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ARE PROBIOTICS EFFECTIVE IN THE TREATMENT OF FUNGAL COLONIZATION OF THE GASTROINTESTINAL TRACT? EXPERIMENTAL AND CLINICAL STUDIES

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The influence of fungal colonization and probiotic treatment on the course of gastric ulcer (GU) and ulcerative colitis (UC) was not explored. Our studies included: 1) clinical investigation of 293 patients with dyspeptic and ulcer complaints and 72 patients with lower gastrointestinal (GI) tract: 60 patients with UC, 12 with irritable bowel syndrome (IBS) - the control group. Significant fungal colonization (SFC), over 10^5 CFU/ml was evaluated. Mycological investigation was performed, including qualitative and quantitative examination, according to Muller method, 2) experimental studies in rats included estimation of the influence of inoculation of *Candida* isolated from human GI tract on the healing process of GU, induced by acetic acid with or without probiotic *Lactobacillus acidophilus* (10^6 CFU/ml) introduced intragastrically (i.g.). At 0, 4, 15 and 25 day after ulcer induction. Weight, damage area, gastric blood flow (GBF) (H2 clearance), expression of mRNA for cytokines IL-β, TNF-α (ELISA) were evaluated. Mycology: qualitative and quantitative examination was performed. MPO serum activity was measured. Results of clinical studies: 1) SFC was more frequent in patients with GU: 54.2% of cases and patients with over 5 years history of UC: 33.3% cases. 2) SFC delayed GU healing and influenced the maintenance of clinical symptoms in both diseases. Results of animal studies: 3) In *Candida* inoculated rats, the GBF was significantly lower than in the vehicle controls (saline administered group). Upregulation of TNF-α, IL-1β was recorded. The GUs were still present till 25 day in all rats inoculated with *Candida*, in contrast to vehicle group (reduction of ulcer in 92% at day 25). Conclusions: 1) Fungal colonization delays process of ulcer and inflammation healing of GI tract mucosa. That effect was attenuated by probiotic therapy. 2) Probiotic therapy seems to be effective in treatment of fungal colonization of GI tract. 3) *Lactobacillus acidophilus* therapy shortens the duration of fungal colonization of mucosa (enhanced *Candida* clearance is associated with IL-4, INF-γ response).

Key words: *Candida, fungal overgrowth, probiotics, gastric ulcer, ulcerative colitis.*

INTRODUCTION

Fungi are important element of the human gastrointestinal (GI) tract flora. They represent only a small fraction of the total microbiota. GI tract constitutes the second body surface area (250-450 m^2). It contains 500 different microbial species, 10^{14} microorganisms. Microflora of the GI tract establishes after birth through time-dependent phases. In adult age it is relatively constant. Factors influencing GI microflora include: stress of modern life, reduced physical activity, processed food, chemicals, and changes in food habits (1). There are several aspects related to the presence of *Candida* (*C.*) in the GI tract. They are considered as a part of normal human flora, but also a risk factor for immunocompromised patients. The GI tract may be the source of dissemination. Mechanisms of bacterial and fungal balance, still explored to a small extent, may play an important role in the pathogenesis of inflammatory bowel diseases. *Candida* spp. are supposed to facilitate permeation of food antigens through the mucosal barrier of GI tract with the involvement of mast cells, via mediators: protease II, TNF-α (2).

Factors predisposing for *Candidiasis* include: old age, broad-spectrum antibiotic or steroid treatment, diabetes mellitus, malignancies, chemotherapy, intravascular and bladder catheters, blood group O, smoking, intravenous hyperalimentation (3).

The balance between the two, i.e. colonization and *Candidiasis* depends on the ability of *Candida* strains to modulate expression of virulence factors in response to environmental change combined with the competence of the host immune system. A combination of different factors contribute to it at each stage of infection, such as: 1) adherence of *Candida* to host surfaces (required for initial colonization): adhesins expression, cell surface proteins with similarity to the mammalian integrins. 2) dynamic composition of *C. albicans* cell surface modulation, synthesis of new proteins, enables yeast strain to escape immune surveillance. 3) morphogenesis: based on switching of fungal forms from non-pathogenic yeast to hyphal-invasive. 4) hydrolytic enzymes including: phospholipase, lipase, phosphomonoesterase, aspartic proteinases (in the most pathogenic *Candida* strains). 5) interaction of *C. albicans* with the host immune system including primary defence mechanisms like the physical barrier of the epithelium (antimicrobial defensins, secretory IgA), second line defence (neutrophils, granulocytes, macrophages, IL-1 beta, TNF-alpha), cell-mediated immunity, humoral immunity (3).

Fungal overgrowth may be the complication of any bacterial imbalance, for instance antibiotic therapy. We may hypothesize that alteration in the balance of bacterial and fungal species in the mucosal microflora reflects a metabolic misbalance of the complex microbial ecosystem with pathophysiological consequences for the mucosal barrier (4). Restoration of balance between bacteria and fungi could probably be achieved with probiotics. Probiotics are living

microorganisms that, on ingestion in sufficient numbers, exert benefits beyond the metabolic effect of nutritional components. They seem to be effective for treatment of inflammatory gastrointestinal barrier disorders such as UC. Probiotics may influence epithelial barrier function and intestinal permeability, reduce proinflammatory cytokines, induce anti-inflammatory cytokine expression. They may also be important in restoration of microbiological balance in the gut (4).

MATERIAL AND METHODS

To evaluate the effect of fungal colonization on the GI tract mucosa, our studies included clinical investigation of upper and lower GI tract. Clinical studies were performed at the Chair and Department of Gastroenterology, Hepatology and Infectious Diseases of the Jagiellonian University Medical College in Cracow (Head – Prof. T. Mach, M.D., Ph.D.).

To examine the influence of fungal colonization on the healing process of upper and lower GI tract mucosal lesions, animal model was introduced. Studies were performed at the Department of Physiology of the Jagiellonian University Medical College in Cracow (Head – Prof. W. Pawlik, M.D., Ph.D.).

CLINICAL STUDIES

Studies of upper GI tract

We investigated group of 293 patients, 142 females and 151 males, aged 20-80 years, They presented dyspeptic symptoms or ulcer complaints. Patients with diabetes, systemic diseases, chemotherapy, also treated with steroids and antibiotics before studies, were excluded from evaluation.

Clinical examination included medical history (in particular duration of disease, medications, risk factors of C*andidiasis*), gastroscopy with biopsy for mycological and histological evaluation, aspiration of gastric juice and brush smear for mycological evaluation. Quantitative mycological investigation was performed by Mueller method. Result $>10^4$ CFU/ml was considered as significant. The cultured fungi were identified from morphological and biochemical features using API system (bioMerieux). Susceptibility of fungi to antifungals was assessed using Fungitest (Sanofi Diagnostics Pasteur). In addition, the MIC for fluconazole was determined with the use of Etest (AB Biodisk, Sweden).

Histopathological examination

Biopsies from gastric mucosa were stained with haematoxylin-eosin, PAS and alcyan blue- PAS method for histological evaluation and for the estimation of the presence of fungi in the gastric tissue.

Studies of the lower GI tract

These included patients suffering from ulcerative colitis (UC). The study involved 72 UC patients aged 18-72 years, including 38 patients at active and 22 patients at non-active phase of disease. The control group included 12 patients with diarrhoeal form of irritable bowel syndrome (IBS) (*Fig. 1*). The patients reported to the outpatient unit of the Clinic of Gastroenterology of the

Profile of investigated group

Diagnosis	No	Disease duration		woman		man	
	N	< 5 years	> 5 years	N	age	N	age
UC- active phase	38	15	23	20	44 (18-72)	18	51 (18-72)
UC- nonactive phase	22	13	9	16	38 (18-72)	6	47 (18-72)
Irritable bowel syndrome(control)	12	-	-	7	35 (18-48)	5	41 (18-45)

Fig. 1. Profile of investigated group.

University Hospital in Cracow with abdominal pains, diarrhoea, sometimes associated with blood. The symptoms were representative at the moment of admission or were reported at history taking. The clinical examination included history taking, in particular: current symptoms, duration of UC and the number of the disease relapses a year. At the beginning, all patients were examined and following 4 weeks, all underwent colonoscopy. Endoscopic picture of the colonic changes was assessed with particular attention paid to the changes indicating UC, their extent and activity of the disease. The assessment of the activity of UC was based on the index of the disease activity scored with a 0-3 scale, according to the criteria specified for chronic pouchitis by Gionchetti (4), such as: 1) the clinical criteria (number of stools, blood presence in the stool, rectal tenesmus, fever); 2) endoscopic criteria (swelling, granulation and fragility of the mucosa, exudates, presence of ulcerations); 3) histological criteria (presence of neutrophilic infiltrations).

Histopathological examinations of colon mucosa

Biopsies of colon mucosa were stained with haematoxylin and eosin and assessed with a 3-point scale regarding presence of inflammatory infiltrations, cryptic abscesses and thickening of the muscular layer (4). The examinations were carried out at the Department of Clinical and Experimental Pathomorphology of the Jagiellonian University Medical College in Cracow (Head - Prof. J. Stachura, M.D., Ph.D.). Patients with UC were treated with mesalazine according to a standard regimen. A group of patients (N=13) with significant fungal colonization of the colon (10^5 CFU g^{-1}) underwent a 2-week antifungal therapy according to antimycograms. In both clinical studies, including upper and lower GI tract, patients were informed of the aim of the study and gave their informed consent to participate. The experiment was approved by the Bioethical Committee of the Jagiellonian University.

ANIMAL MODEL UPPER GI TRACT STUDIES

Studies on the relation of fungal colonization and gastric pathology are limited owing to lack of convenient animal models resembling *Candida* infection in humans. Wistar rats, weighing 180-220 g, were fasted for 24 hours before the study. Gastric ulcers were induced with acetic acid (ulcer area 28 mm^2), according to a modified Okabe *et al.* method. We compared the effect of *C. albicans* and vehicle inoculation on healing of gastric ulcers, treated with ranitidine 30 mg/kg/d sub-cutaneously (s.c.) or aspirin (ASA) 60 mg/kg/d intragastrically (i.g.) with or without *Lactobacillus acidophilus*

(10^6 CFU/ml/d i.g.). Live suspension of *C. albicans* 10^6 CFU/ml was inoculated i.g. at day 2 after induction of ulcer for 25 days. Vehicle: saline 0.2 ml /d i.g.

Studies were performed in the following groups of rats:

A) given vehicle,

B) *C. albicans* alone

C) *C. albicans* + ranitidine, with or without *Lactobacillus acidophilus*

D) *C. albicans* + ASA, with or without *Lactobacillus acidophilus*.

Animals were sacrified after 4, 15 and 25 days upon ulcer induction. The ulcer area was measured with planimetry (Morphomat, Carl Zeiss, Berlin, Germany). The gastric blood flow (GBF) was determined by H2 gas clearance. Mycological evaluation of gastric mucosa from ulcer margin was quantitative – number of yeast cells per millilitre was calculated (>10^4 CFU/ml was considered as significant) and qualititive (Candida ID plates, API Candida strips, bioMerieux). Plasma levels of IL-1β, TNF-α were measured. RT-PCR expression of IL-1β, TNF-α was evaluated in gastric tissue. (MMLV-RT, Stratagene, Heidelberg, Germany & Perkin-Elmer-Cetus, Norwalk, CT, USA).

RESULTS

Clinical studies of upper GI tract

The studies revealed significant fungal colonization >10^4 CFU/ml, found in 54.2% of GU patients and 10.3% patients with chronic gastritis compared with control group: 4.3% (*Fig. 2*).

The most frequently isolated fungal species in GU were *C. glabrata* (42.4%) and *C. albicans* (38.7%) (*Fig. 3*).

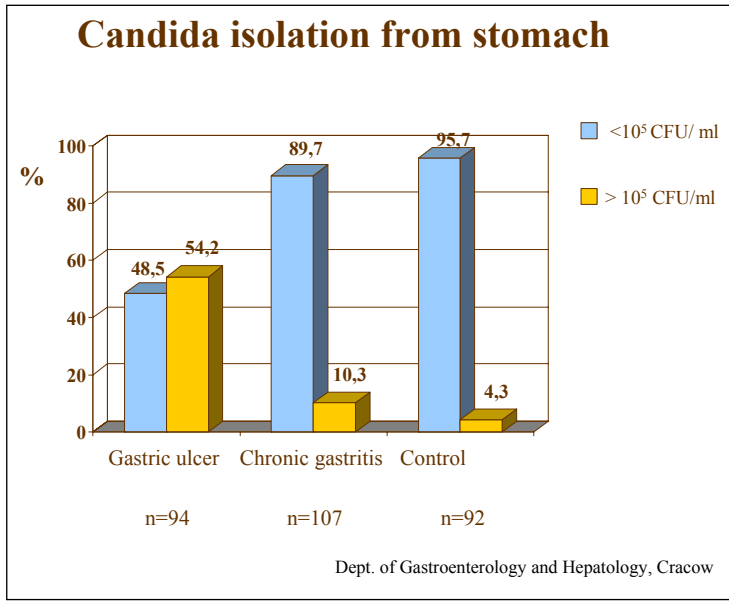

Fig. 2. Frequency of significant fungal colonization in stomach of patients with gastric ulcer, chronic gastritis and in the control group.

Among clinical symptoms reported by patients, epigastric pain and weight loss were significantly more frequent in GU patients with significant fungal colonization (*Fig. 4*). In the 4 week of observation such symptoms as epigastric pain, weakness, weight loss and vomiting were significantly more frequent in GU patients with significant fungal colonization of gastric mucosa (*Fig. 5*). Evaluation of time-related changes in GU diameters revealed a significantly higher rate of changes in patients with non-significant fungal colonization as compared to significant colonization (exponent b: 0.4688 and 0.1792, respectively) (*Fig. 6*). Photo 1 presents the fungal cells in gastric biopsy taken from examined patients.

Clinical investigations of the lower GI tract

The studies revealed more frequent, significant fungal colonization in patients with over 5 years of UC history (33.3%), in comparison to patients with shorter disease history (13.8%) and irritable bowel syndrome (1.3%) (*Fig. 7*).

Mycological examinations revealed that *C. albicans* was the most frequent fungus isolated from the colon (91.7% of cases). Other isolated fungi were: *C. glabrata* (6.7% of cases) and *C. incospicua* (1.6% of cases). Initial analysis of total mean activity indexes of inflammation in UC regarding clinical, endoscopic and histological criteria in patients with significant and insignificant fungal colonization of the colon did not demonstrate significant differences between these groups: 13.8 and 14 ($p>0.05$), respectively.

A group of 13 patients out of the total of 25 UC patients with diagnosed significant fungal colonization were treated with an antimycogram-related

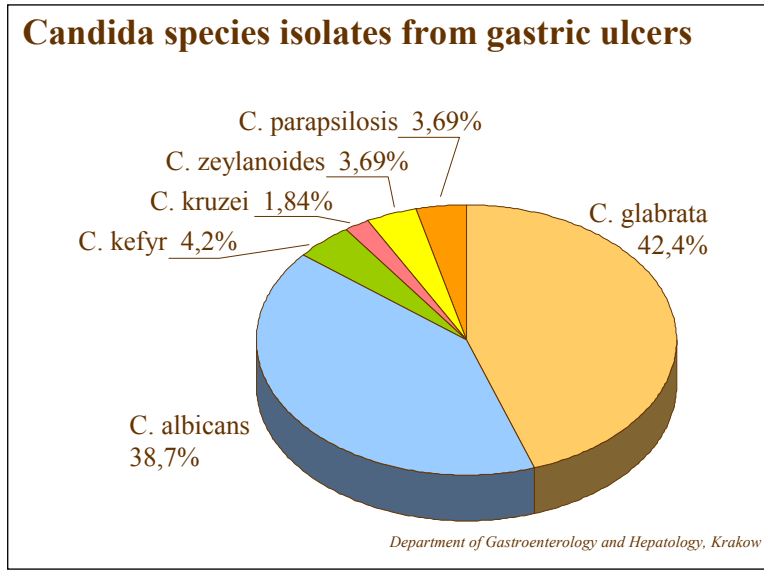

Fig. 3. Candida species isolated from gastric ulcers.

The frequency of symptoms in GU patients at the beginning of observation

Group	N	Epigastric pain	Weakness	Weight loss	Vomiting	Retroster-nal pain	Dyspha-gia
GU significant fungal colonization	38	92%	76%	60%	31%	18%	18%
GU nonsignif. fungal colonization	43	83%	68%	43%	35%	29%	11%
Significance level		< 0,05	NS	< 0,05	NS	NS	NS

NS – statistically non-significant

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Fig. 4. The frequency of symptoms in GU patients at the beginning of observation.

Regression of clinical symptoms in GU patients in 4th week of observation.

Group	N	Epigastric pain	Weakness	Weight loss	Vomiting	Retroster-nal pain	Dyspha-gia
GU, signif.fungal colonization	20	80%	88%	80%	84%	90%	93%
GU non-signif.fungal colonization	43	98%	99%	90%	99%	96%	97%
Significance level		< 0,05	< 0,05	< 0,05	< 0,05	NS	NS

NS – statistically non- significant

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Fig. 5. Regression of clinical symptoms in GU patients in 4th week of observation.

antifungal treatment. After 4 weeks, activity of inflammatory state was evaluated once again. There was a difference between UC patients receiving (group C) and not receiving (group D) the antifungal treatment. Antifungally treated patients

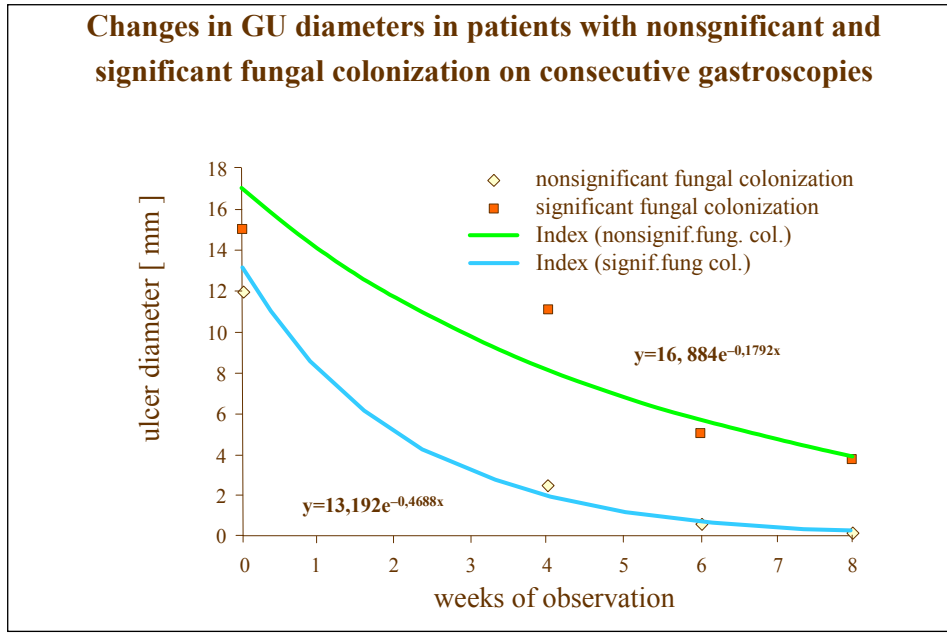

Fig. 6. Changes in GU diameters in patients with non-significant and significant fungal colonization on consecutive gastroscopies.

Results of quantitative mycological stool evaluation of patients with UC and IBS.

Fungal concetntration	< 5 years			> 5 years			Control
	N %	Active phase	Nonactive phase	N %	Active phase	Nonactive phase	IBS
> 10^5 CFU/g	10 13,8%	7 9,7%	3 4,1%	24 33,3%	18 25%	6 8,3%	1 1,3%
10^3 -10^4CFU/g	18 25%	8 11,1%	10 13,8%	8 11,1%	5 6,9%	3 4,1%	11 15,2%

Fig. 7. Results of quantitative mycological stool evaluation of patients with ulcerative colitis (UC) and irritable bowel syndrome (IBS).

presented with a higher drop in the total mean activity index of colonic mucosa inflammation as compared with patients not treated antifungally: 8.0±0.9 (C) and 10.4±1.4 (D), respectively ($p<0,05$).

The differences were particularly significant in case of clinical criteria. Patients receiving an antifungal were found to show more frequent regression of symptoms or a decrease in their intensity. The activity indexes of UC, as referred to clinical criterion, amounted to 2.2±1.5 and 3.3±1.0 for antifungal treated and non-treated patients, respectively ($p<0,05$).

Studies on animal model

The area of ulcers in vehicle-controlled rats decreased significantly from day 8 after ulcer induction, reaching 75% and 92% of reduction at days 15 and 25 respectively. In contrast the ulcers were present till 25 day in all rats inoculated with *Candida* (*Fig. 8*). Co-treatment of ranitidine or ASA with *C. albicans* induced a significantly greater increase in the area of gastric ulcer and a significantly greater fall in GBF at the ulcer margin as compared to these recorded in control rats, treated with vehicle and those treated with ranitidine or ASA alone (*Fig. 7*).

Combined therapy with *L. acidophilus* in *Candida* inoculated rats with ranitidine or ASA reversed the increase in the ulcer area and accompanying fall in GBF at ulcer margin caused by *Candida* as compared to the respective values determined in *Candida*-inoculated rats without addition of probiotic bacteria (*Figs 9, 10, 11*).

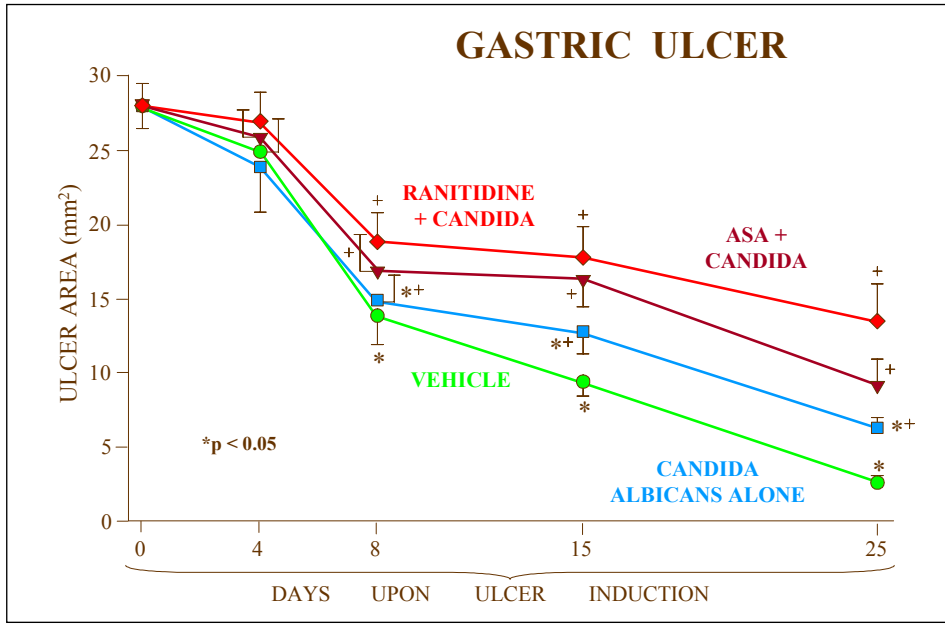

Fig. 8. Mean area of acetic acid ulcers in rats treated with vehicle and those inoculated with *Candida albicans* alone or combined with aspirin (ASA) (60 mg/kg/day i.g.) or ranitidine (30 mg/kg/day s.c.).

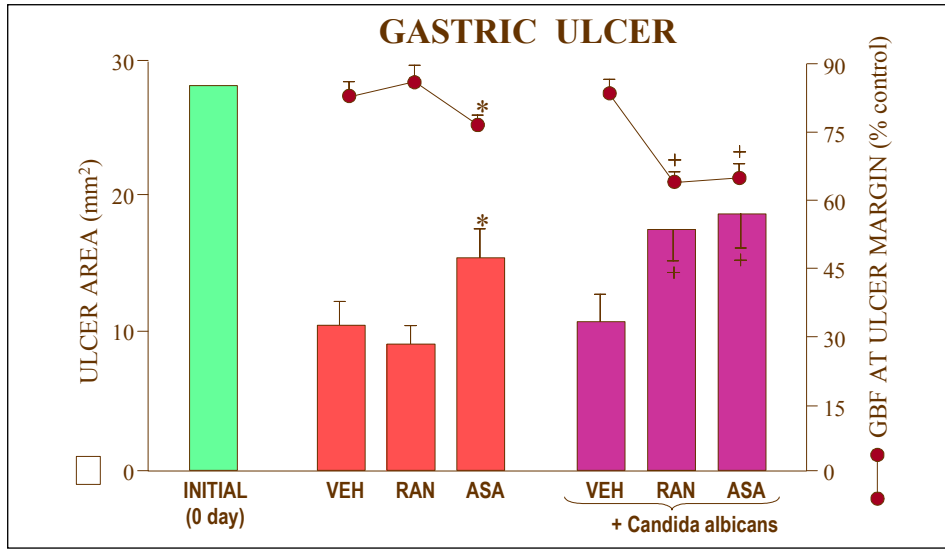

Fig. 9. The influence of *C.albicans* gastric inoculation with- or without ranitidine or ASA on the gastric ulcer area. Co-treatment of ranitidine or ASA with *C.albicans* induced significantly greater increase in the area of gastric ulcer and significantly greater fall in GBF at the ulcer margin as compared with those recorded in control rats treated with vehicle and those treated with ranitidine or ASA alone.

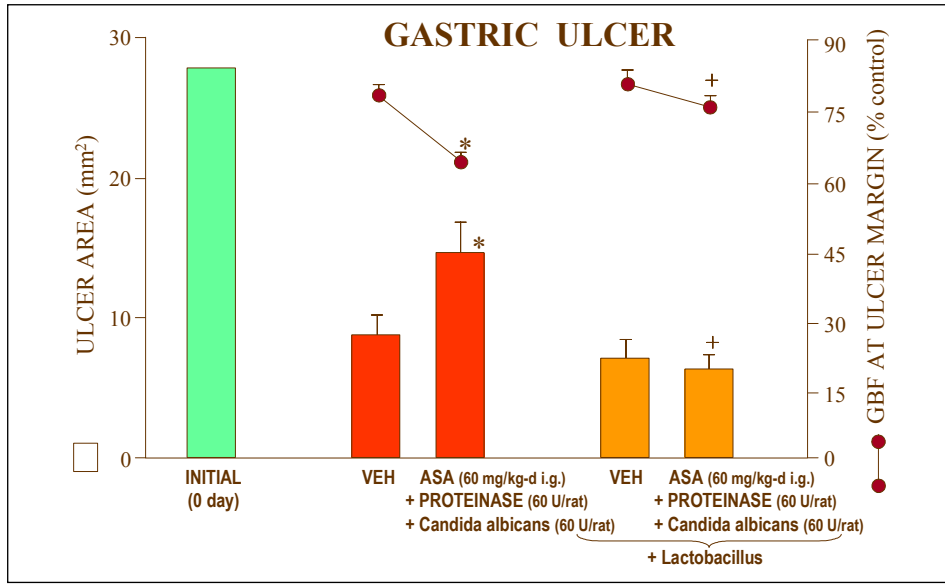

Fig. 10. The influence of therapy with *Lactobacillus* (*L.*) *acidophilus* in *C.albicans* inoculated rats with or with ASA on the ulcer healing and GBF. Combined therapy with *L. acidophilus* in *Candida* inoculated rats reversed the increase in the ulcer area and accompanying fall in GBF at ulcer margin caused by *Candida* as compared to the respective values determined in *Candida*-inoculated rats without addition of probiotic bacteria.

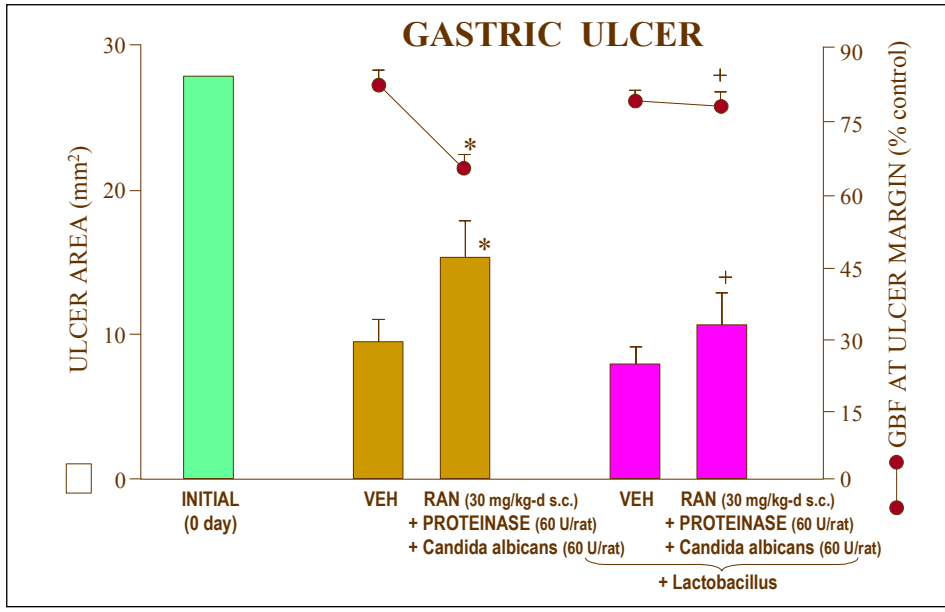

Fig. 11. The influence of therapy with *L. acidophilus* in *C.albicans* inoculated rats with or with ranitidine on the ulcer healing and GBF. Combined therapy with *L. acidophilu*s in *Candida* inoculated rats with ranitidine reversed the increase in the ulcer area and accompanying fall in GBF ulcer margin caused by *Candida* as compared to the respective values determined in *Candida* inoculated rats without addition of probiotic bacteria.

Quantitative mycological evaluation revealed a significant fungal colonization in 85% of animals co-treated with *C. albicans* and ASA and in about 82% of rats receiving *C. albicans* with ranitidine. Treatment with *Lactobacillus* significantly attenuated the number of rats infected with *C. albicans* (*Fig. 12*). Photo 2 shows the fungal material, such as spores and hyphae, identified in gastric tissue stained with haematoxylin-eosin.

Gastric inoculation with *Candida* combined with ASA or ranitidine resulted in a delay in ulcer healing accompanied by a significant rise in plasma Il-1β and TNF-α as compared with vehicle-control animals. Co-treatment with *L. acidophilus,* added to rats inoculated with *Candida* and ASA or ranitidine resulted in decrease in the area of gastric ulcer followed by attenuation in plasma IL– 1β and TNF-α (*Fig. 13*).

DISCUSSION

The role of fungal colonization of the GI tract is explored only to a small extent. Our clinical investigations revealed more frequent significant fungal

Quantitative mycological determination of the C. albicans colonization of gastric mucosa at day 25 after induction of ulcer.

Type of test	Percentage of animals infected with *C. albicans* (concentration of *C. albicans* > 10^5 CFU/g)
Vehicle	**0**
***Candida* alone**	**1,3 ± 1,2**
***Candida* + ASA**	**85 ± 3,2**
***Candida* + ranitidine**	**82 ± 2,8**
Candida* + ASA + *Lactobacillus	**33 ± 2,3**
Candida* + ranitidine + *Lactobacillus	**28 ± 1,4**

Results are means ± SEM of 3 determinations on 6-8 animals.

Fig. 12. Quantitative mycological determination of the *C.albicans* colonization of gastric mucosa at day 25 after induction of ulcer.

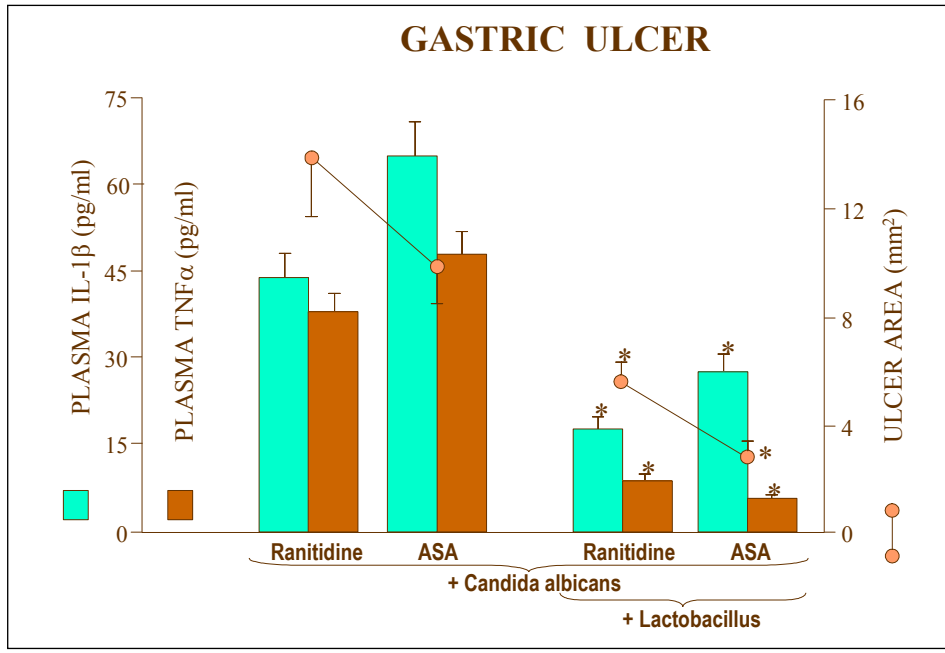

Fig. 13. The mean area of gastric ulcers and plasma Il1-β and TNF-α levels after 15 days of treatment with vehicle or combination of *C.albicans* and ASA (60 mg/kg/day i.g.) or ranitidine (30 mg/kg/day s.c.) with r without concurrent treatment with *L. acidophilus* (10^5CFU/ml i.g.).

colonization in patients with GU, in comparison with chronic gastritis and control group: 54.2%, 10.3%, 4.3%, respectively. In UC patients, increased fungal concentration was found in 33.3% of cases in patients with over 5 years history of disease in comparison with shorter disease duration (13.8%) and irritable bowel syndrome (1.3%).

The results from UC patients indicate the importance of duration of that disease. Our clinical observations seem to confirm studies on animal model, which revealed the delay in the process of GU healing in *Candida* inoculated rats, probably associated with the decrease of GBF which was significantly lower in comparison to vehicle controls. Upregulation of TNF-α ad IL1-β was also recorded.

Introduction of *L .acidophilus* therapy in *Candida* inoculated rats with GU reversed the increase in the ulcer area and accompanying fall in GBF at ulcer margin caused by *Candida* as compared to the respective values determined in *Candida*-inoculated rats, without addition of probiotic bacteria. Noteworthy is emergence of *C. albicans* strains resistant to fluconazole and itraconazole, antifungals that are most commonly used in prophylaxis and treatment of gastrointestinal candidiases. So far, resistance to the above mentioned agents has been observed in some of *C. glabrata* and *C. lusitaniae* strains and as natural feature of *Candida krusei*. Growing resistance of *C. albicans* strains to these antifungals may be a result of the worldwide use of antifungals in empirical therapy and prophylaxis, without any proven justification. In this situation probiotic therapy could be considered instead of antifungal treatment, as a safer alternative to the above therapy. The influence of *Lactobacillus acidophilus* on mucosal *Candida albicans* clearance was evaluated by Elahi *et al.* (5). Infection-prone (DBA/2) mice, 6-8 weeks old, were used in this study. Mice were fed 10^9 lactobacilli in 0.2 ml PBS by gastric intubation with feeding needle every day for 2 weeks. Control mice were fed with phosphate buffered saline (PBS). One day after the last feed, all mice were orally challenged with 10^8 *C. albicans* by topical application. Feeding was continued for an additional 14 days. Cytokine enzyme-linked immunosorbent assay (ELISA) was introduced (cytokine secretion by antigen-stimulated cervical lymph node cells from mice fed lactobacilli). Production of INF-γ and NO in saliva as important factors of antifungal protection, were evaluated. The results of the above studies showed enhanced clearance of *C. albicans* from oral cavities of mice fed lactobacilli. The levels of fungal colonization in the oral cavity in DBA/2 mice fed with lactobacilli were similar to control animals 1 day after infection. Rapid decline in colonization levels at day 2 in the group of mice fed *L. acidophilus* was observed. By day 6, mice fed *L. acidophilus* had undetectable number of yeasts in the oral cavity. The protective effect of *Lactobacilli* against fungal colonization was observed in DBA/2 mice 2 weeks after cessation of oral feeding with *L. acidophilus*. The rate of oral clearance was lower in comparison with mice fed continuously with *L. acidophilus*. By day 6, fungal colonization was undetectable in mice continuously

fed with *L. acidophilus* (5). Enhanced clearance of *C. albicans* correlated with increased expression of IL-4 and IFN-γ in mice fed *L. acidophilus* (5). The influence of microbiological factors is taken into the consideration in the pathogenesis of ulcerative colitis (UC). Alterations of the bacterial microflora are considered as an important factor triggering the disease process and may explain the efficacy of antibiotic treatment in active phase of UC. Bacterial imbalance may lead to fungal overgrowth in the gut (5).

Kuhbacher *et al.* evaluated the influence of probiotic therapy on bacterial and fungal microflora in the group of patients with recurrent or chronic pouchitis. Activity of disease was defined by clinical, endoscopic and histological criteria using the pouchitis disease activity index (PDAI). Patients received probiotic therapy: 6g once daily (VSL#3), 300 billions lyophilized bacteria per gram or placebo for 12 months (lactobacilli, bifidobacteria, streptococci). Mucosal biopsies from colon were frozen in liquid nitrogen (6). Bacterial analysis was performed by polymerase chain reaction (PCR) and fluorescent *in situ* hybridization (FISH). Analysis of fungal microflora was evaluated by denaturing gradient gel electrophoresis (DGGE). Richness and diversity of bacteria and fungi was estimated. The results of the above studied revealed, that all patients treated with probiotic therapy were still in remission after 2 months. Placebo treated patients presented signs of active pouchitis. Bacterial diversity (number of bands) of probiotic group was significantly higher as compared with the starting value (6). It was suggested, that low bacterial diversity could be an important mechanism for mucosal inflammation. Restoration of *Enterobacteriaceae* species in the mucosa and rise in bacterial diversity characterized remission maintenance by probiotic therapy (6). Fungal diversity, indicated as abundance (no. of bands) was obtained from denaturing gradient gel electrophoresis profiles of pouchitis patients in remission, after placebo and two months after probiotic treatment. The DGGE evaluation of fungi revealed in the probiotic group a marked reduction of fungal diversity both in comparison with pretreatment levels and with placebo group (0.002) (6).

The results of the studies performed so far indicate, that the problem of fungal overgrowth, bacterial-fungal interactions and their influence on the onset and maintenance of clinical symptoms of GI tract inflammatory diseases needs further investigations.

CONCLUSIONS

1. Fungal colonization delays process of ulcer and inflammation healing of GI tract mucosa. That effect was attenuated by probiotic therapy.
2. Probiotic therapy seems to be effective in treatment of fungal colonization of GI tract.

3. *Lactobacillus acidophilus* therapy shortens the duration of fungal colonization of mucosa (enhanced *Candida* clearance is associated with IL-4, INF-γ response).
4. Fungal diversity is related to bacterial diversity.
5. The role of fungal overgrowth and the bacterial-fungal interactions need further investigations.

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