

The mixture of bifidobacterium associated with fructo-oligosaccharides reduces the damage of the ocular surface

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Abstract

Background. Despite its high prevalence Dry Eye Syndrome (DES) in frequently under-recognized owing to its negative influence on patients visual function.

Methods. This clinical trial was a pilot study to evaluate the effects of supplementation with mixture (Bifidobacterium lactis and Bifidobacterium bifido) on the tear film. Following the run-in period subjects were randomized in two groups: group A (N°20 subjects) and group B (N°20 subjects). Group A (control) treated only with substitute tear and group B treated with substitute tear + mixture (symbiotic).

Results. The data obtained in the two study groups A and B were, respectively the following: Schirmer 9.1±0.2 vs 12.7±0.4 (p< 0.001); Schirmer II 3.5±0.1 VS 4.7±0.2 (p<0.001); BUT 3.9±0.3 vs 6.3±0.2 (p<0.001). Culture test showed initial bacterial growth in group "A" (placebo) 18 out of 40 samples tested, corresponding to 45.0% and "B" after treatment ((symbiotic) was found positive culture whit growth of bacteria in 12 tests equal to 30.0%. The total numbers of isolations of aerobic and anaerobic bacteria found group A and B after treatment. A reduction of 15 to 11 strains of aerobic and anaerobic isolates from 9 to 5 has been found.

Conclusions. The present study shows that the administration of bifidobacterium may represent a success full treatment in ameliorating dry eye syndrome (DES). The effect of imbalanced microbiota are not restricted by gastrointestinal abnormalities but could have systemic impact on immunity. Commensal bacteria or probiotics interact with the endogenous enteric microbiota and gut cells therein confereing health benefit to the host. *Clin Ter 2017; 168(3):e181-185. doi: 10.7417/CT.2017.2002*

Key words: Dry Eye Syndrome (DES), symbiotic, Bifidobacterium lactis, Bifidobacterium bifido, fructo-oligosaccharides

Introduction

The anterior eye part in contact with the exterior is the ocular surface, today regarded as one functional unit, whose individual anatomical components are coordinated by the neurological control. Dry Eye Syndrome (DES) is a common ocular manifestation in several diseases that results in discomfort, visual disturbance, and tears film instability with damage to the ocular surface (1- 2). The symptoms

of DES include constant irritation, foreign body sensation, and blurred vision. The dry eye is not a negligible disturbance and it is capable of significantly reducing quality of life. Despite its high prevalence DES in frequently under-recognized owing to its negative influence on patients visual function. Population- based studies identify this condition in 5% to 30% of the population aged 50 years or older. These estimated suggest that DES is more prevalent than diabetes, cancer, heart disease, Parkinson disease, and Lupus erythematosus. Despite its high prevalence DES is an under-recognized pathology. Only a handful of therapies are available for DES patients and are used according to the disease severity (3-12). The traditional therapy of DES is a basis of tear substitutes. This type of treatment has the purpose of to restore the normal tear film without intervening on the conditions that caused the lacrimal dysfunction. For this reason, alongside the traditional treatment with tear substitutes, we are developing a number of other therapeutic measures aimed at normalizing the tear film; and reduce the inflammation of the ocular surface (T lymphocytes and cytokines released from these) (13, 14). Recent date demonstrate that intestinal microorganism could influence lipid metabolism and act as environmental factors triggering development of metabolic and cardiovascular diseases. In addition other data have revealed a close relationship between inflammatory and metabolic pathways. Traditionally colonic bacteria were considered as agents activating inflammatory mechanisms. (15, 16). This is supported by multiple data showing the link between the microbiota, inflammation and autoimmunity. (17-21). Based on this knowledge we proposed to evaluate the effects of a new symbiotic with Bifidobacterium on the ecosystem eye.

Materials and methods

Patients

All patients with clinical or pathologic evidence of DES in our department were invited to participate in the study. In all 43 patients were enrolled of these 40 completed the study. T We collected all demographic data, date of first symptoms,

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date of diagnosis. (Table n.1) None of the patients had infections of the ocular surface and appendages or allergic diseases of the ocular surface in the last 30 days. We excluded patients with previous eye surgery, lachrymal disorders, and medical therapy with systemic or topical medications that alter the tearing and / or topical steroids during the 4 weeks preceding the start of the study. Other exclusion criteria are use of any symbiotic product intended to improve gastrointestinal function within the 2 weeks preceding study entry; major chronic and uncontrolled systemic medical conditions; lactose intolerance; chronic diarrhea; gastric bypass surgery or lap band insertion for weight loss; regular laxative use; pregnant or breast -feeding women. Following the run-in period subjects were randomized in two groups: group A (N°20 subjects) and group B (N°20 subjects). Group A (control) treated only with substitute tear and group B treated with substitute tear + mixture symbiotic). The mixture is composed of fructo-oligosaccaridi; Bifidobacterium lactis DSM 25566; Bifidobacterium bifido DSM 25565 (disbiocol cps. – 1 cps/day 30 days). Patients were instructed to not change their routine diet or other life stile habits, and no restrictions were placed on use of routine taken drugs. In all patients, the subjective symptoms and objective signs at the time of enrollment visit and after 30 days of treatment (symbiotic) were considered. Five days after cessation of treatment (washout) clinical parameters were reassessed. At the end of treatment, five days after washing, all of the patients belonging to the two groups A (control) and B (symbiotic) received Schirmer test I, II Schirmer test, BUT Test and bacteriological research. All patients with DLE were recruited and were admitted to the study (29 females and 11 males, age 57.5 ± 11.1 , Table 1 average) showing signs of discomfort and / or dry eyes (burning, foreign body sensation, dryness or itching)

Table 1. Demographic characteristics of patients who completed the study.

Patients n°	Sex		Age (mean)	Range
	M.	F.		
40	11	29	57.5	38-73

Methods

Evaluation of the clinical signs of dry eye considers three features of the tears film and ocular surface tears functions, tear composition, and ocular surface alterations. The simple tests of tear function are performed by direct observation all patients carry out. The questionnaire (OSDI) been administered to all patients. Tear film instability is a valuable sign of dry eye disease and can be produced by either aqueous - deficit dry eye or evaporative dry eye or a combination of both mechanisms.

The method for determining tear film stability is the tear flourescein in break-up time (TF BUT) that is performed by instilling a small amount of flourescein due into the tear film and having the patient blink while being observed through the slit-lamp with incident cobalt blue filtered light.

The uniform greenish hue of the flourescein across the cornea is observed for early breakup as identified by a dark spot forming in the tear film Normal TF BUT range is 10-15 seconds.

Rapid tear film breakup is an indicator of tear instability that can be due to dry eye or ocular surface irregularities. Determination of tear secretion rate differentiates aqueous - deficient dry eye from evaporative dry eye, and is most frequently done clinically by use of the Schirmer tear test strip.

The Schirmer test is performed placing a small strip of filter paper of known dimension (5x35 mm) on the margin of the lower eyelid at the junction of the lateral and middle third of the lid and leaving it in place for 5 minutes, then measuring the length of the strip that is wet with tears.

In this test is done without prior instillation of topical anesthetic, it is a measure of reflex secretion of tear (Schirmer 1 test); if the test is done following instillation of a topical anaesthetics, it is a measure of baseline tear secretion (*basal tear secretion test*).

The normal value of the Schirmer 1 test is greater than 10 mm of wetting, but cut-off referent values for dry eye have been recommended as 5 mm of wetting.

Some clinicians use a value of 7 mm with the Schirmer 1 test and 3 mm for the Schirmer with Anaesthetics.

Bacteriological analysis

It was carried out testing of conjunctiva swab Hess, to search for aerobic and anaerobic bacteria.

Samples from patients were seeded in the appropriate culture medium and incubated in aerobic and anaerobic atmosphere for the isolation and identification of bacteria, with separate counts for aerobic and anaerobic bacteria. After the identification of bacteria has been confirmed through Vitek (Biomerieux, Mercy l'Etoile, Francia) in case of aerobic bacteria and through API 20A (Biomerieux) in case of anaerobic bacteria.

Statistical Analysis

The results are expressed as mean +/- standard deviation. Statistical significance in contingency Tables was evaluated using the chi square and Fisher exact test. Student's test for unpaired data, one way ANOVA, and Mann-Whitney rank sum test were used for comparisons of continuous variables. Statistical analysis was performed using tests for repeated measures as well by controls for multiple comparisons with correction by Duncan Procedure.

Results

Baseline data

Baseline subject characteristics were similar among the two treatment groups.

Frequency of consumption of are food categories was, in general, similar across the treatment groups.

Effect of the Mixture of Probiotics on the DES

The data obtained in the two study groups A and B were, respectively the following: Schirmer 9.1±0.2 vs 12.7±0.4 (p<0.001); Schirmer II 3.5±0.1 VS 4.7±0.2 (p<0.001); BUT 3.9±0.3 vs 6.3±0.2 (p<0.001) (Fig. 1).

Culture test showed initial bacterial growth in group “A” (placebo) 18 out of 40 samples tested, corresponding to 45.0% and “B” after treatment (symbiotic) was found positive culture whit growth of bacteria in 12 tests equal to 30.0% (Table 2). The total numbers of isolations of aerobic and anaerobic bacteria found group A and B after treatment are shown in Table 3. A reduction of 15 to 11 strains of aerobic and anaerobic isolates from 9 to 5 has been found. Table 4 shows the species of aerobic and anaerobic bacteria found in patients A (placebo) and B after symbiotic treatment on the fifth day after discontinuation of therapy: *S. epidermidis* from 25.0% to 56.2%; for the *S. aureus* by 16.7% to 12.5%, while for the rest of isolates showed a reduction of almost homogenous aerobic Gram-negative before and after treatment. In the same table list the species of anaerobic bacteria isolated before and after treatment: for *Peptococcus* spp. from 20.7 to 25.0% for *Peptostreptococcus* from 8.3 to 6.3% and for *Propionibacterium* spp. from 4.2 to 0%.

Discussion and conclusions

The significant change in tear testing (Schirmer I, II and B.U.T.) obtained after treatment with the symbiotic, with the disappearance of symptoms, showed a good activity in the physiological recovery of the ocular ecosystem in patients with DES (22 – 26).

The present study shows that the administration of bifidobacterium may represent a success full treatment in ameliorating DES. Commensal bacteria or probiotics interact

Table 2. Overall incidence of culture positivity of bacteriological tests “A” and “B”.

N° pazients (40)	N°. Eyes (80)	Group A N°. %	Group B N°. %
	80	18 45	12 30

Table 3. Total number of aerobic and anaerobic isolates of cultures group “A” and “B”.

Microrganisms	Group A	Group B
Aerobes	15	11
Anaerobes	9	5

Table 4. Overall incidence of culture positivity of bacteriological tests group “A” and “B”.

Microrganism	Group A N° %	Group B N° %
<i>S. epidermidis</i>	6 25.0	9 56.2
<i>S. aureus</i>	4 16.7	2 12.5
<i>S.pneumoniae</i>	2 8.3	- -
<i>S.pyogens</i>	1 4.2	- -
<i>H. influenzae</i>	1 4.2	- -
<i>E. coli</i>	1 4.2	- -
<i>Peptococcus</i> spp.	5 20.7	4 25.0
<i>Peptostreptococcus</i> spp.	2 8.3	1 6.3
<i>Propionibacterium</i> spp.	1 4.2	- -
<i>Bacteroides</i> spp.	1 4.2	- -
Total strains	24 100	16 100

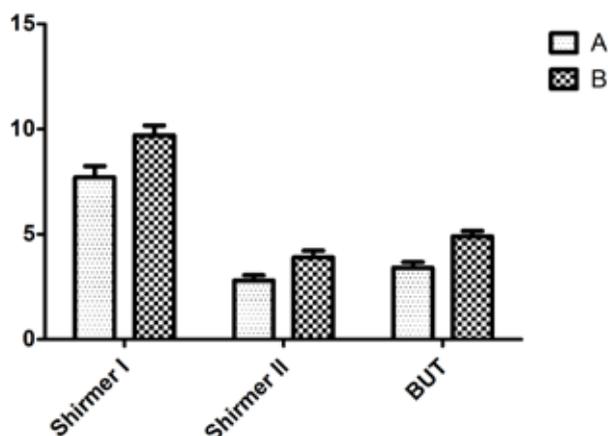


Fig. 1. Statistical analysis of clinical parameters in subjects of the group “A” and “B”.

with the endogenous enteric microbiota and gut cells therein confereing health benefit to the host, infact, the effect of imbalanced microbiota are not restricted by gastrointestinal abnormalities but could have systemic impact on immunity. Although data are somewhat limited, it has been shown that *Bifidobacterium* species have in particular been shown to display potential therapeutic proprieties. *Bifidobacterium* are used as beneficial food supplements in dairy products and play a protective role against pathogenic bacteria and allergies (27, 28). The gut microbiota appears to play role chronic inflammatory disease, through several mechanisms. Colonic microbiota could stimulate infiltration of macrophages in the adipose tissue by providing inflammatory stimuli such as LPS and enhancing energy intake from the food that leads to adipocyte hypertrophy (29-32). Free fatty acids and bacterial LPS act synergistically in stimulation of that inflammation. Although the gut flora contributes to a healthy environment, acute and chronic mucosal inflammation can arise as a result of both commensal and pathogenic bacteria that influence the innate and adaptative immune-systems. Intestinal microbes

can alter host defense mechanisms, leading to the activation of cytokines and the stimulation of adaptive T-cell and B-cell response. Many intervention studies were performed for a shorter periods, thus limiting a correct evolution of effectiveness, tolerability and adverse effect. The good compliance in taking capsules demonstrated by all patient, represent another relevant point for connect study outcome. In the present study no serious adverse events were detected. The intestinal symptoms described by two patients did not represent a limiting occurrence. They lasted for a short time, were mild, were not accompanied by an increase in stool frequency or a physical change in stool, did not interfere with normal activities. Because symptoms occurred at the start if both the probiotic and the placebo supplementation it is plausible to exclude any cause-effect relationship. The mechanisms involved in improvement of ocular surface are not known the probiota contribute to immunomodulation in tandem with both the innate and adaptive immune systems. The components and the cell type from the immune system that participate in the immunomodulatory process includes the gut associated tissues (GALT), effectors and regulatory T cells, IgA producing B cells. The indirect effect of this probiotic in the restoration of normal ocular microbiota, of the arrival of the normal bacterial inhabitants of the ocular surface. In fact the habitat of the ocular surface changes with the increase of saprophytic bacteria allow these bacteria integration with the glycocalyx of ocular epithelial cells by determining a stability and prevention barrier by the possible superficial infections. As has been demonstrated in previous searches, the stable tear film is the result of the balance of a series of complex functions implemented by the system of the ocular surface. an environment suitable for pH, electrolyte concentration, relative humidity and presence of nutritive elements fundamental is indispensable for the ocular surface can perform its main functions along with an integration of the normal bacterial flora that exerts a direct action and indirect defense of same surface (33 - 38). In fact, the function of physical and immunological barrier from the epithelium of the surface eye piece is ensured by the tight junction of epithelial cells that precisely determines the barrier effect versus pathogenic bacteria. It has been shown by several studies that if the lacrimal osmolarity increases chronically can result in damage to the epithelial cells of the ocular surface (39, 40). On the base of these results, we are identified in our Bifidobacterium activity integration with the action of tear substitutes, along with standardization of clinical parameters of the tear film and microbiological activity in restoring of the microbiota ocular surface subject with DES.

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