients with renal failure who are prone to electrolyte imbalance following ileocystoplasty. The latter problem can put question on using of ileal segment as a suitable native tissue for cystoplasty in animals and men. Finally, it was stated that enzyme, as a chemical agent, is able to not only reduce and even omit the absorptive capacity in early days but its effect could also be continued on epithelial cells even for longer time and is constant at least for more than one month.

The intestinal epithelium is mainly responsible for absorption of glucose with sodium (Guyton and Hall, 2000). Normal bladder has neither absorptive nor secretive ability (Ueno *et al*., 2001). The reduction of absorptive function of enzymatically-treated ileal segment was probably due to changes in the ultrastructures of cellular membranes (Turkeri *et al*., 1996). Although this study showed that glucose absorption through the ileal segment used was ceased after 25 min of enzymatic treatment, some extrapolations may be made regarding the associated electrolyte movements. Further detailed biochemical, haematologic and histopathologic evaluation of ileocystoplasty is required to recognize the exact mechanism of enzyme processing of the ileal segment.

Enzymatic treatment of ileal segment by collagenase and trypsin has been used successfully on rats by Turkeri *et al*., (1996). The treatment completely abolished the absorptive function of the epithelium, while BGL increased to about three times the baseline value in the PCG on the 21st day (Turkeri *et al*., 1996). It has been reported that silver nitrate can reduce the absorptive function of the ileal segment (Demirbilek *et al*., 2001). Niku *et al*., (1995) also showed that protamine sulfate and urea were able to de-epithelialize intestinal mucosa up to 90%, which the sequel confirmed that ileal segment was inactive after using chemical agents and it is the reason of electrolyte balances after ileocystoplasty.

The absorptive function of the ileal mucosa reduced following collagenase-trypsin therapy in specific in early phase. According to our findings, the purpose mentioned in the summary can be confirmed that dogs could tolerate ileocystoplasty and over in two separate times, in early days and even 5 weeks post-operation, the capability of neobladder glucose absorption from urine will significantly reduce.

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