Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

# Changes in cholinergic and nitrergic systems of defunctionalized colons after colostomy in rabbits

*Serdar Moralıoğlu, MD,*[a,*] *İsmail Mert Vural, MD,*[b] *İbrahim Onur Özen, MD,*[a] *Gökçe Öztürk, MD,*[b] *Yusuf Sarıoğlu, MD,*[c] *and Abdullah Can Başaklar, MD*[a]

[a] *Faculty of Medicine, Department of Pediatric Surgery, Gazi University, Ankara, Turkey*
[b] *Faculty of Medicine, Department of Pharmacology, Gazi University, Ankara, Turkey*
[c] *Department of Pharmacology, Faculty of Medicine, Istinye University, Istanbul, Turkey*

ARTICLE INFO

*Article history:*
Received 16 March 2016
Received in revised form
4 July 2016
Accepted 4 August 2016
Available online 12 August 2016

*Keywords:*
Diversion colostomy
Enteric nervous system
Cholinergic system
Nitrergic system
Rabbit

ABSTRACT

*Background:* This study was designed to assess smooth muscle function and motility in defunctionalized colonic segments and subsequent changes in pathways responsible for gastrointestinal motility.

*Methods:* Two-month-old New Zealand rabbits were randomly allocated into control and study groups. Sigmoid colostomies were performed in the study group. After a 2-month waiting period, colonic segments were harvested in both groups. For the *in vitro* experiment, the isolated circular muscle strips which were prepared from the harvested distal colon were used. First, contraction responses were detected using KCl and carbachol; relaxation responses were detected using papaverine, sodium nitroprusside, sildenafil, and L-arginine. The neurologic responses of muscle strips to electrical field stimulation (EFS) were evaluated in an environment with guanethidine and indomethacin. EFS studies were then repeated with atropine, Nω-nitro-L-arginine methyl ester, atropine, and Nω-nitro-L-arginine methyl ester–added environments.

*Results:* Although macroscopic atrophy had developed in the distal colonic segment of the colostomy, the contraction and relaxation capacity of the smooth muscle did not change. EFS-induced nitrergic-peptidergic, cholinergic-peptidergic, and noncholinergic nonnitrergic responses significantly decreased at all frequencies (0.5-32 Hz) in the study group compared with those in the control group ($P < 0.05$).

*Conclusions:* Although the contraction capacity of the smooth muscle was not affected, the motility of the distal colon deteriorated owing to the defective secretion of presynaptic neurotransmitters such as acetylcholine, nitric oxide, and neuropeptides.



## Introduction

A colostomy can be performed as a temporary or permanent measure for several reasons in children and adults. A temporary colostomy is typically used in children. At the time of the closure of the temporary colostomy, comparisons of distal *versus* proximal colonic segments have revealed differences in diameter and wall thickness. Kissmeyer-Nielsen *et al.*[1] showed that the wall composition of a defunctionalized colon in rats is changed due to mucosal and muscular

* *Corresponding author*. Department of Pediatric Surgery, Zeynep Kamil Maternity and Children's Training and Research Hospital, Zeynep Kamil Mah. Op. Dr. Burhanettin Üstünel Cad. No: 4/3, 34668 Üsküdar, Istanbul, Turkey. Tel.: +90 216 3910680; fax: +90 216 3439251.
E-mail address: serdarmoralioglu@gmail.com (S. Moralıoğlu).
0022-4804/$ – see front matter 
http://dx.doi.org/10.1016/j.jss.2016.08.032

atrophy. Chaudhury *et al.*[2] demonstrated that there was a significant reduction in the volume and diameter of myenteric neurons in a defunctionalized colon in male Wistar rats that underwent complete diversion colostomy compared with a control group. On the other hand, Violi *et al.*[3] evaluated the ratio of myenteric plexus size to muscularis propria thickness by histopathologic methods and found that the histopathologic parameters were not different between the distal and proximal colons after colostomy.

The indications for a colostomy are Hirschsprung's disease, anorectal malformations and colonic perforations in children. Distal colonic motility affects the long-term results of surgical therapy in Hirschsprung's disease and anorectal malformations.[4-9] In these patients, after definitive surgical therapy, constipation, faecal soiling/incontinence, and enterocolitis can occur. In patients with anorectal malformation, stooling pattern and faecal control can be affected by colonic motility.[6,9]

In a defunctionalized distal colon, it is unknown if any changes occur in neural pathways, which is the enteric nervous system responsible for gastrointestinal motility. From this point of view, we aimed to assess smooth muscle function and motility of atrophic distal colonic segments and, thereby, the effects of temporary colostomy on smooth muscle functions and pathways responsible for gastrointestinal motility.

## Materials and methods

The study was approved by the Animal Ethics Committee (G.Ü.ET-06.003) and performed according to the guidelines of the Research Committee of Gazi University Faculty of Medicine.

### *Animals, groups, and operation*

Two-month-old New Zealand rabbits were randomly allocated into control ($n = 8$) and study ($n = 12$) groups. The rabbits were operated on under sterile conditions and anaesthetized with ketamine hydrochloride (45 mg/kg intramuscular Ketalar; Eczacıbaşı, İstanbul, Turkey) and xylazine hydrochloride (5 mg/kg intramuscular Alfazyne %2; Ege Vet, İzmir, Turkey). Sigmoid colostomies were performed in the study group. A midline incision was made, and the sigmoid colon was divided approximately 15 cm from the anus. A proximal stoma was created 3 cm from the midline incision through a separate incision on the left side using interrupted, 5-0 polyglactin sutures. The distal bowel was closed as a Hartmann's pouch and left in the peritoneal cavity. The abdomen was closed in two layers after intraperitoneal sterile saline instillation. After a 2-month waiting period, colonic segments were harvested and rabbits subsequently killed. In the control group, the segments from the same part of the colon were harvested for *in vitro* experiments when the rabbits were 4 mo old. All animals were kept under controlled temperature (23.2°C) and humidity (55.5%) conditions with a 14-hour light and 10-hour dark cycle. They were fed standard lab chow and given tap water ad libitum.

For the *in vitro* experiment, the isolated circular muscle strips which were prepared from the harvested distal colon were used. The sigmoid colonic ring segments (3 cm in length) were mounted in 10-mL organ baths with a resting tension of 1 g containing a physiological salt solution (Krebs–Henseleit solution) of the following composition (mM): NaCl, 118.0; KCl, 4.7; CaCl, 1.26; $NaHCO_3$, 25.0; MgCl, 0.54; $NaH_2PO_4$, 0.9; glucose, 11.0. The pH of the solution was 7.4 after being bubbled with a gas mixture of 95% $O_2$ and 5% $CO_2$ and the solution was maintained at 37°C.

### *Experimental procedure*

#### *Investigation of the effects of colostomy on tissue weight and smooth muscle function*

All isolated circular muscle strips, which were prepared in equal size, were weighed after drying using precision scales at the end of experiments.

Contractile responses induced by 40-mM KCl were obtained at the beginning of all experiments.[10,11] A high concentration of $K^+$ was used to depolarize the cells, which leads the opening of voltage-gated $Ca^{++}$ channels. Calcium entering the cell from outside the cell through voltage-gated $Ca^{++}$ channels leads to the emergence of the tissue contractile response. The contractile response of tissues was evaluated based on the dry weight of the tissue. Following the contraction induced by 40-mM KCl, relaxation responses induced by papaverine ($10^{-4}$ M) were obtained from all the tissues. Papaverine relaxes the smooth muscle by a direct effect.

Carbachol, a muscarinic acetylcholine receptor agonist, was applied to the sigmoid colonic ring segments cumulatively at concentrations from $10^{-8}$ to $10^{-4}$ M in both groups ($n = 8$ in both groups).[12,13] The contractile response of tissues was evaluated based on the dry weight of the tissue. We investigated the effect of colostomy on the postsynaptic cholinergic system using this experimental protocol.

In different tissues following contraction induced by 40-mM KCl, relaxation responses were induced by the NO-releasing drug sodium nitroprusside (SNP, $10^{-6}$-$10^{-3}$ M; $n = 8$ in both groups); L-arginine, a precursor of NO ($10^{-4}$, $3 \times 10^{-3}$, and $10^{-3}$ M; $n = 8$ in both groups); and sildenafil ($10^{-8}$-$10^{-6}$ M; $n = 8$ in both groups). All relaxant responses were expressed by comparing the relaxation responses to papaverine. We investigated the effect of colostomy on the postsynaptic nitrergic system using this experimental protocol.[10,14,15]

#### *Investigation of the effects of colostomy on electrical field stimulation (EFS) responses*

EFS-evoked responses were recorded using Grass isometric force displacement transducers (FT 03; Grass Instruments, Quincy, MA) connected to an ink-writing oscillograph (Grass 79 E; Grass Instruments, Quincy, MA) via a preamplifier.[3,14,16-18] Tissues were allowed to equilibrate for 60 min at 1 *g* tension before experimental procedures. Isometric contractions were evoked by EFS through a platinum electrode every 2 min with trains of impulses of 0.8 ms duration for 10 s at a voltage of 50 V. To characterize the EFS-evoked contractile responses, the tissues were treated with atropine ($10^{-6}$ M, nonspecific muscarinic acetylcholine receptor antagonist), Nω-nitro-L-arginine methyl ester (L-NAME, $10^{-4}$ M, nitric oxide synthase inhibitor), tetrodotoxin (TTX, $3 \times 10^{-6}$ M, $Na^+$ channel blocker), or MEN 10376 ($10^{-8}$ M, neurokinin receptor antagonist).

In another set of experiments, isometric contractions were evoked by EFS through a platinum electrode, every 2 min with varying stimulation frequencies (0.5, 1, 2, 4, 8, 16, and 32 Hz)