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Development of a murine colonoscopic polypectomy model (with videos)

Furkan Ertem, MD¹, Wan-Mohaiza Dashwood¹, Praveen Rajendran, PhD¹, Gottumukkala Raju, MD², Asif Rashid, MD, PhD³, and Roderick Dashwood, PhD^{1,4,5,6}

¹Center for Epigenetics & Disease Prevention, Texas A&M Health Science Center, Houston, Texas, USA

²Department of Gastroenterology, Hepatology & Nutrition, MD Anderson Cancer Center, Houston, Texas, USA

³Department of Pathology, MD Anderson Cancer Center, Houston, Texas, USA

⁴Department of Nutrition & Food Science, Texas A&M University, College Station, Texas

⁵Department of Molecular & Cellular Medicine, College of Medicine, Texas A&M University, College Station, Texas

⁶Department of Clinical Cancer Prevention, MD Anderson Cancer Center, Houston, Texas, USA

Abstract

Background—Colonoscopy provides a means for screening and removal of colonic adenomas, preventing such lesions from progressing to late-stage carcinoma. No preclinical model currently exists that closely parallels the clinical scenario with respect to polyp resection and recovery post-endoscopy.

Methods—When we used the polyposis in rat colon (Pirc) model, a new polypectomy methodology was developed. A novel “PLC” classification system (Polyp Number/Location/Clockwise orientation) also was devised in order to accurately and reproducibly specify the location of each lesion within the colon.

Results—One week after surgery, injuries to the polypectomy site were confined to the submucosa, indicating that little or no damage occurred to the inner muscle layer of the colon.

Correspondence: Roderick Dashwood, PhD, Director, Center for Epigenetics & Disease Prevention, Texas A&M Health Science Center, 2121 West Holcombe Blvd., Houston, Texas 77030, USA. ; Email: rdashwood@ibt.tamhsc.edu.

Guarantor of the article: Roderick Dashwood, Ph.D.

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Polypectomy sites occasionally continued to show ulcer formation, whereas others exhibited tissue regeneration. A pilot study (n = 6 animals), involving a total of 37 polypectomies, confirmed that the new methodology could be applied using either air insufflation or water-assisted techniques, with either hot or cold snare. As a general observation, polyps tended to be more fully distended and less flattened against the colonic mucosa using the water-assisted protocol, increasing the technical ease of ensnaring and resecting lesions. The PLC system proved to be straightforward, and facilitated longitudinal studies by allowing the investigator to track each polypectomy site upon repeated examination.

Conclusions—The Pirc model was ideally suited to colonoscopy with polypectomy. Because the main cause of morbidity in the Pirc model is blockage of the colon, polypectomy can be used as a preventive strategy, and will likely facilitate long-term investigations of single agent and combination therapies with potential direct clinical relevance.

Keywords

Pirc model; Colonoscopy; Polypectomy; Air-insufflation; Water-assisted

INTRODUCTION

Colonoscopy provides the best current approach for screening and removal of colonic adenomas.¹ Colonoscopies traditionally have been performed with air insufflation, which provides at least 95% cecal intubation success rate.² Water-assisted colonoscopy recently has been discussed as an improved approach,³ requiring less sedation.^{4–9} An increase in the proportion of colonoscopy examinations performed with the patient under sedation¹⁰ suggests that further work is needed to validate the respective procedures.¹¹

We developed a preclinical methodology that parallels the clinical scenario for colonoscopy with polyp resection. The polyposis in rat colon (Pirc) model was chosen based on several favorable features, including the presence of a significant colon tumor burden.¹² A pilot study examined the feasibility of using air insufflation and water-assisted techniques, with hot or cold snare, as described below.

METHODS

Animals

A 9-month time-point arbitrarily was chosen in the Pirc model. Given the importance of adequate bowel preparation,^{13,14} rats were fasted for 24 hours, but had free access to water. On the rare occasion of incomplete bowel preparation, an enema of pre-warmed water was applied with a Pasteur pipette.

System

The basic platform (Figure 1) included a micro-endoscope (Hopkins Forward-Oblique, 30°, diameter 2.7 mm, length 18 cm, Karl Storz, Goleta, Calif), examination sheath with 2 ports (14.5F, 15 cm working length, working channel 5F, dual stopcocks, Karl Storz), camera capture system (Image SPIES, Karl Storz), and light source (Cold Light Fountain Power LED 175 SCB, Karl Storz). For air insufflation, an air pump (Petco Aquarium Air Pump,

Houston, Tex) was connected to one port of the examination sheath (Figure 1), with continuous air flow throughout the procedure.

For water-assisted colonoscopy, a peristaltic pump (3A, 115VAC, Celsep, Brinkmann Instruments, NY, USA) provided pre-warmed water from the water source (WS, Figure 1). Water released during the procedure was collected in a stainless tray placed under the procedure platform (Figure 1). To avoid hypothermia, the animal was placed on a water-proof heating pad (K&H Pet Products, Colorado Springs, Colo). An insulating mat also was used for grounding during electrocautery (Figure 1). Electrocautery was maintained between 5 to 15 W to avoid possible transmural necrosis and microperforation.

Polypectomy

A rigid colonoscope was inserted with an attached snare tube (Figure 1b), allowing the first 10 cm of the colon to be visualized. In addition to the cold snare, hot snare was facilitated by linking the 2.4 mm snare (Karl Storz) to an electrocautery system (Autocon II, Karl Storz). After reaching the most proximal region of the colon, gradual withdrawal of the scope allowed for sequential visualization and resection of polyps. By switching the snare position relative to the colonoscope and repeating the procedure on the same animal, most or all of the polyps were amenable to resection. During polypectomy under air, the water port was switched off at the tee-junction (Figure 1a), and as one person positioned the colonoscope, another handled the snare loop.

To assist in identifying each polyp during repeat procedures, a new “PLC” classification system was developed: P, polyp number from the anus; L, location of polyp from the anus on withdrawal (cm); C, clock-face orientation of polyp within the lumen. The PLC approach requires the animal to be kept in the same orientation during sequential colonoscopy procedure. Because polyp size is a risk factor for bleeding and other adverse events,^{15–19} lesions were size-matched as closely as possible.

After polypectomy using either hot or cold snare, rats were followed daily until 1 week post-surgery. Observations were made for rectal bleeding (fresh bleeding, blood in the feces, melena), general behavior and posture, food consumption, and body weight. One week post-surgery, the entire colon was viewed again by colonoscopy before killing the animal. A full gross pathology examination was performed and tissues were prepared for H&E staining. The pathologist, blinded to the treatment condition, provided an evaluation of injury score based on the previously described system for preclinical specimens.²⁰ Studies were approved by the Institutional Animal Care and Use Committee.

RESULTS

As reported²¹, overnight fasting can provide adequate bowel preparation in murine preclinical models. Occasionally, residual gut contents obscured the view using water-assisted colonoscopy. This could increase the time to snare the polyp from under 30 seconds to about 1 minute.

No differences were noted in behavior, body weight, or food consumption during the respective procedures. Rats that underwent hot snare with electrocoagulation showed no signs of distress, localized peritonitis, or perforation. Active fresh bleeding generally stopped within 15 min of resection. In a pilot study with 6 rats (Table 1), 37 polypectomies were performed in total, using either water-assisted (Video 1) or air insufflation colonoscopy (Video 2). In the latter procedure, polyps tended to be more fully distended within the lumen (Figure 2), which facilitated positioning of the snare.

Animals were examined before, immediately after, and 1 week after polyp resection (Figure 3). One week after surgery, polypectomy sites occasionally continued to show ulcer formation (Figure 4a), whereas others exhibited tissue regeneration (Figure 4b). At the end of the study, the entire-colon view was much improved, even after multiple polyp resections (Video 3). Evidence of residual adenoma or carcinoma at the polypectomy site was extremely rare. A prior scoring system was used for depth of tissue injury²¹, in which the most extreme category 5 designates “Perforation.” Animals in the current investigation were assigned either to category 1 “Submucosa, but not reaching muscularis propria” or to category 2 “Muscularis propria, within the internal muscle layer” (Table 1, final column).

DISCUSSION

We report here the first methodology for polypectomy and surgical recovery in a murine model, with tissue injury scores indicating little or no damage to the inner muscle layer of the colon. The new polypectomy procedure was performed successfully using air-insufflation and water-assisted approaches in the Pirc model, but we are cautious about drawing firm conclusions on the relative merits of each procedure in the context of human clinical practice. A larger investigation appears to be warranted, with more animals and greater statistical power. This could highlight the pros and cons of each procedure, and the relative contributions of inter-individual as well as intra-individual lesion differences.

The new PLC classification system (Figure 2) was straightforward, easily adopted by each trainee, and facilitated longitudinal studies by enabling the investigator to track and categorize the temporal progression of each lesion. However, it is important to use a consistent approach, since size-grading of lesions can differ between air insufflation and water-assisted colonoscopy procedures.²¹

The new preclinical methodology closely parallels the clinical scenario involving colonoscopy and polyp resection, with no apparent deleterious consequences toward the health and wellbeing of the animal. This protocol permitted comparison of hot and cold snare capture using air insufflation or water-assisted approaches. More extensive application will likely help to clarify the pros and cons of different colonoscopy procedures, provide a useful training platform for colonoscopists, and facilitate longitudinal studies of biomarkers for colorectal cancer prevention and treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A, View of water-assisted colonoscopy in the polyposis in rat colon (Pirc) model, showing water port (W) and air port (A) supply lines connected to a tee-junction. **B,** Pirc animal placed on a water-proof heating pad (H) showing position of examination sheath (E), snare tube (S), and grounding for electrocautery (G), as well as peristaltic pump (P) and water source (WS).

Figure 2.
A, Clock-face orientation of lesions with water-assisted (left) and air insufflated colonoscopy (right) in the Pirc model. **B,** Polyp floating during water-assisted colonoscopy (left), and sitting close to the mucosa during air insufflated colonoscopy (right).

Figure 3.

A, air insufflated colonoscopy and **B**, water-assisted colonoscopy in the Pirc model, with sequential views of the lesion site before, immediately after, and 1 week after polyp resection.

Figure 4. One week post-surgery. H&E staining; scale-bar at lower right = 500 μm . **A**, Ulcer **B**, tissue regeneration at the site of polypectomy (arrow) in the Pirc model.

Table 1

Summary of colonoscopy and polyp resection in the Pirc model

Rat Gender	No. of polyps resected	Colonoscopy (air or water)	Polypectomy (hot or cold snare)	Body weight change (%) 1 week after polypectomy	Injury score (mean)
Female	6	Air	Hot	0% (248→248 g)	1.4
Female	6	Water	Hot	-4% (246→236 g)	1.3
Female	5	Air	Cold	+3% (214→222 g)	1.0
Female	4	Water	Cold	+2% (240→246 g)	1.0
Male	5	Air	Hot	+3% (340→351 g)	1.1
Male	11	Water	Hot	+3% (291→300 g)	2.0