

Simultaneous follow-up of mouse colon lesions by colonoscopy and endoluminal ultrasound biomicroscopy

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Abstract

AIM: To evaluate the potential use of colonoscopy and endoluminal ultrasonic biomicroscopy (eUBM) to track the progression of mouse colonic lesions.

METHODS: Ten mice were treated with a single azoxy-

methane intraperitoneal injection (week 1) followed by seven days of a dextran sulfate sodium treatment in their drinking water (week 2) to induce inflammation-associated colon tumors. eUBM was performed simultaneously with colonoscopy at weeks 13, 17-20 and 21. A 3.6-F diameter 40 MHz mini-probe catheter was used for eUBM imaging. The ultrasound mini-probe catheter was inserted into the accessory channel of a pediatric flexible bronchofiberscope, allowing simultaneous acquisition of colonoscopic and eUBM images. During image acquisition, the mice were anesthetized with isoflurane and kept in a supine position over a stainless steel heated surgical waterbed at 37 °C. Both eUBM and colonoscopic images were captured and stored when a lesion was detected by colonoscopy or when the eUBM image revealed a modified colon wall anatomy. During the procedure, the colon was irrigated with water that was injected through a flush port on the mini-probe catheter and that acted as the ultrasound coupling medium between the transducer and the colon wall. Once the acquisition of the last eUBM/colonoscopy section for each animal was completed, the colons were fixed, paraffin-embedded, and stained with hematoxylin and eosin. Colon images acquired at the first time-point for each mouse were compared with subsequent eUBM/colonoscopic images of the same sites obtained in the following acquisitions to evaluate lesion progression.

RESULTS: All 10 mice had eUBM and colonoscopic images acquired at week 13 (the first time-point). Two animals died immediately after the first imaging acquisition and, consequently, only 8 mice were subjected to the second eUBM/colonoscopy imaging acquisition (at the second time-point). Due to the advanced stage of colonic tumorigenesis, 5 animals died after the second time-point image acquisition, and thus, only three were subjected to the third eUBM/colonoscopy imaging acquisition (the third time-point). eUBM was able to detect the four layers in healthy segments of

colon: the mucosa (the first hyperechoic layer moving away from the mini-probe axis), followed by the muscularis mucosae (hypoechoic), the submucosa (the second hyperechoic layer) and the muscularis externa (the second hypoechoic layer). Hypoechoic regions between the mucosa and the muscularis externa layers represented lymphoid infiltrates, as confirmed by the corresponding histological images. Pedunculated tumors were represented by hyperechoic masses in the mucosa layer. Among the lesions that decreased in size between the first and third time-points, one of the lesions changed from a mucosal hyperplasia with ulceration at the top to a mucosal hyperplasia with lymphoid infiltrate and, finally, to small signs of mucosal hyperplasia and lymphoid infiltrate. In this case, while lesion regression and modification were observable in the eUBM images, colonoscopy was only able to detect the lesion at the first and second time-points, without the capacity to demonstrate the presence of lymphoid infiltrate. Regarding the lesions that increased in size, one of them started as a small elevation in the mucosa layer and progressed to a pedunculated tumor. In this case, while eUBM imaging revealed the lesion at the first time-point, colonoscopy was only able to detect it at the second time-point. All colonic lesions (tumors, lymphoid infiltrate and mucosal thickening) were identified by eUBM, while colonoscopy identified just 76% of them. Colonoscopy identified all of the colonic tumors but failed to diagnose lymphoid infiltrates and increased mucosal thickness and failed to differentiate lymphoid infiltrates from small adenomas. During the observation period, most of the lesions (approximately 67%) increased in size, approximately 14% remained unchanged, and 19% regressed.

CONCLUSION: Combining eUBM with colonoscopy improves the diagnosis and the follow-up of mouse colonic lesions, adding transmural assessment of the bowel wall.

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Key words: Ultrasound biomicroscopy; Animal model; Diagnostic imaging; Colonic neoplasm; Longitudinal study

Core tip: This paper employed imaging methods, endoluminal ultrasonic biomicroscopy (eUBM) associated to colonoscopy, in a longitudinal study to evaluate the progression of chemically-induced colonic lesions in mice, during a period of two months. The eUBM method complemented colonoscopy and enhanced the study, once the ultrasonic images allowed the detection of lesions underneath the epithelium. Potential future application of eUBM combined with colonoscopy could be in the monitoring of therapeutic efficacy of chemotherapeutic drugs *in vivo*.

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INTRODUCTION

Colorectal cancer (CRC) has a high incidence in the world as it is the third most common cancer in men and women in developed countries^[1]. It is estimated that more than 142000 people in the United States will be diagnosed with CRC in 2013^[2]. In Europe, CRC is detected in approximately 413000 people each year, half of whom die during the course of the disease. Despite its high incidence and mortality rates, the majority of CRC-related deaths could be prevented through the implementation of powerful tools for CRC early detection and staging.

Currently, colonoscopy is the recommended screening method for CRC screening and follow-up, but it has some limitations. Studies have demonstrated that the detection of adenomas, serrated polyps and sessile serrated adenomas differs significantly among endoscopists^[3]. Furthermore, colorectal neoplasms of a diminutive size (smaller than 10 mm) or nonpolypoid shape may be more easily overlooked during a routine colonoscopy^[4-6]. The miss rate for CRC lesions may explain the high proportion (3.3%-12.4%) of proximal CRC that is diagnosed shortly after a clearing colonoscopy^[7,8]. Therefore, the efforts of some research groups are focused on the development of other imaging methods that complement the results of a colonoscopy.

High frequency endoscopic ultrasonography (EUS) is a relatively new technique in which an ultrasonography probe is inserted into the accessory channel of a regular endoscope. EUS has the capacity to look deep below the lining of the colon and is a useful modality for transmural assessment of the bowel wall^[9,10]. Usually, the ultrasound transducers used in EUS instrumentations operate at low frequencies (7.5-12 MHz), but higher ultrasound frequencies are also employed by using a mini-probe^[11-13]. Higher ultrasound frequencies increase EUS resolution, allowing for the staging of colon tumors and the visualization of small colonic lesions. The use of high frequency mini-probe ultrasound for the diagnosis of mucosal and submucosal colorectal lesions and for the guidance of lesion resection has already been proposed as a safe and effective technique^[13,14]. However, the role for high frequency mini-probe ultrasound in the routine diagnosis of colonic lesions has not been fully established^[15-18].

Animal models of diseases can be used to develop and evaluate new diagnostic tools before they are applied clinically. The development of non-invasive experimental imaging modalities allows for the study of the same ani-

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mal over time, enabling the investigation of disease development and therapeutic interventions. Mouse models of chemically induced CRC are highly reproducible, can be tested on animals with different genetic backgrounds and recapitulate human CRC. The use of an effective and valuable mouse model of chemically induced CRC can help investigators understand colonic tumorigenesis and to probe novel diagnostic platforms for use in clinical practice^[19].

Our group has previously used ultrasonic biomicroscopic (UBM) instrumentation, operating at 45 MHz, for *in vitro* imaging of chemically induced mouse CRC^[20] to demonstrate that UBM is a feasible tool to identify the layers of mouse colon with adequate contrast between them and with sufficient resolution. Afterwards, endoluminal UBM (eUBM), operating at 40 MHz, was performed along with a colonoscopy, and simultaneous eUBM and colonoscopic images were generated *in vivo*^[21].

Recently, studies have verified the efficacy of UBM as a tool for longitudinal studies in mice: Harmon and co-workers^[22] validated the use of 40 MHz extracorporeal UBM for carotid plaque development in mice; Tiwari *et al*^[23] used a 40 MHz UBM for longitudinal monitoring of infliximab treatment efficacy in a mouse model of pancreatic cancer; Fernández-Domínguez *et al*^[24] also used a 40 MHz UBM in a longitudinal study to evaluate the progression of fatty liver disease in mice; and Campos-Junior *et al*^[25] analyzed the efficacy of UBM in the evaluation of induced ovarian follicular growth and ovulation in mice. Despite growing evidence confirming the efficacy of high frequency ultrasound in the monitoring of lesion progression, the ability of eUBM to diagnose colonic tumoral development in animal models has not yet been studied.

The present work comprises the use of eUBM instrumentation associated with colonoscopy in a longitudinal study to evaluate the progression of chemically induced colonic lesions in mice.

MATERIALS AND METHODS

Animals

Ten mice [*Mus musculus* (Linnaeus, 1758)] of both genders, with an average age of 7 wk, an average weight of 25 g, and $p53^{+/+}$ and $p53^{+/-}$ (heterozygous for tumor suppressor gene *Trp53*), were used. The mice were originally purchased from The Jackson Laboratory (Bar Harbor, ME, United States) and kept in the 129/SvJ background. We used the $p53^{+/-}$ mice because *Trp53* mutations accelerate tumorigenesis in several tissues, including the colon^[26].

The animals were maintained at room temperature with the appropriate circadian cycle and diet. The Guide for Care and Use of Laboratory Animals (National Institutes of Health) was also considered.

Colon tumors were induced using a protocol (DAHE-ICB 042) approved by the Animal Care and Use Commit-

tee of the Biological Science Institute/Federal University of Rio de Janeiro. The studies involving colon imaging, such as eUBM combined with colonoscopy, were conducted under a protocol (71/08) approved by the Ethical Committee for Laboratory Animal Research/Federal University of Rio de Janeiro.

Azoxymethane and dextran sulfate sodium carcinogenesis protocol

Inflammation-related colon tumors were induced using azoxymethane (AOM) and dextran sulfate sodium (DSS)^[27-29]. AOM is a colon-specific carcinogen that can be combined with DSS, a mucosal-irritant agent, to mimic inflammation-associated colon carcinogenesis^[29,30]. The animals were subjected to a single intraperitoneal (*ip*) injection of AOM (A5486; Sigma Aldrich, St. Louis, MO, United States) with a concentration of 12.5 mg/kg. One week after AOM administration, the mice were fed with water containing 3% DSS salt, 36000-50000 Da (02160110; MP Biomedicals, Santa Ana, CA, United States), for 1 wk. All of the mice received solid food and water *ad libitum*, with regular water given after the week of DSS intake.

Endoluminal ultrasonic biomicroscopy system

Briefly, images were generated by employing a 3.6-F diameter 40 MHz mini-probe catheter (Atlantis[®] SR Pro Coronary Imaging Catheter; Boston Scientific Corporation, Natick, MA, United States) mechanically driven by a motordrive unit (MD5; Boston Scientific Corporation, Natick, MA, United States). The ultrasonic transducer rotates 360° around its axis, providing cross-sectional ultrasound images of the colon wall. More details concerning the eUBM instrumentation are described in Alves *et al*^[21].

Simultaneous eUBM and colonoscopic image acquisition

Colonoscopy was used simultaneously with eUBM and served to guide the mini-probe through the colon. The ultrasound mini-probe catheter was inserted into the accessory channel of a pediatric flexible bronchofiberscope (FB120P; Fujinon, Tokyo, Japan), allowing simultaneous acquisition of colonoscopy and eUBM images. The bronchofiberscope has a total length of 920 mm and outer diameters of 2.8 and 2.7 mm for the flexible and distal-end portions, respectively.

To ensure that the colonoscopy and eUBM techniques acquired simultaneous images from the same region, the ultrasonic transducer, at the mini-probe imaging core tip, was positioned outside of the distal end accessory channel extremity, while still as close as possible to the bronchofiberscope extremity. The mini-probe telescoping shaft section was used to advance and retract the imaging core, placing it in the correct position.

During image acquisition, the mice were anesthetized with isoflurane (Cristália; São Paulo, Brazil) at 1.5% in 1.5 L/min oxygen, using a laboratory animal anesthe-

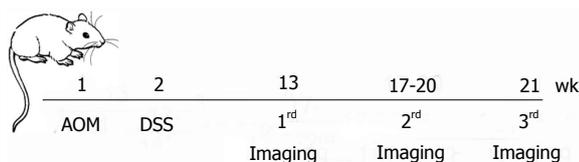


Figure 1 Schematic overview of the azoxymethane and dextran sulfate sodium model and subsequent image acquisition. A single azoxymethane (AOM) *ip* injection was given to 6-wk-old mice (week 1). One week later (week 2), 3% dextran sulfate sodium (DSS) administration was given in the drinking water for 7 d, followed by regular water. The first endoluminal ultrasonic biomicroscopy (eUBM) and colonoscopic images were acquired at week 13, the second acquisition was from weeks 17-20, and the third acquisition was at week 21.

sia system (EZ-7000; Euthanex, Palmer, PA, United States). The animals were kept in a supine position over a stainless steel heated surgical waterbed at 37 °C using the T/Pump System (Gaymar, Orchard Park, NY, United States). Before the examination, an enema was performed with 1 mL of water to remove feces. Subsequently, the flexible bronchofiberscope containing the ultrasound mini-probe catheter was introduced into the descending colon. Both eUBM and colonoscopy images were captured simultaneously and stored when a lesion was detected by colonoscopy or when the eUBM image revealed a modified colon wall anatomy. During the procedure, the colon was irrigated with water that was injected through a flush port of the mini-probe catheter and that acted as the ultrasound coupling medium between the transducer and the colon wall.

Study design

The sequential evaluation of colonic lesions by simultaneous *in vivo* eUBM and colonoscopic imaging started at 13 wk after AOM administration and was performed at three different time-points, according to Figure 1: the first one at week 13, the second one between weeks 17 and 20 and the last one at week 21.

Colon lesion images acquired at the first time-point for each mouse were compared with subsequent eUBM/colonoscopic images of the same sites obtained in the following acquisitions. After the last eUBM examination, the images of each lesion were separated for subsequent comparison with histopathology.

Histological analysis

Once the acquisition of the last image for each animal was completed, each anesthetized mouse was euthanized by cervical dislocation. The distal colon was excised, cleaned and fixed in 4% formaldehyde for 16 h before paraffin embedding. The paraffin-embedded tissues were cross-sectioned (5 µm) stepwise transversally to the colon longitudinal axis and stained with hematoxylin and eosin. All stained sections were analyzed by light microscopy and compared with the ultrasonic images, whose frames were obtained from the same lesions observed with the eUBM and/or colonoscopy.

Table 1 Simultaneous endoluminal ultrasonic biomicroscopy and colonoscopy image acquisition on colon tumor-bearing mice

Mouse number	Weeks after AOM administration		
	1 st eUBM	2 nd eUBM	3 rd eUBM
1	13	-	-
2	13	-	-
3	13	18	-
4	13	20	-
5	13	20	-
6	13	20	-
7	13	20	-
8	13	17	21
9	13	17	21
10	13	17	21

eUBM: Endoluminal ultrasonic biomicroscopy; AOM: Azoxymethane.

RESULTS

The time-points for image acquisition of each animal are presented in Table 1. All 10 mice had eUBM and colonoscopic images acquired at week 13. Two animals died immediately after the first imaging acquisition and, consequently, only eight mice were subjected to the second eUBM/colonoscopy imaging acquisition. Due to the advanced stage of colonic tumorigenesis, five animals died after the second time-point image acquisition, and thus, only three were subjected to the third eUBM/colonoscopy imaging acquisition.

An example of interrelated eUBM and histological images of a healthy section from the mouse colon is presented in Figure 2A. The mucosal layer is seen as a hyperechoic circular layer (the first hyperechoic layer moving away from the mini-probe axis), followed by a hypoechoic layer representing the muscularis mucosae. The submucosa corresponds to a hyperechoic layer, followed by the muscularis externa, the second hypoechoic layer. At the center of the lumen is the ultrasound mini-probe, represented by a gray circle. An eUBM image of a colonic lymphoid infiltrate, represented by a hypoechoic region between the mucosa and muscularis externa layers, is presented in Figure 2B with the corresponding histological image.

An example of an eUBM image of a pedunculated tumor, whose size increased during the 6 wk between the first and second time-points, is presented in Figure 3A. At the first eUBM exam, a small elevation in the mucosa layer is seen, indicating an early adenoma. At this time, colonoscopy was unable to visualize the lesion. Six weeks later, eUBM showed that the adenoma had increased in size, and the lesion was then observed in the colonoscopic image. Figure 3B presents an eUBM image of a pedunculated adenoma, whose size remained virtually unchanged between the first and third image acquisitions. The adenoma was also visualized in all of the colonoscopy sections. Finally, a sequence of three eUBM images of a lesion that decreased in size during the observation period is depicted in Figure 3C. This lesion was identified at the first eUBM exam (Figure

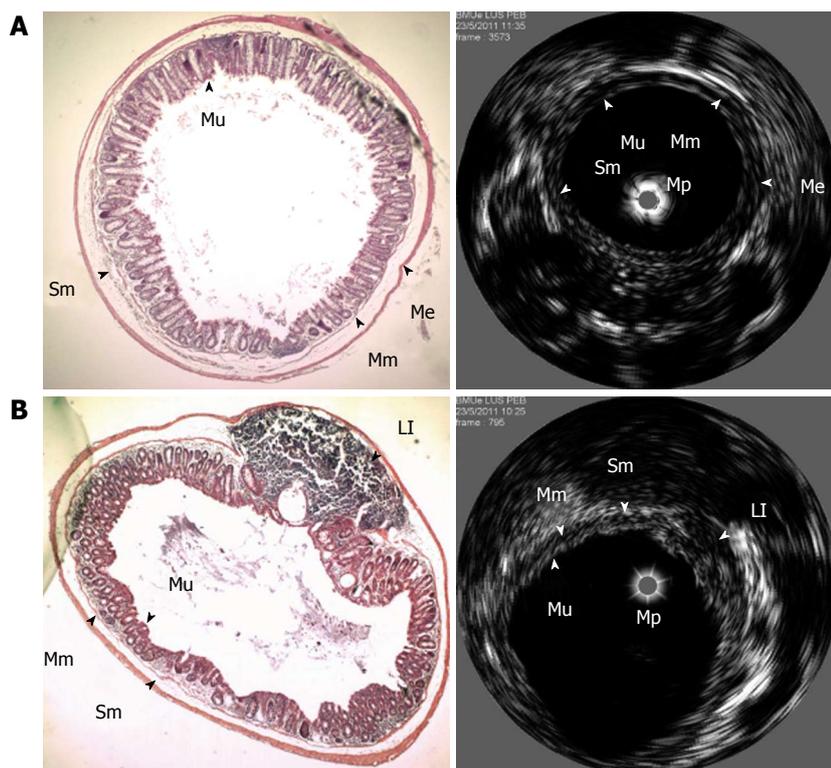


Figure 2 Correlation between endoluminal ultrasonic biomicroscopy and histological images. A: Endoluminal ultrasonic biomicroscopy (eUBM) (right) and the corresponding hematoxylin and eosin-stained histological section (left, $\times 40$ magnification) obtained from a healthy region of a mouse colon. The eUBM image displays two hyperechoic layers: mucosa (Mu) and submucosa (Sm) and two hypoechoic layers: muscularis mucosae (Mm) and muscularis externa (Me). The ultrasound catheter mini-probe (Mp) is at the center of the lumen; B: eUBM (right) and the corresponding hematoxylin and eosin-stained histological section (left, $\times 40$ magnification) obtained from a mouse colon containing a lymphoid infiltrate in the colonic wall. The eUBM image displays the mucosa (Mu), muscularis mucosae (Mm) and submucosa layer (Sm). The lymphoid infiltrate (LI) lesion is seen as a hypoechoic region underneath the mucosa. The ultrasound catheter mini-probe (Mp) is at the center. All layers identified in the ultrasound images are well correlated with the histological images from the same site.

3C-a) as a mucosal hyperplasia with ulceration at the top. The ulceration was also visualized by colonoscopy. Four weeks later, at the second eUBM exam (Figure 3C-b), the mucosal hyperplasia had decreased, and a hypoechoic area underneath the mucosa was observed, indicating the emergence of an inflammatory infiltrate. At this point, colonoscopy showed no alterations in this colonic section. At the last eUBM exam (Figure 3C-c), both the mucosal hyperplasia and lymphoid infiltrate had almost completely disappeared. Histological analysis of the same section confirmed the presence of the remaining diminutive lymphoid infiltrate section (Figure 3C-d).

Colonic lesions detected by either the last eUBM or colonoscopy, and confirmed by *post mortem* histology, are indicated in Table 2. Altogether, eUBM identified all of the lesions (tumors, lymphoid infiltrate and mucosal thickening), while colonoscopy identified just 76% of them. Colonoscopy identified all colonic tumors but failed to diagnose lymphoid infiltrates and increased mucosal thickness and failed to differentiate lymphoid infiltrates from small adenomas.

Additionally, the lesion progression outcomes, based on eUBM image analysis, are presented in Table 2. During the observation period, most of the lesions (approximately 67%) increased in size, approximately 14% remained unchanged and 19% regressed.

DISCUSSION

This report describes the use of a eUBM imaging system for the detection and follow-up of mouse colonic lesions. The simultaneous use of eUBM with colonoscopy was able to detect, diagnose and analyze the progression of tumoral and non-tumoral lesions in a CRC mouse model. Our group has previously demonstrated that two UBM systems, one operating at 45 MHz and the other at 40 MHz, could diagnose mouse colonic lesions *in vitro*^[20] and *in vivo*^[21], respectively. Here, we have demonstrated that a variety of colon lesions can be detected by eUBM in a minimally invasive way. In contrast to histopathological analysis, eUBM can be employed to make repeated measures on the same animal, facilitating the investigation of pathological processes and therapies.

Similar to the previous work, the ultrasound images obtained with eUBM also allowed for the visualization of normal colonic layers: the mucosa, muscularis mucosae, submucosa and muscularis externa (Figure 2A), as well as colon alterations, such as lymphoid infiltrates, ulcerations and tumors (Figure 2B and Figure 3). Confirming our previous findings, lymphoid infiltrates appear as hypoechoic regions underneath a hyperechoic layer representing the mucosa. Colon tumors appear as hyperechoic masses above the mucosa layer. This characteriza-

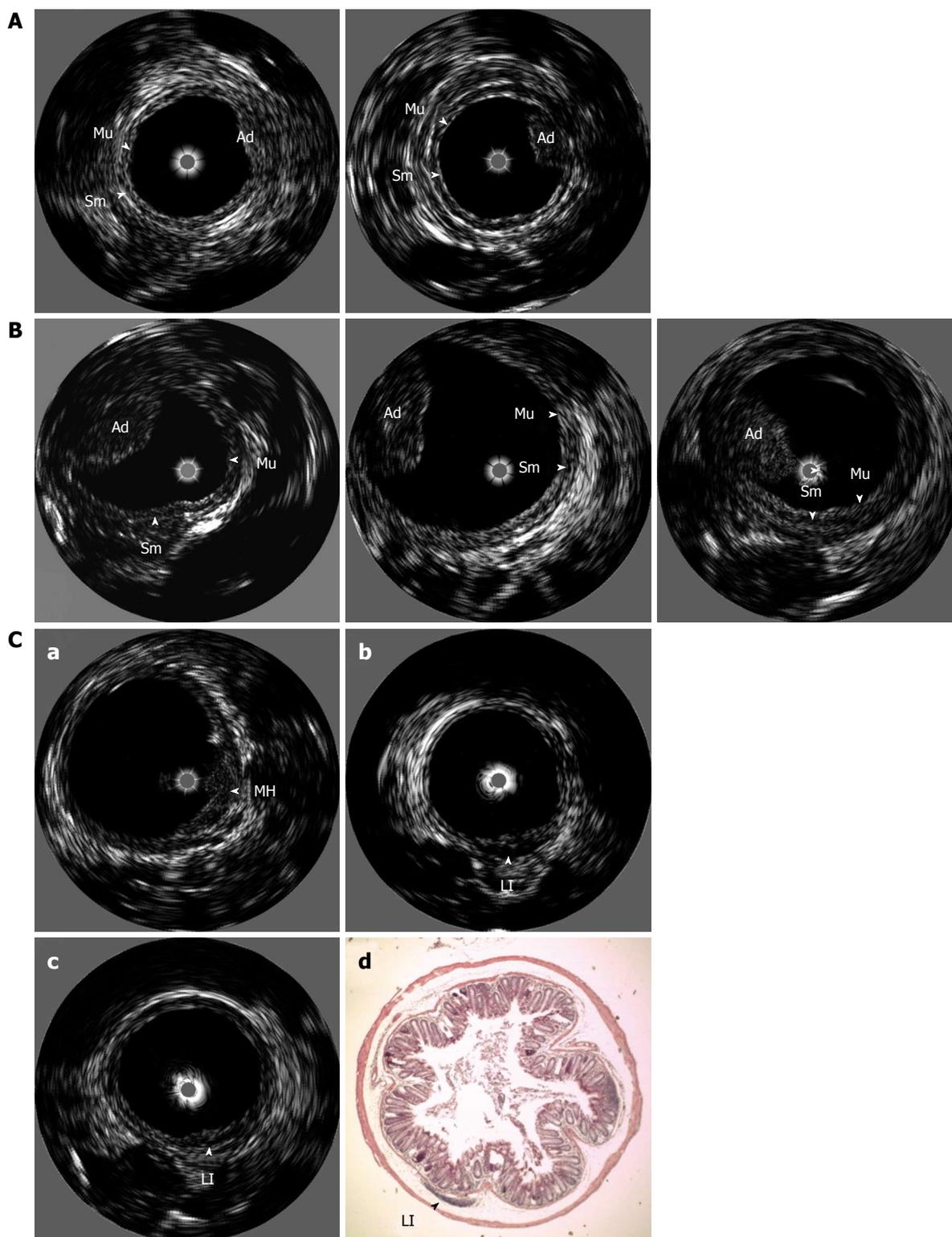


Figure 3 Endoluminal ultrasonic biomicroscopy images. A: Show increase in tumor volume. Endoluminal ultrasonic biomicroscopy (eUBM) colon images acquired at the first (left) and second (right) time-points from azoxymethane (AOM)-dextran sulfate sodium (DSS)-treated mice. The volume of the pedunculated adenoma (Ad) increased between the first and second eUBM examinations; B: Show no alteration in tumor volume. eUBM colon images acquired at the first (left), second (middle) and third (right) time-points from AOM-DSS-treated mice. The lesion observed is a pedunculated tumor. Images show that the tumor volume is unchanged during the observation period; C: Show reduction in lesion size. eUBM colon images acquired at the first (a), second (b) and third (c) time-points from AOM-DSS-treated mice. The lesion observed at the first eUBM image is a mucosa hyperplasia (MH) with ulceration at the top. In the subsequent eUBM image, the MH has decreased, and a lymphoid infiltrate (LI) has appeared in the submucosa layer. In the third and last eUBM image, MH and LI have almost completely disappeared, which is confirmed by histological analysis (d, $\times 40$ magnification). Mu: Mucosa; Sm: Submucosa.

tion is of great importance because it could be used to distinguish small adenomatous polyps from lymphoid

Table 2 Lesion progression observed by longitudinal endoluminal ultrasonic biomicroscopy and colonoscopic imaging

Animals	Animal lesion	Lesion detection					Lesion progression						
		eUBM			Colonoscopy		Size			Lesion type			
		N°	Yes	No	Yes	No	Obs	↑	↓	=	Tu	LI	MT
1	L1-1	1	✓		✓			✓			✓	✓	
2	L1-2	1	✓		✓		✓				✓		
3	L1-3	2	✓		✓		✓				✓		
	L2-3		✓		✓		✓				✓		
4	L1-4	2	✓		✓		✓				✓		
	L2-4		✓		✓				✓			✓	
	L3-4		✓		✓		✓					✓	
5	L4-4	2	✓		✓		✓						✓
	L1-5		✓		✓		✓				✓		
	L2-5		✓		✓		✓					✓	
6	L1-6	2	✓		✓		✓				✓		
	L2-6		✓		✓		✓				✓		
	L3-6		✓		✓				✓		✓		
7	L4-6	2	✓		✓				✓		✓		
	L1-7		✓		✓		✓				✓		
	L2-7		✓		✓		✓				✓		
8	L1-8	3	✓		✓		✓				✓		
	L2-8		✓		✓		✓				✓		
	L3-8		✓		✓				✓		✓		
9	L1-9	3	✓				✓				✓	✓	
10	L1-10	3	✓		✓		✓				✓		

Tu: Tumor; LI: Lymphoid infiltrate; MT: Mucosal thickening; eUBM: Endoluminal ultrasonic biomicroscopy; Obs: impossible to analyze due to colonic hemorrhage or feces. ↑: Increased lesion size; ↓: Decreased lesion size; =: No alteration.

hyperplasias, both seen by colonoscopy as mucosal elevations.

The correct detection and diagnosis of colonic neoplasias during a colonoscopy is essential for CRC prevention. The ranges for adenoma detection rates during a routine colonoscopy could vary up to 37% among endoscopists^[3], increasing the chances to misdiagnose CRC. Most postcolonoscopy cancers have a small macroscopic appearance^[31,32] and in these cases, the simultaneous use of eUBM with colonoscopy could aid in accurately detecting submucosal invasion in colonic lesions.

The small elevation in the mucosa layer observed with eUBM and registered in Figure 3A was not detected by colonoscopy. Perhaps, this fact was due to the poor bronchofiberscope image quality and could be overcome with high-resolution scopes designed specifically for work with rat and mouse models of colonic diseases^[33]. These high-resolution scopes are usually rigid telescopes and a working channel is formed in a space between an operating sheath and the telescope external wall. Although the bronchofiberscope used in the present work is unable to produce high-resolution images, it has the advantage of being flexible. According to the authors' experience, this facility of the bronchofiberscope is important to position the eUBM mini-probe tip close to a lesion, which improves the lesion visualization, or at the center of the colon lumen in order to generate circular eUBM images of the colon.

According to the results obtained with this longitudinal evaluation of inflammation-associated colon tumor

progression, most of the lesions increased in size, mimicking human cancer development. All tumoral lesions were diagnosed at the first analysis (13 wk after AOM administration), even when the size was very diminutive. The diameter of the smallest detected tumoral lesion was 0.45 mm, and eUBM was able to identify even smaller structures, such as mucosal elevations with a height of 0.1 mm. Of all the lesions detected by eUBM, approximately 15% of them (four lesions) showed a reduction in size. Of these lesions, two were lymphoid infiltrates, whose size reduction indicated inflammation resolution; one was an increase in mucosal thickness that regressed; and the last one was a small adenoma whose tumoral mass decreased. Besides pedunculated and depressed lesions, we have also detected flat lesions in animal models of CRC and current work is being conducted using *p53* knockout mice, which develop flat lesions with a higher incidence than wild type mice^[34], to evaluate the eUBM sensitivity.

The data presented here suggest that the use of high-resolution endoluminal ultrasound is a valuable tool to evaluate the progression of colonic lesions. eUBM detected alterations in mouse colonic lesion morphology and in adenoma volume throughout the examination period. Longitudinal high-resolution ultrasound measurements could be helpful in the monitoring of therapeutic efficacy of chemotherapeutic drugs *in vivo*. Additionally, this technique allows for the study of lesion progression in animal models, providing detailed insights into the biology of tumor development.

The potential of the eUBM technique to differentiate malignant from non-malignant lesions is yet to be implemented. Nowadays, the technique of narrow band imaging (NBI) has the capacity to diagnose colon lesion malignancy in real time based in mucosal and superficial vascular structures imaging enhancement^[35]. However, eUBM has the potential to detect lesion penetration depth through submucosal layers. Both methodologies have their advantages and limitations and could be performed simultaneously to complement each other.

Another advantage of longitudinal eUBM imaging is the possibility to use ultrasound contrast agents to target specific molecules involved in tumor development, such as the angiogenic promoter vascular endothelial growth factor, providing a minimally invasive tool for molecular diagnosis. This new modality of molecular imaging is now being tested in preclinical models with successful results in the characterization of tumor response to anti-angiogenic treatment^[36-38].

In summary, the simultaneous use of eUBM with colonoscopy enhances the ability to correctly diagnose and follow-up colonic lesions, offering rapid imaging acquisition and distinct advantages because high-resolution transmural imaging of the bowel wall improves lesion detection and cost-effectiveness.

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COMMENTS

Background

Colonoscopy is the recommended screening method for colorectal cancer screening and follow-up, but it fails to detect some small or nonpolypoid lesions. Therefore, the development of other imaging methods that complement the results of colonoscopy is extremely important. The authors have previously show that endoluminal ultrasonic biomicroscopy (eUBM) associated to colonoscopy improves the detection and diagnose of inflammatory and tumoral colonic lesions in animal models. Here the authors analyze the capacity of eUBM to evaluate the progression of chemically-induced colonic lesions in mice.

Research frontiers

The use of eUBM to diagnose mucosal and submucosal colorectal lesions and to guide lesion resection has already been proposed as a safe and effective clinical technique. However, its significant role in the routine diagnosis of colonic lesions has not yet been established. The use of animal models contributes in the development and evaluation of new diagnostic tools before they are completely clinically applied.

Innovations and breakthroughs

A step forward of previous work done by our group, which now includes the longitudinal study of lesion progression.

Applications

The current results suggest that the use of eUBM simultaneously to colonoscopy enhances the ability to correctly diagnose and follow up colonic lesions. In addition to its potential clinical application, eUBM can aid investigators to study colonic tumorigenesis processes and to evaluate novel therapeutic agents for colorectal cancer.

Terminology

eUBM, also known as high frequency endoscopic ultrasonography is a relatively new technique in which an ultrasound probe is inserted into the accessory channel of a regular endoscope. eUBM is an useful modality for transmural assessment of the bowel wall.

Peer review

The authors describe the evaluation of the potential use of colonoscopy and eUBM to track the progression of mouse colonic lesions. This is a clinically very interesting study.

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