ORIGINAL ARTICLE

APPLICATION OF ANTIBIOTICS AND PROBIOTICS FOR PREVENTION OF ANTIBIOTIC-ASSOCIATED DISBIOSIS IN PATIENTS WITH GENERALIZED PERITONITIS AND ENTERAL DYSFUNCTION SUPPORTS STAFF AWARENESS

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ABSTRACT

The aim: To clarify the efficacy of probiotics use as a preventive measure for post-antibiotic treatment in acute peritonitis and increase staff awareness related to antibiotic and probiotic use.

Materials and methods: The study design included determination of the proper antibiotic and probiotic strain combination and clinical application of probiotic strains. The control group consisted of 63 (48.46%) patients who underwent traditional multimodal treatment of peritonitis and the study group of 67 (51.54%) individuals, with inclusion of different antibiotic/probiotic combinations.

Results: Prior to antimicrobial therapy 67.7% patients of both groups' patients had severe dysbiosis, proving dysbiosis as a sign of peritonitis. *S. boulardii* showed widest resistance spectrum and was used for probiotic therapy in study group. Intestinal dysbiosis grades distribution in control group significantly worsened, while in study group ratio of severe dysbiosis significantly dropped from 58.2% to 38.8% with significant growth of grade II dysbiosis to 61.2%. No visible differences in disease course and clinical picture, duration or complications rate between study and control groups were observed.

Conclusions: Most of probiotic strains lack antibacterial resistance that makes meaningless their use during systemic antibiotic therapy of acute peritonitis. It is characterized by harsh changes of intestinal microbiota (severe intestinal dysbiosis). While probiotic strains showed antibiotic tolerance, their use presented no significant clinical efficacy, though high level of positive influence on intestinal dysbiosis was observed.

KEY WORDS: peritonitis, treatment, antibiotics, probiotics, physiology, microbiota

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INTRODUCTION

Acute peritonitis (AP) is a comparatively common, but deadly condition, generally described as an acute inflammation of the peritoneum resulting mainly from the bacterial infection as well as other causes such as chemicals, irradiation, and foreign-body injury [1, 2]. It is a major contributor to non-trauma deaths despite improvements in diagnosis and surgical and intensive care management. Influence on the peritoneal lining by any of these agents can lead to an inflammatory response, known as AP [3]. The structure of the etiological causes leading to AP consists of sources in the colon (32% of patients), appendix (31%), stomach/duodenum (18%), small bowel (13%), or biliary tract (6%). About 78% patients with AP present with generalized peritonitis and 26% with severe peritonitis. The overall mortality rate significantly decreased (15-25%) over last decades but remains high [4-7].

Multiorgan failure, or multiorgan dysfunction syndrome (MODS) is essential for peritonitis and abdominal sepsis (AS) and is observed in the vast majority of such patients [8]. The role of endotoxemia in the pathogenesis of multiorgan failure is confirmed by data on the homogeneity of systemic changes in different pathologies, which leads to AP and AS, as well as the lack of direct correlation between the type of pathogen and the nature of the disease [9-11]. Moreover, the understanding of the role of bacteremia in the pathogenesis of AS has changed radically. At the present stage, bacteremia is no longer considered as the only decisive component in the diagnosis of abdominal sepsis. It is the high concentration of endotoxins and exotoxins of bacterial cells in the blood that becomes the main factor in the activation of mediator systems, in particular cytokines [12, 13]. Among them, TNF, interleukins, system of complement, and interferons play an important role. Damage to the epithelium in the target organs under the influence of mediators leads to significant functional disorders and forms MODS, which is similar to many inflammatory and immune dependent conditions and considered to have strong genetic background [14-16].

Development of intestinal insufficiency as a part of MODS is often underevaluated, whilst playing an extremely important

role in the pathogenesis of peritonitis and abdominal sepsis. Under the influence of inflammatory mediators, hypercatabolism, impaired coagulation, systemic and visceral blood flow disorders, damage of enterocytes develops rapidly, almost all functions of the digestive tract are disrupted – barrier, metabolic, immunoreactive, endocrine, etc. Intestinal insufficiency is a key moment in the development of the "vicious" circle in AP and AS, because the translocation of microorganisms and their toxins supports the general inflammatory reaction, aggravating metabolic disorders [17-19].

Intestinal dysbiosis is known to be either a background or aggravating factor for multiple conditions including chronic and acute inflammatory processes, inflammatory bowel disease, non-alcoholic fatty liver disease, several types of tumors, etc. Possible mechanisms of microbiota involvement in their pathogenesis are complex and not clearly understood, though immune, metabolic and even genetic factors associated with intestinal microbiota are important in many diseases [20, 21].

Relationship between virulence factors of pathogens and host resistance explains diverse clinical picture of AP and formation of AS in many patients. Furthermore, existing multimodal treatment approach including massive antimicrobial therapy seems to partially add to both developments of intestinal insufficiency and MODS. However, linkage of antibacterial therapies and intestinal insufficiency, including intestinal microbiota changes and translocation is not clearly understood. Moreover, there is related issue of insufficient staff awareness related to antimicrobial therapy application.

THE AIM

The aim of the study was to clarify the efficacy of possible use of different probiotic compositions as a preventive measure for post-antibiotic treatment in acute peritonitis and increase staff awareness related to antibiotic and probiotic use.

MATERIALS AND METHODS

Design of the study included two consecutive components, determination of the proper antibiotic and probiotic strain combination and clinical application of probiotic strains aimed on finding the probiotic activity in patients undergoing treatment. According to the aim of the study, the examination was conducted of 130 patients with acute surgical diseases of the abdominal cavity the course of which was complicated by the development of different forms of peritonitis. The study was conducted in accordance with the principles of the Council of Europe Convention on Human Rights and Biomedicine, Declaration of Helsinki on the ethical principles for medical research involving human subjects, and other valid international and national legislations in bioethics (including GCP, EU directives, etc. The study protocol was approved by the institutional ethics committee. All patients signed an informed consent prior to participating in the study.

Among study individuals, there were 73 men (56.15%) and 57 women (43.85%), indicating frequency of AP in both men and women. The age-related division of AP occurrence: in age categories from 40 to 60 years (41.5%), from 61 to 80 years (28.46%), from 20 to 40 years (23.85%). The number of patients with concomitant somatic pathology (71 patients, 54.62%) significantly exceeded the number of patients without comorbidities (45.38%). Only surviving patients were included into the study.

All patients are divided into two groups. The control group consisted of 63 (48.46%) patients who underwent traditional comprehensive multimodal treatment of peritonitis [4, 17, 22] and patients of the study group consisting of 67 (51.54%) individuals, who underwent multimodal treatment with inclusion of different antibiotic/probiotic combinations. Distribution of patients with peritonitis is presented in Table I.

The peculiarities of the probiotics' and antibiotics' action on the microflora were studied according to the standard microbiological methods: a culture of a probiotic strain was previously grown from a registered biological product and a suspension of 10^9 CFU/ml was made from it using an optical standardization approach. In this study we tested following strains of probiotic microorganisms: *Esherichia coli strain M-17* (Bifikol, Biofarma, Ukraine); *Bacillus subtilis 3, Bacillus licheniformis 31* (Biosporin, Biofarma, Ukraine); *Bacillus clausii* (Enterogermina, Sanofi, France); *Lactobacillus fermentum 90 TC-4* (Lactobacterin, Biofarma, Ukraine); *Lactobacillus acidophilus, Lactobacillus bifidus, Lactobacillus bulgaricus, Streptococcus thermophilus* (Canadian yoghurt, Astrapharm, Ukraine), *Lactobacillus rhamnosus R0011, Lactobacillus rhamnosus R0049, Streptococcus thermophilus, Lactobacillus debrueckii spp. bulgaricus* (Yoghurt Rosell, Astrapharm, Ukraine) *Saccharomyces boulardii* (Enterol 250, Biocodex, France), *Enterococcus faecium* (Bifiform, Ferrosan AS, Denmark), *Lactobacillus GG* (Bifiform Baby, Ferrosan AS, Denmark), and *Aerococcus viridans* (A-bacterin, Biolik, Ukraine). Sensitivity of probiotic strains to chemotherapeutic agents was studied by disc-diffusion (growth retention area size in mm) method on dense medium using commercially available standard disc sets according to their manuals. Dysbiosis severity was determined in colonic content by standard microbiologic methods, and calculated as a 1/3 deviation steps (grades I-III) from normal microbiota values, where 1/3 deviation was grade I, 2/3 deviation was grade II and more than that was grade III. Microsoft Excel 365 and Statsoft Statistica 7.0 (USA) software packages were used for data collection and statistical analysis.

RESULTS

Probiotic strains of L. fermentum 90TS-4 and S. boulardii have been shown to be antagonistic to *Enterobacteria, Pseudomonas, Staphylococci, Proteus* and *Bacilli*, while Enterol 250 is resistant to most antibiotics. Obtained study results are presented in Tables II-IV, while summary of compatible antibiotic and probiotic combinations is shown in Table V.

E. coli M-17, A. viridans, B. subtilis 3, B. licheniformis 31, B. Clausii, and *L. fermentum 90 TC-4* showed insufficient resistance to antibiotics applying choice limits on their use and alleviating possibilities for successful systemic antimicrobial therapy of peritonitis. Probiotic strains

included in Bifiform (*E. faecium*) and lactose-containing drugs (*Lactobacterin, Bifiform Baby, Canadian yoghurt* and *Roselle yoghurt*) are sufficiently resistant to cephalosporins, so they can be used in combination with these drugs. As shown at Table V, *S. boulardii* seems to be a better choice for use in patients undergoing systemic antibiotic treatment.

Prior to antimicrobial therapy, 75 (67.7%) patients of both groups' patients had severe dysbiosis (grade III). Thirty-six (48.0%) of them were in control group, the rest 39 (52.0%) belonged to the study group. Grade II had 46 (35.4%) and grade I – 9 (6.9%) patients. Distribution of grades I-II between both groups was similar (grade II – 25 and 21 patients in study and control groups, respectively), grade I – 3 in study group and 6 in control. No significant correlation of dysbiosis and peritonitis severity before treatment was observed in both groups.

As *S. boulardii* showed widest possible resistance for most of tested antibiotics, it was used for probiotic therapy in study group patients in the form of Enterol-250, 500 mg, twice a day for seven days as a part of early enteral feeding approach.

After the treatment, intestinal dysbiosis grades distribution in control group significantly worsened. No patients had grade I dysbiosis, and 13 (20.6%) had grade II, twice as low compared to the period before the antimicrobial therapy. The majority of control group patients (50 or 79.4%) had grade III dysbiosis after antibiotics use. Study group patients after combined use of antibiotics and selected probiotic demonstrated absence of grade I dysbiosis, too. However, ratio of severe dysbiosis (grade III) significantly dropped from 58.2% to 38.8% (26 patients) with significant growth of grade II dysbiosis to 61.2% (41 patients). No visible differences in disease course and clinical picture, as well as duration of treatment or complications rate between study and control groups were observed in this study.

DISCUSSION

Acute peritonitis and its generalized forms often lead to systemic changes defined as abdominal sepsis (AS) and multiple organ dysfunction syndrome (MODS). While antibiotic use is an essential part of peritonitis managements it may under these circumstances have negative influence, too. This is exactly one of the reasons why the vast majority of hospitals employ different programs for increasing staff awareness towards antibiotic use as an addition to more common antibiotic resistance awareness. However, very few of these programs including globally spread initiatives or organizations like APUA (Alliance for the Prudent Use of Antibiotics) pay attention for the alleviation of the antibiotic use side effects, focusing mainly on preventing unnecessary use only [23]. Comparatively few papers published under the auspices of APUA deal with inter-bacterial relationships as a part of understanding antibiotic resistance [24].

Multiple genetic studies of both intestinal microbiome and proteome, show existence of complex cross-related links between human genomic single nucleotide polymorphisms (SNP) and occurrence of different conditions serving as a background for AS and MODS. Moreover, there are ties connecting intestinal microbiome and human genome. Interestingly, connections of several SNPs, metabolic and endothelial changes are well associated with changes in gut microbiota and vice versa [25]. Such complex interrelations make situation even more complex. While influence of antibiotics on intestinal microflora seems to be well established, the use of probiotics to alleviate negative influence of antibacterial therapy remains generally confusing. There are both supporting and challenging reports on this issue, especially concerning acute conditions like AP and AS [26].

In this study we attempted to add clarification on the problem while finding out the appropriate combination of antibiotic and probiotic to avoid preliminary elimination of probiotic strain by the drug used. In addition, both clinical and microbiologic efficacy of such approach were evaluated. Our findings support the idea, that insufficient efficacy of the probiotic use in AP may be associated with inappropriate selection of probiotic strain and its elimination or neutralization by the applied antibiotic itself. But even when appropriate antibiotic and probiotic composition is used, whilst there is a good impact on microbiota's balance as shown by better dysbiosis grades proportions compared to control, no reliable support in terms of clinical picture or complication rates was observed. It rises multiple questions concerning the possible mechanisms of microflora involvements into the acute inflammatory process, metabolic and immune changes and AS/MODS formation during acute peritonitis. Further study of these mechanisms may produce sufficient data to understand why clinical efficacy of probiotic and antibiotic use is confusing, while being effective in correction of dysbiosis itself. Furthermore, this study supports awareness of medical personnel towards the proper use of antibiotics.

CONCLUSIONS

Most of probiotic strains lack antibacterial resistance that makes meaningless their use during systemic antibiotic therapy of acute peritonitis. Acute peritonitis is characterized by harsh changes of intestinal microbiota as proved by severe intestinal dysbiosis registered in this study. While selected probiotic strains of *S. boulardii* showed good antibiotic tolerance when combined with common antimicrobial drugs, their use presented no significant clinical efficacy, though high level of positive influence on intestinal dysbiosis was observed.

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The Authors declare no conflict of interest

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