Journal of Surgery
Lychkova AE, et al. J Surg 9: 11168
www.doi.org/10.29011/2575-9760.11168
www.gavinpublishers.com
Research Article

Motor Activity of the Hollow Organs of the Digestive System in Pseudomembranous Colitis

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Citation: Lychkova AE, Leontyev AV, Orlyuk MA, Danilov MA, Knyazev OV, et al. (2024) Motor Activity of the Hollow Organs of the Digestive System in Pseudomembranous Colitis. J Surg 9: 11168 DOI: 10.29011/2575-9760.11168

Received Date: 19 October 2024; Accepted Date: 31 October 2024; Published Date: 06 November 2024

Abstract

Materials and Methods: 86 PC patients were examined. Patients complain of liquid stools more than 3-5 times a day, body temperature up to 39 °C, flatulence, rarely nausea, vomiting, spastic abdominal pain. Anamnestically, patients before the development of clinical symptoms may have undergone surgical treatment, the use of antibacterial drugs. The motor function of stomach, small intestine and right and left colon sections, as well as biliary tract was recorded electromyographically using CONAN-M electromyograph with evaluation of amplitude-frequency characteristics of slow waves and spasms, power of phasic and tonic contractions and propulsive activity. Statistical processing was performed by Mann-Whitney test at $p<0.05$.

Results: Increased excitability of longitudinal, circular and partially oblique gastric muscles and the presence of both antegrade and retrograde contractions of gastric smooth muscles were revealed. The unfavourable influence of Clostridii defficile toxins on excitability of longitudinal and circular muscles and neuromuscular apparatus of the small intestine was revealed. Hypermotor dyskinesia of the left colon with a spastic component due to increased excitability of the longitudinal and circular muscle layers caused by hyperexcitability of the intramural nervous apparatus of the small intestine was revealed in PC.

Conclusions: 1. Hypermotor dyskinesia of the left colon with increased excitability of the intermuscular nerve plexus and predominance of the activity of the longitudinal muscle layer, which ensures the development of diarrhoea, is revealed in PC. 2. Hypomotor dyskinesia of choledochus and gallbladder due to proportional increase of excitability of longitudinal and circular muscular layers was revealed in PC. Hypomotor dyskinesia can be caused by increased excitability under the influence of toxins of clostridial infection of sympathetic efferent fibres.

3 Electromyography can be used for objective assessment of the state of the neuromuscular apparatus of the colon.

Introduction

Clostridium difficile-associated infection is a disease that develops when the intestinal microbiome is disturbed with excessive colonisation by Clostridium (Clostridioides) difficile, whose toxins cause inflammation and damage to the colon [1-5]. Pseudomembranous colitis is colitis, usually caused by toxigenic Clostridium (Clostridioides) difficile, characterised by fibrinous deposits on the colonic mucosa [6,7,8]. Clostridium Difficile-Associated Infection (CDI) is a disease that develops when the intestinal microbiome is disturbed with excessive colonisation by Clostridium (Clostridioides) difficile, whose toxins cause inflammation and damage to the colonic mucosa. The pathogenesis of CDI is caused by irrational and uncontrolled use of antibacterial drugs, surgical interventions, taking drugs that cause immunosuppression, biological properties of the pathogen itself. The leading pathogenicity factors of C. difficile are exotoxins A (TcdA), B (TcdB) and binary toxin. TcdA and TcdB are enterotoxins that act on intestinal enterocytes, disrupting the actin cytoskeleton, resulting in inflammation and necrosis of the mucosa, loss of tight contacts between cells, and increased epithelial permeability. Binary toxin of C. difficile of ribotype NAP1/BI/027 forms a complex on the enterocyte membrane, which penetrates into the cytoplasm, disrupts cell functioning by disorganising the cytoskeleton and leads to cell death [9,10], and also increases adhesion and colonisation of C. difficile [11,12,13]. In the absence of rational antibiotic therapy aimed at irradiation of toxigenic strains of C. difficile, CDI can progress and cause extensive inflammatory changes in the colon wall, characterised by superficial necrosis of the mucosa with the formation of 'pseudomembranes' (exudative plaques) in some cases may be accompanied by toxic megacolon, perforation of the intestinal wall, sepsis.

The prevalence of CDI is monitored in many countries [7]. In the USA, out of 500 million patients presenting to the emergency medical system from 2006 to 2010, 500,000 were diagnosed with CDI [14,15]. During the same period in the USA there was a 24% increase in the incidence of CDI, from 34.1 to 42.3 cases per 100,000 population. Mild, moderate, severe and recurrent forms of CDI are distinguished. The mild form of the disease is characterised by diarrhoea as the only clinical manifestation. The moderate form involves diarrhoea and other clinical manifestations (e.g. abdominal pain) without additional symptoms, which require a diagnosis of severe or complicated CDI. Severe CDI is the presence or development during the illness of hypoalbuminaemia (albumin level less than 30 g/L) and one of the following symptoms: 1) white blood cell count greater than 15×109 kL/L, and/or 2) abdominal pain without criteria for complicated illness. Complicated CDI involves the development of one of the following symptoms: need for ICU stay, hypotension with or without the need for vasopressor support, hyperthermia (above 38.5 C), abdominal bloating, signs of intestinal obstruction, psychiatric changes, leukocytosis greater than 35×109 kL/L or leukopenia less than 2×109 kL/L, serum lactate level greater than 2.2 mmol/L, and hollow organ perforation. Although the criteria are not validated, these were the criteria chosen to determine the severity of CDI because they are predictors of the possibility of surgical intervention or patient mortality (81). In the presence of a recurrent form of the disease, clinical symptomatology resumes within 8 weeks after the end of the course of therapy.

Patients most often complain of liquid stools more than 3 times a day or an increase in the amount of intestinal discharge from the ileostomy - more than 1000 ml/day or from the colostomy - more than 500 ml/day, increased body temperature up to 39°C, flatulence, rarely nausea, vomiting, abdominal pain of a spastic nature. Anamnestically, patients before the development of clinical symptoms may have undergone surgical treatment, use of antibacterial drugs, proton pump inhibitors, H2-blockers, anticancer drugs, presence of inflammatory bowel disease, diabetes mellitus, chronic kidney disease, etc Endoscopic examination allows to assess the severity of morphological changes in the intestine. In severe cases of the disease, endoscopic examination reveals diffuse hyperaemia and swelling of the mucosa with thickening of the intestinal wall. On the surface are found characteristic fibrinous plaques of yellowish-white colour (pseudomembranes) from 2 to 10 mm in diameter, tending to merge with the progression of the process. Histological examination reveals that the pseudomembranes consist of fibrin, mucus, epithelial detritus, and destroyed neutrophils. Typical macroscopic picture of changes in the colon allows to confirm the diagnosis. In the early stages of the disease, pseudomembranes are not found in the intestine. Due to poor patient tolerance to colonoscopy, low sensitivity at early stages and risk of intestinal perforation at later stages of the disease, the indications for endoscopic examination in PIC are limited [16]. Since the effects of CDI toxins on smooth muscle contractility are part of their overall impact on clinical manifestations, possible mechanisms of action on the gastrointestinal tract are crucial. Effects on intestinal muscles involved in segment formation along the small intestine (provided that the contractility of longitudinal muscles is intact) should cause changes in segmentation frequency and amplitude. The above may lead to some disturbance of synchronisation of the movement of intestinal contents. This would inevitably lead to atypical retention of intestinal contents in some parts and premature evacuation from other parts in combination with difficulties in homogenisation of masses in the intestinal lumen [17]. The aim was to identify the peculiarities of motor activity of the gastrointestinal tract and biliary tract in PC. Materials and methods. 86 PC patients were examined. Patients complain of liquid stools more than 3-5 times

a day, body temperature up to 39 °C, flatulence, rarely nausea, vomiting, spastic abdominal pain. Anamnestically, patients before the development of clinical symptoms may have undergone surgical treatment, the use of antibacterial drugs. At endoscopic examination diffuse hyperaemia and swelling of the mucosa with thickening of the intestinal wall are noted. Characteristic fibrinous plaques of yellowish-white colour (pseudomembranes) from 2 to 10 mm in diameter are found on the surface. Histological examination reveals that pseudomembranes consist of fibrin, mucus, epithelial detritus, destroyed neutrophils. The control group consisted of 12 patients with C gastritis. Motor function of the stomach, small intestine and right and left colon sections, as well as biliary tract was recorded electromyographically using CONAN-M electromyograph with evaluation of amplitude-frequency characteristics of slow waves and adhesions, power of phasic and tonic contractions and propulsive activity. Statistical processing was performed by Mann-Whitney test at $p<0.05$.

Results and their Discussion

Electromyographically, hypermotor gastric dyskinesia is noted: slow wave frequency increased from 5.0±0.3 in control to 9.5±0.7 / per min (89.9%, $p<0.05$) with a change in slow wave amplitude from 0.15 ±0, 03 to 0.12±0.02mV (20%, $p < 0.05$), spike frequency increased from 1.0±0.3 to 4.3±0.3 (330%, $p < 0.001$), and spike amplitude decreased from 0.1 ±0.03 to 0.03±0.002mV. (69.8 %, $p < 0.05$), tonic contraction power increases from 0.5 ±0.04 to 1.14 ±0.006 (21.8 %, $p < 0.05$), phasic contraction power increases from 0.1 ±0.02 to 0.129 ±0.006 (29 %, $p < 0.05$), propulsive activity changes from 8.25 ±0.15 in control to 8, 8 ±0.006 (6.6 %, $p < 0.05$), which generally indicates increased excitability of longitudinal, circular and partially oblique gastric muscles and the presence of both antegrade and retrograde contractions of gastric smooth muscles. This leads to the development of GERD with ingestion of acidic gastric contents into the oesophagus with the development of heartburn. In this case, the excitability of cholinergic and serotoninergic fibres and ganglia increases. Electromyographically, hypomotor dyskinesia of the duodenum is noted: slow wave frequency changes from 22.0±1.3 in control to 20.3±1.7 /v min (7.9%, $p < 0.05$) with a change in slow wave amplitude from 0.1 ±0.03 to 0, 14±0.006 mV (39.8%, $p < 0.05$), spike frequency increased from 1.0±0.4 to 4.9±0.6 (390%, $p < 0.001$), and spike amplitude increased from 0.1 ±0.012 to 0.4±0.005 mV. (299.8 %, $p < 0.001$), tonic contraction power increases from 2.2 ±0.4 to 2.84 ±0.06 (27.2 %, $p < 0.05$), phasic contraction power increases from 0.1 ±0.02 to 0.129 ±0.006 (29 %, $p < 0.05$), propulsive activity changes from 22±2.1 to 14.1 ±0.006 (22 % %, $p < 0.05$) compared to control , which in general indicates the toxic effect of Clostridii defficile toxins on the excitability of longitudinal and circular muscles and neuromuscular apparatus of duodenum due to the fact that TcdB inactivates Rho proteins [18] involved in the modulation of the contractile apparatus; regulates the activity of phospholipase D, having a minor effect on the activity of protein kinase C, as well as a pronounced inhibitory effect on the activity of muscarinic receptors [19].

Electromyographically, hypomotor dyskinesia of the jejunum was noted: slow wave frequency remained stable at 20.0±0.7 per min ($p < 0.5$) with a change in slow wave amplitude from 0.1 ±0.015 to 0.07±0.003 mV. (29.8%, $p < 0.05$), spike frequency increased from 1.0±0.2 to 3.2±0.6 (220%, $p < 0.001$), and spike amplitude decreased from 0.1 ±0.01 to 0.03±0.002mV. (69.8%, $p < 0.05$), tonic contraction power decreases from 2.0 ±0.5 to 1.4 ±0.06 (30.2%, $p < 0.05$), phasic contraction power changes from 0.1 ±0.02 to 0.96 ±0.006 (4%, $p < 0.05$), propulsive activity decreases from 20±2, 15 to 14.5 ±0.006 (37.8 % decrease, $p < 0.05$) , which indicates an adverse effect of Clostridii defficile toxins on the excitability of longitudinal and circular muscles and neuromuscular apparatus of the jejunum. In general, the effect of Clostridial toxins in the small intestine is due to increased excitability of adrenergic fibres and possibly extra-organ sympathetic ganglia. The slow-wave activity of the ascending colon was found to change from 11.0±0.2 in control to 13.0±0.9 per min (12.7%, $p < 0.05$), while the amplitude of slow waves remained the same - 0.1± 0.03 mV, There was an increase in the frequency of adhesions from 1.0 ± 0.03 to 4.4± 0.6, while their amplitude decreased from 0, 1 ± 0.04 to 0.03 ± 0.005 mV, power of tonic contractions increased from 1.1 ± 0.5 to 1.3 ± 0.04 (18.2%, $p < 0.05$) phase activity increased from 0.1 ± 0.06 to 0.132 ± 0.0012 (32%, $p < 0.05$) while propulsive activity was slightly changed from 11.0±0.9 in control to 9.9±0.7 (by 10.1%, $p < 0.05$). That is, there is spastic dyskinesia of the longitudinal muscular layer of the ascending colon. There was an increase in the frequency of slow-wave activity of the descending colon from 6.0 ± 0.3 to 11.5 ± 1.3 per min (91.7%, $p > 0.05$) and amplitude - from 0.1 ± 0.02 to 0.11 ± 0.07 mV (10%, $p < 0.05$), while the frequency of spasms increased from 1.0 ± 0.04 to 3.0 ± 0.2 (199%, $p < 0.001$), and the amplitude was halved. At the same time, the power of tonic contractions was increased from 0.6 ± 0.05 to 1.265 ± 0.11 (118.3%, $p < 0.001$), and phasic power from 0.1 ± 0.004 to 0.15 ± 0.005 (50.1%, $p < 0.05$), which may indicate the presence of hypermotor dyskinesia, as evidenced by an increase in propulsive activity from 6.0 ± 0.4 to 12, 65 ± 1.6 (by 84.3%, $p < 0.05$).

That is, hypermotor dyskinesia of the left colon with spastic component due to increased excitability of the longitudinal and circular muscle layers caused by hyperexcitability of the intramural nervous apparatus of the LOTC was revealed in PC. Since the effect of Clostridium difficile toxins on the contractility of smooth muscles is a component of their general effect on clinical manifestations, possible mechanisms of the effect on the gastrointestinal tract with regard to the formation of pseudomembranes and hypermotor dyskinesia consist in the

increased excitability of neurons of submucosal and intermuscular nerve plexus. Thus, there is a progressively increasing hypermotor dyskinesia of the colon in general in the distal direction due to the action of TcdA and TcdB produced by Clostridium difficile on smooth muscles and nerve plexuses. Hypermotor dyskinesia may be associated with hyperexcitability of cholinergic and serotoninergic [20,21,22] neurons of intramural nerve plexuses.

Indices of electromotor activity of biliary tract are given in Tables 1,2.

Table 1 Indices of electromyography of smooth muscles of choledochal intestinal smooth muscles in patients with pseudomembranous colitis in different conditions

	Slow waves			Adhesion activity			Propulsive activity
Study group	frequency	Wave amplitude	Power of tonic contractions	frequency	Wave amplitude	Power of phasic contractions	
Pseudomembranous colitis	12.1 ± 1.5	0.11 ± 0.004	1.331 ± 0.125	3.8 ± 0.4	0.33 ± 0.005	1.254± 0.16	1.064 ± 0.15
Contol	9.0 ± 0.7	0.1 ± 0.003	0.9 ± 0.06	1.0 ± 0.1	0.1 ± 0.002	0.1 ± 0.003	9.0±0.5

Table 1: Shows that the propulsive activity of the choledochus in PC patients was reduced by 87.2% ($p < 0.05$), the power of tonic contractions of longitudinal muscles was reduced by 47.8% compared to control ($p < 0.05$), the power of phasic contractions - by 25.4% ($p < 0.05$). That is, hypomotor dyskinesia of choledochus was revealed in PC due to decrease of excitability of mainly longitudinal muscle layer and, probably, activation of adrenergic system.

The results of the study of gallbladder motor activity in pseudomembranous colitis are presented in Table 2.

Table 2.

Electromyography indices of smooth muscles of the gallbladder in patients with pseudomembranous colitis in different conditions

Study group	Slow waves			Adhesion activity			Propulsive activity
	frequency	Wave amplitude	Power of tonic contractions	frequency	Wave amplitude	Power of phasic contractions	
Pseudomembranous colitis	9.8 ± 0.7	0.1 ± 0.002	0.98 ± 0.013	3.7 ± 0.4	0.3 ± 0.004	1.11± 0.004	0.88± 0.04
Contol	8.0 ± 0.6	0.1 ± 0.003	0.8 ± 0.04	1.0 ± 0.04	0.1 ± 0.005	0.1 ± 0.003	8.0 ± 0.6

Table 2: that propulsive activity of gallbladder in PC patients is reduced by 89,0% ($p < 0,05$), power of tonic contractions of longitudinal muscles is increased by 122,5% in comparison with control ($p < 0,05$), power of phasic contractions - by 101% ($p < 0,05$). That is, hypomotor dyskinesia of the gallbladder due to proportional increase of excitability of longitudinal and circular muscle layers was revealed in PC. Hypomotor dyskinesia can be caused by increased excitability under the influence of toxins of clostridial infection of sympathetic efferent fibres.

Conclusion

Clostridioides Difficile Infection (CDI) is a major cause of gastrointestinal infections associated with pathology of the large and small intestine. The pronounced inflammation of the colon caused by C. difficile toxins, such as toxin B (TcdB), damages tissues and promotes C. difficile colonisation 3,4,5,6 but the mechanism of TcdB damage to the colon with the development of inflammation is unclear. What is known is that TcdB induces neurogenic inflammation by acting on afferent neurons and pericytes innervating the gut via receptors including Frizzled Receptors (FZD1, FZD2 and FZD7) in neurons and Chondroitin Sulfate Proteoglycan 4 (CSPG4) in pericytes. TcdB stimulates the secretion of neuropeptide Substance P (SP) and Calcitonin Gene-Related Peptide (CGRP) from neurons and pro-inflammatory cytokines from pericytes. Targeted delivery of the enzymatic domain of TcdB by fusion with detoxified diphtheria toxin to peptidergic sensory neurons that express the exogenous <diphtheria toxin receptor (an approach termed toxogenetics) is sufficient to induce

neurogenic inflammation and recapitulate the underlying colon histopathology associated with CDI. Conversely, mice lacking SP, CGRP, or the <SP receptor (neurokinin 1 receptor) showed reduced pathology in both TcdB blind injection and CDI models. Blocking SP or CGRP signalling reduces tissue damage and burden of C. difficile in mice infected with a standard strain of C. difficile or hypervirulent strains expressing the TcdB2 variant. Thus, targeting neurogenic inflammation provides a patient-centred therapeutic approach for the treatment of CDI. The lack of significant changes in ACh-induced responses in TCdB-incubated preparations should be analysed with the strong possibility that TcdB inactivates Rho proteins involved in the modulation of contractile apparatus; regulation of phospholipase D activity, with minor effects on protein kinase C activity, and a pronounced inhibitory effect on muscarinic receptor activity [12]. Tests to determine the effect of TCdA and TCdB on 5-HT-mediated contractile responses in the presence of TCdA and TCdB showed that circular preparations of Colonic Smooth Muscle (SM) demonstrated resistance to 5-HT-induced responses in the presence of TCdA and TCdB.

In contrast, longitudinal preparations showed enhanced contractile responses in the presence of TCdA. Enteroendocrine cells are known to act as pressure sensors secreting 5-HT, which initiates peristaltic reflexes [20]. The contraction-relaxation processes induced by 5-HT through interneuronal interactions include acetylcholine, substance P, Nitric Oxide (NO), Vasoactive Intestinal Peptide (VIP) and calcitonin gene regulatory peptide [21]. Activation of Rho-protein and Rho-kinase is most likely the leading factor in the generation of 5-HT-induced contractions [22]. Meanwhile, the main regulatory link in the contractility chain, protein kinase C, plays a minor role in the development of these processes [22]. Our observations in this aspect contradict Lucius' hypothesis of a negative effect of 5-HT on the contractile activity of longitudinal intestinal muscles in experimental animals [23]. Gastrointestinal expression and function of 5-HT receptors are specific at different levels of the gastrointestinal tract in rats. Activation of the 5-HT7 receptor causes SM relaxation, probably through activation of cyclic nucleotides, whereas 5-HT2B receptors mediate contractions. Another expressed type, the 5-HT4 receptor, is probably involved in both SM inhibition and activation [24,25]. Parallel neural and myogenic processes are involved in the above-mentioned effects. changes in contractile activity after TcdA and TcdB treatment. Under these conditions, autogenous regulation of contractility (stimulated contraction in areas with excessive stretching of the muscle wall) is impaired. This reflects the superimposition of myogenically generated peristaltic rhythms.

Conclusions

1. In PC hypermotor dyskinesia of the left colon with increased excitability of the intermuscular nerve plexus and predominance of the activity of the longitudinal muscle layer, which ensures the development of diarrhoea.

2. Hypomotor dyskinesia of choledochus and gallbladder due to proportional increase of excitability of longitudinal and circular muscular layers was revealed in PC. Hypomotor dyskinesia can be caused by increased excitability under the influence of toxins of clostridial infection of sympathetic efferent fibres.

3 Electromyography can be used for objective assessment of the state of the neuromuscular apparatus of the colon.

Reference

1. Ivashkin VT, Shelygin YA, Khalif IL (2015) Recommendations of the Russian Gastroenterological Association and the Association of Coloproctologists of Russia on the Diagnosis and treatment of adult patients with ulcerative colitis // Russian Journal of Gastroenterology, Gastroenterology, Gerontology, and Coloproctology. Gastroenterology, Hepatology, Coloproctology 25: 48-65.

2. Ivashkin VT, Shelygin YA, Abdulganieva DI (2013) Draft clinical recommendations for the diagnosis and treatment of adult patients with Crohn's disease. Recommendations on diagnostics and treatment of adult patients with Crohn's disease. Coloproctology 2013: 4-21.

3. Korneeva ON, Ivashkin VT (2007) Antibiotic-associated colitis: pathomorphology, Clinic, treatment // Russian Journal of Gastroenterology, Hepatology, Coloproctology 17: 65-71.

4. Lobzin Yu V, Zakharenko SM, Ivanov GA (2002) Modern ideas about infection with Clostridium difficile // Clinical microbiology and antimicrobial chemotherapy. Clostridium difficile // Clinical Microbiology and Antimicrobial Chemotherapy 4: 200-232.

5. Ivashkin VT (2007) Rational pharmacotherapy of diseases of the digestive organs: A guide for practicing physicians / V. T. Ivashkin, Ivashkina N. Yu. et al. - Moscow: Litterra 2007: 884 c.

6. Sheptulin AA (2011) Refractory and recurrent forms of colitis associated with Clostridium difficile // Russian Journal of Gastroenterology, Hepatology and Hepatology. Clostridium difficile // Russian Journal of Gastroenterology, Hepatology and Coloproctology. Coloproctology 2: 50-53.

7. Wright Donna FR (2014) Largest ever European clinician consensus report on Clostridium difficile infection provides recommendations for improved management of CDI pressemitteilung astellas //EMEA - 2014.

8. Surawicz CM, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, et al. (2013) Guidelines for diagnosis, treatment, and prevention of Clostridium difficile. prevention of Clostridium difficile infections: Journal Article, Review . Am. J. Gastroenterol 4: 478-499.

9. Safin AL, Achkasov SI, Sukhina MA, Sushkov OI (2017) Risk factors for the development of diarrhoea, associated with Slostridium difficile in coloproctological patients (review of the literature). literature). Coloproctology 1: 59-67.

10. Manion J (2023) C. difficile intoxicates neurons and pericytes to drive neurogenic inflammation //Nature 622: 611-618.

11. Norman JM, Handley SA, Baldridge MT (2016) Disease-specific Alterations in the enteric virome in inflammatory bowel disease. NIH Public Access 160: 447-460.

12. Spinlera JK, Ross CL, Savidgea TC (2016) Probiotics as adjunctive therapy for preventing Clostridium difficile infection – What are we waiting for? Anaerobe 41: 51-57.

13. Aguado JM, Anttila VJ, Galperine T, Goldenberg SD (2015) Highlighting clinical needs in Clostridium difficile infection: the views of European healthcare professionals at the front line. J. Hosp. Infect 90: 117-125.

14. Kazanowski M, Smolarek S, Kinnarney F (2014) Clostridium difficile: epidemiology, diagnostic and therapeutic possibilities – a systematic review/ – Techniques in coloproctology 18: 223-232.

15. Shah DN, Aitken SL, Barragan LF (2016) Economic burden of primary compared with recurrent Clostridium difficile infection in hospitalized patients: a prospective cohort study. J. Hosp. Infect 93: 286-289.

16. Brien JA (2007) The emerging infectious challenge of Clostridium difficile-associated disease in Massachusetts hospitals: clinical and economic consequences. //Infect. Control & Hosp. Epidemiol 28: 1219-1227.

17. Vassilev P (2023) Clostridium difficile toxins impact on rat colon smooth muscle reactivity //Folia Medica 65: 116-123.

18. Schmidt M, Rümenapp U, Bienek Ch (1996) Inhibition of receptor signaling to phospholipase D by Clostridium difficile toxin B: Role of Rho proteins. J Biol Chem 271: 2422-2426.

19. Schmidt M, Hüwe SM, Fasselt B (1994) Mechanisms of phospholipase D stimulation by M3 muscarinic acetylcholine receptors: evidence for involvement of tyrosine phosphorylation. Eur J Biochem 225: 667-675.

20. Gershon MD (2013) 5-Hydroxytryptamine (serotonin) in the gastrointestinal tract. Curr Opin Endocrinol Diabetes Obes 20: 14-21.

21. Hansen MB (2008) The role of serotonin in intestinal luminal sensing and secretion. Acta Physiol (Oxf) 193: 311-323.

22. Nishikawa Y, Doi M, Koji T (2003) The role of Rho and Rho-dependent kinase in serotonin-induced contraction observed in bovine middle cerebral artery. Tohoku J Exp Med 201: 239-249.

23. Lucius C, Arner A, Steusloff A (1998) Clostridium difficile toxin B inhibits carbachol-induced force and myosin light chain phosphorylation in guinea-pig smooth muscle: role of Rho proteins. J Physiol 506: 83-93.

24. Wouters MM, Farrugia G, Schemann M (2007) 5□HT receptors on interstitial cells of Cajal, smooth muscle and enteric nerves. Neurogastroentero Motil 19: 5-12.

25. Ek BA, Bjellin LA, Lundgren BT (1986) Beta-adrenergic control of motility in the rat colon. I. Evidence for functional separation of the beta 1- and beta 2-adrenoceptor-mediated inhibition of colon activity. Gastroenterology 90: 400-407.