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Original Article

Impaired Colonic Contractility and Intestinal Permeability in Symptomatic Uncomplicated Diverticular Disease

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Background/Aims
Impaired intestinal motility seems to play a crucial role in symptomatic uncomplicated diverticular disease (SUDD), although the mechanism is not clear. The aim of the present study is to explore the contractility patterns of colonic smooth muscle strips (MS) and smooth muscle cells (SMCs) and to assess mucosal integrity in SUDD patients.

Methods
MS or SMCs were isolated from specimens of human distal colon of 18 patients undergoing surgery for non-obstructive colonic cancer, among them 9 with SUDD. Spontaneous phasic contractions on strips and morpho-functional parameters on cells were evaluated in basal conditions and in response to acetylcholine (ACh). Mucosal integrity of SUDD colonic biopsies was evaluated by the Ussing Chamber system. Immunohistochemical staining for tight junction protein complex and for Toll-like receptor 4 (TLR4) was performed.

Results
Colonic MS of SUDD group showed a significant reduced basal tone and ACh-elicited contraction, compared to the control group (9.5 g and 47.0% in the SUDD group; 14.16 g and 69.0% in the control group; $P < 0.05$). SMCs of SUDD group showed a maximal contractile response to ACh significantly reduced compared to control group (8.8% vs 16.5%, $P < 0.05$). SUDD patients displayed lower transepithelial electrical resistance and increased paracellular permeability compared to control group. Immunohistochemical expression of TLR4 was not different in both groups, while tight junction protein complex expression was lower in SUDD patients compared to control group patients.

Conclusion
It could be hypothesized that in SUDD, in absence of severe inflammation, an increased intestinal mucosal permeability is related to altered colonic motility probably responsible for symptoms genesis.
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Key Words
Diverticular disease; Gastrointestinal motility; Humans; Muscle, smooth; Permeability

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Introduction

Colon diverticulosis is a frequent condition in adults in Western countries and several patients report clinical symptoms (abdominal pain, bloating, and altered bowel habits) even when diverticulosis is not complicated by perforation, fistula, obstruction, and/or bleeding: this condition being referred to as symptomatic uncomplicated diverticular disease (SUDD).[1-3]

Impaired intestinal motility seems to play an important role in this condition, although it is unclear if the altered colonic motor activity is the first event, which leads to the formation of diverticula, or if it is a consequence.[2-5]

Colonic transit studies reported intestinal higher pressures and raised electrical activity and an abnormal response to cholinergic stimulus in the left colon in most of SUDD patients.[2,4-6] Therefore, data from in vitro studies mainly reported an increased excitatory response that might account for the spasticity observed in these patients.[2]

In SUDD patients, undergoing surgery with uncomplicated disease, it has been reported an incremented in vitro sensitivity of colonic circular smooth muscle to exogenous acetylcholine (ACh)[5,7] and tachykinergic agonists[6] in combination with a reduction of smooth muscle choline acetyltransferase activity and upregulation of postsynaptic muscarinic M_3 receptors. These results raised the hypothesis of a type of hypersensitivity to cholinergic denervation in SUDD,[7] which is a mechanism also recognized following a damage to skeletal muscle motor innervation. Nevertheless, Tomita et al[8] did not show a significant increase in sensitivity to exogenous ACh of longitudinal muscle strips (MS), although observing an up-regulation of muscarinic M_3 receptors in circular and longitudinal muscles. On the other hand, most of these in vitro studies were performed in patients undergoing surgery for diverticulitis,[6,9] thus suggesting the doubt that the effect could be related to the inflammatory state. Furthermore, the current mechanisms underlying these alterations have not been fully clarified yet and data on smooth muscle cells (SMCs), which are the last effectors of enteric neuromuscular units, are lacking in diverticular disease (DD) patients undergoing surgery for diverticulitis.[9] A recent paper explored the expression patterns of molecular factors involved in the contractile activity of SMCs of colonic samples from patients with complicated DD, focusing on the role of Ras homolog family member A (RhoA)/Rho-associated protein kinase (ROCK), and protein kinase C/C-kinase-potentiated protein phosphatase 1 inhibitor of 17 kDa that seems to be strategic in the control of the intestinal SMC contraction. The results of this study showed an altered phenotype compatible with an inhibition of the RhoA/ROCK pathway, such a condition has been shown to directly affect the relaxation of SMCs.[9]

Moreover, previous studies have demonstrated, in case of sepsis, the role of macrophages present in the intestinal muscularis externa that, after the identification of the bacterial endotoxin lipopolysaccharide (LPS) of Gram-negative bacteria, being due primarily to Toll-like receptor 4 (TLR4) activation, produce inflammatory cytokines responsible for the impairment of smooth muscle contractility[10,11] and increase trans-membrane permeability. In a recent investigation, it has been demonstrated that SUDD causes significant modifications of TLR2 and TLR4 expression on various immune cell subpopulations obtained by both peripheral blood and affected mucosa.[12]

In SUDD, it cannot be excluded that neuromuscular changes could be associated to an impaired muscle contractility due to products released by the local colonic bacterial population. Therefore, in this study the contractility patterns of colonic SMCs and MS isolated from human circular colonic muscle layers of SUDD patients were explored and their mucosal permeability, trans-epithelial electrical resistance and tight junction (TJ) protein complex integrity were assessed, as a measurable feature of the intestinal barrier function, as well as the expression of TLRs.

Materials and Methods

Patients and Tissue Preparation

Specimens of sigmoid colon were obtained from 9 patients with SUDD undergoing elective left hemi-colectomy for uncomplicated colonic cancer; all patients had previously reported symptoms as abdominal pain and/or alterations of bowel habits (SUDD group). Documented evidence of DD was detected with CT scan or during endoscopy. Specimens of sigmoid were taken also from 9 patients undergoing surgery for uncomplicated colonic cancer as controls (control group). In both groups, samples were obtained at least 10 cm away from any macroscopically evident alteration due to inflammation or cancer. The average age in the control group was 63 years (range 47-76 years). The average age for SUDD patients was 76 years (range 68-83 years, $P = 0.003$). Clinical data regarding the characteristics of all patients are summarized in Table.

Informed consent was obtained from all patients and the clinical protocol was approved by the Ethical Committee (Ref. No. 29/14 PAR). Exclusion criteria were: previous radiotherapy or chemotherapy; treatment with calcium channel blockers or opioids.

Table. Clinical and Demographic Characteristics of Symptomatic Uncomplicated Diverticular Disease Patients and Controls

Characteristics	SUDD patients (n = 9)	Control group (n = 9)
Gender (male/female)	5/4	4/5
Age (yr)	76 (68-83)	63 (47-76)
Smoking habit (yes)	7	5
Body mass index (kg/m^2)	22.5 (21.0-24.0)	21.8 (19.0-23.0)
Abdominal pain	7/9	0/9
Bowel habits alterations	9/9	0/9

Variables are expressed as n or median (25%-75% interquartile range).

Patients had not been treated with steroids or opioids before surgery. The pathologist reports were reviewed to be sure regarding the absence/presence of colonic diverticula and to exclude samples with other histological abnormalities or inflammation.

Fresh samples were transported to the laboratory in chilled oxygenated Krebs solution (consisting of 116.6 NaCl, 21.9 $NaHCO_3$, 1.2 KH_2PO_4, 5.4 glucose, 1.2 $MgCl_2$, 3.4 KCl, and 2.5 $CaCl_2$ in mM).

Experimental Set-up

Ex vivo experiments on human colonic muscle strip motility

Preparation of human smooth muscle strips. Colonic circular smooth muscle was obtained after removing mucosa and submucosa layers; afterwards small strips (10 mm long by 2 mm wide) were isolated by sharp dissection. The strips were positioned in separate 10 mL chambers with a continuous perfusion of oxygenated Krebs solution, as previously described.[13] The solution was maintained with a gas mixture (95% O_2 and 5% CO_2) at 7.4 pH and at the temperature of 37℃. Strips were at first stretched to 10.0 g of force until they reached the conditions of optimum force development and then, they were stabilized for 1 hour. During this period, spontaneous phasic contractions gradually increased and stabilized following a 1-hour period of equilibration. Isometric contractions were detected using force displacement transducers using a computer with MacLab system software (Oxford, UK).

Muscle strip contractile activity. Therefore, strips from both control patients and SUDD group were maintained in Krebs solution (control) for 1 hour and then stimulated with a maximally effective dose of ACh (10^{-5} M) as previously described.[14] The baseline contraction and the response to cholinergic stimulation were recorded.

Ex vivo experiments on human colonic smooth muscle cell motility

Isolation of smooth muscle cells. Following the isolation of colonic smooth muscle tissue from the submucosa by sharp dissection, macroscopic blood vessels of muscle tissue were removed. The specimens of the circular muscle layer were then cut into small samples (about 1 mm wide) and, through an enzymatic digestion (as described elsewhere[14]), the muscle cells were isolated obtaining homogeneous primary human colonic SMCs, without any neuroimmune contamination. Briefly, 10 mM of glucose were added to HEPES buffer (10 mM HEPES, 1 mM $CaCl_2$, 4 mM KCl, 125 mM NaCl, and 1 mM $MgCl_2$): 20 mL of this solution were added with 0.25 mM EDTA, 1 mg/mL bovine serum albumin,) and 1 mg/mL papain from Papaia latex. Following the adjustment of this solution to pH 7.2, 0.5 mg/mL collagenase type F from *Clostridium histolyticum* were added. The tissue was equilibrated in an enzyme solution at 4℃ for 3 hours, kept at room temperature for 30 minutes, then maintained in a water bath at 31℃ for 30 minutes in order to obtain single SMCs. Finally, single SMCs were isolated filtering the suspension on a 200 μm Nitex mesh (Tetko Inc, Elmsford, NY, USA). The obtained SMCs were rinsed with collagenase-free HEPES-buffered solution to remove any presence of collagenase (approximately 3-4 times with 3-4 mL of solution). The purified SMCs were saved into clean beakers with fresh HEPES-buffered solution (usually 1 mL for every sample to be analyzed) and then mixed together to obtain a homogeneous cell suspension. All reagents used for the solutions were purchased from Sigma-Aldrich (Milan, Italy).

Measurement of SMC contractile response. Isolated SMCs, from both control patients and SUDD study group, were maintained in Krebs solution for 1 hour before the stimulation with a maximum dose of ACh (10^{-6} M)[15,16] for 30 seconds to measure the biological activity. Therefore, the cells were fixed in acrolein at a 1% final concentration and refrigerated.

Ussing Chambers Experiments

Subjects for Ussing Chambers experiments

Human biopsy samples were obtained from distal colon of 9 consecutive patients with SUDD, who were consecutively recruited among the outpatients and inpatients attending the Gastrointestinal Disease Unit of Campus Bio-Medico. Control group was composed of 9 healthy volunteers age- and gender-matched, undergoing colonoscopy for colorectal cancer screening or follow-

up of polyposis. Exclusion criteria were: assumption of antibiotics or probiotics/prebiotics within the last 4 weeks, current infectious diarrhea, use of drugs altering gastrointestinal motility and secretions (anticholinergics and prokinetics agents) and recent NSAIDs use. After obtaining informed consent, colonic mucosa integrity and function was assessed by collecting 4 biopsies for each patient from the sigmoid colon. Abdominal pain, meteorism, comorbidity, and smoking habits of healthy subjects or patients with SUDD were recorded.

Ussing Chambers experiments for mucosal integrity

Mucosal integrity of colonic biopsy specimens was analyzed in Ussing Chambers (Mussler Scientific Instruments, Aachen, Germany) by detecting and comparing paracellular permeability to fluorescein isothiocyanate-dextran (FD-4) and transepithelial electrical resistance (TEER) between 9 SUDD patients and 9 healthy controls. Transmural alternating current impedance analysis was carried out as described earlier.[17,18] Biopsies were positioned in modified 3 mL Ussing Chambers as previously described,[19] exposing a surface of 0.017 cm^2. Briefly, mucosal and serosal layers were filled with 3 mL of 10 mM mannitol and 10 mM glucose in Krebs–Ringer bicarbonate buffer at pH 7.4, respectively. Solutions were maintained at 37℃ and continuously carbogenated with O_2/CO_2 (95%/5%). After reaching a stable TEER baseline, the mucosal side of the biopsies was exposed to FD-4 (molecular mass = 4000 Da, 1 mg/mL; Sigma-Aldrich, St Louis, MO, USA) to measure paracellular permeability. Samples (300 μL/each) were collected from the serosal side during 2 hours at 30-minute intervals, the fluorescence level of which was detected using a fluorescence reader (Tecan Infinite M200-Pro multiplate reader; Tecan, Männedorf, Switzerland). Fluorescence values were then converted in concentrations of fluorescein (pmol) through a standard curve. Time points 0 minute and 30 minutes were excluded from the analysis, as the paracellular probe takes time to accumulate on the serosal side. The average of time points 60, 90, and 120 minutes of the biopsy specimens used in each experiment was calculated. TEER was measured from the voltage deflections induced by bipolar constant-current pulses of 16 mA every 60 seconds with duration of 200 milliseconds and was recorded every 30 minutes over 2 hours. The average of all time points (from 30 minutes to 120 minutes) of the biopsy samples used in each experiment was calculated and results were expressed in $\Omega \times cm^2$. Each experiment was performed in quadruplicate.

Immunohistochemistry

Zonulin, claudin, and TLR4 expression were studied by immunohistochemistry performed on 3-μm thick consecutive sections obtained from formalin-fixed tissue embedded in paraffin. Sixteen samples from SUDD patients and 16 samples from control group patients were analyzed and quantified for TLR4 and TJs. After antigen retrieval with citrate buffer (pH 6.0), sections were incubated at room temperature with the following primary antibodies: mouse monoclonal antibody anti-claudin diluted 1:100 (sc81796; Santa Cruz, Dallas, TX, USA), rabbit polyclonal antibody anti-zonulin diluted 1:100 (21773-I-AP; Proteintech, Rosemont, IL, USA), and mouse monoclonal antibody anti-TLR4 (clone C-18) diluted 1:100 (sc8594; Santa Cruz). Negative controls were obtained by omitting primary antibodies. Immunohistochemical reactions were visualized by 3'3-diaminobezidine tetrahydrochloride as the chromogen from MACH 1 Universal HRP-Polymer Detection (Biocare Medical, Concord, MA, USA). Hematoxylin was used as counterstain in order to visualize nuclei.

Immunoreactivity in unaffected mucosa of SUDD patients and control group subjects was scored. Images were acquired with an automated digital scanning system (Hamamatsu NanoZoomer 2.0 RS, Hamamatsu, Hamamatsu City, Japan). The TLR4 and TJ immunostaining were analyzed in 3 fields for each sample (magnification ×100), and a specific Image J (National Institutes of Health, Bethesda, Maryland, USA) plugin (IHC Profiler as described in Varghese et al[20]) was used to quantify the immunoreactivity. For correlative purpose, an average score was derived for each sample.

Statistical Methods

All statistical analyses were performed by GraphPad Prism ver. 4.02 (GraphPad Software Inc, CA, USA). Variables are expressed as median and 25%-75% interquartile range. Differences were evaluated by the Kruskal–Wallis test followed by *post hoc* testing using unpaired Mann-Whitney *U* test. A $P < 0.05$ was considered statistically significant.

Results

Histopathological Evaluation of the Specimens

Histopathological analysis, carried out by conventional hematoxylin and eosin staining, did not reveal significant changes in the healthy mucosa between patients with and without diverticula. TLR4 immunohistochemical expression was localized in epithelial cells and endocrine-like cells, present in the intestinal glands and in inflammatory cells present in lamina propria.

Immunohistochemical expression of TJ protein complex (clau-

din and zonulin) was lower in the colonic healthy mucosa of SUDD patients compared to control group subjects. Claudin immunohistochemical expression was 41.62 (21.02-89.67) in SUDD vs 72.24 (46.61-108.88) in the control group ($P = 0.038$). Zonulin immunohistochemical expression was 15.44 (9.06-81.06) in SUDD vs 57.85 (36.06-107.63) in the control group ($P = 0.019$) (Fig. 1).

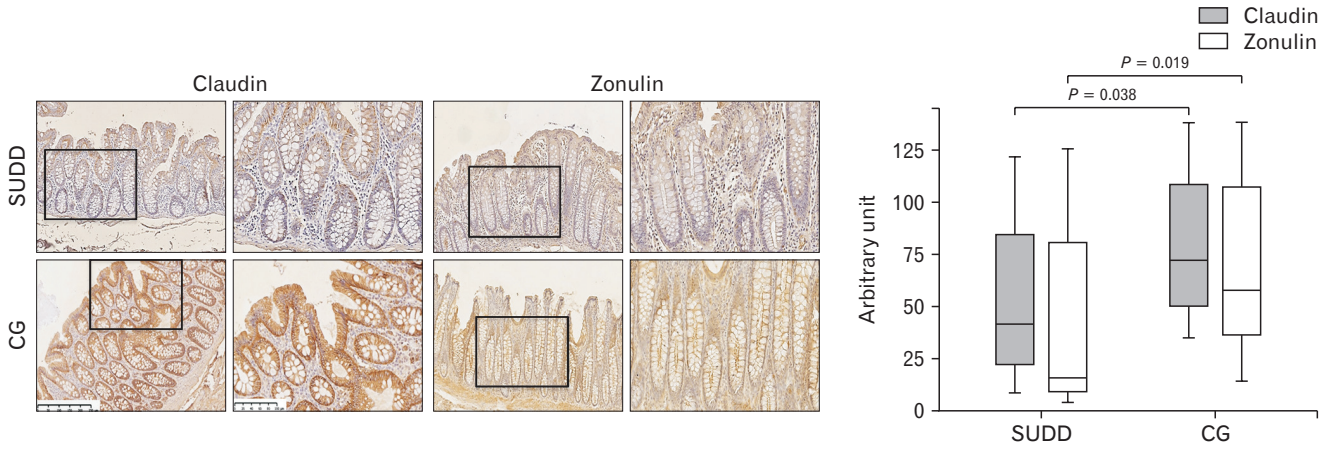

Figure 1. Immunohistochemical expression of tight junction (TJ) protein complex in unaffected colonic mucosa of symptomatic uncomplicated diverticular disease (SUDD) patients and controls (control group [CG]). Immunohistochemical expression of TJ protein complex (claudin and zonulin) was lower in the colonic healthy mucosa of 16 SUDD patients compared to 16 CG subjects. Bars in the graphic represents the median average score of immunohistochemical positivity calculated in 3 ×100 field for each sample using an ImageJ plugin. Box plots represent median, 25%-75% interquartile range and minimum-maximum values. Original magnification: ×100; high power field: ×200. Scale bars, 250 μm and 100 μm.

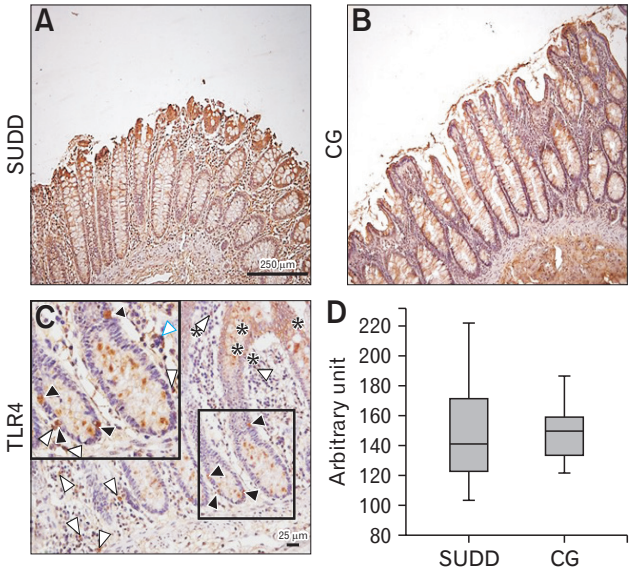

Figure 2. Immunohistochemical expression of Toll-like receptor 4 (TLR4) in unaffected colonic mucosa of symptomatic uncomplicated diverticular disease (SUDD) patients and controls (control group [CG]). (A-B) TLR4 immunohistochemical expression does not show significant changes in SUDD patients (A) and in CG subjects (B), and involves scattered epithelial cells and inflammatory cells in lamina propria of the mucosa. (C) TLR4 expression was localized in epithelial cells (*) and endocrine-like cells (black arrows) present in the intestinal glands and in inflammatory cells (white arrows) in lamina propria. (D) Bars in the graphic represent the median average score of TLR4 immunohistochemical positivity calculated in 3 ×100 field for each sample using an ImageJ plugin. Box plots represent median, 25%-75% interquartile range and minimum-maximum values. Original magnification: ×100 (A-B), ×200 (C); high power field ×400. Scale bars, 250 μm (A-B), 25 μm (C).

The average score of TLR4 immunohistochemical expression positivity did not show significant difference between SUDD patients, 133.82 (113.29-167.47) and control group, 143.66 (125.28-153.81) ($P = 0.890$) (Fig. 2).

Contractility Patterns of Colonic Smooth Muscle Strip of Symptomatic Uncomplicated Diverticular Disease Patients Versus Controls

Following 60 minutes of stabilization, colonic MS of SUDD patients and controls developed basal tone and spontaneous activity, consisting in small amplitude rhythmic contractions of myogenic origin (Fig. 3). In SUDD group we found a significant reduced basal tone and ACh-elicited contraction over basal compared to the control group being 9.5 g (minimum 4.2-maximum 21 g), and 47.0% in the SUDD group, and 14.16 g (minimum 7.2-maximum 25.77 g), and 69.0% in the control group, $P < 0.05$ (Fig. 4).

Contractility Patterns of SMCs of Symptomatic Uncomplicated Diverticular Disease Patients Versus Controls

SMCs of control group presented a resting cell length of 178.7 ± 12.1 μm and a maximal contractile response to ACh of 16.49% ± 1.2 over basal. We found that in the SUDD group the resting cell length was higher, even if not statistically different from the controls of 184.8 ± 12.9. Similarly to MS, the ACh-elicited contraction of SMCs was significantly reduced when compared to controls, being 8.83% ± 1.8 (Fig. 5).

Colonic Mucosal Integrity in Patients With Symptomatic Uncomplicated Diverticular Disease Compared to Controls

Compared to healthy controls, patients with SUDD showed lower TEER (median 15.0 Ω × cm^2, minimum 11.0-maximum

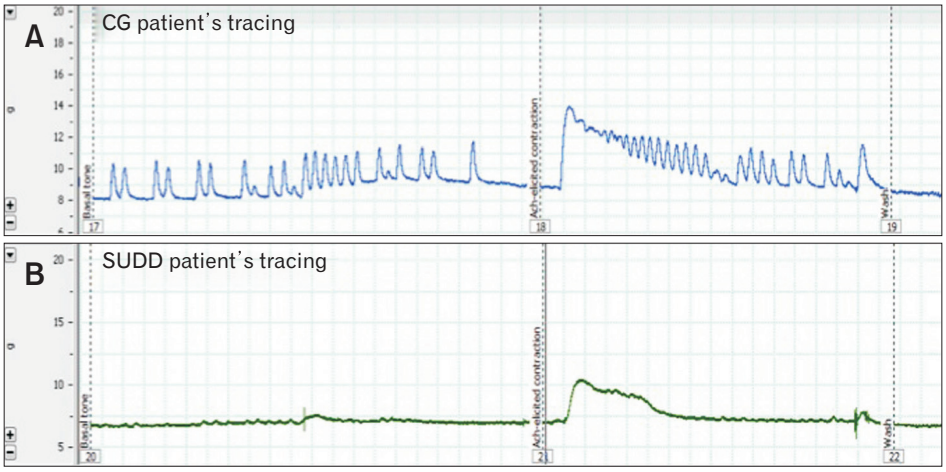

Figure 3. Selected representative traces showing colonic strip muscle contractility in a control subject (control group [CG]) (A) and in a symptomatic uncomplicated diverticular disease (SUDD) patient (B).

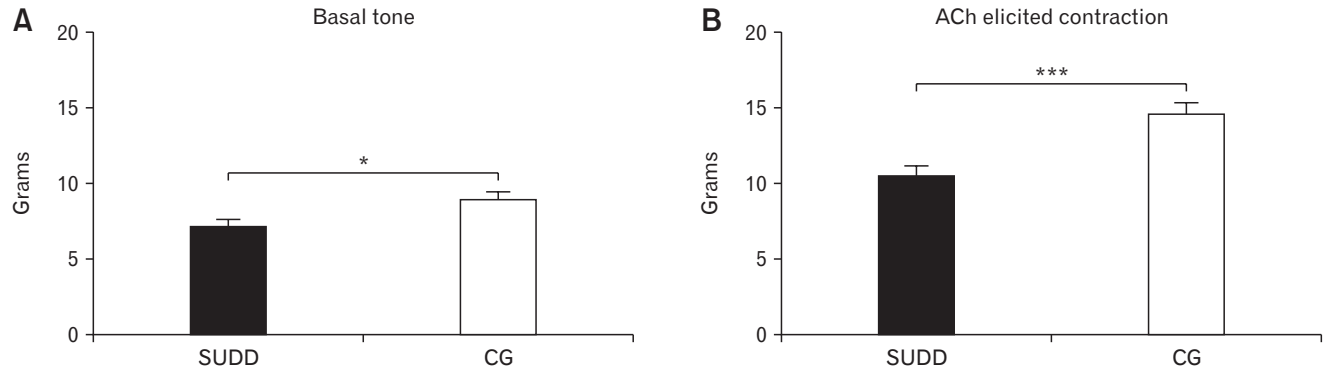

Figure 4. Colonic muscle strips contractility in symptomatic uncomplicated diverticular disease (SUDD) patients versus controls (control group [CG]). In the SUDD group we found a significant reduced basal tone (A) and a reduced acetylcholine (ACh)-elicited contraction over basal (B), compared to the results obtained in the CG (being 7.16 g in the SUDD group and 8.82 g in the CG, $P < 0.05$). *$P < 0.05$, ***$P < 0.001$.

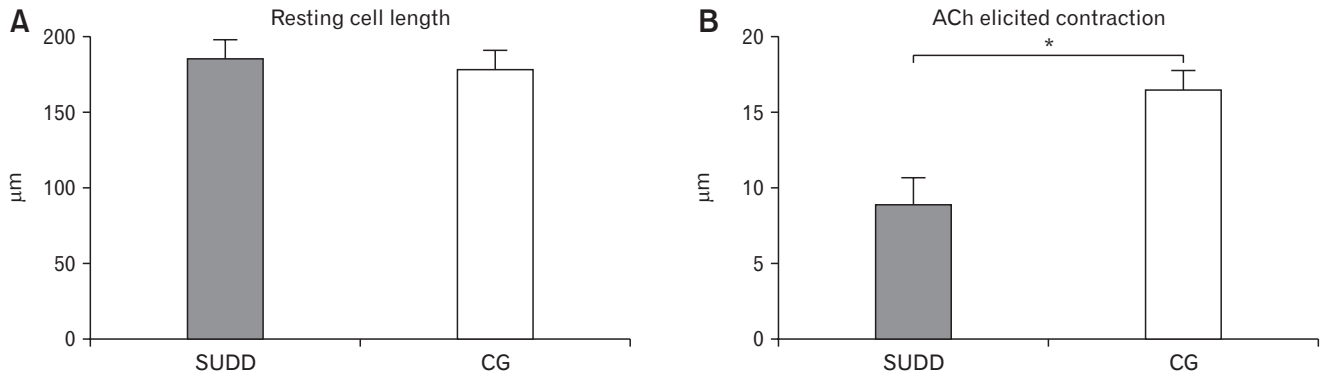

Figure 5. Colonic smooth muscle cells contractility in symptomatic uncomplicated diverticular disease (SUDD) patients vs controls (control group [CG]). In SUDD patients the resting cell length was higher, even if not statistically different from CG 184.8 ± 12.9 (A). Similarly to muscle strips, the acetylcholine (ACh)-elicited contraction of smooth muscle cells (SMCs) was significantly reduced when compared to CG, being 8.83% ± 1.8 (B). *$P < 0.05$.

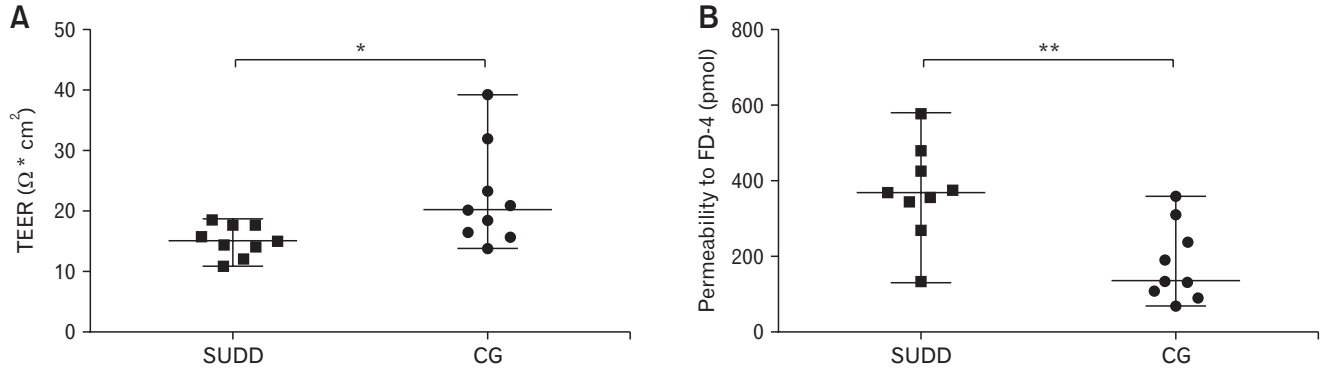

Figure 6. Transepithelial electrical resistance (TEER) and paracellular permeability to fluorescein isothiocyanate-dextran (FD-4) measured on colonic mucosa of symptomatic uncomplicated diverticular disease (SUDD) patients and controls (control group [CG]). (A) TEER was measured every 30 minutes over 2 hours and expressed as $\Omega \times cm^2$ in colonic biopsies of patients with SUDD (n = 9, black squares) and CG (n = 9, black dots); *$P < 0.05$ vs CG. Data are presented as median and range. (B) Paracellular permeability to FD-4 (expressed in pmol) was measured every 30 minutes over 2 hours in colonic biopsies of patients with SUDD (n = 9, black squares) and CG (n = 9, black dots); **$P < 0.01$ vs CG. Data are presented as median and 25%-75% range.

18.7 in SUDD vs 20.3 $\Omega \times cm^2$, minimum 13.9-maximum 39.3 in the control group, $P = 0.024$) (Fig. 6A), and higher permeability to FD-4 (median 368.6 pmol, minimum 131.7-maximum 579.0 in SUDD vs 134.0 pmol, minimum 70.2-maximum 357.5 in the control group, $P = 0.004$) (Fig. 6B), which are indicative of impaired mucosal integrity.

Discussion

In the present study, the contractile activity of SUDD colonic SM strips showed a significant alteration of both basal tone and ACh-elicited contraction, compared to controls. Previous studies support the evidence that, in patients with DD, the colon exhibits an altered motor pattern showing an increased tone and enhanced postprandial motility with the presence of spastic motor activity and excessive segmentary contractions.[21,22] It seems that the predisposition to mucosal herniation at areas of weakness in the colonic wall could be determined by these consistent high-pressure contractions.

Differently from our investigation, which analyze the isolated colonic muscle contraction, most of previous studies have investigated the longitudinal muscle contraction or were mainly conducted by in vivo evaluation using manometric techniques, thus analyzing the whole colonic motility.[21,23] These conditions could explain the apparent disagreement with the results of our study, which analyzed circular muscle specimens in an in vitro model. Our data are in accordance with an interesting investigation, conducted by Gallego

et al[24] in 2013, which demonstrated a significant altered in vitro motor pattern, in sigmoid and left colon strips from patients with SUDD, showing a decrease in the amplitude and area under the curve of rhythmic phasic contractions.[24]

Colonic motor alterations in DD have been suggested to be due to changes in enteric nerves, smooth muscle function and/or mucosal and myenteric inflammation.[25] An interesting study, which demonstrated some structural and functional abnormalities of the enteric musculature in DD patients, consisted in a disturbed muscular architecture, connective tissue replacement and loss of specific myofilaments, suggests a primitive muscle disorder responsible for the colonic contractile alteration, which could be involved in the pathogenesis and development of DD.[26]

Moreover, an immunohistochemical analysis, performed in colonic samples from patients with DD who underwent elective abdominal surgery following the third or fourth episode of diverticulitis, revealed significant abnormalities in both the expression and distribution patterns of several proteins involved in the functions of gap junctions and the contractile activity of SMCs; the specimens analyzed did not have microscopically abnormalities and they did not show the presence of leukocyte infiltrates.[9]

Supporting this hypothesis, we found that, similarly to the contractile impairment of MS, the analysis performed on colonic SMCs showed a reduced ACh-elicited contraction. Most of the in vitro studies conducted on colonic smooth muscle tissue of DD patients regards DD, complicated with acute diverticulitis; interestingly the present study is focused on SUUD, for which little evidence is present regarding the possible mechanism responsible for the presence of symptoms in the absence of macroscopically alterations of the intestinal mucosa.

Moreover, colonic SMC contractility was not previously investigated in SUDD patients, although it has been shown to be impaired in other pathological gastrointestinal conditions such as irritable bowel syndrome (IBS) and symptomatic gallstone disease.[27,28] The impairment of contractility reported in these disorders has been mainly related to a state of low grade inflammation; in IBS, inflammatory mediators, produced by colonic mucosal layer, seem to be responsible for the impaired contractility, which is similar to our study as represented by a significant decrease in basal tone and ACh-elicited contraction of MS and a significant shortening and impairment of ACh-elicited contraction of SMCs.[27,29] The similarity between IBS and our patients in terms of symptoms is very interesting as both conditions are characterized by absence of macroscopic or histological inflammation; this evidence could suggest a common pathogenic mechanism related to a muscle disorder.

In IBS, alterations in gut microbiota significantly modify mucosal barrier integrity, provoking an important modulation of gut neuromuscular junction probably responsible for the impairment of intestinal motility.[30] In the present investigation, colonic mucosal analysis performed with the Ussing Chamber system has shown, for the first time, a significant increase of permeability in SUDD patients, compared to controls, characterized by a significant reduction of TEER and a significant increase in paracellular permeability to FD-4. Intestinal epithelial barrier function plays a key role in maintaining the right equilibrium between selective permeability of needed nutrients and the possible passage of harmful entities including microorganisms and luminal pro-inflammatory factors.[30-32]

Interestingly, in this investigation, alterations in TJs were detected with immunohistochemistry, supporting the findings of altered permeability detected by the Ussing Chamber system. It is well known that the alteration of TJ barrier under inflammatory conditions is strictly linked to the pathogenesis of intestinal disease. Impaired TJ barrier determines a higher epithelial permeability contributing to intestinal epithelial damage.[33,34] Both in human inflammatory bowel disease and in experimental models of intestinal inflammation, similar structural and functional changes in TJ has been observed,[35-36] which were associated with decreased main TJ proteins. However, no specific studies have been extensively conducted to explore TJ complex and intestinal barrier integrity in SUDD, in which mucosal alteration and inflammation have not be found with routine histological analysis, while the presence of an impaired expression of TJ protein complex was previously observed in other GI diseases in which no histological features of a significant inflammation were detected.[17,19,31,32]

It could be hypothesized that, in SUDD patients, in conditions of altered permeability, a low-grade translocation of intestinal microbes and immune-stimulatory bio-products from the gut lumen through the mucosa, could be responsible for a chronic immune activation that, if prolonged, could be responsible for disease progression and onset of complications.[37]

In conditions of severe inflammation, such as sepsis, the altered intestinal motor function resulted to be associated with inflammation-induced changes of intestinal muscle and with an increased colonic permeability.[38] We have previously demonstrated, in an in vitro model, that human colonic mucosal exposure to LPS alters muscle cell contractility and that this impairment could be determined either to LPS translocation, which directly damages the smooth muscle contractility, or to the mucosal production of free radicals and inflammatory cytokines.[14]

It is well known that intestinal bacteria, which translocate

through the mucosa, are able to activate immune cells binding TLRs on their surface and result in the activation of NF-kB with secretion of pro-inflammatory mediators. This is one of the main mechanisms that mediates the suppression of muscle cell contractility in conditions such as inflammatory bowel disease.[39] It has also been previously described of a vast immunological pattern of innate and adaptive cell markers, including TLRs, which could play a role in the pathogenesis and clinical development of the DD; Cianci et al[12] showed its variations before and following the antibiotic treatment with rifaximin in DD patients. Despite these evidences, in the present investigation the TLR4 mucosal expression did not reveal any differences in patients with SUDD compared to controls. These controversial results could be determined by the different disease stages of DD patients (diverticulosis without symptoms vs DD in patients with abdominal pain and diarrhea and/or diverticulitis). Indeed, unlike the present study, previous investigations on colonic MS motility of DD were mainly conducted in patients with diverticulitis in which inflammation plays a prominent role in the motor abnormalities.

In conclusion, the results of the present study support the hypothesis that SUDD, in absence of clear inflammation, could have a pathogenic mechanism more similar to functional bowel disorders, such as IBS, in which an increased intestinal mucosal permeability is related to altered colonic motility probably responsible for symptoms genesis. Further studies are needed to clarify the exact pathogenic mechanism underlying SUDD, maybe correlating the effect of Western diet and altered microbiota to intestinal permeability changes in these patients.

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